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(54) Title: COMPOUNDS AND COMPOSITIONS FOR INTRACELLULAR DELIVERY OF AGENTS

(57) Abstract: The disclosure features amino lipids and compositions involving the same. Nanoparticle compositions include an amino lipid as well as additional lipids such as phospholipids, structural lipids, PEG lipids, or a combination thereof. Nanoparticle compositions further including therapeutic and/or prophylactic agents such as RNA are useful in the delivery of therapeutic and/or prophylactic agents to mammalian cells or organs to, for example, regulate polypeptide, protein, or gene expression.

COMPOUNDS AND COMPOSITIONS FOR INTRACELLULAR DELIVERY OF AGENTS

RELATED APPLICATIONS

[001] This application claims priority to, and the benefit of, U.S. Provisional Application No. 62/519,826, filed June 14, 2017, the entire contents of which are incorporated herein by reference in their entireties.

TECHNICAL FIELD

[002] The present disclosure provides compounds, compositions comprising such compounds, and methods involving lipid nanoparticle compositions to deliver one or more therapeutic and/or prophylactic agents to and/or produce polypeptides in mammalian cells or organs. In addition to an amino lipid, lipid nanoparticle compositions of the disclosure may include one or more cationic and/or ionizable amino lipids, phospholipids including polyunsaturated lipids, PEG lipids, structural lipids, and/or therapeutic and/or prophylactic agents in specific fractions.

BACKGROUND

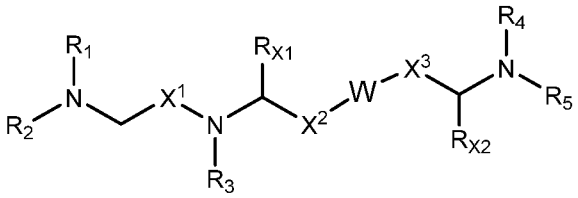
[003] The effective targeted delivery of biologically active substances such as small molecule drugs, proteins, and nucleic acids represents a continuing medical challenge. In particular, the delivery of nucleic acids to cells is made difficult by the relative instability and low cell permeability of such species. Thus, there exists a need to develop methods and compositions to facilitate the delivery of therapeutic and/or prophylactic agents such as nucleic acids to cells.

[004] Lipid-containing nanoparticle compositions, liposomes, and lipoplexes have proven effective as transport vehicles into cells and/or intracellular compartments for biologically active substances such as small molecule drugs, proteins, and nucleic acids. Such compositions generally include one or more "cationic" and/or amino (ionizable) lipids, phospholipids including polyunsaturated lipids, structural lipids (e.g., sterols), and/or lipids containing polyethylene glycol (PEG lipids). Cationic and/or ionizable lipids include, for example, amine-containing lipids that can be readily protonated. Though a variety of such lipid-containing nanoparticle compositions have been demonstrated, improvements in safety, efficacy, and specificity are still lacking.

SUMMARY

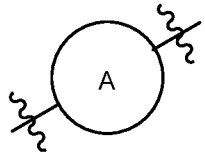
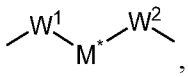
[005] The present disclosure provides compounds and compositions and methods involving the same.

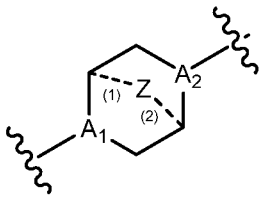
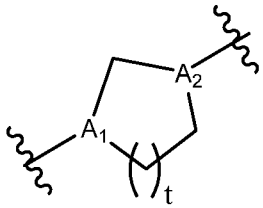
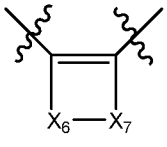
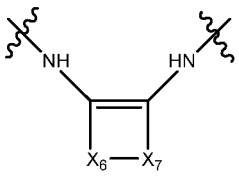
[006] In one aspect, the disclosure provides a compound having the formula (I)



(I),

or a salt or isomer thereof, wherein

W is  or  ,

ring A is  ,  ,  , or  ;

t is 1 or 2;

A_1 and A_2 are each independently selected from CH or N;

Z is CH_2 or absent wherein when Z is CH_2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

R_1, R_2, R_3, R_4, and R_5 are independently selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

R_{X1} and R_{X2} are each independently H or C1-3 alkyl;

each M is independently selected from the group consisting of -C(O)O-, -OC(O)-, -OC(O)O-, -C(O)N(R')-, -N(R')C(O)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(O)(OR')O-, $-S(O)_2-$, -C(O)S-, -SC(O)-, an aryl group, and a heteroaryl group;

M* is -O-, or C_1-C_6 alkyl,

W^1 and W^2 are each independently selected from the group consisting of a bond, **-O-** and **-N(R_6)-;**

each R_6 is independently selected from the group consisting of H and C1-5 alkyl;

X^1, X^2, and X^3 are independently selected from the group consisting of a bond, -CH2-, **-(CH_2)$_2$-, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, -(CH_2)n-C(0)-, -C(0)-(CH_2)n-, -(CH_2)$_n$-C(0)0-, -OC(0)-(CH_2)$_n$-, -(CH_2)$_n$-OC(0)-, -C(0)0-(CH_2)n-, -CH(OH)-, -C(S)-,** and -CH(SH)-;

each **Y** is independently a C3-6 carbocycle;

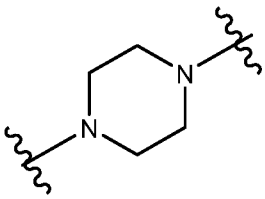
each **R*** is independently selected from the group consisting of **Ci-i$_2$** alkyl and **C_2-i$_2$** alkenyl;

each **R** is independently selected from the group consisting of C1-3 alkyl and a C3-6 carbocycle;

each **R'** is independently selected from the group consisting of C1-18 alkyl, **C_2-is** alkenyl, and H;

each **R"** is independently selected from the group consisting of **C 3-i$_2$** alkyl, ***C3-12*** alkenyl and **-R*MR';** and

n is an integer from **1**-6;



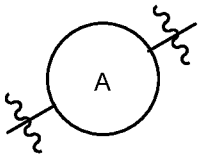
wherein when ring A is , then

i) at least one of X^1, X^2, and X^3 is not -CH_2-; and/or

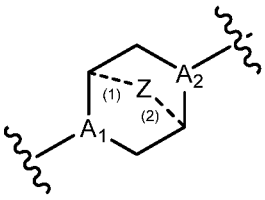
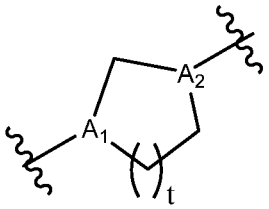
ii) at least one of **Ri, R_2,** R3, R4, and R 5 is **-R"MR'.**

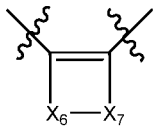
[007] The compounds of formula (I) may include one or more of the following features when applicable.

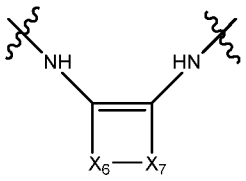
[008] In some embodiments, M* is C1-C6 alkyl and W^1 and W^2 are each independently selected from the group consisting of **-O-** and -N(R6)-.

[009] In some embodiments, W is 

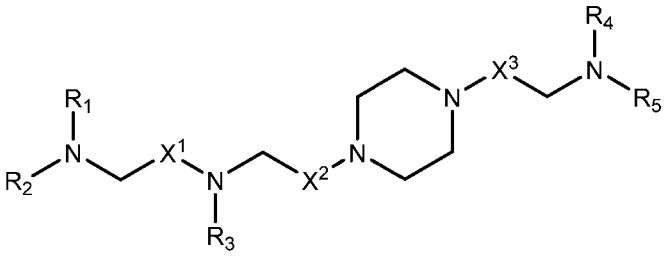
.

[0010] In some embodiments, ring A is  or  .

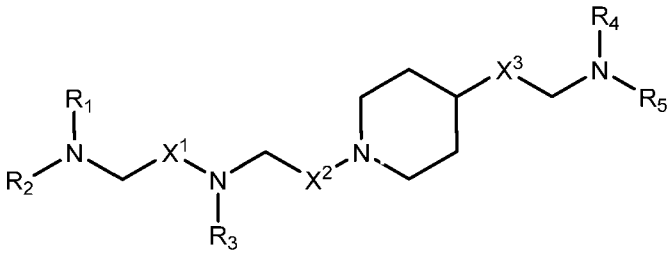
[0011] In some embodiments, ring A is  .

[0012] In some embodiments, ring A is  .

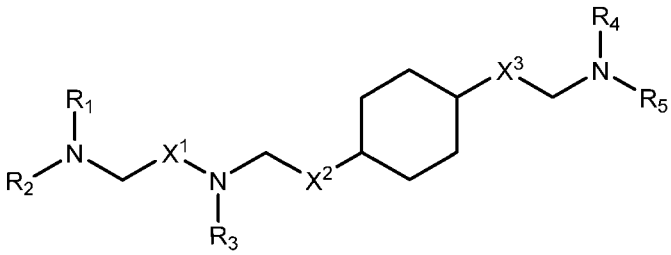
[0013] In some embodiments, the compound is of any of formulae (Ial)-(Ia7):



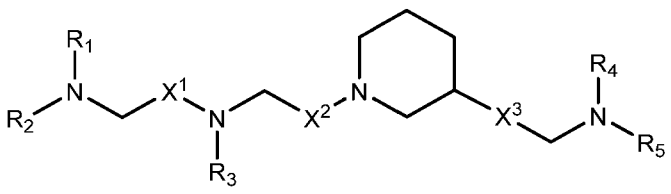
(Ia1),



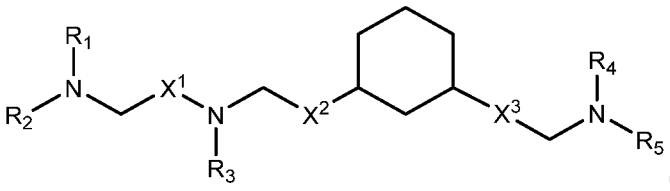
(Ia2),

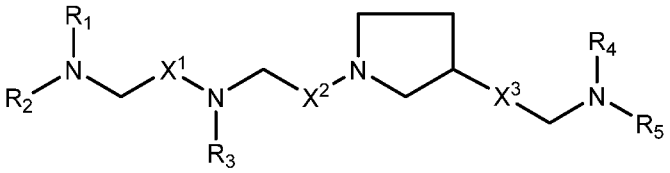


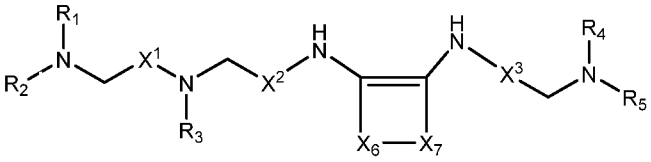
(Ia3),

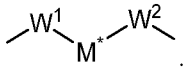


(Ia4),

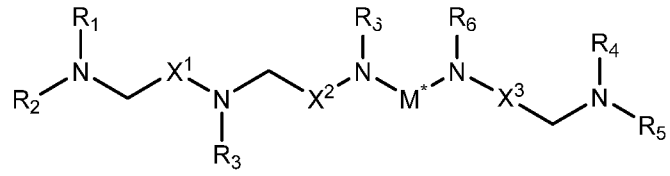

(Ia5),

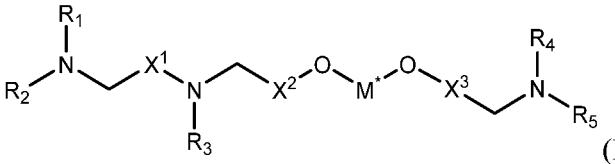

(Ia6), or

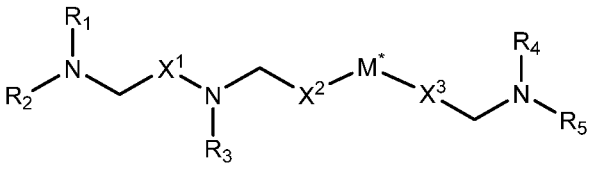

(Ia7).

[0014] In some embodiments, W is  .

[0015] In some embodiments, the compound is of any of formulae (Ia8)-(Ial0):


(Ia8),


(Ia9), or


(Ia10).

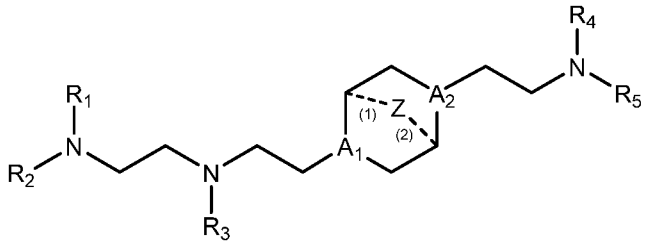
[0016] In some embodiments, at least one of X^1, X^2, and X^3 is not -CH2-. For example, in certain embodiments, X^1 is not -CH2-. In some embodiments, at least one of X^1, X^2, and X^3 is -C(O)-. In some embodiments, X^3 is a bond while each of X^1 and X^2 is not a bond. In some embodiments, none of X^1, X^2, and X^3 is a bond.

[0017] In some embodiments, R_i and R_2 are the same. In certain embodiments, Ri, R2, and R_3 are the same. In some embodiments, R_4 and R_5 are the same. In certain embodiments, Ri, R2, R3, R4, and R_5 are the same.

[0018] In some embodiments, at least one of **Ri,** R2, R3, R4, and R5 is **-R"MR'.** In some embodiments, at most one of **Ri,** R2, R3, R4, and R5 is **-R"MR'.** For example, at least one of **Ri,** R2, and R3 may be **-R"MR',** and/or at least one of R4 and R5 is **-R"MR'.** In certain embodiments, at least one M is -C**(0**)O-. In some embodiments, each M is -C**(0**)O-. In some embodiments, at least one M is -OC(O)-. In some embodiments, each M is -OC(O)- In some embodiments, at least one **R"** is C3 alkyl. In certain embodiments, each **R"** is C3 alkyl. In some embodiments, at least one **R"** is C5 alkyl. In certain embodiments, each **R"** is C5 alkyl. In some embodiments, at least one **R"** is C6 alkyl. In certain embodiments, each **R"** is C6 alkyl. In some embodiments, at least one **R"** is C7 alkyl. In certain embodiments, each **R"** is C7 alkyl. In some embodiments, at least one **R'** is C5 alkyl. In certain embodiments, each **R'** is C5 alkyl. In other embodiments, at least one **R'** is **Ci** alkyl. In certain embodiments, each **R'** is **Ci** alkyl. In some embodiments, at least one **R'** is C2 alkyl. In certain embodiments, each **R'** is C2 alkyl.

[0019] In some embodiments, at least one of **Ri,** R2, R3, R4, and R5 is C12 alkyl. In certain embodiments, each of **Ri,** R2, R3, R4, and R5 are C12 alkyl.

[0020] In another aspect, the disclosure provides a compound having formula (II):



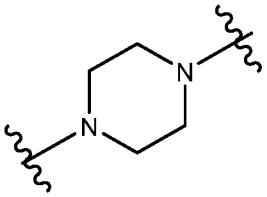
(Π),

or a salt or isomer thereof, wherein

Ai and A2 are each independently selected from CH or N and at least one of **Ai** and A2 is N;

Z is CH2 or absent wherein when Z is CH2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

Ri, R2, R3, R4, and R5 are independently selected from the group consisting of C6-20 alkyl and C6-20 alkenyl;

wherein when ring **A** is 

, then

i) **Ri, R2, R3, R4,** and **R 5** are the same, wherein **R i** is not **C12** alkyl, **Cis** alkyl, or **Cis** alkenyl;

ii) only one of **Ri, R2, R3, R4,** and **R 5** is selected from **C6-20** alkenyl;

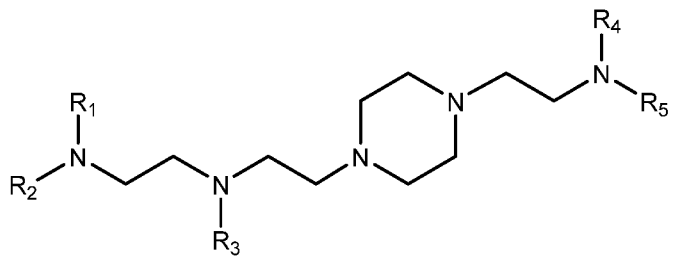
iii) at least one of **Ri, R2, R3, R4,** and **R 5** have a different number of carbon atoms than at least one other of **Ri, R2, R3, R4,** and **R5;**

iv) **Ri, R2,** and **R 3** are selected from **C6-20** alkenyl, and **R 4** and **R 5** are selected from **C6-20** alkyl; or

v) **Ri, R2,** and **R 3** are selected from **C6-20** alkyl, and **R 4** and **R 5** are selected from **C6-20** alkenyl.

[0021] The compounds of formula (II) may include one or more of the following features when applicable.

[0022] In some embodiments, the compound is of formula (Ila):



(Ila).

[0023] In some embodiments, **Ri, R2, R3, R4,** and **R 5** are the same, and are not **C12** alkyl, **Cis** alkyl, or **Cis** alkenyl. In some embodiments, **Ri, R2, R3, R4,** and **R 5** are the same and are **C 9** alkyl or **Ci4** alkyl.

[0024] In some embodiments, only one of **Ri, R2, R3, R4,** and **R 5** is selected from **C6-20** alkenyl. In certain such embodiments, **Ri, R2, R3, R4,** and **R 5** have the same number of carbon atoms. In some embodiments, **R 4** is selected from **C5-20** alkenyl. For example, **R 4** may be **C12** alkenyl or **Cis** alkenyl.

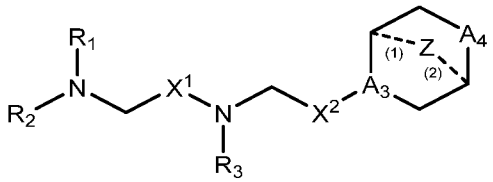
[0025] In some embodiments, at least one of **Ri, R2, R3, R4,** and **R 5** have a different number of carbon atoms than at least one other of **Ri, R2, R3, R4,** and **R5.**

[0026] In certain embodiments, **Ri, R2,** and **R 3** are selected from **C6-20** alkenyl, and **R 4** and **R 5** are selected from **C6-20** alkyl. In other embodiments, **Ri, R2,** and **R 3** are selected from **C6-20** alkyl, and **R 4** and **R 5** are selected from **C6-20** alkenyl. In some embodiments, **Ri, R2,** and **R 3** have the same number of carbon atoms, and/or **R 4** and **R 5** have the same number of carbon atoms. For example, **Ri, R2,** and **R3,** or **R 4** and **R5,** may have 6, 8, 9, 12, 14, or 18 carbon atoms. In some

embodiments, **Ri, R2,** and **R3,** or **R4** and **R5,** are **Cis** alkenyl (e.g., linoleyl). In some embodiments, **Ri, R2,** and **R3,** or **R4** and **R5,** are alkyl groups including 6, 8, 9, 12, or 14 carbon atoms.

[0027] In some embodiments, **Ri** has a different number of carbon atoms than **R2, R3, R4,** and **R5.** In other embodiments, **R3** has a different number of carbon atoms than **Ri, R2, R4,** and **R5.** In further embodiments, **R4** has a different number of carbon atoms than **Ri, R2, R3,** and **R5.**

[0028] In another aspect, the disclosure provides a compound according to formula (III):



(HI),

or a salt or isomer thereof, in which

A_3 is CH or N;

A_4 is **CH2** or NH; and at least one of A_3 and A_4 is N or NH;

Z is **CH2** or absent wherein when Z is **CH2,** the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

Ri, R2, and **R3** are independently selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, **-R"MR', -R*YR", -YR",** and **-R*OR";**

each M is independently selected from -C(0)0-, **-OC(O)-, -C(0)N(R')-,** -N**(R')**C(0)-, **-C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0** -, **-S(0)2-,** an aryl group, and a heteroaryl group;

X^1 and X^2 are independently selected from the group consisting of **-CH2-,** **-$(CH_2)_2$-, -CHR-, -CHY-, -C(O)-,** -C(0)0-, **-OC(O)-, -C(0)-CH_2-, -CH_2-C(0)-,** -C(0)0-CH_2-, -OC(0)-CH_2-, -CH_2-C(0)0-, -CH_2-OC(0)-, -CH(OH)-, -C(S)-, and -CH(SH)-;

each **Y** is independently a C_{3-6} carbocycle;

each **R*** is independently selected from the group consisting of C_{i-12} alkyl and C_{2-12} alkenyl;

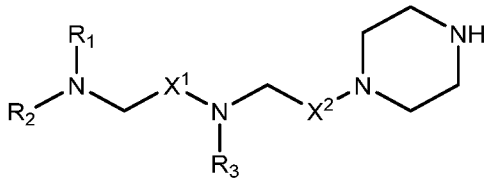
each **R** is independently selected from the group consisting of C_{1-3} alkyl and a C_{3-6} carbocycle;

each **R'** is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, and H; and

each R" is independently selected from the group consisting of C3-12 alkyl and C 3-12 alkenyl.

[0029] The compounds of formula (III) may include one or more of the following features when applicable.

[0030] In some embodiments, the compound is of formula (Ilia):

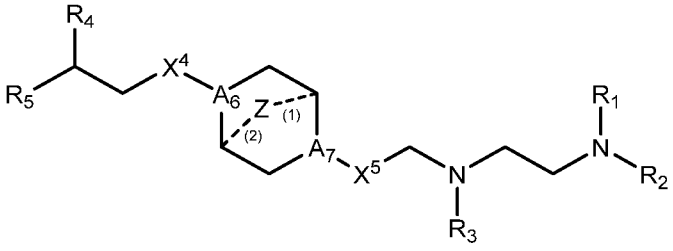


(Ilia).

[0031] In some embodiments, Ri, R2, and R 3 are independently selected from the group consisting of C 5-20 alkyl and C 5-20 alkenyl. In some embodiments, Ri, R2, and R 3 are the same. In certain embodiments, Ri, R2, and R 3 are C_6, C9, C12, or C14 alkyl. In other embodiments, Ri, R2, and R 3 are Cis alkenyl. For example, Ri, R2, and R 3 may be linoleyl.

[0032] In some embodiments, at least one of X^1 and X^2 is not -CH2-. For example, in certain embodiments, X^1 is not -CH2-. In some embodiments, at least one of X^1 and X^2 is -C(O)-.

[0033] In another aspect, the disclosure provides a compound according to formula (lb):



(Ib),

or a salt or isomer thereof, in which

A_6 and A_7 are each independently selected from CH or N, wherein at least one of A_6 and A_7 is N;

Z is CH2 or absent wherein when Z is CH2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

X^4 and X^5 are independently selected from the group consisting of -CH2-, $-(CH_2)_2-$, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, -C(0)-CH_2-, -CH_2-C(0)-, -C(0)0-CH_2-, -OC(0)-CH_2-, -CH_2-C(0)0-, -CH_2-OC(0)-, -CH(OH)-, -C(S)-, and -CH(SH)-;

Ri, R2, R3, R4, and R 5 each are independently selected from the group consisting of C 5-20 alkyl and C 5-20 alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

each M is independently selected from the group consisting of **-C(0)0-, -OC(O)-, -C(0)N(R')-, -N(R')C(0)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0-, -S(0)2-**, an aryl group, and a heteroaryl group;

each Y is independently a $_{C3-6}$ carbocycle;

each **R*** is independently selected from the group consisting of **Ci-**$_{12}$ alkyl and $_{C2-12}$ alkenyl;

each **R** is independently selected from the group consisting of $_{C1-3}$ alkyl and a $_{C3-6}$ carbocycle;

each **R'** is independently selected from the group consisting of $_{C1-18}$ alkyl, $_{C2-18}$ alkenyl, and H; and

each **R"** is independently selected from the group consisting of $_{C\ 3-12}$ alkyl and $_{C\ 3-12}$ alkenyl.

[0034] The compounds of formula (lb) may include one or more of the following features when applicable.

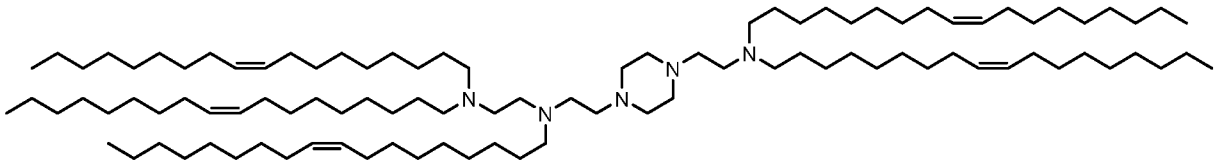
[0035] In some embodiments, **Ri** and $_{R\,2}$ are the same. In certain embodiments, **Ri,** R2, and $_{R\ 3}$ are the same. In some embodiments, $_{R\ 4}$ and $_{R\ 5}$ are the same. In certain embodiments, **Ri,** R2, $_{R3,\ R4,}$ and $_{R\ 5}$ are the same.

[0036] In some embodiments, at least one of **Ri,** R2, $_{R3,\ R4,}$ and $_{R\ 5}$ is $_{C\ 9-12}$ alkyl. In certain embodiments, each of **Ri,** R2, $_{R3,\ R4,}$ and $_{R\ 5}$ independently is $_{C9,}$ C12 or $_{C14}$ alkyl. In certain embodiments, each of **Ri,** R2, $_{R3,\ R4,}$ and $_{R\ 5}$ is $_{C\ 9}$ alkyl.

[0037] In some embodiments, $_{A\ 6}$ is **N** and A7 is **N.** In some embodiments, $_{A\ 6}$ is **CH** and A7 is **N.**

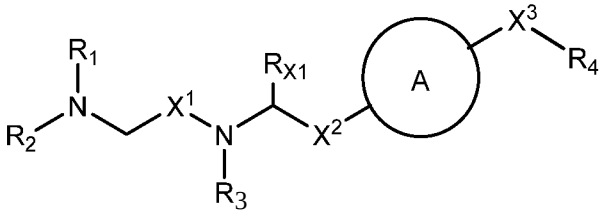
[0038] In some embodiments, **X**^{4} $_{iS}$-CH2- and **X**^{5} is **-C(O)-.** In some embodiments, **X**^{4} and **X**^{5} are **-C(O)-.**

[0039] In an embodiment, the compound has the formula (IV)



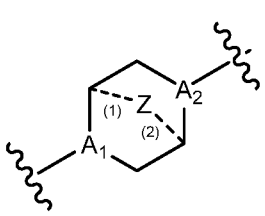
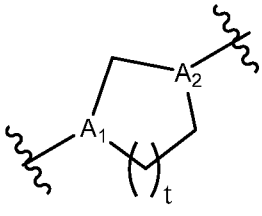
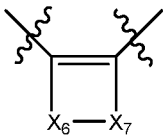
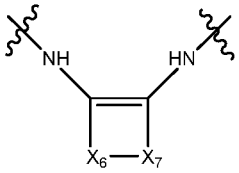
(IV).

[0040] In another aspect, the disclosure provides a compound according to formula (Ic):



(Ic),

or a salt or isomer thereof, wherein

ring A is  ,  ,  , or  ;

t is 1 or 2;

A_1 and A_2 are each independently selected from CH or N;

Z is CH_2 or absent wherein when Z is CH_2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

R_1, R_2, R_3, and R_4 are independently selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

R_{X1} is H or C1-3 alkyl;

each M is independently selected from the group consisting of -C(0)0-, -OC(O)-, -OC(0)0-, -C(0)N(R')-, -N(R')C(0)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0-, $-S(0)_2-$, -C(0)S-, -SC(O)-, an aryl group, and a heteroaryl group;

X^1, X^2, and X^3 are independently selected from the group consisting of a bond, -CH2-, $-(CH_2)_2-$, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, $-(CH_2)_n$-C(0)-, -C(0)-$(CH_2)_n$-,-$(CH_2)_n$-C(0)0-, -OC(0)-$(CH_2)_n$-, $-(CH_2)_n$-OC(0)-, -C(0)0-$(CH_2)_n$-, -CH(OH)-, -C(S)-, and -CH(SH)-;

each Y is independently a C3-6 carbocycle;

each R* is independently selected from the group consisting of Ci-12 alkyl and C2-12 alkenyl;

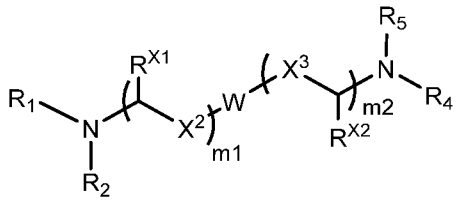
each R is independently selected from the group consisting of C1-3 alkyl and a C3-6 carbocycle;

each R' is independently selected from the group consisting of Ci-is alkyl, C2-18 alkenyl, and H;

each R'' is independently selected from the group consisting of C 3-12 alkyl, C 3-12 alkenyl and -R*MR'; and

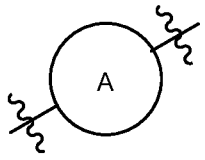
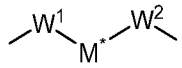
n is an integer from 1-6.

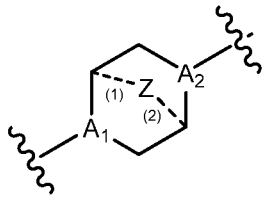
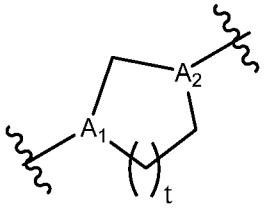
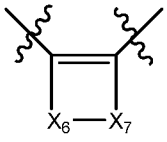
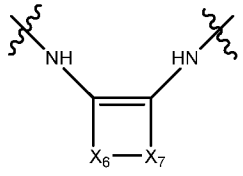
[0041] In another aspect, the disclosure provides a compound according to formula (Id):



(Id),

or a salt or isomer thereof, wherein

W is  or  ;

ring A is  ,  ,  , or  ;

t is 1 or 2;

Ai and A2 are each independently selected from CH or N;

Z is CH2 or absent wherein when Z is CH2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

Ri, R2, R4, and R 5 are independently selected from the group consisting of C 5-20 alkyl, C 5-20 alkenyl, -R''MR', -R*YR'', -YR'', and -R*OR'';

Rxi and Rx2 are each independently H or C1-3 alkyl;

each M is independently selected from the group consisting of -C(0)0-, -OC(O)-, -OC(0)0-, -C(0)N(R')-, -N(R')C(0)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0-, -S(0)$_2$-, -C(0)S-, -SC(O)-, an aryl group, and a heteroaryl group;

M* is -0-, Ci-Ce alkyl, -((CH$_2$)pCH(R7)(CH2)qCH(R7)(CH$_2$)r)s-, or -((CH$_2$)tC(=R8)(CH2)uC(=R8)(CH$_2$)v)s-;

W^1 and W^2 are each independently selected from the group consisting of a bond, -O- and -N(R$_6$)-;

each R$_6$ is independently selected from the group consisting of H and C1-3 alkyl;

each R$_7$ is independently selected from the group consisting of C1-3 alkyl, (CH2)oCN, C1-3 hydroxy alkyl, and C1-C3 haloalkyl;

each R$_8$ is independently selected from the group consisting of CH(CH2)oCN, CH(CH$_2$)oCH$_3$, CH(CH$_2$)oOH, and CHX^{Hal};

X^{Hal} is C1-C3 haloalkyl;

w is an integer from 1-3;

X^2 and X^3 are independently selected from the group consisting of a bond, -CH2-, -(CH$_2$)$_2$-, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, -(CH$_2$)n-C(0)-, -C(0)-(CH$_2$)n-, -(CH$_2$)n-C(0)0-, -OC(0)-(CH$_2$)n-, -(CH$_2$)n-OC(0)-, -C(0)0-(CH$_2$)n-, -CH(OH)-, -C(S)-, and -CH(SH)-;

X^6 and X^7 are each independently selected from the group consisting of -C(O)-, -C(S)-, -C((CH$_2$)wOH)-, and -C(X^{Ha1})-;

each Y is independently a C3-6 carbocycle;

each R* is independently selected from the group consisting of Ci-12 alkyl and C2-12 alkenyl;

each R is independently selected from the group consisting of C1-3 alkyl and a C3-6 carbocycle;

each R' is independently selected from the group consisting of C1-18 alkyl, C2-18 alkenyl, and H;

each R" is independently selected from the group consisting of C3-12 alkyl, C3-12 alkenyl and -R*MR';

m1 and m2 are each independently 0 or 1;

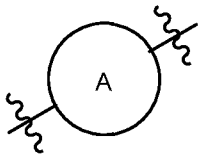
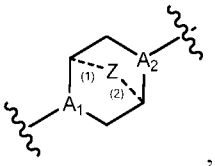
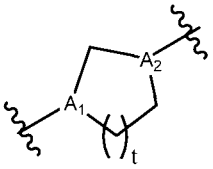
n is an integer from 1-6;

0 is an integer from 0-3;

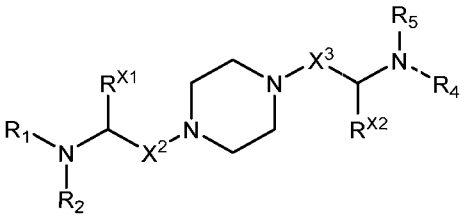
p, q, r, t, u, and v are each independently an integer from 0-3;

w is an integer from 0-3; and

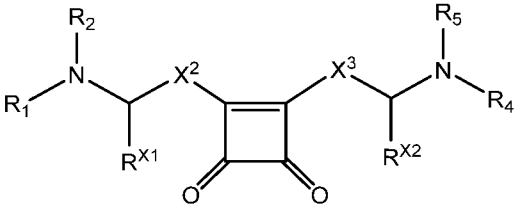
s is an integer from 1-3.

[0042] In some embodiments of formula (Id), W is  and ring A is  , or  ; and m1 and m2 are each 1.

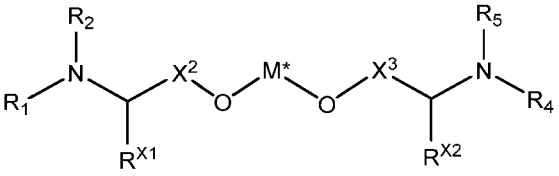
[0043] In some embodiments, the compound is of any of formulae (Idl)-(IdlO):



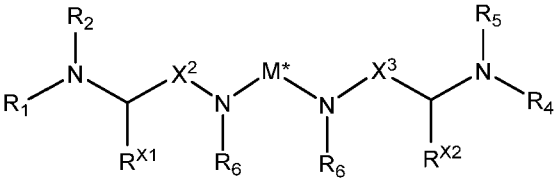
(Id1),



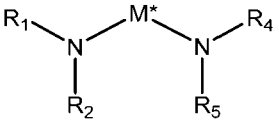
(Id2),



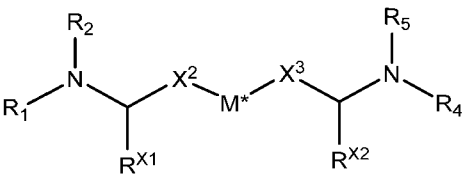
(Id3),



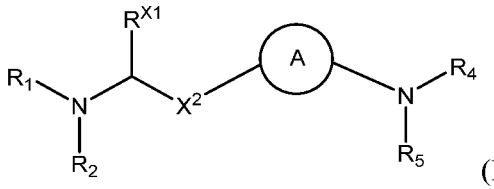
(Id4),



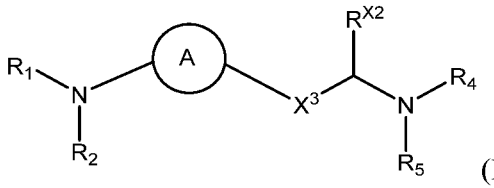
(Id5),



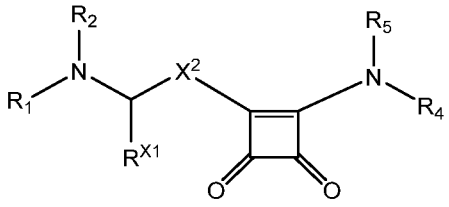
(Id6),



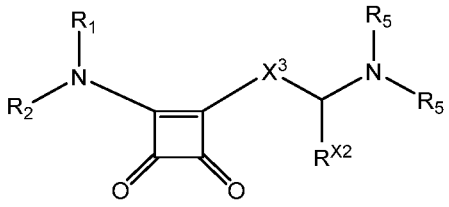
(Id7),



(Id8),

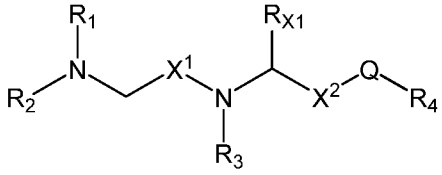


(Id9), or



(Id10).

[0044] In another aspect, the disclosure provides a compound according to formula (Ie):



(Ie), or a salt or isomer thereof, wherein

Ri, R2, $_{R3,}$ and $_{R\ 4}$ are independently selected from the group consisting of $_{C\ 5\text{-}20}$ alkyl, $_{C5\text{-}20}$ alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

Rxi is H or C1-3 alkyl;

each M is independently selected from the group consisting of -C(0)0-, -OC(O)-, -OC(0)0-, -C(0)N(R')-, -N(R')C(0)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0-, -S(0) $_{2}$-, -C(0)S-, -SC(O)-, an aryl group, and a heteroaryl group;

X^1, and X^2 are independently selected from the group consisting of a bond, -CH2-, -$(CH_2)_2$-, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, -$(CH_2)_n$-C(0)-, -C(0)-$(CH_2)_n$-,-$(CH_2)_n$-C(0)0-, -OC(0)-$(CH_2)_n$-, -$(CH_2)_n$-OC(0)-, -C(0)0-$(CH_2)_n$-, -CH(OH)-, -C(S)-, and -CH(SH)-;

Q is **-NR9-,** or a bond;

$_{R\ 9}$ is H or Ci-3 alkyl;

each Y is independently a $_{C3\text{-}6}$ carbocycle;

each R^* is independently selected from the group consisting of C_{1-12} alkyl and C_{2-12} alkenyl;

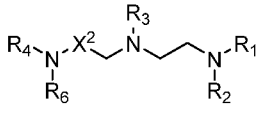
each R is independently selected from the group consisting of C_{1-3} alkyl and a C_{3-6} carbocycle;

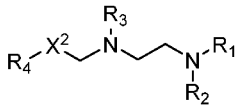
each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, and H;

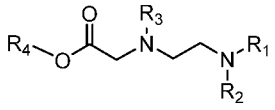
each R'' is independently selected from the group consisting of C_{3-12} alkyl, C_{3-12} alkenyl and $-R^*MR'$; and

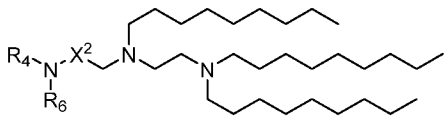
n is an integer from 1-6.

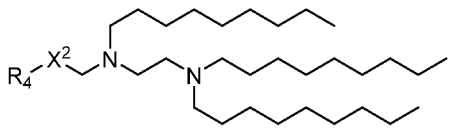
[0045] In some embodiments, the compound is of any of formulae (Iel)-(Ie6):

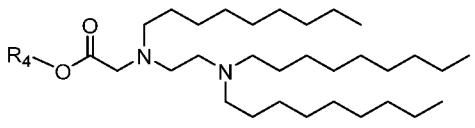

(Ie1),


(Ie2),


(Ie3),

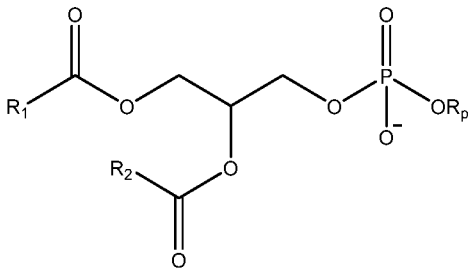

(Ie4),


(Ie5), or


(Ie6).

[0046] In a further aspect, the disclosure features a nanoparticle composition including a lipid component comprising a compound as described above (e.g., a compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia)). In some embodiments, the lipid component of the nanoparticle composition includes a

phospholipid. In certain embodiments, a phospholipid of a nanoparticle composition includes a phospholipid moiety and one or more fatty acid moieties, one or more of which may be unsaturated. For example, a nanoparticle composition may include a lipid according to formula (V)



(V)

in which R_p represents a phospholipid moiety and Ri and R2 represent unsaturated fatty acid moieties that may be the same or different.

[0047] A phospholipid moiety may be selected from the non-limiting group consisting of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl serine, phosphatidic acid, 2-lysophosphatidyl choline, and a sphingomyelin. A fatty acid moiety may be selected from the non-limiting group consisting of lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, erucic acid, arachidic acid, arachidonic acid, phytanic acid, eicosapentaenoic acid, behenic acid, docosapentaenoic acid, and docosahexaenoic acid. For example, in certain embodiments, a phospholipid is selected from the group consisting of
l,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC),
1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), l,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), l,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC),
l,2-distearoyl-sn-glycero-3-phosphocholine (DSPC),
1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC),
l-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC),
l,2-di-0-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC),
l-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC),
l-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC),
l,2-dilinolenoyl-sn-glycero-3-phosphocholine,
l,2-diarachidonoyl-sn-glycero-3-phosphocholine,
l,2-didocosahexaenoyl-sn-glycero-3-phosphocholine,l,2-dioleoyl-sn-glycero-3-phosphoethanol amine (DOPE), l,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE),

l,2-distearoyl-sn-glycero-3-phosphoethanolamine,

l,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine,

l,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine,

l,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine,

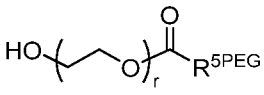
l,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine,

l,2-dioleoyl-sn-glycero-3-phospho-rac-(l -glycerol) sodium salt (DOPG), and sphingomyelin. In certain embodiments, the phospholipid is DOPE. In other embodiments, the phospholipid is DSPC. Non-natural species including natural species with modifications and substitutions including branching, oxidation, cyclization, and alkynes are also contemplated.

[0048] In some embodiments, the lipid component of the nanoparticle composition includes a structural lipid. In certain embodiments, a structural lipid is selected from the group consisting of cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, and alpha-tocopherol. In certain embodiments, the structural lipid is cholesterol.

[0049] In some embodiments, the lipid component of the nanoparticle composition includes a PEG lipid. In certain embodiments, the PEG lipid is selected from the group consisting of a PEG-modified phosphatidylethanolamine, a PEG-modified phosphatidic acid, a PEG-modified ceramide, a PEG-modified dialkylamine, a PEG-modified diacylglycerol, and a PEG-modified dialkylglycerol.

[0050] In certain embodiments, a PEG lipid may be of Formula (VI):



(VI), or a salt or isomer thereof, wherein:

R^{3PEG} is-OR^{0};

R° is hydrogen, Ci-6 alkyl or an oxygen protecting group;

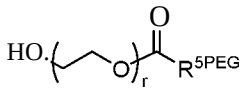
r is an integer between 1 and 100;

R^{5PEG} is Cio-40 alkyl, Cio-40 alkenyl, or Cio-40 alkynyl; and optionally one or more methylene groups of R^{5PEG} are independently replaced with C_{3-10} carbocyclylene, 4 to 10 membered heterocyclylene, C_{6-10} arylene, 4 to 10 membered heteroarylene, -N(R^{N})-, -0-, -S-, -C(O)-, -C(0)N(R^{N})-, -NR^{N}C(0)-, -NR^{N}C(0)N(R^{N})-, -C(0)0-, -OC(O)-, -OC(0)0-, -OC(0)N(R^{N})-, -NR^{N}C(0)0-, -C(0)S-, -SC(O)-, -C(=NR^{N})-, -C(=NR^{N})N(R^{N})-, -NR^{N}C(=NR^{N})-, -NR^{N}C(=NR^{N})N(R^{N})-, -C(S)-, -C(S)N(R^{N})-, -NR^{N}C(S)-, -NR^{N}C(S)N(R^{N})-, -S(O)-, -OS(O)-, -S(0)0-, -OS(0)0-, -OS(0)$_2$-, -S(0)$_2$0-, -OS(0)$_2$0-, -N(R^{N})S(0)-, -

$-S(O)N(R^N)-$, $-N(R^N)S(O)N(R^N)-$, $-OS(O)N(R^N)-$, $-N(R^N)S(O)O-$, $-S(O)_2-$, $-N(R^N)S(O)_2-$, $-S(O)_2N(R^N)-$, $-N(R^N)S(O)_2N(R^N)-$, $-OS(O)_2N(R^N)-$, or $-N(R^N)S(O)_2O-$; and

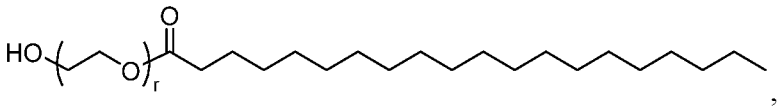
each instance of R^N is independently hydrogen, C_{1-6} alkyl, or a nitrogen protecting group.

[0051] In certain embodiments, the compound of Formula (VI) is of Formula (Vl-a):



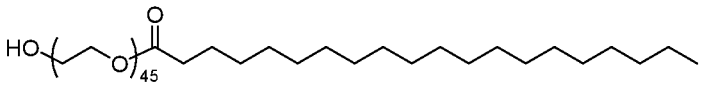
(VI-a), or a salt or isomer thereof.

[0052] In certain embodiments, a compound of Formula (VI) is of Formula (Vl-b):



, (VI-b) or a salt or isomer thereof.

[0053] In certain embodiments, the compound of Formula (Vl-b) is a compound having the formula:



(PEG 1) or a salt or isomer thereof.

[0054] In certain embodiments, the incorporation of lipids of one of formulae (VI), (VI-a) or (Vl-b) in the nanoparticle formulation can improve the pharmacokinetics and/or biodistribution of the lipid nanoparticle formulations. For example, incorporation of **PEG-OH** lipids in the nanoparticle formulation can reduce the accelerated blood clearance (ABC) effect.

[0055] In some embodiments, the nanoparticle composition includes a lipid component comprising a compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(Idl O), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), a phospholipid (which may or may not be unsaturated), a **PEG** lipid, and a structural lipid. In certain embodiments, the lipid component of the nanoparticle composition includes about 30 mol % to about 60 mol % compound of one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(Idl O), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), about 0 mol % to about 30 mol % phospholipid, about 18.5 mol % to about 48.5 mol % structural lipid, and about 0 mol % to about 10 mol % of **PEG** lipid. In some embodiments, the lipid component of the nanoparticle composition includes about 30 mol % to about 45 mol % compound of one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(Idl O), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), about 5 mol % to about 25 mol % phospholipid, about 30 mol % to about 40 mol % structural lipid, and about 0 mol % to about 10 mol % of **PEG** lipid. In some

embodiments, the lipid component of the nanoparticle composition includes about 35 mol % to about 55 mol % compound of one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), about 5 mol % to about 25 mol % phospholipid, about 30 mol % to about 40 mol % structural lipid, and about 0 mol % to about 10 mol % of PEG lipid. In certain embodiments, the lipid component includes about 50 mol % said compound, about 10 mol % phospholipid, about 38.5 mol % structural lipid, and about 1.5 mol % of PEG lipid. In other embodiments, the lipid component includes about 40 mol % said compound, about 20 mol % phospholipid, about 38.5 mol % structural lipid, and about 1.5 mol % of PEG lipid. In some of these embodiments, the phospholipid is DOPE, while in other embodiments the phospholipid is DSPC. In certain embodiments, the structural lipid is cholesterol. In certain embodiments, the PEG lipid is PEG-DMG. In certain embodiments, the PEG lipid is a compound of one of formulae (VI), (Vl-a) or (Vl-b). In any of the above, the total content of the lipid component may not exceed 100%.

[0056] In some embodiments, the nanoparticle composition includes more than one phospholipid, PEG lipid, structural lipid, or other lipid. In certain embodiments, the nanoparticle composition further includes a cationic and/or ionizable lipid such as an amino-lipid. In certain embodiments, a cationic and/or ionizable lipid is selected from the group consisting of 3-(didodecylanTino)-Nl,Nl,4-tridodecyl-l-piperazineethanamine (KL10), N142-(didodecylainino)ethyl]-Nl,N4,N4-tridodecyl-l,4-piperazinediethanamine (KL22), 14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontane (KL25), l,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[l,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (DLin-MC3 -DMA or MC3), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[l,3]-dioxolane (DLin-KC2-DMA), l,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), 2-({8-[(3p)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dime%l-3-[(9Z,12Z)-octadeca-9,12 -dien-l-yloxy]propan- l-amine (Octy 1-CLinDM A), (2R)-2-({8-|(3 P)-cholest-5-en-3 -yloxy]octy l}oxy)-N,N-dime1hyl-3-[(9Z, l2Z)-octadeca-9, 12-dien-l-yloxy] propan-l-amine (Octyl-CLinDMA (2R)), (2S)-2-({8-[(3P)-(&olest-5-en-3-yloxy]o^ }oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-l-yloxy]propan-l-amine (Octyl-CLinDMA (28)), (12Z, 15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-l-amine, and N,N-dimethyl-l-{(lS,2R)-2-octylcyclopropyl}heptadecan-8-amine.

[0057] In some embodiments, the nanoparticle composition includes a therapeutic and/or prophylactic agent. In certain embodiments, the therapeutic and/or prophylactic agent may be selected from the group consisting of a protein, a small molecule drug, a cytotoxic agent, a radioactive ion, a chemotherapeutic agent, a vaccine, a compound that elicits an immune response, and/or a nucleic acid (such as a deoxyribonucleic acid or a ribonucleic acid). In certain embodiments, the therapeutic and/or prophylactic agent is a ribonucleic acid (RNA). An RNA may be selected from the group consisting of a small interfering RNA (siRNA), an asymmetrical interfering RNA (aiRNA), a microRNA (miRNA), a Dicer-substrate RNA (dsRNA), a small hairpin RNA (shRNA), a messenger RNA (niRNA), and mixtures thereof. In certain embodiments, the therapeutic and/or prophylactic agent is a messenger RNA (mRNA). An RNA of a nanoparticle composition may be naturally or non-naturally occurring and may include one or more of a stem loop, a chain terminating nucleoside, a polyA sequence, a polyadenylation signal, and/or a 5' cap structure.

[0058] In some embodiments, the nanoparticle composition includes more than one therapeutic and/or prophylactic agent, such as one or more RNAs. The therapeutic and/or prophylactic agents may be of the same or different types (e.g., two mRNAs, two siRNAs, one mRNA and one siRNA, one mRNA and one small molecule drug, etc.).

[0059] In some embodiments, the encapsulation efficiency of a therapeutic and/or prophylactic agent of a nanoparticle composition is at least 50%. In certain embodiments, the encapsulation efficiency is at least 80%. In certain embodiments, the encapsulation efficiency is greater than 90%.

[0060] In some embodiments, the wt/wt ratio of the lipid component to a therapeutic and/or prophylactic agent in the nanoparticle composition is from about 10: 1 to about 60: 1. In certain embodiments, the wt/wt ratio is about 20: 1.

[0061] In some embodiments, the N:P ratio of the nanoparticle composition is from about 2:1 to about 30:1. In certain embodiments, the N:P ratio is from about 2:1 to about 8:1. In certain embodiments, the N:P ratio is from about 5:1 to about 8:1. For example, the N:P ratio may be about 5.0:1, about 5.5:1, about 5.67:1, about 6.0:1, about 6.5:1, or about 7.0:1. In some embodiments, the mean size of a nanoparticle composition is from about 40 nm to about 150 nm. In certain embodiments, the mean size is from about 70 nm to about 100 nm. In one embodiment, the mean size may be about 80 to about 100 nm. In certain embodiments, the mean size may be about 80 nm. In other embodiments, the mean size may be about 100 nm.

[0062] The polydispersity index of the nanoparticle composition is from about 0 to about 0.25 in certain embodiments. In certain embodiments, the polydispersity index is from about 0.10 to about 0.20.

[0063] In some embodiments, the nanoparticle composition has a zeta potential of about -10 mV to about +20 mV.

[0064] In some embodiments, upon contacting the compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(Idl O), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) (e.g., any of Compounds 1-189) or a nanoparticle composition thereof with a mammalian cell, the cell uptake of the compound or nanoparticle composition is LDLR-independent. In some embodiments, the cell uptake of the compound or nanoparticle composition is LDLR-dependent. In some embodiments, the cell uptake of the compound or nanoparticle composition is apoE-independent. In some embodiments, the cell uptake of the compound or nanoparticle composition is apoE-dependent. In some embodiments, the cell uptake of the compound or nanoparticle composition is LDLR-apoE-interaction independent. In some embodiments, the cell uptake of the compound or nanoparticle composition is LDLR-apoE-interaction dependent.

[0065] In some embodiments, upon contacting the compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(Idl O), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) (e.g., any of Compounds 1-189) or the nanoparticle composition thereof with a mammalian cell to produce a polypeptide, the production of the polypeptide is higher in mammalian hepatocytes than cells from a different tissue (e.g., spleen or kidney).

[0066] In some embodiments, upon contacting the compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(Idl O), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) (e.g., any of Compounds 1-189) or the nanoparticle composition thereof with a mammalian cell to produce a polypeptide, the production of the polypeptide occurs substantively in mammalian hepatocytes (e.g., little or no production of the polypeptide in other cells, e.g., spleen cells or renal cells).

[0067] In some embodiments, the nanoparticle composition includes one or more other components including, but not limited to, one or more pharmaceutically acceptable excipients, small hydrophobic molecules, therapeutic and/or prophylactic agents, carbohydrates, polymers, permeability enhancing molecules, buffers, and surface altering agents.

[0068] In yet another aspect, the disclosure features a pharmaceutical composition comprising a nanoparticle composition according to the preceding aspects and a pharmaceutically acceptable carrier. For example, the pharmaceutical composition is refrigerated or frozen for storage and/or shipment (e.g., being stored at a temperature of 4 °C or lower, such as a temperature between

about -150 °C and about 0 °C or between about -80 °C and about -20 °C (e.g., about -5 °C, -10 °C, -15 °C, -20 °C, -25 °C, -30 °C, -40 °C, -50 °C, -60 °C, -70 °C, -80 °C, -90 °C, -130 °C or -150 °C). For example, the pharmaceutical composition is a solution that is refrigerated for storage and/or shipment at, for example, about -20° C, -30 °C, -40 °C, -50 °C, -60 °C, -70 °C, or -80 °C.

[0069] In a further aspect, the disclosure provides a method of delivering a therapeutic and/or prophylactic agent (e.g., an mRNA) to a cell (e.g., a mammalian cell). This method includes the step of administering to a subject (e.g., a mammal, such as a human) a nanoparticle composition including (i) a lipid component including a phospholipid (such as a polyunsaturated lipid), a PEG lipid, a structural lipid, and a compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), and (ii) a therapeutic and/or prophylactic agent, in which administering involves contacting the cell with the nanoparticle composition, whereby the therapeutic and/or prophylactic agent is delivered to the cell.

[0070] In another aspect, the disclosure provides a method of producing a polypeptide of interest in a cell (e.g., a mammalian cell). The method includes the step of contacting the cell with a nanoparticle composition including (i) a lipid component including a phospholipid (such as a polyunsaturated lipid), a PEG lipid, a structural lipid, and a compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), and (ii) an mRNA encoding the polypeptide of interest, whereby the mRNA is capable of being translated in the cell to produce the polypeptide.

[0071] In yet another aspect, the disclosure provides a method of treating a disease or disorder in a mammal (e.g., a human) in need thereof. The method includes the step of administering to the mammal a therapeutically effective amount of a nanoparticle composition including (i) a lipid component including a phospholipid (such as a polyunsaturated lipid), a PEG lipid, a structural lipid, and a compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), and (ii) a therapeutic and/or prophylactic agent (e.g., an mRNA). In some embodiments, the disease or disorder is characterized by dysfunctional or aberrant protein or polypeptide activity. For example, the disease or disorder is selected from the group consisting of rare diseases, infectious diseases, cancer and proliferative diseases, genetic diseases (e.g., cystic fibrosis), autoimmune diseases, diabetes, neurodegenerative diseases, cardio- and reno-vascular diseases, and metabolic diseases.

[0072] In a further aspect, the disclosure provides a method of delivering (e.g., specifically delivering) a therapeutic and/or prophylactic agent to a mammalian organ (e.g., a liver, spleen, lung, or femur). This method includes the step of administering to a subject (e.g., a mammal) a nanoparticle composition including (i) a lipid component including a phospholipid, a PEG lipid, a structural lipid, and a compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), and (ii) a therapeutic and/or prophylactic agent (e.g., an mRNA), in which administering involves contacting the cell with the nanoparticle composition, whereby the therapeutic and/or prophylactic agent is delivered to the organ such as liver.

[0073] In a further aspect, the disclosure features a method for the enhanced delivery of a therapeutic and/or prophylactic agent (e.g., an mRNA) to a target tissue (e.g., a liver, spleen, lung, or femur). This method includes administering to a subject (e.g., a mammal) a nanoparticle composition, the composition including (i) a lipid component including a compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), a phospholipid, a structural lipid, and a PEG lipid; and (ii) a therapeutic and/or prophylactic agent, the administering including contacting the target tissue with the nanoparticle composition, whereby the therapeutic and/or prophylactic agent is delivered to the target tissue. In some embodiments, the delivery is enhanced as compared to a reference composition which comprises a reference lipid instead of a compound of one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

[0074] In yet another aspect, the disclosure features a method of lowering immunogenicity comprising introducing the nanoparticle composition of the disclosure into cells, wherein the nanoparticle composition reduces the induction of the cellular immune response of the cells to the nanoparticle composition, as compared to the induction of the cellular immune response in cells induced by a reference composition which comprises a reference lipid instead of a compound of one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia). For example, the cellular immune response is an innate immune response, an adaptive immune response, or both.

[0075] In certain embodiments of the above aspects, a cell contacted in a method is in a mammal.

[0076] In any of the preceding aspects, a mammal may be, for example, a rodent, non-human primate, or a human. In certain embodiments, the mammal is a human. In certain embodiments,

the mammal is LDLR-deficient, or apoE-deficient, or both. In certain embodiments, the mammal is not LDLR-deficient. In certain embodiments, the mammal is not apoE-deficient. In certain embodiments, the mammal is neither LDLR-deficient nor apoE-deficient. In certain embodiments, the mammal has an abnormal LDLR-apoE interaction. In certain embodiments, the mammal has a normal LDLR-apoE interaction.

[0077] In any of the preceding aspects, a therapeutic and/or prophylactic agent may be an mRNA.

[0078] In some embodiments of the above methods, the therapeutic and/or prophylactic agent may be specifically delivered to a target tissue of interest (e.g., a mammalian liver, spleen, lung, or femur).

[0079] In some embodiments of the above methods, a polypeptide of interest may be specifically produced in a target cell or tissue of interest (e.g., a hepatocyte, a mammalian liver, spleen, lung, or femur), e.g., the production of polypeptide is substantively higher in the target cell or tissue than in a non-target cell/tissue.

[0080] In some embodiments, the nanoparticle composition is administered intravenously, intramuscularly, intradermally, subcutaneously, intra-arterially, intra-tumor, or by inhalation. A dose of about 0.001 mg/kg to about 10 mg/kg of therapeutic and/or prophylactic agent (e.g., mRNA) is administered to a mammal in certain embodiments.

[0081] In any of the preceding aspects, in some embodiments, the delivery (e.g., delivery efficiency) of the therapeutic and/or prophylactic agent to the mammalian cell is LDLR-independent. In some embodiments, the delivery of the therapeutic and/or prophylactic agent to the mammalian cell is LDLR-dependent. In some embodiments, the delivery of the therapeutic and/or prophylactic agent to the mammalian cell is apoE-independent. In some embodiments, the delivery of the therapeutic and/or prophylactic agent to the mammalian cell is apoE-dependent. In some embodiments, the delivery of the therapeutic and/or prophylactic agent to the mammalian cell is LDLR-apoE-interaction independent. In some embodiments, the delivery of the therapeutic and/or prophylactic agent to the mammalian cell is LDLR-apoE-interaction dependent.

[0082] In any of the preceding aspects, in some embodiments, the production (e.g., yield) of the polypeptide of interest in the mammalian cell is LDLR-independent. In some embodiments, the production of the polypeptide of interest in the mammalian cell is LDLR-dependent. In some embodiments, the production of the polypeptide of interest in the mammalian cell is apoE-independent. In some embodiments, the production of the polypeptide of interest in the

mammalian cell is apoE-dependent. In some embodiments, the production of the polypeptide of interest in the mammalian cell is LDLR-apoE-interaction independent. In some embodiments, the production of the polypeptide of interest in the mammalian cell is LDLR-apoE-interaction dependent.

[0083] In the preceding aspects, one or more nanoparticle compositions each including one or more therapeutic and/or prophylactic agents may be used in combination. In some embodiments, one or more nanoparticle compositions each including one or more therapeutic and/or prophylactic agents may be simultaneously contacted with a cell or delivered to a mammalian cell or organ. In other embodiments, the one or more nanoparticle compositions are contacted with a cell or delivered to a mammalian cell or organ at different times.

[0084] In the preceding aspects, one or more additional therapeutic and/or prophylactic agents or compounds may be used in combination with a nanoparticle composition including a therapeutic and/or prophylactic agent. In some embodiments, an additional therapeutic and/or prophylactic agent or compound may be administered at or near the same time as a nanoparticle composition (e.g., within one hour). In other embodiments, an additional therapeutic and/or prophylactic agent or compound may be administered before or after (e.g., one or more hours before or after) a nanoparticle composition as a pretreatment or post-treatment therapy. In some embodiments, an additional therapeutic and/or prophylactic agent or compound is selected from the group consisting of an anti-inflammatory compound, a steroid (e.g., a corticosteroid), a statin, an estradiol, a BTK inhibitor, an SIP1 agonist, a glucocorticoid receptor modulator (GRM), or an anti-histamine. In certain embodiments, an additional therapeutic and/or prophylactic agent or compound is selected from the group consisting of dexamethasone, methotrexate, acetaminophen, an H1 receptor blocker, or an H2 receptor blocker.

BRIEF DESCRIPTION OF THE DRAWINGS

[0085] The skilled artisan will understand that the drawings primarily are for illustrative purposes and are not intended to limit the scope of the inventive subject matter described herein. The drawings are not necessarily to scale; in some instances, various aspects of the inventive subject matter disclosed herein may be shown exaggerated or enlarged in the drawings to facilitate an understanding of different features. In the drawings, like reference characters generally refer to like features (e.g., functionally similar and/or structurally similar elements).

[0086] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0087] The above and further features will be more clearly appreciated from the following detailed description when taken in conjunction with the accompanying drawings.

[0088] Figure 1A is a graph comparing luciferase expression levels in mice (whole body) after administration of nanoparticle compositions containing compounds of the disclosure over time.

[0089] Figure IB is a graph comparing luciferase expression levels in mice (whole body) after administration of nanoparticle compositions containing compounds of the disclosure over time.

[0090] Figure 2 is a graph summarizing luciferase expression levels at 3 h after administration of nanoparticle compositions containing compounds of the disclosure. Total light flux values were acquired via body luminescent imaging (BLI) 3 h after administration. In this Figure, the numbers 1-12 refer to the compositions containing Compounds 42-52 and MC3 respectively.

[0091] Figure 3 is a graph summarizing luciferase expression levels at 6 hr after administration of nanoparticle compositions containing compounds of the disclosure. Total light flux values were acquired via BLI 6 h after administration. In this Figure, the numbers 1-12 refer to the compositions containing Compounds 42-52 and MC3 respectively.

[0092] Figure 4 is graph summarizing luciferase expression levels at 24 h after administration of nanoparticle compositions containing compounds of the disclosure. Total light flux values were acquired via BLI 24 h after administration. In this Figure, the numbers 1-12 refer to the compositions containing Compounds 42-52 and MC3 respectively.

[0093] Figure 5 is graph summarizing expression levels of luciferase in mouse liver 6 hours after administration of nanoparticle compositions including compounds of the disclosure. In this Figure, the numbers 1-12 refer to the compositions containing Compounds 42-52 and MC3 respectively.

[0094] Figure 6 is a graph summarizing expression levels of luciferase in mouse lungs 6 hours after administration of nanoparticle compositions including compounds of the disclosure. In this Figure, the numbers 1-12 refer to the compositions containing Compounds 42-52 and MC3 respectively.

[0095] Figure 7 is a graph summarizing expression levels of luciferase in mouse spleen 6 hours after administration of nanoparticle compositions including compounds of the disclosure. In this Figure, the numbers 1-12 refer to the compositions containing Compounds 42-52 and MC3 respectively.

[0096] Figures 8A and 8B are a pair of graphs illustrating hEPO expression levels in rats dosed with compounds of the disclosure as compared to with KL22, showing that KL22 and its chain length derivatives (previously showing improved protein expression in mice), do not express hEPO in rats. PBS (phosphate buffered saline) is used as control. Figure 8A compares the hEPO concentration after administration of nanoparticle compositions containing Compound 23, Compound 11, KL22, and MC3 at 2 mpk, i.v. administration. Compound 11 LNP showed hEPO expression comparable to MC3 and improved tolerability as compared to KL22. Figure 8B illustrates the results of a dose response study using Compound 20, KL22, and MC3 at dose of 0.2, 0.5 and 1 mpk. KL22 and Compound 23 were shown to be toxic at the 2 mg/kg dose. In Figure 8A, the numbers 1-5 refer to compositions containing the following: 1: Compound 23, 2: Compound 11, 3: KL22; 4: MC3; 5: PBS. In Figure 8B, the numbers 1-9 refer to compositions containing the following: 1: Compound 20, 0.2 mpk; 2: Compound 20, 0.5 mpk; 3: Compound 20, 1 mpk; 4: KL22, 0.2 mpk; 5: KL22, 0.5 mpk; 6: KL22, 1 mpk; 7: MC3, 0.2 mpk; 8: MC3, 0.5 mpk; 9: MC3, 1 mpk; 10: PBS.

[0097] Figures 9A and B are a pair of graphs illustrating performance of Compound 11 as an LDLr independent lipid. Figure 9A is a bar graph showing expression of luciferase induced by administration of nanoparticle compositions including Compound 11 at dosages of 0.05 mpk, 0.25 mpk, and 0.5 mpk to LDLR -/- knockout and wild-type mice. Figure 9B shows LDL-c levels in LDLR knockout mice after administration of a control mRNA, i.e., non-translating Factor IX ("NT-FIX") and various compositions comprising mRNAs encoding the LDL receptor in mice, with KL22 at 0.5 mpk, or with Compound 11 at 0.5 and 1 mpk. LDL-c levels in mice were found to drop with nanoparticle composition containing Compound 11.

[0098] Figure 10 is a graph showing hEPO levels in non-human primates up to -50 h after administration of a nanoparticle composition containing Compound 4, compared to a composition containing MC3. The Compound 4 LNP demonstrated 3-fold expression of hEPO compared to MC3, establishing Compound 4 as an LDLr independent lipid that translates to higher species.

[0099] Figures 11A and 1IB are a pair of images comparing the results of a mouse liver immunohistochemistry (IHC) using mRNA expressing green fluorescent protein (GFP) after administration of nanoparticle compositions containing Compound 4 and MC3. Figure 11A shows CD-I mouse liver cells after administration of GFP mRNA in a MC3 LNP, 6h after intravenous administration at a dose of 0.5 mpk. GFP mRNA Protein expression from the MC3 LNP composition was observed in both hepatocytes and Kupffer cells. Figure 11B shows

LDLR knockout mouse liver cells after administration of GFP mRNA in a Compound 4 LNP, 8h after intravenous administration at a dose of 0.5 mpk. In contrast to MC3, the LNP containing Compound 4 appears to show less protein expression in Kupffer cells.

[00100] Figures 12A-12C are a set of graphs illustrating hEPO expression levels in CD-I mice dosed with compounds of the disclosure, compared to MC3. PBS is used as control. Figure 12A shows the hEPO concentration 3 h after administration of the nanoparticle compositions. Figure 12B shows the hEPO concentration 6 h after administration of the nanoparticle compositions. Figure 12C shows the hEPO concentration 24 h after administration of the nanoparticle compositions. In Figures 12A-12C numbers 1-14 refer to compositions containing the following: 1: Compound 73, 2: Compound 80, 3: Compound 70; 4: Compound 81; 5: Compound 69; 6: Compound 82; 7: Compound 83; 8: Compound 62; 9: Compound 84; 10: Compound 85; 11: Compound 86; 12: Compound 87; 13: MC3; 14: PBS.

[00101] Figure 13 is a graph showing hEPO levels (pg/mL) in CD-I mice up to ~25 h after administration of a nanoparticle composition containing compounds of the disclosure, compared to a composition containing MC3. Numbers 1-13 refer to compositions containing the following: 1: Compound 73, 2: Compound 80, 3: Compound 70; 4: Compound 81; 5: Compound 69; 6: Compound 82; 7: Compound 83; 8: Compound 62; 9: Compound 84; 10: Compound 85; 11: Compound 86; 12: Compound 87; 13: MC3.

[00102] Figures 14A and 14B are a pair of graphs showing percentages of activated B-cells in the spleens of CD-I mice dosed with compounds of the disclosure, compared to MC3, and compared to mice not having received any treatment (naive test subject). PBS is used as control. Figure 14A shows the percentage of CD19+ cells. Figure 14B shows the percentage of CD19+ CD69+ CD86+ cells. Numbers 1-13 refer to compositions containing the following: 1: Compound 73, 2: Compound 80, 3: Compound 70; 4: Compound 81; 5: Compound 69; 6: Compound 82; 7: Compound 83; 8: Compound 62; 9: Compound 84; 10: Compound 85; 11: Compound 86; 12: Compound 87; 13: MC3; 14: PBS; 15: treatment naive subject.

[00103] Figure 15 is a graph summarizing luciferase expression levels at 6 h after administration of nanoparticle compositions containing compounds of the disclosure to CD-I mice at a dose of 0.5 mpk. Total light flux values were acquired via body luminescent imaging (BLI) 6 h after administration. In this Figure, the numbers 1-8 refer to the compositions containing Compounds 4-10 and MC3 respectively.

[00104] Figure 16 is a graph showing GFP levels in the livers of LDLR knockout mice at 30 min to 24 h after intravenous administration of an eGFP RNA in a lipid composition

containing Compound 4. The liver GFP levels were determined via IHC. The square markers represent the number of GFP positive cells following administration of a dose of 0.1 mpk of the composition. The circular markers represent the number of GFP positive cells following administration of a dose of 0.5 mpk of the composition.

[00105] Figures 17A and B are a pair of graphs showing the ApoE dependence of luciferase ("Luc") expression following administration of a composition containing Luc mRNA and Compound 4 to mice at a dose 0.5 mpk. The expression following administration of a composition containing Luc mRNA and MC3 is presented for comparison. Figure 17A shows the total flux in the liver 6h after administration. The % change in Luc expression in livers of ApoE knockout vs. wild-type mice (i.e., (WT mean expression-KO mean expression)AVT mean expression]* 100%) was 91.9 % for Compound 4 and 97.5 % for MC3. Figure 17B shows the total flux in the spleen 6h after administration. The % change in expression in spleens of ApoE knockout vs. wild-type mice was 4.34 % for Compound 4 and 72.2 % for MC3. Numbers 1-4 refer to the following: 1: Composition containing Compound 4, administered to ApoE knockout mice; 2: Composition containing Compound 4, administered to wild-type mice; 3: Composition containing MC3, administered to ApoE knockout mice; 4: Composition containing MC3, administered to wild-type mice.

[00106] Figures 18A and 18B are a pair of graphs showing the effect of a composition containing Compound 4 on liver enzymes. The composition was administered to rats at 0.1 mpk and 1 mpk. The effects of MC3 are shown for comparison. PBS is used as a control. Figure 18A shows the effect on aspartate aminotransferase (AST). Figure 18B shows the effect on alanine aminotransferase (ALT).

[00107] Figures 19A - 19C are a set of graphs showing immune cell activation by a composition containing Compound 4. The effects of MC3 are shown for comparison. PBS (phosphate buffered saline) is used as control. Compositions were administered to rats at 0.1 mpk or 1 mpk. Figure 19A shows the effect on activation of neutrophil. Figure 19B shows the effect on activation of lymphocytes. Figure 19C shows the effect on activation of monocytes. Numbers 1-5 in Figures 19A-19C refer to the following: 1: Compound 4, 0.1 mpk; 2: Compound 4; 1 mpk; 3: MC3, 0.1 mpk 4: MC3, 1 mpk; 5: PBS.

[00108] Figure 20 is a graph showing the expression of Stefin A Quadruple Mutant-Tracy (SQT) protein in mouse liver determined via FLAG IHC at different time points following intravenous administration of various nanoparticle compositions comprising SQT mRNA and lipids disclosed herein. Numbers 1-11 in the figure refer to the following: 1: 0 h, PBS; 2: 0 h,

Compound 4; 3: 0.5 h, Compound 4; 4: 4 h, Compound 4; 5: 8 h, Compound 4; 6: 24 h, Compound 4; 7: Oh, MC3; 8: 0.5 h, MC3; 9: 4 h, MC3; 10: 8 h, MC3; 11: 24 h, MC3.

[00109] Figure 21 is a graph illustrating hEPO expression levels in CD-I mice dosed with compositions of the disclosure. Compositions were administered intravenously, once weekly for 4 weeks at a dose of 0.5 mpk. Samples were collected 6h after each administration. In the graph, numbers 1-1 through 1-4 refer to hEPO levels at 1, 2, 3, and 4 weeks of administration of a composition comprising Compound 4, DOPE, and PEG DMG. Numbers 2-1 through 2-4 refer to hEPO levels at 1, 2, 3, and 4 weeks of administration of a composition comprising Compound 4, DOPE, and PEG 1.

[00110] Figures 22A and 22B are a pair of graphs illustrating the anti-PEG IgM response in CD-I mice dosed with compositions of the disclosure. Compositions were administered intravenously, once weekly for 4 weeks at a dose of 0.5 mpk. Anti-PEG IgM levels were measured at 96 h after administration. PBS was used as a control. The numbers 1-3 refer to 1: a composition comprising Compound 4, DOPE and PEG DMG, 2: a composition comprising Compound 4, DOPE and PEG 1, and 3: PBS. Figure 22A shows the anti-PEG IgM levels after dose 2, and Figure 22B shows the anti-PEG IgM levels after dose 3.

[00111] Figures 23A and 23B are a pair of images of immunohistochemistries (IHC) illustrating hepatocyte distributions after administration of a nanoparticle composition comprising Compound 4, DOPE and PEG DMG. Figure 23A shows a CD-I mouse liver (high natural IgM). Figure 23B shows a Cynomolgus monkey liver (high anti-PEG IgM). The composition was administered to the CD-I mouse at a dose of 0.2 mpk and to the Cynomolgus monkey at a dose of 0.5 mpk.

[00112] Figure 24 is a graph showing the decrease of LNP-bound PEG over time, comparing a nanoparticle composition comprising Compound 4 to a composition comprising MC3.

[00113] Figure 25 is a graph illustrating serum LDL levels LDLr knockout (KO) mice following administration of nanoparticle compositions comprising compounds of the disclosure intravenously at 0.5 mpk. PBS was used as a control and administered to LDLr KO mice as well as wild type mice. Serum LDL levels were measured 24 h after administration. In the graph, the numbers 1-16 refer to 1: Compound 4; 2: Compound 56; 3: Compound 57; 4: Compound 58; 5: Compound 61; 6: Compound 71; 7: Compound 80; 8: Compound 81; 9: Compound 82; 10: Compound 83; 11: Compound 156; 12: Compound 84; 13: Compound 85; 14: Compound 87; 15: PBS (WT mouse); 16: PBS (LDLr KO mouse).

[00114] Figure 26 is a graph demonstrating the efficacy of nanoparticles comprising compounds of the disclosure in LDLR knockout (KO) mice. Compositions were administered intravenously at 0.5 mpk. PBS was used as a control and administered to C57/BI6 and LDLr KO mice. LDL-c levels were measured 24 h after administration. In the graph, the numbers 1-18 refer to 1: PBS (C57/BI6 mouse); 2: PBS (LDLr KO mouse); 3: Compound 4; 4: Compound 111; 5: Compound 91; 6: Compound 90; 7: Compound 89; 8: Compound 109; 9: Compound 117; 10: Compound 94; 11: Compound 93; 12: Compound 92; Compound 13: Compound 116; 14: Compound 114; 15: Compound 102; 16: Compound 115; 17: Compound 107; 18: Compound 119.

[00115] Figures 27A-27C are a set of graphs illustrating hEPO expression levels in CD-I mice dosed with mRNA expressing hEPO in compounds of the disclosure (0.5 mg/kg; mRNA dose), compared to MC3. PBS is used as control. Figure 27A shows the hEPO concentration 3 h after administration of the nanoparticle compositions. Figure 27B shows the hEPO concentration 6 h after administration of the nanoparticle compositions. Figure 27C shows the hEPO concentration 24 h after administration of the nanoparticle compositions. In Figures 27A-27C numbers 1-13 refer to compositions containing the following: 1: Compound 146, 2: Compound 140, 3: Compound 141; 4: Compound 155; 5: Compound 157; 6: Compound 153; 7: Compound 158; 8: Compound 154; 9: Compound 159; 10: Compound 160; 11: Compound 4; 12: MC3; 13: PBS.

[00116] Figure 28 is a graph illustrating hEPO expression levels up to -24 h after administration of mRNA expressing hEPO in compounds of the disclosure (0.5 mg/kg; mRNA dose), to CD-I mice, compared to MC3. The numbers 1-12 refer to compositions containing the following: 1: Compound 146, 2: Compound 140, 3: Compound 141; 4: Compound 155; 5: Compound 157; 6: Compound 153; 7: Compound 158; 8: Compound 154; 9: Compound 159; 10: Compound 160; 11: Compound 4; 12: MC3.

[00117] Figures 29A and 29B are a pair of graphs showing percentages of activated B-cells in the spleens of CD-I mice dosed with compounds of the disclosure, compared to MC3, and compared to mice not having received any treatment (naive test subject). PBS is used as control. Figure 29A shows the percentage of CD19+ cells. Figure 29B shows the percentage of CD19+ CD69+ CD86+ cells. The numbers 1-12 refer to compositions containing the following: 1: Compound 146, 2: Compound 140, 3: Compound 141; 4: Compound 155; 5: Compound 157; 6: Compound 153; 7: Compound 158; 8: Compound 154; 9: Compound 159; 10: Compound 160; 11: Compound 4; 12: MC3, 13: PBS, 14: treatment naive subject.

[00118] Figure 30 is a graph showing ApoE binding to the nanoparticles in nanoparticle compositions of the disclosure after incubation in two types of human serum and against the pure recombinant ApoE protein, relative to ApoE binding to Compound 4. Compound 4 was used in a composition comprising PEG 1 and DOPE. Numbers 1-13 refer to compositions containing the following: 1: MC3 in PEG-DMG, 2: Compound 146, 3: Compound 140, 4: Compound 141; 5: Compound 155; 6: Compound 157; 7: Compound 153; 8: Compound 158; 9: Compound 154; 10: Compound 159; 11: Compound 160; 12: Compound 4; 13: MC3.

[00119] Figure 31 is a graph showing the IgM binding to the nanoparticles in nanoparticle compositions of the disclosure after incubation in C57BI/6 serum and serum with a high concentration of anti-PEG IgM antibodies, relative to the concentration of ApoE binding to Compound 4. Compound 4 was used in a composition comprising PEG 1 and DOPE. Numbers 1-13 refer to compositions containing the following: 1: MC3 in PEG-DMG, 2: Compound 146, 3: Compound 140, 4: Compound 141; 5: Compound 155; 6: Compound 157; 7: Compound 153; 8: Compound 158; 9: Compound 154; 10: Compound 159; 11: Compound 160; 12: Compound 4; 13: MC3.

[00120] Figures 32A-32C are a set of graphs illustrating hEPO expression levels in CD-I mice dosed with mRNA expressing hEPO in compounds of the disclosure (0.5 mg/kg; mRNA dose), compared to MC3. PBS is used as control. Figure 32A shows the hEPO concentration 3 h after administration of the nanoparticle compositions. Figure 32B shows the hEPO concentration 6 h after administration of the nanoparticle compositions. Figure 32C shows the hEPO concentration 24 h after administration of the nanoparticle compositions. In Figures 32A-32C numbers 1-12 refer to compositions containing the following: 1: Compound 161; 2: Compound 162, 3: Compound 163; 4: Compound 164; 5: Compound 165; 6: Compound 166; 7: Compound 167; 8: Compound 168; 9: Compound 169; 10: Compound 4; 11: MC3; 12: PBS.

[00121] Figure 33 is a graph illustrating hEPO expression levels up to ~24 h after administration of mRNA expressing hEPO in compounds of the disclosure (0.5 mg/kg; mRNA dose), to CD-I mice, compared to MC3. The numbers 1-12 refer to compositions containing the following: 1: Compound 161; 2: Compound 162, 3: Compound 163; 4: Compound 164; 5: Compound 165; 6: Compound 166; 7: Compound 167; 8: Compound 168; 9: Compound 169; 10: Compound 4; 11: MC3; 12: PBS.

[00122] Figures 34A and 34B are a pair of graphs showing percentages of activated B-cells in the spleens of CD-I mice dosed with compounds of the disclosure, compared to MC3, and compared to mice not having received any treatment (naive test subject). PBS is used as

control. Figure 29A shows the percentage of CD19+ cells. Figure 29B shows the percentage of CD19+ CD69+ CD86+ cells. The numbers 1-13 refer to the following: 1: Compound 161; 2: Compound 162, 3: Compound 163; 4: Compound 164; 5: Compound 165; 6: Compound 166; 7: Compound 167; 8: Compound 168; 9: Compound 169; 10: Compound 4; 11: MC3; 12: PBS; 13: treatment naive subject.

[00123] Figure 35 shows the binding of compounds of the disclosure to the Heparin column in a Heparin Sepharose binding assay. The numbers 1-11 refer to the following: 1: Compound 161; 2: Compound 162, 3: Compound 163; 4: Compound 164; 5: Compound 165; 6: Compound 166; 7: Compound 167; 8: Compound 168; 9: Compound 169; 10: Compound 4; 11: MC3.

[00124] Figure 36 is a graph showing ApoE binding to the nanoparticles in nanoparticle compositions of the disclosure after incubation in two types of human serum and against the pure recombinant ApoE protein, relative to ApoE binding to Compound 4. Compound 4 was used in a composition comprising PEG 1 and DOPE. Numbers 1-12 refer to compositions containing the following: 1: MC3 in DSPC, cholesterol and PEG-DMG; 2: Compound 161; 3: Compound 162, 4: Compound 163; 5: Compound 164; 6: Compound 165; 7: Compound 166; 8: Compound 167; 9: Compound 168; 10: Compound 169; 11: Compound 4; 12: MC3.

[00125] Figure 37 is a graph showing the IgM binding to the nanoparticles in nanoparticle compositions of the disclosure after incubation in C57BI/6 serum and serum with a high concentration of anti-PEG IgM antibodies, relative to the concentration of ApoE binding to Compound 4. Compound 4 was used in a composition comprising PEG 1 and DOPE. Numbers 1-12 refer to compositions containing the following: 1: MC3 in DSPC, cholesterol and PEG-DMG; 2: Compound 161; 3: Compound 162, 4: Compound 163; 5: Compound 164; 6: Compound 165; 7: Compound 166; 8: Compound 167; 9: Compound 168; 10: Compound 169; 11: Compound 4; 12: MC3.

[00126] Figures 38A-38C are a set of graphs illustrating hEPO expression levels in CD-I mice dosed with mRNA expressing hEPO in compounds of the disclosure (0.5 mg/kg; mRNA dose), compared to MC3. PBS is used as control. Figure 38A shows the hEPO concentration 3 h after administration of the nanoparticle compositions. Figure 38B shows the hEPO concentration 6 h after administration of the nanoparticle compositions. Figure 38C shows the hEPO concentration 24 h after administration of the nanoparticle compositions. In Figures 38A-38C numbers 1-12 refer to compositions containing the following: 1: Compound 181; 2:

Compound 182, 3: Compound 183; 4: Compound 184; 5: Compound 185; 6: Compound 186; 7: Compound 187; 8: Compound 188; 9: Compound 189; 10: Compound 4; 11: MC3; 12: PBS.

[00127] Figure 39 is a graph illustrating hEPO expression levels up to -24 h after administration of mRNA expressing hEPO in compounds of the disclosure (0.5 mg/kg; mRNA dose), to CD-I mice, compared to MC3. The numbers 1-12 refer to compositions containing the following: : 1: Compound 181; 2: Compound 182, 3: Compound 183; 4: Compound 184; 5: Compound 185; 6: Compound 186; 7: Compound 187; 8: Compound 188; 9: Compound 189; 10: Compound 4; 11: MC3; 12: PBS.

[00128] Figures 40A and 40B are a pair of graphs showing percentages of activated B-cells in the spleens of CD-I mice dosed with compounds of the disclosure, compared to MC3, and compared to mice not having received any treatment (naive test subject). PBS is used as control. Figure 29A shows the percentage of CD 19+ cells. Figure 29B shows the percentage of CD19+ CD69+ CD86+ cells. The numbers 1-13 refer to the following: : 1: Compound 181; 2: Compound 182, 3: Compound 183; 4: Compound 184; 5: Compound 185; 6: Compound 186; 7: Compound 187; 8: Compound 188; 9: Compound 189; 10: Compound 4; 11: MC3; 12: PBS; 13: treatment naive subject.

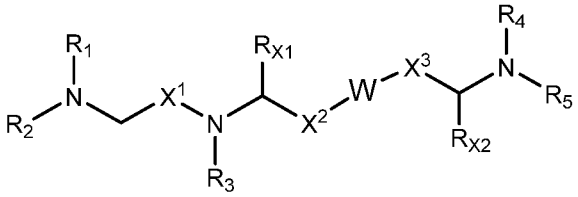
DETAILED DESCRIPTION

[00129] The disclosure relates to novel lipids and lipid nanoparticle compositions including a novel lipid. The disclosure also provides methods of delivering a therapeutic and/or prophylactic agent to a mammalian cell, specifically delivering a therapeutic and/or prophylactic agent to a mammalian organ, producing a polypeptide of interest in a mammalian cell, and treating a disease or disorder in a mammal in need thereof. For example, a method of producing a polypeptide of interest in a cell involves contacting a nanoparticle composition comprising an mRNA with a mammalian cell, whereby the mRNA may be translated to produce the polypeptide of interest. A method of delivering a therapeutic and/or prophylactic agent to a mammalian cell or organ may involve administration of a nanoparticle composition including the therapeutic and/or prophylactic agent to a subject, in which the administration involves contacting the cell or organ with the composition, whereby the therapeutic and/or prophylactic agent is delivered to the cell or organ.

Lipids

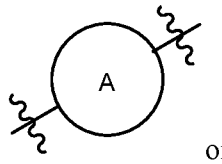
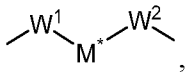
[00130] The present disclosure provides lipids that may be advantageously used in lipid nanoparticle compositions for the delivery of therapeutic and/or prophylactic agents to mammalian cells or organs. For example, the lipids described herein have little or no immunogenicity. For example, the lipid compound of any of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) has a lower immunogenicity as compared to a reference lipid (e.g., MC3, KC2, or DLinDMA). For example, a formulation comprising a lipid disclosed herein and a therapeutic or prophylactic agent has an increased therapeutic index as compared to a corresponding formulation which comprises a reference lipid *(e.g.,* MC3, KC2, or DLinDMA) and the same therapeutic or prophylactic agent.

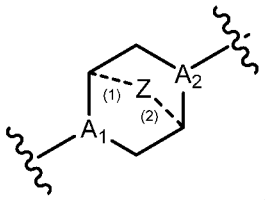
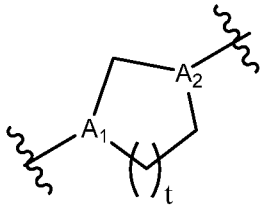
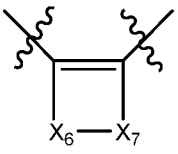
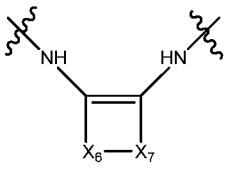
[00131] Lipids may be compounds of formula (I),



(I),

or salts or isomers thereof, wherein

W is  or  ,

ring A is  ,  ,  , or  ;

t is 1 or 2;

A i and **A 2** are each independently selected from **CH** or N;

Z is **CH2** or absent wherein when Z is **CH2,** the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

Ri, R.2, R3, R4, and **R 5** are independently selected from the group consisting of **C 5-20** alkyl, **C 5-20** alkenyl, **-R"MR', -R*YR", -YR",** and **-R*OR";**

Rxi and Rx2 are each independently **H** or C1-3 alkyl;

each M is independently selected from the group consisting of **-C(0)0-, -OC(O)-, -OC(0)0-, -C(0)N(R')-, -N(R')C(0)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0-, -S(0)$_2$-, -C(0)S-, -SC(O)-,** an aryl group, and a heteroaryl group;

M* is **-0-**, or **Ci-Ce** alkyl,

W^1 and W^2 are each independently selected from the group consisting of a bond, **-O-** and **-N(R_6)-;**

each R_6 is independently selected from the group consisting of H and C1-5 alkyl;

X^1, X^2, and X^3 are independently selected from the group consisting of a bond, -CH2-, **-(CH_2)$_2$-, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, -(CH_2)$_n$-C(0)-, -C(0)-(CH_2)$_n$-,** -(CH_2)$_n$-C**(0)0-**, -OC**(0)**-(CH_2)$_n$-, -(CH_2)$_n$-OC**(0)-**, -C**(0)0** -(CH_2)$_n$-, -CH(OH)-, -C(S)-, and -CH(SH)-;

each **Y** is independently a $_{C3-6}$ carbocycle;

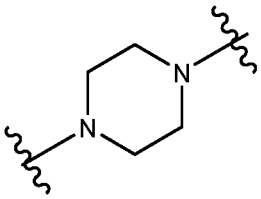
each **R*** is independently selected from the group consisting of **Ci-i$_2$** alkyl and **C_2-i$_2$** alkenyl;

each **R** is independently selected from the group consisting of C1-3 alkyl and a $_{C3-6}$ carbocycle;

each **R'** is independently selected from the group consisting of C1-18 alkyl, **C_2-is** alkenyl, and H;

each **R"** is independently selected from the group consisting of **$_{C3}$-i$_2$** alkyl, ***C3-12*** alkenyl and **-R*MR';** and

n is an integer from 1-6;

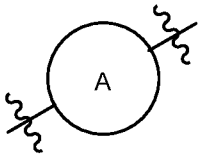


wherein when ring A is , then

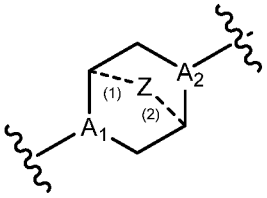
i) at least one of X^1, X^2, and X^3 is not -CH_2-; and/or

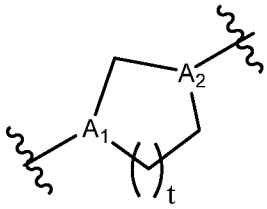
ii) at least one of **Ri, R_2,** $_{R3,}$ $_{R4,}$ and **R_5** is **-R"MR'.**

[00132] In some embodiments, M* is C1-C6 alkyl and W^1 and W^2 are each independently selected from the group consisting of -O- and **-N(R6)-.**

[00133] In some embodiments, **W** is 

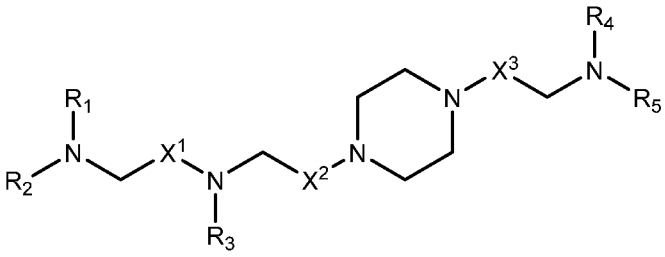
.

[00134] In some embodiments, ring A is 

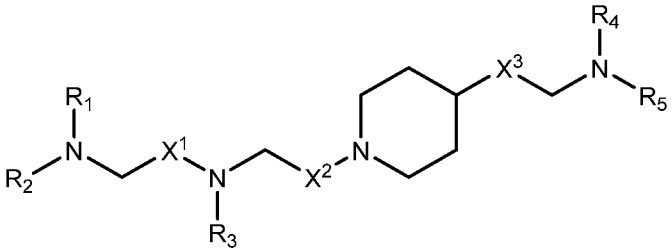
or 

.

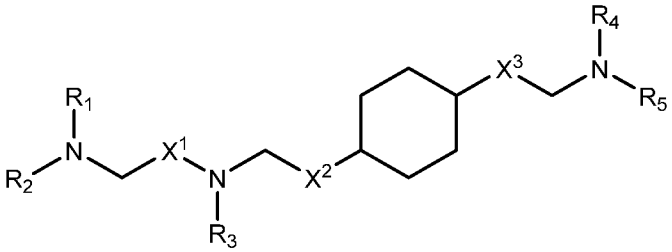
[00135] In some embodiments, the compound is of any of formulae (Ial)-(Ia7) :



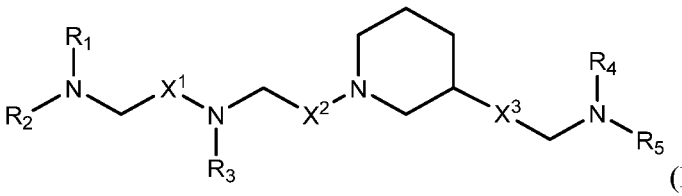
(Ia1),



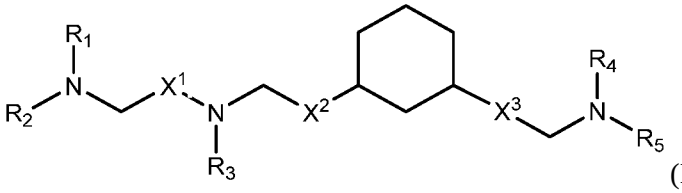
(Ia2),



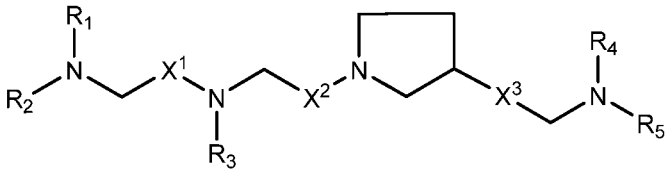
(Ia3),



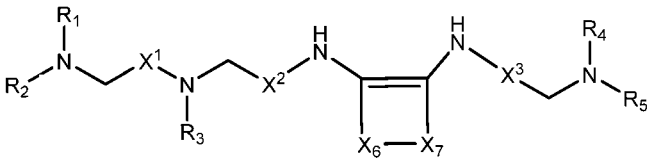
(Ia4),



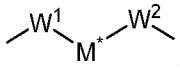
(Ia5), or



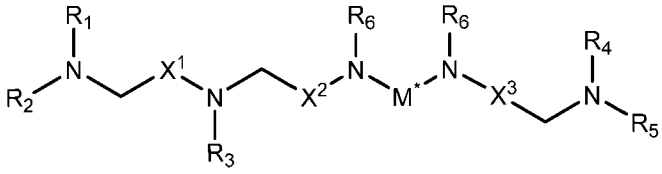
(Ia6), or



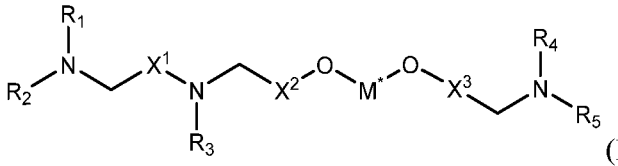
(Ia7).

[00136] In some embodiments, W is  .

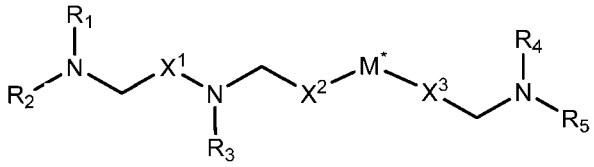
[00137] In some embodiments, the compound is of any of formulae (Ia8)-(Ial0):



(Ia**8**),

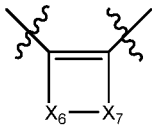


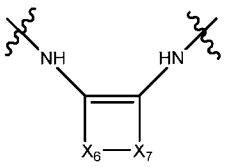
(Ia9), or

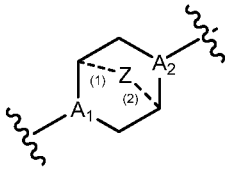


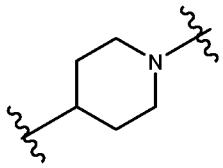
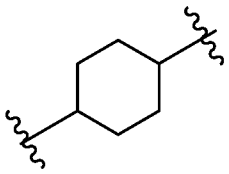
(Ia10).

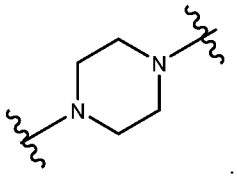
[00138] The compounds of Formula (I) or any of (Ial)-(Ial O) include one or more of the following features when applicable.

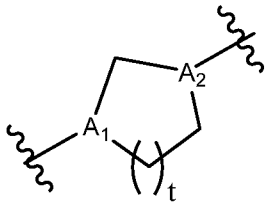
[00139] In some embodiments, ring A is  .

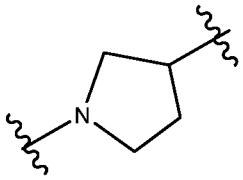
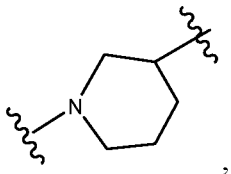
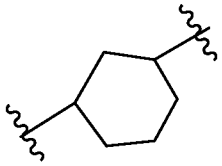
[00140] In some embodiments, ring A is  .

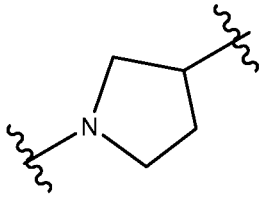
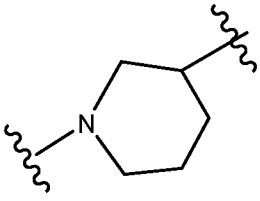
[00141] In some embodiments, ring **A** is  .

[00142] In some embodiments, ring **A** is  or  .

[00143] In some embodiments, ring **A** is  .

[00144] In some embodiments, ring A is  .

[00145] In some embodiments, ring A is  ,  , or  .

[00146] In some embodiments, ring A is  or 

wherein ring, in which the N atom is connected with X^2.

[00147] In some embodiments, **Z** is **CH2.**

[00148] In some embodiments, **Z** is absent.

[00149] In some embodiments, at least one of **Ai** and **A2** is **N.**

[00150] In some embodiments, each of A_1 and A_2 is N.

[00151] In some embodiments, each of A_1 and A_2 is CH.

[00152] In some embodiments, A_1 is N and A_2 is CH.

[00153] In some embodiments, A_1 is CH and A_2 is N.

[00154] In some embodiments, at least one of W^1 and W^2 is **–0-.**

[00155] In some embodiments, W^1 and W^2 are each a bond.

[00156] In some embodiments, W^1 and W^2 are each **-0-.**

[00157] In some embodiments, at least one of W^1 and W2 is -N(R_6)-.

[00158] In some embodiments, W^1 and W^2 are each -N(R.6)-.

[00159] In some embodiments, at least one R_6 is H.

[00160] In some embodiments, each R_6 is H.

[00161] In some embodiments, at least one R_6 is C_1 alkyl. In certain embodiments, each R_6 is C_1 alkyl. In some embodiments, at least one R_6 is C_2 alkyl. In certain embodiments, each R_6 is C_2 alkyl. In some embodiments, at least one R_6 is C_3 alkyl. In certain embodiments, each Re is C_3 alkyl.

[00162] In some embodiments, M* is C_1 alkyl. In some embodiments, M* is C_2 alkyl. In some embodiments, M* is C_3 alkyl. In some embodiments, M* is C_4 alkyl. In some embodiments, M* is C_5 alkyl. In some embodiments, M* is C_6 alkyl.

[00163] In some embodiments, M* is **-0-.**

[00164] In some embodiments, at least one of X^1, X^2, and X^3 is not -CH2-. For example, in certain embodiments, X^1 is not -CH2-. In some embodiments, at least one of X^1, X^2, and X^3 is -C(O)-.

[00165] In some embodiments, X^1 is -CH2-.

[00166] In some embodiments, X^2 is -(CH_2)n-C **(0)-,** -C**(0**)-(CH_2)n-, -(CH_2)n-C **(0)0-,** -OC**(0**)-(CH_2)n-, -(CH_2)n-OC **(0)-,** -C**(0)0** -(CH_2)n-. In some embodiments, X^2 is -CH2-. In some embodiments, X^2 is -(CH_2)$_2$-.

[00167] In some embodiments, X^3 is -(CH_2)n-C **(0)-,** -C**(0**)-(CH_2)n-, -(CH_2)n-C **(0)0-,** -OC**(0**)-(CH_2)n-, -(CH_2)n-OC **(0)-,** -C**(0)0** -(CH_2)n-.

[00168] In some embodiments, n is 1, 2, 3, 4, or 5. In certain embodiments, n is 1. In certain embodiments, n is 2. In certain embodiments, n is 3.

[00169] In some embodiments, X^2 is -C(O)-, **-C(0)0-,** -OC(O)-, -C**(0**)-CH_2-, **-CH_2-C(0)-,** -C**(0)0** -CH_2-, -OC **(0**)-CH_2-, **-CH_2-C(0)0-,** or -CH_2-OC **(0)-.**

[00170] In some embodiments, X^3 is -C(O)-, **-C(0)0-,** -OC(O)-, -C**(0**)-CH_2-, **-CH_2-C(0)-,** -C**(0)0** -CH_2-, -OC**(0**)-CH_2-, **-CH_2-C(0)0-,** or -CH_2-OC**(0)-.** In other embodiments, X^3 is -CH_2-. In some embodiments, X^3 is -$(CH_2)_2$-.

[00171] In some embodiments, X^2 and X^3 are each -C(O)-.

[00172] In some embodiments, X^3 is a bond or -$(CH_2)_2$-.

[00173] In some embodiments, **Ri** and **R_2** are the same. In certain embodiments, **Ri, R_2,** and R3 are the same. In some embodiments, R4 and R_5 are the same. In certain embodiments, **Ri, R_2,** R3, R4, and R_5 are the same.

[00174] In some embodiments, at least one of **Ri, R_2,** and R3 is **R*YR".** In some embodiments, **Ri** is **R*YR".** In some embodiments, **R_2** is **R*YR".** In some embodiments R3 is **R*YR".**

[00175] In some embodiments, at least one of R4 and R_5 is **R*YR".** In some embodiments, R4 is **R*YR".** In some embodiments, R_5 is **R*YR".**

[00176] In some embodiments, **Y** is a C_3 carbocycle.

[00177] In some embodiments, **R"** is **-R*MR'.**

[00178] In some embodiments, **Rxi** and **Rx_2** are each independently C1-3 alkyl. For example, **Rxi** and **Rx_2** are each **Ci** alkyl. For example, **Rxi** and **Rx_2** are each C_2 alkyl. For example, **Rxi** and **Rx_2** are each C_3 alkyl. For example, **Rxi** is **Ci** alkyl and **Rx_2** is **C_2** alkyl. For example, **Rxi** is **C_2** alkyl and **Rx_2** is **Ci** alkyl. For example, **Rxi** is **Ci** alkyl and **Rx_2** is C_3 alkyl. For example, **Rxi** is C_3 alkyl and **Rx_2** is **Ci** alkyl. For example, **Rxi** is **C_2** alkyl and **Rx_2** is C_3 alkyl. For example, **Rxi** is C_3 alkyl and **Rx_2** is **C_2** alkyl.

[00179] In some embodiments, **Rxi** is H and **Rx_2** is C1-3 alkyl. For example, **Rxi** is H and **Rx_2** is **Ci** alkyl. For example, **Rxi** is H and **Rx_2** is C_2 alkyl. For example, **Rxi** is H and **Rx_2** is C_3 alkyl.

[00180] In some embodiments, **Rxi** is C1-3 alkyl and **Rx_2** is H. For example, **Rxi** is **Ci** alkyl and **Rx_2** is H. For example, **Rxi** is C_2 alkyl and **Rx_2** is H. For example, **Rxi** is C_3 alkyl and **Rx_2** is H.

[00181] In some embodiments, **Rxi** and **Rx_2** are each H.

[00182] In some embodiments, at least one of **Ri, R_2,** R3, R4, and R_5 is **-R"MR'.** In some embodiments, at most one of **Ri, R_2,** R3, R4, and R_5 is **-R"MR'.** For example, at least one of **Ri, R_2,** and R3 may be **-R"MR',** and/or at least one of R4 and R_5 is **-R"MR'.** In certain embodiments, at least one M is -C**(0**)O-. In some embodiments, each M is -C**(0**)O-. In some embodiments, at least one M is -OC(O)-. In some embodiments, each M is -OC(O)-. In some

embodiments, at least one M is -0C(0)O. In some embodiments, each M is -0C(0)O. In some embodiments, at least one M is -C(0)S-. In some embodiments, each M is -C(0)S-. In some embodiments, at least one M is -SC(O)-. In some embodiments, each M is -SC(O)-.

[00183] In some embodiments, at least one R" is C2-5 alkyl. In some embodiments, each R" is C2-5 alkyl. In some embodiments, at least one R" is C3-7 alkyl. In some embodiments, each R" is C3-7 alkyl. In some embodiments, at least one R" is C_3 alkyl. In certain embodiments, each R" is C_3 alkyl. In some embodiments, at least one R" is C_5 alkyl. In certain embodiments, each R" is C_5 alkyl. In some embodiments, at least one R" is C_6 alkyl. In certain embodiments, each R" is C_6 alkyl. In some embodiments, at least one R" is C_7 alkyl. In certain embodiments, each R" is C7 alkyl. In some embodiments, at least one R" is Cs alkyl. In certain embodiments, each R" is Cs alkyl. In some embodiments, at least one R" is C_9 alkyl. In certain embodiments, each R" is C_9 alkyl. In some embodiments, at least one R" is C10 alkyl. In certain embodiments, each R" is C10 alkyl. In some embodiments, at least one R" is C11 alkyl. In certain embodiments, each R" is C11 alkyl. In some embodiments, at least one R" is branched C3-12 alkyl or C3-12 alkenyl. In some embodiments, each R" is branched C3-12 alkyl or C3-12 alkenyl.

[00184] In other embodiments, at least one R' is Cio-is alkyl. In some embodiments, each R' is Cio-is alkyl. In some embodiments, at least one R' is C1-9 alkyl. In certain embodiments, each R' is C1-9 alkyl. In some embodiments, at least one R' is C1-5 alkyl. In certain embodiments, each R' is C1-5 alkyl. In certain embodiments, at least one R' is C i alkyl. In certain embodiments, each R' is C i alkyl. In some embodiments, at least one R' is C 2 alkyl. In certain embodiments, each R' is C 2 alkyl. In some embodiments, at least one R' is C_5 alkyl. In certain embodiments, each R' is C_5 alkyl. In some embodiments, at least one R' is C_6 alkyl. In certain embodiments, each R' is C_6 alkyl. In some embodiments, at least one R' is C7 alkyl. In certain embodiments, each R' is C7 alkyl. In some embodiments, at least one R' is Cs alkyl. In certain embodiments, each R' is Cs alkyl. In some embodiments, at least one R' is C_9 alkyl. In certain embodiments, each R' is C_9 alkyl.

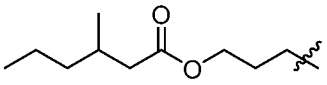
[00185] In some embodiments, at least one R' is branched C3-12 alkyl or C3-12 alkenyl. In some embodiments, each R' is branched C3-18 alkyl or C3-18 alkenyl. In some embodiments, each R' is branched C3-18 alkyl or C3-18 alkenyl. In some embodiments, at least one R' is branched C_{17} alkyl. In some embodiments, each R' is branched C_{17} alkyl. In some embodiments, at least one R' is branched C17 alkenyl. In some embodiments, each R' is branched C17 alkenyl.

[00186] In some embodiments at least one R" is C3-7 alkenyl. In some embodiments, each R" is C3-7 alkenyl. In some embodiments at least one R" is C4-10 alkenyl. In some embodiments, each R" is C4-10 alkenyl. In some embodiments at least one R" is C11-18 alkenyl. In some embodiments, each R" is C11-18 alkenyl. In certain embodiments, at least one R" is C 3 alkenyl. In certain embodiments, each R" is C 3 alkenyl. In certain embodiments, at least one R" is C 4 alkenyl. In certain embodiments, each R" is C 4 alkenyl. In certain embodiments, at least one R" is C 5 alkenyl. In certain embodiments, each R" is C 5 alkenyl. In certain embodiments, at least one R" is C_6 alkenyl. In certain embodiments, each R" is C_6 alkenyl. In certain embodiments, at least one R" is C 7 alkenyl. In certain embodiments, each R" is C 7 alkenyl. In certain embodiments, at least one R" is **Cs** alkenyl. In certain embodiments, each R" is **Cs** alkenyl. In certain embodiments, at least one R" is C9 alkenyl. In certain embodiments, each R" is C9 alkenyl.

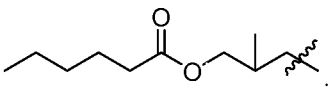
[00187] In some embodiments, at least one R" is branched C3-12 alkyl or C3-12 alkenyl.

[00188] In some embodiments, at least one R' is C4-10 alkenyl. In some embodiments, each R' is C4-10 alkenyl. In certain embodiments, at least one R' is C4 alkenyl. In certain embodiments, each R' is C4 alkenyl. In certain embodiments, at least one R' is C 5 alkenyl. In certain embodiments, each R' is C 5 alkenyl. In certain embodiments, at least one R' is C_6 alkenyl. In certain embodiments, each R' is C_6 alkenyl. In certain embodiments, at least one R' is C7 alkenyl. In certain embodiments, each R' is C7 alkenyl. In certain embodiments, at least one R' is **Cs** alkenyl. In certain embodiments, each R' is **Cs** alkenyl. In certain embodiments at least one R' is C9 alkenyl. In certain embodiments, each R' is **Cs** alkenyl. In certain embodiments, at least one R' is C10 alkenyl. In certain embodiments, each R' is C10 alkenyl.

[00189] In some embodiments, M is - O-**C**(O)- or **-C(0)O-**, and at least one R' is **Ci**-12 alkyl or C2-12 alkenyl. For example, -R"MR' is

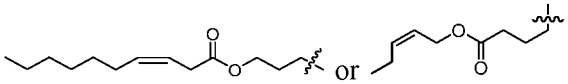


[00190] In some embodiments, M is -O-C(O)- or - **C(0)0-** and at least one R" is branched C3-12 alkyl, C3-12 alkenyl. For example, -R"MR' is

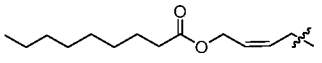
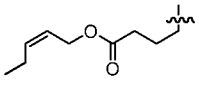


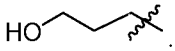
[00191] In some embodiments, R' is C2-12 alkenyl (e.g., R' is non **-2**-enyl or pent **-2**-enyl). In some embodiments, R' is non **-2**-enyl or pent **-2**-enyl and M' is -OC(O)-. In other

embodiments, R' is non-2-enyl or pent-2-enyl and M' is -C(O)O-. For example R"MR' is

 or .

[00192] In some embodiments, R" is or. In some embodiments, R' is but-2-ene and M' is -OC(O)-. In other embodiments, R' is but-2-ene and M' is -C(O)O-. For example R"MR' is

 or  .

[00193] In some embodiments, at least one of Ri, R2, R3, R4, and R_5 is substituted C_{5-20} alkyl or substituted C_{5-20} alkenyl. For example, one of R_4, and R_5 is substituted C_{5-20} alkyl or substituted C_{5-20} alkenyl (e.g. substituted with a C_{1-3} alkyl or substituted with OH or alkoxy). For example, R_4 or R_5 is  .

[00194] In some embodiments, Ri, R2, R3, R4, and R_5 are each independently selected from -R"MR' and C_{5-10} alkyl. In certain embodiments, Ri, R2, R3, and R_4 are each independently selected from -R"MR' and C_5 alkyl. In certain embodiments, Ri, R2, R3, and R_4 are each independently selected from -R"MR' and C_6 alkyl. In certain embodiments, Ri, R2, R3, and R_4 are each independently selected from -R"MR' and C_7 alkyl. In certain embodiments, Ri, R2, R3, and R_4 are each independently selected from -R"MR' and C_8 alkyl. In certain embodiments, Ri, R2, R3, and R_4 are each independently selected from -R"MR' and C_9 alkyl. In certain embodiments, Ri, R2, R3, and R_4 are each independently selected from -R"MR' and C10 alkyl.

[00195] In some embodiments, at least one of R_4 and R_5 is C_9 alkyl. In some embodiments, one of R_4 and R_5 is C_9 alkyl. In some embodiments, at least one of R_4 and R_5 is Cio alkyl. In some embodiments, one of R_4 and R_5 is C10 alkyl. In some embodiments, at least one of R_4 and R_5 is C11 alkyl. In some embodiments, one of R_4 and R_5 is C11 alkyl. In some embodiments, at least one of R_4 and R_5 is C12 alkyl. In some embodiments one of R_4 and R_5 is C12 alkyl. In some embodiments, at least one of R_4 and R_5 is C13 alkyl. In some embodiments, one of R_4 and R_5 is C_{13} alkyl. In some embodiments, at least one of R_4, and R_5 is C14 alkyl. In some embodiments, one of R_4, and R_5 is C_{14} alkyl.

[00196] In some embodiments, at least one of R_4 and R_5 is -R"MR' . In some embodiments, each of R_4 and R_5 is -R"MR'. In some embodiments, one of R_4 and R_5 is -R"MR' .

[00197] In some embodiments, R_4 and R_5 have the same number of carbon atoms. In some embodiments, R_4 and R_5 have 6, 8, 9, 12, 14, or 18 carbon atoms.

[00198] In some embodiments, **R4** and $_{R\,5}$ are C_6 alkyl. In some embodiments, $_{R\,4}$ and $_{R\,5}$ are C7 alkyl. In some embodiments, $_{R\,4}$ and $_{R\,5}$ are **Cs** alkyl. In some embodiments, $_{R\,4}$ and $_{R\,5}$ are C9 alkyl. In some embodiments, $_{R\,4}$ and $_{R\,5}$ are C10 alkyl. In some embodiments, $_{R\,4}$ and $_{R\,5}$ are C11 alkyl. In some embodiments, $_{R\,4}$ and $_{R\,5}$ are C12 alkyl. In some embodiments, $_{R\,4}$ and $_{R\,5}$ are C12 alkyl. In some embodiments, $_{R\,4}$ and $_{R\,5}$ are C_{14} alkyl. In some embodiments, $_{R\,4}$ and $_{R\,5}$ are Ci6 alkyl. In some embodiments, $_{R\,4}$ and $_{R\,5}$ are **Cis** alkenyl. In some embodiments, $_{R\,4}$ and $_{R\,5}$ are linoleyl.

[00199] The compound of any one of the preceding claims, wherein $_{R\,4}$ and $_{R\,5}$ are C14

[00200] In some embodiments, **Ri,** R2, and $_{R\,3}$ have the same number of carbon atoms. In some embodiments, **Ri,** R2, and $_{R\,3}$ have 6, 8, 9, 12, 14, or 18 carbon atoms. In some embodiments, **Ri,** R2, and $_{R\,3}$ are linoleyl. In some embodiments, at least one of **Ri,** R2, and $_{R\,3}$ is $_{C5\text{-}10}$ alkenyl. In some embodiments, **Ri,** R2, and $_{R\,3}$ each independently are $_{C5\text{-}10}$ alkenyl.

[00201] In some embodiments, at least one of **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ is C12 alkyl. In certain embodiments, each of **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ are C12 alkyl. In some embodiments, at least one of **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ is C9, C12, or C14 alkyl.

[00202] In some embodiments, **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ each independently are $_{C5\text{-}10}$ alkyl. In certain embodiments, **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ each are **Cs** alkyl. In certain embodiments, **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ each are C9 alkyl. In certain embodiments, **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ each are C10 alkyl. In certain embodiments, **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ each are C11 alkyl. In certain embodiments, **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ each are C12 alkyl. In certain embodiments, **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ each are C13 alkyl. In certain embodiments, **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ each are C14 alkyl. In some embodiments, **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ each independently are C9, C12, or C14 alkyl.

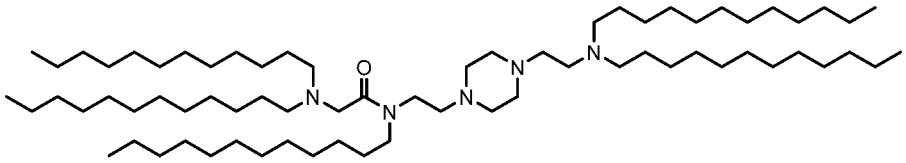
[00203] In some embodiments, at least one of **Ri,** R2, and $_{R\,3}$ is $_{C5\text{-}10}$ alkenyl. In some embodiments, **Ri,** R2, and $_{R\,3}$ each independently are $_{C5\text{-}10}$ alkenyl. In certain embodiments, at least one of **Ri,** R2, and $_{R\,3}$ is C9 alkenyl. In certain embodiments, **Ri,** R2, and $_{R\,3}$ each are C9 alkenyl.

[00204] In some embodiments, at least one of **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ is $_{C5\text{-}10}$ alkenyl. In some embodiments, at least one of **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ is $_{C\,5}$ alkenyl. In some embodiments, at least one of **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ is C_6 alkenyl. In some embodiments, at least one of **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ is C7 alkenyl. In some embodiments, at least one of **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ is **Cs** alkenyl. In some embodiments, at least one of **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ is C9 alkenyl. In some embodiments, at least one of **Ri,** R2, $_{R3,}$ $_{R4,}$ and $_{R\,5}$ is C10 alkenyl.

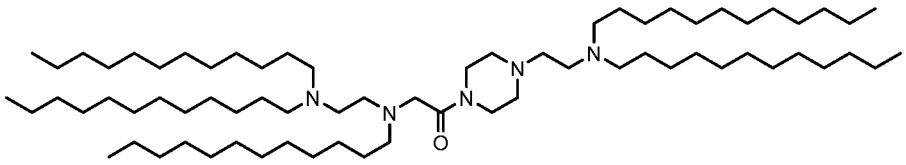
[00205] In some embodiments, R_4 is selected from C_{5-20} alkenyl. In some embodiments, R_4 is **Cio** alkenyl. In some embodiments, R_4 is C_{11} alkenyl. In some embodiments, R_4 is C_{12} alkenyl. In some embodiments, R_4 is C_{13} alkenyl. In some embodiments, R_4 is C_{14} alkenyl. In some embodiments, R_4 is C_{15} alkenyl. In some embodiments, R_4 is Ci6 alkenyl. In some embodiments, R_4 is C_{17} alkenyl. In some embodiments, R_4 is **Cis** alkenyl.

[00206] In some embodiments at least one of R_4 and R_5 is C_{14} alkenyl.

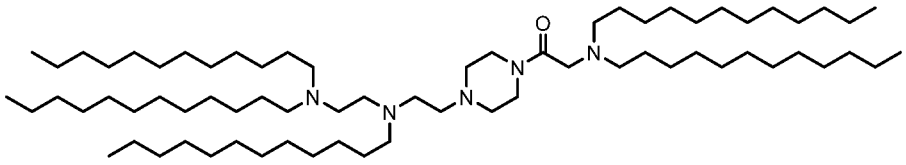
[00207] In certain embodiments, the compound is selected from the group consisting of:



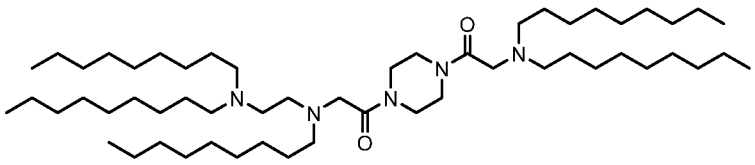
(Compound 1),



(Compound 2),



(Compound 3),



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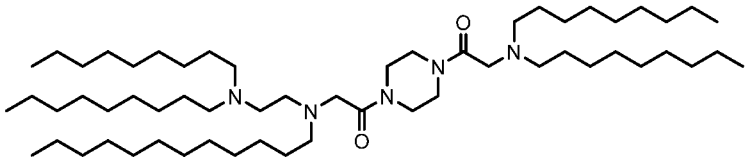
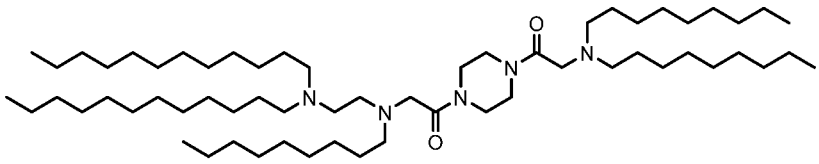
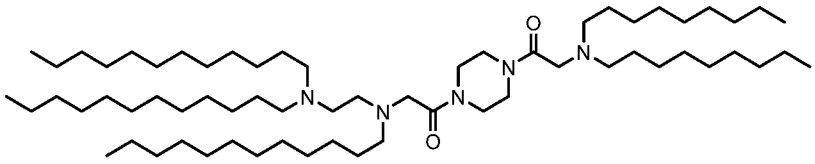


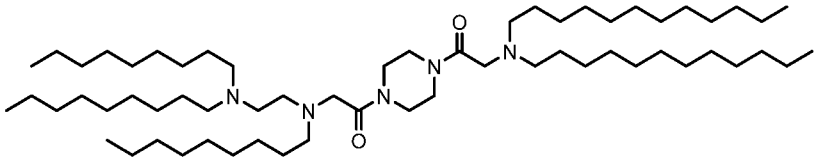
(Compound 5),



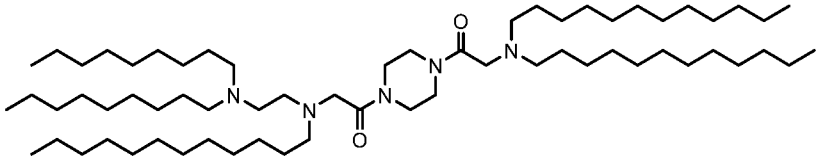
(Compound 6),



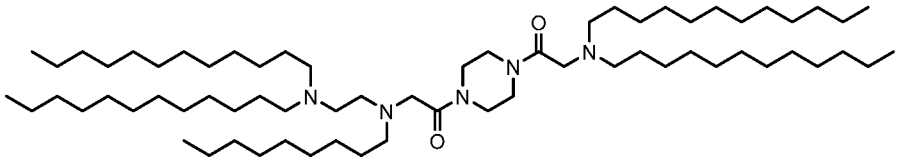
(Compound **7),**



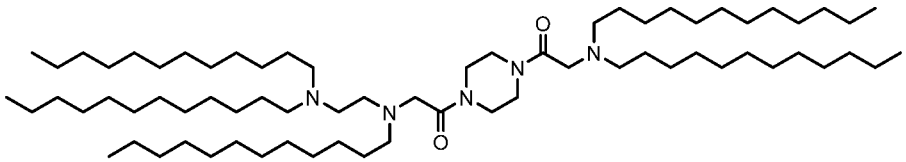
(Compound 8),



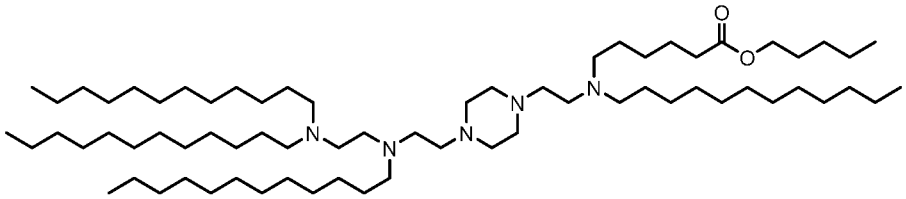
(Compound 9),



(Compound 10),



(Compound 11),



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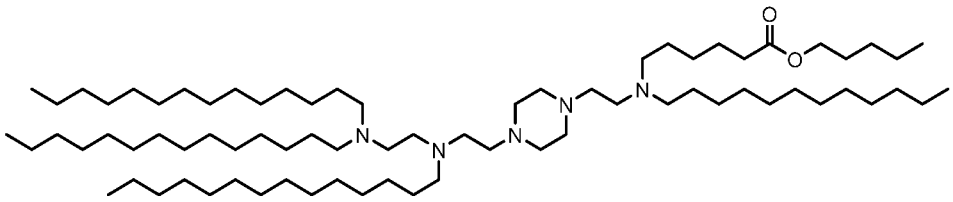

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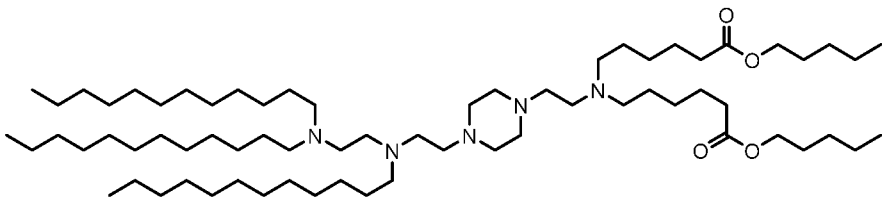

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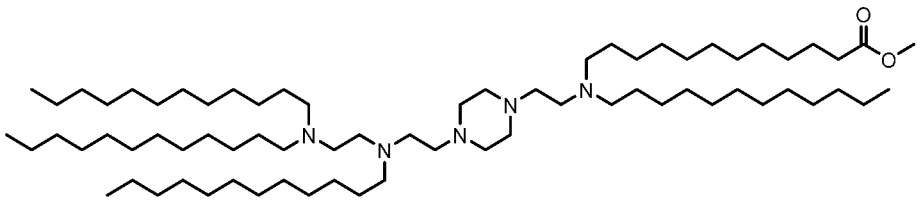

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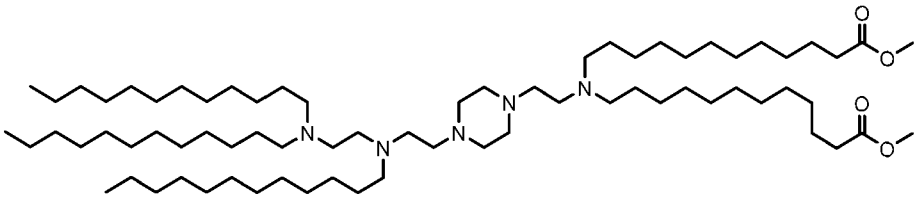

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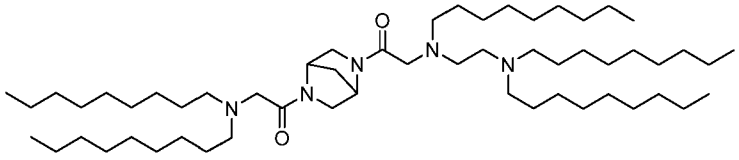

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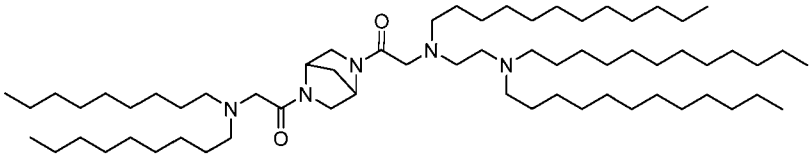

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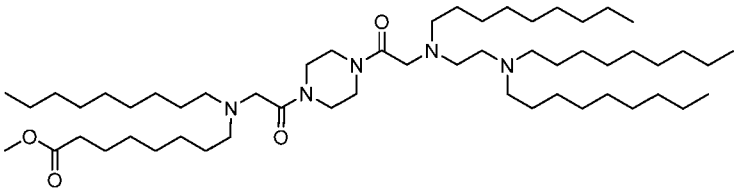

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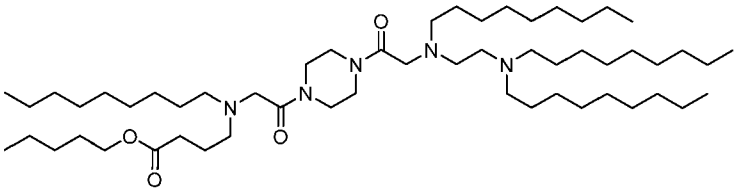

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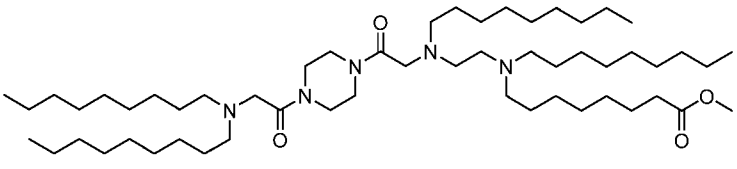

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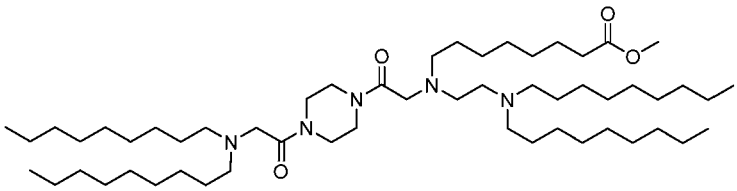

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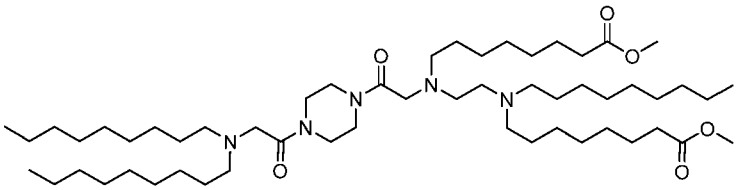

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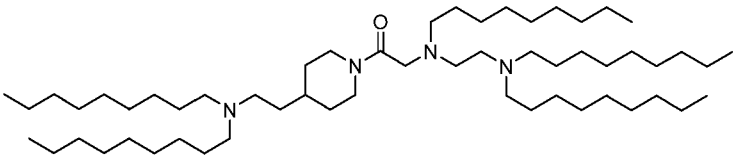

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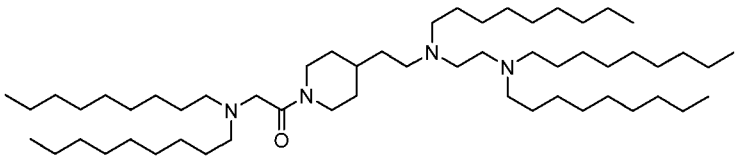

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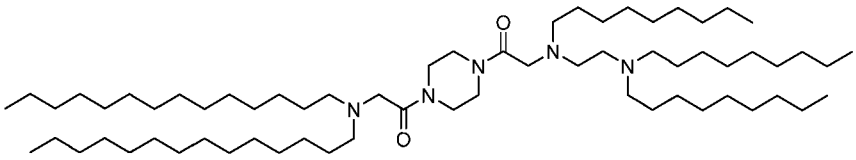

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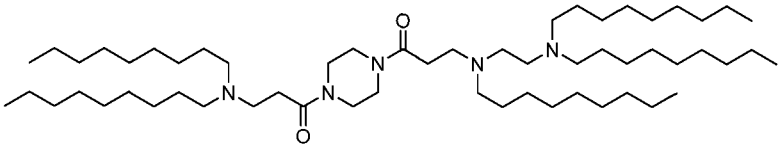

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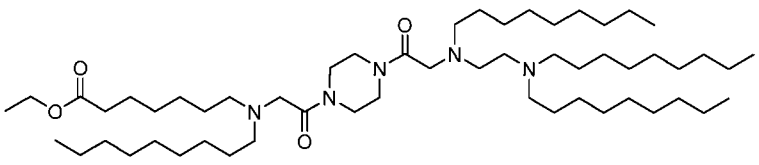

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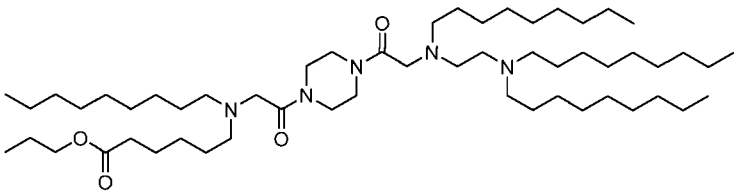

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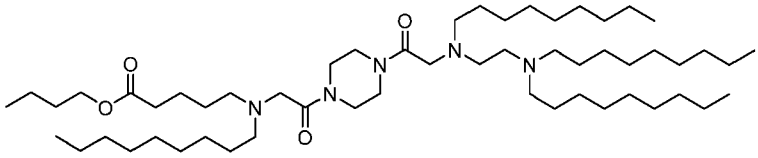

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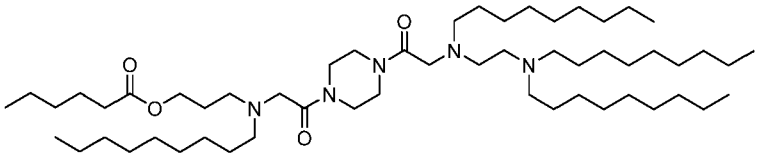

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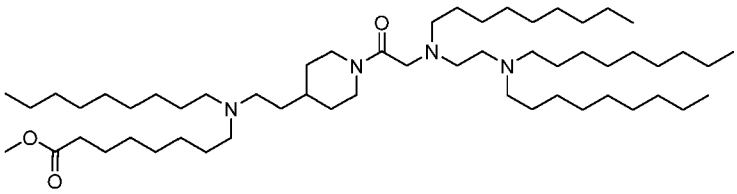

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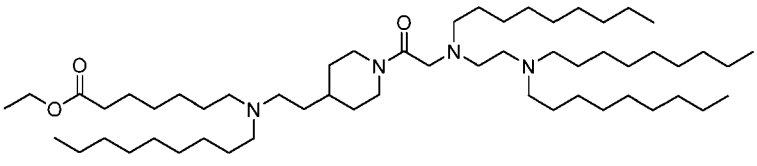

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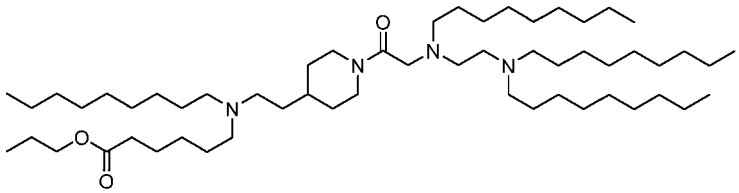

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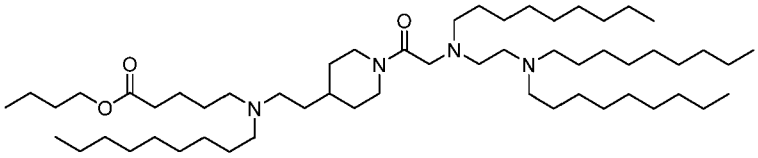

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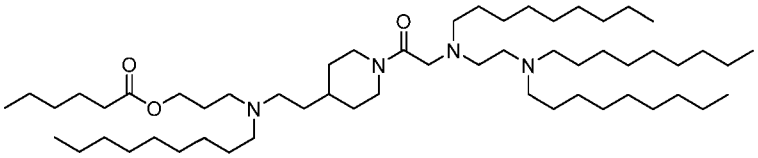

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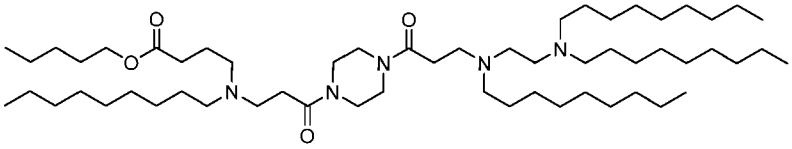

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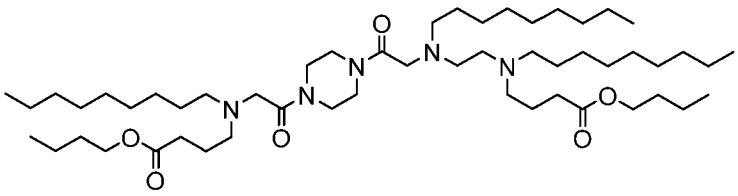

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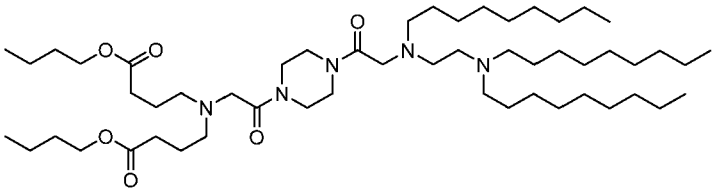

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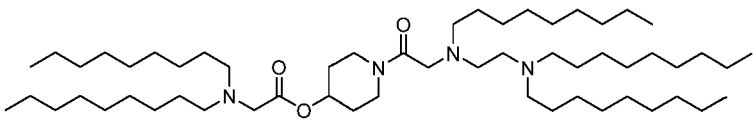

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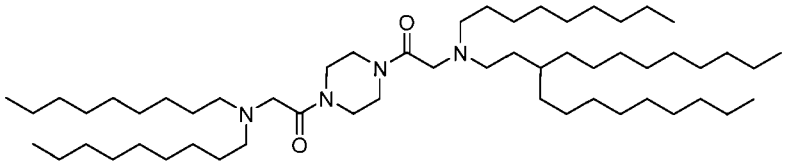

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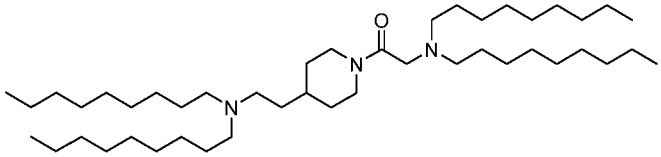

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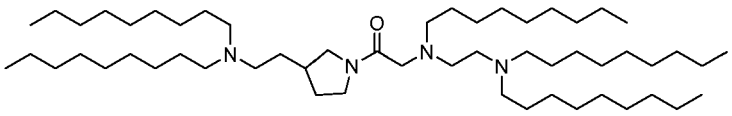

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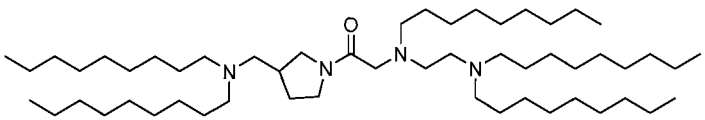

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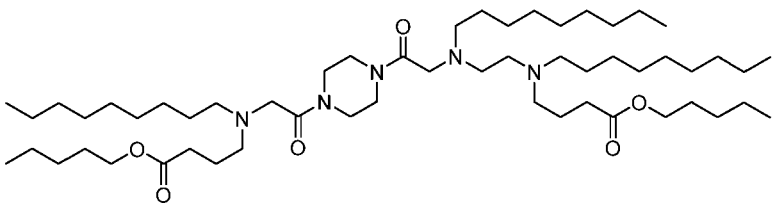

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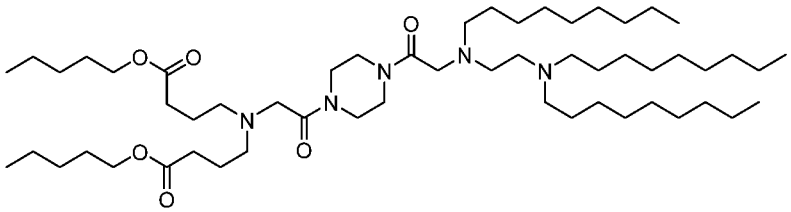

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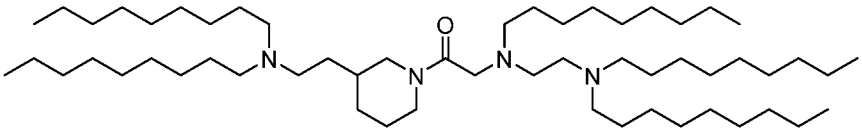

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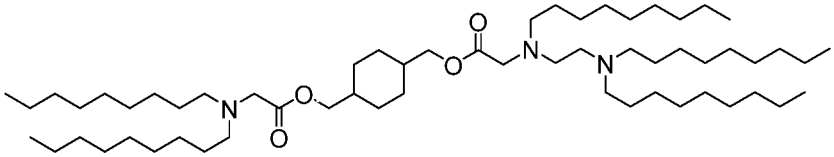

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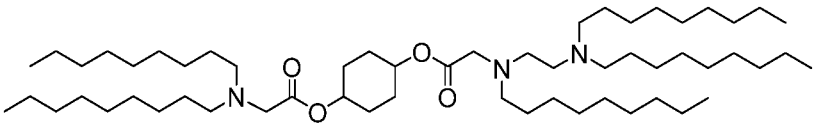

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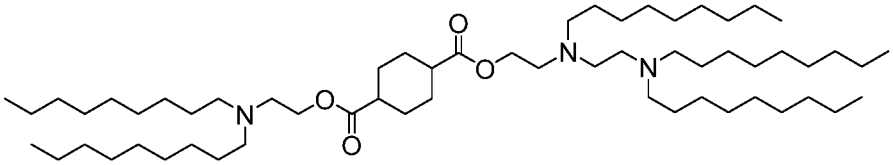

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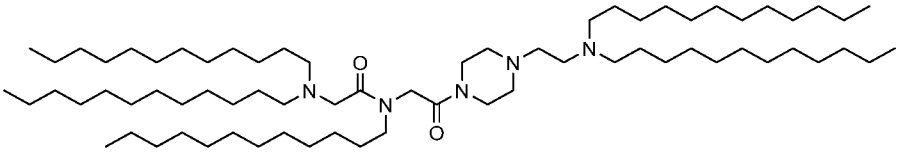

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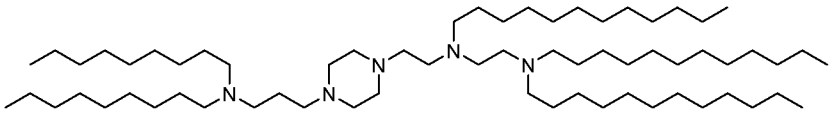

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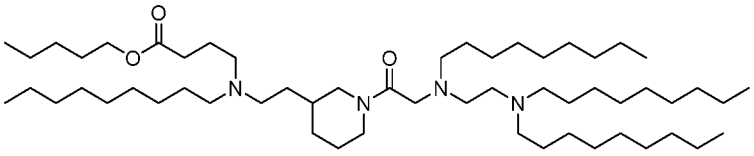

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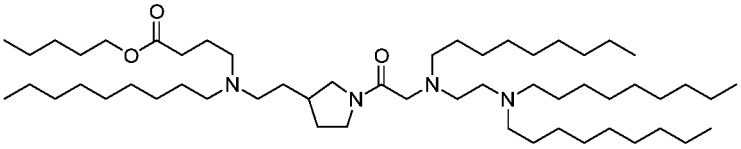

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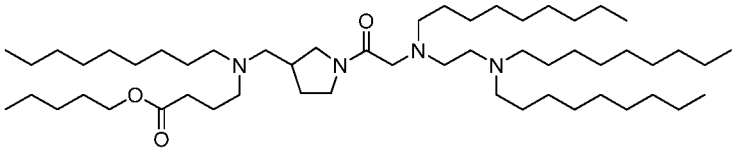

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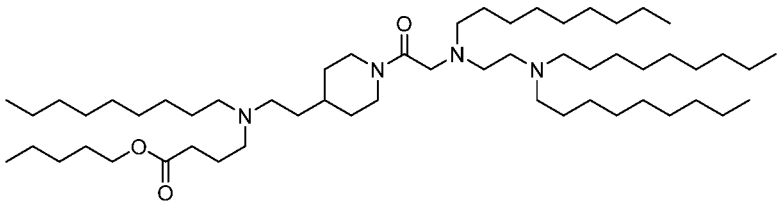

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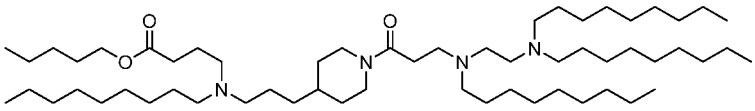

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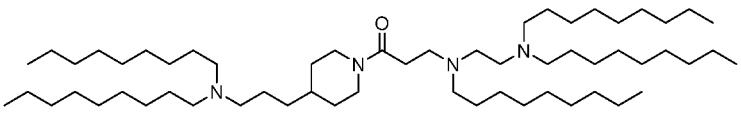

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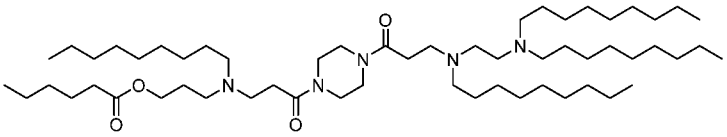

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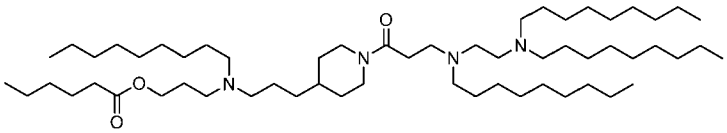

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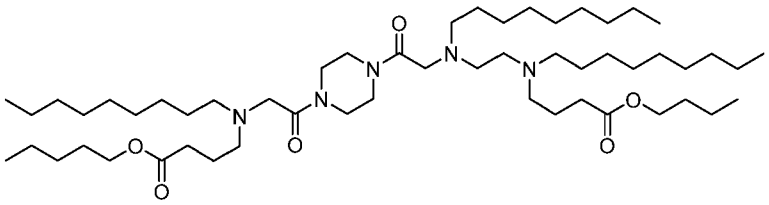

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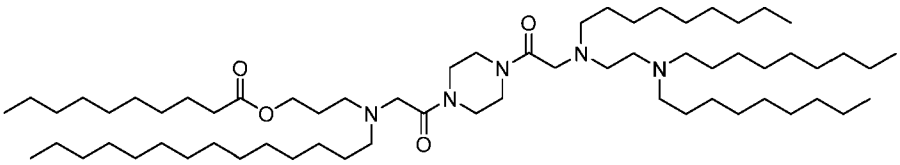

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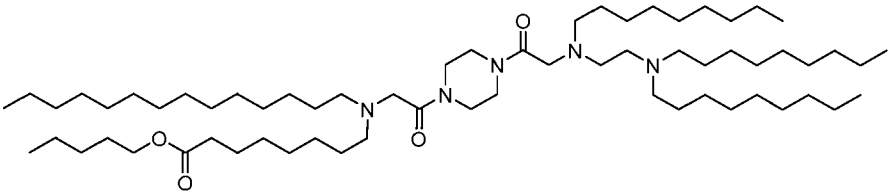

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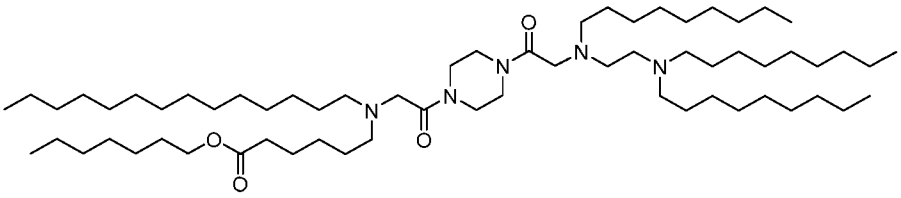

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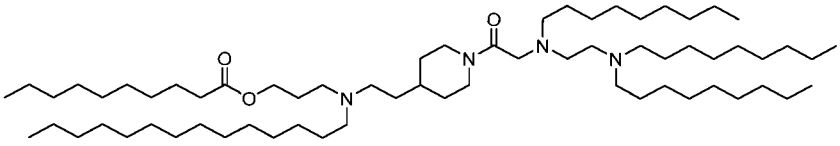

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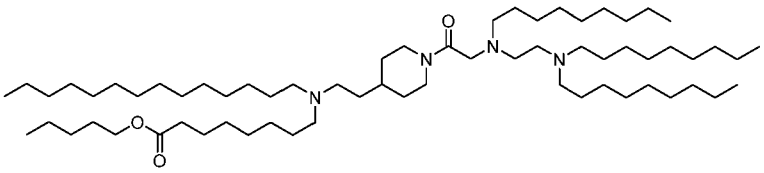

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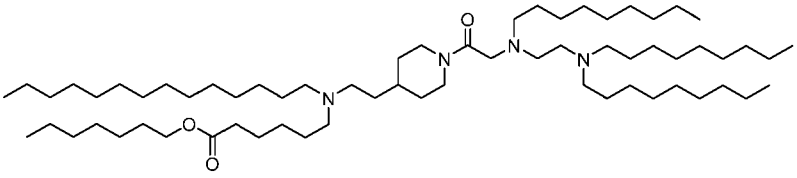

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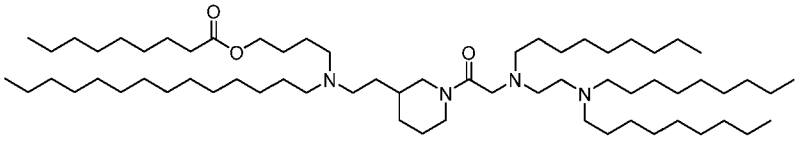

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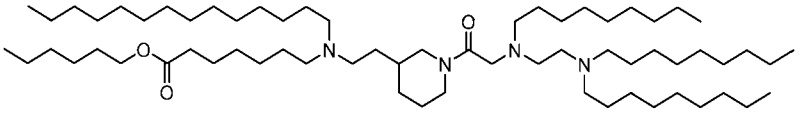

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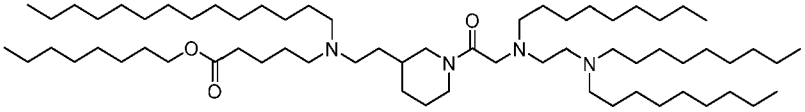

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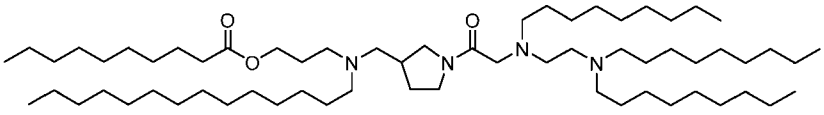

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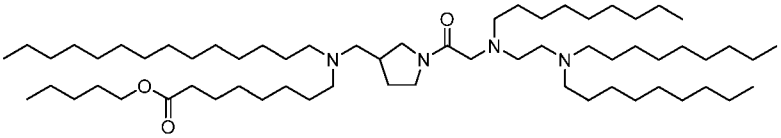

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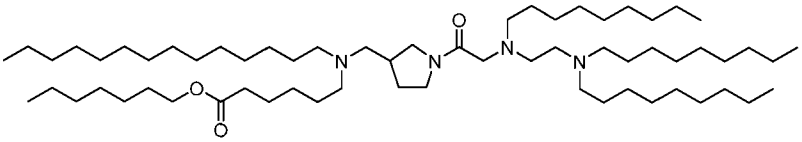

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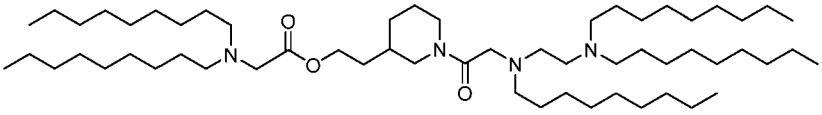

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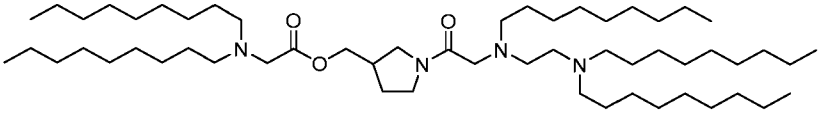

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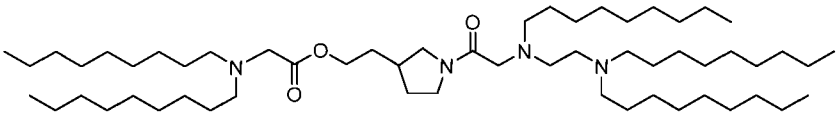

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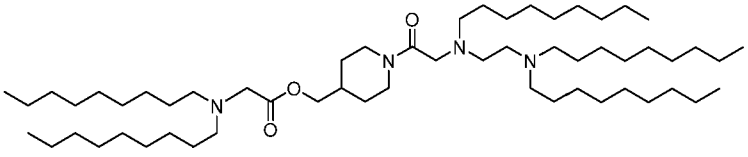

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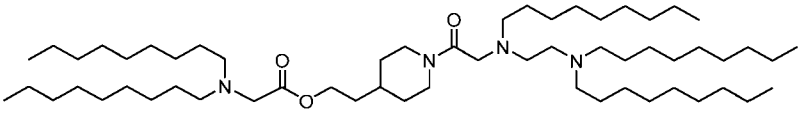

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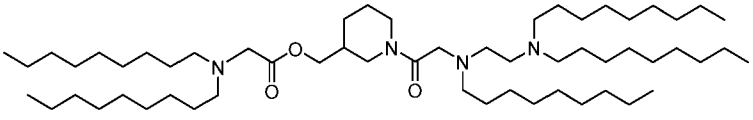

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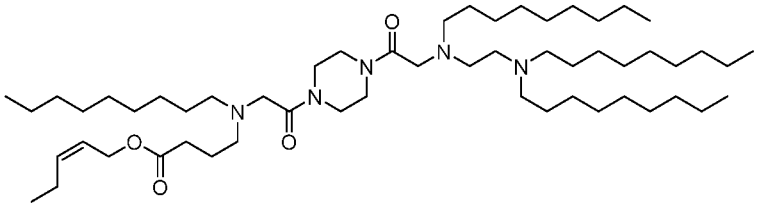

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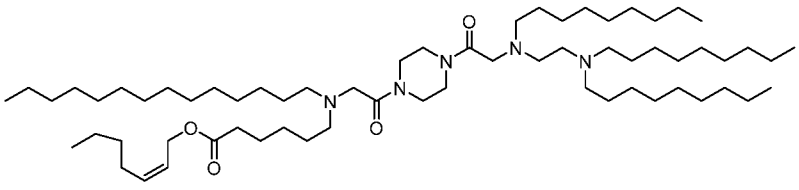

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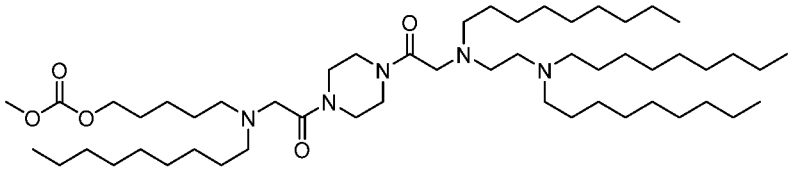

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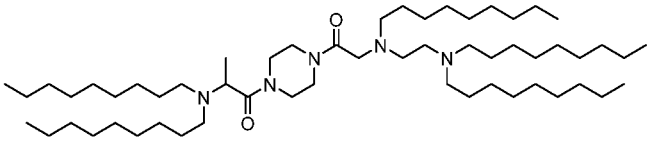

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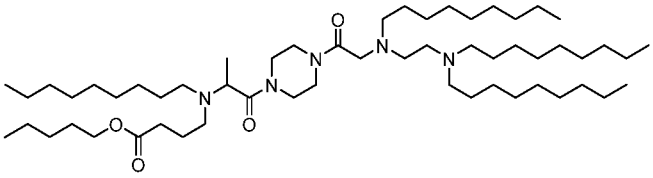

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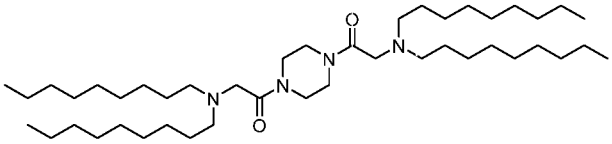

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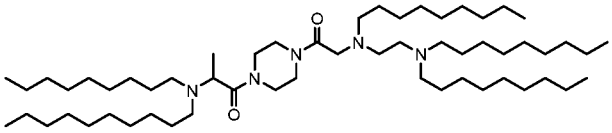

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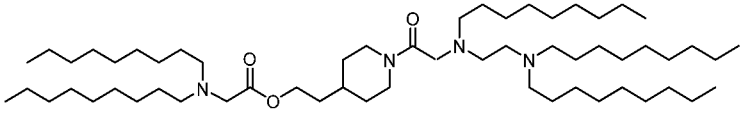

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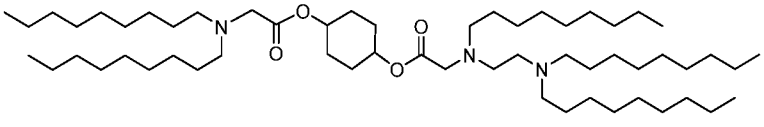

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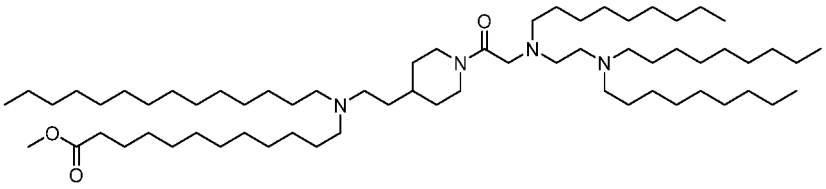

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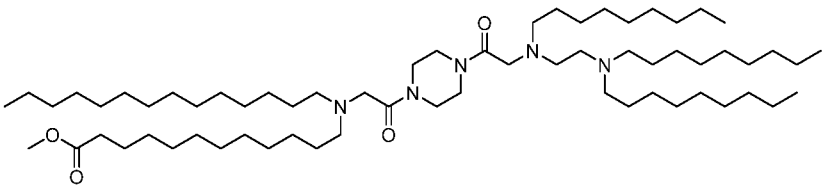

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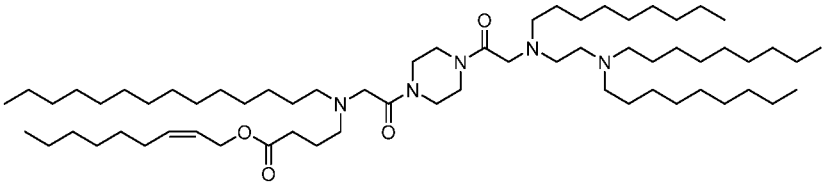

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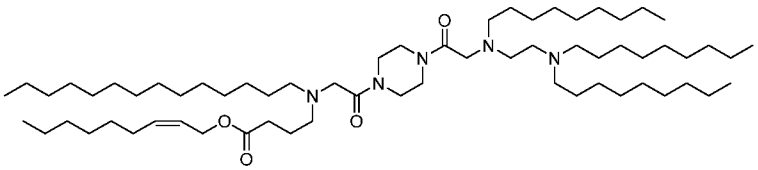

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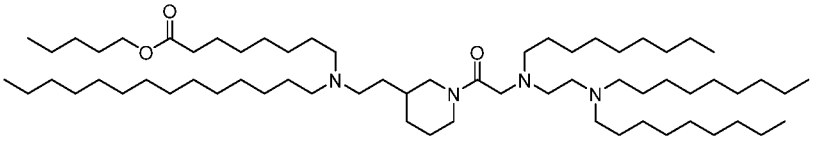

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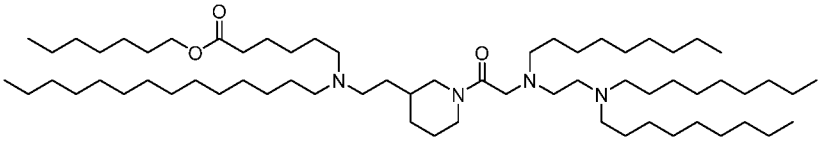

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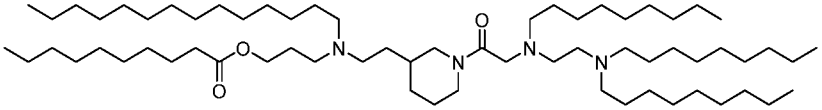

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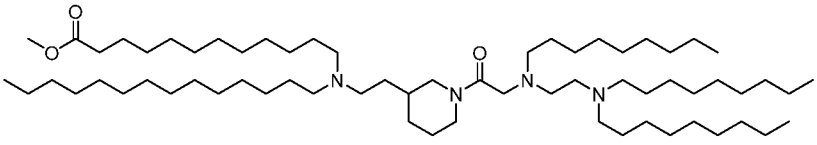

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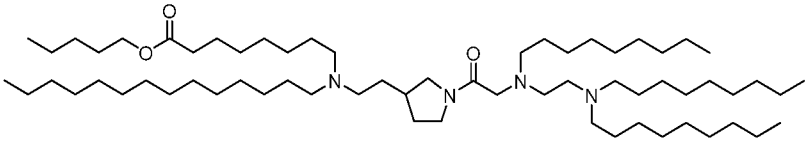

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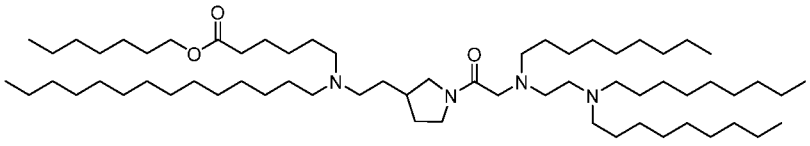

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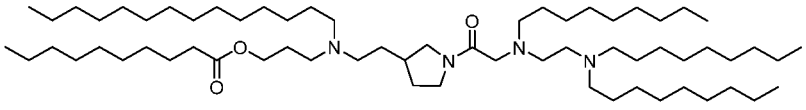

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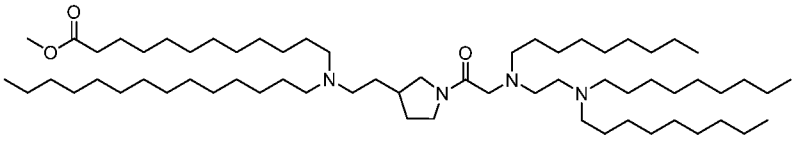

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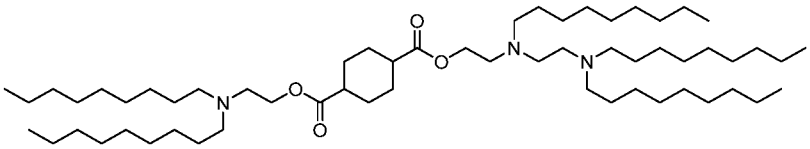

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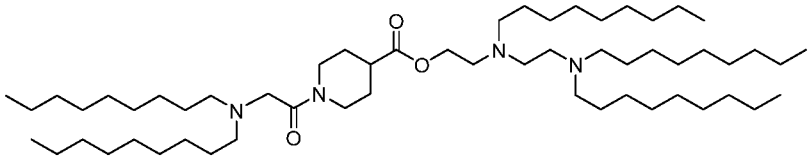

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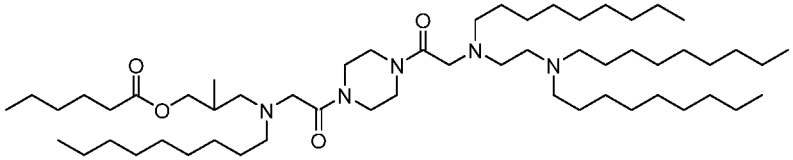

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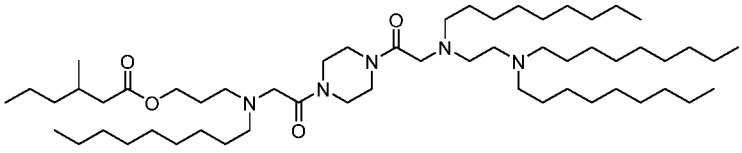

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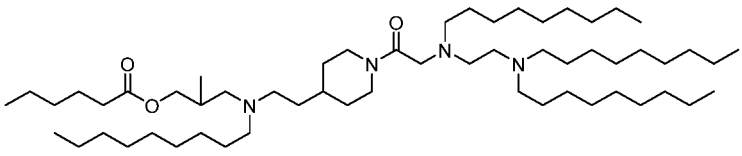

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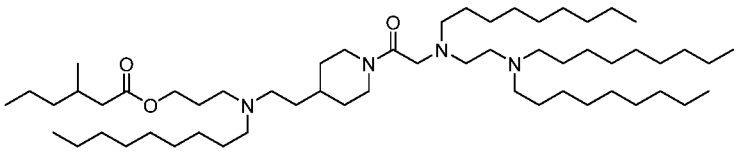

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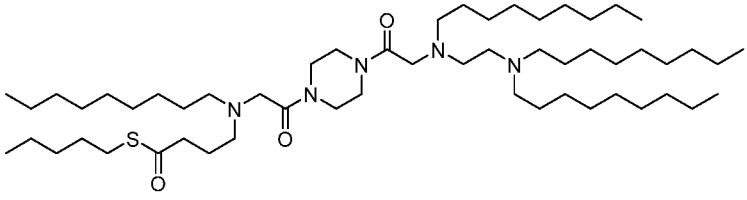

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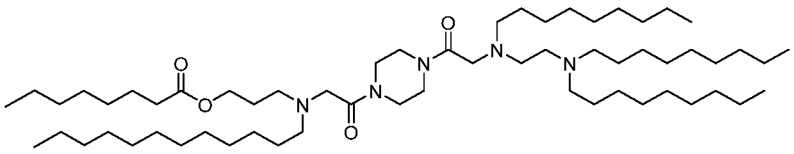

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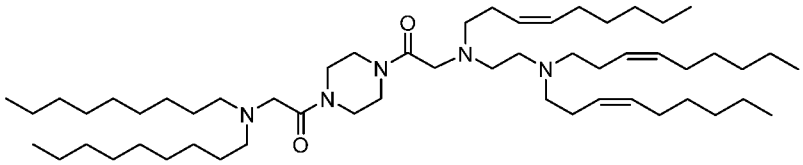

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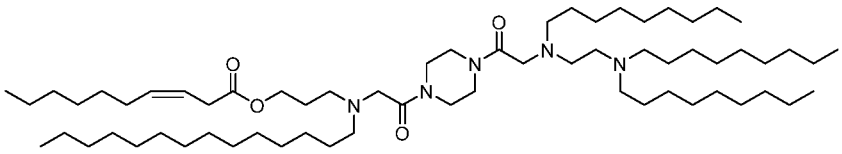

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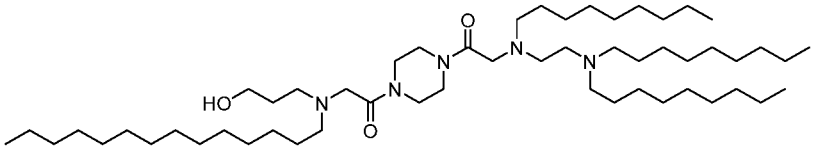

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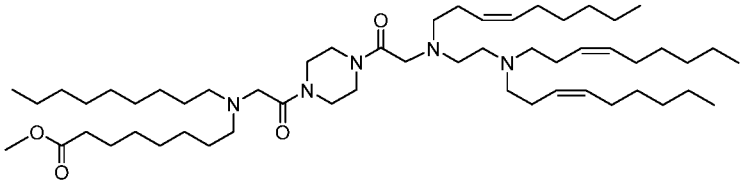

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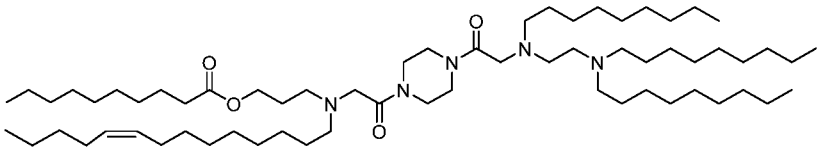

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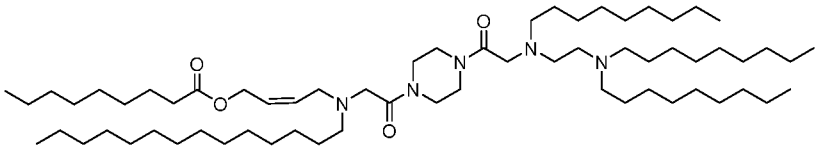

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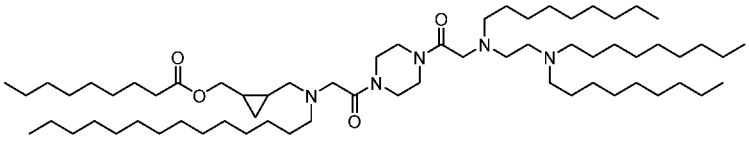

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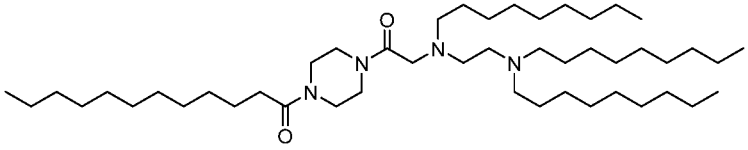

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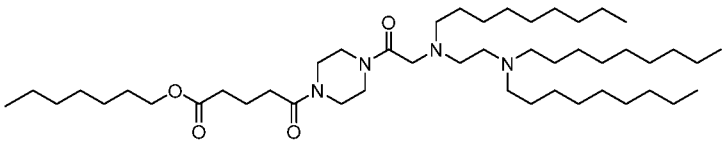

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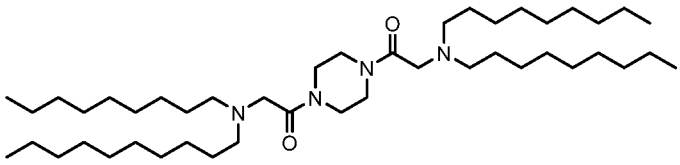

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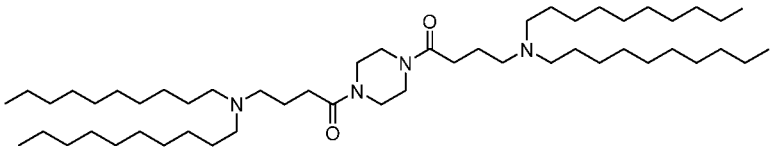

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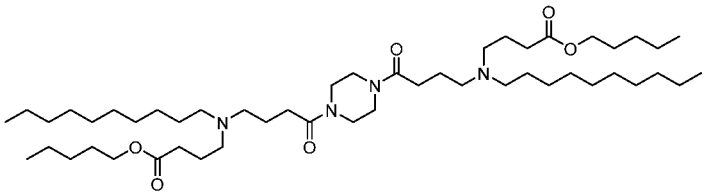

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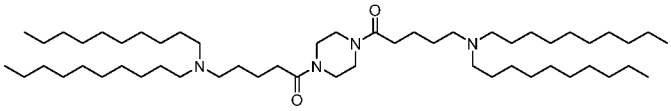

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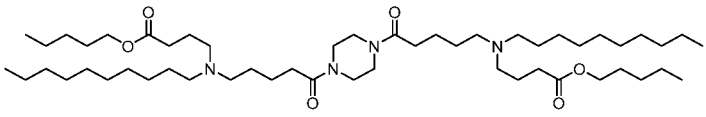

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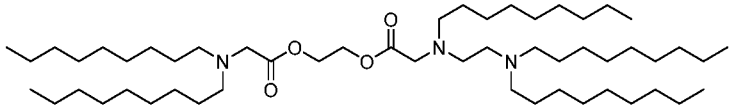

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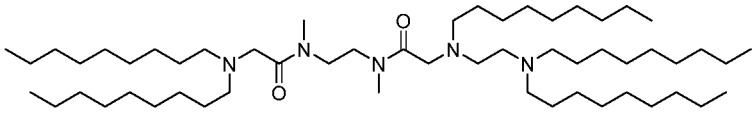

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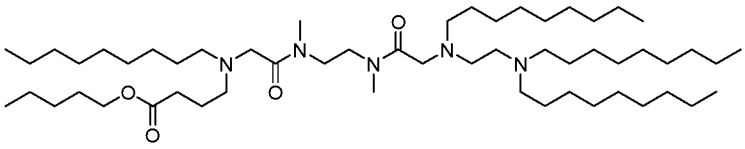

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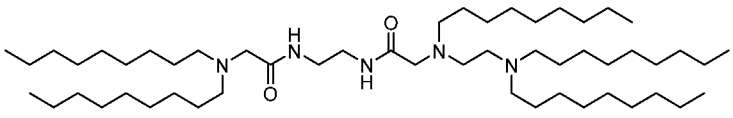

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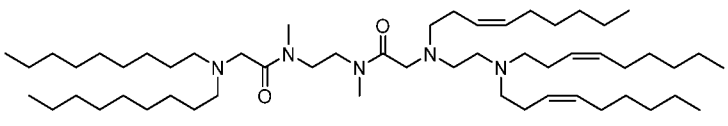

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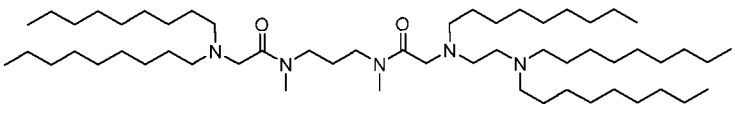
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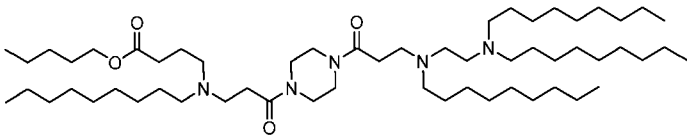
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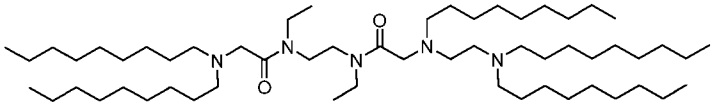
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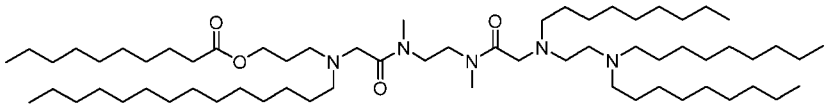
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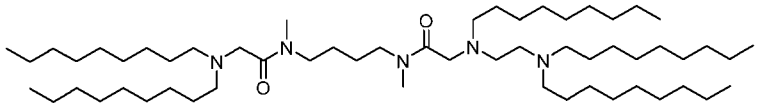
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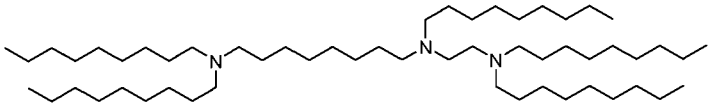
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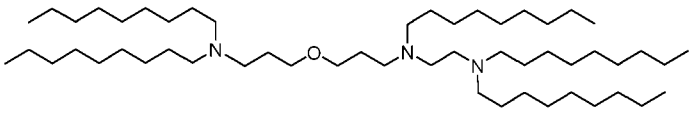
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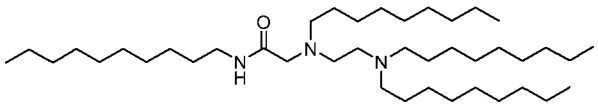
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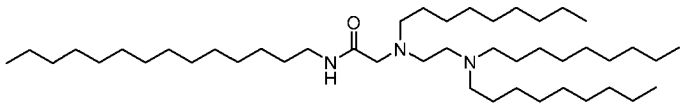
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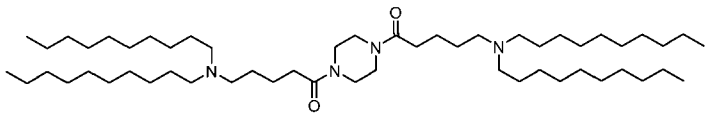
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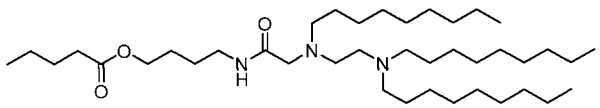
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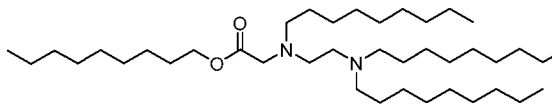
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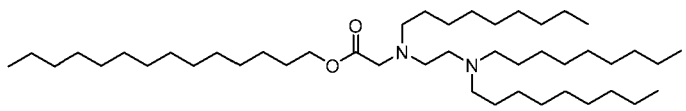
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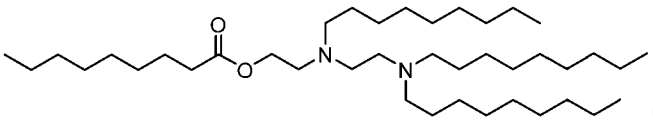

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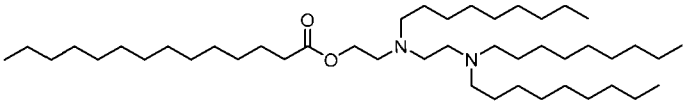

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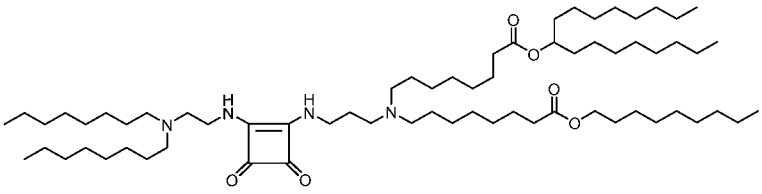

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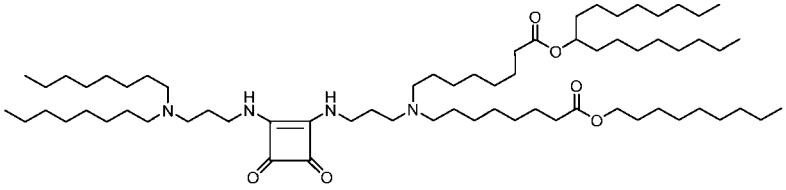

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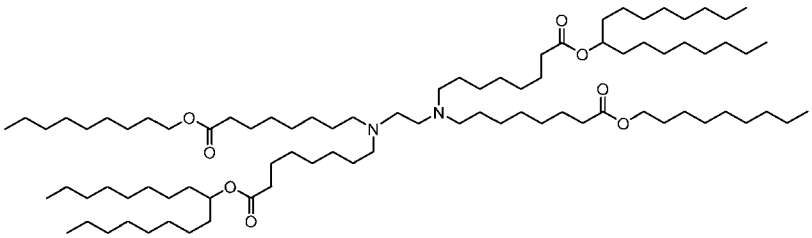

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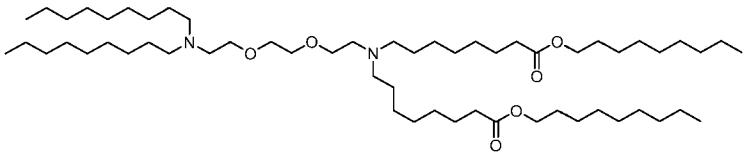

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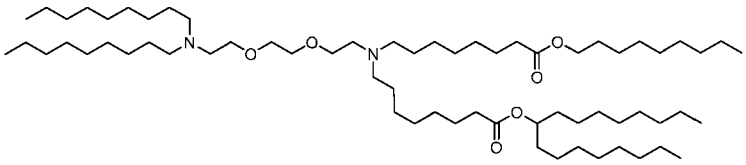

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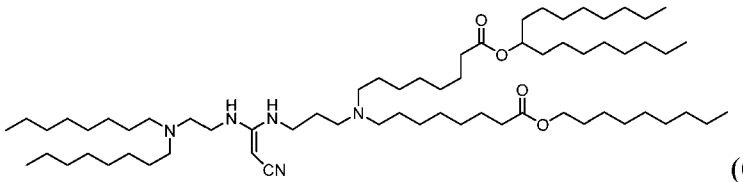

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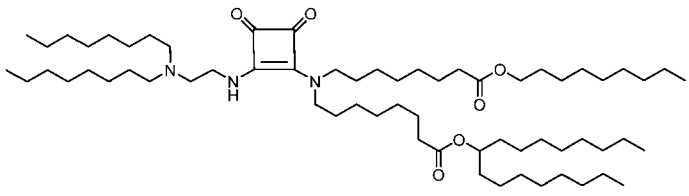

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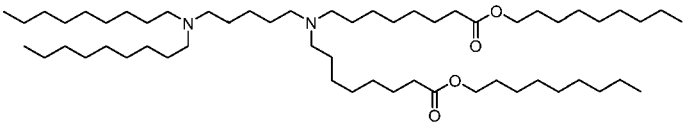

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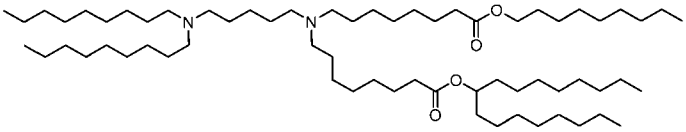

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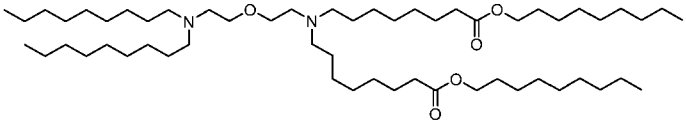

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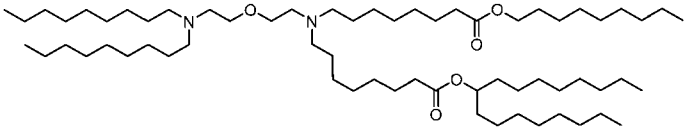

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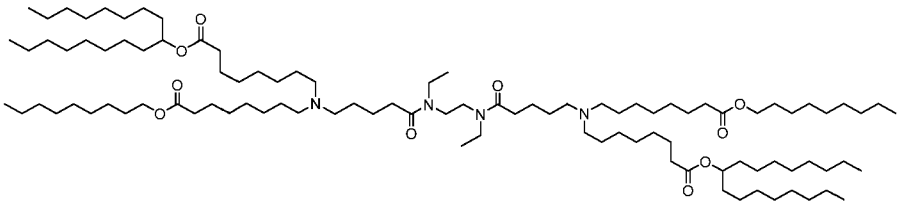

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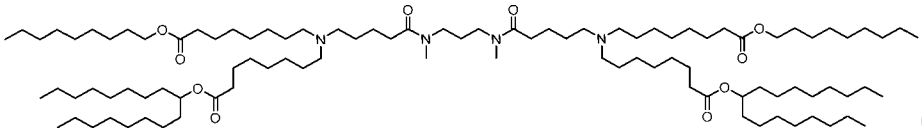

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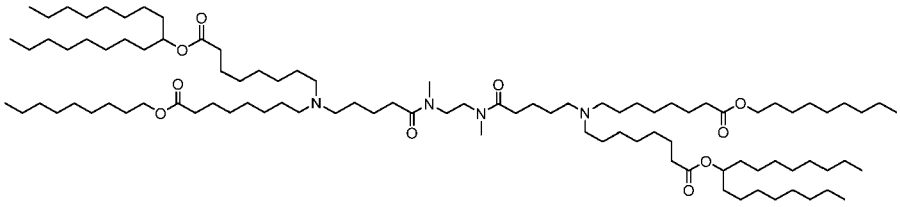

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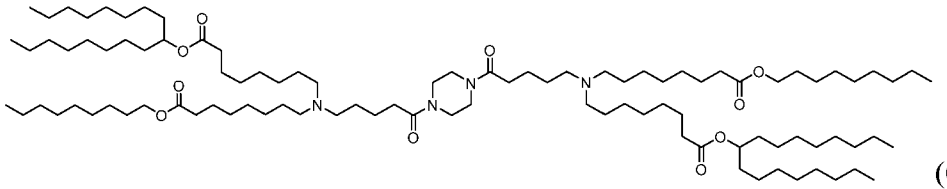

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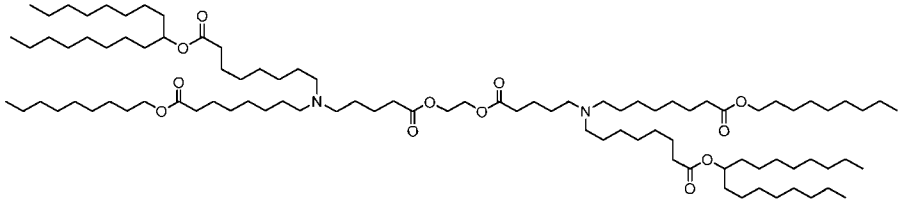
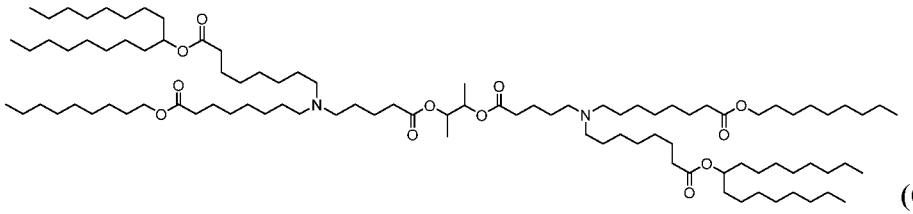
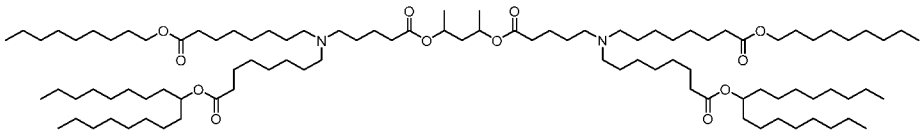


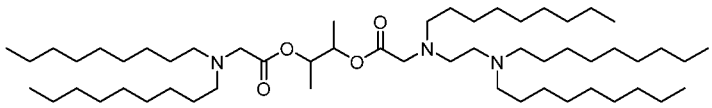
(Compound 185),



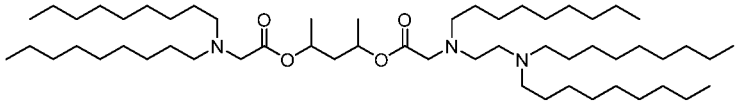
(Compound 186),



(Compound 187),

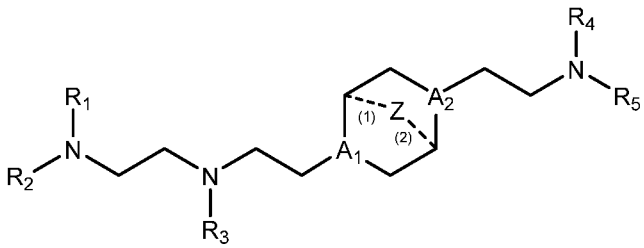


(Compound 188), and



(Compound 189).

[00208] In other embodiments, a lipid has the formula (II)



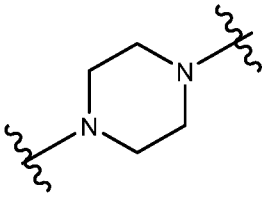
(II),

or a salt or isomer thereof, wherein

A_1 and A_2 are each independently selected from CH or N and at least one of A_1 and A_2 is N;

Z is CH_2 or absent wherein when Z is CH_2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

R_1, R_2, R_3, R_4, and R_5 are independently selected from the group consisting of C_{6-20} alkyl and C_{6-20} alkenyl;

wherein when ring A is  , then

i) **Ri,** R2, R3, R4, and R 5 are the same, wherein **Ri** is not C12 alkyl, **Cis** alkyl, or **Cis** alkenyl;

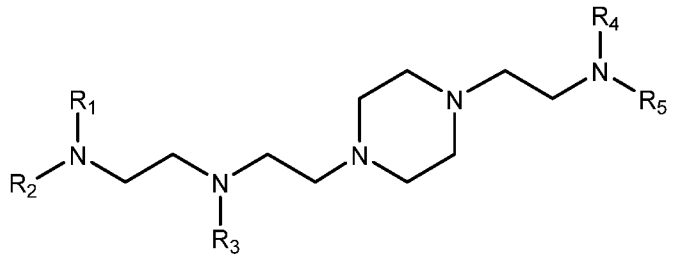
ii) only one of **Ri,** R2, R3, R4, and R 5 is selected from C 6-20 alkenyl;

iii) at least one of **Ri,** R2, R3, R4, and R 5 have a different number of carbon atoms than at least one other of **Ri,** R2, R3, R4, and R5;

iv) **Ri,** R2, and R 3 are selected from C 6-20 alkenyl, and R 4 and R 5 are selected from C 6-20 alkyl; or

v) **Ri,** R2, and R 3 are selected from C 6-20 alkyl, and R 4 and R 5 are selected from C 6-20 alkenyl.

[00209] In some embodiments, the compound is of formula (Ila):



(Ila).

[00210] The compounds of Formula (II) or (Ila) include one or more of the following features when applicable.

[00211] In some embodiments, Z is CH2.

[00212] In some embodiments, Z is absent.

[00213] In some embodiments, at least one of **Ai** and A 2 is N.

[00214] In some embodiments, each of **Ai** and A 2 is N.

[00215] In some embodiments, each of **Ai** and A 2 is **CH.**

[00216] In some embodiments, **Ai** is N and A 2 is CH.

[00217] In some embodiments, **Ai** is CH and A 2 is N.

[00218] In some embodiments, **Ri,** R2, R3, R4, and R 5 are the same, and are not C12 alkyl, **Cis** alkyl, or **Cis** alkenyl. In some embodiments, **Ri,** R2, R3, R4, and R 5 are the same and are C 9 alkyl or C14 alkyl.

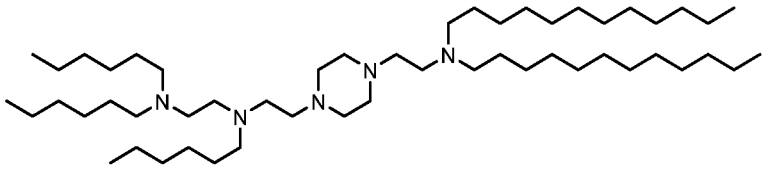
[00219] In some embodiments, only one of **Ri,** R2, R3, R4, and R 5 is selected from C 6-20 alkenyl. In certain such embodiments, **Ri,** R2, R3, R4, and R 5 have the same number of carbon atoms. In some embodiments, R 4 is selected from C 5-20 alkenyl. For example, R 4 may be C12 alkenyl or **Cis** alkenyl.

[00220] In some embodiments, at least one of Ri, R2, R3, R4, and R_5 have a different number of carbon atoms than at least one other of Ri, R2, R3, R4, and R5.

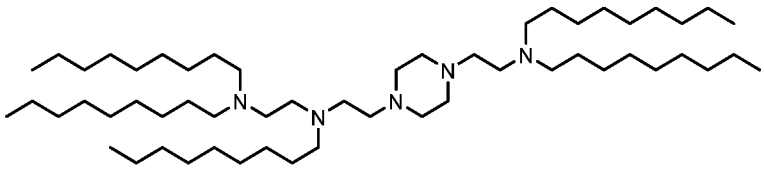
[00221] In certain embodiments, Ri, R2, and R_3 are selected from C_{6-20} alkenyl, and R_4 and R_5 are selected from C_{6-20} alkyl. In other embodiments, Ri, R2, and R_3 are selected from C_{6-20} alkyl, and R_4 and R_5 are selected from C_{6-20} alkenyl. In some embodiments, Ri, R2, and R_3 have the same number of carbon atoms, and/or R_4 and R_5 have the same number of carbon atoms. For example, Ri, R2, and R3, or R_4 and R5, may have 6, 8, 9, 12, 14, or 18 carbon atoms. In some embodiments, Ri, R2, and R3, or R_4 and R5, are Cis alkenyl (e.g., linoleyl). In some embodiments, Ri, R2, and R3, or R_4 and R5, are alkyl groups including 6, 8, 9, 12, or 14 carbon atoms.

[00222] In some embodiments, Ri has a different number of carbon atoms than R2, R3, R4, and R5. In other embodiments, R_3 has a different number of carbon atoms than Ri, R2, R4, and R5. In further embodiments, R_4 has a different number of carbon atoms than Ri, R2, R3, and R5.

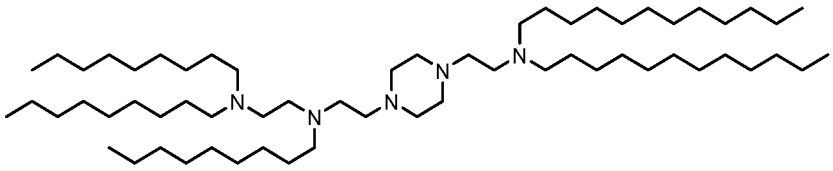
[00223] In some embodiments, the compound is selected from the group consisting of:



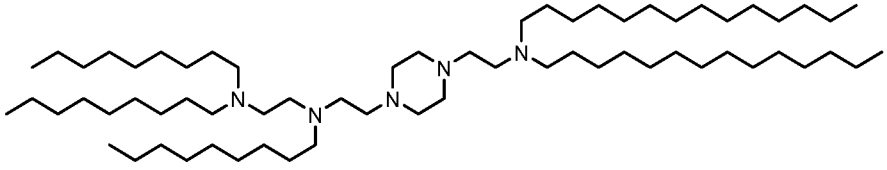
(Compound 17),



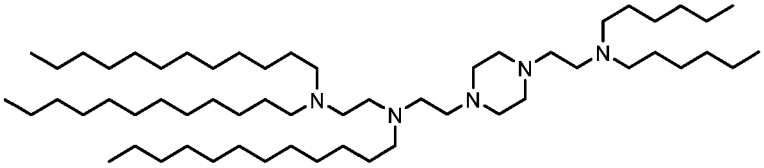
(Compound 18),



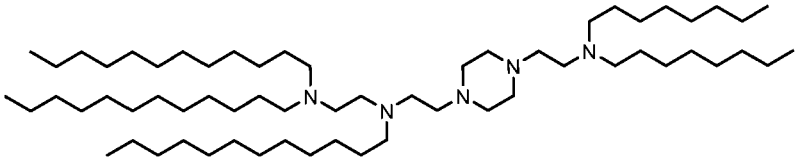
(Compound 19),



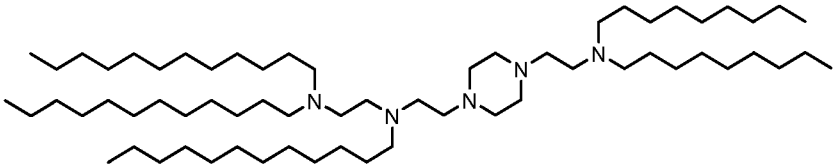
(Compound 20),



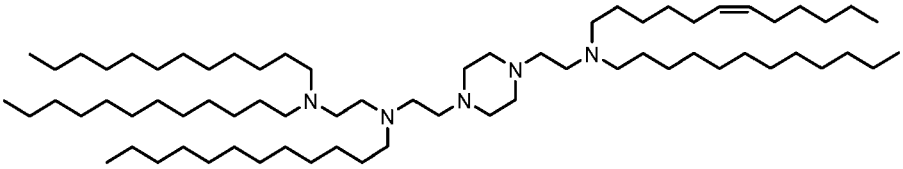
(Compound 21),



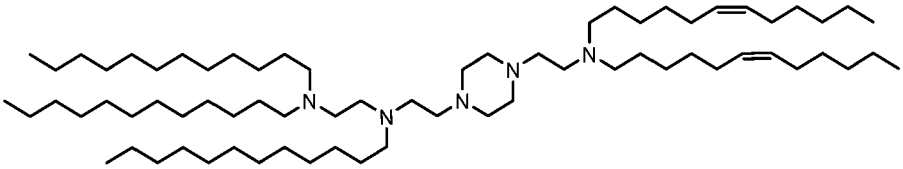
(Compound 22),



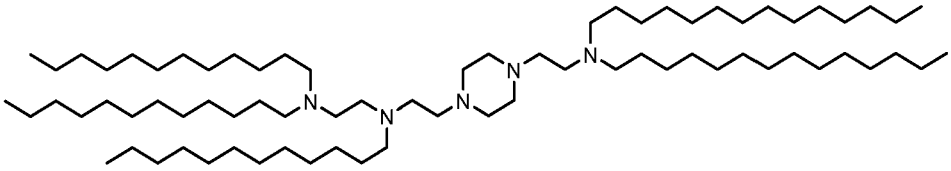
(Compound 23),



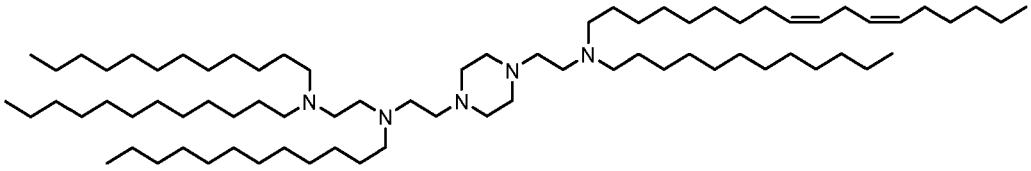
(Compound 24),



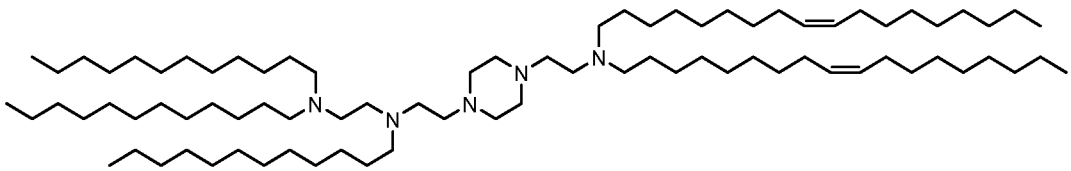
(Compound 25),



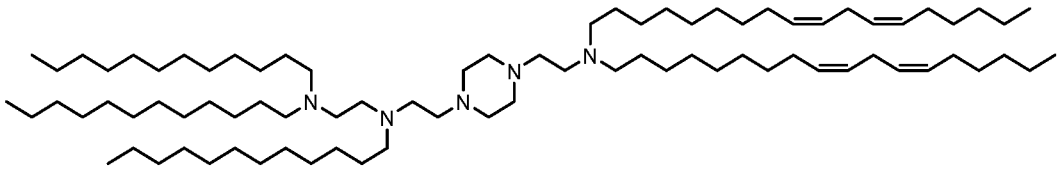
(Compound 26),



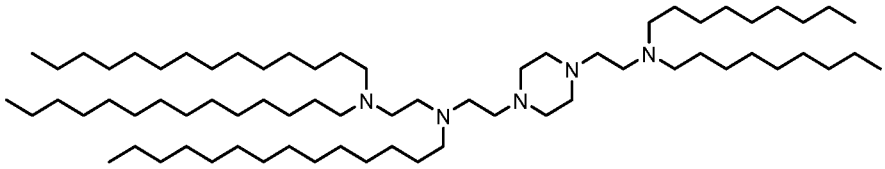
(Compound 27),



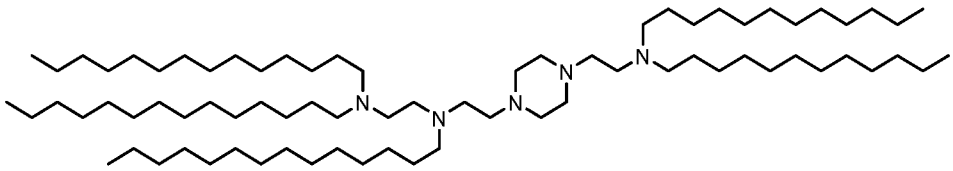
(Compound 28),



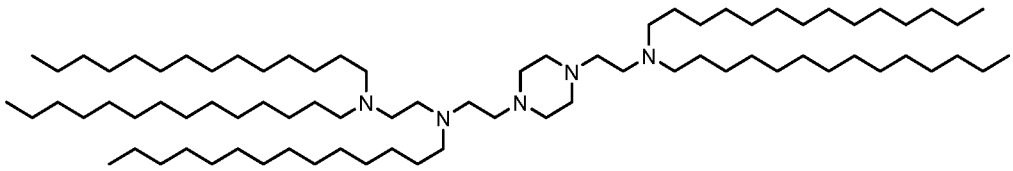
(Compound 29),



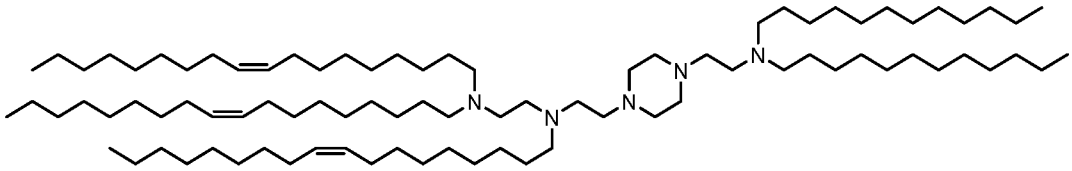
(Compound 30),



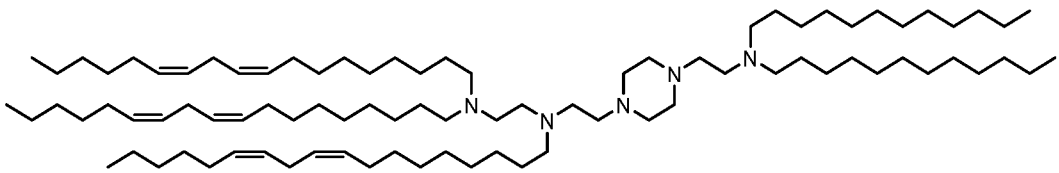
(Compound 31),



(Compound 32),

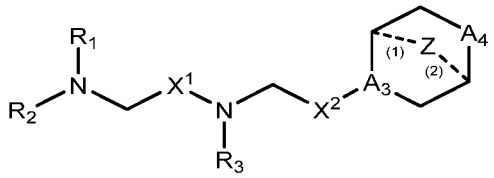


(Compound 33), and



(Compound 34).

[00224] In other embodiments, the compound has the formula (III)



(HI),

or a salt or isomer thereof, in which

A_3 is CH orN;

A_4 is CH_2 or NH; and at least one of A_3 and A_4 is N or NH;

Z is CH_2 or absent wherein when Z is CH_2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

Ri, R2, and R_3 are independently selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

each M is independently selected from -C(0)0-, -OC(O)-, -C(0)N(R')-, -N(R')C(0)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0-, -S(0)2-, an aryl group, and a heteroaryl group;

X^1 and X^2 are independently selected from the group consisting of -CH2-, $-(CH_2)_2-$, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, -C(0)-CH_2-, -CH_2-C(0)-, -C(0)0-CH_2-, -OC(0)-CH_2-, -CH_2-C(0)0-, -CH_2-OC(0)-, -CH(OH)-, -C(S)-, and -CH(SH)-;

each Y is independently a C_{3-6} carbocycle;

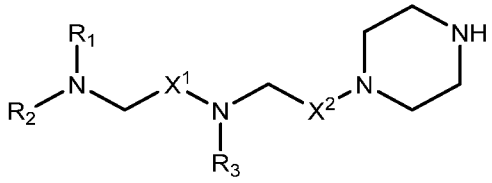
each R* is independently selected from the group consisting of Ci-12 alkyl and C2-12 alkenyl;

each R is independently selected from the group consisting of C1-3 alkyl and a C3-6 carbocycle;

each R' is independently selected from the group consisting of C1-18 alkyl, C2-18 alkenyl, and H; and

each R" is independently selected from the group consisting of C_{3-12} alkyl and C_{3-12} alkenyl.

[00225] In some embodiments, the compound is of formula (Ilia):



(Ilia).

[00226] The compounds of Formula (III) or (Ilia) include one or more of the following features when applicable.

[00227] In some embodiments, Z is **CH2.**

[00228] In some embodiments, Z is absent.

[00229] In some embodiments, at least one of A_3 and A_4 is N or NH.

[00230] In some embodiments, A_3 is N and A_4 is NH.

[00231] In some embodiments, A_3 is N and A_4 is **CH2.**

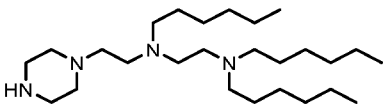
[00232] In some embodiments, A_3 is CH and A_4 is NH.

[00233] In some embodiments, at least one of X^1 and X^2 is not **-CH2-.** For example, in certain embodiments, X^1 is not **-CH2-.** In some embodiments, at least one of X^1 and X^2 is -C(O)-.

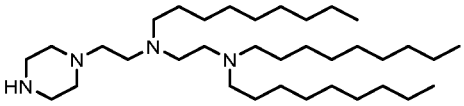
[00234] In some embodiments, X^2 is -C(O)-, -C(0)0-, -OC(O)-, -C(0)-CH_2-, -CH_2-C(O)-, -C(0)0-CH_2-, -OC(0)-CH_2-, -CH_2-C(0)0-, or -CH_2-OC(0)-.

[00235] In some embodiments, **Ri, R2,** and **R3** are independently selected from the group consisting of C_{5-20} alkyl and C_{5-20} alkenyl. In some embodiments, **Ri, R2,** and **R3** are the same. In certain embodiments, **Ri, R2,** and **R3** are C_6, **C9, C12,** or **C14** alkyl. In other embodiments, **Ri, R2,** and **R3** are **Cis** alkenyl. For example, **Ri, R2,** and **R3** may be linoleyl.

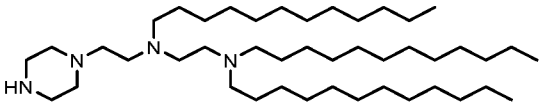
[00236] In some embodiments, the compound is selected from the group consisting of:



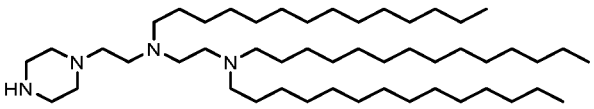
(Compound 35),



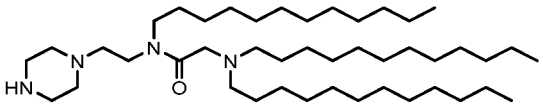
(Compound 36),



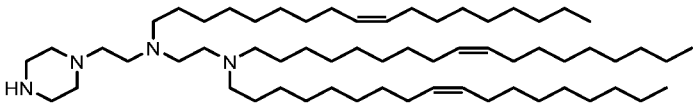
(Compound 37),



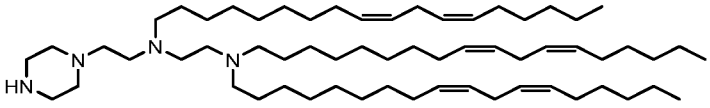
(Compound 38),



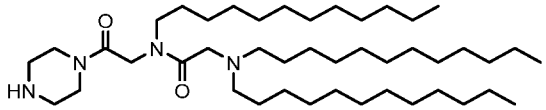
(Compound 39),



(Compound 40),

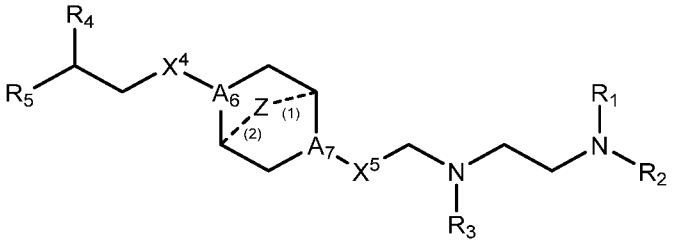


(Compound 41), and



(Compound 77).

[00237] In another aspect, the disclosure provides a compound according to formula (lb):



(lb),

or a salt or isomer thereof, in which

A_6 and A_7 are each independently selected from CH or N, wherein at least one of A_6 and A_7 is N;

Z is CH_2 or absent wherein when Z is CH_2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

X^4 and X^5 are independently selected from the group consisting of -CH2-, $-(CH_2)_2-$, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, -C(0)-CH_2-, -CH_2-C(0)-, -C(0)0-CH_2-, -OC(0)-CH_2-, -CH_2-C(0)0-, -CH_2-OC(0)-, -CH(OH)-, -C(S)-, and -CH(SH)-;

Ri, R2, R3, R4, and R_5 each are independently selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

each M is independently selected from the group consisting of -C(0)0-, -OC(O)-, -C(0)N(R')-, -N(R')C(0)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0-, -S(0)2-, an aryl group, and a heteroaryl group;

each Y is independently a C3-6 carbocycle;

each R* is independently selected from the group consisting of Ci-12 alkyl and C2-12 alkenyl;

each R is independently selected from the group consisting of C1-3 alkyl and a C3-6 carbocycle;

each R' is independently selected from the group consisting of C1-18 alkyl, C2-18 alkenyl, and H; and

each R" is independently selected from the group consisting of C_{3-12} alkyl and C_{3-12} alkenyl.

[00238] In some embodiments, Ri, R2, $R_{3,}$ $R_{4,}$ and R_5 each are independently selected from the group consisting of C_{6-20} alkyl and C_{6-20} alkenyl.

[00239] In some embodiments, Ri and R2 are the same. In certain embodiments, Ri, R2, and R_3 are the same. In some embodiments, R_4 and R_5 are the same. In certain embodiments, Ri, R2, $R_{3,}$ $R_{4,}$ and R_5 are the same.

[00240] In some embodiments, at least one of Ri, R2, $R_{3,}$ $R_{4,}$ and R_5 is C_{9-12} alkyl. In certain embodiments, each of Ri, R2, $R_{3,}$ $R_{4,}$ and R_5 independently is C_9, C12 or C_{14} alkyl. In certain embodiments, each of Ri, R2, $R_{3,}$ $R_{4,}$ and R_5 is C_9 alkyl.

[00241] In some embodiments, R2, $R_{3,}$ $R_{4,}$ and R_5 are Cio-20 alkenyl. In some embodiments, R2, $R_{3,}$ $R_{4,}$ and R_5 are Cis alkenyl.

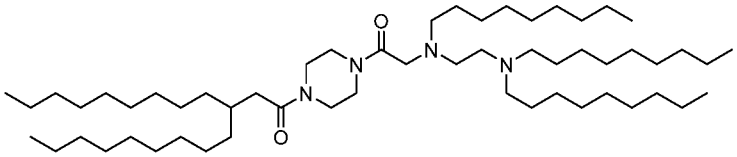
[00242] In some embodiments, A_6 is N and A_7 is N. In some embodiments, A_6 is CH and A_7 is N.

[00243] In some embodiments, X^4 is -CH2- and X^5 is -C(O)-. In some embodiments, X^4 and X^5 are -C(O)-.

[00244] In some embodiments, when A_6 is N and A_7 is N, at least one of X^4 and X^5 is not -CH2-, e.g., at least one of X^4 and X^5 is -C(O)-. In some embodiments, when A_6 is N and A_7 is N, at least one of Ri, R2, $R_{3,}$ $R_{4,}$ and R_5 is -R"MR' .

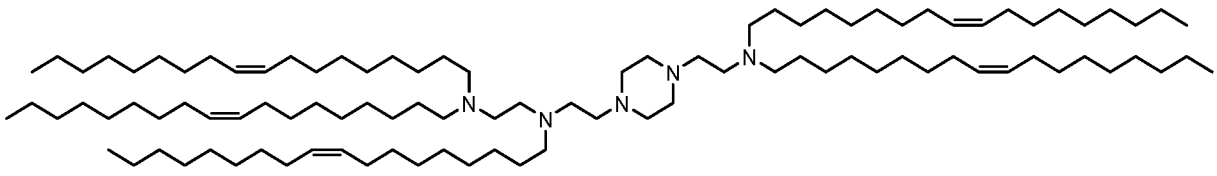
[00245] In some embodiments, at least one of Ri, R2, $R_{3,}$ $R_{4,}$ and R_5 is not -R"MR'.

[00246] In some embodiments, the compound is



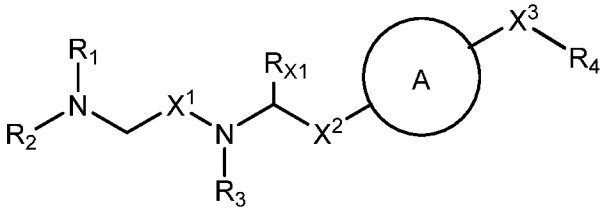
(Compound 67).

[00247] In an embodiment, the compound has the formula (IV)



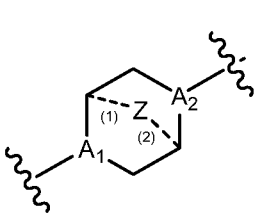
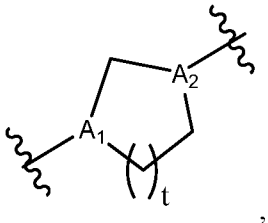
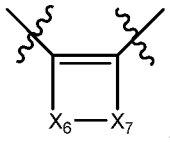
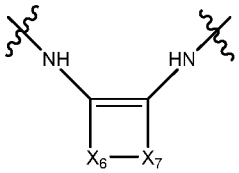
(IV).

[00248] In another aspect, the disclosure provides a compound according to formula (Ic):



(Ic),

or a salt or isomer thereof, wherein

ring A is  ,  ,  , or  ;

t is 1 or 2;

A_1 and A_2 are each independently selected from CH or N;

Z is CH_2 or absent wherein when Z is CH_2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

R_1, R_2, R_3, and R_4 are independently selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

R_{X1} is H or C1-3 alkyl;

each M is independently selected from the group consisting of -C(0)0-, -OC(O)-, -OC(0)0-, -C(0)N(R')-, -N(R')C(0)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0-, $-S(0)_2-$, -C(0)S-, -SC(O)-, an aryl group, and a heteroaryl group;

X^1, X^2, and X^3 are independently selected from the group consisting of a bond, -CH2-, $-(CH_2)_2-$, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, $-(CH_2)_n$-C(0)-, -C(0)-$(CH_2)_n$-,-$(CH_2)_n$-C(0)0-, -OC(0)-$(CH_2)_n$-, $-(CH_2)_n$-OC(0)-, -C(0)0-$(CH_2)_n$-, -CH(OH)-, -C(S)-, and -CH(SH)-;

each Y is independently a C3-6 carbocycle;

each R* is independently selected from the group consisting of Ci-12 alkyl and C2-12 alkenyl;

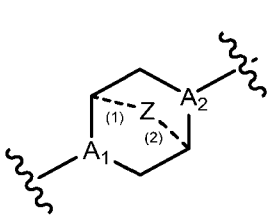
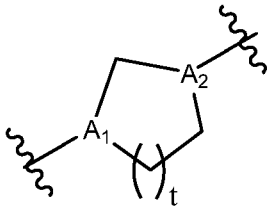
each R is independently selected from the group consisting of C1-3 alkyl and a C3-6 carbocycle;

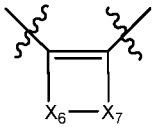
each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-18} alkenyl, and H;

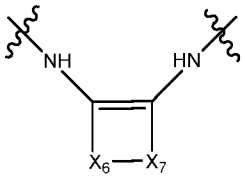
each R" is independently selected from the group consisting of C_{3-12} alkyl, C_{3-12} alkenyl and -R*MR'; and

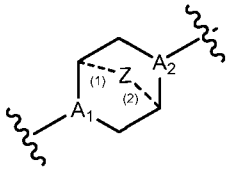
n is an integer from **1-6.**

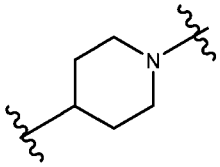
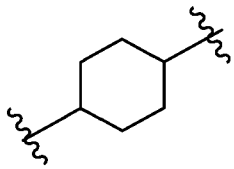
[00249] The compounds of formula (Ic) may include one or more of the following features when applicable.

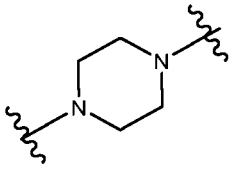
[00250] In some embodiments, ring A is  or  .

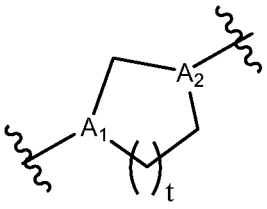
[00251] In some embodiments, ring A is  .

[00252] In some embodiments ring A is  .

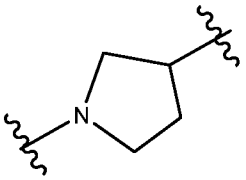
[00253] In some embodiments, ring A is  .

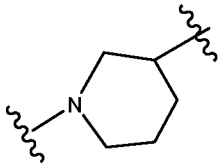
[00254] In some embodiments, ring A is  or  .

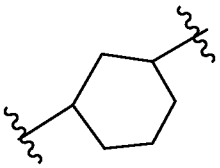
[00255] In some embodiments, ring A is  .

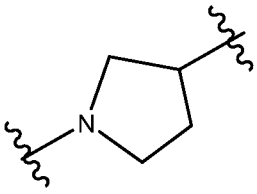
[00256] In some embodiments, ring A is 

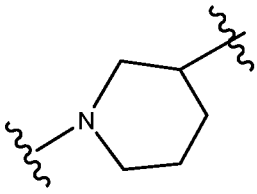
.

[00257] In some embodiments, ring A is 

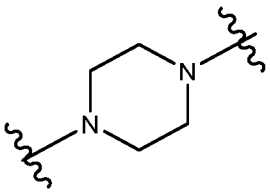
, 

, or 
.

[00258] In some embodiments, ring A is 

or 

wherein ring, in which the N atom is connected with X^2.

[00259] In some embodiments, when ring **A** is 

, then

i) at least one of **X 1, X 2,** and **X 3** is not **-CH2-;**

ii) at least one of **X 2** and **X 3** is - **C**(O)-; and/or

iii) at least one of **Ri, R2, R3,** and **R 4** is **-R"MR' .**

[00260] In some embodiments, **Z** is **CH2.**

[00261] In some embodiments, **Z** is absent.

[00262] In some embodiments, at least one of **A i** and **A 2** is **N .**

[00263] In some embodiments, each of **A i** and **A 2** is **N .**

[00264] In some embodiments, each of **A i** and **A 2** is **CH.**

[00265] In some embodiments, **A i** is **N** and **A 2** is **CH.**

[00266] In some embodiments, **A i** is **CH** and **A 2** is **N .**

[00267] In some embodiments, **X 1** is **-CH2-.**

[00268] In some embodiments, X^2 and X^3 are independently selected from the group consisting of -C(O)-, **-C(0)0-,** -OC(O)-, -C**(0**)-CH_2-, **-CH_2-C(0)-,** -C**(0)0** -CH_2-, -OC(O)-CH2-, **-CH_2-C(0)0-,** and -CH_2-OC**(0)-.**

[00269] In some embodiments, at least one of X^2 and X^3 is -C(O)-. In some embodiments, X^2 and X^3 are each -C(O)-.

[00270] In some embodiments, X^3 is a bond or -$(CH_2)_2$-.

[00271] In some embodiments, **Ri** and **R_2** are the same. In certain embodiments, **Ri, R_2,** and R_3 are the same. In some embodiments, R_4 and R_5 are the same. In certain embodiments, **Ri, R_2,** R_3, R_4, and R_5 are the same.

[00272] In some embodiments, **Ri, R_2,** and R_3 are each C_9 alkyl. In some embodiments, **Ri, R_2,** and R_3 are each C_{10} alkyl.

[00273] In some embodiments, R_4 is C_9 alkyl. In some embodiments, R_4 is C_{10} alkyl. In some embodiments, R_4 is C_{11} alkyl. In some embodiments, R_4 is **Ci_2** alkyl. In some embodiments, R_4 is C_{13} alkyl. In some embodiments, R_4 is C_{14} alkyl.

[00274] In some embodiments, R_4 is **-R"MR'.**

[00275] In some embodiments, R_4 is **-R"MR'** and **Ri, R_2,** and R_3 are each C_9 alkyl. In some embodiments, R_4 is **-R"MR'** and **Ri, R_2,** and R_3 are each C_{10} alkyl.

[00276] In some embodiments, at least one of **Ri, R_2,** R_3, and R_4, is **-R"MR'.** In some embodiments, at most one of **Ri, R_2,** R_3, and R_4, is **-R"MR'.** For example, at least one of **Ri, R_2,** and R_3 may be **-R"MR',** and/or R_4 is **-R"MR'.** In certain embodiments, at least one **M** is -C**(0**)O-. In some embodiments, each M is -C**(0**)O-. In some embodiments, at least one M is -OC(O)-. In some embodiments, each M is -OC(O)-. In some embodiments, at least one M is **-0C(0**)O-. In some embodiments, each M is **-0C(0**)O-. In some embodiments, at least one M is -C**(0**)S-. In some embodiments, each M is -C**(0**)S-. In some embodiments, at least one M is -SC(O)-. In some embodiments, each M is -SC(O)-.

[00277] In some embodiments, at least one **R"** is ***C2-5*** alkyl. In some embodiments, each **R"** is ***C2-5*** alkyl. In some embodiments, at least one **R"** is C_3 alkyl. In certain embodiments, each **R"** is C_3 alkyl. In some embodiments, at least one **R"** is C_5 alkyl. In certain embodiments, each **R"** is C_5 alkyl. In some embodiments, at least one **R"** is C_6 alkyl. In certain embodiments, each **R"** is C_6 alkyl. In some embodiments, at least one **R"** is C_7 alkyl. In certain embodiments, each **R"** is C_7 alkyl. In some embodiments, at least one **R"** is C_8 alkyl. In certain embodiments, each **R"** is C_8 alkyl. In some embodiments, at least one **R"** is C_9 alkyl. In certain embodiments, each **R"** is C_9 alkyl. In some embodiments, at least one **R"** is C_{10} alkyl. In certain embodiments,

each R" is Cio alkyl. In some embodiments, at least one R" is C_{11} alkyl. In certain embodiments, each R" is C_{11} alkyl.

In other embodiments, at least one R' is C1-9 alkyl. In certain embodiments, each R' is C1-9 alkyl. In certain embodiments, R' is Ci alkyl. In certain embodiments, each R' is Ci alkyl. In some embodiments, at least one R' is C2 alkyl. In certain embodiments, each R' is C2 alkyl. In some embodiments, at least one R' is C_5 alkyl. In certain embodiments, each R' is C_5 alkyl. In some embodiments, at least one R' is C_6 alkyl. In certain embodiments, each R' is C_6 alkyl. In some embodiments, at least one R' is C7 alkyl. In certain embodiments, each R' is C7 alkyl. In some embodiments, at least one R' is Cs alkyl. In certain embodiments, each R' is Cs alkyl. In some embodiments, at least one R' is C9 alkyl. In certain embodiments, each R' is C9 alkyl.

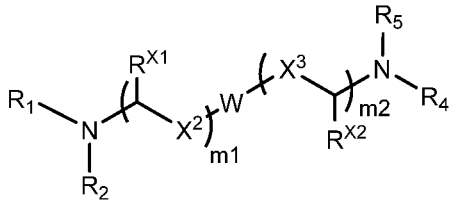
[00278] In some embodiments at least one R" is C3-7 alkenyl. In some embodiments, each R" is C3-7 alkenyl. In certain embodiments, at least one R" is C3 alkenyl. In certain embodiments, each R" is C3 alkenyl. In certain embodiments, at least one R" is C_4 alkenyl. In certain embodiments, each R" is C_4 alkenyl. In certain embodiments, at least one R" is C_5 alkenyl. In certain embodiments, each R" is C_5 alkenyl. In certain embodiments, at least one R" is C_6 alkenyl. In certain embodiments, each R" is C_6 alkenyl. In certain embodiments, at least one R" is C7 alkenyl. In certain embodiments, each R" is C7 alkenyl.

[00279] In some embodiments, at least one R" is branched C3-12 alkyl, C3-12 alkenyl.

[00280] In some embodiments, at least one R' is C4-10 alkenyl. In certain embodiments, at least one R' is C4 alkenyl. In certain embodiments, at least one R' is C_5 alkenyl. In certain embodiments, at least one R' is C_6 alkenyl. In certain embodiments, at least one R' is C7 alkenyl. In certain embodiments, at least one R' is Cs alkenyl. In certain embodiments at least one R' is C9 alkenyl. In certain embodiments, at least one R' is Cio alkenyl.

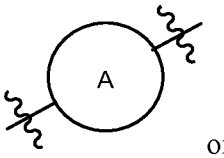
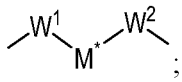
[00281] In some embodiments, Ri, R2, $_{R3,}$ and $_{R4}$ are each independently selected from -R"MR' and $_{C5-10}$ alkyl. In certain embodiments, Ri, R2, $_{R3,}$ and $_{R4}$ are each independently selected from -R"MR' and $_{C5}$ alkyl. In certain embodiments, Ri, R2, $_{R3,}$ and $_{R4}$ are each independently selected from -R"MR' and C_6 alkyl. In certain embodiments, Ri, R2, $_{R3,}$ and $_{R4}$ are each independently selected from -R"MR' and C7 alkyl. In certain embodiments, Ri, R2, $_{R3,}$ and $_{R4}$ are each independently selected from -R"MR' and Cs alkyl. In certain embodiments, Ri, R2, $_{R3,}$ and $_{R4}$ are each independently selected from -R"MR' and C9 alkyl. In certain embodiments, Ri, R2, $_{R3,}$ and $_{R4}$ are each independently selected from -R"MR' and Cio alkyl.

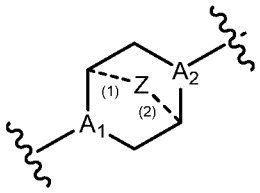
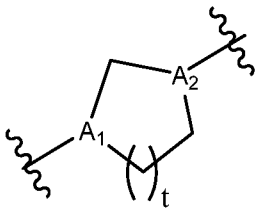
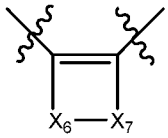
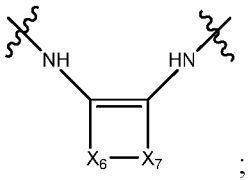
[00282] In another aspect, the disclosure provides a compound according to formula (Id):



(Id),

or a salt or isomer thereof, wherein

W is  or  ;

ring A is  ,  ,  , or  ;

t is 1 or 2;

A_1 and A_2 are each independently selected from CH or N;

Z is CH_2 or absent wherein when Z is CH_2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

R_1, R_2, R_4, and R_5 are independently selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

R_{x1} and R_{x2} are each independently H or C_{1-3} alkyl;

each M is independently selected from the group consisting of -C(0)0-, -OC(O)-, -OC(0)0-, -C(0)N(R')-, -N(R')C(0)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0-, $-S(0)_2-$, -C(0)S-, -SC(O)-, an aryl group, and a heteroaryl group;

M* is -0-, Ci-Ce alkyl, $-((CH_2)_pCH(R_7)(CH_2)_qCH(R_7)(CH_2)_r)_s-$, or $-((CH_2)_tC(=R_8)(CH_2)_uC(=R_8)(CH_2)_v)_s-$;

W^1 and W^2 are each independently selected from the group consisting of a bond, -O- and -N(Re)-;

each R_6 is independently selected from the group consisting of H and C_{1-3} alkyl;

each $R_{.7}$ is independently selected from the group consisting of C_{1-3} alkyl, $(CH_2)_oCN$, C_{i-3} hydroxyalkyl, and C_{1-C3} haloalkyl;

each R_s is independently selected from the group consisting of $CH(CH_2)_oCN$, $CH(CH_2)_oCH_3$, $CH(CH_2)_oOH$, and CHX^{Hal};

X^{Hal} is C_{1-C3} haloalkyl;

X^2 and X^3 are independently selected from the group consisting of a bond, -CH2-, $-(CH_2)_2-$, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, $-(CH_2)_n$-C(0)-, -C(0)-$(CH_2)_n$-, $-(CH_2)_n$-C(0)0-, -OC(0)-$(CH_2)_n$-, $-(CH_2)_n$-OC(0)-, -C(0)0-$(CH_2)_n$-, -CH(OH)-, -C(S)-, and -CH(SH)-;

X^6 and X^7 are each independently selected from the group consisting of -C(O)-, -C(S)-, $-C((CH_2)_wOH)-$, and $-C(X^{Ha1})-$;

each Y is independently a C_{3-6} carbocycle;

each R^* is independently selected from the group consisting of C_{i-i2} alkyl and C_{2-i2} alkenyl;

each R is independently selected from the group consisting of C_{1-3} alkyl and a C_{3-6} carbocycle;

each R' is independently selected from the group consisting of C_{1-18} alkyl, C_{2-i8} alkenyl, and H;

each R'' is independently selected from the group consisting of C_{3-i2} alkyl, C_{3-12} alkenyl and $-R^*MR'$;

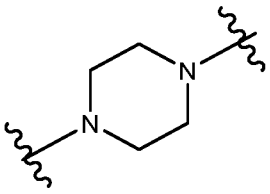
m1 and m2 are each independently 0 or 1;

n is an integer from 1-6;

o is an integer from 0-3;

p, q, r, t, u, and v are each independently an integer from 0-3; and

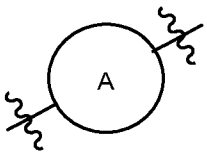
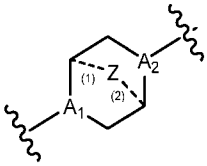
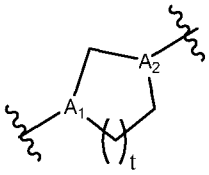
s is an integer from 1-3.

[00283] In some embodiments, when ring A is  , then

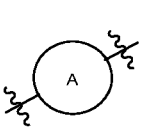
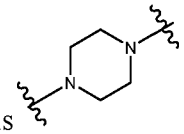
i) at least one of X^2, and X^3 is not $-CH_2-$;

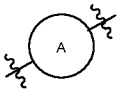
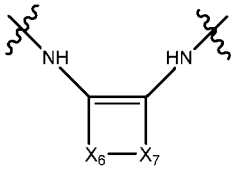
ii) at least one of X^2 and X^3 is -C(O)-; and/or

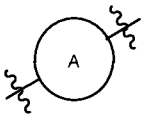
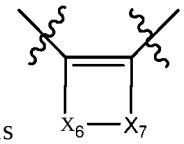
iii) at least one of R_i, $R^\wedge$, R_4, and R_5 is $-R''MR'$.

[00284] In some embodiments, W is  and ring A is  or  .

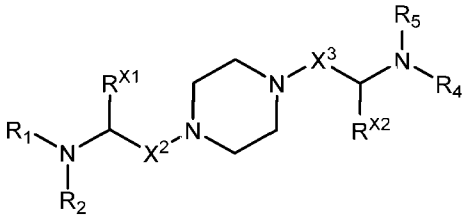
[00285] In some embodiments, m1 and m2 are each 1. In some embodiments, m1 and m2 are each 0. In some embodiments, m1 is 1 and m2 is 0. In some embodiments, m1 is 0 and m1 is 1.

[00286] In some embodiments,  is  .

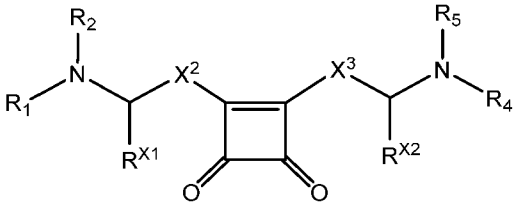
[00287] In some embodiments,  is  .

[00288] In some embodiments,  is  .

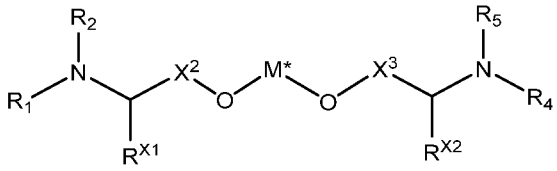
[00289] In some embodiments, the compound is of any of formulae (Idl)-(IdlO):

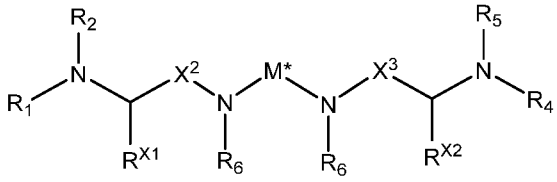


(Id1),



(Id2),


(Id3),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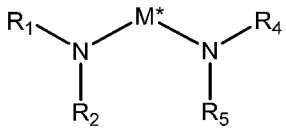
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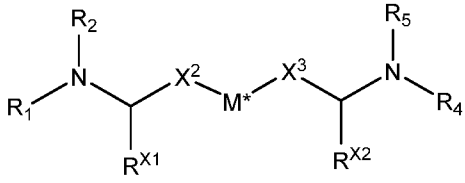
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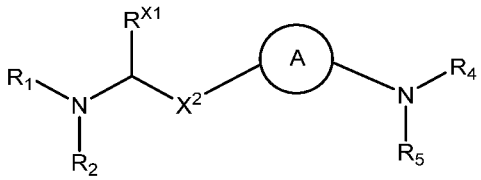
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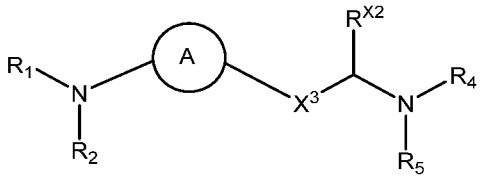
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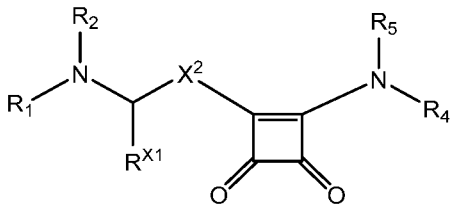
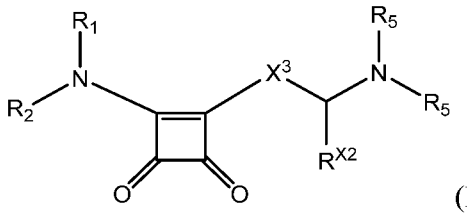

(Id9), or



(Id10).

[00290] In some embodiments, Z is CH2.

[00291] In some embodiments, Z is absent.

[00292] In some embodiments, at least one of A i and A 2 is N .

[00293] In some embodiments, each of A i and A 2 is N .

[00294] In some embodiments, each of A i and A 2 is CH.

[00295] In some embodiments, A i is N and A 2 is CH.

[00296] In some embodiments, A i is CH and A 2 is N .

[00297] In some embodiments, X^2 and X^3 are independently selected from the group consisting of -C(O)-, -C(0)0-, -OC(O)-, -C(0)-CH $_2$-, -CH $_2$-C(0)-, -C(0)0-CH $_2$-, -OC(O)-CH2-, -CH $_2$-C(0)0-, and -CH $_2$-OC(0)-.

[00298] In some embodiments, at least one of X^2 and X^3 is -C(O)-. In some embodiments, X^2 and X^3 are each -C(O)-.

[00299] In some embodiments X^2 and X^3 are independently selected from the group consisting of -C(O)-, C(0)-(CH $_2$)n-, and -(CH $_2$)n-C(0)-.

[00300] In some embodiments, X^2 is -CH2-.

[00301] In some embodiments, X^2 is -(CH $_2$)n-.

[00302] In some embodiments, X^2 is a bond.

[00303] In some embodiments, X^2 is -C(O)-.

[00304] In some embodiments, X^2 is $-(CH_2)_nC(O)-$.

[00305] In some embodiments, X^3 is $-CH_2-$.

[00306] In some embodiments, X^3 is $-(CH_2)_n-$. In some embodiments, X^3 is a bond.

[00307] In some embodiments, X^3 is -C(O)-.

[00308] In some embodiments, wherein X^3 is $-(CH_2)_nC(O)-$.

[00309] In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5.

[00310] In some embodiments, R i and R 2 are the same. In certain embodiments, Ri, and R 3 are the same. In some embodiments, R 4 and R 5 are the same. In certain embodiments, Ri, R2, R4, and R 5 are the same.

[00311] In some embodiments, **Rxi** and Rx2 are each independently C1-3 alkyl. For example, **Rxi** and Rx2 are each **Ci** alkyl. For example, **Rxi** and Rx2 are each C2 alkyl. For example, **Rxi** and Rx2 are each $_{C\,3}$ alkyl. For example, **Rxi** is **Ci** alkyl and Rx2 is C2 alkyl. For example, **Rxi** is C2 alkyl and Rx2 is **Ci** alkyl. For example, **Rxi** is **Ci** alkyl and Rx2 is $_{C\,3}$ alkyl. For example, **Rxi** is $_{C\,3}$ alkyl and Rx2 is **Ci** alkyl. For example, **Rxi** is C2 alkyl and Rx2 is $_{C\,3}$ alkyl. For example, **Rxi** is $_{C\,3}$ alkyl and Rx2 is C2 alkyl.

[00312] In some embodiments, **Rxi** is H and Rx2 is C1-3 alkyl. For example, **Rxi** is H and Rx2 is **Ci** alkyl. For example, **Rxi** is H and Rx2 is C2 alkyl. For example, **Rxi** is H and Rx2 is $_{C\,3}$ alkyl.

[00313] In some embodiments, **Rxi** is C1-3 alkyl and Rx2 is H. For example, **Rxi** is **Ci** alkyl and Rx2 is H. For example, **Rxi** is C2 alkyl and Rx2 is H. For example, **Rxi** is $_{C\,3}$ alkyl and Rx2 is H.

[00314] In some embodiments, **Rxi** and Rx2 are each H.

[00315] In some embodiments, at least one of **Ri,** R2, $_{R4,}$ and $_{R\,5}$ is -R"MR' . In some embodiments, at most one of **Ri,** R2, $_{R4,}$ and $_{R\,5}$ is -R"MR' . For example, at least one of **Ri,** R2, and $_{R\,3}$ may be -R"MR', and/or at least one of $_{R\,4}$ and $_{R\,5}$ is -R"MR' . In certain embodiments, at least one M is -C**(0**)O-. In some embodiments, each M is -C**(0**)O-. In some embodiments, at least one M is -OC(O)-. In some embodiments, each M is -OC(O)-. In some embodiments, at least one M is **-0C(0**)O-. In some embodiments, each M is **-0C(0**)O-. In some embodiments, at least one M is -C**(0**)S-. In some embodiments, each M is -C**(0**)S-. In some embodiments, at least one M is -SC(O)-. In some embodiments, each M is -SC(O)-.

[00316] In some embodiments, at least one R" is $_{C3\text{-}7}$ alkyl. In some embodiments, each R" is C2-5 alkyl. In some embodiments, at least one R" is $_{C\,3}$ alkyl. In certain embodiments, each R" is $_{C\,3}$ alkyl. In some embodiments, at least one R" is $_{C\,5}$ alkyl. In certain embodiments, each R" is $_{C\,5}$ alkyl. In some embodiments, at least one R" is C_6 alkyl. In certain embodiments, each R" is $_{C\,6}$ alkyl. In some embodiments, at least one R" is $_{C\,7}$ alkyl. In certain embodiments, each R" is $_{C\,7}$ alkyl. In some embodiments, at least one R" is Cs alkyl. In certain embodiments, each R" is Cs alkyl. In some embodiments, at least one R" is $_{C\,9}$ alkyl. In certain embodiments, each R" is $_{C\,9}$ alkyl. In some embodiments, at least one R" is C10 alkyl. In certain embodiments, each R" is **Cio** alkyl. In some embodiments, at least one R" is C11 alkyl. In certain embodiments, each R" is C11 alkyl.

[00317] In other embodiments, at least one R' is C1-5 alkyl. In certain embodiments, each R' is **Ci-5** alkyl. In certain embodiments, at least one R' is **Ci** alkyl. In certain embodiments,

each **R'** is **Ci** alkyl. In some embodiments, at least one **R'** is C2 alkyl. In certain embodiments, each **R'** is C2 alkyl. In some embodiments, at least one **R'** is $_{C5}$ alkyl. In certain embodiments, each **R'** is $_{C5}$ alkyl. In some embodiments, at least one **R'** is ***C***$_6$ alkyl. In certain embodiments, each **R'** is **C**$_6$ alkyl. In some embodiments, at least one **R'** is C7 alkyl. In certain embodiments, each **R'** is C7 alkyl. In some embodiments, at least one **R'** is **Cs** alkyl. In certain embodiments, each **R'** is **Cs** alkyl. In some embodiments, at least one **R'** is C9 alkyl. In certain embodiments, each **R'** is C9 alkyl.

[00318] In some embodiments, each **R'** is C9 alkyl. In some embodiments, one **R'** is $_{C5-10}$ alkyl. In some embodiments, two **R'** are $_{C5-10}$ alkyl. In some embodiments, one **R'** is C9 alkyl. In some embodiments, two **R'** are C9 alkyl. In some embodiments, at least one **R'** is **Cn-is** alkyl. In some embodiments, one **R'** is C17 alkyl. In some embodiments, two **R'** are C17 alkyl. In some embodiments, **Ri,** $_{R2,}$ $_{R4,}$ and $_{R5}$ are each C9 alkyl.

[00319] In some embodiments, at least one **R'** is C10 alkyl. In certain embodiments, each **R'** is **Cio** alkyl. In some embodiments, at least one **R'** is **Cn-is** alkyl. In some embodiments, each **R'** is **C**$_{11-18}$ alkyl. In some embodiments, at least one **R'** is C11 alkyl. In certain embodiments, each **R'** is C11 alkyl. In some embodiments, at least one **R'** is C12 alkyl. In certain embodiments, each **R'** is C12 alkyl. In some embodiments, at least one **R'** is C13 alkyl. In certain embodiments, each **R'** is C13 alkyl. In some embodiments, at least one **R'** is C14 alkyl. In certain embodiments, each **R'** is C14 alkyl. In some embodiments, at least one **R'** is $_{C15}$ alkyl. In certain embodiments, each **R'** is $_{C15}$ alkyl. In some embodiments, at least one **R'** is Ci6 alkyl. In certain embodiments, each **R'** is Ci6 alkyl. In some embodiments, at least one **R'** is C17 alkyl. In certain embodiments, each **R'** is $_{C17}$ alkyl. In some embodiments, at least one **R'** is **Cis** alkyl. In certain embodiments, each **R'** is **Cis** alkyl.

[00320] In some embodiments, at least one **R'** is a branched alkyl. In some embodiments, each **R'** is a branched alkyl. In some embodiments, at least one **R'** is a branched alkyl. In some embodiments, at least one **R'** is branched **C**$_{17}$ alkyl. In some embodiments, each **R'** is branched Ci7 alkyl. In some embodiments, one **R'** is branched **C**$_{17}$ alkyl. In some embodiments, two **R'** are branched **C**$_{17}$ alkyl.

[00321] In some embodiments at least one of **Ri,** $_{R2,}$ $_{R4,}$ and $_{R5}$ is $_{C5-10}$ alkyl. In some embodiments at least one of **Ri,** $_{R2,}$ $_{R4,}$ and $_{R5}$ is **Cio** alkyl. In some embodiments, at least **Ri** and R2, and one of $_{R4,}$ and $_{R5}$ are $_{C5-10}$ alkyl. In some embodiments, one of **Ri** and R2, and one of $_{R4}$ and $_{R5}$ are **Cio** alkyl. In some embodiments at least one **Ri,** $_{R2,}$ $_{R4,}$ and $_{R5}$ are each $_{C5-10}$

alkyl. In some embodiments, Ri, R2, R4, and R_5 are each C_9 alkyl. In some embodiments, Ri, R2, R4, and R_5 are each C10 alkyl.

[00322] In some embodiments, Ri and R2 are each C_{5-10} alkyl. In some embodiments, Ri and R2 are each Cs alkyl. In some embodiments, Ri and R2 are each C_9 alkyl. In some embodiments, R3 and R_4 are each C_{5-10} alkyl. In some embodiments, R3 and R4 are each Cs alkyl. In some embodiments, R3 and R4 are each C_9 alkyl.

[00323] In some embodiments, ml is 1 and ml is 0. In some embodiments, ml is 0 and ml is 1. In some embodiments, ml is 0 and ml are each 0. In some embodiments, ml and ml are each 1.

[00324] In some embodiments, M* is **-0-.** The compound of any one of the preceding claims, wherein M* is C1-C6 alkyl. In some embodiments, M* is Ci alkyl In some embodiments, M* is C2 alkyl. In some embodiments, M* is C_3 alkyl. In some embodiments, M* is -((CH2)pCH(R7)(CH2)qCH(R7)(CH_2)r)s-. In some embodiments, M* is -((CH2)tC(=R8)(CH2)uC(=R8)(CH_2)v)s-.

[00325] In some embodiments, W^1 and W^2 are each a bond. In some embodiments, W^1 and W^2 are each **-0-.** In some embodiments, W^1 and W^2 are each -N(R6)-. In some embodiments, W^1 is a bond and W^2 is -N(R6)-. In some embodiments, W^1 is -N(R6)- and W^2 is a bond. In some embodiments, at least one R_6 is H. In some embodiments, each R_6 is H. In some embodiments, at least one R_6 is Ci alkyl. In some embodiments, each R_6 is Ci alkyl. In some embodiments, at least one R_6 is C2 alkyl. In some embodiments, each R_6 is C2 alkyl.

[00326] In some embodiments wherein M* is -((CH2)pCH(R7)(CH2)qCH(R7)(CH_2)r)s-, p is 0, 1 or 2. In some embodiments, p is 0. In some embodiments, p is 1.

[00327] In some embodiments wherein M* is -((CH2)pCH(R7)(CH2)qCH(R7)(CH_2)r)s-, q is 0, 1 or 2. In some embodiments, q is 0. In some embodiments, q is 1.

[00328] In some embodiments wherein M* is -((CH2)pCH(R7)(CH2)qCH(R7)(CH_2)r)s-, r is 0, 1 or 2. In some embodiments, r is 0. In some embodiments, r is 1.

[00329] In some embodiments wherein M* is -((CH2)pCH(R7)(CH2)qCH(R7)(CH_2)r)s-, p, q, and r are each 0. In some embodiments, p and r are each 0 and q is 1.

[00330] In some embodiments wherein M* is -((CH2)tC(=R8)(CH2)uC(=R8)(CH_2)v)s-, t is 0, 1 or 2. In some embodiments, t is 0. In some embodiments, t is 1.

[00331] In some embodiments wherein M* is -((CH2)tC(=R8)(CH2)uC(=R8)(CH_2)v)s-, u is 0, 1, or 2. In some embodiments, u is 0. In some embodiments, u is 1.

[00332] In some embodiments wherein **M*** is **-((CH $_2$)tC**(=R8)(CH2)**uC**(=R8)**(CH$_2$**)v)s-, v is 0, 1, or 2. In some embodiments, v is 0. In some embodiments, v is 1.

[00333] In some embodiments wherein **M*** is -((CH2)**tC**(=R8)(CH2)**uC**(=R8)**(CH$_2$**)v)s-, t, u, and v are each 0.

[00334] In some embodiments, s is 1, 2, or 3. In some embodiments, s is 1.

[00335] In some embodiments wherein **M*** is **-((CH $_2$)$_P$CH(R7)(CH $_2$)$_q$CH(R7)(CH $_2$)r)$_s$-,** s is 1 and p, q, and r are each 0. In some embodiments, s is 1, p and r are each 0, and q is 1.

[00336] In some embodiments wherein **M*** is **-((CH $_2$)tC**(=R8)(CH2)**uC**(=R8)**(CH$_2$**)v)s-, s is 1 and t, u, and v are each 0.

[00337] In some embodiments wherein **M*** is **-((CH $_2$)tC**(=**R8)(CH $_2$)$_u$C**(=**R8)(CH $_2$)y)$_s$**- and **Rs** is **CH(CH$_2$)oCN,** o is 0. For example **Rs** is **CHCN,** In some embodiments, o is 1. In some embodiments, o is 2.

[00338] In some embodiments wherein **M*** is **-((CH $_2$)tC**(=**R8)(CH $_2$)$_u$C**(=**R8)(CH $_2$)y)$_s$**, **Rs** is **CH(CH$_2$)oCN** and o is 0, 1, or 2. In some embodiments, **Rs** is **CHCN.** In some embodiments, **Rs** is **CHCH$_2$CN.** In some embodiments, **Rs** is **CH(CH$_2$)$_2$CN.**

[00339] In some embodiments wherein **M*** is **-((CH $_2$)$_t$C**(=**R8)(CH $_2$)uC(=Rs)(CH$_2$)v)s**, **Rs** is **CH(CH$_2$)oOH** and o is 0, 1, or 2. In some embodiments, **Rs** is **CHCH$_2$OH.** In some embodiments, **Rs** is **CH(CH$_2$)$_2$OH.** In some embodiments, **Rs** is **CH(CH$_2$)30H.**

[001] In some embodiments wherein **M*** is **-((CH $_2$)$_t$C**(=**R8)(CH $_2$)uC(=Rs)(CH$_2$)v)s**, **Rs** is **CHX^{Hal}.** In some embodiments, **X^{Hal}** is fluoromethyl, fluoroethyl, fluoropropyl, difluoromethyl, difluoroethyl, difluoropropyl, trifluoromethyl, trifluoroethyl, trifluoropropyl, chloromethyl, chloroethyl, chloropropyl, dichloromethyl, dichloroethyl, dichloropropyl, trichloromethyl, trichloroethyl, trichloropropyl, bromomethyl, bromoethyl, bromopropyl, dibromomethyl, dibromoethyl, dibromopropyl, tribromomethyl, tribromoethyl, tribromopropyl, iodomethyl, iodoethyl, iodopropyl, diiodomethyl, diiodoethyl, diiodopropyl, triiodomethyl, triiodoethyl, or triiodopropyl. In some embodiments **X^{Hal}** is trifluoromethyl.

[00340] In some embodiments wherein **M*** is **-((CH $_2$)$_P$CH(R7)(CH $_2$)$_q$CH(R7)(CH $_2$)r)$_s$-, R**^{7} is **Ci** alkyl. In some embodiments, R^7 is **C$_2$** alkyl. In some embodiments, R^7 is C$_3$ alkyl.

[00341] In some embodiments wherein **M* is-((CH $_2$)$_P$CH(R7)(CH $_2$)$_q$CH(R7)(CH $_2$)r)$_s$, R**^{7} is **(CH$_2$)oCN** and 0 is 0, 1, or 2. In some embodiments, R^7 is **CN.** In some embodiments, R^7 is **CH$_2$CN.** In some embodiments, R^7 is **(CH$_2$)$_2$CN.**

[00342] In some embodiments wherein M* is -((CH $_2$)pCH(R7)(CH2)qCH(R7)(CH $_2$)r)s, **R** $_7$ is **Ci** $_{-3}$ hydroxy alkyl. In some embodiments, R 7 is CH2OH. In some embodiments, R 7 is (CH2)20H. In some embodiments, R 7 is (**CH2)30H.**

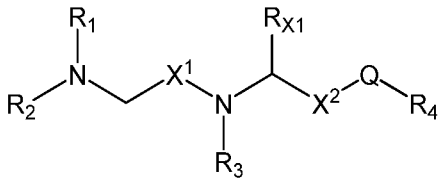
[00343] In some embodiments wherein M* is -((CH2)pCH(R7)(CH2)qCH(R7)(CH $_2$)r)s, **Rv** is **Ci** $_{-3}$ haloalkyl. For example, in some embodiments, R 7 is fluoromethyl, fluoroethyl, fluoropropyl, difluoromethyl, difluoroethyl, difluoropropyl, trifluoromethyl, trifluoroethyl, trifluoropropyl, chloromethyl, chloroethyl, chloropropyl, dichloromethyl, dichloroethyl, dichloropropyl, trichloromethyl, trichloroethyl, trichloropropyl, bromomethyl, bromoethyl, bromopropyl, dibromomethyl, dibromoethyl, dibromopropyl, tribromomethyl, tribromoethyl, tribromopropyl, iodomethyl, iodoethyl, iodopropyl, diiodomethyl, diiodoethyl, diiodopropyl, triiodomethyl, triiodoethyl, or triiodopropyl. In some embodiments R 7 is trifluoromethyl.

[00344] In some embodiments X $_6$ and X 7 are each-C(O)-. In some embodiments X $_6$ and **X** $_7$ are each -C(S)-.

[00345] In some embodiments, X $_6$ and X 7 are each -C((CH2)wOH)- and w is 0, 1, or 2. In some embodiments, X $_6$ and X 7 are each-COH-. In some embodiments, X $_6$ and X 7 are each-C((CH $_2$)OH)-. In some embodiments, **X** $_6$ and **X** $_7$ are each-C((CH $_2$)20H)-.

[002] In some embodiments, X $_6$ and X 7 are each **-C(X^{Ha1})-.** In some embodiments, **X^{Hal}** is fluoromethyl, fluoroethyl, fluoropropyl, difluoromethyl, difluoroethyl, difluoropropyl, trifluoromethyl, trifluoroethyl, trifluoropropyl, chloromethyl, chloroethyl, chloropropyl, dichloromethyl, dichloroethyl, dichloropropyl, trichloromethyl, trichloroethyl, trichloropropyl, bromomethyl, bromoethyl, bromopropyl, dibromomethyl, dibromoethyl, dibromopropyl, tribromomethyl, tribromoethyl, tribromopropyl, iodomethyl, iodoethyl, iodopropyl, diiodomethyl, diiodoethyl, diiodopropyl, triiodomethyl, triiodoethyl, or triiodopropyl. In some embodiments **X^{Hal}** is trifluoromethyl.

[00346] In another aspect, the disclosure provides a compound according to formula (Ie):



(Ie), or a salt or isomer thereof, wherein

Ri, R2, R3, and R 4 are independently selected from the group consisting of C 5-20 alkyl, C5-20 alkenyl, **-R"MR', -R*YR", -YR",** and **-R*OR";**

Rxi is **H** or C1-3 alkyl;

each M is independently selected from the group consisting of -C(0)0-, -OC(O)-, -OC(0)0-, -C(0)N(R')-, -N(R')C(0)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0-, -S(0)$_2$-, -C(0)S-, -SC(O)-, an aryl group, and a heteroaryl group;

X^1, and X^2 are independently selected from the group consisting of a bond, -CH2-, -(CH$_2$)$_2$-, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, -(CH$_2$)n-C(0)-, -C(0)-(CH$_2$)$_n$-,-(CH$_2$)$_n$-C(0)0-, -OC(0)-(CH$_2$)$_n$-, -(CH$_2$)$_n$-OC(0)-, -C(0)0-(CH$_2$)$_n$-, -CH(OH)-, -C(S)-, and -CH(SH)-;

Q is -NR9-, or a bond;

R_9 is H or Ci-3 alkyl;

each Y is independently a C3-6 carbocycle;

each R* is independently selected from the group consisting of Ci-i$_2$ alkyl and C_2-i$_2$ alkenyl;

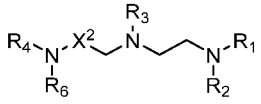
each R is independently selected from the group consisting of C1-3 alkyl and a C3-6 carbocycle;

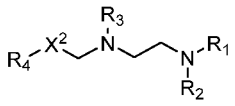
each R' is independently selected from the group consisting of C1-18 alkyl, C_2-is alkenyl, and H;

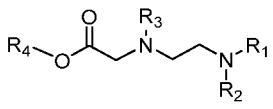
each R" is independently selected from the group consisting of C 3-i$_2$ alkyl, C3-12 alkenyl and -R*MR' ; and

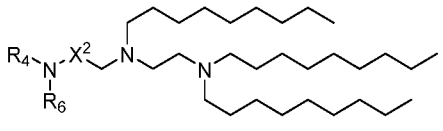
n is an integer from 1-6.

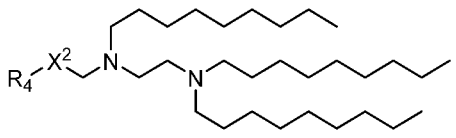
[00347] In some embodiments, the compound is of any of formulae (Iel)-(Ie6):

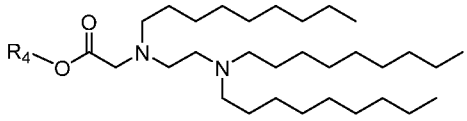

(Ie1),


(Ie2),


(Ie3),


(Ie4),


(Ie5), or



(Ie6).

[00348] In some embodiments, X^1 is -CH2-.

[00349] In some embodiments, X^2 is -C(O)-. In some embodiments, X^2 is -OC(O)-. In some embodiments, X^2 is -C(0)O. In some embodiments, X^2 is -CH20C(0)-. In some embodiments, X^2 is **-CH_2C(0)0-.**

[00350] In some embodiments, **Rxi** is **H.** In some embodiments, **Rxi** is C1-3 alkyl. In some embodiments, **Rxi** is **Ci** alkyl.

[00351] In some embodiments, **Ri,** R2, $_{R\ 3}$ and $_{R\ 4}$ are the same. In some embodiments, $_{R\ 4}$ is different from **Ri,** R2, and $_{R3.}$

[00352] In some embodiments, $_{R\ 4}$ is $_{C\ 5\text{-}10}$ alkyl. In some embodiments, $_{R\ 4}$ is **Cs** alkyl. In some embodiments, **R 4** is C 9 alkyl. In some embodiments, **R 4** is C10 alkyl. In some embodiments, $_{R\ 4}$ is **Cii -20** alkyl. In some embodiments, $_{R\ 4}$ is C13 alkyl. In some embodiments, **R 4** is Ci4 alkyl.

[00353] In some embodiments, **Ri** is **-R"MR'.** In some embodiments, R2 is **-R"MR'.** In some embodiments, $_{R\ 3}$ is **-R"MR'.** In some embodiments, at least one of **Ri,** R2 and $_{R\ 3}$ is -**R"MR'.** In some embodiments, wherein **Ri,** R2 and $_{R\ 3}$ are each **-R"MR'.** In some embodiments, **R_4** is **-R"MR'.**

[00354] In some embodiments, **M** is **-C(0)O.** In some embodiments, **M** is **-OC(O)-.**

[00355] In some embodiments, at least one **R"** is C3-7 alkyl. In some embodiments, at least one **R"** is C 4 alkyl. In some embodiments, at least one **R"** is C1-5 alkyl. In some embodiments, at least one **R"** is C 4 alkyl. In some embodiments, each **R"** is C3-7 alkyl. In some embodiments, each **R"** is C 4 alkyl.

[00356] In some embodiments, at least one **R'** is C1-5 alkyl. In some embodiments, each **R'** is **Ci-5** alkyl. In some embodiments, at least one **R'** is C 4 alkyl. In some embodiments, each **R'** is **C_4** alkyl.

[00357] In some embodiments, at least one of **Ri,** R2, and $_{R\ 3}$ is $_{C\ 5\text{-}10}$ alkyl. In some embodiments, at least one of **Ri,** R2, and $_{R\ 3}$ is C10 alkyl. In some embodiments, at least one of **Ri,** R2, and $_{R\ 3}$ is C 9 alkyl. In some embodiments, at least one of **Ri,** R2, and $_{R\ 3}$ is **Cs** alkyl. In some embodiments, at least one of **Ri,** R2, and $_{R\ 3}$ is $_{C\ 7}$ alkyl. In some embodiments, at least one of **Ri,** R2, and $_{R\ 3}$ is C_6 alkyl. In some embodiments, at least one of **Ri,** R2, and $_{R\ 3}$ is $_{C\ 5}$ alkyl.

[00358] In some embodiments, Ri, R^2, and R^3 are each C_{5-10} alkyl. In some embodiments, Ri, R^2, and R^3 are each C_{10} alkyl. In some embodiments, Ri, R^2, and R^3 are each C_9 alkyl. In some embodiments, Ri, R^2, and R^3 are each C_8 alkyl. In some embodiments, are each Ri, R^2, and R^3 is C_7 alkyl. In some embodiments, are each Ri, R^2, and R^3 is C_6 alkyl. In some embodiments, are each Ri, R^2, and R^3 is C_5 alkyl.

[00359] In some embodiments, Q is NR9. In some embodiments, R9 is H. In some embodiments, Q is a bond.

[00360] As used herein, the term "alkyl" or "alkyl group" means a linear or branched, saturated hydrocarbon including one or more carbon atoms (e.g., one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or more carbon atoms), which is optionally substituted. For example, the notation "C_{i-24} alkyl" means an optionally substituted linear or branched, saturated hydrocarbon including 1-24 carbon atoms. An alkyl group described herein refers to both unsubstituted and substituted alkyl group unless otherwise specified.

[00361] As used herein, the term "alkenyl" or "alkenyl group" means a linear or branched hydrocarbon including two or more carbon atoms (e.g., two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or more carbon atoms) and at least one double bond, which is optionally substituted. The notation "C_{2-24} alkenyl" means an optionally substituted linear or branched hydrocarbon including 2 to 24 carbon atoms and at least one carbon-carbon double bond. An alkenyl group may include one, two, three, four, or more carbon-carbon double bonds. For example, Cis alkenyl may include one or more double bonds. A Cis alkenyl group including two double bonds may be a linoleyl group. An alkenyl group described herein refers to both unsubstituted and substituted unless otherwise specified.

[00362] As used herein, the term "carbocycle" or "carbocyclic group" means an optionally substituted mono- or multi-cyclic system including one or more rings of carbon atoms. Rings may be three, four, five, six, seven, eight, nine, ten, eleven, or twelve membered rings. The notation "C_{3-6} carbocycle" means a carbocycle including a single ring having 3-6 carbon atoms. Carbocycles may include one or more carbon-carbon double or triple bonds and may be non-aromatic or aromatic (e.g., cycloalkyl or aryl groups). Examples of carbocycles include cyclopropyl, cyclopentyl, cyclohexyl, phenyl, naphthyl, and 1,2-dihydronaphthyl groups. Carbocycles described herein refers to both unsubstituted and substituted carbocycles unless otherwise specified. The term "cycloalkyl" as used herein means a non-aromatic

carbocycle and may or may not include any double or triple bond. Unless otherwise specified, carbocycles described herein refers to both unsubstituted and substituted carbocycle groups, i.e., optionally substituted carbocycles.

[00363] As used herein, the term "heterocycle" or "heterocyclic group" means an optionally substituted mono- or multi-cyclic system including one or more rings, where at least one ring includes at least one heteroatom. Heteroatoms may be, for example, nitrogen, oxygen, or sulfur atoms. Rings may be three, four, five, six, seven, eight, nine, ten, eleven, or twelve membered rings. Heterocycles may include one or more double or triple bonds and may be non-aromatic or aromatic. Examples of heterocycles include imidazolyl, imidazolidinyl, oxazolyl, oxazolidinyl, thiazolyl, thiazolidinyl, pyrazolidinyl, pyrazolyl, isoxazolidinyl, isoxazolyl, isothiazolidinyl, isothiazolyl, morpholinyl, pyrrolyl, pyrrolidinyl, furyl, tetrahydrofuryl, thiophenyl, pyridinyl, piperidinyl, quinolyl, and isoquinolyl groups. Heterocycles may be optionally substituted.

[00364] Affixing the suffix "-ene" to a group indicates the group is a divalent moiety, *e.g.,* alkylene is the divalent moiety of alkyl, alkenylene is the divalent moiety of alkenyl, alkynylene is the divalent moiety of alkynyl, heteroalkylene is the divalent moiety of heteroalkyl, heteroalkenylene is the divalent moiety of heteroalkenyl, heteroalkynylene is the divalent moiety of heteroalkynyl, carbocyclylene is the divalent moiety of carbocyclyl, heterocyclylene is the divalent moiety of heterocyclyl, arylene is the divalent moiety of aryl, and heteroarylene is the divalent moiety of heteroaryl.

[00365] As used herein, a "biodegradable group" is a group that may facilitate faster metabolism of a lipid in a mammalian entity. A biodegradable group may be selected from the group consisting of, but is not limited to, **-C(0)0-,** -OC(O)-, -C**(0**)N(R')-, -N(R')C **(0)-,** -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, **-P(0**)(**OR**')**0-, -S(0)** $_2$**-,** an aryl group, and a heteroaryl group. As used herein, an "aryl group" is a carbocyclic group including one or more aromatic rings. Examples of aryl groups include phenyl and naphthyl groups. As used herein, a "heteroaryl group" is a heterocyclic group including one or more aromatic rings. Examples of heteroaryl groups include pyrrolyl, furyl, thiophenyl, imidazolyl, oxazolyl, and thiazolyl. Aryl and heteroaryl groups may be optionally substituted. For example, each M, M^b, M^c, or M^d can be independently selected from the non-limiting group consisting of phenyl, oxazole, and thiazole. In the formulae above, each M, M^b, M^c, or M^d can be independently selected from the list of biodegradable groups above.

[00366] Alkyl, alkenyl, and cyclyl (e.g., carbocyclyl and heterocyclyl) groups may be optionally substituted unless otherwise specified. Optional substituents may be selected from the group consisting of, but are not limited to, a halogen atom (e.g., a chloride, bromide, fluoride, or iodide group), a carboxylic acid (e.g., -C(O)OH), an alcohol (e.g., ahydroxyl, -OH), an ester (e.g., -C(**0**)OR or -OC(O)R), an aldehyde (e.g.,-C(**0**)H), a carbonyl (e.g., -C(**0**)R, alternatively represented by **C=0),** an acyl halide (e.g.,-C(**0**)X, in which X is ahalide selected from bromide, fluoride, chloride, and iodide), a carbonate (e.g., -OC(O)OR), an alkoxy (e.g., -OR), an acetal (e.g.,-C(OR)2R"", in which each OR are alkoxy groups that can be the same or different and R"" is an alkyl or alkenyl group), a phosphate (e.g., P(**0**)4^{3-}), a thiol (e.g., -SH), a sulfoxide (e.g., -S(O)R), a sulfinic acid (e.g., -S(O)OH), a sulfonic acid (e.g., -S(**0**)$_2$OH), a thial (e.g., -C(S)H), a sulfate (e.g., **S(0)** 4^{2-}**),** a sulfonyl (e.g., -**S(0)** $_2$-**),** an amide (e.g., -C(**0**)NR2, or -N(R)C(**0**)R), an azido (e.g., -N3), anitro (e.g., -NO2), a cyano (e.g., -CN), an isocyano (e.g., -NC), an acyloxy (e.g.,-OC(**0**)R), an amino (e.g., -NR2, -NRH, or -NH2), a carbamoyl (e.g., -OC(**0**)NR$_2$, -OC(**0**)NRH, or -OC(**0**)NH$_2$), a sulfonamide (e.g., -S(**0**)$_2$NR$_2$, -S(**0**)$_2$NRH, -S(**0**)$_2$NH$_2$, -N(R)S(**0**)$_2$R, -N(H)S(**0**)$_2$R, -N(R)S(**0**)$_2$H, or -N(H)S(**0**)2H), a cyclyl (e.g., carbocyclyl or heterocyclyl) group, an alkyl group, and an alkenyl group. In any of the preceding, R is an alkyl or alkenyl group, as defined herein. In some embodiments, the substituent groups themselves may be further substituted with, for example, one, two, three, four, five, or six substituents as defined herein. For example, a C 5-20 alkyl group may be further substituted with one, two, three, four, five, six, or more substituents as described herein.

[00367] An amine moiety of a lipid according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) may be protonated at a physiological pH. Thus, a lipid may have a positive or partial positive charge at physiological pH. Such lipids may be referred to as cationic or ionizable (amino)lipids. Lipids may be zwitterionic, i.e., neutral molecules having both a positive and a negative charge.

Nanoparticle compositions

[00368] The disclosure also features nanoparticle compositions comprising a lipid component comprising a compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) as described herein. In some embodiments, the largest dimension of a nanoparticle composition is 1 μιτι or shorter (e.g., 1 μιη, 900 nm, 800 nm, 700 nm, 600 nm, 500 nm, 400 nm, 300 nm, 200 nm, 175 nm, 150 nm, 125

nm, 100 nm, 75 nm, 50 nm, or shorter), *e.g.*, when measured by dynamic light scattering (DLS), transmission electron microscopy, scanning electron microscopy, or another method. Nanoparticle compositions include, for example, lipid nanoparticles (LNPs), liposomes, lipid vesicles, and lipoplexes. In some embodiments, nanoparticle compositions are vesicles including one or more lipid bilayers. In certain embodiments, a nanoparticle composition includes two or more concentric bilayers separated by aqueous compartments. Lipid bilayers may be functionalized and/or crosslinked to one another. Lipid bilayers may include one or more ligands, proteins, or channels.

[00369] Nanoparticle compositions comprise a lipid component including at least one lipid, such as a compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), as described herein. For example, in some embodiments, a nanoparticle composition may include a lipid component including one of Compounds 1 through 88, Compounds 17-1 through 17-13, Compounds 19-1 through 19-6, Compounds 20-1 through 20-25 and Compounds 21-1 through 21-6. Nanoparticle compositions may also include a variety of other components. For example, the lipid component of a nanoparticle composition may include one or more other lipids in addition to a lipid according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

Cationic/ionizable lipids

[0001] A nanoparticle composition may include one or more cationic and/or ionizable lipids (e.g., lipids that may have a positive or partial positive charge at physiological pH) in addition to a lipid according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia). Cationic and/or ionizable lipids may be selected from the non-limiting group consisting of

3-(didodecy lamino)-N 1,N 1,4-tridodecy 1- 1-piperazineethanamine (KL 10),

Nl-[2-(didodecylamino)ethyl]-Nl,N4,N4-tridodecyl-l,4-piperazinediethanamine (KL22),

14,25-ditridecyl-l 5, 18,21 ,24-tetraaza-octatriacontane (KL25),

l,2-dilinoleyloxy-N,N-dimethylaminopropane (DLinDMA),

2,2-dilinoleyl-4-dimethylaminomethyl-[l,3]-dioxolane (DLin-K-DMA),

heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (DLin-MC3-DMA),

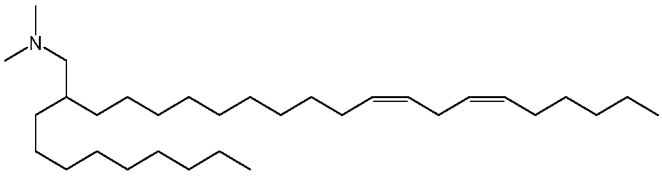
2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[l,3]-dioxolane (DLin-KC2-DMA),

l,2-dioleyloxy-N,N-dimethylaminopropane (DODMA),

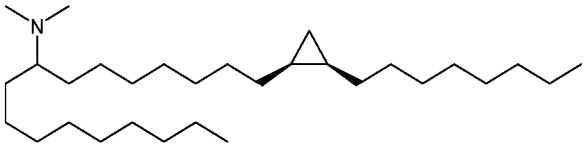
2-({8-[(3P)-cholest-5-en-3-ylox>']octyl}oxy)-N,N-dime%l-3-[(9Z,12Z)-octadeca-9,12-dien-l-y loxy]propan-1-amine (Oety1-CLinDMA),

(2R)-2<{8-[(3P)-cholest-5-es^3-yloxy]octyl}o^ y)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-die n-1-y1oxy]propan-1-amine (Octy1-CLinDMA (2R)),

(2S)-2-({8-[(3p)-cholest-5-en-3-yloxy]octyl}o xy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-die n-1-yloxy]propan-1-amine (Octyl-CLinDMA (2S)),



(i.e., (12Z, 15Z)-N,N-dimethyl-2-nonylhenicosa-12,15-dien-1-amine), and



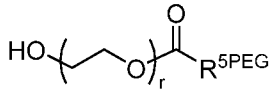
(i.e., N,N-dimethyl-l-{(lS,2R)-2-octylcyclopropyl}heptadecan-8-amine).

[00370] In addition to these, a cationic lipid may also be a lipid including a cyclic amine group. Additional cationic and/or ionizable lipids that are suitable for the formulations and methods disclosed herein include those described in WO2015 199952, WO201 6 176330, and WO2015011633, the entire contents of each of which are hereby incorporated by reference in their entireties.

PEG lipids

[00371] The lipid component of a nanoparticle composition may include one or more PEG or PEG-modified lipids. Such species may be alternately referred to as PEGylated lipids. A PEG lipid is a lipid modified with polyethylene glycol. A PEG lipid may be selected from the non-limiting group consisting of PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, PEG-modified dialkylglycerols, and mixtures thereof. For example, a PEG lipid may be PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, or a PEG-DSPE lipid.

[00372] In certain embodiments, a PEG lipid may be of Formula (VI):



(VI), or a salt or isomer thereof, wherein:

R^{3PEG} is -OR^{0};

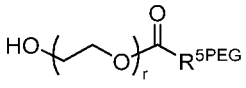
R^{0} is hydrogen, Ci-6 alkyl or an oxygen protecting group;

r is an integer between 1 and 100;

R^{5PEG} is Cio-40 alkyl, Cio-40 alkenyl, or Cio-40 alkynyl; and optionally one or more methylene groups of R^{5PEG} are independently replaced with C_{3-10} carbocyclylene, 4 to 10 membered heterocyclylene, C_{6-10} arylene, 4 to 10 membered heteroarylene, -N(R^{N})-, -0-, -S-, -C(O)-, -C(0)N(R^{N})-, -NR^{N}C(0)-, -NR^{N}C(0)N(R^{N})-, -C(0)0-, -OC(O)-, -OC(0)0-, -OC(0)N(R^{N})-, -NR^{N}C(0)0-, -C(0)S-, -SC(O)-, -C(=NR^{N})-, -C(=NR^{N})N(R^{N})-, -NR^{N}C(=NR^{N})-, -NR^{N}C(=NR^{N})N(R^{N})-, -C(S)-, -C(S)N(R^{N})-, -NR^{N}C(S)-, -NR^{N}C(S)N(R^{N})-, -S(O)-, -OS(O)-, -S(0)0-, -OS(0)0-, -OS(0)$_2$-, -S(0)$_2$0-, -OS(0)$_2$0-, -N(R^{N})S(0)-, -S(0)N(R^{N})-, -N(R^{N})S(0)N(R^{N})-, -OS(0)N(R^{N})-, -N(R^{N})S(0)0-, -S(0)$_2$-, -N(R^{N})S(0)$_2$-, -S(0)$_2$N(R^{N})-, -N(R^{N})S(0)$_2$N(R^{N})-, -OS(0)$_2$N(R^{N})-, or -N(R^{N})S(0)$_2$0-; and

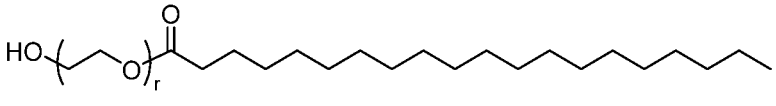
each instance of R^{N} is independently hydrogen, Ci-6 alkyl, or a nitrogen protecting group.

In certain embodiments, the compound of Formula (VI) is of Formula (Vl-a):



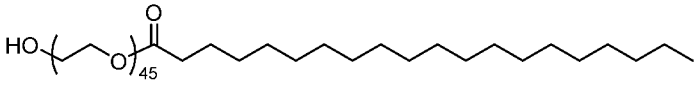
(VI-a), or a salt or isomer thereof.

[00373] In certain embodiments, a compound of Formula (VI) is of Formula (Vl-b):



, (VI-b) or a salt or isomer thereof.

[00374] In certain embodiments, the compound of Formula (Vl-b) is a compound having the formula:



(PEG 1) or a salt or isomer thereof.

[00375] In some embodiments of the compositions provided herein, the PEG lipid is a PEG lipid described in International Patent Application No. PCT/US2016/000129, filed December 10, 201 6, the entire contents of each of which are incorporated herein by reference.

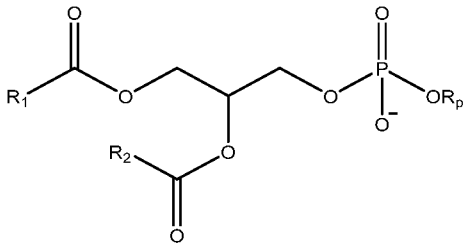
Structural lipids

[00376] The lipid component of a nanoparticle composition may include one or more structural lipids. Structural lipids can be selected from the group consisting of, but are not

limited to, cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alpha-tocopherol, and mixtures thereof. In certain embodiments, the structural lipid is cholesterol. In some embodiments, the structural lipid includes cholesterol and a corticosteroid (such as prednisolone, dexamethasone, prednisone, and hydrocortisone), or a combination thereof.

Phospholipids

[00377] The lipid component of a nanoparticle composition may include one or more phospholipids, such as one or more (poly)unsaturated lipids. Phospholipids may assemble into one or more lipid bilayers. In general, phospholipids may include a phospholipid moiety and one or more fatty acid moieties. For example, a phospholipid may be a lipid according to formula (V)



(V),

in which R_p represents a phospholipid moiety and Ri and R2 represent fatty acid moieties with or without unsaturation that may be the same or different. A phospholipid moiety may be selected from the non-limiting group consisting of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl serine, phosphatidic acid, 2-lysond a sphingomyelin. A fatty acid moiety may be selected from the non-limiting group consisting of lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, alpha-linolenic acid, erucic acid, phytanic acid, arachidic acid, arachidonic acid, eicosapentaenoic acid, behenic acid, docosapentaenoic acid, and docosahexaenoic acid. Non-natural species including natural species with modifications and substitutions including branching, oxidation, cyclization, and alkynes are also contemplated. For example, a phospholipid may be functionalized with or cross-linked to one or more alkynes (e.g., an alkenyl group in which one or more double bonds is replaced with a triple bond). Under appropriate reaction conditions, an alkyne group may undergo a copper-catalyzed cycloaddition upon exposure to an azide. Such reactions may be useful in functionalizing a lipid bilayer of a nanoparticle composition to facilitate membrane permeation or cellular recognition or in

conjugating a nanoparticle composition to a useful component such as a targeting or imaging moiety (e.g., a dye).

[00378] Phospholipids useful in the compositions and methods described herein may be selected from the non-limiting group consisting of l,2-distearoyl-s«-glycero-3-phosphocholine (DSPC), l,2-dioleoyl-OT-glycero-3-phosphoethanolamine (DOPE), l,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), l,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), l,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), l-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), l,2-di-0-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), l-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), l-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), l,2-dilinolenoyl-sn-glycero-3-phosphocholine, l,2-diarachidonoyl-sn-glycero-3-phosphocholine, l,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, l,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), l,2-distearoyl-sn-glycero-3-phosphoethanolamine, l,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, l,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, l,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, l,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, l,2-dioleoyl-sn-glycero-3-phospho-rac-(l-glycerol) sodium salt (DOPG), and sphingomyelin. In certain embodiments, a nanoparticle composition includes DSPC. In certain embodiments, a nanoparticle composition includes DOPE. In some embodiments, a nanoparticle composition includes both DSPC and DOPE.

Adjuvants

[00379] In some embodiments, a nanoparticle composition that includes one or more lipids described herein may further include one or more adjuvants, e.g., Glucopyranosyl Lipid Adjuvant (GLA), CpG oligodeoxynucleotides (e.g., Class A or B), poly(I:C), aluminum hydroxide, and Pam3CSK4.

Therapeutic agents

[00380] Nanoparticle compositions may include one or more therapeutic and/or prophylactic agents. The disclosure features methods of delivering a therapeutic and/or prophylactic agent to a mammalian cell or organ, producing a polypeptide of interest in a mammalian cell, and treating a disease or disorder in a mammal in need thereof comprising administering to a mammal and/or contacting a mammalian cell with a nanoparticle composition including a therapeutic and/or prophylactic agent.

[00381] Therapeutic and/or prophylactic agents include biologically active substances and are alternately referred to as "active agents." A therapeutic and/or prophylactic agent may be a substance that, once delivered to a cell or organ, brings about a desirable change in the cell, organ, or other bodily tissue or system. Such species may be useful in the treatment of one or more diseases, disorders, or conditions. In some embodiments, a therapeutic and/or prophylactic agent is a small molecule drug useful in the treatment of a particular disease, disorder, or condition. Examples of drugs useful in the nanoparticle compositions described herein include, but are not limited to, antineoplastic agents (e.g., vincristine, doxorubicin, mitoxantrone, camptothecin, cisplatin, bleomycin, cyclophosphamide, methotrexate, and streptozotocin), antitumor agents (e.g., actinomycin D, vincristine, vinblastine, cytosine arabinoside, anthracyclines, alkylating agents, platinum compounds, antimetabolites, and nucleoside analogs, such as methotrexate and purine and pyrimidine analogs), anti-infective agents, local anesthetics (e.g., dibucaine and chlorpromazine), beta-adrenergic blockers (e.g., propranolol, timolol, and labetalol), antihypertensive agents (e.g., clonidine and hydralazine), anti-depressants (e.g., imipramine, amitriptyline, and doxepin), anti-convulsants (e.g., phenytoin), antihistamines (e.g., diphenhydramine, chlorpheniramine, and promethazine), antibiotic/antibacterial agents (e.g., gentamycin, ciprofloxacin, and cefoxitin), antifungal agents (e.g., miconazole, terconazole, econazole, isoconazole, butaconazole, clotrimazole, itraconazole, nystatin, naftifine, and amphotericin B), antiparasitic agents, hormones, hormone antagonists, immunomodulators, neurotransmitter antagonists, antiglaucoma agents, vitamins, narcotics, and imaging agents.

[00382] In some embodiments, a therapeutic and/or prophylactic agent is a cytotoxin, a radioactive ion, a chemotherapeutic, a vaccine, a compound that elicits an immune response, and/or another therapeutic and/or prophylactic agent. A cytotoxin or cytotoxic agent includes any agent that may be detrimental to cells. Examples include, but are not limited to, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxyanthracinedione,

mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, maytansinoids, e.g., maytansinol, rachelmycin (CC-1065), and analogs or homologs thereof. Radioactive ions include, but are not limited to iodine (e.g., iodine 125 or iodine 131), strontium 89, phosphorous, palladium, cesium, iridium, phosphate, cobalt, yttrium 90, samarium 153, and praseodymium. Vaccines include compounds and preparations that are capable of providing immunity against one or more conditions related to infectious diseases such as influenza, measles, human papillomavirus (HPV), rabies, meningitis, whooping cough, tetanus, plague, hepatitis, and tuberculosis and can include mRNAs encoding infectious disease derived antigens and/or epitopes. Vaccines also include compounds and preparations that direct an immune response against cancer cells and can include mRNAs encoding tumor cell derived antigens, epitopes, and/or neoepitopes. Compounds eliciting immune responses may include vaccines, corticosteroids (e.g., dexamethasone), and other species. In some embodiments, a vaccine and/or a compound capable of eliciting an immune response is administered intramuscularly via a composition including a compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia). Other therapeutic and/or prophylactic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil, dacarbazine), alkylating agents (e.g., mechlorethamine, thiotepa chlorambucil, rachelmycin (CC-1065), melphalan, carmustine (BSNU), lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine, vinblastine, taxol and maytansinoids).

[00383] In other embodiments, a therapeutic and/or prophylactic agent is a protein. Therapeutic proteins useful in the nanoparticles of the disclosure include, but are not limited to, gentamycin, amikacin, insulin, erythropoietin (EPO), granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), Factor VIR, luteinizing hormone-releasing hormone (LHRH) analogs, interferons, heparin, Hepatitis B surface antigen, typhoid vaccine, and cholera vaccine.

Polynucleotides and Nucleic Acids

[00384] In some embodiments, a therapeutic and/or prophylactic agent is a polynucleotide or nucleic acid (e.g., ribonucleic acid or deoxyribonucleic acid). The term "polynucleotide," in its broadest sense, includes any compound and/or substance that is or can be incorporated into an oligonucleotide chain. Exemplary polynucleotides for use in accordance with the present disclosure include, but are not limited to, one or more of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) including messenger mRNA (mRNA), hybrids thereof, RNAi-inducing agents, RNAi agents, siRNAs, shRNAs, miRNAs, antisense RNAs, ribozymes, catalytic DNA, RNAs that induce triple helix formation, aptamers, vectors, etc. In certain embodiments, a therapeutic and/or prophylactic agent is an RNA. RNAs useful in the compositions and methods described herein can be selected from the group consisting of, but are not limited to, shortmers, antagomirs, antisense, ribozymes, small interfering RNA (siRNA), asymmetrical interfering RNA (aiRNA), microRNA (miRNA), Dicer-substrate RNA (dsRNA), small hairpin RNA (shRNA), transfer RNA (tRNA), messenger RNA (mRNA), and mixtures thereof. In certain embodiments, the RNA is an mRNA.

[00385] In certain embodiments, a therapeutic and/or prophylactic agent is an mRNA. An mRNA may encode any polypeptide of interest, including any naturally or non-naturally occurring or otherwise modified polypeptide. A polypeptide encoded by an mRNA may be of any size and may have any secondary structure or activity. In some embodiments, a polypeptide encoded by an mRNA may have a therapeutic effect when expressed in a cell.

[00386] In other embodiments, a therapeutic and/or prophylactic agent is an siRNA. An siRNA may be capable of selectively knocking down or down regulating expression of a gene of interest. For example, an siRNA could be selected to silence a gene associated with a particular disease, disorder, or condition upon administration to a subject in need thereof of a nanoparticle composition including the siRNA. An siRNA may comprise a sequence that is complementary to an mRNA sequence that encodes a gene or protein of interest. In some embodiments, the siRNA may be an immunomodulatory siRNA.

[00387] In some embodiments, a therapeutic and/or prophylactic agent is an shRNA or a vector or plasmid encoding the same. An shRNA may be produced inside a target cell upon delivery of an appropriate construct to the nucleus. Constructs and mechanisms relating to shRNA are well known in the relevant arts.

[00388] Nucleic acids and polynucleotides useful in or suitable for the compounds and methods of the disclosure typically include a first region of linked nucleosides encoding a

polypeptide of interest (e.g., a coding region), a first flanking region located at the 5'-terminus of the first region (e.g., a 5'-UTR), a second flanking region located at the 3'-terminus of the first region (e.g., a 3'-UTR), at least one 5'-cap region, and a 3'-stabilizing region. In some embodiments, a nucleic acid or polynucleotide further includes a poly-A region or a Kozak sequence (e.g., in the 5'-UTR). In some cases, polynucleotides may contain one or more intronic nucleotide sequences capable of being excised from the polynucleotide. In some embodiments, a polynucleotide or nucleic acid (e.g., an mRNA) may include a 5' cap structure, a chain terminating nucleotide, a stem loop, a polyA sequence, and/or a polyadenylation signal. Any one of the regions of a nucleic acid may include one or more alternative components (e.g., an alternative nucleoside). For example, the 3'-stabilizing region may contain an alternative nucleoside such as an L-nucleoside, an inverted thymidine, or a 2'-0-methyl nucleoside and/or the coding region, 5'-UTR, 3'-UTR, or cap region may include an alternative nucleoside such as a 5-substituted uridine (e.g., 5-methoxyuridine), a 1-substituted pseudouridine (e.g., 1-methyl-pseudouridine or 1-ethyl-pseudouridine), and/or a 5-substituted cytidine (e.g., 5-methyl-cytidine).

[00389] Generally, the shortest length of a polynucleotide can be the length of the polynucleotide sequence that is sufficient to encode for a dipeptide. In another embodiment, the length of the polynucleotide sequence is sufficient to encode for a tripeptide. In another embodiment, the length of the polynucleotide sequence is sufficient to encode for a tetrapeptide. In another embodiment, the length of the polynucleotide sequence is sufficient to encode for a pentapeptide. In another embodiment, the length of the polynucleotide sequence is sufficient to encode for a hexapeptide. In another embodiment, the length of the polynucleotide sequence is sufficient to encode for a heptapeptide. In another embodiment, the length of the polynucleotide sequence is sufficient to encode for an octapeptide. In another embodiment, the length of the polynucleotide sequence is sufficient to encode for a nonapeptide. In another embodiment, the length of the polynucleotide sequence is sufficient to encode for a decapeptide.

[00390] Examples of dipeptides that the alternative polynucleotide sequences can encode for include, but are not limited to, camosine and anserine.

[00391] In some cases, a polynucleotide is greater than 30 nucleotides in length. In another embodiment, the polynucleotide molecule is greater than 35 nucleotides in length. In another embodiment, the length is at least 40 nucleotides. In another embodiment, the length is at least 45 nucleotides. In another embodiment, the length is at least 55 nucleotides. In another embodiment, the length is at least 50 nucleotides. In another embodiment, the length is at least

60 nucleotides. In another embodiment, the length is at least 80 nucleotides. In another embodiment, the length is at least 90 nucleotides. In another embodiment, the length is at least 100 nucleotides. In another embodiment, the length is at least 120 nucleotides. In another embodiment, the length is at least 140 nucleotides. In another embodiment, the length is at least 160 nucleotides. In another embodiment, the length is at least 180 nucleotides. In another embodiment, the length is at least 200 nucleotides. In another embodiment, the length is at least 250 nucleotides. In another embodiment, the length is at least 300 nucleotides. In another embodiment, the length is at least 350 nucleotides. In another embodiment, the length is at least 400 nucleotides. In another embodiment, the length is at least 450 nucleotides. In another embodiment, the length is at least 500 nucleotides. In another embodiment, the length is at least 600 nucleotides. In another embodiment, the length is at least 700 nucleotides. In another embodiment, the length is at least 800 nucleotides. In another embodiment, the length is at least 900 nucleotides. In another embodiment, the length is at least 1000 nucleotides. In another embodiment, the length is at least 1100 nucleotides. In another embodiment, the length is at least 1200 nucleotides. In another embodiment, the length is at least 1300 nucleotides. In another embodiment, the length is at least 1400 nucleotides. In another embodiment, the length is at least 1500 nucleotides. In another embodiment, the length is at least 1600 nucleotides. In another embodiment, the length is at least 1800 nucleotides. In another embodiment, the length is at least 2000 nucleotides. In another embodiment, the length is at least 2500 nucleotides. In another embodiment, the length is at least 3000 nucleotides. In another embodiment, the length is at least 4000 nucleotides. In another embodiment, the length is at least 5000 nucleotides, or greater than 5000 nucleotides.

[00392] Nucleic acids and polynucleotides may include one or more naturally occurring components, including any of the canonical nucleotides A (adenosine), G (guanosine), C (cytosine), U (uridine), or T (thymidine). In one embodiment, all or substantially all of the nucleotides comprising (a) the 5'-UTR, (b) the open reading frame (ORF), (c) the 3'-UTR, (d) the poly A tail, and any combination of (a, b, c, or d above) comprise naturally occurring canonical nucleotides A (adenosine), G (guanosine), C (cytosine), U (uridine), or T (thymidine).

[00393] Nucleic acids and polynucleotides may include one or more alternative components, as described herein, which impart useful properties including increased stability and/or the lack of a substantial induction of the innate immune response of a cell into which the polynucleotide is introduced. For example, an alternative polynucleotide or nucleic acid exhibits reduced degradation in a cell into which the polynucleotide or nucleic acid is

introduced, relative to a corresponding unaltered polynucleotide or nucleic acid. These alternative species may enhance the efficiency of protein production, intracellular retention of the polynucleotides, and/or viability of contacted cells, as well as possess reduced immunogenicity .

[00394] Polynucleotides and nucleic acids may be naturally or non-naturally occurring. Polynucleotides and nucleic acids may include one or more modified (e.g., altered or alternative) nucleobases, nucleosides, nucleotides, or combinations thereof. The nucleic acids and polynucleotides useful in the nanoparticle compositions described herein can include any useful modification or alteration, such as to the nucleobase, the sugar, or the internucleoside linkage (e.g., to a linking phosphate / to a phosphodiester linkage / to the phosphodiester backbone). In certain embodiments, alterations (e.g., one or more alterations) are present in each of the nucleobase, the sugar, and the internucleoside linkage. Alterations according to the present disclosure may be alterations of ribonucleic acids (RNAs) to deoxyribonucleic acids (DNAs), e.g., the substitution of the 2′-OH of the ribofuranosyl ring to 2′-H, threose nucleic acids (TNAs), glycol nucleic acids (GNAs), peptide nucleic acids (PNAs), locked nucleic acids (LNAs), or hybrids thereof. Additional alterations are described herein.

[00395] Polynucleotides and nucleic acids may or may not be uniformly altered along the entire length of the molecule. For example, one or more or all types of nucleotide (e.g., purine or pyrimidine, or any one or more or all of A, G, U, C) may or may not be uniformly altered in a polynucleotide or nucleic acid, or in a given predetermined sequence region thereof. In some instances, all nucleotides X in a polynucleotide (or in a given sequence region thereof) are altered, wherein X may any one of nucleotides A, G, U, C, or any one of the combinations A+G, A+U, A+C, G+U, G+C, U+C, A+G+U, A+G+C, G+U+C or A+G+C.

[00396] Different sugar alterations and/or internucleoside linkages (e.g., backbone structures) may exist at various positions in a polynucleotide. One of ordinary skill in the art will appreciate that the nucleotide analogs or other alteration(s) may be located at any position(s) of a polynucleotide such that the function of the polynucleotide is not substantially decreased. An alteration may also be a 5′- or 3′-terminal alteration. In some embodiments, the polynucleotide includes an alteration at the 3′-terminus. The polynucleotide may contain from about 1% to about 100% alternative nucleotides (either in relation to overall nucleotide content, or in relation to one or more types of nucleotide, i.e., any one or more of A, G, U or C) or any intervening percentage (e.g., from 1% to 20%, from 1% to 25%, from 1% to 50%, from 1% to 60%, from 1% to 70%, from 1% to 80%, from 1% to 90%, from 1% to 95%, from 10% to 20%,

from 10% to 25%, from 10% to 50%, from 10% to 60%, from 10% to 70%, from 10% to 80%, from 10% to 90%, from 10% to 95%, from 10% to 100%, from 20% to 25%, from 20% to 50%, from 20% to 60%, from 20% to 70%, from 20% to 80%, from 20% to 90%, from 20% to 95%, from 20% to 100%, from 50% to 60%, from 50% to 70%, from 50% to 80%, from 50% to 90%, from 50% to 95%, from 50% to 100%, from 70% to 80%, from 70% to 90%, from 70% to 95%, from 70% to 100%, from 80% to 90%, from 80% to 95%, from 80% to 100%, from 90% to 95%, from 90% to 100%, and from 95% to 100%). It will be understood that any remaining percentage is accounted for by the presence of a canonical nucleotide (e.g., A, G, U, or C).

[00397] Polynucleotides may contain at a minimum zero and at maximum 100% alternative nucleotides, or any intervening percentage, such as at least 5% alternative nucleotides, at least 10% alternative nucleotides, at least 25% alternative nucleotides, at least 50% alternative nucleotides, at least 80% alternative nucleotides, or at least 90% alternative nucleotides. For example, polynucleotides may contain an alternative pyrimidine such as an alternative uracil or cytosine. In some embodiments, at least 5%, at least 10%, at least 25%, at least 50%, at least 80%, at least 90% or 100% of the uracil in a polynucleotide is replaced with an alternative uracil (e.g., a 5-substituted uracil). The alternative uracil can be replaced by a compound having a single unique structure, or can be replaced by a plurality of compounds having different structures (e.g., 2, 3, 4 or more unique structures). In some instances, at least 5%, at least 10%, at least 25%, at least 50%, at least 80%, at least 90% or 100% of the cytosine in the polynucleotide is replaced with an alternative cytosine (e.g., a 5-substituted cytosine). The alternative cytosine can be replaced by a compound having a single unique structure, or can be replaced by a plurality of compounds having different structures (e.g., 2, 3, 4 or more unique structures).

[00398] In some instances, nucleic acids do not substantially induce an innate immune response of a cell into which the polynucleotide (e.g., mRNA) is introduced. Features of an induced innate immune response include 1) increased expression of pro-inflammatory cytokines, 2) activation of intracellular PRRs (RIG-I, MDA5, etc., and/or 3) termination or reduction in protein translation.

[00399] The nucleic acids can optionally include other agents (e.g., RNAi-inducing agents, RNAi agents, siRNAs, shRNAs, miRNAs, antisense RNAs, ribozymes, catalytic DNA, tRNA, RNAs that induce triple helix formation, aptamers, and vectors). In some embodiments, the nucleic acids may include one or more messenger RNAs (mRNAs) having one or more alternative nucleoside or nucleotides (i.e., alternative mRNA molecules).

[00400] In some embodiments, a nucleic acid (e.g. mRNA) molecule, formula, composition or method associated therewith comprises one or more polynucleotides comprising features as described in WO2002/098443, WO2003/051401, WO2008/052770, WO2009127230, WO2006122828, WO2008/083949, WO201 0088927, WO201 0/037539, WO2004/004743, WO2005/016376, WO2006/024518, WO2007/095976, WO2008/014979, WO2008/077592, WO2009/030481, WO2009/095226, WO201 1069586, WO20 11026641, WO201 1/144358, WO2012019780, WO2012013326, WO2012089338, WO20121 13513, WO20121 1681 1, WO20121 16810, WO20131 13502, WO2013113501, WO20131 13736, WO201 3143698, WO2013 143699, WO2013143700, WO2013/120626, WO201 3120627, WO201 3120628, WO20 13 120629, WO201 3174409, WO2014127917, WO20 15/024669, WO20 15/024668, WO20 15/024667, WO20 15/024665, WO20 15/024666, WO20 15/024664, WO2015101415, WO2015101414, WO201 5024667, WO2015062738, WO2015101416, all of which are incorporated by reference herein.

[00401] In some embodiments the agent that inhibits immune responses by the LNP comprises a miR binding site. In other embodiments the miR binding site is selected from miR 126, miR 155, and miR 142 3p. The miR binding site is incorporated into a mRNA in some embodiments. In other embodiments the miR binding site is separate from the mRNA.

[00402] In some embodiments the agent that inhibits immune responses by the LNP comprises an mRNA comprising a miR binding site. In various embodiments, the mRNA comprises, one, two, three or four miR binding sites, wherein at least one of the miR binding sites is a miR-126 binding site. In one embodiment, the mRNA, comprises at least two microRNA binding sites, wherein at least one of the microRNA binding sites is a miR-126 binding site. In one embodiment, the mRNA, e.g., mmRNA, comprises a miR-126 binding site and a second microRNA binding site for a miR selected from the group consisting of miR- 142-3p, miR-142-5p, miR-146-3p, miR-146-5p, miR-155, miR-16, miR-21, miR-223, miR-24 and miR-27. In another embodiment, the mRNA, comprises a miR-126 (e.g., miR-126-3p) binding site and a miR-142 (e.g., miR-142-3p) binding site. A miR referred to by number herein can refer to either of the two mature microRNAs originating from opposite arms of the same pre-miRNA (e.g., either the 3p or 5p microRNA). All miRs referred to by number herein are intended to include both the 3p and 5p arms/sequences. It has now been discovered that incorporation of at least one microRNA binding site for a microRNA expressed in immune cells (e.g., miR-126, miR-142, miR-155 and combinations thereof) into an mRNA construct can reduce or inhibit accelerated blood clearance (ABC) when the lipid-comprising compound or

composition comprising the mRNA is administered to a subject. In one embodiment, the mechanism of action of the miRNA binding site(s) is a microRNA "sponge", wherein the miRNA binding site(s) in the construct or LNP "soaks up" microRNAs that bind to the binding site(s).

Nucleobase Alternatives

[00403] The alternative nucleosides and nucleotides can include an alternative nucleobase. A nucleobase of a nucleic acid is an organic base such as a purine or pyrimidine or a derivative thereof. A nucleobase may be a canonical base (e.g., adenine, guanine, uracil, thymine, and cytosine). These nucleobases can be altered or wholly replaced to provide polynucleotide molecules having enhanced properties, e.g., increased stability such as resistance to nucleases. Non-canonical or modified bases may include, for example, one or more substitutions or modifications including but not limited to alkyl, aryl, halo, oxo, hydroxyl, alkyloxy, and/or thio substitutions; one or more fused or open rings; oxidation; and/or reduction.

[00404] Alternative nucleotide base pairing encompasses not only the standard adenine-thymine, adenine-uracil, or guanine-cytosine base pairs, but also base pairs formed between nucleotides and/or alternative nucleotides including non-standard or alternative bases, wherein the arrangement of hydrogen bond donors and hydrogen bond acceptors permits hydrogen bonding between a non-standard base and a standard base or between two complementary non-standard base structures. One example of such non-standard base pairing is the base pairing between the alternative nucleotide inosine and adenine, cytosine, or uracil.

[00405] In some embodiments, the nucleobase is an alternative uracil. Exemplary nucleobases and nucleosides having an alternative uracil include pseudouridine (ψ), pyridin-4-one ribonucleoside, 5-aza-uracil, 6-aza-uracil, 2-thio-5-aza-uracil, 2-thio-uracil (s^2U), 4-thio-uracil (s^4U), 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxy-uracil (ho^5U), 5-aminoallyl-uracil, 5-halo-uracil (e.g., 5-iodo-uracil or 5-bromo-uracil), 3-methyl-uracil (m^3U), 5-methoxy-uracil (mo^5U), uracil 5-oxyacetic acid (cmo^5U), uracil 5-oxyacetic acid methyl ester ($mcmo^5U$), 5-carboxymethyl-uracil (cm^5U), 1-carboxymethyl-pseudouridine, 5-carboxyhydroxymethyl-uracil (chm^5U), 5-carboxyhydroxymethyl-uracil methyl ester ($mchm^5U$), 5-methoxycarbonylmethyl-uracil (mcm^5U), 5-methoxycarbonylmethyl-2-thio-uracil (mcm^5s^2U), 5-aminomethyl-2-thio-uracil (nm^5s^2U), 5-methylaminomethyl-uracil (mnm^5U), 5-methylaminomethyl-2-thio-uracil (mnm^5s^2U), 5-methylaminomethyl-2-seleno-uracil

(mnm^{5}se^{2}U), 5-carbamoylmethyl-uracil (ncm^{5}U), 5-carboxymethylaminomethyl-uracil (cmnm^{5}U), 5-carboxymethylaminomethyl-2-thio-uracil (cmnm^{5}s^{2}U), 5-propynyl-uracil, 1-propynyl-pseudouracil, 5-taurinomethyl-uracil (xm^{5}U), 1-taurinomethyl-pseudouridine, 5-taurinomethyl-2-thio-uracil(xm^{5}s^{2}U), 1-taurinomethyl-4-thio-pseudouridine, 5-methyl-uracil (m^{5}U, i.e., having the nucleobase deoxythymine), 1-methyl-pseudouridine (mV), 1-ethyl-pseudouridine (EtV), 5-methyl-2-thio-uracil (m^{5}s^{2}U), 1-methyl-4-thio-pseudouridine (m^{1}s$^4\psi$), 4-thio-l -methyl-pseudouridine, 3-methyl-pseudouridine ($\eta_1{}^3\psi$), 2-thio-l -methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-l -methyl-1-deaza-pseudouridine, dihydrouracil **(D),** dihydropseudouridine, 5,6-dihydrouracil, 5-methyl-dihydrouracil (**m^{5}D),** 2-thio-dihydrouracil, 2-thio-dihydropseudouridine, 2-methoxy-uracil, 2-methoxy-4-thio-uracil, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, N1-methyl-pseudouridine, 3-(3-amino-3-carboxypropyl)uracil (acp^{3}U), l-methyl-3-(3-amino-3-carboxypropyl)pseudouridine (acp$^3\psi$), 5-(isopentenylaminomethyl)uracil (inm^{5}U), 5-(isopentenylaminomethyl)-2-thio-uracil (inm^{5}s^{2}U), 5,2'-0-dimethyl-uridine (m^{5}Um), 2-thio-2'-0_methyl-uridine (s^{2}Um), 5-methoxycarbonylmethyl-2'-0-methyl-uridine (mcm^{5}Um), 5-carbamoylmethyl-2'-0-methyl-uridine (ncm^{5}Um), 5-carboxymethylaminomethyl-2'-0-methyl-uridine (cmnm^{5}Um), 3,2'-0-dimethyl-uridine (m^{3}Um), and 5-(isopentenylaminomethyl)-2'-0-methyl-uridine (inm^{5}Um), 1-thio-uracil, deoxythymidine, 5-(2-carbomethoxyvinyl)-uracil, 5-(carbamoylhydroxymethyl)-uracil, 5-carbamoylmethyl-2-thio-uracil, 5-carboxymethyl-2-thio-uracil, 5-cyanomethyl-uracil, 5-methoxy-2-thio-uracil, and 5-[3-(l-E-propenylamino)]uracil.

[00406] In some embodiments, the nucleobase is an alternative cytosine. Exemplary nucleobases and nucleosides having an alternative cytosine include 5-aza-cytosine, 6-aza-cytosine, pseudoisocytidine, 3-methyl-cytosine (m3C), N4-acetyl-cytosine (ac4C), 5-formyl-cytosine (f5C), N4-methyl-cytosine (m4C), 5-methyl-cytosine (m5C), 5-halo-cytosine (e.g., 5-iodo-cytosine), 5-hydroxymethyl-cytosine (hm5C), 1-methyl-pseudoisocytidine, pyrrolo-cytosine, pyrrolo-pseudoisocytidine, 2-thio-cytosine (s2C), 2-thio-5-methyl-cytosine, 4-thio-pseudoisocytidine, 4-thio-1-methyl-pseudoisocytidine, 4-thio-l -methyl-1-deaza-pseudoisocytidine, 1-methyl-l-deaza-pseudoisocytidine, zebularine, 5-aza-zebularine, 5-methyl-zebularine, 5-aza-2-thio-zebularine, 2-thio-zebularine, 2-methoxy-cytosine, 2-methoxy-5-methyl-cytosine, 4-methoxy-pseudoisocytidine, 4-methoxy-l -methyl-pseudoisocytidine, lysidine (k2C), 5,2'-0-dimethyl-cytidine (m5Cm), N4-acetyl-2'-0-methyl-cytidine (ac4Cm), N4,2'-0-dimethyl-cytidine (m4Cm), 5-formyl-2'-0-methyl-cytidine (f5Cm), N4,N4,2'-0-

trimethyl-cytidine (m42Cm), 1-thio-cytosine, 5-hydroxy-cytosine, 5-(3-azidopropyl)-cytosine, and 5-(2-azidoethyl)-cytosine.

[00407] In some embodiments, the nucleobase is an alternative adenine. Exemplary nucleobases and nucleosides having an alternative adenine include 2-amino-purine, 2,6-diaminopurine, 2-amino-6-halo-purine (e.g., 2-amino-6-chloro-purine), 6-halo-purine (e.g., 6-chloro-purine), 2-amino-6-methyl-purine, 8-azido-adenine, 7-deaza-adenine, 7-deaza-8-aza-adenine, 7-deaza-2-amino-purine, 7-deaza-8-aza-2-amino-purine, 7-deaza-2,6-diaminopurine, 7-deaza-8-aza-2,6-diaminopurine, 1-methyl-adenine (mlA), 2-methyl-adenine (m2A), N6-methyl-adenine (m6A), 2-methylthio-N6-methyl-adenine (ms2m6A), N6-isopentenyl-adenine (i6A), 2-methylthio-N6-isopentenyl-adenine (ms2i6A), N6-(cis-hydroxyisopentenyl)adenine (io6A), 2-methylthio-N6-(cis-hydroxyisopentenyl)adenine (ms2io6A), N6-glycinylcarbamoyl-adenine (g6A), N6-threonylcarbamoyl-adenine (t6A), N6-methyl-N6-threonylcarbamoyl-adenine (m6t6A), 2-methylthio-N6-threonylcarbamoyl-adenine (ms2g6A), N6,N6-dimethyl-adenine (m62A), N6-hydroxynorvalylcarbamoyl-adenine (hn6A), 2-methylthio-N6-hydroxynorvalylcarbamoyl-adenine (ms2hn6A), N6-acetyl-adenine (ac6A), 7-methyl-adenine, 2-methylthio-adenine, 2-methoxy-adenine, N6,2'-0-dimethyl-adenosine (m6Am), N6,N6,2'-0-trimethyl-adenosine (m62Am), 1,2'-0-dimethyl-adenosine (ml Am), 2-amino-N6-methyl-purine, 1-thio-adenine, 8-azido-adenine, N6-(19-amino-pentaoxanonadecyl)-adenine, 2,8-dimethyl-adenine, N6-formyl-adenine, and N6-hydroxymethyl-adenine.

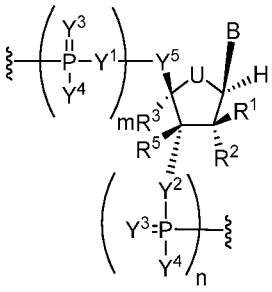
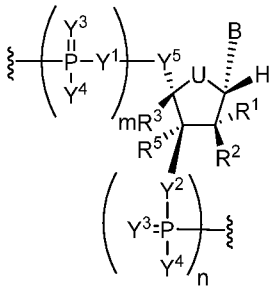
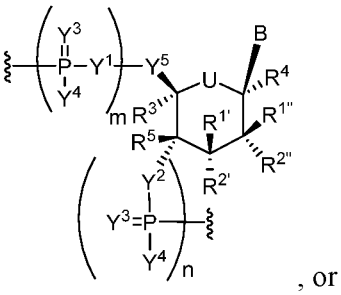
[00408] In some embodiments, the nucleobase is an alternative guanine. Exemplary nucleobases and nucleosides having an alternative guanine include inosine (I), 1-methyl-inosine (mil), wyosine (imG), methylwyosine (mimG), 4-demethyl-wyosine (imG-14), isowyosine (imG2), wybutosine (yW), peroxywybutosine (o2yW), hydroxywybutosine (OHyW), undermodified hydroxywybutosine (OHyW*), 7-deaza-guanine, queuosine (Q), epoxyqueuosine (oQ), galactosyl-queuosine (galQ), mannosyl-queuosine (manQ), 7-cyano-7-deaza-guanine (preQO), 7-aminomethyl-7-deaza-guanine (preQl), archaeosine (G+), 7-deaza-8-aza-guanine, 6-thio-guanine, 6-thio-7-deaza-guanine, 6-thio-7-deaza-8-aza-guanine, 7-methyl-guanine (m7G), 6-thio-7-methyl-guanine, 7-methyl-inosine, 6-methoxy-guanine, 1-methyl-guanine (mlG), N2-methyl-guanine (m2G), N2,N2-dimethyl-guanine (m22G), N2,7-dimethyl-guanine (m2,7G), N2, N2,7-dimethyl-guanine (m2,2,7G), 8-oxo-guanine, 7-methyl-8-oxo-guanine, 1-methyl-6-thio-guanine, N2-methyl-6-thio-guanine, N2,N2-dimethyl-6-thio-guanine, N2-methyl-2'-0-methyl-guanosine (m2Gm), N2,N2-dimethyl-2'-0-methyl-guanosine (m22Gm), l-methyl-2'-0-methyl-

guanosine (mlGm), N2,7-dimethyl-2 '-**0** -methyl-guanosine (m2,7Gm), 2'-**0** -methyl-inosine (Im), l,2 '-**0** -dimethyl-inosine (mllm), 1-thio-guanine, and O-6-methyl-guanine.

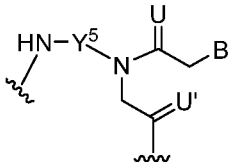
[00409] The alternative nucleobase of a nucleotide can be independently a purine, a pyrimidine, a purine or pyrimidine analog. For example, the nucleobase can be an alternative to adenine, cytosine, guanine, uracil, or hypoxanthine. In another embodiment, the nucleobase can also include, for example, naturally-occurring and synthetic derivatives of a base, including pyrazolo[3,4-d]pyrimidines, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo *(e.g.,* 8-bromo), 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxy and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, deazaguanine, 7-deazaguanine, 3-deazaguanine, deazaadenine, 7-deazaadenine, 3-deazaadenine, pyrazolo [3,4-d] pyrimidine, imidazo[l,5-a] 1,3,5 triazinones, 9-deazapurines, imidazo[4,5-d]pyrazines, thiazolo[4,5-d]pyrimidines, pyrazin-2-ones, 1,2,4-triazine, pyridazine; or 1,3,5 triazine. When the nucleotides are depicted using the shorthand A, G, C, T or U, each letter refers to the representative base and/or derivatives thereof, e.g., A includes adenine or adenine analogs, e.g., 7-deaza adenine).

Alterations on the Sugar

[00410] Nucleosides include a sugar molecule (e.g., a 5-carbon or 6-carbon sugar, such as pentose, ribose, arabinose, xylose, glucose, galactose, or a deoxy derivative thereof) in combination with a nucleobase, while nucleotides are nucleosides containing a nucleoside and a phosphate group or alternative group (e.g., boranophosphate, thiophosphate, selenophosphate, phosphonate, alkyl group, amidate, and glycerol). A nucleoside or nucleotide may be a canonical species, e.g., a nucleoside or nucleotide including a canonical nucleobase, sugar, and, in the case of nucleotides, a phosphate group, or may be an alternative nucleoside or nucleotide including one or more alternative components. For example, alternative nucleosides and nucleotides can be altered on the sugar of the nucleoside or nucleotide. In some embodiments, the alternative nucleosides or nucleotides include the structure:




, , , or

Formula VI **Formula VII** **Formula VIII**



Formula IX

In each of the Formulae VI, VII, VIII, and IX,

each of m and n is independently, an integer from 0 to 5,

each of U and U' independently, is O, S, $N(R^u)_{nu}$, or $C(R^u)_{nu}$, wherein nu is an integer from 0 to 2 and each R^u is, independently, H, halo, or optionally substituted alkyl;

each of $R^{1'}$, $R^{2'}$, $R^{1''}$, $R^{2''}$, R^1, R^2, R^3, R^4, and R^5 is, independently, if present, H, halo, hydroxy, thiol, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted aminoalkoxy, optionally substituted alkoxyalkoxy, optionally substituted hydroxyalkoxy, optionally substituted amino, azido, optionally substituted aryl, optionally substituted aminoalkyl, optionally substituted aminoalkenyl, optionally substituted aminoalkynyl, or absent; wherein the combination of R^3 with one or more of $R^{1'}$, $R^{1''}$, $R^{2'}$, $R^{2''}$, or R^5 (e.g., the combination of $R^{1'}$ and R^3, the combination of $R^{1''}$ and R^3, the combination of $R^{2'}$ and R^3, the combination of $R^{2''}$ and R^3, or the combination of R^5 and R^3) can join together to form optionally substituted alkylene or optionally substituted heteroalkylene and, taken together with the carbons to which they are attached, provide an optionally substituted heterocyclyl (e.g., a bicyclic, tricyclic, or tetracyclic heterocyclyl); wherein the combination of R^5 with one or more of $R^{1'}$, $R^{1''}$, $R^{2'}$, or $R^{2''}$ (e.g., the combination of $R^{1'}$ and R^5, the combination of $R^{1''}$ and R^5, the combination of $R^{2'}$ and R^5, or the combination of $R^{2''}$ and R^5) can join together to form optionally substituted alkylene or optionally substituted heteroalkylene and, taken together with the carbons to which they are attached, provide an optionally substituted heterocyclyl (e.g., a bicyclic, tricyclic, or tetracyclic heterocyclyl); and wherein the combination of R^4 and one or more of $R^{1'}$, $R^{1''}$, $R^{2'}$, $R^{2''}$, R^3, or R^5 can join together to

form optionally substituted alkylene or optionally substituted heteroalkylene and, taken together with the carbons to which they are attached, provide an optionally substituted heterocyclyl (e.g., a bicyclic, tricyclic, or tetracyclic heterocyclyl); each of m' and m" is, independently, an integer from 0 to 3 (e.g., from 0 to 2, from 0 to 1, from 1 to 3, or from 1 to 2);

each of Y^1, Y^2, and Y^3, is, independently, O, S, Se, —NR^{N1}—, optionally substituted alkylene, or optionally substituted heteroalkylene, wherein R^{N1} is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, or absent;

each Y^4 is, independently, H, hydroxy, thiol, boranyl, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted alkoxy, optionally substituted alkenyloxy, optionally substituted alkynyloxy, optionally substituted thioalkoxy, optionally substituted alkoxyalkoxy, or optionally substituted amino;

each Y^5 is, independently, O, S, Se, optionally substituted alkylene (e.g., methylene), or optionally substituted heteroalkylene; and

B is a nucleobase, either modified or unmodified.In some embodiments, the 2'-hydroxy group (OH) can be modified or replaced with a number of different substituents. Exemplary substitutions at the 2'-position include, but are not limited to, H, azido, halo (e.g., fluoro), optionally substituted Ci-6 alkyl (e.g., methyl); optionally substituted Ci-6 alkoxy (e.g., methoxy or ethoxy); optionally substituted C_{6-10} aryloxy; optionally substituted C_{3-8} cycloalkyl; optionally substituted C_{6-10} aryl-Ci-6 alkoxy, optionally substituted Ci-12 (heterocyclyl)oxy; a sugar (e.g., ribose, pentose, or any described herein); a polyethyleneglycol (PEG), -$(X CH_2CH_2O)_nCH_2CH_2OR$, where R is H or optionally substituted alkyl, and n is an integer from 0 to 20 (e.g., from 0 to 4, from 0 to 8, from 0 to 10, from 0 to 16, from 1 to 4, from 1 to 8, from 1 to 10, from 1 to 16, from 1 to 20, from 2 to 4, from 2 to 8, from 2 to 10, from 2 to 16, from 2 to 20, from 4 to 8, from 4 to 10, from 4 to 16, and from 4 to 20); "locked" nucleic acids (LNA) in which the 2'-hydroxy is connected by a Ci-6 alkylene or Ci-6 heteroalkylene bridge to the 4'-carbon of the same ribose sugar, where exemplary bridges included methylene, propylene, ether, or amino bridges; aminoalkyl, as defined herein; aminoalkoxy, as defined herein; amino as defined herein; and amino acid, as defined herein.

[00412] Generally, RNA includes the sugar group ribose, which is a 5-membered ring having an oxygen. Exemplary, non-limiting alternative nucleotides include replacement of the oxygen in ribose (e.g., with S, Se, or alkylene, such as methylene or ethylene); addition of a double bond (e.g., to replace ribose with cyclopentenyl or cyclohexenyl); ring contraction of

ribose (e.g., to form a 4-membered ring of cyclobutane or oxetane); ring expansion of ribose (e.g., to form a 6- or 7-membered ring having an additional carbon or heteroatom, such as for anhydrohexitol, altritol, mannitol, cyclohexanyl, cyclohexenyl, and morpholino (that also has a phosphoramidate backbone)); multicyclic forms (e.g., tricyclo and "unlocked" forms, such as glycol nucleic acid (GNA) (e.g., R-GNA or S-GNA, where ribose is replaced by glycol units attached to phosphodiester bonds), threose nucleic acid (TNA, where ribose is replace with a-L-threofuranosyl-(3' →2')), and peptide nucleic acid (PNA, where 2-amino-ethyl-glycine linkages replace the ribose and phosphodiester backbone).

[00413] In some embodiments, the sugar group contains one or more carbons that possess the opposite stereochemical configuration of the corresponding carbon in ribose. Thus, a polynucleotide molecule can include nucleotides containing, e.g., arabinose or L-ribose, as the sugar.

[00414] In some embodiments, the polynucleotide includes at least one nucleoside wherein the sugar is L-ribose, 2'-O-methyl-ribose, 2'-fluoro-ribose, arabinose, hexitol, an LNA, or a PNA.

Alterations on the Internucleoside Linkage

[00415] Alternative nucleotides can be altered on the internucleoside linkage (e.g., phosphate backbone). Herein, in the context of the polynucleotide backbone, the phrases "phosphate" and "phosphodiester" are used interchangeably. Backbone phosphate groups can be altered by replacing one or more of the oxygen atoms with a different substituent.

[00416] The alternative nucleotides can include the wholesale replacement of an unaltered phosphate moiety with another internucleoside linkage as described herein. Examples of alternative phosphate groups include, but are not limited to, phosphorothioate, phosphoroselenates, boranophosphates, boranophosphate esters, hydrogen phosphonates, phosphoramidates, phosphorodiamidates, alkyl or aryl phosphonates, and phosphotriesters. Phosphorodithioates have both non-linking oxygens replaced by sulfur. The phosphate linker can also be altered by the replacement of a linking oxygen with nitrogen (bridged phosphoramidates), sulfur (bridged phosphorothioates), and carbon (bridged methylene-phosphonates).

[00417] The alternative nucleosides and nucleotides can include the replacement of one or more of the non-bridging oxygens with a borane moiety (BH_3), sulfur (thio), methyl, ethyl, and/or methoxy. As a non-limiting example, two non-bridging oxygens at the same position

(e.g., the alpha (a), beta (β) or gamma (γ) position) can be replaced with a sulfur (thio) and a methoxy.

[00418] The replacement of one or more of the oxygen atoms at the a position of the phosphate moiety (e.g., a-thio phosphate) is provided to confer stability (such as against exonucleases and endonucleases) to RNA and DNA through the unnatural phosphorothioate backbone linkages. Phosphorothioate DNA and RNA have increased nuclease resistance and subsequently a longer half-life in a cellular environment.

[00419] Other intemucleoside linkages that may be employed according to the present disclosure, including intemucleoside linkages which do not contain a phosphorous atom, are described herein.

Internal ribosome entry sites

[00420] Polynucleotides may contain an internal ribosome entry site (IRES). An IRES may act as the sole ribosome binding site, or may serve as one of multiple ribosome binding sites of an mRNA. A polynucleotide containing more than one functional ribosome binding site may encode several peptides or polypeptides that are translated independently by the ribosomes (e.g., multicistronic mRNA). When polynucleotides are provided with an IRES, further optionally provided is a second translatable region. Examples of IRES sequences that can be used according to the present disclosure include without limitation, those from picornaviruses (e.g., FMDV), pest viruses (CFFV), polio viruses (PV), encephalomyocarditis viruses (ECMV), foot-and-mouth disease viruses (FMDV), hepatitis C viruses (HCV), classical swine fever viruses (CSFV), murine leukemia virus (MLV), simian immune deficiency viruses (SIV) or cricket paralysis viruses (CrPV).

5 '-cap structure

[00421] A polynucleotide (e.g., an mRNA) may include a **5**'-cap structure. The **5**'-cap structure of a polynucleotide is involved in nuclear export and increasing polynucleotide stability and binds the mRNA Cap Binding Protein (CBP), which is responsible for polynucleotide stability in the cell and translation competency through the association of CBP with poly-A binding protein to form the mature cyclic mRNA species. The cap further assists the removal of **5**'-proximal introns removal during mRNA splicing.

[00422] Endogenous polynucleotide molecules may be **5**'-end capped generating a **5**′-ppp-**5**'-triphosphate linkage between a terminal guanosine cap residue and the **5**'-terminal transcribed sense nucleotide of the polynucleotide. This **5**'-guanylate cap may then be

methylated to generate an N7-methyl-guanylate residue. The ribose sugars of the terminal and/or anteterminal transcribed nucleotides of the 5′ end of the polynucleotide may optionally also be 2'-**0** -methylated. 5′-decapping through hydrolysis and cleavage of the guanylate cap structure may target a polynucleotide molecule, such as an mRNA molecule, for degradation.

[00423] Alterations to polynucleotides may generate a non-hydrolyzable cap structure preventing decapping and thus increasing polynucleotide half-life. Because cap structure hydrolysis requires cleavage of 5′-ppp-5′ phosphorodi ester linkages, alternative nucleotides may be used during the capping reaction. For example, a Vaccinia Capping Enzyme from New England Biolabs (Ipswich, MA) may be used with a-thio-guanosine nucleotides according to the manufacturer's instructions to create a phosphorothioate linkage in the 5′-ppp-5′ cap. Additional alternative guanosine nucleotides may be used such as a-methyl-phosphonate and seleno-phosphate nucleotides.

[00424] Additional alterations include, but are not limited to, 2'-**0** -methylation of the ribose sugars of 5′-terminal and/or 5′-anteterminal nucleotides of the polynucleotide (as mentioned above) on the 2'-hydroxy group of the sugar. Multiple distinct 5′-cap structures can be used to generate the 5′-cap of a polynucleotide, such as an mRNA molecule.

[00425] 5′-Cap structures include those described in International Patent Publication Nos. WO2008127688, WO 2008016473, and WO 2011015347, the cap structures of each of which are incorporated herein by reference.

[00426] Cap analogs, which herein are also referred to as synthetic cap analogs, chemical caps, chemical cap analogs, or structural or functional cap analogs, differ from natural (i.e., endogenous, wild-type, or physiological) 5′-caps in their chemical structure, while retaining cap function. Cap analogs may be chemically (i.e., non-enzymatically) or enzymatically synthesized and/linked to a polynucleotide.

[00427] For example, the Anti-Reverse Cap Analog (ARCA) cap contains two guanosines linked by a 5′-5′-triphosphate group, wherein one guanosine contains an N7-methyl group as well as a 3'-**0** -methyl group (i.e., N7,3'-**0** -dimethyl-guanosine-5′-triphosphate-5′-guanosine, m^7G-3'mppp-G, which may equivalently be designated 3' **0** -Me-m7G(5′)ppp(5′)G). The **3'-0** atom of the other, unaltered, guanosine becomes linked to the 5′-terminal nucleotide of the capped polynucleotide (e.g., an mRNA). The N7- and 3'-**0** -methlyated guanosine provides the terminal moiety of the capped polynucleotide (e.g., mRNA).

[00428] Another exemplary cap is mCAP, which is similar to ARCA but has a **2'-0-**methyl group on guanosine (i.e., N7,2**'-0** -dimethyl-guanosine-5'-triphosphate-5'-guanosine, m^7Gm-ppp-G).

[00429] A cap may be a dinucleotide cap analog. As a non-limiting example, the dinucleotide cap analog may be modified at different phosphate positions with a boranophosphate group or a phophoroselenoate group such as the dinucleotide cap analogs described in US Patent No. 8,519,110, the cap structures of which are herein incorporated by reference.

[00430] Alternatively, a cap analog may be a N7-(4-chlorophenoxy ethyl) substituted dinucleotide cap analog known in the art and/or described herein. Non-limiting examples of N7-(4-chlorophenoxy ethyl) substituted dinucleotide cap analogs include a N7-(4-chlorophenoxyethyl)-G(5′)ppp(5')G and a N7-(4-chlorophenoxyethyl)-m3 '-OG(5′)ppp(5')G cap analog (see, e.g., the various cap analogs and the methods of synthesizing cap analogs described in Kore et al. Bioorganic & Medicinal Chemistry 2013 21:4570-4574; the cap structures of which are herein incorporated by reference). In other instances, a cap analog useful in the polynucleotides of the present disclosure is a 4-chloro/bromophenoxy ethyl analog.

[00431] While cap analogs allow for the concomitant capping of a polynucleotide in an *in vitro* transcription reaction, up to 20% of transcripts remain uncapped. This, as well as the structural differences of a cap analog from endogenous 5'-cap structures of polynucleotides produced by the endogenous, cellular transcription machinery, may lead to reduced translational competency and reduced cellular stability.

[00432] Alternative polynucleotides may also be capped post-transcriptionally, using enzymes, in order to generate more authentic 5'-cap structures. As used herein, the phrase "more authentic" refers to a feature that closely mirrors or mimics, either structurally or functionally, an endogenous or wild type feature. That is, a "more authentic" feature is better representative of an endogenous, wild-type, natural or physiological cellular function, and/or structure as compared to synthetic features or analogs of the prior art, or which outperforms the corresponding endogenous, wild-type, natural, or physiological feature in one or more respects. Non-limiting examples of more authentic 5'-cap structures useful in the polynucleotides of the present disclosure are those which, among other things, have enhanced binding of cap binding proteins, increased half-life, reduced susceptibility to 5'-endonucleases, and/or reduced 5'-decapping, as compared to synthetic 5'-cap structures known in the art (or to a wild-type, natural or physiological 5'-cap structure). For example, recombinant Vaccinia Virus Capping Enzyme

and recombinant 2'-0-methy transferase enzyme can create a canonical 5'-5'-triphosphate linkage between the 5'-terminal nucleotide of a polynucleotide and a guanosine cap nucleotide wherein the cap guanosine contains an N7-methylation and the 5'-terminal nucleotide of the polynucleotide contains a 2'-0-methyl. Such a structure is termed the Capl structure. This cap results in a higher translational-competency, cellular stability, and a reduced activation of cellular pro-inflammatory cytokines, as compared, e.g., to other 5'cap analog structures known in the art. Other exemplary cap structures include 7mG(5')ppp(5')N,pN2p (Cap 0), 7mG(5')ppp(5')NlmpNp (Cap 1), 7mG(5')-ppp(5')NlmpN2mp (Cap 2), and m(7)Gpppm(3)(6,6,2 ')Apm(2')Apm(2')Cpm(2)(3,2')Up (Cap 4).

[00433] Because the alternative polynucleotides may be capped post-transcriptionally, and because this process is more efficient, nearly 100% of the alternative polynucleotides may be capped. This is in contrast to -80% when a cap analog is linked to an polynucleotide in the course of an in vitro transcription reaction.

[00434] 5'-terminal caps may include endogenous caps or cap analogs. A 5'-terminal cap may include a guanosine analog. Useful guanosine analogs include inosine, Nl-methyl-guanosine, 2'-fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

[00435] In some cases, a polynucleotide contains a modified 5'-cap. A modification on the 5'-cap may increase the stability of polynucleotide, increase the half-life of the polynucleotide, and could increase the polynucleotide translational efficiency. The modified 5'-cap may include, but is not limited to, one or more of the following modifications: modification at the 2'- and/or 3'-position of a capped guanosine triphosphate (GTP), a replacement of the sugar ring oxygen (that produced the carbocyclic ring) with a methylene moiety **(CH2),** a modification at the triphosphate bridge moiety of the cap structure, or a modification at the nucleobase (G) moiety.

5′-UTRs

[00436] A 5′-UTR may be provided as a flanking region to polynucleotides (e.g., niRNAs). A 5'-UTR may be homologous or heterologous to the coding region found in a polynucleotide. Multiple 5'-UTRs may be included in the flanking region and may be the same or of different sequences. Any portion of the flanking regions, including none, may be codon optimized and any may independently contain one or more different structural or chemical alterations, before and/or after codon optimization.

[00437] Shown in Table 21 in US Provisional Application No 61/775,509, and in Table 21 and in Table 22 in US Provisional Application No. 61/829,372, of which are incorporated herein by reference, is a listing of the start and stop site of alternative polynucleotides (e.g., mRNA). In Table 21 each 5'-UTR (5'-UTR-005 to 5'-UTR 68511) is identified by its start and stop site relative to its native or wild type (homologous) transcript (ENST; the identifier used in the ENSEMBL database).

[00438] To alter one or more properties of a polynucleotide (e.g., mRNA), 5'-UTRs which are heterologous to the coding region of an alternative polynucleotide (e.g., mRNA) may be engineered. The polynucleotides (e.g., mRNA) may then be administered to cells, tissue or organisms and outcomes such as protein level, localization, and/or half-life may be measured to evaluate the beneficial effects the heterologous 5'-UTR may have on the alternative polynucleotides (mRNA). Variants of the 5'-UTRs may be utilized wherein one or more nucleotides are added or removed to the termini, including A, T, C or G. 5'-UTRs may also be codon-optimized, or altered in any manner described herein.

5′-UTRs, 3′-UTRs, and Translation Enhancer Elements (TEEs)

[00439] The 5'-UTR of a polynucleotides (e.g., mRNA) may include at least one translation enhancer element. The term "translational enhancer element" refers to sequences that increase the amount of polypeptide or protein produced from a polynucleotide. As a non-limiting example, the TEE may be located between the transcription promoter and the start codon. The polynucleotides (e.g., mRNA) with at least one TEE in the 5'-UTR may include a cap at the 5'-UTR. Further, at least one TEE may be located in the 5'-UTR of polynucleotides (e.g., mRNA) undergoing cap-dependent or cap-independent translation.

[00440] In one aspect, TEEs are conserved elements in the UTR which can promote translational activity of a polynucleotide such as, but not limited to, cap-dependent or cap-independent translation. The conservation of these sequences has been previously shown by Panek et al. (Nucleic Acids Research, 2013, 1-10) across 14 species including humans.

[00441] In one non-limiting example, the TEEs known may be in the 5′-leader of the Gtx homeodomain protein (Chappell et al, Proc. Natl. Acad. Sci. USA 101:9590-9594, 2004, the TEEs of which are incorporated herein by reference).

[00442] In another non-limiting example, TEEs are disclosed in US Patent Publication Nos. 2009/0226470 and 2013/0177581, International Patent Publication Nos. WO2009/075886, WO20 12/009644, and WO1999/024595, US Patent Nos. 6,310,197 and 6,849,405, the TEE sequences disclosed in each of which are incorporated herein by reference.

[00443] In yet another non-limiting example, the TEE may be an internal ribosome entry site (IRES), HCV-IRES or an IRES element such as, but not limited to, those described in US Patent No. 7,468,275, US Patent Publication Nos. 2007/0048776 and 2011/0124100 and International Patent Publication Nos. WO2007/025008 and WO2001/055369, the IRES sequences of each of which are incorporated herein by reference. The IRES elements may include, but are not limited to, the Gtx sequences (e.g., Gtx9-nt, Gtx8-nt, Gtx7-nt) described by Chappell et al. (Proc. Natl. Acad. Sci. USA 101:9590-9594, 2004) and Zhou et al. (PNAS 102:6273-6278, 2005) and in US Patent Publication Nos. 2007/0048776 and 2011/0124100 and International Patent Publication No. WO2007/025008, the IRES sequences of each of which are incorporated herein by reference.

[00444] "Translational enhancer polynucleotides" are polynucleotides which include one or more of the specific TEE exemplified herein and/or disclosed in the art (see e.g., U.S. Patent Nos. 6,310,197, 6,849,405, 7,456,273, 7,183,395, U.S. Patent Publication Nos. 20090/226470, 2007/0048776, 2011/0124100, 2009/0093049, 2013/0177581, International Patent Publication Nos. WO2009/075886, WO2007/025008, WO20 12/009644, WO2001/055371, WO1999/024595, and European Patent Nos. 2610341 and 2610340; the TEE sequences of each of which are incorporated herein by reference) or their variants, homologs or functional derivatives. One or multiple copies of a specific TEE can be present in a polynucleotide (e.g., mRNA). The TEEs in the translational enhancer polynucleotides can be organized in one or more sequence segments. A sequence segment can harbor one or more of the specific TEEs exemplified herein, with each TEE being present in one or more copies. When multiple sequence segments are present in a translational enhancer polynucleotide, they can be homogenous or heterogeneous. Thus, the multiple sequence segments in a translational enhancer polynucleotide can harbor identical or different types of the specific TEEs exemplified herein, identical or different number of copies of each of the specific TEEs, and/or identical or different organization of the TEEs within each sequence segment.

[00445] A polynucleotide (e.g., mRNA) may include at least one TEE that is described in International Patent Publication Nos. WO1999/024595, WO20 12/009644, WO2009/075886, WO2007/025008, WO1999/024595, European Patent Publication Nos. 2610341 and 2610340, US Patent Nos. 6,310,197, 6,849,405, 7,456,273, 7,183,395, and US Patent Publication Nos. 2009/0226470, 2011/0124100, 2007/0048776, 2009/0093049, and 2013/0177581 the TEE sequences of each of which are incorporated herein by reference. The TEE may be located in the 5'-UTR of the polynucleotides (e.g., mRNA).

[00446] A polynucleotide (e.g., mRNA) may include at least one TEE that has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identity with the TEEs described in US Patent Publication Nos. 2009/0226470, 2007/0048776, 2013/0177581 and 2011/0124100, International Patent Publication Nos. WO1999/024595, WO2012/009644, WO2009/075886 and WO2007/025008, European Patent Publication Nos. 2610341 and 2610340, US Patent Nos. 6,310,197, 6,849,405, 7,456,273, 7,183,395, the TEE sequences of each of which are incorporated herein by reference.

[00447] The 5'-UTR of a polynucleotide (e.g., mRNA) may include at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55 or more than 60 TEE sequences. The TEE sequences in the 5'-UTR of a polynucleotide (e.g., mRNA) may be the same or different TEE sequences. The TEE sequences may be in a pattern such as ABABAB, AABBAABBAABB, or ABCABCABC, or variants thereof, repeated once, twice, or more than three times. In these patterns, each letter, A, B, or C represent a different TEE sequence at the nucleotide level.

[00448] In some cases, the 5′-UTR may include a spacer to separate two TEE sequences. As anon-limiting example, the spacer may be a 15 nucleotide spacer and/or other spacers known in the art. As another non-limiting example, the 5'-UTR may include a TEE sequence-spacer module repeated at least once, at least twice, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times, or more than 9 times in the 5′-UTR

[00449] In other instances, the spacer separating two TEE sequences may include other sequences known in the art which may regulate the translation of the polynucleotides (e.g., mRNA) of the present disclosure such as, but not limited to, miR sequences (e.g., miR binding sites and miR seeds). As a non-limiting example, each spacer used to separate two TEE sequences may include a different miR sequence or component of a miR sequence (e.g., miR seed sequence).

[00450] In some instances, the TEE in the 5′-UTR of a polynucleotide (e.g., mRNA) may include at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% or more

than 99% of the TEE sequences disclosed in US Patent Publication Nos. 2009/0226470, 2007/0048776, 2013/0177581 and 201 1/0124100, International Patent Publication Nos. WO1999/024595, WO20 12/009644, WO2009/075886 and WO2007/025008, European Patent Publication Nos. 2610341 and 2610340, and US Patent Nos. 6,310,197, 6,849,405, 7,456,273, and 7,183,395 the TEE sequences of each of which are incorporated herein by reference. In another embodiment, the TEE in the 5'-UTR of the polynucleotides (e.g., mRNA) of the present disclosure may include a 5-30 nucleotide fragment, a 5-25 nucleotide fragment, a 5-20 nucleotide fragment, a 5-15 nucleotide fragment, a 5-10 nucleotide fragment of the TEE sequences disclosed in US Patent Publication Nos. 2009/0226470, 2007/0048776, 2013/0177581 and 2011/0124100, International Patent Publication Nos. WO1999/024595, WO20 12/009644, WO2009/075886 and WO2007/025008, European Patent Publication Nos. 2610341 and 2610340, and US Patent Nos. 6,310,197, 6,849,405, 7,456,273, and 7,183,395; the TEE sequences of each of which are incorporated herein by reference.

[00451] In certain cases, the TEE in the 5'-UTR of the polynucleotides (e.g., mRNA) of the present disclosure may include at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99% or more than 99% of the TEE sequences disclosed in Chappell et al. (Proc. Natl. Acad. Sci. USA 101:9590-9594, 2004) and Zhou et al. (PNAS 102:6273-6278, 2005), in Supplemental Table 1 and in Supplemental Table 2 disclosed by Wellensiek et al (Genome-wide profiling of human cap-independent translation-enhancing elements, Nature Methods, 2013; DOI:10.1038/NMETH.2522); the TEE sequences of each of which are herein incorporated by reference. In another embodiment, the TEE in the 5'-UTR of the polynucleotides (e.g., mRNA) of the present disclosure may include a 5-30 nucleotide fragment, a 5-25 nucleotide fragment, a 5-20 nucleotide fragment, a 5-15 nucleotide fragment, a 5-10 nucleotide fragment of the TEE sequences disclosed in Chappell et al. (Proc. Natl. Acad. Sci. USA 101:9590-9594, 2004) and Zhou et al. (PNAS 102:6273-6278, 2005), in Supplemental Table 1 and in Supplemental Table 2 disclosed by Wellensiek et al (Genome-wide profiling of human cap-independent translation-enhancing elements, Nature Methods, 2013; DOI:10.1038/NMETH.2522); the TEE sequences of each of which is incorporated herein by reference.

[00452] In some cases, the TEE used in the 5'-UTR of a polynucleotide (e.g., mRNA) is an IRES sequence such as, but not limited to, those described in US Patent No. 7,468,275 and

International Patent Publication No. WO2001/055369, the TEE sequences of each of which are incorporated herein by reference.

[00453] In some instances, the TEEs used in the 5′-UTR of a polynucleotide (e.g., mRNA) may be identified by the methods described in US Patent Publication Nos. 2007/0048776 and 201 1/0124100 and International Patent Publication Nos. WO2007/025008 and WO2012/009644, the methods of each of which are incorporated herein by reference.

[00454] In some cases, the TEEs used in the 5'-UTR of a polynucleotide (e.g., mRNA) of the present disclosure may be a transcription regulatory element described in US Patent Nos. 7,456,273 and 7,183,395, US Patent Publication No. 2009/0093049, and International Publication No. WO2001/055371, the TEE sequences of each of which is incorporated herein by reference. The transcription regulatory elements may be identified by methods known in the art, such as, but not limited to, the methods described in US Patent Nos. 7,456,273 and 7,183,395, US Patent Publication No. 2009/0093049, and International Publication No. WO2001/055371, the methods of each of which is incorporated herein by reference.

[00455] In yet other instances, the TEE used in the 5′-UTR of a polynucleotide (e.g., mRNA) is a polynucleotide or portion thereof as described in US Patent Nos. 7,456,273 and 7,183,395, US Patent Publication No. 2009/0093049, and International Publication No. WO2001/055371, the TEE sequences of each of which are incorporated herein by reference.

[00456] The 5′-UTR including at least one TEE described herein may be incorporated in a monocistronic sequence such as, but not limited to, a vector system or a polynucleotide vector. As a non-limiting example, the vector systems and polynucleotide vectors may include those described in US Patent Nos. 7,456,273 and 7,183,395, US Patent Publication Nos. 2007/0048776, 2009/0093049 and 201 1/0124100, and International Patent Publication Nos. WO2007/025008 and WO2001/055371, the TEE sequences of each of which are incorporated herein by reference.

[00457] The TEEs described herein may be located in the 5′-UTR and/or the 3′-UTR of the polynucleotides (e.g., mRNA). The TEEs located in the 3'-UTR may be the same and/or different than the TEEs located in and/or described for incorporation in the 5'-UTR.

[00458] In some cases, the 3'-UTR of a polynucleotide (e.g., mRNA) may include at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55 or more than 60 TEE sequences. The TEE sequences

in the 3'-UTR of the polynucleotides (e.g., mRNA) of the present disclosure may be the same or different TEE sequences. The TEE sequences may be in a pattern such as ABABAB, AABBAABBAABB, or ABCABCABC, or variants thereof, repeated once, twice, or more than three times. In these patterns, each letter, A, B, or C represent a different TEE sequence at the nucleotide level.

[00459] In one instance, the 3′-UTR may include a spacer to separate two TEE sequences. As anon-limiting example, the spacer may be a 15 nucleotide spacer and/or other spacers known in the art. As another non-limiting example, the 3'-UTR may include a TEE sequence-spacer module repeated at least once, at least twice, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, at least 9 times, or more than 9 times in the 3′-UTR

[00460] In other cases, the spacer separating two TEE sequences may include other sequences known in the art which may regulate the translation of the polynucleotides (e.g., mRNA) of the present disclosure such as, but not limited to, miR sequences described herein (e.g., miR binding sites and miR seeds). As a non-limiting example, each spacer used to separate two TEE sequences may include a different miR sequence or component of a miR sequence (e.g., miR seed sequence).

[00461] In yet other cases, the incorporation of a miR sequence and/or a TEE sequence changes the shape of the stem loop region which may increase and/or decrease translation. (see e.g., Kedde et al. A Pumilio-induced RNA structure switch in p27-3'UTR controls miR-221 and miR-22 accessibility. Nature Cell Biology. 2010).

Stem Loops

[00462] Polynucleotides (e.g., mRNAs) may include a stem loop such as, but not limited to, a histone stem loop. The stem loop may be a nucleotide sequence that is about 25 or about 26 nucleotides in length such as, but not limited to, those as described in International Patent Publication No. WO2013/103659, which is incorporated herein by reference. The histone stem loop may be located 3'-relative to the coding region (e.g., at the 3'-terminus of the coding region). As a non-limiting example, the stem loop may be located at the 3'-end of a polynucleotide described herein. In some cases, a polynucleotide (e.g., an mRNA) includes more than one stem loop (e.g., two stem loops). Examples of stem loop sequences are described in International Patent Publication Nos. WO2012/019780 and WO201502667, the stem loop sequences of which are herein incorporated by reference. In some instances, a polynucleotide includes the stem loop sequence CAAAGGCTCTTTTCAGAGCCACCA (SEQ ID NO: 1). In

others, a polynucleotide includes the stem loop sequence CAAAGGCUCUUUUCAGAGCCACCA (SEQ ID NO: 2).

[00463] A stem loop may be located in a second terminal region of a polynucleotide. As anon-limiting example, the stem loop may be located within an untranslated region (e.g., 3'-UTR) in a second terminal region.

[00464] In some cases, a polynucleotide such as, but not limited to mRNA, which includes the histone stem loop may be stabilized by the addition of a 3'-stabilizing region (e.g., a 3'-stabilizing region including at least one chain terminating nucleoside). Not wishing to be bound by theory, the addition of at least one chain terminating nucleoside may slow the degradation of a polynucleotide and thus can increase the half-life of the polynucleotide.

[00465] In other cases, a polynucleotide such as, but not limited to mRNA, which includes the histone stem loop may be stabilized by an alteration to the 3'-region of the polynucleotide that can prevent and/or inhibit the addition of oligio(U) (see e.g., International Patent Publication No. WO201 3/1 03659).

[00466] In yet other cases, a polynucleotide such as, but not limited to mRNA, which includes the histone stem loop may be stabilized by the addition of an oligonucleotide that terminates in a 3'-deoxynucleoside, 2',3'-dideoxynucleoside 3'-**0** -methylnucleosides, 3′-**0**-ethylnucleosides, 3'-arabinosides, and other alternative nucleosides known in the art and/or described herein.

[00467] In some instances, the polynucleotides of the present disclosure may include a histone stem loop, a poly-A region, and/or a 5'-cap structure. The histone stem loop may be before and/or after the poly-A region. The polynucleotides including the histone stem loop and a poly-A region sequence may include a chain terminating nucleoside described herein.

[00468] In other instances, the polynucleotides of the present disclosure may include a histone stem loop and a 5'-cap structure. The 5'-cap structure may include, but is not limited to, those described herein and/or known in the art.

[00469] In some cases, the conserved stem loop region may include a miR sequence described herein. As a non-limiting example, the stem loop region may include the seed sequence of a miR sequence described herein. In another non-limiting example, the stem loop region may include a miR-122 seed sequence.

[00470] In certain instances, the conserved stem loop region may include a miR sequence described herein and may also include a TEE sequence.

[00471] In some cases, the incorporation of a miR sequence and/or a TEE sequence changes the shape of the stem loop region which may increase and/or decrease translation. (see e.g., Kedde et al. A Pumilio-induced RNA structure switch in p27-3'UTR controls miR-221 and miR-22 accessibility. Nature Cell Biology. 2010, herein incorporated by reference in its entirety).

[00472] Polynucleotides may include at least one histone stem-loop and a poly-A region or polyadenylation signal. Non-limiting examples of polynucleotide sequences encoding for at least one histone stem-loop and a poly-A region or a polyadenylation signal are described in International Patent Publication No. WO2013/120497, WO2013/120629, WO2013/120500, WO2013/120627, WO2013/120498, WO2013/120626, WO2013/120499 and WO2013/120628, the sequences of each of which are incorporated herein by reference. In certain cases, the polynucleotide encoding for a histone stem loop and a poly-A region or a polyadenylation signal may code for a pathogen antigen or fragment thereof such as the polynucleotide sequences described in International Patent Publication No WO2013/120499 and WO2013/120628, the sequences of both of which are incorporated herein by reference. In other cases, the polynucleotide encoding for a histone stem loop and a poly-A region or a polyadenylation signal may code for a therapeutic protein such as the polynucleotide sequences described in International Patent Publication No WO2013/120497 and WO2013/120629, the sequences of both of which are incorporated herein by reference. In some cases, the polynucleotide encoding for a histone stem loop and a poly-A region or a polyadenylation signal may code for a tumor antigen or fragment thereof such as the polynucleotide sequences described in International Patent Publication No WO2013/120500 and WO2013/120627, the sequences of both of which are incorporated herein by reference. In other cases, the polynucleotide encoding for a histone stem loop and a poly-A region or a polyadenylation signal may code for a allergenic antigen or an autoimmune self-antigen such as the polynucleotide sequences described in International Patent Publication No WO2013/120498 and WO20 13/1 20626, the sequences of both of which are incorporated herein by reference.

Poly-A Regions

[00473] A polynucleotide or nucleic acid (e.g., an mRNA) may include a polyA sequence and/or polyadenylation signal. A polyA sequence may be comprised entirely or mostly of adenine nucleotides or analogs or derivatives thereof. A polyA sequence may be a tail located adjacent to a 3' untranslated region of a nucleic acid.

[00474] During RNA processing, a long chain of adenosine nucleotides (poly-A region) is normally added to messenger RNA (mRNA) molecules to increase the stability of the molecule. Immediately after transcription, the 3'-end of the transcript is cleaved to free a 3'-hydroxy. Then poly-A polymerase adds a chain of adenosine nucleotides to the RNA. The process, called polyadenylation, adds a poly-A region that is between 100 and 250 residues long.

[00475] Unique poly-A region lengths may provide certain advantages to the alternative polynucleotides of the present disclosure.

[00476] Generally, the length of a poly-A region of polynucleotides of the present disclosure is at least 30 nucleotides in length. In another embodiment, the poly-A region is at least 35 nucleotides in length. In another embodiment, the length is at least 40 nucleotides. In another embodiment, the length is at least 45 nucleotides. In another embodiment, the length is at least 55 nucleotides. In another embodiment, the length is at least 60 nucleotides. In another embodiment, the length is at least 70 nucleotides. In another embodiment, the length is at least 80 nucleotides. In another embodiment, the length is at least 90 nucleotides. In another embodiment, the length is at least 100 nucleotides. In another embodiment, the length is at least 120 nucleotides. In another embodiment, the length is at least 140 nucleotides. In another embodiment, the length is at least 160 nucleotides. In another embodiment, the length is at least 180 nucleotides. In another embodiment, the length is at least 200 nucleotides. In another embodiment, the length is at least 250 nucleotides. In another embodiment, the length is at least 300 nucleotides. In another embodiment, the length is at least 350 nucleotides. In another embodiment, the length is at least 400 nucleotides. In another embodiment, the length is at least 450 nucleotides. In another embodiment, the length is at least 500 nucleotides. In another embodiment, the length is at least 600 nucleotides. In another embodiment, the length is at least 700 nucleotides. In another embodiment, the length is at least 800 nucleotides. In another embodiment, the length is at least 900 nucleotides. In another embodiment, the length is at least 1000 nucleotides. In another embodiment, the length is at least 1100 nucleotides. In another embodiment, the length is at least 1200 nucleotides. In another embodiment, the length is at least 1300 nucleotides. In another embodiment, the length is at least 1400 nucleotides. In another embodiment, the length is at least 1500 nucleotides. In another embodiment, the length is at least 1600 nucleotides. In another embodiment, the length is at least 1700 nucleotides. In another embodiment, the length is at least 1800 nucleotides. In another embodiment, the length is at least 1900 nucleotides. In another embodiment, the length is at least 2000 nucleotides. In

another embodiment, the length is at least 2500 nucleotides. In another embodiment, the length is at least 3000 nucleotides.

[00477] In some instances, the poly-A region may be 80 nucleotides, 120 nucleotides, 160 nucleotides in length on an alternative polynucleotide molecule described herein.

[00478] In other instances, the poly-A region may be 20, 40, 80, 100, 120, 140 or 160 nucleotides in length on an alternative polynucleotide molecule described herein.

[00479] In some cases, the poly-A region is designed relative to the length of the overall alternative polynucleotide. This design may be based on the length of the coding region of the alternative polynucleotide, the length of a particular feature or region of the alternative polynucleotide (such as mRNA), or based on the length of the ultimate product expressed from the alternative polynucleotide. When relative to any feature of the alternative polynucleotide (e.g., other than the mRNA portion which includes the poly-A region) the poly-A region may be 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% greater in length than the additional feature. The poly-A region may also be designed as a fraction of the alternative polynucleotide to which it belongs. In this context, the poly-A region may be 10, 20, 30, 40, 50, 60, 70, 80, or 90% or more of the total length of the construct or the total length of the construct minus the poly-A region.

[00480] In certain cases, engineered binding sites and/or the conjugation of polynucleotides (e.g., mRNA) for poly-A binding protein may be used to enhance expression. The engineered binding sites may be sensor sequences which can operate as binding sites for ligands of the local microenvironment of the polynucleotides (e.g., mRNA). As a non-limiting example, the polynucleotides (e.g., mRNA) may include at least one engineered binding site to alter the binding affinity of poly-A binding protein (PABP) and analogs thereof. The incorporation of at least one engineered binding site may increase the binding affinity of the PABP and analogs thereof.

[00481] Additionally, multiple distinct polynucleotides (e.g., mRNA) may be linked together to the PABP (poly-A binding protein) through the 3'-end using alternative nucleotides at the 3'-terminus of the poly-A region. Transfection experiments can be conducted in relevant cell lines at and protein production can be assayed by ELISA at 12 hours, 24 hours, 48 hours, 72 hours, and day 7 post-transfection. As a non-limiting example, the transfection experiments may be used to evaluate the effect on PABP or analogs thereof binding affinity as a result of the addition of at least one engineered binding site.

[00482] In certain cases, a poly-A region may be used to modulate translation initiation. While not wishing to be bound by theory, the poly-A region recruits PABP which in turn can interact with translation initiation complex and thus may be essential for protein synthesis.

[00483] In some cases, a poly-A region may also be used in the present disclosure to protect against 3'-5'-exonuclease digestion.

[00484] In some instances, a polynucleotide (e.g., mRNA) may include a polyA-G Quartet. The G-quartet is a cyclic hydrogen bonded array of four guanosine nucleotides that can be formed by G-rich sequences in both DNA and RNA. In this embodiment, the G-quartet is incorporated at the end of the poly-A region. The resultant polynucleotides (e.g., mRNA) may be assayed for stability, protein production and other parameters including half-life at various time points. It has been discovered that the polyA-G quartet results in protein production equivalent to at least 75% of that seen using a poly-A region of 120 nucleotides alone.

[00485] In some cases, a polynucleotide (e.g., mRNA) may include a poly-A region and may be stabilized by the addition of a 3'-stabilizing region. The polynucleotides (e.g., mRNA) with a poly-A region may further include a 5'-cap structure.

[00486] In other cases, a polynucleotide (e.g., mRNA) may include a poly-A-G Quartet. The polynucleotides (e.g., mRNA) with a poly-A-G Quartet may further include a 5'-cap structure.

[00487] In some cases, the 3'-stabilizing region which may be used to stabilize a polynucleotide (e.g., mRNA) including a poly-A region or poly-A-G Quartet may be, but is not limited to, those described in International Patent Publication No. WO2013/103659, the poly-A regions and poly-A-G Quartets of which are incorporated herein by reference. In other cases, the 3'-stabilizing region which may be used with the polynucleotides of the present disclosure include a chain termination nucleoside such as 3'-deoxyadenosine (cordycepin), 3'-deoxyuridine, 3'-deoxycytosine, 3'-deoxyguanosine, 3'-deoxythymine, 2',3'-dideoxynucleosides, such as 2',3'- dideoxyadenosine, 2',3'-dideoxyuridine, 2',3'-dideoxycytosine, 2',3'- dideoxyguanosine, 2',3'-dideoxythymine, a 2'-deoxynucleoside, or an O-methylnucleoside.

[00488] In other cases, a polynucleotide such as, but not limited to mRNA, which includes a polyA region or a poly-A-G Quartet may be stabilized by an alteration to the 3'-region of the polynucleotide that can prevent and/or inhibit the addition of oligio(U) (see e.g., International Patent Publication No. WO2013/103659).

[00489] In yet other instances, a polynucleotide such as, but not limited to mRNA, which includes a poly-A region or a poly-A-G Quartet may be stabilized by the addition of an oligonucleotide that terminates in a 3'-deoxynucleoside, 2',3'-dideoxynucleoside 3'-**0**-methylnucleosides, 3'-**0** -ethylnucleosides, 3'-arabinosides, and other alternative nucleosides known in the art and/or described herein.

Chain terminating nucleosides

[00490] A nucleic acid may include a chain terminating nucleoside. For example, a chain terminating nucleoside may include those nucleosides deoxygenated at the 2' and/or 3' positions of their sugar group. Such species may include 3'-deoxyadenosine (cordycepin), 3'-deoxyuridine, 3'-deoxycytosine, 3'-deoxyguanosine, 3'-deoxythymine, and 2',3'-dideoxynucleosides, such as 2',3'-dideoxy adenosine, 2',3'-dideoxyuridine, 2',3'-dideoxycytosine, 2',3'-dideoxyguanosine, and 2',3'-dideoxythymine.

Other components

[00491] A nanoparticle composition may include one or more components in addition to those described in the preceding sections. For example, a nanoparticle composition may include one or more small hydrophobic molecules such as a vitamin (e.g., vitamin A or vitamin E) or a sterol.

[00492] Nanoparticle compositions may also include one or more permeability enhancer molecules, carbohydrates, polymers, surface altering agents, or other components. A permeability enhancer molecule may be a molecule described by U.S. patent application publication No. 2005/0222064, for example. Carbohydrates may include simple sugars (e.g., glucose) and polysaccharides (e.g., glycogen and derivatives and analogs thereof).

[00493] A polymer may be included in and/or used to encapsulate or partially encapsulate a nanoparticle composition. A polymer may be biodegradable and/or biocompatible. A polymer may be selected from, but is not limited to, polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, polystyrenes, polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyleneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. For example, a polymer may include poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-co-

glycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacralate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethyleneglycol, poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as polyvinyl chloride) (PVC), polyvinylpyrrolidone (PVP), polysiloxanes, polystyrene (PS), polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fumarate, polyoxymethylene, poloxamers, polyoxamines, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), trimethylene carbonate, poly(*N*-acryloylmorpholine) (PAcM), poly(2-methyl-2-oxazoline) (PMOX), poly(2-ethyl-2-oxazoline) (PEOZ), and polyglycerol.

[00494] Surface altering agents may include, but are not limited to, anionic proteins (e.g., bovine serum albumin), surfactants (e.g., cationic surfactants such as dimethyldioctadecyl-ammonium bromide), sugars or sugar derivatives (e.g., cyclodextrin), nucleic acids, polymers (e.g., heparin, polyethylene glycol, and poloxamer), mucolytic agents (e.g., acetylcysteine, mugwort, bromelain, papain, clerodendrum, bromhexine, carbocisteine, eprazinone, mesna, ambroxol, sobrerol, domiodol, letosteine, stepronin, tiopronin, gelsolin, thymosin β4, dornase alfa, neltenexine, and erdosteine), and DNases (e.g., rhDNase). A surface altering agent may be disposed within a nanoparticle and/or on the surface of a nanoparticle composition (e.g., by coating, adsorption, covalent linkage, or other process).

[00495] A nanoparticle composition may also comprise one or more functionalized lipids. For example, a lipid may be functionalized with an alkyne group that, when exposed to an azide under appropriate reaction conditions, may undergo a cycloaddition reaction. In particular, a lipid bilayer may be functionalized in this fashion with one or more groups useful in facilitating

membrane permeation, cellular recognition, or imaging. The surface of a nanoparticle composition may also be conjugated with one or more useful antibodies. Functional groups and conjugates useful in targeted cell delivery, imaging, and membrane permeation are well known in the art.

[00496] In addition to these components, nanoparticle compositions may include any substance useful in pharmaceutical compositions. For example, the nanoparticle composition may include one or more pharmaceutically acceptable excipients or accessory ingredients such as, but not limited to, one or more solvents, dispersion media, diluents, dispersion aids, suspension aids, granulating aids, disintegrants, fillers, glidants, liquid vehicles, binders, surface active agents, isotonic agents, thickening or emulsifying agents, buffering agents, lubricating agents, oils, preservatives, and other species. Excipients such as waxes, butters, coloring agents, coating agents, flavorings, and perfuming agents may also be included. Pharmaceutically acceptable excipients are well known in the art (see for example Remington's *The Science and Practice of Pharmacy*, 21st Edition, A. R. Gennaro; Lippincott, Williams & Wilkins, Baltimore, MD, 2006).

[00497] Examples of diluents may include, but are not limited to, calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, and/or combinations thereof. Granulating and dispersing agents may be selected from the non-limiting list consisting of potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked poly(vinyl-pyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (VEEGUM®), sodium lauryl sulfate, quaternary ammonium compounds, and/or combinations thereof.

[00498] Surface active agents and/or emulsifiers may include, but are not limited to, natural emulsifiers (e.g. acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (e.g. bentonite [aluminum silicate] and VEEGUM® [magnesium aluminum silicate]), long chain amino acid derivatives, high molecular weight alcohols (e.g. stearyl

alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene glycol monostearate, polyvinyl alcohol), carbomers (e.g. carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxyvinyl polymer), carrageenan, cellulosic derivatives (e.g. carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (e.g. polyoxyethylene sorbitan monolaurate [TWEEN®20], polyoxyethylene sorbitan [TWEEN® 60], polyoxyethylene sorbitan monooleate [TWEEN®80], sorbitan monopalmitate [SPAN®40], sorbitan monostearate [SPAN®60], sorbitan tristearate [SPAN®65], glyceryl monooleate, sorbitan monooleate [SPAN®80]), polyoxyethylene esters (e.g. polyoxyethylene monostearate [MYRJ® 45], polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and SOLUTOL®), sucrose fatty acid esters, polyethylene glycol fatty acid esters (e.g. CREMOPHOR®), polyoxyethylene ethers, (e.g. polyoxyethylene lauryl ether [BRIJ® 30]), poly(vinyl-pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, PLURONIC®F 68, POLOXAMER® 188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, and/or combinations thereof.

[00499] A binding agent may be starch (e.g. cornstarch and starch paste); gelatin; sugars (e.g. sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol); natural and synthetic gums (e.g. acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxy ethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, poly(vinyl-pyrrolidone), magnesium aluminum silicate (VEEGUM®), and larch arabogalactan); alginates; polyethylene oxide; polyethylene glycol; inorganic calcium salts; silicic acid; polymethacrylates; waxes; water; alcohol; and combinations thereof, or any other suitable binding agent.

[00500] Examples of preservatives may include, but are not limited to, antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and/or other preservatives. Examples of antioxidants include, but are not limited to, alpha tocopherol, ascorbic acid, acorbyl palmitate, butylated hydroxyanisole, butylated hydroxy toluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and/or sodium sulfite. Examples of chelating agents include ethylenediaminetetraacetic acid (EDTA), citric acid

monohydrate, disodium edetate, dipotassium edetate, edetic acid, fumaric acid, malic acid, phosphoric acid, sodium edetate, tartaric acid, and/or trisodium edetate. Examples of antimicrobial preservatives include, but are not limited to, benzalkonium chloride, benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorobutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and/or thimerosal. Examples of antifungal preservatives include, but are not limited to, butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and/or sorbic acid. Examples of alcohol preservatives include, but are not limited to, ethanol, polyethylene glycol, benzyl alcohol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and/or phenylethyl alcohol. Examples of acidic preservatives include, but are not limited to, vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic acid, dehydroascorbic acid, ascorbic acid, sorbic acid, and/or phytic acid. Other preservatives include, but are not limited to, tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabisulfite, potassium sulfite, potassium metabisulfite, GLYDANT PLUS®, PHENONIP®, methylparaben, GERMALL® 115, GERMABEN®II, NEOLONE™, KATHON™, and/or EUXYL®.

[00501] Examples of buffering agents include, but are not limited to, citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium glubionate, calcium gluceptate, calcium gluconate, d-gluconic acid, calcium glycerophosphate, calcium lactate, calcium lactobionate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, amino-sulfonate buffers (e.g. HEPES), magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, and/or combinations thereof. Lubricating agents may selected from the non-limiting group consisting of magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behenate, hydrogenated vegetable oils, polyethylene glycol, sodium

benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, and combinations thereof.

[00502] Examples of oils include, but are not limited to, almond, apricot kernel, avocado, babassu, bergamot, black current seed, borage, cade, camomile, canola, caraway, carnauba, castor, cinnamon, cocoa butter, coconut, cod liver, coffee, corn, cotton seed, emu, eucalyptus, evening primrose, fish, flaxseed, geraniol, gourd, grape seed, hazel nut, hyssop, isopropyl myristate, jojoba, kukui nut, lavandin, lavender, lemon, litsea cubeba, macademia nut, mallow, mango seed, meadowfoam seed, mink, nutmeg, olive, orange, orange roughy, palm, palm kernel, peach kernel, peanut, poppy seed, pumpkin seed, rapeseed, rice bran, rosemary, safflower, sandalwood, sasquana, savoury, sea buckthorn, sesame, shea butter, silicone, soybean, sunflower, tea tree, thistle, tsubaki, vetiver, walnut, and wheat germ oils as well as butyl stearate, caprylic triglyceride, capric triglyceride, cyclomethicone, diethyl sebacate, dimethicone 360, simethicone, isopropyl myristate, mineral oil, octyldodecanol, oleyl alcohol, silicone oil, and/or combinations thereof.

Formulations

[00503] Nanoparticle compositions may include a lipid component and one or more additional components, such as a therapeutic and/or prophylactic agent. A nanoparticle composition may be designed for one or more specific applications or targets. The elements of a nanoparticle composition may be selected based on a particular application or target, and/or based on the efficacy, toxicity, expense, ease of use, availability, or other feature of one or more elements. Similarly, the particular formulation of a nanoparticle composition may be selected for a particular application or target according to, for example, the efficacy and toxicity of particular combinations of elements.

[00504] The lipid component of a nanoparticle composition may include, for example, a lipid according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(Idl O), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), a phospholipid (such as an unsaturated lipid, e.g., DOPE or DSPC), a PEG lipid, and a structural lipid. The elements of the lipid component may be provided in specific fractions.

[00505] In some embodiments, the lipid component of a nanoparticle composition includes a lipid according to one of formulae (I), (Ial)-(Ial O), (lb), (Ic), (Id), (Idl)-(Idl O), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), a phospholipid, a PEG lipid, and a structural lipid. In certain embodiments, the lipid component of the nanoparticle composition includes about 30

mol % to about 60 mol % compound according to one of formulae (I), (Ial)-(Ial O), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), about 0 mol % to about 30 mol % phospholipid, about 18.5 mol % to about 48.5 mol % structural lipid, and about 0 mol % to about 10 mol % of PEG lipid, provided that the total mol % does not exceed 100%. In some embodiments, the lipid component of the nanoparticle composition includes about 35 mol % to about 55 mol % compound according to one of formulae (I), (Ial)-(Ial O), (lb), (Ic), (Id), (Idl)-(Idl O), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), about 5 mol % to about 25 mol % phospholipid, about 30 mol % to about 40 mol % structural lipid, and about 0 mol % to about 10 mol % of PEG lipid. In certain embodiments, the lipid component includes about 50 mol % said compound, about 10 mol % phospholipid, about 38.5 mol % structural lipid, and about 1.5 mol % of PEG lipid. In other embodiments, the lipid component includes about 40 mol % said compound, about 20 mol % phospholipid, about 38.5 mol % structural lipid, and about 1.5 mol % of PEG lipid. In some embodiments, the phospholipid may be DOPE or DSPC. In other embodiments, the PEG lipid may be PEG-DMG or a PEG lipid according to one of formulae (VI), (Vl-a), or (Vl-b), and/or the structural lipid may be cholesterol.

[00506] Nanoparticle compositions may be designed for one or more specific applications or targets. For example, a nanoparticle composition may be designed to deliver a therapeutic and/or prophylactic agent such as an RNA to a particular cell, tissue, organ, or system or group thereof in a mammal's body. Physiochemical properties of nanoparticle compositions may be altered in order to increase selectivity for particular bodily targets. For instance, particle sizes may be adjusted based on the fenestration sizes of different organs. The therapeutic and/or prophylactic agent included in a nanoparticle composition may also be selected based on the desired delivery target or targets. For example, a therapeutic and/or prophylactic agent may be selected for a particular indication, condition, disease, or disorder and/or for delivery to a particular cell, tissue, organ, or system or group thereof (e.g., localized or specific delivery). In certain embodiments, a nanoparticle composition may include an mRNA encoding a polypeptide of interest capable of being translated within a cell to produce the polypeptide of interest. Such a composition may be designed to be specifically delivered to a particular organ. In certain embodiments, a composition may be designed to be specifically delivered to a mammalian liver.

[00507] The amount of a therapeutic and/or prophylactic agent in a nanoparticle composition may depend on the size, composition, desired target and/or application, or other properties of the nanoparticle composition as well as on the properties of the therapeutic and/or prophylactic agent. For example, the amount of an RNA useful in a nanoparticle composition

may depend on the size, sequence, and other characteristics of the RNA. The relative amounts of a therapeutic and/or prophylactic agent and other elements (e.g., lipids) in a nanoparticle composition may also vary. In some embodiments, the wt/wt ratio of the lipid component to a therapeutic and/or prophylactic agent in a nanoparticle composition may be from about 5:1 to about 60:1, such as 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, and 60:1. For example, the wt/wt ratio of the lipid component to a therapeutic and/or prophylactic agent may be from about 10:1 to about 40:1. In certain embodiments, the wt/wt ratio is about 20:1. The amount of a therapeutic and/or prophylactic agent in a nanoparticle composition may, for example, be measured using absorption spectroscopy (e.g., ultraviolet-visible spectroscopy).

[00508] In some embodiments, a nanoparticle composition includes one or more RNAs, and the one or more RNAs, lipids, and amounts thereof may be selected to provide a specific N:P ratio. The N:P ratio of the composition refers to the molar ratio of nitrogen atoms in one or more lipids to the number of phosphate groups in an RNA. In general, a lower N:P ratio is preferred. The one or more RNA, lipids, and amounts thereof may be selected to provide an N:P ratio from about 2:1 to about 30:1, such as 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 12:1, 14:1, 16:1, 18:1, 20:1, 22:1, 24:1, 26:1, 28:1, or 30:1. In certain embodiments, the N:P ratio may be from about 2:1 to about 8:1. In other embodiments, the N:P ratio is from about 5:1 to about 8:1. For example, the N:P ratio may be about 5.0:1, about 5.5:1, about 5.67:1, about 6.0:1, about 6.5:1, or about 7.0:1. For example, the N:P ratio may be about 5.67:1.

Physical properties

[00509] The characteristics of a nanoparticle composition may depend on the components thereof. For example, a nanoparticle composition including cholesterol as a structural lipid may have different characteristics than a nanoparticle composition that includes a different structural lipid. Similarly, the characteristics of a nanoparticle composition may depend on the absolute or relative amounts of its components. For instance, a nanoparticle composition including a higher molar fraction of a phospholipid may have different characteristics than a nanoparticle composition including a lower molar fraction of a phospholipid. Characteristics may also vary depending on the method and conditions of preparation of the nanoparticle composition.

[00510] Nanoparticle compositions may be characterized by a variety of methods. For example, microscopy (e.g., transmission electron microscopy or scanning electron microscopy) may be used to examine the morphology and size distribution of a nanoparticle composition.

Dynamic light scattering or potentiometry (e.g., potentiometric titrations) may be used to measure zeta potentials. Dynamic light scattering may also be utilized to determine particle sizes. Instruments such as the Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, Worcestershire, UK) may also be used to measure multiple characteristics of a nanoparticle composition, such as particle size, polydispersity index, and zeta potential.

[00511] The mean size of a nanoparticle composition may be between 10s of nm and 100s of nm. For example, the mean size may be from about 40 nm to about 150 nm, such as about 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, or 150 nm. In some embodiments, the mean size of a nanoparticle composition may be from about 50 nm to about 100 nm, from about 50 nm to about 90 nm, from about 50 nm to about 80 nm, from about 50 nm to about 70 nm, from about 50 nm to about 60 nm, from about 60 nm to about 100 nm, from about 60 nm to about 90 nm, from about 60 nm to about 80 nm, from about 60 nm to about 70 nm, from about 70 nm to about 100 nm, from about 70 nm to about 90 nm, from about 70 nm to about 80 nm, from about 80 nm to about 100 nm, from about 80 nm to about 90 nm, or from about 90 nm to about 100 nm. In certain embodiments, the mean size of a nanoparticle composition may be from about 70 nm to about 100 nm. In some embodiments, the mean size may be about 80 nm. In other embodiments, the mean size may be about 100 nm.

[00512] A nanoparticle composition may be relatively homogenous. A polydispersity index may be used to indicate the homogeneity of a nanoparticle composition, e.g., the particle size distribution of the nanoparticle compositions. A small (e.g., less than 0.3) polydispersity index generally indicates a narrow particle size distribution. A nanoparticle composition may have a polydispersity index from about 0 to about 0.25, such as 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.20, 0.21, 0.22, 0.23, 0.24, or 0.25. In some embodiments, the polydispersity index of a nanoparticle composition may be from about 0.10 to about 0.20.

[00513] The zeta potential of a nanoparticle composition may be used to indicate the electrokinetic potential of the composition. For example, the zeta potential may describe the surface charge of a nanoparticle composition. Nanoparticle compositions with relatively low charges, positive or negative, are generally desirable, as more highly charged species may interact undesirably with cells, tissues, and other elements in the body. In some embodiments, the zeta potential of a nanoparticle composition may be from about -10 mV to about +20 mV, from about -10 mV to about +15 mV, from about -10 mV to about +10 mV, from about -10 mV

to about +5 mV, from about -10 mV to about 0 mV, from about -10 mV to about -5 mV, from about -5 mV to about +20 mV, from about -5 mV to about +15 mV, from about -5 mV to about +10 mV, from about -5 mV to about +5 mV, from about -5 mV to about 0 mV, from about 0 mV to about +20 mV, from about 0 mV to about +15 mV, from about 0 mV to about +10 mV, from about 0 mV to about +5 mV, from about +5 mV to about +20 mV, from about +5 mV to about +15 mV, or from about +5 mV to about +10 mV.

[00514] The efficiency of encapsulation of a therapeutic and/or prophylactic agent describes the amount of therapeutic and/or prophylactic agent that is encapsulated or otherwise associated with a nanoparticle composition after preparation, relative to the initial amount provided. The encapsulation efficiency is desirably high (e.g., close to 100%). The encapsulation efficiency may be measured, for example, by comparing the amount of therapeutic and/or prophylactic agent in a solution containing the nanoparticle composition before and after breaking up the nanoparticle composition with one or more organic solvents or detergents. Fluorescence may be used to measure the amount of free therapeutic and/or prophylactic agent (e.g., RNA) in a solution. For the nanoparticle compositions described herein, the encapsulation efficiency of a therapeutic and/or prophylactic agent may be at least 50%, for example 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the encapsulation efficiency may be at least 80%. In certain embodiments, the encapsulation efficiency may be at least 90%.

[00515] A nanoparticle composition may optionally comprise one or more coatings. For example, a nanoparticle composition may be formulated in a capsule, film, or tablet having a coating. A capsule, film, or tablet including a composition described herein may have any useful size, tensile strength, hardness, or density.

Pharmaceutical compositions

[00516] Nanoparticle compositions may be formulated in whole or in part as pharmaceutical compositions. Pharmaceutical compositions may include one or more nanoparticle compositions. For example, a pharmaceutical composition may include one or more nanoparticle compositions including one or more different therapeutic and/or prophylactic agents. Pharmaceutical compositions may further include one or more pharmaceutically acceptable excipients or accessory ingredients such as those described herein. General guidelines for the formulation and manufacture of pharmaceutical compositions and agents are available, for example, in Remington's *The Science and Practice of Pharmacy*, 21st Edition, A.

R. Gennaro; Lippincott, Williams & Wilkins, Baltimore, MD, 2006. Conventional excipients and accessory ingredients may be used in any pharmaceutical composition, except insofar as any conventional excipient or accessory ingredient may be incompatible with one or more components of a nanoparticle composition. An excipient or accessory ingredient may be incompatible with a component of a nanoparticle composition if its combination with the component may result in any undesirable biological effect or otherwise deleterious effect.

[00517] In some embodiments, one or more excipients or accessory ingredients may make up greater than 50% of the total mass or volume of a pharmaceutical composition including a nanoparticle composition. For example, the one or more excipients or accessory ingredients may make up 50%, 60%, 70%, 80%, 90%, or more of a pharmaceutical convention. In some embodiments, a pharmaceutically acceptable excipient is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In some embodiments, an excipient is approved for use in humans and for veterinary use. In some embodiments, an excipient is approved by United States Food and Drug Administration. In some embodiments, an excipient is pharmaceutical grade. In some embodiments, an excipient meets the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[00518] Relative amounts of the one or more nanoparticle compositions, the one or more pharmaceutically acceptable excipients, and/or any additional ingredients in a pharmaceutical composition in accordance with the present disclosure will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, a pharmaceutical composition may comprise between 0.1% and 100% (wt/wt) of one or more nanoparticle compositions.

[00519] In certain embodiments, the nanoparticle compositions and/or pharmaceutical compositions of the disclosure are refrigerated or frozen for storage and/or shipment (e.g., being stored at a temperature of 4 °C or lower, such as a temperature between about -150 °C and about 0 °C or between about -80 °C and about -20 °C (e.g., about -5 °C, -10 °C, -15 °C, -20 °C, -25 °C, -30 °C, -40 °C, -50 °C, -60 °C, -70 °C, -80 °C, -90 °C, -130 °C or -150 °C). For example, the pharmaceutical composition comprising a compound of any of Formulae (I)-(IV) is a solution that is refrigerated for storage and/or shipment at, for example, about -20 °C, -30 °C, -40 °C, -50 °C, -60 °C, -70 °C, or -80 °C. In certain embodiments, the disclosure also relates to a method of increasing stability of the nanoparticle compositions and/or pharmaceutical compositions comprising a compound of any of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-

(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) by storing the nanoparticle compositions and/or pharmaceutical compositions at a temperature of 4 °C or lower, such as a temperature between about -150 °C and about 0 °C or between about -80 °C and about -20 °C, e.g., about -5 °C, -10 °C, -15 °C, -20 °C, -25 °C, -30 °C, -40 °C, -50 °C, -60 °C, -70 °C, -80 °C, -90 °C, -130 °C or -150 °C). For example, the nanoparticle compositions and/or pharmaceutical compositions disclosed herein are stable for about at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 1 month, at least 2 months, at least 4 months, at least 6 months, at least 8 months, at least 10 months, at least 12 months, at least 14 months, at least 16 months, at least 18 months, at least 20 months, at least 22 months, or at least 24 months, e.g., at a temperature of 4 °C or lower (e.g., between about 4 °C and -20 °C). In one embodiment, the formulation is stabilized for at least 4 weeks at about 4 °C. In certain embodiments, the pharmaceutical composition of the disclosure comprises a nanoparticle composition disclosed herein and a pharmaceutically acceptable carrier selected from one or more of Tris, an acetate (e.g., sodium acetate), an citrate (e.g., sodium citrate), saline, PBS, and sucrose. In certain embodiments, the pharmaceutical composition of the disclosure has a pH value between about 7 and 8 (e.g., 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9 or 8.0, or between 7.5 and 8 or between 7 and 7.8). For example, a pharmaceutical composition of the disclosure comprises a nanoparticle composition disclosed herein, Tris, saline and sucrose, and has a pH of about 7.5-8, which is suitable for storage and/or shipment at, for example, about -20 °C. For example, a pharmaceutical composition of the disclosure comprises a nanoparticle composition disclosed herein and PBS and has a pH of about 7-7.8, suitable for storage and/or shipment at, for example, about 4 °C or lower. "Stability," "stabilized," and "stable" in the context of the present disclosure refers to the resistance of nanoparticle compositions and/or pharmaceutical compositions disclosed herein to chemical or physical changes (e.g., degradation, particle size change, aggregation, change in encapsulation, etc.) under given manufacturing, preparation, transportation, storage and/or in-use conditions, e.g., when stress is applied such as shear force, freeze/thaw stress, etc.

[00520] Nanoparticle compositions and/or pharmaceutical compositions including one or more nanoparticle compositions may be administered to any patient or subject, including those patients or subjects that may benefit from a therapeutic effect provided by the delivery of a therapeutic and/or prophylactic agent to one or more particular cells, tissues, organs, or systems or groups thereof, such as the renal system. Although the descriptions provided herein of nanoparticle compositions and pharmaceutical compositions including nanoparticle

compositions are principally directed to compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to any other mammal. Modification of compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the compositions is contemplated include, but are not limited to, humans, other primates, and other mammals, including commercially relevant mammals such as cattle, pigs, hoses, sheep, cats, dogs, mice, and/or rats.

[00521] A pharmaceutical composition including one or more nanoparticle compositions may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include bringing the active ingredient into association with an excipient and/or one or more other accessory ingredients, and then, if desirable or necessary, dividing, shaping, and/or packaging the product into a desired single- or multi-dose unit.

[00522] A pharmaceutical composition in accordance with the present disclosure may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient (e.g., nanoparticle composition). The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[00523] Pharmaceutical compositions may be prepared in a variety of forms suitable for a variety of routes and methods of administration. For example, pharmaceutical compositions may be prepared in liquid dosage forms (e.g., emulsions, microemulsions, nanoemulsions, solutions, suspensions, syrups, and elixirs), injectable forms, solid dosage forms (e.g., capsules, tablets, pills, powders, and granules), dosage forms for topical and/or transdermal administration (e.g., ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants, and patches), suspensions, powders, and other forms.

[00524] Liquid dosage forms for oral and parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, nanoemulsions, solutions, suspensions, syrups, and/or elixirs. In addition to active ingredients, liquid dosage forms may comprise inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate,

ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, oral compositions can include additional therapeutic and/or prophylactic agents, additional agents such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and/or perfuming agents. In certain embodiments for parenteral administration, compositions are mixed with solubilizing agents such as Cremophor®, alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and/or combinations thereof.

[00525] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing agents, wetting agents, and/or suspending agents. Sterile injectable preparations may be sterile injectable solutions, suspensions, and/or emulsions in nontoxic parenterally acceptable diluents and/or solvents, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P., and isotonic sodium chloride solution. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid can be used in the preparation of injectables.

[00526] Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, and/or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[00527] In order to prolong the effect of an active ingredient, it is often desirable to slow the absorption of the active ingredient from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsulated matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are

prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

[00528] Compositions for rectal or vaginal administration are typically suppositories which can be prepared by mixing compositions with suitable non-irritating excipients such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active ingredient.

[00529] Solid dosage forms for oral administration include capsules, tablets, pills, films, powders, and granules. In such solid dosage forms, an active ingredient is mixed with at least one inert, pharmaceutically acceptable excipient such as sodium citrate or dicalcium phosphate and/or fillers or extenders (e.g. starches, lactose, sucrose, glucose, mannitol, and silicic acid), binders (e.g. carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia), humectants (e.g. glycerol), disintegrating agents (e.g. agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate), solution retarding agents (e.g. paraffin), absorption accelerators (e.g. quaternary ammonium compounds), wetting agents (e.g. cetyl alcohol and glycerol monostearate), absorbents (e.g. kaolin and bentonite clay, silicates), and lubricants (e.g. talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate), and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may comprise buffering agents.

[00530] Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. Solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally comprise opacifying agents and can be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

[00531] Dosage forms for topical and/or transdermal administration of a composition may include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants, and/or patches. Generally, an active ingredient is admixed under sterile conditions with a

pharmaceutically acceptable excipient and/or any needed preservatives and/or buffers as may be required. Additionally, the present disclosure contemplates the use of transdermal patches, which often have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms may be prepared, for example, by dissolving and/or dispensing the compound in the proper medium. Alternatively or additionally, rate may be controlled by either providing a rate controlling membrane and/or by dispersing the compound in a polymer matrix and/or gel.

[00532] Suitable devices for use in delivering intradermal pharmaceutical compositions described herein include short needle devices such as those described in U.S. Patents 4,886,499; 5,190,521; 5,328,483; 5,527,288; 4,270,537; 5,015,235; 5,141,496; and 5,417,662. Intradermal compositions may be administered by devices which limit the effective penetration length of a needle into the skin, such as those described in PCT publication WO 99/34850 and functional equivalents thereof. Jet injection devices which deliver liquid compositions to the dermis via a liquid jet injector and/or via a needle which pierces the stratum corneum and produces a jet which reaches the dermis are suitable. Jet injection devices are described, for example, in U.S. Patents 5,480,381; 5,599,302; 5,334,144; 5,993,412; 5,649,912; 5,569,189; 5,704,911; 5,383,851; 5,893,397; 5,466,220; 5,339,163; 5,312,335; 5,503,627; 5,064,413; 5,520,639; 4,596,556; 4,790,824; 4,941,880; 4,940,460; and PCT publications WO 97/37705 and WO 97/13537. Ballistic powder/particle delivery devices which use compressed gas to accelerate vaccine in powder form through the outer layers of the skin to the dermis are suitable. Alternatively or additionally, conventional syringes may be used in the classical mantoux method of intradermal administration.

[00533] Formulations suitable for topical administration include, but are not limited to, liquid and/or semi liquid preparations such as liniments, lotions, oil in water and/or water in oil emulsions such as creams, ointments and/or pastes, and/or solutions and/or suspensions. Topically-administrable formulations may, for example, comprise from about 1% to about 10% (wt/wt) active ingredient, although the concentration of active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

[00534] A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for pulmonary administration via the buccal cavity. Such a formulation may comprise dry particles which comprise the active ingredient. Such compositions are conveniently in the form of dry powders for administration using a device comprising a dry

powder reservoir to which a stream of propellant may be directed to disperse the powder and/or using a self propelling solvent/powder dispensing container such as a device comprising the active ingredient dissolved and/or suspended in a low-boiling propellant in a sealed container. Dry powder compositions may include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

[00535] Low boiling propellants generally include liquid propellants having a boiling point of below 65 °F at atmospheric pressure. Generally the propellant may constitute 50% to 99.9% (wt/wt) of the composition, and active ingredient may constitute 0.1% to 20% (wt/wt) of the composition. A propellant may further comprise additional ingredients such as a liquid non-ionic and/or solid anionic surfactant and/or a solid diluent (which may have a particle size of the same order as particles comprising the active ingredient).

[00536] Pharmaceutical compositions formulated for pulmonary delivery may provide an active ingredient in the form of droplets of a solution and/or suspension. Such formulations may be prepared, packaged, and/or sold as aqueous and/or dilute alcoholic solutions and/or suspensions, optionally sterile, comprising active ingredient, and may conveniently be administered using any nebulization and/or atomization device. Such formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, and/or a preservative such as methylhydroxybenzoate. Droplets provided by this route of administration may have an average diameter in the range from about 1 nm to about 200 nm.

[00537] Formulations described herein as being useful for pulmonary delivery are useful for intranasal delivery of a pharmaceutical composition. Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an average particle from about 0.2 μιτι to 500 μιτι. Such a formulation is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close to the nose.

[00538] Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (wt/wt) and as much as 100% (wt/wt) of active ingredient, and may comprise one or more of the additional ingredients described herein. A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets and/or lozenges made using conventional methods, and may, for example, 0.1% to 20% (wt/wt) active ingredient, the balance comprising an orally dissolvable and/or degradable composition and,

optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder and/or an aerosolized and/or atomized solution and/or suspension comprising active ingredient. Such powdered, aerosolized, and/or aerosolized formulations, when dispersed, may have an average particle and/or droplet size in the range from about 0.1 nm to about 200 nm, and may further comprise one or more of any additional ingredients described herein.

[00539] A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for ophthalmic administration. Such formulations may, for example, be in the form of eye drops including, for example, a 0.1/1.0% (wt/wt) solution and/or suspension of the active ingredient in an aqueous or oily liquid excipient. Such drops may further comprise buffering agents, salts, and/or one or more other of any additional ingredients described herein. Other ophthalmically-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form and/or in a liposomal preparation. Ear drops and/or eye drops are contemplated as being within the scope of this present disclosure.

Methods of producing polypeptides in cells

[00540] The present disclosure provides methods of producing a polypeptide of interest in a mammalian cell. Methods of producing polypeptides involve contacting a cell with a nanoparticle composition including an mRNA encoding the polypeptide of interest. Upon contacting the cell with the nanoparticle composition, the mRNA may be taken up and translated in the cell to produce the polypeptide of interest.

[00541] In general, the step of contacting a mammalian cell with a nanoparticle composition including an mRNA encoding a polypeptide of interest may be performed *in vivo*, *ex vivo*, in culture, or *in vitro*. The amount of nanoparticle composition contacted with a cell, and/or the amount of mRNA therein, may depend on the type of cell or tissue being contacted, the means of administration, the physiochemical characteristics of the nanoparticle composition and the mRNA (e.g., size, charge, and chemical composition) therein, and other factors. In general, an effective amount of the nanoparticle composition will allow for efficient polypeptide production in the cell. Metrics for efficiency may include polypeptide translation (indicated by polypeptide expression), level of mRNA degradation, and immune response indicators.

[00542] The step of contacting a nanoparticle composition including an mRNA with a cell may involve or cause transfection. A phospholipid including in the lipid component of a nanoparticle composition may facilitate transfection and/or increase transfection efficiency, for

example, by interacting and/or fusing with a cellular or intracellular membrane. Transfection may allow for the translation of the mRNA within the cell.

[00543] In some embodiments, the nanoparticle compositions described herein may be used therapeutically. For example, an mRNA included in a nanoparticle composition may encode a therapeutic polypeptide (e.g., in a translatable region) and produce the therapeutic polypeptide upon contacting and/or entry (e.g., transfection) into a cell. In other embodiments, an mRNA included in a nanoparticle composition may encode a polypeptide that may improve or increase the immunity of a subject. For example, an mRNA may encode a granulocyte-colony stimulating factor or trastuzumab.

[00544] In certain embodiments, an mRNA included in a nanoparticle composition may encode a recombinant polypeptide that may replace one or more polypeptides that may be substantially absent in a cell contacted with the nanoparticle composition. The one or more substantially absent polypeptides may be lacking due to a genetic mutation of the encoding gene or a regulatory pathway thereof. Alternatively, a recombinant polypeptide produced by translation of the mRNA may antagonize the activity of an endogenous protein present in, on the surface of, or secreted from the cell. An antagonistic recombinant polypeptide may be desirable to combat deleterious effects caused by activities of the endogenous protein, such as altered activities or localization caused by mutation. In another alternative, a recombinant polypeptide produced by translation of the mRNA may indirectly or directly antagonize the activity of a biological moiety present in, on the surface of, or secreted from the cell. Antagonized biological moieties may include, but are not limited to, lipids (e.g., cholesterol), lipoproteins (e.g., low density lipoprotein), nucleic acids, carbohydrates, and small molecule toxins. Recombinant polypeptides produced by translation of the mRNA may be engineered for localization within the cell, such as within a specific compartment such as the nucleus, or may be engineered for secretion from the cell or for translocation to the plasma membrane of the cell.

[00545] In some embodiments, contacting a cell with a nanoparticle composition including an mRNA may reduce the innate immune response of a cell to an exogenous nucleic acid. A cell may be contacted with a first nanoparticle composition including a first amount of a first exogenous mRNA including a translatable region and the level of the innate immune response of the cell to the first exogenous mRNA may be determined. Subsequently, the cell may be contacted with a second composition including a second amount of the first exogenous mRNA, the second amount being a lesser amount of the first exogenous mRNA compared to the first amount. Alternatively, the second composition may include a first amount of a second

exogenous mRNA that is different from the first exogenous mRNA. The steps of contacting the cell with the first and second compositions may be repeated one or more times. Additionally, efficiency of polypeptide production (e.g., translation) in the cell may be optionally determined, and the cell may be re-contacted with the first and/or second composition repeatedly until a target protein production efficiency is achieved.

[00546] In some embodiments, a method of producing a polypeptide of interest in a mammalian cell involves contacting the cell with a nanoparticle composition including (i) a lipid component including a phospholipid, a structural lipid, a PEG lipid, and a compound of one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), as described herein; and (ii) an mRNA encoding the polypeptide of interest, whereby the mRNA is capable of being translated in the cell to produce the polypeptide of interest.

Methods of delivering therapeutic agents to cells and organs

[00547] The present disclosure provides methods of delivering a therapeutic and/or prophylactic agent to a mammalian cell or organ. Delivery of a therapeutic and/or prophylactic agent to a cell involves administering a nanoparticle composition including the therapeutic and/or prophylactic agent to a subject, where administration of the composition involves contacting the cell with the composition. For example, a protein, cytotoxic agent, radioactive ion, chemotherapeutic agent, or nucleic acid (such as an RNA, e.g., mRNA) may be delivered to a cell or organ. In the instance that a therapeutic and/or prophylactic agent is an mRNA, upon contacting a cell with the nanoparticle composition, a translatable mRNA may be translated in the cell to produce a polypeptide of interest. However, mRNAs that are substantially not translatable may also be delivered to cells. Substantially non-translatable mRNAs may be useful as vaccines and/or may sequester translational components of a cell to reduce expression of other species in the cell.

[00548] In some embodiments, a nanoparticle composition may target a particular type or class of cells (e.g., cells of a particular organ or system thereof). For example, a nanoparticle composition including a therapeutic and/or prophylactic agent of interest may be specifically delivered to a mammalian liver, kidney, spleen, femur, or lung. Specific delivery to a particular class of cells, an organ, or a system or group thereof implies that a higher proportion of nanoparticle compositions including a therapeutic and/or prophylactic agent are delivered to the destination (e.g., tissue) of interest relative to other destinations, e.g., upon administration of a nanoparticle composition to a mammal. In some embodiments, specific delivery may result in a

greater than 2 fold, 5 fold, 10 fold, 15 fold, or 20 fold increase in the amount of therapeutic and/or prophylactic agent per 1 g of tissue of the targeted destination (e.g., tissue of interest, such as a liver) as compared to another destination (e.g., the spleen). In certain embodiments, the tissue of interest is selected from the group consisting of a liver, kidney, a lung, a spleen, a femur, vascular endothelium in vessels (e.g., intra-coronary or intra-femoral) or kidney, and tumor tissue (e.g., via intratumoral injection).

[00549] As another example of targeted or specific delivery, an mRNA that encodes a protein-binding partner (e.g., an antibody or functional fragment thereof, a scaffold protein, or a peptide) or a receptor on a cell surface may be included in a nanoparticle composition. An mRNA may additionally or instead be used to direct the synthesis and extracellular localization of lipids, carbohydrates, or other biological moieties. Alternatively, other therapeutic and/or prophylactic agents or elements (e.g., lipids or ligands) of a nanoparticle composition may be selected based on their affinity for particular receptors (e.g., low density lipoprotein receptors) such that a nanoparticle composition may more readily interact with a target cell population including the receptors. For example, ligands may include, but are not limited to, members of a specific binding pair, antibodies, monoclonal antibodies, Fv fragments, single chain Fv (scFv) fragments, Fab' fragments, F(ab')2 fragments, single domain antibodies, camelized antibodies and fragments thereof, humanized antibodies and fragments thereof, and multivalent versions thereof; multivalent binding reagents including mono- or bi-specific antibodies such as disulfide stabilized Fv fragments, scFv tandems, diabodies, tridobdies, or tetrabodies; and aptamers, receptors, and fusion proteins.

[00550] In some embodiments, a ligand may be a surface-bound antibody, which can permit tuning of cell targeting specificity. This is especially useful since highly specific antibodies can be raised against an epitope of interest for the desired targeting site. In one embodiment, multiple antibodies are expressed on the surface of a cell, and each antibody can have a different specificity for a desired target. Such approaches can increase the avidity and specificity of targeting interactions.

[00551] A ligand can be selected, e.g., by a person skilled in the biological arts, based on the desired localization or function of the cell. For example an estrogen receptor ligand, such as tamoxifen, can target cells to estrogen-dependent breast cancer cells that have an increased number of estrogen receptors on the cell surface. Other non-limiting examples of ligand/receptor interactions include CCR1 (e.g., for treatment of inflamed joint tissues or brain in rheumatoid arthritis, and/or multiple sclerosis), CCR7, CCR8 (e.g., targeting to lymph node

tissue), CCR6, CCR9,CCR1 0 (e.g., to target to intestinal tissue), CCR4, CCR1 0 (e.g., for targeting to skin), CXCR4 (e.g., for general enhanced transmigration), HCELL (e.g., for treatment of inflammation and inflammatory disorders, bone marrow), Alpha4beta7 (e.g., for intestinal mucosa targeting), and VLA-4NCAM-1 (e.g., targeting to endothelium). In general, any receptor involved in targeting (e.g., cancer metastasis) can be harnessed for use in the methods and compositions described herein.

[00552] Targeted cells may include, but are not limited to, hepatocytes, epithelial cells, hematopoietic cells, epithelial cells, endothelial cells, lung cells, bone cells, stem cells, mesenchymal cells, neural cells, cardiac cells, adipocytes, vascular smooth muscle cells, cardiomyocytes, skeletal muscle cells, beta cells, pituitary cells, synovial lining cells, ovarian cells, testicular cells, fibroblasts, B cells, T cells, reticulocytes, leukocytes, granulocytes, and tumor cells.

[00553] In certain embodiments, a nanoparticle composition may target hepatocytes. Apolipoproteins such as apolipoprotein E (apoE) have been shown to associate with neutral or near neutral lipid-containing nanoparticle compositions in the body, and are known to associate with receptors such as low-density lipoprotein receptors (LDLRs) found on the surface of hepatocytes. See, e.g., Akinc, A. *et al.*, *Mol. Ther.* 2010, 18, 1357-1364 and Dong, Y. *et al*, *PNAS* 2014, 111, 3955-3960, the contents of each of which are incorporated herein by reference in their entireties. Thus, a nanoparticle composition including a lipid component with a neutral or near neutral charge that is administered to a subject may acquire apoE in a subj ect's body and may subsequently deliver a therapeutic and/or prophylactic agent (e.g., an RNA) to hepatocytes including LDLRs in a targeted manner.

[00554] In certain embodiments, cell uptake of a compound of one of formulae (I), (Ial)-(Ia6), (lb), (II), (Ila), (III), (Ilia), and (IV)as described herein or a nanoparticle composition comprising the compound may be dependent on levels and/or activities of LDLRs, or cell uptake of the nanoparticle composition is LDLR-dependent. For example, if the cell is LDLR-deficient (e.g., having an aberrant LDLR activity and/or an abnormally low level of LDLRs), the cell uptake of the compound or nanoparticle composition may decrease as compared to the uptake by a normal cell.

[00555] In certain embodiments, cell uptake of a compound of one of formulae (I), (Ial)-(Ia6), (lb), (II), (Ila), (III), (Ilia), and (IV) as described herein or a nanoparticle composition comprising the compound may be independent on levels and/or activities of LDLRs, or cell uptake of the nanoparticle composition is LDLR-independent. For example, if the cell is LDLR-

deficient (e.g., having an aberrant LDLR activity and/or an abnormally low level of LDLRs), the cell uptake of the compound or nanoparticle composition is substantively the same as the uptake by a normal cell.

[00556] In certain embodiments, cell uptake of a compound of one of formulae (I), (Ial)-(Ia6), (lb), (II), (Ila), (III), (Ilia), and (IV)= as described herein or a nanoparticle composition comprising the compound may be dependent on levels and/or activities of apoE, or cell uptake of the nanoparticle composition is apoE-dependent. For example, if the cell is apoE-deficient (e.g., having an aberrant apoE activity and/or an abnormally low level of apoE), the cell uptake of the compound or nanoparticle composition may decrease as compared to the uptake by a normal cell.

[00557] In certain embodiments, cell uptake of a compound of one of formulae (I), (Ial)-(Ia6), (lb), (II), (Ila), (III), (Ilia), and (IV) as described herein or a nanoparticle composition comprising the compound may be independent on levels and/or activities of apoE, or cell uptake of the nanoparticle composition is apoE-independent. For example, if the cell is apoE-deficient (e.g., having an aberrant apoE activity and/or an abnormally low level of apoE), the cell uptake of the compound or nanoparticle composition is substantively the same as the uptake by a normal cell.

[00558] In certain embodiments, cell uptake of the compound or nanoparticle composition disclosed herein may be both LDLR-dependent and apoE-dependent.

[00559] In certain embodiments, cell uptake of the compound or nanoparticle composition disclosed herein may be dependent on the interaction of LDLR and apoE. For example, if the interaction of LDLR and apoE is abnormal (e.g., leading to an abnormally low level of downstream signaling), the cell uptake of the compound or nanoparticle composition may decrease as compared to the uptake by a normal cell.

[00560] In certain embodiments, cell uptake of the compound or nanoparticle composition disclosed herein may be both LDLR-independent and apoE-independent.

[00561] In certain embodiments, cell uptake of the compound or nanoparticle composition disclosed herein may be independent on the interaction of LDLR and apoE. For example, if the interaction of LDLR and apoE is abnormal (e.g., leading to an abnormally low level of downstream signaling), the cell uptake of the compound or nanoparticle composition is substantively the same as the uptake by a normal cell.

[00562] In certain embodiments, the apoE is apoE3.

[00563] In some embodiments, a method of delivering a therapeutic and/or prophylactic agent to a mammalian cell involves administering to a subject a nanoparticle composition including (i) a lipid component including a phospholipid, a structural lipid, a PEG lipid, and a compound of one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), as described herein; and (ii) a therapeutic and/or prophylactic agent (e.g., an mRNA), where administering involves contacting the cell with the nanoparticle composition, whereby the therapeutic and/or prophylactic agent is delivered to the cell.

[00564] In further embodiments, a method of specifically delivering a therapeutic and/or prophylactic agent to a mammalian organ involves administering to a mammal a nanoparticle composition including (i) a lipid component including a phospholipid, a structural lipid, a PEG lipid, and a compound of one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), as described herein; and (ii) a therapeutic and/or prophylactic agent (e.g., an mRNA), where administering involves contacting the mammalian organ with the nanoparticle composition, whereby the therapeutic and/or prophylactic agent is delivered to the organ.

[00565] In certain embodiments, the delivery efficiency of the therapeutic and/or prophylactic agent is LDLR-independent or apoE-independent, or both. In certain embodiments, the delivery efficiency of the therapeutic and/or prophylactic agent is LDLR-dependent or apoE-dependent, or both. In certain embodiments, the delivery efficiency of the therapeutic and/or prophylactic agent is independent of LDLR-apoE interaction. In certain embodiments, the delivery efficiency of the therapeutic and/or prophylactic agent is dependent on LDLR-apoE interaction.

Methods of treating diseases and disorders

[00566] Nanoparticle compositions may be useful for treating a disease, disorder, or condition. In particular, such compositions may be useful in treating a disease, disorder, or condition characterized by missing or aberrant protein or polypeptide activity. For example, a nanoparticle composition comprising an mRNA encoding a missing or aberrant polypeptide may be administered or delivered to a cell. Subsequent translation of the mRNA may produce the polypeptide, thereby reducing or eliminating an issue caused by the absence of or aberrant activity caused by the polypeptide. Because translation may occur rapidly, the methods and compositions may be useful in the treatment of acute diseases, disorders, or conditions such as sepsis, stroke, and myocardial infarction. A therapeutic and/or prophylactic agent included in a

nanoparticle composition may also be capable of altering the rate of transcription of a given species, thereby affecting gene expression.

[00567] Diseases, disorders, and/or conditions characterized by dysfunctional or aberrant protein or polypeptide activity for which a composition may be administered include, but are not limited to, rare diseases, infectious diseases (as both vaccines and therapeutics), cancer and proliferative diseases, genetic diseases (e.g., cystic fibrosis), autoimmune diseases, diabetes, neurodegenerative diseases, cardio- and reno-vascular diseases, and metabolic diseases. Multiple diseases, disorders, and/or conditions may be characterized by missing (or substantially diminished such that proper protein function does not occur) protein activity. Such proteins may not be present, or they may be essentially non-functional. A specific example of a dysfunctional protein is the missense mutation variants of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which produce a dysfunctional protein variant of CFTR protein, which causes cystic fibrosis. The present disclosure provides a method for treating such diseases, disorders, and/or conditions in a subj ect by administering a nanoparticle composition including an RNA and a lipid component including a lipid according to one of formulae (I), (Ial)-(Ial O), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), a phospholipid (optionally unsaturated), a PEG lipid, and a structural lipid, wherein the RNA may be an mRNA encoding a polypeptide that antagonizes or otherwise overcomes an aberrant protein activity present in the cell of the subject.

[00568] In some embodiments, a method of treating a disease or disorder in a mammal in need involves administering to the mammal a therapeutically effective amount of a nanoparticle composition including (i) a lipid component including a phospholipid, a structural lipid, a PEG lipid, and a compound of formula (I), (Ial)-(Ia8), (lb), (Ic), (Id), (II), (Ila), (III), or (Ilia), as described herein; and (ii) a therapeutic and/or prophylactic agent (e.g., an mRNA).

[00569] The disclosure provides methods involving administering nanoparticle compositions including one or more therapeutic and/or prophylactic agents and pharmaceutical compositions including the same. The terms therapeutic and prophylactic can be used interchangeably herein with respect to features and embodiments of the present disclosure. Therapeutic compositions, or imaging, diagnostic, or prophylactic compositions thereof, may be administered to a subject using any reasonable amount and any route of administration effective for preventing, treating, diagnosing, or imaging a disease, disorder, and/or condition and/or any other purpose. The specific amount administered to a given subject may vary depending on the species, age, and general condition of the subject; the purpose of the administration; the

particular composition; the mode of administration; and the like. Compositions in accordance with the present disclosure may be formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of a composition of the present disclosure will be decided by an attending physician within the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or otherwise appropriate dose level (e.g., for imaging) for any particular patient will depend upon a variety of factors including the severity and identify of a disorder being treated, if any; the one or more therapeutic and/or prophylactic agents employed; the specific composition employed; the age, body weight, general health, sex, and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific pharmaceutical composition employed; the duration of the treatment; drugs used in combination or coincidental with the specific pharmaceutical composition employed; and like factors well known in the medical arts.

[00570] **A** nanoparticle composition including one or more therapeutic and/or prophylactic agents may be administered by any route. In some embodiments, compositions, including prophylactic, diagnostic, or imaging compositions including one or more nanoparticle compositions described herein, are administered by one or more of a variety of routes, including oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, subcutaneous, intraventricular, trans- or intra-dermal, interdermal, rectal, intravaginal, intraperitoneal, intraocular, subretinal, intravitreal, topical *(e.g.* by powders, ointments, creams, gels, lotions, and/or drops), mucosal, nasal, buccal, enteral, vitreal, intratumoral, sublingual, intranasal; by intratracheal instillation, bronchial instillation, and/or inhalation; as an oral spray and/or powder, nasal spray, and/or aerosol, and/or through a portal vein catheter. In some embodiments, a composition may be administered intravenously, intramuscularly, intradermally, intra-arterially, intratumorally, subcutaneously, intraocular, subretinal, intravitreal, or by inhalation. However, the present disclosure encompasses the delivery or administration of compositions by any appropriate route taking into consideration likely advances in the sciences of drug delivery. In general, the most appropriate route of administration will depend upon a variety of factors including the nature of the nanoparticle composition including one or more therapeutic and/or prophylactic agents (e.g., its stability in various bodily environments such as the bloodstream and gastrointestinal tract), the condition of the patient (e.g., whether the patient is able to tolerate particular routes of administration), etc.

[00571] In certain embodiments, compositions in accordance with the present disclosure may be administered at dosage levels sufficient to deliver from about 0.0001 mg/kg to about 10

mg/kg, from about 0.001 mg/kg to about 10 mg/kg, from about 0.005 mg/kg to about 10 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.05 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, from about 1 mg/kg to about 10 mg/kg, from about 2 mg/kg to about 10 mg/kg, from about 5 mg/kg to about 10 mg/kg, from about 0.0001 mg/kg to about 5 mg/kg, from about 0.001 mg/kg to about 5 mg/kg, from about 0.005 mg/kg to about 5 mg/kg, from about 0.01 mg/kg to about 5 mg/kg, from about 0.05 mg/kg to about 5 mg/kg, from about 0.1 mg/kg to about 5 mg/kg, from about 1 mg/kg to about 5 mg/kg, from about 2 mg/kg to about 5 mg/kg, from about 0.0001 mg/kg to about 2.5 mg/kg, from about 0.001 mg/kg to about 2.5 mg/kg, from about 0.005 mg/kg to about 2.5 mg/kg, from about 0.01 mg/kg to about 2.5 mg/kg, from about 0.05 mg/kg to about 2.5 mg/kg, from about 0.1 mg/kg to about 2.5 mg/kg, from about 1 mg/kg to about 2.5 mg/kg, from about 2 mg/kg to about 2.5 mg/kg, from about 0.0001 mg/kg to about 1 mg/kg, from about 0.001 mg/kg to about 1 mg/kg, from about 0.005 mg/kg to about 1 mg/kg, from about 0.01 mg/kg to about 1 mg/kg, from about 0.05 mg/kg to about 1 mg/kg, from about 0.1 mg/kg to about 1 mg/kg, from about 0.0001 mg/kg to about 0.25 mg/kg, from about 0.001 mg/kg to about 0.25 mg/kg, from about 0.005 mg/kg to about 0.25 mg/kg, from about 0.01 mg/kg to about 0.25 mg/kg, from about 0.05 mg/kg to about 0.25 mg/kg, or from about 0.1 mg/kg to about 0.25 mg/kg of a therapeutic and/or prophylactic agent (e.g., an mRNA) in a given dose, where a dose of 1 mg/kg (mpk) provides 1 mg of a therapeutic and/or prophylactic agent per 1 kg of subject body weight. In certain embodiments, a dose of about 0.001 mg/kg to about 10 mg/kg of a therapeutic and/or prophylactic agent (e.g., mRNA) of a nanoparticle composition may be administered. In other embodiments, a dose of about 0.005 mg/kg to about 2.5 mg/kg of a therapeutic and/or prophylactic agent may be administered. In certain embodiments, a dose of about 0.1 mg/kg to about 1 mg/kg may be administered. In other embodiments, a dose of about 0.05 mg/kg to about 0.25 mg/kg may be administered. A dose may be administered one or more times per day, in the same or a different amount, to obtain a desired level of mRNA expression and/or therapeutic, diagnostic, prophylactic, or imaging effect. The desired dosage may be delivered, for example, three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, or every four weeks. In certain embodiments, the desired dosage may be delivered using multiple administrations (e.g., two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations). In some embodiments, a single dose may be administered, for example, prior to or after a surgical procedure or in the instance of an acute disease, disorder, or condition.

[00572] Nanoparticle compositions including one or more therapeutic and/or prophylactic agents may be used in combination with one or more other therapeutic, prophylactic, diagnostic, or imaging agents. By "in combination with," it is not intended to imply that the agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope of the present disclosure. For example, one or more nanoparticle compositions including one or more different therapeutic and/or prophylactic agents may be administered in combination. Compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. In some embodiments, the present disclosure encompasses the delivery of compositions, or imaging, diagnostic, or prophylactic compositions thereof in combination with agents that improve their bioavailability, reduce and/or modify their metabolism, inhibit their excretion, and/or modify their distribution within the body.

[00573] It will further be appreciated that therapeutically, prophylactically, diagnostically, or imaging active agents utilized in combination may be administered together in a single composition or administered separately in different compositions. In general, it is expected that agents utilized in combination will be utilized at levels that do not exceed the levels at which they are utilized individually. In some embodiments, the levels utilized in combination may be lower than those utilized individually.

[00574] The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, a composition useful for treating cancer may be administered concurrently with a chemotherapeutic agent), or they may achieve different effects (e.g., control of any adverse effects, such as infusion related reactions).

[00575] A nanoparticle composition may be used in combination with an agent to increase the effectiveness and/or therapeutic window of the composition. Such an agent may be, for example, an anti-inflammatory compound, a steroid (e.g., a corticosteroid), a statin, an estradiol, a BTK inhibitor, an S1P1 agonist, a glucocorticoid receptor modulator (GRM), or an anti-histamine. In some embodiments, a nanoparticle composition may be used in combination with dexamethasone, methotrexate, acetaminophen, an H1 receptor blocker, or an H2 receptor blocker. In certain embodiments, a method of treating a subject in need thereof or of delivering

a therapeutic and/or prophylactic agent to a subject (e.g., a mammal) may involve pre-treating the subject with one or more agents prior to administering a nanoparticle composition. For example, a subject may be pre-treated with a useful amount (e.g., 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, or any other useful amount) of dexamethasone, methotrexate, acetaminophen, an H1 receptor blocker, or an H2 receptor blocker. Pre-treatment may occur 24 or fewer hours (e.g., 24 hours, 20 hours, 16 hours, 12 hours, 8 hours, 4 hours, 2 hours, 1 hour, 50 minutes, 40 minutes, 30 minutes, 20 minutes, or 10 minutes) before administration of the nanoparticle composition and may occur one, two, or more times in, for example, increasing dosage amounts.

[00576] In any method or use described herein, in certain embodiments, the subject in need thereof is LDLR-deficient or apoE-deficient or both. In certain embodiments, the subject in need thereof is not LDLR-deficient or has normal LDLR levels and/or activities. In certain embodiments, the subject in need thereof is not apoE-deficient or has normal apoE levels and/or activities. In certain embodiments, the subject in need thereof has an abnormal interaction of LDLR and apoE. In certain embodiments, the subject in need thereof has a normal interaction of LDLR and apoE.

[00577] About, Approximately: As used herein, the terms "approximately" and "about," as applied to one or more values of interest, refer to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value). For example, when used in the context of an amount of a given compound in a lipid component of a nanoparticle composition, "about" may mean +/- 10% of the recited value. For instance, a nanoparticle composition including a lipid component having about 40% of a given compound may include 30-50% of the compound.

[00578] Compound: As used herein, the term "compound," is meant to include all isomers and isotopes of the structure depicted. "Isotopes" refers to atoms having the same atomic number but different mass numbers resulting from a different number of neutrons in the nuclei. For example, isotopes of hydrogen include tritium and deuterium. Further, a compound, salt, or complex of the present disclosure can be prepared in combination with solvent or water molecules to form solvates and hydrates by routine methods.

[00579] Contacting: As used herein, the term "contacting" means establishing a physical connection between two or more entities. For example, contacting a mammalian cell with a nanoparticle composition means that the mammalian cell and a nanoparticle are made to share a physical connection. Methods of contacting cells with external entities both in vivo and ex vivo are well known in the biological arts. For example, contacting a nanoparticle composition and a mammalian cell disposed within a mammal may be performed by varied routes of administration (e.g., intravenous, intramuscular, intradermal, and subcutaneous) and may involve varied amounts of nanoparticle compositions. Moreover, more than one mammalian cell may be contacted by a nanoparticle composition.

[00580] Delivering: As used herein, the term "delivering" means providing an entity to a destination. For example, delivering a therapeutic and/or prophylactic agent to a subject may involve administering a nanoparticle composition including the therapeutic and/or prophylactic agent to the subject (e.g., by an intravenous, intramuscular, intradermal, or subcutaneous route). Administration of a nanoparticle composition to a mammal or mammalian cell may involve contacting one or more cells with the nanoparticle composition.

[00581] Enhanced delivery: As used herein, the term "enhanced delivery" means delivery of more (e.g., at least 1.5 fold more, at least 2-fold more, at least 3-fold more, at least 4-fold more, at least 5-fold more, at least 6-fold more, at least 7-fold more, at least 8-fold more, at least 9-fold more, at least 10-fold more) of a therapeutic and/or prophylactic agent by a nanoparticle to a target tissue of interest (e.g., mammalian liver) compared to the level of delivery of a therapeutic and/or prophylactic agent by a control nanoparticle to a target tissue of interest (e.g., MC3, KC2, or DLinDMA). The level of delivery of a nanoparticle to a particular tissue may be measured by comparing the amount of protein produced in a tissue to the weight of said tissue, comparing the amount of therapeutic and/or prophylactic agent in a tissue to the weight of said tissue, comparing the amount of protein produced in a tissue to the amount of total protein in said tissue, or comparing the amount of therapeutic and/or prophylactic agent in a tissue to the amount of total therapeutic and/or prophylactic agent in said tissue. It will be understood that the enhanced delivery of a nanoparticle to a target tissue need not be determined in a subject being treated, it may be determined in a surrogate such as an animal model (e.g., a rat model).

[00582] Specific delivery: As used herein, the term "specific delivery," "specifically deliver," or "specifically delivering" means delivery of more (e.g., at least 1.5 fold more, at least 2-fold more, at least 3-fold more, at least 4-fold more, at least 5-fold more, at least 6-fold more, at least 7-fold more, at least 8-fold more, at least 9-fold more, at least 10-fold more) of a

therapeutic and/or prophylactic agent by a nanoparticle to a target tissue of interest (e.g., mammalian liver) compared to an off-target tissue (e.g., mammalian spleen). The level of delivery of a nanoparticle to a particular tissue may be measured by comparing the amount of protein produced in a tissue to the weight of said tissue, comparing the amount of therapeutic and/or prophylactic agent in a tissue to the weight of said tissue, comparing the amount of protein produced in a tissue to the amount of total protein in said tissue, or comparing the amount of therapeutic and/or prophylactic agent in a tissue to the amount of total therapeutic and/or prophylactic agent in said tissue. For example, for renovascular targeting, a therapeutic and/or prophylactic agent is specifically provided to a mammalian kidney as compared to the liver and spleen if 1.5, 2-fold, 3-fold, 5-fold, 10-fold, 15 fold, or 20 fold more therapeutic and/or prophylactic agent per 1 g of tissue is delivered to a kidney compared to that delivered to the liver or spleen following systemic administration of the therapeutic and/or prophylactic agent. It will be understood that the ability of a nanoparticle to specifically deliver to a target tissue need not be determined in a subject being treated, it may be determined in a surrogate such as an animal model (e.g., a rat model).

[00583] Encapsulation efficiency: As used herein, "encapsulation efficiency" refers to the amount of a therapeutic and/or prophylactic agent that becomes part of a nanoparticle composition, relative to the initial total amount of therapeutic and/or prophylactic agent used in the preparation of a nanoparticle composition. For example, if 97 mg of therapeutic and/or prophylactic agent are encapsulated in a nanoparticle composition out of a total 100 mg of therapeutic and/or prophylactic agent initially provided to the composition, the encapsulation efficiency may be given as 97%. As used herein, "encapsulation" may refer to complete, substantial, or partial enclosure, confinement, surrounding, or encasement.

[00584] Expression: As used herein, "expression" of a nucleic acid sequence refers to translation of an mRNA into a polypeptide or protein and/or post-translational modification of a polypeptide or protein.

[00585] In vitro: As used herein, the term "in vitro" refers to events that occur in an artificial environment, e.g., in a test tube or reaction vessel, in cell culture, in a Petri dish, etc., rather than within an organism (e.g., animal, plant, or microbe).

[00586] In vivo: As used herein, the term "in vivo" refers to events that occur within an organism (e.g., animal, plant, or microbe or cell or tissue thereof).

[00587] Ex vivo: As used herein, the term "ex vivo" refers to events that occur outside of an organism (e.g., animal, plant, or microbe or cell or tissue thereof). Ex vivo events may take place in an environment minimally altered from a natural (e.g., in vivo) environment.

[00588] Isomer: As used herein, the term "isomer" means any geometric isomer, tautomer, zwitterion, stereoisomer, enantiomer, or diastereomer of a compound. Compounds may include one or more chiral centers and/or double bonds and may thus exist as stereoisomers, such as double-bond isomers (i.e., geometric E/Z isomers) or diastereomers (e.g., enantiomers (i.e., (+) or (-)) or *cis*/*trans* isomers). The present disclosure encompasses any and all isomers of the compounds described herein, including stereomerically pure forms (e.g., geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric and stereoisomeric mixtures, e.g., racemates. Enantiomeric and stereomeric mixtures of compounds and means of resolving them into their component enantiomers or stereoisomers are well-known.

[00589] Lipid component: As used herein, a "lipid component" is that component of a nanoparticle composition that includes one or more lipids. For example, the lipid component may include one or more cationic/ionizable, PEGylated, structural, or other lipids, such as phospholipids.

[00590] Linker: As used herein, a "linker" is a moiety connecting two moieties, for example, the connection between two nucleosides of a cap species. A linker may include one or more groups including but not limited to phosphate groups (e.g., phosphates, boranophosphates, thiophosphates, selenophosphates, and phosphonates), alkyl groups, amidates, or glycerols. For example, two nucleosides of a cap analog may be linked at their 5' positions by a triphosphate group or by a chain including two phosphate moieties and a boranophosphate moiety.

[00591] Methods of administration: As used herein, "methods of administration" may include intravenous, intramuscular, intradermal, subcutaneous, or other methods of delivering a composition to a subject. A method of administration may be selected to target delivery (e.g., to specifically deliver) to a specific region or system of a body.

[00592] Modified: As used herein, "modified" means non-natural. For example, an RNA may be a modified RNA. That is, an RNA may include one or more nucleobases, nucleosides, nucleotides, or linkers that are non-naturally occurring. A "modified" species may also be referred to herein as an "altered" species. Species may be modified or altered chemically, structurally, or functionally. For example, a modified nucleobase species may include one or more substitutions that are not naturally occurring.

[00593] N:P ratio: As used herein, the "N:P ratio" is the molar ratio of ionizable (in the physiological pH range) nitrogen atoms in a lipid to phosphate groups in an RNA, e.g., in a nanoparticle composition including a lipid component and an RNA.

[00594] Nanoparticle composition: As used herein, a "nanoparticle composition" is a composition comprising one or more lipids. Nanoparticle compositions are typically sized on the order of micrometers or smaller and may include a lipid bilayer. Nanoparticle compositions encompass lipid nanoparticles (LNPs), liposomes (e.g., lipid vesicles), and lipoplexes. For example, a nanoparticle composition may be a liposome having a lipid bilayer with a diameter of 500 nm or less.

[00595] Naturally occurring: As used herein, "naturally occurring" means existing in nature without artificial aid.

[00596] Patient: As used herein, "patient" refers to a subject who may seek or be in need of treatment, requires treatment, is receiving treatment, will receive treatment, or a subject who is under care by a trained professional for a particular disease or condition.

[00597] PEG lipid: As used herein, a "PEG lipid" or "PEGylated lipid" refers to a lipid comprising a polyethylene glycol component.

[00598] Pharmaceutically acceptable: The phrase "pharmaceutically acceptable" is used herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[00599] Pharmaceutically acceptable excipient: The phrase "pharmaceutically acceptable excipient," as used herein, refers to any ingredient other than the compounds described herein (for example, a vehicle capable of suspending, complexing, or dissolving the active compound) and having the properties of being substantially nontoxic and non-inflammatory in a patient. Excipients may include, for example: anti-adherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspending or dispersing agents, sweeteners, and waters of hydration. Exemplary excipients include, but are not limited to: butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine,

methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E (alpha-tocopherol), vitamin C, xylitol, and other species disclosed herein.

[00600] In the present specification, the structural formula of the compound represents a certain isomer for convenience in some cases, but the present disclosure includes all isomers, such as geometrical isomers, optical isomers based on an asymmetrical carbon, stereoisomers, tautomers, and the like, it being understood that not all isomers may have the same level of activity. In addition, a crystal polymorphism may be present for the compounds represented by the formula. It is noted that any crystal form, crystal form mixture, or anhydride or hydrate thereof is included in the scope of the present disclosure.

[00601] The term "crystal polymorphs", "polymorphs" or "crystal forms" means crystal structures in which a compound (or a salt or solvate thereof) can crystallize in different crystal packing arrangements, all of which have the same elemental composition. Different crystal forms usually have different X-ray diffraction patterns, infrared spectral, melting points, density hardness, crystal shape, optical and electrical properties, stability and solubility. Recrystallization solvent, rate of crystallization, storage temperature, and other factors may cause one crystal form to dominate. Crystal polymorphs of the compounds can be prepared by crystallization under different conditions.

[00602] Pharmaceutically acceptable salts: Compositions may also include salts of one or more compounds. Salts may be pharmaceutically acceptable salts. As used herein, "pharmaceutically acceptable salts" refers to derivatives of the disclosed compounds wherein the parent compound is altered by converting an existing acid or base moiety to its salt form (e.g., by reacting a free base group with a suitable organic acid). Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate,

palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. The pharmaceutically acceptable salts of the present disclosure include the conventional non-toxic salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. The pharmaceutically acceptable salts of the present disclosure can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418, Pharmaceutical Salts: Properties, Selection, and Use, P.H. Stahl and C.G. Wermuth (eds.), Wiley-VCH, 2008, and Berge et al., Journal of Pharmaceutical Science, 66, 1-19 (1977), each of which is incorporated herein by reference in its entirety.

[00603] Phospholipid: As used herein, a "phospholipid" is a lipid that includes a phosphate moiety and one or more carbon chains, such as unsaturated fatty acid chains. A phospholipid may include one or more multiple (e.g., double or triple) bonds (e.g., one or more unsaturations). Particular phospholipids may facilitate fusion to a membrane. For example, a cationic phospholipid may interact with one or more negatively charged phospholipids of a membrane (e.g., a cellular or intracellular membrane). Fusion of a phospholipid to a membrane may allow one or more elements of a lipid-containing composition to pass through the membrane permitting, e.g., delivery of the one or more elements to a cell.

[00604] Polydispersity index: As used herein, the "polydispersity index" is a ratio that describes the homogeneity of the particle size distribution of a system. A small value, e.g., less than 0.3, indicates a narrow particle size distribution.

[00605] Polypeptide: As used herein, the term "polypeptide" or "polypeptide of interest" refers to a polymer of amino acid residues typically joined by peptide bonds that can be produced naturally (e.g., isolated or purified) or synthetically.

[00606] RNA: As used herein, an "RNA" refers to a ribonucleic acid that may be naturally or non-naturally occurring. For example, an RNA may include modified and/or non-naturally occurring components such as one or more nucleobases, nucleosides, nucleotides, or linkers. An RNA may include a cap structure, a chain terminating nucleoside, a stem loop, a polyA sequence, and/or a polyadenylation signal. An RNA may have a nucleotide sequence encoding a polypeptide of interest. For example, an RNA may be a messenger RNA (mRNA). Translation of an mRNA encoding a particular polypeptide, for example, in vivo translation of an mRNA inside a mammalian cell, may produce the encoded polypeptide. RNAs may be selected from the non-liming group consisting of small interfering RNA (siRNA), asymmetrical interfering RNA (aiRNA), microRNA (miRNA), Dicer-substrate RNA (dsRNA), small hairpin RNA (shRNA), mRNA, and mixtures thereof.

[00607] Single unit dose: As used herein, a "single unit dose" is a dose of any therapeutic administered in one dose/at one time/single route/single point of contact, i.e., single administration event.

[00608] Split dose: As used herein, a "split dose" is the division of single unit dose or total daily dose into two or more doses.

[00609] Total daily dose: As used herein, a "total daily dose" is an amount given or prescribed in 24 hour period. It may be administered as a single unit dose.

[00610] Size: As used herein, "size" or "mean size" in the context of nanoparticle compositions refers to the mean diameter of a nanoparticle composition.

[00611] Subject: As used herein, the term "subject" or "patient" refers to any organism to which a composition in accordance with the disclosure may be administered, e.g., for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (e.g., mammals such as mice, rats, rabbits, non-human primates, and humans) and/or plants.

[00612] Targeted cells: As used herein, "targeted cells" refers to any one or more cells of interest. The cells may be found in vitro, in vivo, in situ, or in the tissue or organ of an organism. The organism may be an animal, preferably a mammal, more preferably a human and most preferably a patient.

[00613] Target tissue: As used herein "target tissue" refers to any one or more tissue types of interest in which the delivery of a therapeutic and/or prophylactic agent would result in a desired biological and/or pharmacological effect. Examples of target tissues of interest include specific tissues, organs, and systems or groups thereof. In particular applications, a target tissue

may be a kidney, a lung, a spleen, vascular endothelium in vessels (e.g., intra-coronary or intra-femoral), or tumor tissue (e.g., via intratumoral injection). An "off-target tissue" refers to any one or more tissue types in which the expression of the encoded protein does not result in a desired biological and/or pharmacological effect. In particular applications, off-target tissues may include the liver and the spleen.

[00614] Therapeutic and/or prophylactic agent: The term "therapeutic agent" refers to any agent that, when administered to a subject, has a therapeutic and/or diagnostic effect and/or elicits a desired biological and/or pharmacological effect. The term "prophylactic agent" refers to any agent that, when administered to a subject, has a prophylactic effect. Therapeutic and/or prophylactic agents are also referred to as "actives" or "active agents." Such agents include, but are not limited to, cytotoxins, radioactive ions, chemotherapeutic agents, small molecule drugs, proteins, and nucleic acids.

[00615] Therapeutically effective amount: As used herein, the term "therapeutically effective amount" means an amount of an agent to be delivered (e.g., nucleic acid, drug, composition, therapeutic agent, diagnostic agent, prophylactic agent, etc.) that is sufficient, when administered to a subject suffering from or susceptible to an infection, disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition.

[00616] Transfection: As used herein, "transfection" refers to the introduction of a species (e.g., an RNA) into a cell. Transfection may occur, for example, in vitro, ex vivo, or in vivo.

[00617] Treating: As used herein, the term "treating" refers to partially or completely alleviating, ameliorating, improving, relieving, delaying onset of, inhibiting progression of, reducing severity of, and/or reducing incidence of one or more symptoms or features of a particular infection, disease, disorder, and/or condition. For example, "treating" cancer may refer to inhibiting survival, growth, and/or spread of a tumor. Treatment may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition and/or to a subject who exhibits only early signs of a disease, disorder, and/or condition for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition.

[00618] Zeta potential: As used herein, the "zeta potential" is the electrokinetic potential of a lipid e.g., in a particle composition.

[00619] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments in accordance with the

present disclosure. The scope of the present disclosure is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

[00620] In the claims, articles such as "a," "an," and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The disclosure includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The disclosure includes embodiments in which more than one, or all, of the group members are present in, employed in, or otherwise relevant to a given product or process. As used herein, the expressions "one or more of A, B, or C," "one or more A, B, or C," "one or more of A, B, and C," "one or more A, B, and C", "selected from A, B, and C," "selected from the group consisting of A, B, and C," and the like are used interchangeably and all refer to a selection from a group consisting of A, B, and /or C, i.e., one or more As, one or more Bs, one or more Cs, or any combination thereof, unless otherwise specified.

[00621] It is also noted that the term "comprising" is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term "comprising" is used herein, the terms "consisting essentially of and "consisting of are thus also encompassed and disclosed. Throughout the description, where compositions are described as having, including, or comprising specific components, it is contemplated that compositions also consist essentially of, or consist of, the recited components. Similarly, where methods or processes are described as having, including, or comprising specific process steps, the processes also consist essentially of, or consist of, the recited processing steps. Further, it should be understood that the order of steps or order for performing certain actions is immaterial so long as the invention remains operable. Moreover, two or more steps or actions can be conducted simultaneously.

[00622] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or sub-range within the stated ranges in different embodiments of the disclosure, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[00623] The synthetic processes of the disclosure can tolerate a wide variety of functional groups, therefore various substituted starting materials can be used. The processes generally provide the desired final compound at or near the end of the overall process, although it may be desirable in certain instances to further convert the compound to a pharmaceutically acceptable salt thereof.

[00624] Compounds of the present disclosure can be prepared in a variety of ways using commercially available starting materials, compounds known in the literature, or from readily prepared intermediates, by employing standard synthetic methods and procedures either known to those skilled in the art, or which will be apparent to the skilled artisan in light of the teachings herein. Standard synthetic methods and procedures for the preparation of organic molecules and functional group transformations and manipulations can be obtained from the relevant scientific literature or from standard textbooks in the field. Although not limited to any one or several sources, classic texts such as Smith, M. B., March, *J., March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, 5th edition, John Wiley & Sons: New York, 2001; Greene, T.W., Wuts, P.G. M., *Protective Groups in Organic Synthesis*, 3rd edition, John Wiley & Sons: New York, 1999; R. Larock, *Comprehensive Organic Transformations*, VCH Publishers (1989); L. Fieser and M. Fieser, *Fieser andFieser's Reagents for Organic Synthesis*, John Wiley and Sons (1994); and L. Paquette, ed., *Encyclopedia of Reagents for Organic Synthesis*, John Wiley and Sons (1995), incorporated by reference herein, are useful and recognized reference textbooks of organic synthesis known to those in the art. The following descriptions of synthetic methods are designed to illustrate, but not to limit, general procedures for the preparation of compounds of the present disclosure.

[00625] The compounds of this disclosure having any of the formulae described herein may be prepared according to the procedures illustrated in Schemes 1-3 below, from commercially available starting materials or starting materials which can be prepared using literature procedures. The variables in the Schemes (e.g., Ri, R2, R3, R4, and $_{R5)}$ are as defined herein, e.g., Ri, R2, R3, R4, and $_{R\ 5}$ are each independently alkyl. One of ordinary skill in the art will note that, during the reaction sequences and synthetic schemes described herein, the order of certain steps may be changed, such as the introduction and removal of protecting groups.

[00626] One of ordinary skill in the art will recognize that certain groups may require protection from the reaction conditions via the use of protecting groups. Protecting groups may also be used to differentiate similar functional groups in molecules. A list of protecting groups

and how to introduce and remove these groups can be found in Greene, T.W., Wuts, P.G. M., *Protective Groups in Organic Synthesis,* 3rd edition, John Wiley & Sons: New York, 1999. Preferred protecting groups include, but are not limited to:

For a hydroxyl moiety: TBS, benzyl, THP, Ac;

For carboxylic acids: benzyl ester, methyl ester, ethyl ester, allyl ester;

For amines: Fmoc, Cbz, BOC, DMB, Ac, Bn, Tr, Ts, trifluoroacetyl, phthalimide, benzylideneamine;

For diols: Ac (x2) TBS (x2), or when taken together acetonides;

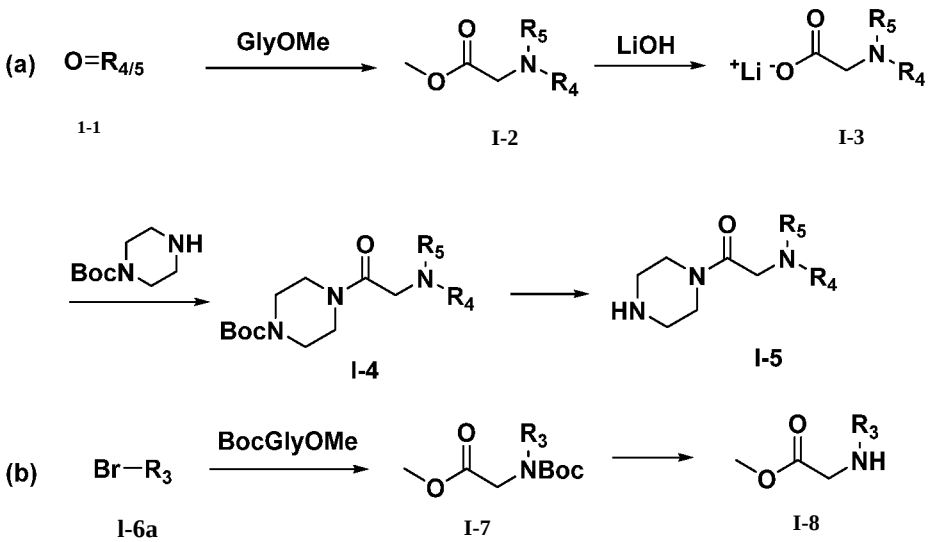
For thiols: Ac;

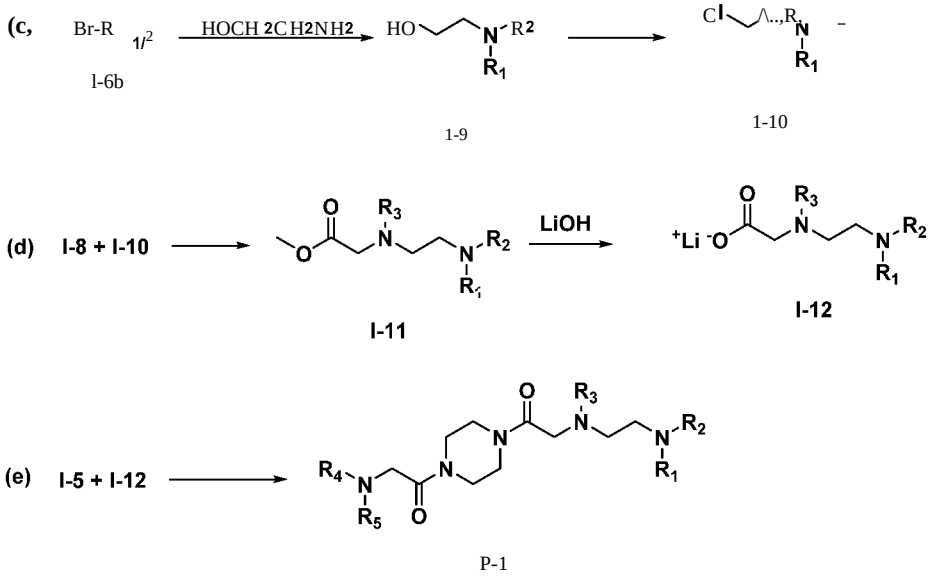
For benzimidazoles: SEM, benzyl, PMB, DMB;

For aldehydes: di-alkyl acetals such as dimethoxy acetal or diethyl acetyl.

[00627] In the reaction schemes described herein, multiple stereoisomers may be produced. When no particular stereoisomer is indicated, it is understood to mean all possible stereoisomers that could be produced from the reaction. A person of ordinary skill in the art will recognize that the reactions can be optimized to give one isomer preferentially, or new schemes may be devised to produce a single isomer. If mixtures are produced, techniques such as preparative thin layer chromatography, preparative HPLC, preparative chiral HPLC, or preparative SFC may be used to separate the isomers.

Scheme 1

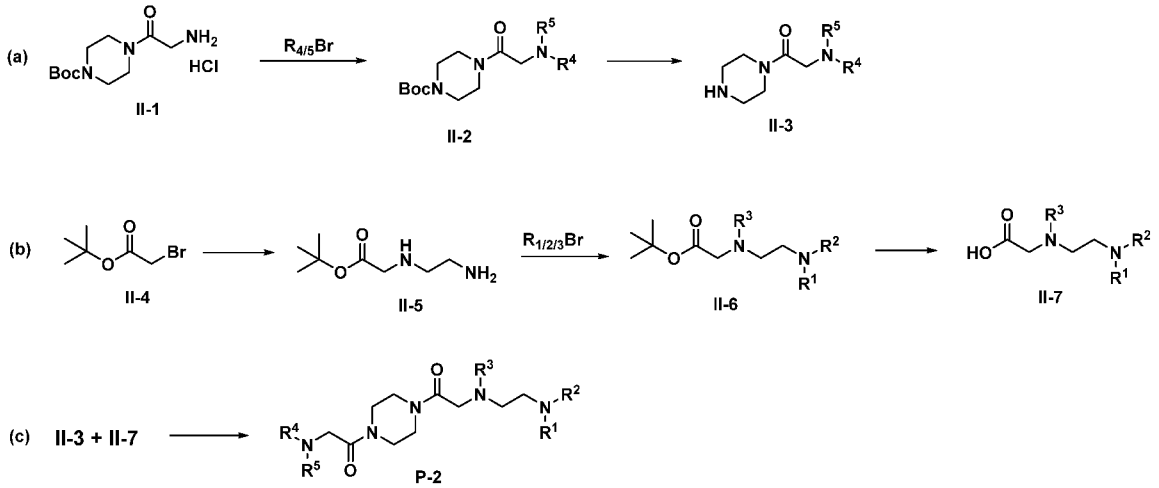




[00628] Scheme **1** above illustrates an **11**-step procedure for the synthesis of lipids of the disclosure. (a) Aldehyde (1-1) is reacted with glycine methyl ester hydrochloride in the presence of a reducing agent, e.g. NaBH(OAc)3, and a base, e.g. NEt3, in an appropriate solvent, e.g. AcOH, to yield methyl dialkylglycinate (1-2). This reaction can take place in an organic solvent, e.g. dichloroethane. Methyl dialkylglycinate (1-2) is hydrolyzed using lithium hydroxide, e.g. in THF, to produce the respective lithium alkyl glycinate (1-3). A solution of (1-3), e.g. in THF, is reacted with l-fert-butyl-piperazine in the presence of a base, e.g. diisopropylethylamine (DIPEA), and a coupling agent, e.g. propylphosphonic acid anhydride, to form *tert*-butyl **4**-(alkyl)piperazine-l-carboxy late (1-4), which is then deprotected, using e.g. trifluoroacetic acid (TFA), to yield 2-(alkyl)-l-(piperazine-l-yl)ethan-l-one (1-5). This reaction can take place in an organic solvent, e.g. dichloromethane (DCM). (b) Bromoalkane (1-6) is reacted with *tert*-butyl-methyl alkyl glycinate in the presence of a strong base, e.g. NaH, and in an appropriate solvent, e.g. dimethylformamide (DMF), to form methyl N-(tert-butyl)-N-alkylycinate (1-7), which is then deprotected, using e.g. TFA, to yield methyl alkylglycinate (1-8). The deprotection reaction can take place in an organic solvent, e.g. dichloromethane. (c) Bromoalkane (1-6) is reacted with ethanol-**1**-amine under alkaline conditions (e.g. K2CO3) and in the presence of a catalyst, e.g. **KI** in an appropriate solvent (e.g. acetonitrile), to form 2-(dialkylamino)ethanol (1-9), which is converted to *N*-(2-chloroethyl)-*N*-alkylalkan-l-amine (I-10) using a suitable reagent, e.g. mesylchloride in the presence of a base, e.g. triethylamine, and an appropriate solvent, e.g. DCM. (d) methyl alkylglycinate (1-8), obtained according to (b) and *N*-(2-chloroethyl)-*N*-alkylalkan-**1**-amine (I-10) obtained according to (c) are coupled in the presence of a base, **e.g.K2C03** and a nucleophilic catalyst, e.g. **KI** in an appropriate solvent (e.g. acetonitrile), to

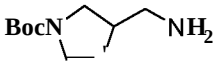
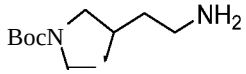
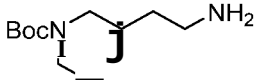
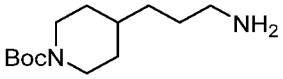
form the methyl glycinate intermediate (1-11), which is then hydrolized using lithium hydroxide in an appropriate solvent, e.g. tetrahydrofuran (THF), to yield the lithium glycinate compound (I-12).(e) 2-(alkyl)-l-(piperazine-l-yl)ethan-l-one (1-5) obtained according to (a) and compound (1-12) obtained according to (d) are reacted in the presence of a base, e.g. diisopropylethylamine (DIPEA), and a coupling agent, e.g. propylphosphonic acid anhydride, to yield the product (P-1). This reaction can take place in an organic solvent, e.g. THF.

Scheme 2



[00629] Scheme 2 above illustrates a 6-step procedure for the synthesis of lipids of the disclosure. (a) Commercially available tert-butyl 4-glycylpiperazine-l-carboxylate hydrochloride (II-l) is reacted with bromoalkane in the presence of a base, e.g.K_2CO_3 and a nucleophilic catalyst, e.g. KI in an appropriate solvent, e.g. cyclopentyl methyl ether/acetonitrile, and deprotected using, e.g. trifluoroacetic (TFA) to yield 2-(dialkylamino)-l-(piperazin-l-yl)ethan-l-one. The deprotection step can take place in an organic solvent, e.g. dichloromethane (DCM) (b) tert-Butyl 2-bromoacetate (II-4) is reacted with 1,2-diamino ethane, in an appropriate solvent, e.g. DCM to yield tert-butyl (2-aminoethyl)glycinate (П-5) which is coupled with bromoalkane in the presence of a base, e.g. K2CO3 and a nucleophilic catalyst, e.g. KI in an appropriate solvent, e.g. acetonitrile, to yield tert-butyl N-(2-(dialkylamino)ethyl)-N-alkylglycinate (II-6). Deprotection of II-6, using e.g. TFA, yields the corresponding glycine compound (П-7). (c) The reaction of (II-3), obtained according to (a), and (II-7), obtained according to (b), in the presence of a base, e.g. diisopropylethylamine (DIPEA), and a coupling agent, e.g. propylphosphonic acid anhydride, yields the product (P-2). This reaction can take place in an organic solvent, e.g. 2-methyltetrahydrofuran.

Scheme 3

[00630] As illustrated in Scheme 3 above, intermediates for the synthesis of certain compounds of the disclosure may be obtained by alkylating the amino group of tert-butyl 4-(2-aminoethyl)piperidine-1-carboxylate. Similar reactions can be performed with a different starting material such as  ,  ,  , or  .

[00631] In addition, it is to be understood that any particular embodiment of the present disclosure that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein.

[00632] All cited sources, for example, references, publications, databases, database entries, and art cited herein, are incorporated into this application by reference, even if not expressly stated in the citation. In case of conflicting statements of a cited source and the instant application, the statement in the instant application shall control. Citation of publications and patent documents is not intended as an admission that any is pertinent prior art, nor does it constitute any admission as to the contents or date of the same. The invention having now been described by way of written description, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description and examples below are for purposes of illustration and not limitation of the claims that follow.

Examples

Example 1: Synthesis of compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

A. General Considerations

[00633] All solvents and reagents used were obtained commercially and used as such unless noted otherwise. ¾ NMR spectra were recorded in CDCh, at 300 K using a Bruker Ultrashield 300 MHz instrument or a Varian Unity Inova 400MHz Instrument. Chemical shifts are reported as parts per million (ppm) relative to TMS (0.00) for ¾. Silica gel

chromatographies were performed on ISCO CombiFlash Rf+ Lumen Instruments using ISCO RediSep Rf Gold Flash Cartridges (particle size: 20-40 microns). Reverse phase chromatographies were performed on ISCO CombiFlash Rf+ Lumen Instruments using RediSep Rf Gold CI8 High Performance columns. All final compounds were determined to be greater than 85% pure *via* analysis by reverse phase UPLC-MS (retention times, RT, in minutes) using Waters Acquity UPLC instrument with DAD and ELSD and a ZORBAX Rapid Resolution High Definition (RRHD) SB-C18 LC column, 2.1 mm, 50 mm, 1.8 μm, and a gradient of 65 to 100% acetonitrile in water with 0.1% TFA over 5 minutes at 1.2 mL/min. Injection volume was 5 μL and the column temperature was 80 °C. Detection was based on electrospray ionization (ESI) in positive mode using Waters SQD mass spectrometer (Milford, MA, USA) and evaporative light scattering detector.

[00634] The procedures described for the synthesis of Compounds 12 and 19 are applicable to the synthesis of compounds according to formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) generally.

The following abbreviations are employed herein:

rt: Room Temperature

MeOH: Methanol

DCM: Dichloromethane

DCE: Dichloroethane

DMAP: 4-Dimethylaminopyridine

DMF: *N*^V-Dimethylformamide

EtOAc: Ethylacetate

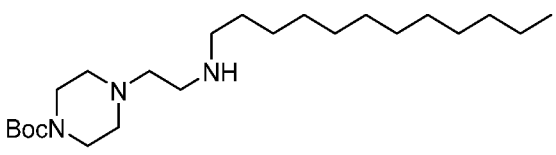
MeCN: Acetonitrile

THF: Tetrahydrofuran

EDC·HCl: N-(3-Dimethylaminopropyl)-N'-ethylcarbodiirnide hydrochloride

B. Compound 1: 2-(Didodecylamino)-*N*-(2-(4-(2-(didodecylamino)ethyl)piperazin-l-yl)ethyl)-*N*-dodecylacetamide

Step 1: *tert*-Butyl 4-(2-(dodecylamino)ethyl)piperazine-l-carboxylate



Chemical Formula: C23H47N3O2

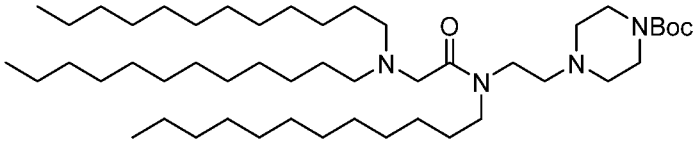
Molecular Weight: 397.65

[00635] In the same manner as Step 3 for Compound 18, *tert*-butyl 4-(2-(dodecylamino)ethyl)piperazine-l-carboxylate was synthesized from 1-bromododecane (3.3 g, 13.1 mmol), 4-(2-aminoethyl)-l-boc-piperazine (3.0 g, 13.1 mmol), K2CO3 (3.62 g, 26.2 mmol), and KI (217 mg, 1.31 mmol) in MeCN (60 mL). Yield (1.42 g, 27%).

UPLC/ELSD: RT = 1.18 min. MS (ES): *m/z* (MH^+) 398.56 for C23H47N3O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.45 (br. m, 4H); 2.75 (br. m, 2H); 2.65 (br. m, 2H); 2.55 (br. m, 2H); 2.42 (br. m, 4H); 1.60-1.22 (br. m, 29H); 0.91 (br. m, 3H).

Step 2: *tert*-Butyl 4-(2-(2-(didodecylamino)-*N*-dodecylacetamido)ethyl)piperazine-l-carboxylate



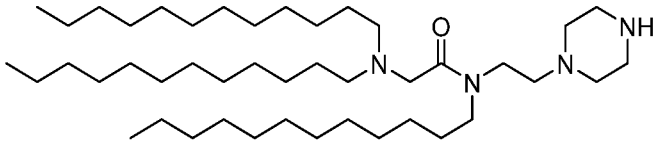
Chemical Formula: C49H98N4O3

Molecular Weight: 791.35

[00636] In the same manner as Step 3 for Compound 11, tot-butyl 4-(2-(2-(didodecylamino)-*N*-dodecylacetamido)ethyl)piperazine-l-carboxylate was synthesized from tot-butyl 4-(2-(dodecylamino)ethyl)piperazine-l-carboxylate (100 mg, 0.25 mmol), lithium didodecylglycine (0.10 g, 0.25 mmol), propylphosphonic acid anhydride (50% EtOAc solution, 0.45 mL, 0.75 mmol), and z-Pr_2EtN (0.044 mL, 0.25 mmol) in THF (2 mL). Yield (0.12 g, 63%).

UPLC/ELSD: RT = 3.36 min. MS (ES): *m/z* (MH^+) 792.082 for C49H98N4O3

Step 3: 2-(Didodecylamino)-*N*-dodecyl-*N*-(2-(piperazin-1-yl)ethyl)acetamide



Chemical Formula: C44H90N4O

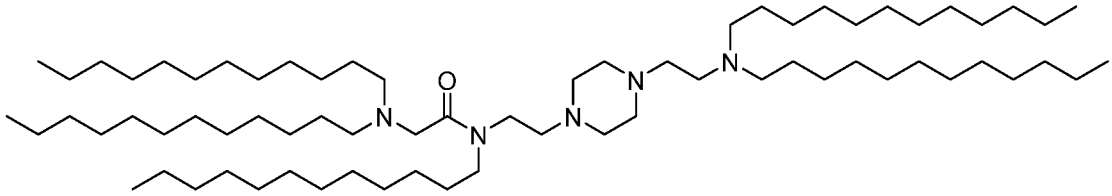
Molecular Weight: 691.23

[00637] In the same manner as Step 4 for Compound 11, 2-(didodecylamino)-*N*-dodecyl-*N*-(2-(piperazin-l-yl)ethyl)acetamide was synthesized from tot-butyl 4-(2-(2-(didodecylamino)-

N-dodecylacetamido)ethyl)piperazine-l-carboxylate (0.12 g, 0.16 mmol) and TFA (0.25 mL, 3.2 mmol) in 0.25 mL DCM.

UPLC/ELSD: RT = 3.06 min. MS (ES): *m/z* (MH^+) 692.984 for C44H90N4O

Step 4: Compound 1: 2-(Didodecylamino *)-N*-(2-(4-(2-(didodecylamino)ethyl)piperazin-l-yl)ethyl *)-N*-dodecylacetamide



Chemical Formula: C70H143N5O

Molecular Weight: 1070.95

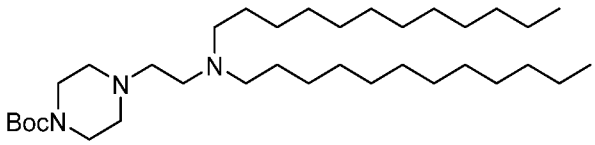
[00638] In the same manner as Step 6 for Compound 18, 2-(didodecylamino *)-N*-(2-(4-(2-(didodecylamino)ethyl)piperazin-l-yl)ethyl *)-N*-dodecylacetamide was synthesized from 2-(didodecylamino *)-N*-dodecyl-*N*-(2-(piperazin-l-yl)ethyl)acetamide (65 mg, 0.094 mmol), *N*-(2-chloroethyl *)-N*-dodecyldodecan-l-amine (42 mg, 0.10 mmol), K2CO3 (13 mg, 0.094 mmol) and KI (2 mg, 0.0094 mmol) in 0.5 mL MeCN to afford 58.5 mg for 58% yield.

UPLC/ELSD: RT = 3.75 min. MS (ES): *m/z* (MH^+) 1072.585 for C70H143N5O

¾ NMR (300 MHz, CDCh) δ: ppm 3.82-3.23 (br. m. 8H); 3.04-2.90 (br. m , 2H); 2.47 (m, 18H); 1.24 (m, 100H); 0.96 (m, 15H).

C. Compound 2: 2-((2-(Didodecylamino)ethyl)(dodecyl)amino)-l-(4-(2-(didodecylamino)ethyl)piperazin-1-yl)ethan-1-one

Step 1: tot-Butyl 4-(2-(didodecylamino)ethyl)piperazine-l-carboxylate



Chemical Formula: C35H71N3O2

Molecular Weight: 565.97

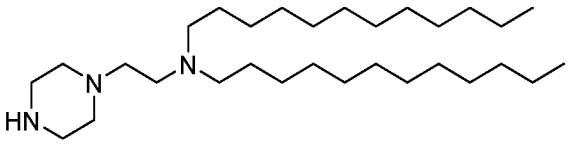
[00639] A mixture of 1-bromododecane (1.1 mL, 4.6 mmol), 4-(2-aminoethyl)-1-boc-piperazine (1.0 g, 4.4 mmol), K2CO3 (0.61 g, 4.4 mmol), in 10 mL MeCN was allowed to stir at rt for 12 h. After this time the reaction was filtered and concentrated. The crude material was

purified by silica gel chromatography (0-20% MeOH in DCM with 1% NH4OH to afford *tert*-butyl 4-(2-(didodecylamino)ethyl)piperazine-l-carboxylate (450 mg, 0.80 mmol, 18%).

UPLC/ELSD: RT = 2.87 min. MS (ES): *m/z* (MH^+) 566.655 for C35H71N3O2

¾ NMR (400 MHz, CDCh) δ: ppm 3.40 (m, 4H); 2.56 (m, 2H); 2.40 (m, 10H); 1.44 (s, 9H); 1.40-1.24 (m, 40H); 0.86 (t, 6H).

Step 2: *N*-Dodecyl-*N*-(2-(piperazin-l-yl)ethyl)dodecan-l-amine



Chemical Formula: C30H63N3

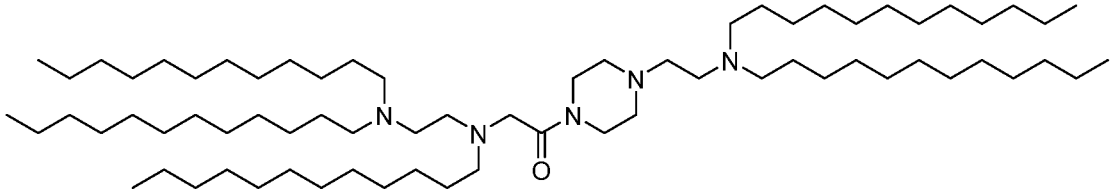
Molecular Weight: 465.86

[00640] In the same manner as Step 5 for Compound 18, *N*-dodecyl-*N*-(2-(piperazin-l-yl)ethyl)dodecan-l-amine was synthesized from *tert*-butyl 4-(2-(didodecylamino)ethyl)piperazine-l-carboxylate (0.92 g, 1.63 mmol), TFA (6.2 mL, 82 mmol) in 6 mL DCM to afford 490 mg for 65% yield.

UPLC/ELSD: RT = 2.10 min. MS (ES): *m/z* (MH^+) 466.379 for C30H63N3

¾ NMR (400 MHz, CDCh) δ: ppm 2.88 (t, 4H); 2.61 (m, 2H); 2.45 (m, 10H); 1.43-1.24 (m, 40H); 0.86 (t, 6H).

Step 3: Compound 2: 2-((2-(Didodecylamino)ethyl)(dodecyl)amino)-l-(4-(2-(didodecylamino)ethyl)piperazin-1-yl)ethan-1-one



Chemical Formula: C70H143N5O

Molecular Weight: 1070.95

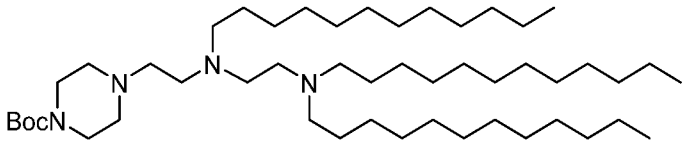
In the same manner as Step 11 for Compound 11, 2-((2-(didodecylamino)ethyl)(dodecyl)amino)-1-(4-(2-(didodecylamino)ethyl)piperazin-1-yl)ethan-1-one was synthesized from *N*-dodecyl-*N*-(2-(piperazin-l-yl)ethyl)dodecan-l-amine (32 mg, 0.069 mmol), *N*-(2-(didodecylamino)ethyl)-*N*-dodecylglycine (43 mg, 0.069 mmol), propylphosphonic acid anhydride (50% EtOAc solution, 0.12 mL, 0.21 mmol) and z-Pr_2EtN (0.024 mL, 0.14 mmol) in 0.5 mL THF to provide 17.7 mg (17%).

UPLC: RT = 3.90 min. MS (ES): *m/z* (MH^+) 1071.475 for C70H143N5O

¾ NMR (400 MHz, CDCh) δ: ppm 3.65 (m, 2H); 3.57 (m, 2H); 3.26 (s, 2H); 2.33-2.57 (m, 22H); 1.24-1.39 (m, 100H); 0.88 (t, 15H).

D. Compound 3: 2-(Didodecylamino)-l-(4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazin-l-yl)ethan-l-one

Step 1: *tert*-Butyl 4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazine-l-carboxylate



Chemical Formula: C49H100N4O2

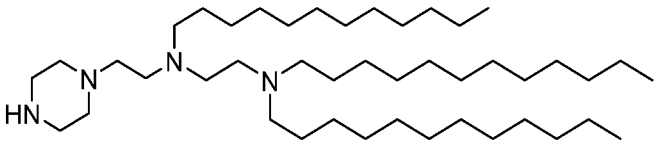
Molecular Weight: 777.37

[00641] In the same manner as Step 4 for Compound 18, *tert*-butyl 4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazine-1-carboxylate was synthesized from *tert*-butyl 4-(2-(dodecylamino)ethyl)piperazine-l-carboxylate (700 mg, 1.76 mmol), *N*-(2-chloroethyl)-*N*-dodecyldodecan-l-amine (806 mg, 1.93 mmol), K2CO3 (486 mg, 3.52 mmol), and KI (29 mg, 0.176 mmol) in THF (15 mL). Yield (683 mg, 50%).

UPLC/ELSD: RT = 3.35 min. MS (ES): *m/z* (MH^+) 778.16 for C49H100N4O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.44 (t, 4H); 3.11-2.86 (br. m, 4H); 2.78-2.32 (br. m, 14H); 1.80-1.05 (br. m, 69H); 0.91 (t, 9H).

Step 2: N^1,N^1,N^2-Tridodecyl-N^2-(2-(piperazin-1-yl)ethyl)ethane-1,2-diamine



Chemical Formula: C44H92N4

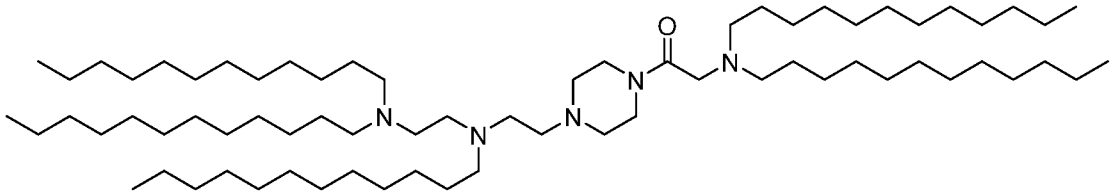
Molecular Weight: 677.25

[00642] In the same manner as Step 5 for Compound 18, N^1,N^1,N^2-tridodecyl-N^2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine was synthesized from *tert*-butyl 4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazine-l-carboxylate (683 mg, 0.879 mmol), and TFA (3.4 mL, 43.9 mmol) in DCM (3.4 mL). Yield (595 mg, 99%).

UPLC/ELSD: RT = 2.94 min. MS (ES): *m/z* (MH^+) 678.16 for C44H92N4

Step 3: Compound 3:

2-(Didodecylamino)-l-(4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazin-l-yl)ethan-1-one



Chemical Formula: C70H143N5O

Molecular Weight: 1070.95

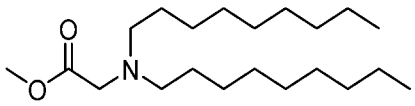
[00643] In the same manner as Step 11 for Compound 11, 2-(didodecylamino)-l-(4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazin-l-yl)ethan-l-one was from N^1,N^1,^-tridodecyl-N^2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine (50 mg, 0.074 mmol), lithium didodecylglycine (33 mg, 0.078 mmol), propylphosphonic acid anhydride (50% in EtOAc, 0.13 mL, 0.22 mmol) and z-PnEtN (0.026 mL) in 0.5 mL THF to afford 33.9 mg (43%).

UPLC: RT = 3.90 min. MS (ES): *m/z* (MH^+) 1071.475 for C70H143N5O

¾ NMR (400 MHz, CDCh) δ: ppm 3.65 (m, 2H); 3.57 (m, 2H); 3.26 (s, 2H); 2.33-2.57 (m, 22H); 1.24-1.39 (m, 100H); 0.88 (t, 15H).

E. Compound 4: 2-(Dinonylamino)-l-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)ethan-l-one

Step 1: Methyl dinonylglycinate



Chemical Formula: C21H43NO2

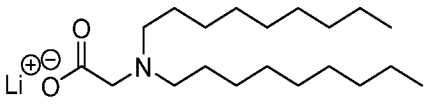
Molecular Weight: 341.58

[00644] In the same manner as Step 1 for Compound 11, methyl dinonylglycinate was synthesized from glycine methyl ester hydrochloride (5.0 g, 39.8 mmol), triethylamine (8.3 mL, 59.7 mmol), 95% nonanal (15.0 g, 99.6 mmol), sodium triacetoxyborohydride (21.1 g, 99.6 mmol), and acetic acid (5.7 mL, 99.6 mmol) in DCE (50 mL). Yield (3.5 g, 26%).

UPLC/ELSD: RT = 1.82 min. MS (ES): *m/z* (MH^+) 343.62 for C21H43NO2

^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.72 (s, 3H); 3.35 (s, 2H); 2.57 (t, 4H); 1.46 (br. m, 4H); 1.29 (br. m, 24H); 0.90 (t, 6H).

Step 2: Lithium dinonylglycinate



Chemical Formula: C20H40L1NO2

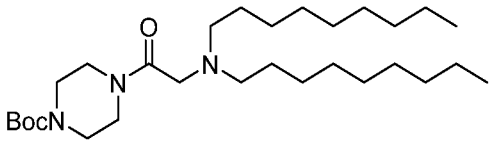
Molecular Weight: 333.49

[00645] In the same manner as Step 2 for Compound 11, lithium dinonylglycinate was synthesized from methyl dinonylglycinate (3.5 g, 10.2 mmol) and 1M LiOH (50 mL, 50 mmol) in THF (50 mL). Yield (3.0 g, 88%).

UPLC/ELSD: RT = 1.71 min. MS (ES): *m/z* (MH^+) 328.37 for C20H41NO2

¾-NMR (300 MHz, CD3OD) δ: ppm 3.13 (s, 2H); 2.59 (t, 4H); 1.51 (br. m, 4H); 1.32 (br. m, 24H); 0.92 (t, 6H).

Step 3: *tert*-Butyl 4-(dinonylglycyl)piperazine-l-carboxylate



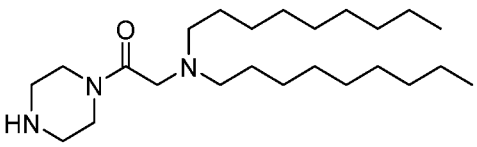
Chemical Formula: C29H57N3O3

Molecular Weight: 495.79

[00646] In the same manner as Step 3 for Compound 11, /er/-butyl 4-(dinonylglycyl)piperazine-l-carboxylate was synthesized from lithium dinonylglycinate (2.0 g, 6.00 mmol), 1-boc-piperazine (1.23 g, 6.58 mmol), *i*-Pr_2EtN (2.3 mL, 13.2 mmol), and propylphosphonic acid anhydride (50% EtOAc solution, 10.7 mL, 17.9 mmol). Yield (824 mg, 28%).

UPLC/ELSD: RT = 2.19 min. MS (ES): *m/z* (MH^+) 496.72 for C29H57N3O3

Step 4: 2-(Dinonylamino)-l-(piperazin-l-yl)ethan-l-one



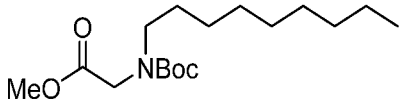
Chemical Formula: C24H49N3O

Molecular Weight: 395.68

[00647] In the same manner as Step 4 for Compound 11, 2-(dinonylamino)-1-(piperazin-1-yl)ethan-1-onewas synthesized from *tert*-butyl 4-(dinonylglycyl)piperazine-1-carboxylate (824 mg, 1.66 mmol) and TFA (6.4 mL, 83.1 mmol) in DCM (6.4 mL). Yield (246 mg, 37%). UPLC/ELSD: RT = 1.25 min. MS (ES): *m/z* (MH^+) 396.68 for $C_{24}H_{49}N_3O$

¾-NMR (300 MHz, CDCh) δ: ppm 3.63 (br. m, 4H); 3.28 (s, 2H); 2.89 (br. m, 4H); 2.48 (t, 4H); 1.45 (br. m, 4H); 1.28 (br. m, 24H); 0.90 (t, 6H).

Step 5: Methyl *N*-(fer/-butoxycarbonyl)-*N*-nonylglycinate



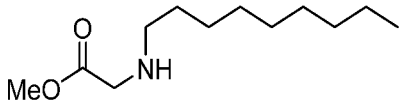
Chemical Formula: $C_{17}H_{33}NO_4$

Molecular Weight: 315.45

[00648] In the same manner as Step 5 for Compound 11, methyl *N*-(fer/-butoxycarbonyl)-*N*-nonylglycinate was synthesized from *N*-(fer/-butoxycarbonyl)glycine methyl ester (7.7 g, 40.7 mmol) and NaH (60%, 1.71 g, 42.7 mmol) in DMF (100 mL). Yield (3.32 g, 26%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.02-3.84 (br. m, 2H); 3.75 (s, 3H); 3.26 (br. m, 2H); 1.65-1.39 (br. m, 11H); 1.28 (br. m, 12H); 0.90 (t, 3H).

Step 6: Methyl nonylglycinate



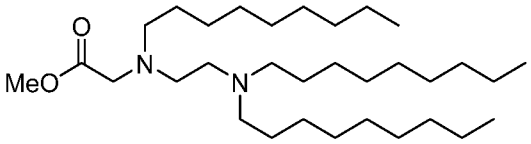
Chemical Formula: $C_{12}H_{25}NO_2$

Molecular Weight: 215.34

[00649] In the same manner as Step 6 for Compound 11, methyl nonylglycinate was synthesized from methyl *N*-(fer/-butoxycarbonyl)-*N*-nonylglycinate (3.32 g, 10.5 mmol) and TFA (16 mL, 210 mmol) in DCM (16 mL). Yield (2.23 g, 98%).

¾-NMR (300 MHz, CDCh) δ: ppm 3.75 (s, 3H); 3.44 (s, 2H); 2.61 (t, 2H); 1.69 (br. m, 1H); 1.51 (br. m, 2H); 1.28 (br. m, 12H); 0.90 (t, 3H).

Step 7: Methyl *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycinate

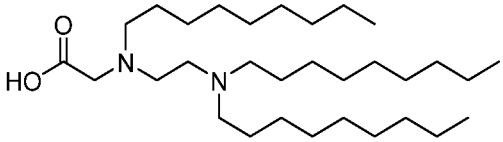


Chemical Formula: C32H66N2O2

Molecular Weight: 510.89

[00650] In the same manner as Step 9 for Compound 11, methyl *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycinate was synthesized from methyl nonylglycinate (449 mg, 2.08 mmol), *N*-(2-chloroethyl)-*N*-nonylnonan-1-amine (830 mg, 2.50 mmol), K2CO3 (576 mg, 4.16 mmol), and KI (35 mg, 0.208 mmol) in MeCN (13 mL). Yield (958 mg, 90%). UPLC/ELSD: RT = 3.11 min. MS (ES): *m/z* (MH^+) 511.97 for C32H66N2O2 ¾-NMR (300 MHz, CDCh) δ: ppm 3.72 (s, 3H); 3.42 (s, 2H); 2.95-2.15 (br. m, 10H); 1.85-1.00 (br. m, 42H); 0.90 (t, 9H).

Step 8: *N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycine

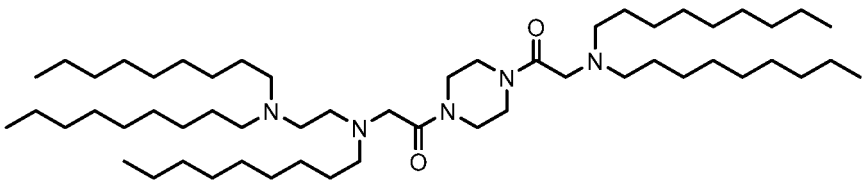


Chemical Formula: C31H64N2O2

Molecular Weight: 496.87

[00651] In the same manner as Step 10 for Compound 11, *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine was synthesized from methyl *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycinate (958 mg, 1.88 mmol), and 1M LiOH (10 mL, 10 mmol) in THF (10 mL). Yield (514 mg, 55%). UPLC/ELSD: RT = 2.75 min. MS (ES): *m/z* (MH^+) 497.95 for C31H64N2O2 ¾-NMR (300 MHz, CDCh) δ: ppm 3.92 (br. m, 6H); 3.14 (br. m, 6H); 1.77 (br. m, 6H); 1.45-1.13 (br. m, 36H); 0.90 (t, 9H).

Step 9: Compound 4: 2-(Dinonylamino)-1-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)ethan-1-one



Chemical Formula: C55H111N5O2

Molecular Weight: 874.53

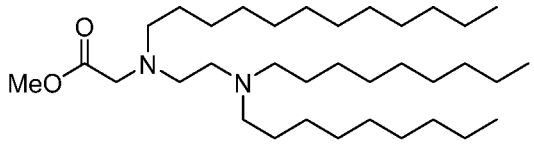
[00652] In the same manner as Step 11 for Compound 11, 2-(dinonylamino)-l-(4 -(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)ethan-l-one was synthesized from 2-(dinonylamino)-l-(piperazin-l-yl)ethan-l-one (61.5 mg, 0.155 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (85 mg, 0.171 mmol), *i*-Pr_2EtN (60 μL, 0.342 mmol), and propylphosphonic acid anhydride (50% EtOAc solution, 0.278 mL, 0.466 mmol). Yield (38 mg, 28%).

UPLC/ELSD: RT = 3.13 min. MS (ES): *m/z* (MH^+) 875.76 for **C55H111N5O2**

¾-NMR (300 MHz, CDCh) δ: ppm 3.82-3.49 (br. m, 8H); 3.33 (s, 2H); 3.27 (s, 2H); 2.68-2.18 (br. m, 14H); 1.82-1.02 (br. m, 70H); 0.90 (t, 15H).

F. Compound 5: 2-(Dinonylamino)-l-(4-(N-(2-(dinonylamino)ethyl)-N-dodecylgly cyl)piperazin- 1-yl)ethan- 1-one

Step 1: Methyl *N*-(2-(dinonylamino)ethyl)-*N*-dodecylglycinate



Chemical Formula: C35H72N2O2

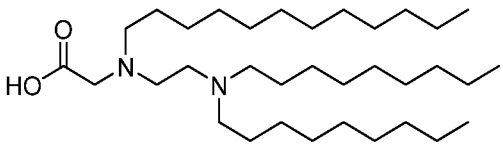
Molecular Weight: 552.97

[00653] In the same manner as Step 9 for Compound 11, methyl *N*-(2-(dinonylamino)ethyl)-*N*-dodecylglycinate was synthesized from methyl dodecylglycinate (535 mg, 2.08 mmol), *N*-(2-chloroethyl)-*N*-nonylnonan-l -amine (830 mg, 2.50 mmol), K2CO3 (576 mg, 4.16 mmol), and KI (35 mg, 0.208 mmol) in MeCN (13 mL). Yield (385 mg, 34%).

UPLC/ELSD: RT = 3.34 min. MS (ES): *m/z* (MH^+) 553.96 for C35H72N2O2

^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.72 (s, 3H); 3.41 (s, 2H); 2.90-2.20 (br. m, 10H); 1.85-1.05 (br. m, 48H); 0.90 (t, 9H).

Step 2: *N*-(2-(Dinonylamino)ethyl)-*N*-dodecylglycine



Chemical Formula: C34H70N2O2

Molecular Weight: 538.95

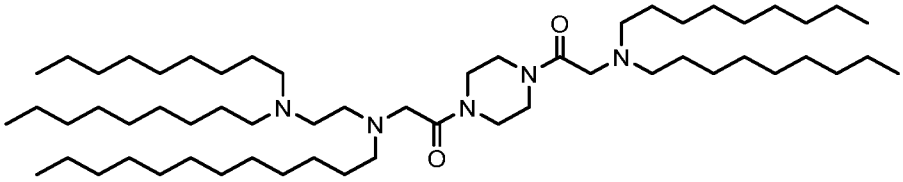
[00654] In the same manner as Step 10 for Compound 11, *N*-(2-(dinonylamino)ethyl)-*N*-dodecylglycine was synthesized from methyl *N*-(2-(dinonylamino)ethyl)-*N*-dodecylglycinate (385 mg, 0.696 mmol), and 1M LiOH (3.5 mL, 3.5 mmol) in THF (3.5 mL). Yield (225 mg, 60%).

UPLC/ELSD: RT = 3.13 min. MS (ES): *m/z* (MH^+) 539.93 for C34H70N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.73 (s, 2H); 3.62-3.39 (br. m, 4H); 3.09 (br. m, 6H); 1.76 (br. m, 6H); 1.28 (br, 42H); 0.90 (t, 9H).

Step 3: Compound 5:

2-(Dinonylamino)-1-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-dodecylglycyl)piperazin-1-yl)ethan-1-one



Chemical Formula: **C58H117N5O2**

Molecular Weight: 916.61

[00655] In the same manner as Step 11 for Compound 11, 2-(dinonylamino)-1-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-dodecylglycyl)piperazin-1-yl)ethan-1-one was synthesized from 2-(dinonylamino)-1-(piperazin-1-yl)ethan-1-one (62 mg, 0.155 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-dodecylglycine (92 mg, 0.171 mmol), *i*-Pr_2EtN (60 µL, 0.342 mmol), and propylphosphonic acid anhydride (50% EtOAc solution, 0.278 mL, 0.466 mmol). Yield (38 mg, 26%).

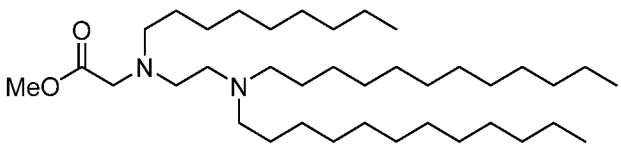
UPLC/ELSD: RT = 3.32 min. MS (ES): *m/z* (MH^+) 917.67 for **C58H117N5O2**

¾-NMR (300 MHz, CDCh) δ: ppm 3.86-3.45 (br. m, 8H); 3.33 (s, 2H); 3.28 (s, 2H); 2.73-2.27 (br. m, 14H); 1.86-1.00 (76H); 0.91 (t, 15H).

G. Compound 6:

2-((2-(Didodecylamino)ethyl)(nonyl)amino)-1-(4-(dinonylglycyl)piperazin-1-yl)ethan-1-one

Step 1: Methyl *N*-(2-(didodecylamino)ethyl)-*N*-nonylglycinate



Chemical Formula: **C38H78N2O2**

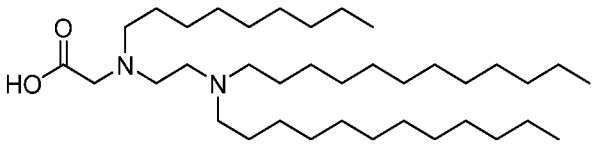
Molecular Weight: 595.05

[00656] In the same manner as Step 9 for Compound 11, methyl *N*-(2-(didodecylamino)ethyl)-*N*-nonylglycinate was synthesized from methyl nonylglycinate (355 mg, 1.65 mmol), *N*-(2-chloroethyl)-*N*-dodecyldodecan-1-amine (825 mg, 1.98 mmol), K_2CO_3 (457 mg, 3.30 mmol), and KI (27 mg, 0.165 mmol) in MeCN (10 mL). Yield (460 mg, 47%).

UPLC/ELSD: RT = 3.62 min. MS (ES): *m/z* (MH^+) 596.03 for $C_{38}H_{78}N_2O_2$

¾-NMR (300 MHz, CDCh) δ: ppm 3.72 (s, 3H); 3.42 (s, 2H); 2.80-2.24 (br. m, 10H); 1.56-1.00 (br. m, 54H); 0.90 (t, 9H).

Step 2: *N*-(2-(Didodecylamino)ethyl)-*N*-nonylglycine



Chemical Formula: $C_{37}H_{76}N_2O_2$

Molecular Weight: 581.03

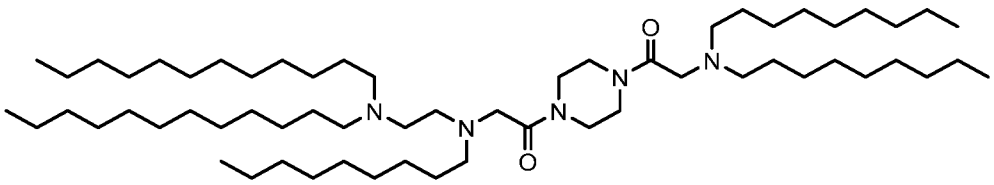
[00657] In the same manner as Step 10 for Compound 11, *N*-(2-(didodecylamino)ethyl)-*N*-nonylglycine was synthesized from methyl *N*-(2-(didodecylamino)ethyl)-*N*-nonylglycinate (460 mg, 0.773 mmol), and 1M LiOH (3.9 mL, 3.9 mmol) in THF (3.9 mL). Yield (323 mg, 72%).

UPLC/ELSD: RT = 3.37 min. MS (ES): *m/z* (MH^+) 582.00 for $C_{37}H_{76}N_2O_2$

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.17 (s, 2H); 4.00 (br. m, 2H); 3.84 (br. m, 2H); 3.34 (br. m, 2H); 3.18 (br. m, 4H); 1.82 (br. m, 6H); 1.27 (br. m, 48H); 0.91 (t, 9H).

Step 3: Compound 6:

2-((2-(Didodecylamino)ethyl)(nonyl)amino)-1-(4-(dinonylglycyl)piperazin-1-yl)ethan-1-one



Chemical Formula: $C_{61}H_{123}N_5O_2$

Molecular Weight: 958.69

[00658] In the same manner as Step 11 for Compound 11, 2-((2-(didodecylamino)ethyl)(nonyl)amino)-1-(4-(dinonylglycyl)piperazin-1-yl)ethan-1-one was

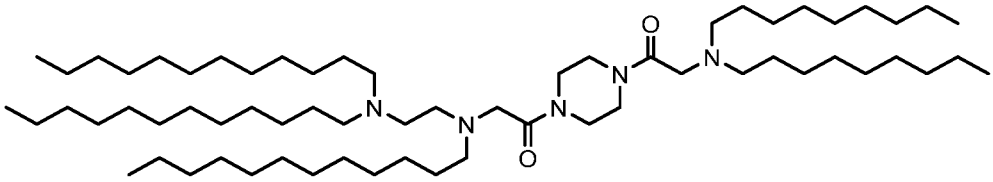
synthesized from 2-(dinonylamino)-l-(piperazin-l-yl)ethan-l-one (62 mg, 0.155 mmol), *N*-(2-(didodecylamino)ethyl)-*N*-nonylglycine (99 mg, 0.171 mmol), z-PrcEtN (60 μL, 0.342 mmol), and propylphosphonic acid anhydride (50% EtOAc solution, 0.278 mL, 0.466 mmol). Yield (45 mg, 30%).

UPLC/ELSD: RT = 3.46 min. MS (ES): *m/z* (MH^+) 959.98 for $C_{6}iH_{123}N_5O_2$

¾-NMR (300 MHz, CDCh) δ: ppm 3.81-3.49 (br. m, 8H); 3.33 (s, 2H); 3.27 (s, 2H); 2.70-2.25 (br. m, 14H); 1.90-1.00 (br. m, 82H); 0.90 (t, 15H).

H. Compound 7:

2-((2-(Didodecylamino)ethyl)(dodecyl)amino)-1-(4-(dinonylglycyl)piperazin-1-yl)ethan-1-one



Chemical Formula: C64H129N5O2

Molecular Weight: 1000.77

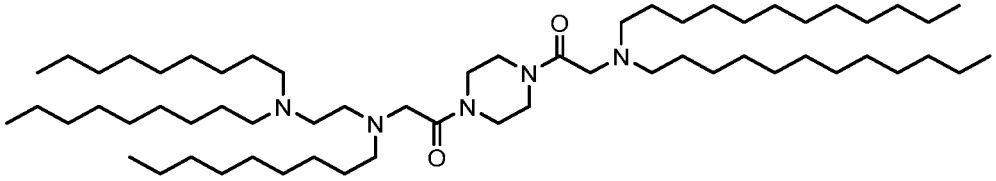
[00659] In the same manner as Step 11 for Compound 11, 2-((2-(didodecylamino)ethyl)(dodecyl)amino)-1-(4-(dinonylglycyl)piperazin-1-yl)ethan-1-one was synthesized from 2-(dinonylamino)-l-(piperazin-l-yl)ethan-l-one (62 mg, 0.155 mmol), *N*-(2-(didodecylamino)ethyl)-*N*-dodecylglycine (107 mg, 0.171 mmol), *i*-Pr_2EtN (60 μL, 0.342 mmol), and propylphosphonic acid anhydride (50% EtOAc solution, 0.278 mL, 0.466 mmol). Yield (34 mg, 20%).

UPLC/ELSD: RT = 3.60 min. MS (ES): *m/z* (MH^+) 1001.97 for C64H129N5O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.85-2.18 (br. m, 26H); 1.91-1.00 (br. m, 88H); 0.90 (t, 15H).

I. Compound 8:

2-(Didodecylamino)-1-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)ethan-1-one



Chemical Formula: C61H123N5O2

Molecular Weight: 958.69

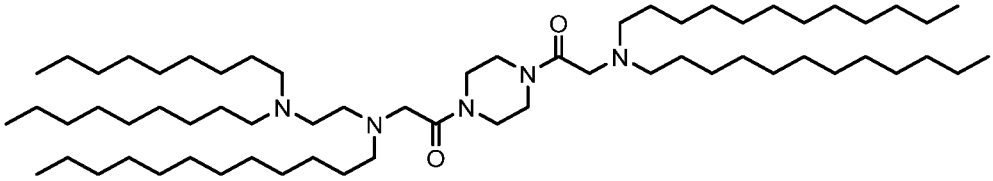
[00660] In the same manner as Step 11 for Compound 11, 2-(didodecylamino)-1-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)ethan-1-one was synthesized from 2-(didodecylamino)-1-(piperazin-1-yl)ethan-1-one (202 mg, 0.421 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (230 mg, 0.463 mmol), z-PnEtN (0.162 mL, 0.926 mmol), and propylphosphonic acid anhydride (50% EtOAc solution, 0.752 mL, 1.26 mmol). Yield (148 mg, 37%).

UPLC/ELSD: RT = 3.41 min. MS (ES): *m/z* (MH^+) 959.74 for $C_{61}H_{123}N_5O_2$

¾-NMR (300 MHz, CDCh) δ: ppm 3.82-3.49 (br. m, 8H); 3.33 (s, 2H); 3.27 (s, 2H); 2.66-2.30 (br. m, 14H); 1.85-1.02 (br. m, 82H), 0.90 (t, 15H).

J. Compound 9:

2-(Didodecylamino)-1-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-dodecylglycyl)piperazin-1-yl)ethan-1-one



Chemical Formula: C64H129N5O2

Molecular Weight: 1000.77

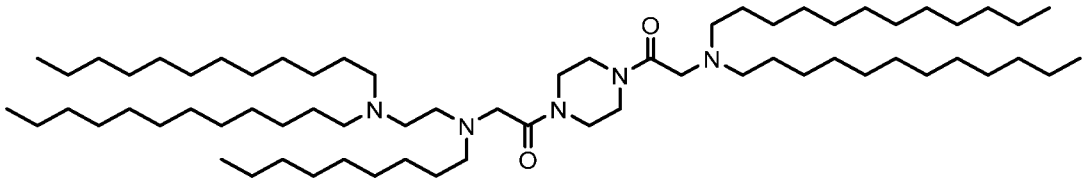
[00661] In the same manner as Step 11 for Compound 11, 2-(didodecylamino)-1-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-dodecylglycyl)piperazin-1-yl)ethan-1-one was synthesized from (76 mg, 0.157 mmol), (93 mg, 0.173 mmol), *i*-Pr_2EtN (60 μL, 0.342 mmol), and propylphosphonic acid anhydride (50% EtOAc solution, 0.278 mL, 0.466 mmol). Yield (59 mg, 37%).

UPLC/ELSD: RT = 3.57 min. MS (ES): *m/z* (MH^+) 1001.65 for C64H129N5O2

^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.95-2.23 (br. m, 26H); 2.05-1.00 (br. m, 88H); 0.90 (t, 15H).

K. Compound 10:

2-(Didodecylamino)-1-(4-(*N*-(2-(didodecylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)ethan-1-one



Chemical Formula: C67H135N5O2

Molecular Weight: 1042.85

[00662] In the same manner as Step 11 for Compound 11, 2-(didodecylamino)-l-(4 -*(N*-(2-(didodecylamino)ethyl *)-N*-nonylglycyl)piperazin-l-yl)ethan-l-one was synthesized from 2-(didodecylamino)-l-(piperazin-l-yl)ethan-l-one (76 mg, 0.157 mmol), *N*-(2-(didodecylamino)ethyl *)-N*-nonylglycine (101 mg, 0.173 mmol), *i*-Pr_2EtN (60 μL, 0.342 mmol), and propylphosphonic acid anhydride (50% EtOAc solution, 0.278 mL, 0.466 mmol). Yield (56 mg, 34%).

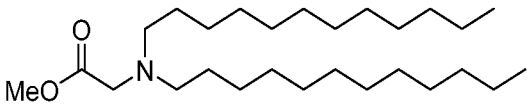
UPLC/ELSD: RT = 3.72 min. MS (ES): *m/z* (MH^+) 1043.88 for C67H135N5O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.95-2.15 (br. m, 26H); 1.90-1.05 (br. m, 94H); 0.90 (t, 15H).

L. Compound 11:

2-(Didodecylamino)-l-(4 -*(N*-(2-(didodecylamino)ethyl *)-N*-dodecylglycyl)piperazin-l-yl)ethan-1-one

Step 1: Methyl didodecylglycinate



Chemical Formula: C27H55NO2

Molecular Weight: 425.74

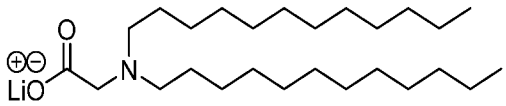
[00663] A solution of glycine methyl ester hydrochloride (5.0 g, 39.8 mmol) and triethylamine (8.3 mL, 59.7 mmol) in DCE (50 mL) was allowed to stir at room temperature for 15 minutes. A solution of 92% dodecanol (20.0 g, 99.6 mmol) in DCE (50 mL) was added and the mixture was cooled to 0 °C. Sodium triacetoxyborohydride (21.1 g, 99.6 mmol) and acetic acid (5.7 mL, 99.6 mmol) were added and the reaction was allowed to return to room temperature and stir for 16 hours. The reaction was quenched by slow addition of saturated sodium bicarbonate and extracted with DCM. The combined extracts were washed with brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica

flash chromatography (0-30% EtOAc/hexanes) provided methyl didodecylglycinate (7.7 g, 45%).

UPLC/ELSD: RT = 2.82 min. MS (ES): *m/z* (MH^+) 426.69 for C27H55NO2

¾-NMR (300 MHz, CDCh) δ: ppm 3.72 (s, 3H); 3.35 (s, 2H); 2.57 (t, 4H); 1.46 (m, 4H); 1.28 (br. m, 36H); 0.91 (t, 6H).

Step 2: Lithium didodecylglycinate



Chemical Formula: C26H52L1NO2

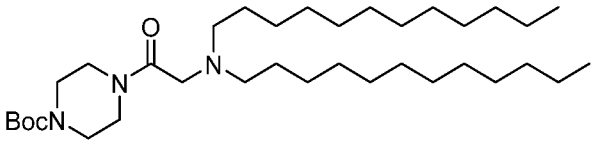
Molecular Weight: 417.65

[00664] A solution of methyl didodecylglycinate (7.7 g, 18.1 mmol) in THF (100 mL) and 1M LiOH (90.4 mL, 90.4 mmol) was allowed to stir at 65 °C for 16 hours. The reaction was cooled to room temperature and concentrated to a white powder. The powder was suspended in water, filtered, washed with water and diethyl ether, and dried under vacuum to provide lithium didodecylglycinate (7.0 g, 93%).

UPLC/ELSD: RT = 2.74 min. MS (ES): *m/z* (MH^+) 412.83 for C26H53NO2

^{1}H-NMR (300 MHz, CD3OD) δ: ppm 3.14 (s, 2H); 2.60 (t, 4H); 1.51 (m, 4H); 1.31 (br. m, 36H); 0.92 (t, 6H).

Step 3: /er/-Butyl 4-(didodecylglycyl)piperazine-l-carboxylate



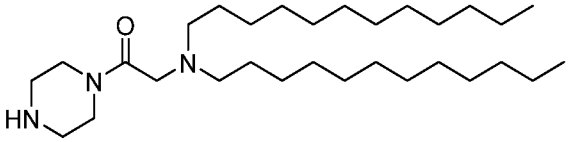
Chemical Formula: C35H69N3O3

Molecular Weight: 579.96

[00665] A solution of lithium didodecylglycinate (2.0 g, 4.79 mmol), 1-boc-piperazine (978 mg, 5.25 mmol), *i*-Pr_2EtN (1.84 mL, 10.5 mmol), and propylphosphonic acid anhydride (50% EtOAc solution, 8.53 mL, 14.3 mmol) in THF (24 mL) was allowed to stir at room temperature for 48 hours. The reaction was diluted with water and extracted with EtOAc. The organics were washed with brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo*. Purification by ISCO silica flash chromatography (0-20% MeOH/DCM) provided *tert*-butyl 4-(didodecylglycyl)piperazine-l-carboxylate (983 mg, 35%).

UPLC/ELSD: RT = 3.06 min. MS (ES): *m/z* (MH^+) 581.02 for C35H69N3O3

Step 4: 2-(Didodecylamino)-l-(piperazin-l-yl)ethan-l-one



Chemical Formula: C30H61N3O

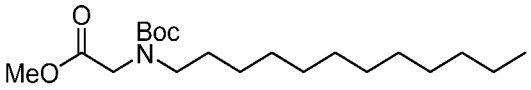
Molecular Weight: 479.84

[00666] To a 0 °C solution of *tert*-butyl 4-(didodecylglycyl)piperazine-l-carboxylate (983 mg, 1.69 mmol) in DCM (6.5 mL) was added dropwise TFA (6.5 mL, 84.7 mmol). The reaction was allowed to return to room temperature and stir for 16 hours. The reaction mixture was concentrated *in vacuo* and the crude material was dissolved in CHCh. The solution was washed with 5% Na2C03, brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo* to provide 2-(didodecylamino)-l-(piperazin-l-yl)ethan-l-one (163 mg, 20%).

UPLC/ELSD: RT = 2.07 min. MS (ES): *m/z* (MH^+) 480.89 for C30H61N3O

Ťi-NMR (300 MHz, CDCh) δ: ppm 3.67 (br. m, 4H); 3.32 (s, 2H); 2.92 (br. m, 4H); 2.53 (br. m, 4H); 1.48 (br. m, 4H); 1.28 (br. m, 36H); 0.91 (t, 6H).

Step 5: Methyl *N*-(/er/-butoxycarbonyl *)-N*-dodecylglycinate



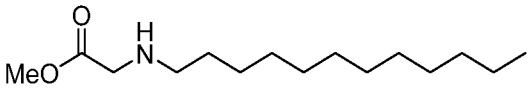
Chemical Formula: C20H39NO4

Molecular Weight: 357.54

[00667] A 0 °C solution of *N*-(fert-butoxycarbonyl)glycine methyl ester (7.7 g, 40.7 mmol) in DMF (100 mL) was treated with NaH (60%, 1.71 g, 42.7 mmol) and the mixture was allowed to stir for 30 minutes. The solution was allowed to return to room temperature before 1-bromododecane (15.2 g, 61.0 mmol) was added. The reaction was quenched with water and extracted with EtOAc. The organics were washed with brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-20% EtOAc/hexanes) provided methyl *N*-(/er/-butoxycarbonyl *)-N*-dodecylglycinate (4.03 g, 28%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.01-3.84 (br. m, 2H); 3.75 (s, 3H); 3.27 (br. m, 2H); 1.67-1.39 (br. m, 11H); 1.28 (br. m, 18H); 0.90 (t, 3H).

Step 6: Methyl dodecylglycinate



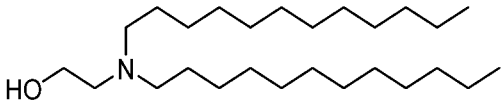
Chemical Formula: C15H31NO2

Molecular Weight: 257.42

[00668] To a 0 °C solution of methyl N-(tert-butoxycarbonyl)-N-dodecylglycinate (4.03 g, 11.3 mmol) in DCM (17 mL) was added dropwise TFA (17 mL, 226 mmol). The reaction was allowed to return to room temperature and stir for 6 hours. The reaction mixture was concentrated in vacuo and the crude material was dissolved in DCM. The solution was washed with 10% NaOH, brine, dried over anhydrous Na2S04, filtered, and concentrated in vacuo to provide methyl dodecylglycinate (2.84 g, 98%).

Ṫi-NMR (300 MHz, CDCh) δ: ppm 3.75 (s, 3H); 3.44 (s, 2H); 2.62 (t, 2H); 1.70 (br, 1H); 1.51 (m, 2H); 1.29 (br. m, 18H); 0.90 (t, 3H).

Step 7: 2-(Didodecylamino)ethan-l-ol



Chemical Formula: C26H55NO

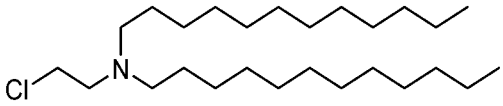
Molecular Weight: 397.73

[00669] In the same manner as Step 1 for Compound 18, 2-(didodecylamino)ethan-l-ol was synthesized from 1-bromododecane (10 g, 40.1 mmol), ethanolamine (1.10 mL, 18.2 mmol), K2CO3 (11.1 g, 80.1 mmol), and KI (302 mg, 1.82 mmol) in MeCN (84 mL). Yield (3.87 g, 53%).

UPLC/ELSD: RT = 2.69 min. MS (ES): *m/z* (MH^+) 398.56 for C26H55NO

¾-NMR (300 MHz, CDCh) δ: ppm 3.57 (t, 2H); 2.63 (t, 2H); 2.49 (br. m, 4H); 1.48 (br. m, 4H); 1.29 (br. m, 36H); 0.91 (t, 6H).

Step 8: *N*-(2-Chloroethyl)-*N*-dodecyldodecan-l-amine



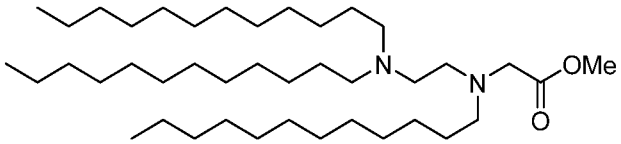
Chemical Formula: C26H54CIN

Molecular Weight: 416.18

[00670] In the same manner as Step 2 for Compound 18, *N*-(2-chloroethyl *)-N*-dodecyldodecan-1 -amine was synthesized from 2-(didodecylamino)ethan-l-ol (3.87 g, 9.73 mmol), triethylamine (1.76 mL, 12.6 mmol), and methanesulfonyl chloride (0.941 mL, 12.2 mmol) in DCM (50 mL). Yield (1.92 g, 47%).

¾-NMR (300 MHz, CDCh) δ: ppm 3.51 (t, 2H); 2.78 (t, 2H); 2.47 (br. m, 4H); 1.44 (br. m, 4H); 1.28 (br. m, 36H); 0.90 (t, 6H).

Step 9: Methyl *N*-(2-(didodecylamino)ethyl *)-N*-dodecylglycinate



Chemical Formula: C41H84N2O2

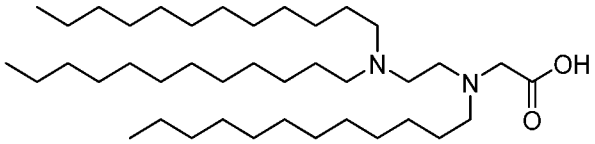
Molecular Weight: 637.14

[00671] To a solution of methyl dodecylglycinate (425 mg, 1.65 mmol) in MeCN (10 mL) was added *N*-(2-chloroethyl *)-N*-dodecyldodecan-l -amine (825 mg, 1.98 mmol), K2CO3 (457 mg, 3.30 mmol), and KI (27 mg, 0.165 mmol). The reaction was allowed to stir at 82 °C for 72 hours. The reaction mixture was filtered and the solids were washed with hexanes. The filtrate was concentrated *in vacuo* to provide the crude product. Purification by ISCO silica flash chromatography (0-20% MeOH/DCM) provided methyl *N*-(2-(didodecylamino)ethyl *)-N*-dodecylglycinate (652 mg, 62%).

UPLC/ELSD: RT = 3.77 min. MS (ES): *m/z* (MH^+) 638.18 for C41H84N2O2

Ṫi-NMR (300 MHz, CDCh) δ: ppm 3.72 (s, 3H); 3.41 (s, 2H); 2.90-2.20 (br. m, 10H); 1.60-1.00 (br. m, 60H); 0.90 (t, 9H).

Step 10: *N*-(2-(Didodecylamino)ethyl *)-N*-dodecylglycine



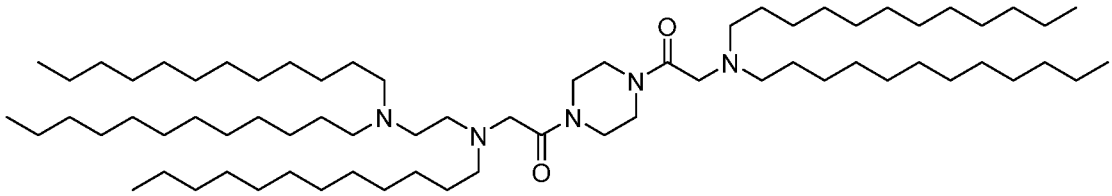
Chemical Formula: C40H82N2O2

Molecular Weight: 623.11

[00672] A solution of methyl *N*-(2-(didodecylamino)ethyl *)-N*-dodecylglycinate (652 mg, 1.02 mmol) in THF (6 mL) and 1M LiOH (5 mL, 5 mmol) was allowed to stir at 65 °C for 16 hours. The reaction was cooled to room temperature and acidified with 10% HCl. The mixture

was extracted with chloroform, and the organics were washed with brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo*. Purification by ISCO silica flash chromatography (0-20% MeOH/DCM) provided *N*-(2-(didodecylamino)ethyl *)-N*-dodecylglycine (153 mg, 24%).
UPLC/ELSD: RT = 3.60 min. MS (ES): *m/z* (MH^+) 624.07 for C40H82N2O2
¾-NMR (300 MHz, CDCh) δ: ppm 4.02-3.40 (br. m, 6H); 3.16 (br. m, 6H); 1.78 (br. m, 6H); 1.46-1.01 (br. m, 54H); 0.90 (t, 9H).

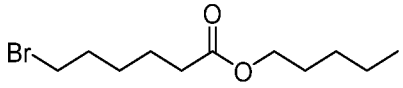
Step 11: Compound 11: 2-(Didodecylamino)-l-(4 *-(N*-(2-(didodecylamino)ethyl *)-N*-dodecylgly cyl)piperazin- 1-yl)ethan- 1-one



Chemical Formula: C70H141N5O2
Molecular Weight: 1084.93

[00673] To a solution of *N*-(2-(didodecylamino)ethyl *)-N*-dodecylglycine (212 mg, 0.340 mmol) and 2-(didodecylamino)-l-(piperazin-l-yl)ethan-l-one (163 mg, 0.340 mmol) in THF (4 mL) was added z-PnEtN (0.119 mL, 0.680 mmol), and propylphosphonic acid anhydride (50% EtOAc solution, 0.606 mL, 1.02 mmol). The reaction was allowed to stir at room temperature overnight. The reaction mixture was diluted with water and extracted with EtOAc. The organics were washed with brine, dried over anhydrous Na2S04, and concentrated *in vacuo*. Purification by ISCO silica flash chromatography (0-100% [DCM, 20% MeOH, 1% NH4OH] /MeOH) provided 2-(didodecylamino)-l-(4 *-(N*-(2-(didodecylamino)ethyl *)-N*-dodecylglycyl)piperazin-l-yl)ethan-l-one (148 mg, 37%).
UPLC/ELSD: RT = 3.81 min. MS (ES): *m/z* (MH^+) 1086.94 for C70H141N5O2
¾-NMR (300 MHz, CDCh) δ: ppm 4.00-2.20 (br. m, 26H); 1.77 (br. m, 6H); 1.54-1.02 (br. m, 94H); 0.90 (t, 15H).

M. Compound 12: Pentyl 6-((2-(4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazin-l-yl)ethyl)(dodecyl)amino)hexanoate
Step 1: Pentyl 6-bromohexanoate



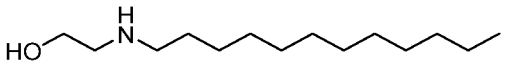
Chemical Formula: $C_{11}H_{21}BrO_2$

Molecular Weight: 265.19

[00674] To a solution of 6-bromohexanoic acid (2 g, 10.3 mmol) and pentan-l-ol (2.2 mL, 20.5 mmol) in 26 mL DCM, EDC-HCl (1.97 g, 10.3 mmol) and DMAP (0.26 g, 2.1 mmol) were added. The solution was allowed to stir at rt overnight. After this time the reaction was quenched by the addition of water. The mixture was extracted three times with DCM. The organics were pooled and washed with saturated NaHCCb, 10% citric acid and brine. The organics were then then dried over $MgSO_4$, filtered and concentrated in vacuo. The crude material was purified via silica gel chromatography (0-30% EtOAc in hexanes) to afford the desired product (2.3 g, 8.67 mmol).

¾ NMR (400 MHz, CDCh) δ: ppm 4.06 (t, 2H); 3.39 (t, 2H); 2.30 (t, 2H); 1.84 (m, 2H); 1.62 (m, 4H); 1.46 (m, 2H); 1.31 (m, 4H); 0.88 (t, 3H).

Step 2: 2-(Dodecylamino)ethan-l-ol



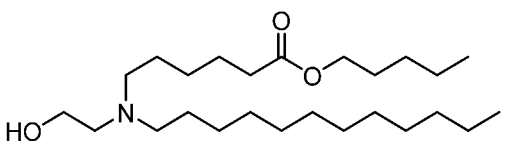
Chemical Formula: C14H31NO

Molecular Weight: 229.41

[00675] Methyl dodecylglycinate (3.4 g, 13.2 mmol) was dissolved in 2 mL THF under N_2 atmosphere and the reaction flask was allowed to cool in an ice bath. To the solution $LiAlH_4$ (0.55 g, 14.5 mmol) was slowly added. The reaction was allowed to stir at the same temperature for 1 h. After this time the reaction was quenched by the subsequent addition of 0.55 mL H_2O, 0.55 mL 10% NaOH and then 1.65 mL of H_2O. The reaction was then filtered and the filtrate was concentrated *in vacuo.* The crude material was purified *via* silica gel chromatography (0-20% MeOH in DCM, with 1% NaOH) to afford the desired alcohol (1.9 g, 8.28 mmol, 63% yield).

¾ NMR (400 MHz, CDCh) δ: ppm 3.63 (t, 2H); 2.78 (t, 2H); 2.63 (t, 2H); 1.48 (m, 2H); 2.14 (m, 18H); 0.88 (t, 3H).

Step 3: Pentyl 6-(dodecyl(2-hydroxyethyl)amino)hexanoate



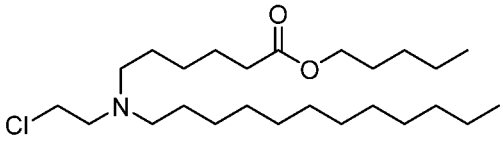
Chemical Formula: C25H51NO3

Molecular Weight: 4 13.69

[00676] In the same manner as Step 1 for Compound 18, pentyl 6-(dodecyl(2-hydroxyethyl)amino)hexanoate was synthesized from pentyl 6-bromohexanoate (0.87 g, 3.27 mmol), 2-(dodecylamino)ethan-l-ol (0.50 g, 2.18 mmol), K2C03 (0.60 g, 4.36 mmol) and KI (36 mg, 0.22 mmol) in 10 mL THF to afford 0.30 g of the desired product (33%).

¾ NMR (400 MHz, CDCh) δ: ppm 4.04 (t, 2H); 3.5 1 (m, 2H); 2.56 (m, 2H); 2.42 (m, 4H); 2.28 (t, 2H); 1.60 (m, 4H); 1.42 (m, 4H); 1.30-1 .24 (m, 24); 0.87 (m, 6H).

Step 4: Pentyl 6-((2-chloroethyl)(dodecyl)amino)hexanoate



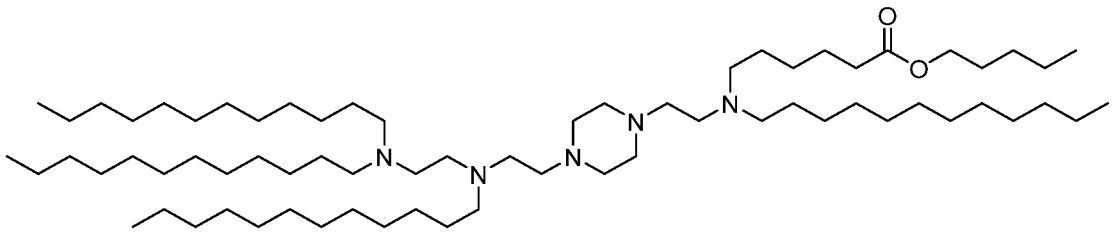
Chemical Formula: C25H50CINO2

Molecular Weight: 432. 13

[00677] In the same manner as Step 2 for Compound 18, pentyl 6-((2-chloroethyl)(dodecyl)amino)hexanoate was synthesized from pentyl 6-(dodecyl(2-hydroxyethyl)amino)hexanoate (300 mg, 0.73 mmol), methanesulfonyl chloride (0.062 mL, 0.80 mmol) and triethylamine (0. 13 mL, 1.3 mmol) in 2 mL DCM to afford 285 mg of the desired product (66%).

¾ NMR (400 MHz, CDCh) δ: ppm 4.04 (t, 2H); 3.45 (t, 2H); 2.74 (t, 2H); 2.43 (m, 4H); 2.28 (t, 2H); 1.65-1 .59 (m, 4H); 1.3 1- 1.24 (m, 32H); 0.88 (m, 6H).

Step 5: Compound 12: Pentyl 6-((2-(4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazin-l -yl)ethyl)(dodecyl)amino)hexanoate



Chemical Formula: C69H141N5O2

Molecular Weight: 1072.92

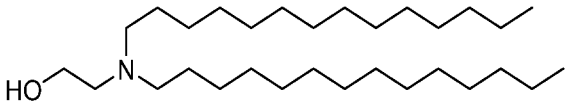
In the same manner as Step 6 for Compound 18, pentyl 6-((2-(4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazin-l-yl)ethyl)(dodecyl)amino)hexan oate was synthesized from pentyl 6-((2-chloroethyl)(dodecyl)amino)hexanoate (75 mg, 0.17 mmol), N^1,N^1,N^2-tridodecyl -N^2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine (107 mg, 0.16 mmol), K2CO3 (23 mg, 0.17 mmol) and KI (2.7 mg, 0.1 mmol) in 1 mL MeCN to afford 99 mg of the desired product (58%).

UPLC: RT = 3.53 min. MS (ES): *m/z* (MH^+) 1073.325 for C69H141N5O2

¾ NMR (400 MHz, CDCh) δ: ppm 4.03 (t, 2H); 2.56-2.37 (br. m , 30H); 2.27 (t, 2H); 1.61 (m, 4H); 1.40-1.23 (br. m ; 90H); 0.87 (m, 15H).

N. Compound 13: Pentyl 6-((2-(4-(2-((2-(ditetradecy lamino)ethy l)(tetradecy l)amino)ethy l)piperazin- 1-yl)ethyl)(dodecyl)amino)hexanoate

Step 1: 2-(Ditetradecylamino)ethan-1-ol



Chemical Formula: C30H63NO

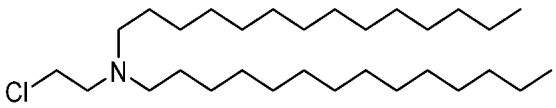
Molecular Weight: 453.84

[00678] In the same manner as Step 1 for Compound 18, 2-(ditetradecylamino)ethan-l-ol was synthesized from 1-bromotetradecane (21.6 mL, 72.8 mmol), ethanolamine (2 mL, 33.1 mmol), K2C03 (20 g, 145.5 mmol), and KI (549 mg, 3.31 mmol) in MeCN (165 mL). Yield (12 g, 81%).

UPLC/ELSD: RT = 3.30 min. MS (ES): *m/z* (MH^+) 454.46 for $C_{30}H_{63}NO$

^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.54 (br. m, 2H); 2.59 (br. m, 2H); 2.46 (br. m, 4H); 1.56-1.17(br. m, 48H); 0.90 (br. m, 6H).

Step 2: *N*-(2-Chloroethyl)-*N*-tetradecyltetradecan-l -amine



Chemical Formula: C30H62CIN

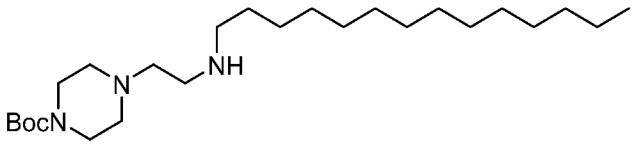
Molecular Weight: 472.28

[00679] In the same manner as Step 2 for Compound 18, *N*-(2-chloroethyl)-*N*-tetradecyltetradecan-1-amine was synthesized from 2-(ditetradecylamino)ethan-l-ol (10 g, 22.0 mmol), triethylamine (4.0 mL, 28.6 mmol), and methanesulfonyl chloride (2.75 mL, 27.5 mmol) in DCM (110 mL). Crude material was carried onto next step without purification. Yield (10.2 g, 98%).

UPLC/ELSD: RT = 3.37 min. MS (ES): *m/z* (MH^+) 472.45 for $C_{30}H_{62}ClN$

¾-NMR (300 MHz, CDCh) δ: ppm 4.27-2.20 (br. m, 8H); 1.96-1.17 (br. m, 48H); 0.90 (br. m, 6H).

Step 3: to/-Butyl 4-(2-(tetradecylamino)ethyl)piperazine-l-carboxylate



Chemical Formula: C25H51N3O2

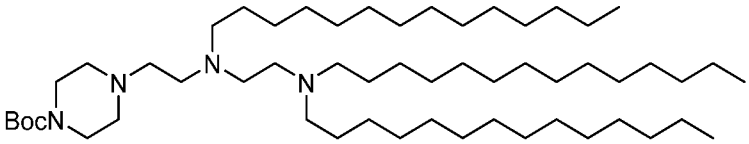
Molecular Weight: 425.70

[00680] In the same manner as Step 3 for Compound 18, to/-butyl 4-(2-(tetradecylamino)ethyl)piperazine-l-carboxylate was synthesized from 1-bromotetradecane (3.63 g, 13.1 mmol), 4-(2-aminoethyl)-l-boc-piperazine (3.0 g, 13.1 mmol), K2CO3 (3.62 g, 26.2 mmol), and KI (217 mg, 1.31 mmol) in MeCN (60 mL). Yield (1.42 g, 27%).

UPLC/ELSD: RT = 1.58 min. MS (ES): *m/z* (MH^+) 426.61 for C25H51N3O2

^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.45 (t, 4H); 2.75 (t, 2H) 2.65 (t, 2H); 2.54 (t, 2H); 2.42 (t, 4H); 1.61-1.41 (br. m, 11H); 1.40-1.20 (br. m, 22H); 0.90 (t, 3H).

Step 4: to/-Butyl 4-(2-((2-(ditetradecylandno)ethyl)(tetradecyl)amino)ethyl)piperazine-l-carboxylate



Chemical Formula: C55H112N4O2

Molecular Weight: 861.53

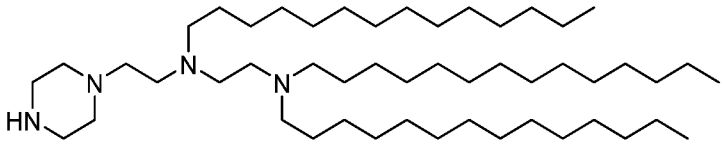
[00681] In the same manner as Step 4 for Compound 18, tot-butyl 4-(2-((2-(ditetradecylamino)ethyl)(tetradecyl)amino)ethyl)piperazine-1-carboxylate was synthesized from tot-butyl 4-(2-(tetradecylamino)ethyl)piperazine-l-carboxylate (700 mg, 1.64 mmol), *N*-

(2-chloroethyl)-*N* -tetradecyltetradecan-l -amine (1.01 g, 2.14 mmol), K2CO3 (455 mg, 3.29 mmol), and KI (27 mg, 0.164 mmol) in THF (15 mL). Yield (740 mg, 52%).

UPLC/ELSD: RT = 3.81 min. MS (ES): *m/z* (MH^+) 862.47 for C55H112N4O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.45 (br. m, 4H); 3.10-2.83 (br. m, 4H); 2.74-2.34 (br. m, 14H); 1.75-1.20 (br. m, 81H); 0.91 (t, 9H).

Step 5: N^1-(2-(Piperazin-1-yl)ethyl)-N^1,N^2,N^2-tritetradecylethane-1,2-diamine



Chemical Formula: C50H104N4

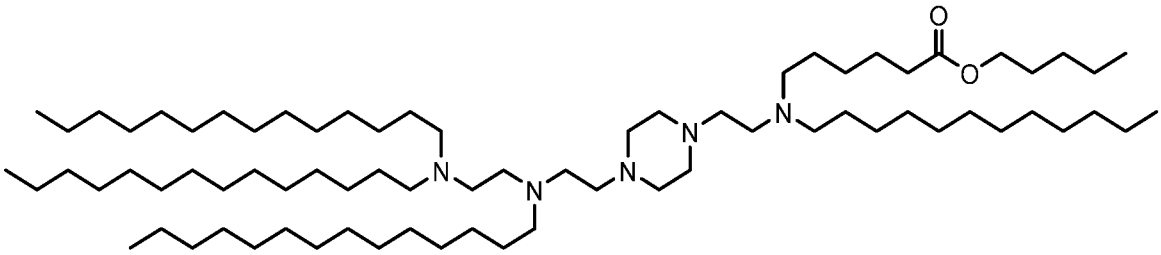
Molecular Weight: 761.41

[00682] In the same manner as Step 5 for Compound 18, N^1-(2-(piperazin-l-yl)ethyl)-N^1,N^2,*N* Mritetradecylethane-l,2-diamine was synthesized from *tert*-butyl 4-(2-((2-(ditetradecylainino)ethyl)(tetradecyl)amino)ethyl)piperazine-l-carboxylate (740 mg, 0.859 mmol), and TFA (3.3 mL, 42.9 mmol) in DCM (3.3 mL). Yield (661 mg, 99%).

UPLC/ELSD: RT = 3.38 min. MS (ES): *m/z* (MH^+) 762.42 for C50H104N4

¾-NMR (300 MHz, CDCh) δ: ppm 2.92 (t, 4H); 2.70-2.30 (br. m, 18H); 1.46 (br. m, 6H); 1.37-1.20 (br. m, 66H); 0.90 (t, 9H).

Step 6: Compound 13: Pentyl 6-((2-(4-(2-((2-(ditetradecy lamino)ethy l)(tetradecy l)amino)ethy l)piperazin- 1-yl)ethyl)(dodecyl)amino)hexanoate



Chemical Formula: C75H153N5O2

Molecular Weight: 1157.08

[00683] In the same manner as Step 6 for Compound 18, pentyl 6-((2-(4-(2-((2-(ditetradecy lamino)ethy l)(tetradecy l)amino)ethy l)piperazin- 1-yl)ethyl)(dodecyl)amino)hexanoate was synthesized from N^1-(2-(piperazin-l-yl)ethyl)-N^1,N^2,N^2-

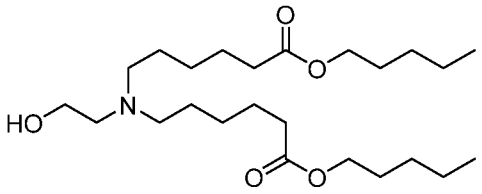
tritetradecylethane-l,2-diamine (66 mg, 0.087 mmol), pentyl 6-((2-chloroethyl)(dodecyl)amino)hexanoate (42 mg, 0.095 mmol) K2CO3 (24 mg, 0.17 mmol), and KI (2 mg, 0.012 mmol) in THF (2 mL). Yield (38 mg, 38%).

UPLC/ELSD: RT = 3.81 min. MS (ES): *m/z* (MH^+) 1157.70 for C75H153N5O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (m, 2H); 3.16-2.15 (br. m, 32H); 1.65 (br. m, 4H); 1.54-1.00 (br. m, 100H); 0.91 (br. m, 15H).

O. Compound 14: Dipentyl 6,6'-((2-(4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazin-l-yl)ethyl)azanediyl)dihexanoate

Step 1: Dipentyl 6,6'-((2-hydroxyethyl)azanediyl)dihexanoate



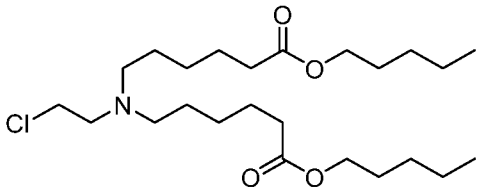
Chemical Formula: C24H47NO5

Molecular Weight: 429.64

[00684] In the same manner as Step 1 for Compound 18, dipentyl 6,6'-((2-hydroxyethyl)azanediyl)dihexanoate was synthesized from pentyl 6-bromohexanoate (0.50 g, 1.89 mmol), ethanolamine (0.052 mL, 0.86 mmol), K2CO3 (0.52 g, 3.77 mmol) and KI (14 mg, 0.098 mmol) in 4 mL MeCN to provide 234 mg (55%).

¾ NMR (400 MHz, CDCh) δ: ppm 4.08(t, 4H); 3.62 (m, 2H); 2.68-2.56 (br. m , 6H); 2.33 (t, 4H); 1.64-1.54 (m, 13H); 1.35 (m, 12H); 0.93 (t, 6H).

Step 2: Dipentyl 6,6'-((2-chloroethyl)azanediyl)dihexanoate



Chemical Formula: C24H46ClNO4

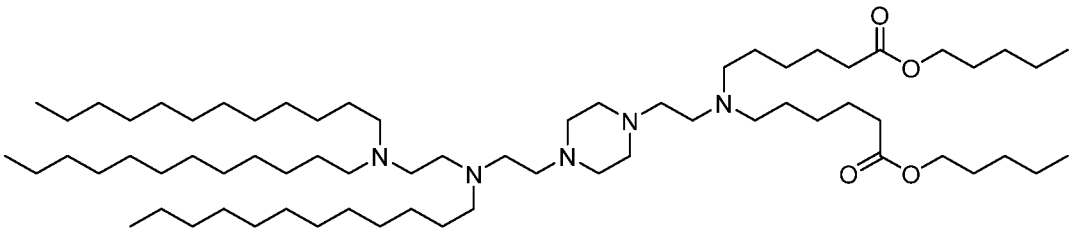
Molecular Weight: 448.09

[00685] In the same manner as Step 2 for Compound 18, dipentyl 6,6'-((2-chloroethyl)azanediyl)dihexanoate was synthesized from dipentyl 6,6'-((2-hydroxyethyl)azanediyl)dihexanoate (124 mg, 0.29 mmol), methanesulfonyl chloride (0.025

mL, 0.32 mmol) and triethylamine (0.060 mL, 0.44 mmol) in 1.5 mL DCM to provide 84 mg (65%).

¾ NMR (400 MHz, CDCb) δ: ppm 4.04 (t, 4H); 3.46 (t, 2H); 2.73 (t, 2H); 2.43 (t, 4H); 2.28 (t, 4H); 1.60 (m, 8H); 1.40 (m, 4H); 1.29 (m, 12H); 0.89 (t, 6H).

Step 3: Compound 14: Dipentyl 6,6'-((2-(4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazin-l -yl)ethyl)azanediyl)dihexan oate



Chemical Formula: $C_{68}H_{137}N_5O_4$

Molecular Weight: 1088.88

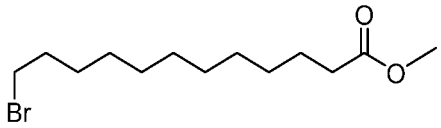
[00686] In the same manner as Step 6 for Compound 18, dipentyl 6,6'-((2-(4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazin-l -yl)ethyl)azanediyl)dihexanoate was synthesized from N^1,N^1,N^2-tridodecyl -N^2-(2-(piperazin-l -yl)ethyl)ethane-l ,2-diamine (105 mg, 0.16 mmol), dipentyl 6,6'-((2-chloroethyl)azanediyl)dihexanoate (84 mg, 0.19 mmol) and K2CO3 (22 mg, 0.16 mmol) in 1 mL MeCN. Yield (53 mg, 0.049 mmol, 30%).

UPLC: RT = 3.47 min. MS (ES): *m/z* (MH^+) 1089.53 for $C_{68}H_{137}N_5O_4$

¾ NMR (400 MHz, CDCb) δ: ppm 4.04 (t, 4H); 2.89-2.98 (m, 4H); 2.39-2.68 (m, 26H); 2.27 (t, 4H); 1.57-1.71 (m, 10H); 1.35 (m, 4H); 1.28-1.35 (m, 74H); 0.90 (m, 15H).

P. Compound 15: Methyl 12-((2-(4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazin-l -yl)ethyl)(dodecyl)amino)dodecanoate

Step 1: Methyl 12-bromododecanoate



Chemical Formula: Ci3H25Br02

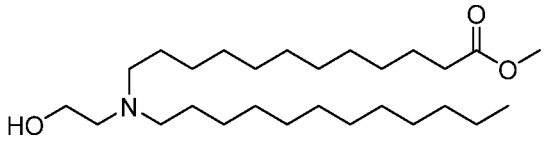
Molecular Weight: 293.25

[00687] To a solution of 12-bromododecanoic acid (2.5 g, 8.95 mmol) in THF (7 mL) was added methanol (7.2 mL, 179 mmol). Sulfuric acid (0.50 mL, 8.95 mmol) was added dropwise

and the reaction was allowed to stir at 65 °C for two hours. The reaction mixture was washed with 5% NaHCCb and brine. The organic layer was dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-20% EtOAc/hexanes) provided methyl 12-bromododecanoate (2.40 g, 92%).

ŧTi-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.44 (t, 2H); 2.33 (t, 2H); 1.88 (br. m, 2H); 1.64 (br. m, 2H); 1.45 (br. m, 2H); 1.31 (br. m, 12H).

Step 2: Methyl 12-(dodecyl(2-hydroxyethyl)amino)dodecanoate



Chemical Formula: C27H55NO3

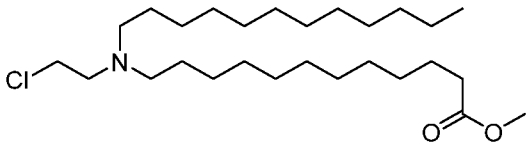
Molecular Weight: 441.74

[00688] To a solution of methyl 12-((2-hydroxyethyl)amino)dodecanoate (413 mg, 1.51 mmol) in MeCN (5 mL) was added 1-bromododecane (452 mg, 1.81 mmol), K2CO3 (418 mg, 3.02 mmol), and KI (25 mg, 0.151 mmol). The reaction was allowed to stir at 82 °C for 16 hours. The reaction mixture was cooled to room temperature, diluted with H2O, and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-100% [DCM, 20% MeOH, 1% NH_4OH]/MeOH) provided methyl 12-(dodecyl(2-hydroxyethyl)amino)dodecanoate (409 mg, 61%).

UPLC/ELSD: RT = 2.39 min. MS (ES): *m/z* (MH^+) 442.60 for C27H55NO3

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.61 (t, 2H); 2.68 (t, 2H); 2.54 (t, 4H); 2.32 (t, 2H); 1.64 (m, 2H); 1.50 (br. m, 4H); 1.28 (br. m, 32H); 0.90 (t, 3H).

Step 3: Methyl 12-((2-chloroethyl)(dodecyl)amino)dodecanoate



Chemical Formula: C27H54CINO2

Molecular Weight: 460.18

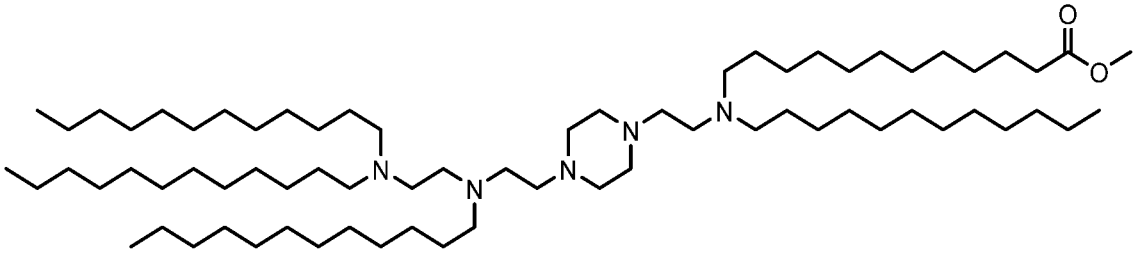
[00689] In the same manner as Step 2 for Compound 18, methyl 12-((2-chloroethyl)(dodecyl)amino)dodecanoate was synthesized from methyl 12-((2-

hydroxyethyl)amino)dodecanoate (409 mg, 0.926 mmol), triethylamine (0.168 mL, 1.20 mmol), and methanesulfonyl chloride (0.090 mL, 1.16 mmol) in DCM (5 mL). Yield (307 mg, 72%).

UPLC/ELSD: RT = 4.30 min. MS (ES): *m/z* (MH^+) 460.80 for C27H54ClNO2

¾-NMR (300 MHz, CDCh) δ: ppm 3.59 (s, 3H); 3.42 (br. m, 2H); 2.70 (br. m, 2H); 2.38 (br. m, 4H); 2.30 (t, 2H); 1.55 (m, 2H); 1.36 (br. m, 4H); 1.27-0.96 (br. m, 32H); 0.81 (t, 3H).

Step 4: Compound 15: Methyl 12-((2-(4-(2-((2-(didodecylamino)ethyl)(dodecyl)ainino)ethy l)piperazin-1-yl)ethyl)(dodecyl)amino)dodecanoate



Chemical Formula: C71H145N5O2

Molecular Weight: 1100.97

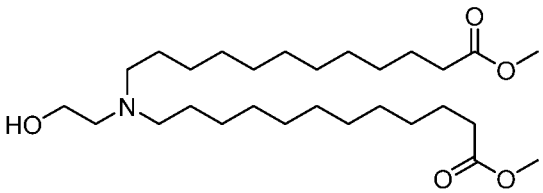
[00690] In the same manner as Step 6 for Compound 18, methyl 12-((2-(4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazin-l -yl)ethyl)(dodecyl)amino)dodecano ate was synthesized from N^1,N^1,N^2-tridodecyl -N^2-(2-(piperazin-l -yl)ethyl)ethane-l,2-diamine (150 mg, 0.221 mmol), methyl 12-((2-chloroethyl)(dodecyl)amino)dodecanoate (134 mg, 0.266 mmol) K2CO3 (61 mg, 0.443 mmol), and KI (4 mg, 0.024 mmol) in THF (5 mL). Yield (32 mg, 15%).

UPLC/ELSD: RT = 4.83 min. MS (ES): *m/z* (MH^+) 1102.11 for C71H145N5O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 2.75-2.24 (br. m, 32H); 1.64 (m, 2H); 1.52-1.00 (br. m, 96H); 0.90 (t, 12H).

Q. Compound 16: Dimethyl 12,12'-((2-(4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazin-l -yl)ethyl)azanediyl)didodecanoate

Step 1: Dimethyl 12,12'-((2-hydroxyethyl)azanediyl)didodecanoate



Chemical Formula: C28H55NO5

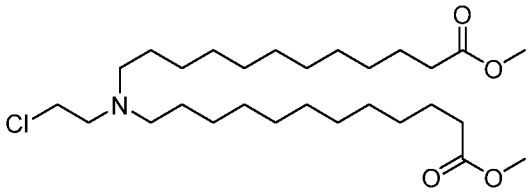
Molecular Weight: 485.75

[00691] In the same manner as Step 1 for Compound 18, dimethyl 12,12'-((2-hydroxyethyl)azanediyl)didodecanoate was synthesized from methyl 12-bromododecanoate (1.5 g, 5.12 mmol), ethanolamine (0.310 mL, 5.12 mmol), K2CO3 (1.42 g, 10.2 mmol), and KI (85 mg, 0.512 mmol) in MeCN (11 mL). Yield (563 mg, 45%).

UPLC/ELSD: RT = 1.81 min. MS (ES): *m/z* (MH^+) 486.63 for C28H55NO5

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 6H); 3.59 (br. m, 2H); 2.75-2.40 (br. m, 6H); 2.32 (t, 4H); 1.64 (m, 4H); 1.48 (br. m, 4H); 1.29 (br. m, 28H).

Step 2: Dimethyl 12,12'-((2-chloroethyl)azanediyl)didodecanoate



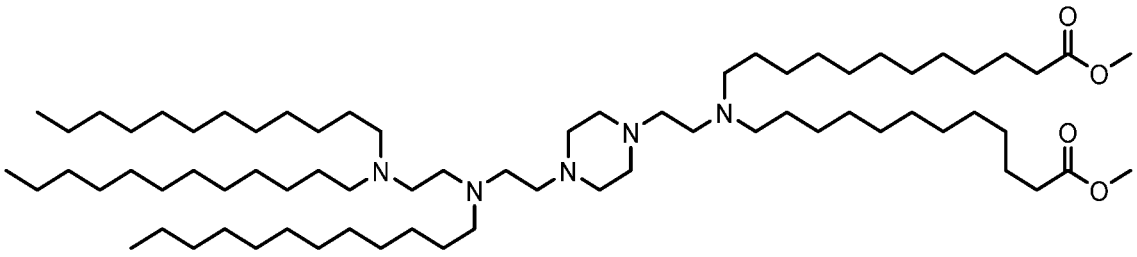
Chemical Formula: C28H54ClNO4

Molecular Weight: 504.19

[00692] In the same manner as Step 2 for Compound 18, dimethyl 12,12'-((2-chloroethyl)azanediyl)didodecanoate was synthesized from dimethyl 12,12'-((2-hydroxyethyl)azanediyl)didodecanoate (518 mg, 1.07 mmol), triethylamine (0.193 mL, 1.39 mmol), and methanesulfonyl chloride (0.103 mL, 1.33 mmol) in DCM (5.5mL). Yield (376 mg, 70%).

UPLC/ELSD: RT = 2.17 min. MS (ES): *m/z* (MH^+) 504.75 for C28H54ClNO4

Step 3: Compound 16: Dimethyl 12,12'-((2-(4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazin-l-yl)ethyl)azanediyl)didodecanoate



Chemical Formula: C72H145N5O4

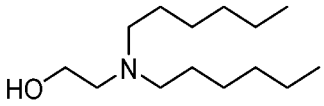
Molecular Weight: 1144.98

[00693] In the same manner as Step 6 for Compound 18, dimethyl 12,12'-((2-(4-(2-((2-(didodecylamino)ethyl)(dodecyl)amino)ethyl)piperazin-l-yl)ethyl)azanediyl)didodecanoate was synthesized from N^1,N^1,N^2-tridodecyl-N^2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine (150 mg, 0.221 mmol), dimethyl 12,12'-((2-chloroethyl)azanediyl)didodecanoate (134 mg, 0.266 mmol) K2CO3 (61 mg, 0.443 mmol), and KI (4 mg, 0.024 mmol) in THF (5 mL). Yield (32 mg, 15%). UPLC/ELSD: RT = 3.46 min. MS (ES): *m/z* (MH^+) 1146.07 for C72H145N5O4

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 6H); 2.75-2.24 (br. m, 34H); 1.64 (m, 4H); 1.52-1.00 (br. m, 92H); 0.90 (t, 9H).

R. Compound 17: N^1-(2-(4-(2-(Didodecylamino)ethyl)piperazin-l-yl)ethyl)-N^1,N^2,N^2-trihexylethane-1,2-diamine

Step 1: 2-(Dihexylamino)ethan-l-ol



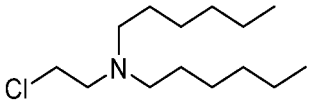
Chemical Formula: C14H31NO

Molecular Weight: 229.41

[00694] In the same manner as Step 1 for Compound 18, 2-(dihexylamino)ethan-l-ol was synthesized from 1-bromohexane (5 g, 82 mmol), ethanolamine (11.5 mL, 82 mmol), K2CO3 (22.7 g, 164 mmol), and KI (1.36 g, 8.2 mmol) in MeCN (380mL). Yield (2.58g, 14%). UPLC/ELSD: RT = 0.41 min. MS (ES): *m/z* (MH^+) 229.95 for C14H31NO

¾-NMR (300 MHz, CDCh) δ: ppm 3.62 (t, 2H); 2.70 (t, 2H), 2.57 (t, 4H); 1.50 (br. m, 4H); 1.30 (br, 12H); 0.91 (t, 6H).

Step 2: N-(2-Chloroethyl)-N-hexylhexan-1-amine



Chemical Formula: C14H30CIN

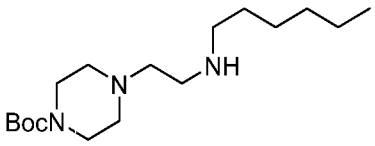
Molecular Weight: 247.85

[00695] In the same manner as Step 2 for Compound 18, N-(2-chloroethyl)-N-hexylhexan-1-amine was synthesized from 2-(dihexylamino)ethan-l-ol (2.50 g, 10.9 mmol), triethylamine (2.0 mL, 14.2 mmol), and methanesulfonyl chloride (1.0 mL, 13.6 mmol) in DCM (56 mL). Yield (1.93 g, 71%).

UPLC/ELSD: RT = 0.42 min. MS (ES): *m/z* (MH^+) 247.86 for C14H30ClN

¾-NMR (300 MHz, CDCh) δ: ppm 3.50 (t, 2H); 2.77 (t, 2H); 2.51 (t, 4H); 1.42 (br. m, 4H); 1.27 (br, 12H); 0.89 (t, 6H).

Step 3: *tert*-Butyl 4-(2-(hexylamino)ethyl)piperazine-l-carboxylate



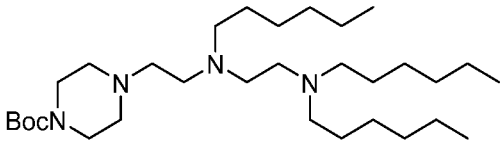
Chemical Formula: C17H35N3O2

Molecular Weight: 313.49

[00696] In the same manner as Step 3 for Compound 18, *tert*-butyl 4-(2-(hexylamino)ethyl)piperazine-l-carboxylate was synthesized from 1-bromohexane (1.44 g, 8.72 mmol), 4-(2-aminoethyl)-l-boc-piperazine (2.0 g, 8.72 mmol), K2CO3 (2.4 g, 17.4 mmol), and KI (145 mg, 0.872 mmol). Yield (446 mg, 16%).

¾-NMR (300 MHz, CDCh) δ: ppm 3.44 (br. m, 4H); 2.75 (br. m, 2H); 2.65 (br. m, 2H); 2.54 (br. m, 2H); 2.42 (br. m, 4H); 1.60-1.43 (br. m, 11H); 1.40-1.05 (br. m, 6H); 0.91 (br. m, 3H).

Step 4: *tert*-Butyl 4-(2-((2-(dihexylamino)ethyl)(hexyl)amino)ethyl)piperazine-l-carboxylate



Chemical Formula: C31H64N4O2

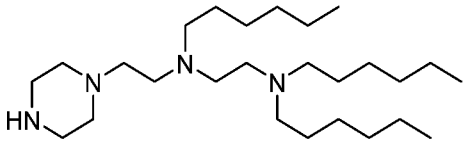
Molecular Weight: 524.88

[00697] In the same manner as Step 4 for Compound 18, tot-butyl 4-(2-((2-(dihexylamino)ethyl)(hexyl)amino)ethyl)piperazine-l-carboxylate was synthesized from *tert*-butyl 4-(2-(hexylamino)ethyl)piperazine-l-carboxylate (250 mg, 0.797 mmol), *N*-(2-chloroethyl)-*N*-hexylhexan-l-amine (217 mg, 0.877 mmol), K2CO3 (220 mg, 1.59 mmol), and KI (13 mg, 0.0797 mmol) in THF (5 mL). Yield 308 mg, 74%).

UPLC/ELSD: RT = 1.40 min. MS (ES): *m/z* (MH^+) 525.83 for C31H64N4O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.45 (br. m, 4H); 3.15-2.15 (br. m, 18H); 1.85-1.00 (br. m, 33H); 0.91 (9H).

Step 5: N^1,N^1,N^2-Trihexyl-N^2-(2-(piperazin-1-yl)ethyl)ethane-1,2-diamine



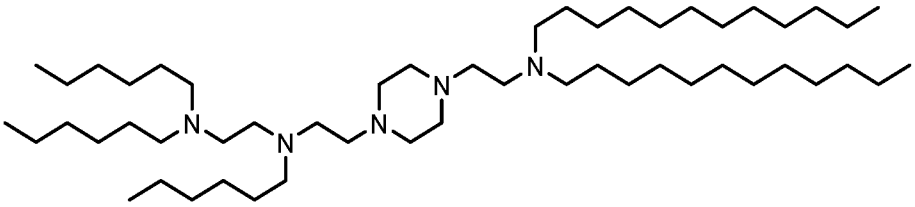
Chemical Formula: $C_{26}H_{56}N_4$

Molecular Weight: 424.76

[00698] In the same manner as Step 5 for Compound 18, N^1,N^1,N^2-trihexyl-N^2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine was synthesized from *tert-b*utyl 4-(2-((2-(dihexylamino)ethyl)(hexyl)amino)ethyl)piperazine-l-carboxylate (308 mg, 0.587 mmol), and TFA (2.25 ml, 29.3 mmol) in DCM (2.5 mL). Yield (220 mg, 88%).

¾-NMR (300 MHz, CDCh) δ: ppm 2.92 (br. m, 4H); 2.70-2.20 (br. m, 18H), 1.54-1.22 (br. m, 24H); 0.91 (br. m, 9H).

Step 6: Compound 17: N^1-(2-(4-(2-(Didodecylamino)ethyl)piperazin-l-yl)ethyl)-N^1,N^2,N^2-trihexylethane-1,2-diamine



Chemical Formula: C52H109N5

Molecular Weight: 804.48

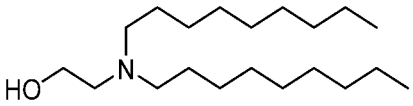
[00699] In the same manner as Step 6 for Compound 18, N^1-(2-(4-(2-(didodecylamino)ethyl)piperazin-1-yl)ethyl)-N^1,N^2,N^2-trihexylethane-1,2-diamine was synthesized from N^1,N^1,N^2-trihexyl-N^2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine (110 mg, 0.259 mmol), N-(2-chloroethyl)-N-dodecyldodecan-l-amine (162 mg, 0.388 mmol), K2CO3 (72 mg, 0.518 mmol), and KI (5 mg, 0.0259 mmol) in THF (6 mL). Yield (81 mg, 39%).

UPLC/ELSD: RT = 2.79 min. MS (ES): *m/z* (MH^+) 806.30 for C52H109N5

¾-NMR (300 MHz, CDCh) δ: ppm 3.05-2.10 (br. m, 30H); 1.80-1.05 (br. m, 64H); 0.91 (br. m, 15H).

S: Compound 18: N^1-(2-(4-(2-(Dinonylamino)ethyl)piperazin-l-yl)ethyl)-N^1,N^2,N^2-trinonylethane-1,2-diamine

Step 1: 2-(Dinonylamino)ethan-l-ol



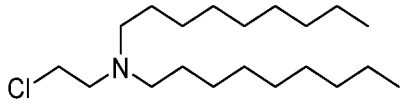
Chemical Formula: C20H43NO

Molecular Weight: 313.57

[00700] To a solution of 1-bromononane (8.31 g, 40. 1 mmol) in MeCN (84 mL) was added ethanolamine (1.10 mL, 18.2 mmol), K2CO3 (11.1 g, 80.1 mmol), and KI (302 mg, 1.82 mmol). The reaction was allowed to stir at 82 °C for 48 hours. The reaction mixture was cooled to room temperature, filtered, and the solids were washed with hexanes. The filtrate was extracted with hexanes, and the combined extracts were concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-20% MeOH/DCM) provided 2-(dinonylamino)ethan-l-ol (4.06 g, 71%).

Ti-NMR (300 MHz, CDCh) δ: ppm 3.57 (t, 2H); 2.63 (t, 2H); 2.49 (br. m, 4H); 1.48 (br. m, 4H); 1.29 (br. m, 24H); 0.91 (t, 6H).

Step 2: *N*-(2-Chloroethyl)-*N*-nonylnonan-l -amine



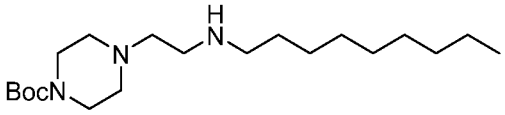
Chemical Formula: C20H42CIN

Molecular Weight: 332.01

[00701] To a 0 °C solution of 2-(dinonylamino)ethan-l-ol (4.06 g, 12.9 mmol) and triethylamine (2.35 ml, 16.8 mmol) in DCM (65 mL) was added dropwise a solution of methanesulfonyl chloride (1.25 mL, 16.18 mmol) in DCM (5 mL). The reaction was allowed to return to room temperature and stir for 16 hours. The mixture was quenched by the addition of water and extracted with DCM. The organic layer was washed with saturated NaHCCb, brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-10% EtOAc/hexanes) provided *N*-(2-chloroethyl)-*N*-nonylnonan-l -amine (2.58 g, 60%).

Ti-NMR (300 MHz, CDCh) δ: ppm 3.51 (t, 2H); 2.78 (t, 2H); 2.47 (br. m, 4H); 1.44 (br. m, 4H); 1.28 (br. m, 24H); 0.90 (t, 6H).

Step 3: *ter*/-Butyl 4-(2-(nonylamino)ethyl)piperazine-l-carboxylate



Chemical Formula: C20H41N3O2

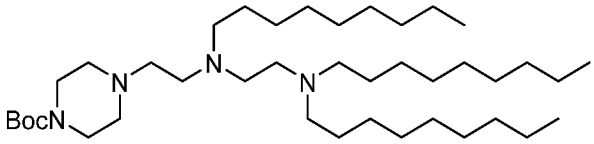
Molecular Weight: 355.57

[00702] To a solution of 1-bromononane (1.81 g, 8.72 mmol) in MeCN (44 mL) was added 4-(2-aminoethyl)-l -boc-piperazine (2.0 g, 8.72 mmol), K2CO3 (2.4 g, 17.4 mmol), and KI (145 mg, 0.872 mmol). The reaction was allowed to stir at 65 °C for 16 hours. The reaction mixture was cooled to room temperature, filtered, and the solids were washed with hexanes. The filtrate was extracted with hexanes, and the combined extracts were concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-20% MeOH/DCM) provided *tert*-butyl 4-(2-(nonylamino)ethyl)piperazine-l -carboxylate (775 mg, 25%).

UPLC/ELSD: RT = 0.47 min. MS (ES): *m/z* (MH^+) 356.41 for C20H41N3O2

Ti-NMR (300 MHz, CDCh) δ: ppm 3.44 (br. m, 4H); 2.74 (t, 2H); 2.63 (t, 2H); 2.53 (t, 2H); 2.41 (br. m, 4H); 1.48 (br. m, 9H); 1.30 (br. m, 14H); 0.90 (t, 3H).

Step 4: *tert*-Butyl 4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)ethyl)piperazine-l -carboxylate



Chemical Formula: C40H82N4O2

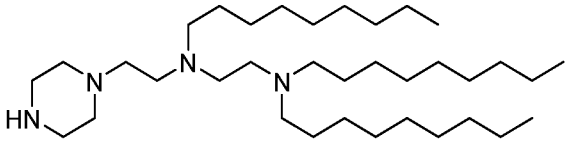
Molecular Weight: 651.12

[00703] To a solution of t*er*/-butyl 4-(2-(nonylamino)ethyl)piperazine-l-carboxylate (500 mg, 1.41 mmol) in THF (9 mL) was added *N*-(2-chloroethyl)-*N*-nonylnonan-l -amine (514 mg, 1.55 mmol), K2CO3 (390 mg, 2.82 mmol), and KI (23 mg, 0.141 mmol). The reaction was allowed to stir at 65 °C for 72 hours. The reaction mixture was cooled to room temperature, diluted with water, and extracted with EtOAc. The combined extracts were washed with brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-15% MeOH/DCM) provided *tert*-butyl 4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)ethyl)piperazine-l -carboxylate (763 mg, 83%).

UPLC/ELSD: RT = 2.61 min. MS (ES): *m/z* (MH^+) 651.91 for C40H82N4O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.45 (br. m, 4H); 2.75-2.30 (br. m, 18H); 1.55-1.20 (br. m, 51H); 0.91 (br. m, 9H).

Step 5: N^1,N^1,N^2-Trinonyl-N^2-(2-(piperazin-l -yl)ethyl)ethane- 1,2-diamine



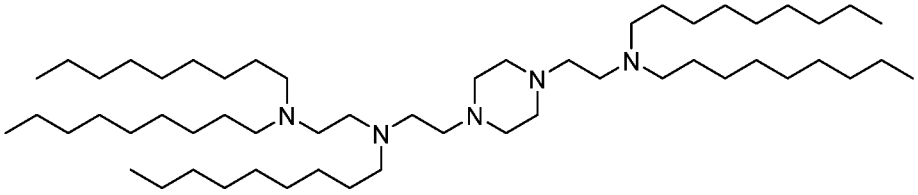
Chemical Formula: C35H74N4

Molecular Weight: 551.01

[00704] To a 0 °C solution of *tert*-Butyl 4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)ethyl)piperazine-l-carboxylate (763 mg, 1.17 mmol) in DCM (4.5 mL) was added dropwise TFA (4.5 mL, 58.5 mmol). The reaction was allowed to return to room temperature and stir for 16 hours. The reaction mixture was concentrated *in vacuo* and the crude material was dissolved in $CHCl_3$. The solution was washed with 5% Na2CCb, brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-100% [DCM, 20% MeOH, 1% NH_4OH]/MeOH) provided N^1,N^1,N^2-trinonyl -N^2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine (218 mg, 34%). UPLC/ELSD: RT = 1.81 min. MS (ES): *m/z* (MH^+) 551.78 for C35H74N4

Ti-NMR (300 MHz, CDCh) δ: ppm 2.91 (br. m, 4H); 2.70-2.35 (br. m, 18H); 1.46 (br. m, 6H); 1.29 (br. m, 36H); 0.91 (br. m, 9H)

Step 6: Compound 18: N^1-(2-(4-(2-(Dinonylamino)ethyl)piperazin-l-yl)ethyl)-N^1,N^2,N^2-trinonylethane-1,2-diamine



Chemical Formula: C55H115N5

Molecular Weight: 846.56

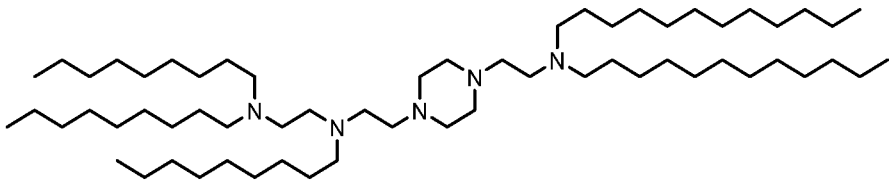
[00705] To a solution of N^1,N^1,N^2-trinonyl -N^2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine (74 mg, 0.134 mmol) and *N*-(2-chloroethyl)-*N*-nonylnonan-l -amine (58 mg, 0.175 mmol) in THF (4 mL) was added K2CO3 (37 mg, 0.269 mmol), and KI (3 mg, 0.0134 mmol). The reaction allowed to stir at 65 °C for 48 hours. The reaction mixture was cooled to room temperature, diluted with water, and extracted with EtOAc. The combined extracts were washed with brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by

ISCO C18 flash chromatography (50-100% [MeCN 0.1% TFA]/[H_20 0.1% TFA]) afforded the desired product as a TFA salt. The salt was dissolved in CHCh and the solution was washed with 5% Na2CCb, brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo* to provide N^1-(2-(4-(2-(dinonylamino)ethyl)piperazin-l-yl)ethyl)-N^1,N^2,N^2-trinonylethane-l,2-diamine (66 mg, 58%).

UPLC/ELSD: RT = 2.91 min. MS (ES): *m/z* (MH^+) 847.30 for C55H115N5

Ti-NMR (300 MHz, CDCh) δ: ppm 3.10-2.25 (br. m, 30H); 1.90-1.35 (br. m, 10H); 1.29 (br. m, 60H); 0.91 (br. m, 15H).

T: Compound 19: N^1-(2-(4-(2-(Didodecylamino)ethyl)piperazin-l-yl)ethyl)-N ^{1},N^2,N^2-trinony lethane-1,2-diamine



Chemical Formula: C61H127N5

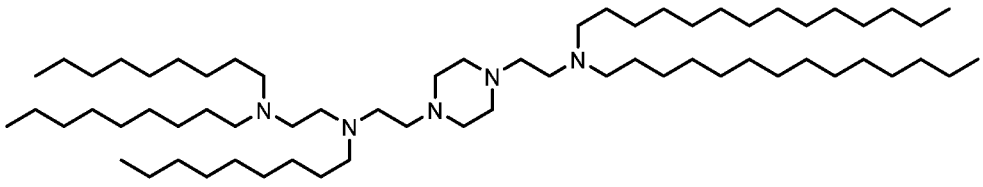
Molecular Weight: 930.72

[00706] In the same manner as Step 6 for Compound 18, N^1-(2-(4-(2-(didodecy lamino)ethy l)piperazin- 1-yl)ethy l)-N^1,N^2,N^trinony lethane- 1,2-diamine was synthesized from N^1,N^1,N^2-trinonyl -N^2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine (70 mg, 0.127 mmol), N-(2-chloroethyl)-N-dodecyldodecan-l -amine (79 mg, 0.191 mmol), K2CO3 (35 mg, 0.254 mmol), and KI (2 mg, 0.0127 mmol) in THF (3 mL). Yield (52 mg, 44%).

UPLC/ELSD: RT = 3.35 min. MS (ES): *m/z* (MH^+) 931.61 for $C_6iH_{127}N_5$

¾-NMR (300 MHz, CDCh) δ: ppm 2.70 (br. m, 30H); 1.56-1.02 (br. m, 82H); 0.90 (t, 15H).

U: Compound 20: N^1-(2-(4-(2-(Ditetradecylamino)ethyl)piperazin-l-yl)ethyl)-N ^{1},N^2,N^2-trinony lethane-1,2-diamine

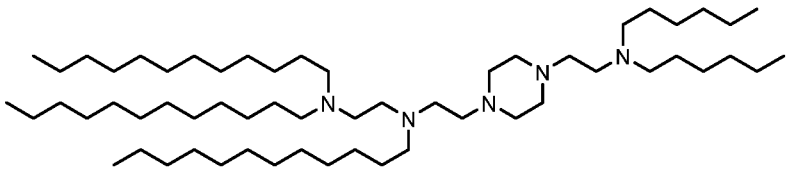


Chemical Formula: C65H135N5

Molecular Weight: 986.83

[00707] In the same manner as Step 6 for Compound 18, ^-(2-(4-(2-(ditetradecylamino)ethyl)piperazin- 1-y^ethy ^-*N*^A^^-trinonylethane- 1,2-diamine was synthesized from *N*1,*N*1,*N*2-trinonyl-*N*2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine (74 mg, 0.134 mmol), *N*-(2-chloroethyl)-*N*-tetradecyltetradecan-l -amine (95 mg, 0.201 mmol), K2CO3 (37 mg, 0.269 mmol), and KI (3 mg, 0.0134 mmol) in THF (2 mL). Yield (50 mg, 38%) UPLC/ELSD: RT = 3.55 min. MS (ES): *m/z* (MH^+) 987.87 for C65H135N5 ¾-NMR (300 MHz, CDCh) δ: ppm 3.20-2.25 (br. m, 30H); 1.85-1.00 (br. m, 90H); 0.91 (t, 15H).

V: Compound 21: *N*1-(2-(4-(2-(Dihexylamino)ethyl)piperazin-l-yl)ethyl)-N 1,N2,N2-tridodecylethane-1 ,2-diamine



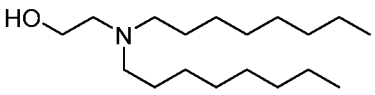
Chemical Formula: C58H121N5

Molecular Weight: 888.64

[00708] In the same manner Step 6 for Compound 18, ^-(2-(4-(2-(dihexylamino)ethyl)piperazin- 1-y^ethy ^-*N*^A^^-tridodecylethane- 1,2-diamine was synthesized from *N*1,*N*1,*N*2-tridodecyl-*N*2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine (67 mg, 0.099 mmol), *N*-(2-chloroethyl)-*N*-hexylhexan-l -amine (32 mg, 0.129 mmol), K2CO3 (28 mg, 0.198 mmol), and KI (2 mg, 0.0099 mmol) in THF (2 mL). Yield (30 mg, 34%).
UPLC/ELSD: RT = 3.24 min. MS (ES): *m/z* (MH^+) 890.58 for $C_{58}H_{121}N_5$ ¾-NMR (300 MHz, CDCh) δ: ppm 3.15-2.20 (br. m, 30H); 1.85-1.00 (br. m, 76H); 0.91 (br. m, 15H).

W: Compound 22: *N*1-(2-(4-(2-(Dioctylamino)ethyl)piperazin-l-yl)ethyl)-*N*1,*N*2,*N*2-tridodecylethane-l,2-diamine

Step 1: 2-(Dioctylamino)ethan-l-ol



Chemical Formula: C18H39NO

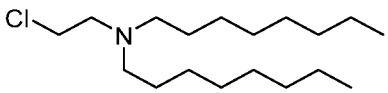
Molecular Weight: 285.52

[00709] In the same manner as Step 1 for Compound 18, compound was synthesized from ethanolamine (5 g, 82 mmol), 1-bromooctane (14 rriL, 82 mmol), and K2CO3 (11 g, 82 mmol) in 200 mL MeCN. Yield (3.13 g, 11 mmol, 13%).

UPLC/ELSD: RT = 1.78 min. MS (ES): *m/z* (MH^+) 286.22 for C18H39NO

¾-NMR (300 MHz, CDCh) δ: ppm 3.54 (m, 2H); 2.60-2.47 (m, 6H); 1.44 (m, 4H); 1.26 (m, 21H); 0.86 (t, 6H).

Step 2: *N*-(2-Chloroethyl *)-N*-octyloctan-l -amine



Chemical Formula: C18H38CIN

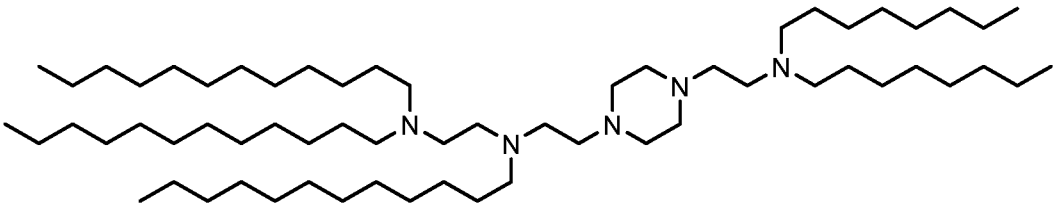
Molecular Weight: 303.96

[00710] In the same manner as Step 2 for Compound 18, *N*-(2-chloroethyl *)-N*-octyloctan-1-amine was synthesized from2-(dioctylamino)ethan-l-ol (3.13 g, 11 mmol), methanesulfonyl chloride (0.85 mL, 11 mmol) and Et_3N (1.5 mL, 11 mmol) in 30 mL DCM. Yield (1.55 g, 5.1 mmol, 46%).

UPLC/ELSD: RT = 3.86 min. MS (ES): *m/z* (MH^+) 304.43 for CisftsCIN

¾-NMR (300 MHz, CDCh) δ: ppm 3.48 (2, 2H); 2.75 (t, 2H); 2.43 (t, 4H); 1.40-1.25 (m, 24H); 0.86 (t, 6H).

Step 3: Compound 22: *N*1-(2-(4-(2-(Dioctylamino)ethyl)piperazin-l-yl)ethyl *)-N*1,*N*2,*N*2-tridodecylethane-1 ,2-diamine



Chemical Formula: C62H129N5

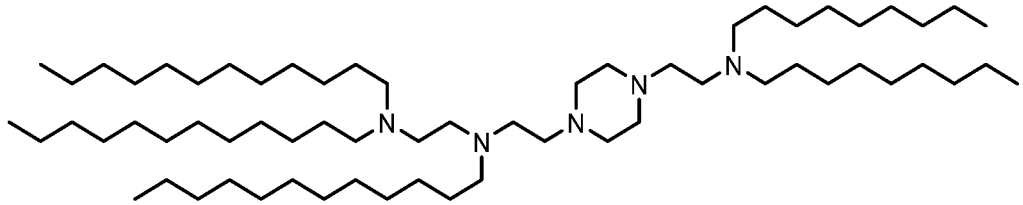
Molecular Weight: 944.75

[00711] In the same manner as Step 6 for Compound 18, *N*1-(2-(4-(2-(dioctylainino)ethyl)piperazin-l-yl)ethyl^ -*N*1,*N*2,*N*2-tridodecylethane-1,2-diamine was synthesized from *N*1,*N*1,*N*2-tridodecyl -*N*2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine (150 mg, 0.221 mmol), *N*-(2-chloroethyl *)-N*-octyloctan-l -amine 54 (81 mg, 0.266 mmol), K2CO3 (61 mg, 0.443 mmol), and KI (4 mg, 0.024 mmol) in THF (5 mL). Yield (200 mg, 96%).

UPLC/ELSD: RT = 3.41 min. MS (ES): *m/z* (MH^+) 945.96 for $C_{62}H_{129}N_5$

¾-NMR (300 MHz, CDCh) δ: ppm 2.76-2. 10 (br. m, 30H); 1.56-1 .00 (br. m, 84H); 0.90 (t, 15H).

X: Compound 23: N^1-(2-(4-(2-(Dinonylamino)ethyl)piperazin-l-yl)ethyl)-N^1,N^2,N^2-tridodecylethane-1 ,2-diamine



Chemical Formula: $C_{64}H_{133}N_5$

Molecular Weight: 972. 80

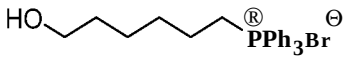
[00712] In the same manner as Step 6 for Compound 18, N^1-(2-(4-(2-(dinonylainino)ethyl)piperazin-l -yl)eth^ l)-N^1,N^2,N^2-tridodecylethane-1,2-diamine was synthesized from N^1,N^1,N^2-tridodecyl -N^2-(2-(piperazin-l -yl)ethyl)ethane-l ,2-diamine (595 mg, 0.879 mg), N-(2-chloroethyl)-N-nonylnonan-l -amine (350 mg, 1.05 mmol), K2CO3 (243 mg, 1.76 mmol), and KI (15 mg, 0.0879 mmol) in THF (13 mL). Yield (534 mg, 62%).

UPLC/ELSD: RT = 3.50 min. MS (ES): *m/z* (MH^+) 973.60 for $C_{64}H_{133}N_5$

¾-NMR (300 MHz, CDCh) δ: ppm 2.70-2.30 (br. m, 30H); 1.56-1 .37 (br. m, 10H); 1.28 (br. m, 78H); 0.90 (t, 15H).

Y: Compound 24: (Z)-N^1-(2-(4-(2-(Dodec-6-en-l-yl(dodecyl)amino)ethyl)piperazin- 1-yl)ethyl)-N^1^^-tridodecy lethane- 1,2-diamine

Step 1: (6-Hydroxyhexyl)triphenylphosphonium bromide



Chemical Formula: C24H2sBrOP

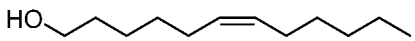
Molecular Weight: 443.36

[00713] 6-Bromo-l-hexanol (4.89 g, 27 mmol) and triphenylphosphine (7.87 g, 30 mmol) and 50 mL MeCN were combined in a round bottomed flask. The flask was fitted with a condenser and placed in a heating mantel and the reaction was allowed to stir at 82 °C for 48 h. After this time the reaction was allowed to cool to rt and the solution was cannulated into 200

mL Et20, producing a white precipitate. The solids were allowed to settle and the solvent was decanted off. 20 mL DCM was added to dissolve the solids and then 100 mL Et^O was slowly added to afford a white precipitate. The solvent was then removed *in vacuo* to afford clean (6-hydroxyhexyl)triphenylphosphonium bromide (9.4 g, 21.2 mmol, for 78% yield).

Ṫi-NMR (300 MHz, CDCh) δ: ppm 7.80 (m, 15H); 3.80 (m, 2H); 3.65 (m, 2H); 2.23 (m, 2H); 1.68 (m, 4H); 1.52 (m, 4H).

Step 2: (Z)-Dodec-6-en-l-ol



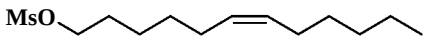
Chemical Formula: C12H24O

Molecular Weight: 184.32

[00714] A solution of (6-hydroxyhexyl)triphenylphosphonium bromide (3.0 g, 6.77 mmol) in 25 mL THF was allowed to cool in a -78 °C dry ice/acetone bath. Once cool «-BuLi (2.5 M in hexanes) (5.7 mL, 14.2 mmol) was added dropwise. After 1 h, an additional 10 mL THF and «-BuLi (1.35 mL) were added and stirring was continued at the same temperature for 1 h. After this time 1-hexanal (1.6 mL, 13.5 mmol) was added and the reaction was allowed to warm to rt and stir for 3 h. After this time the reaction was quenched by addition of excess saturated NH4CI. The solution was extracted three times with EtOAc. The pooled organics were washed with brine, dried over MgS04, filtered and concentrated *in vacuo.* The crude material was purified by silica gel chromatography (0-50% EtOAc in hexanes) to afford the desired product as a clear oil (0.76 g, 4.1 mmol, 61%).

Ṫi-NMR (300 MHz, CDCh) δ: ppm 5.34 (m, 2H); 3.62 (t, 2H); 2.01 (m, 4H); 1.56 (m, 2H); 1.35-1.27 (m, 11H); 0.87 (t, 3H).

Step 3: (Z)-Dodec-6-en-l-yl methanesulfonate



Chemical Formula: C13H26O3S

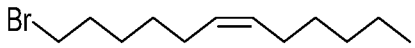
Molecular Weight: 262.41

[00715] To a 0 °C solution of (Z)-dodec-6-en-l-ol (1.81 g, 9.3 mmol) in 20 mL DCM, was added Et3N (1.7 mL, 12.1 mmol) and methanesulfonyl chloride (0.80 mL, 10.2 mmol). The reaction was allowed to slowly warm to rt and stir ovemight. The reaction was quenched by the addition of water and the mixture was extracted two times with DCM. The organics were pooled, washed with brine, dried over MgS04, filtered and concentrated. The crude material

was purified by silica gel chromatography (0-30% EtOAc in hexanes) to afford clean desired product (2.2 g, 8.4 mmol, 90%).

¾-NMR (300 MHz, CDCh) δ: ppm 5.34 (m, 2H); 4.20 (t, 2H); 2.98 (s, 3H); 2.01 (m, 4H); 1.74 (m, 2H); 1.38-1.27 (m, 10H); 0.87 (t, 3H).

Step 4: (Z)-l -Bromododec-6-ene



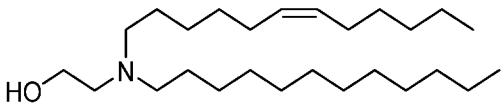
Chemical Formula: $C_{12}H_{23}Br$

Molecular Weight: 247.22

[00716] In a round bottomed flask, under N_2, (Z)-dodec-6-en-l -yl methanesulfonate (2.2 g, 8.3 mmol) was dissolved in 40 mL Et20. $MgBr_2$-Et_20 (6.5 g, 25 mmol) was added and the reaction was allowed to stir for 48 h. After this time the reaction was quenched by the addition of ice. The mixture was then extracted with Et^O three times. The pooled organics were washed with brine, dried over MgS04, filtered and concentrated. The crude material was purified by silica gel chromatography (0-30% EtOAc in hexanes) to afford the desired product (1.8 g, 7.28 mmol, 88%).

Ti-NMR (300 MHz, CDCh) δ: ppm 5.34 (m, 2H); 3.39 (t, 2H); 2.01-1.84 (m, 6H); 1.28 (m, 10H); 0.87 (t, 3H).

Step 5: (Z)-2-(Dodec-6-en-l-yl(dodecyl)amino)ethan-l -ol



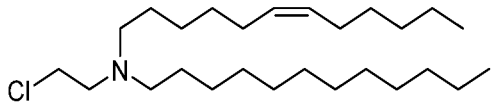
Chemical Formula: C26H53NO

Molecular Weight: 395.72

[00717] In the same manner as Step 1 for Compound 18, (Z)-2-(Dodec-6-en-l -yl(dodecyl)amino)ethan-l-ol was synthesized from (Z)-l -bromododec-6-ene (0.25 g, 1.0 mmol), 2-(dodecylamino)ethan-l-ol (0.23 g, 1.0 mmol), K2CO3 (0.14 g, 1.0 mmol) and KI (2 mg, 0.01 mmol) in 5 mL MeCN.

¾-NMR (300 MHz, CDCh) δ: ppm 5.34 (m, 2H); 3.65 (br. m , 2H); 2.64 (br. m, 6H); 2.00 (m, 4H); 1.55 (m, 6H); 1.24 (m, 26H); 0.86 (t, 6H).

Step 6: (Z)-*N*-(2-Chloroethyl)-*N*-dodecyldodec-6-en-1-amine



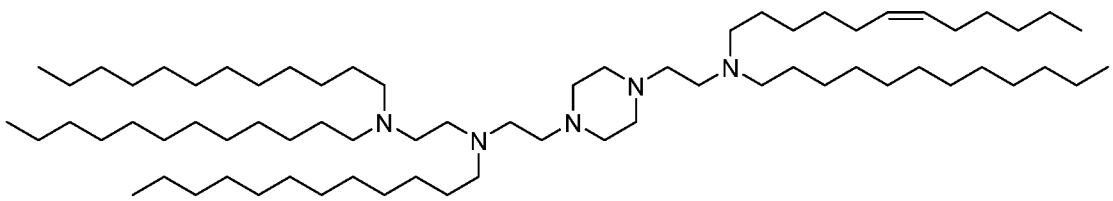
Chemical Formula: C26H52ClN

Molecular Weight: 414.16

[00718] In the same manner as Step 2 for Compound 18, (Z)-*N*-(2-chloroethyl)-*N*-dodecyldodec-6-en-1-amine was synthesized from (Z)-2-(dodec-6-en-1-yl(dodecyl)amino)ethan-1-ol (35 mg, 0.088 mmol), methanesulfonyl chloride (0.008 mL, 0.097 mmol) and triethylamine (0.018 mL, 0.13 mmol) in 0.5 mL DCM. Yield (17.3 mg, 47%).

¾-NMR (300 MHz, CDCh) δ: ppm 5.34 (m, 2H); 3.47 (t, 2H); 2.74 (t, 2H); 2.43 (t, 4H); 2.0 (m, 4H); 1.24 (m, 32H); 0.86 (t, 6H).

Step 7: Compound 24: (Z)-N^1-(2-(4-(2-(Dodec-6-en-1-yl(dodecyl)amino)ethyl)piperazin-1-yl)ethyl)-N^1,N^2,N^2-tridodecylethane-1,2-diamine



Chemical Formula: C70H143N5

Molecular Weight: 1054.95

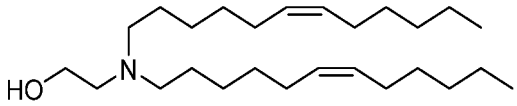
[00719] In the same manner as Step 6 for Compound 18, (Z)-N^1-(2-(4-(2-(dodec-6-en-1-yl(dodecyl)amino)ethyl)piperazin-1-yl)ethyl)-N^2,N^2-tridodecylethane-1,2-diamine was synthesized from N^1,N^1,N^2-tridodecyl-N^2-(2-(piperazin-1-yl)ethyl)ethane-1,2-diamine (27 mg, 0.040 mmol) and (Z)-*N*-(2-chloroethyl)-*N*-dodecyldodec-6-en-1-amine (17.3 mg, 0.042 mmol), K_2CO_3 (6 mg, 0.040 mmol) in 0.5 mL DCM. Yield (24 mg, 0.023 mmol, 57%).

UPLC: RT = 3.78 min. MS (ES): *m/z* (MH^+) 1056.376 for C70H143N5

¾ NMR (400 MHz, CDCh) δ: ppm 5.36 (m, 2H); 2.53-2.35 (m, 30H); 2.00 (m, 4H); 1.39-1.24 (m, 92H); 0.86 (m, 15H).

Z: Compound 25: N^1-(2-(4-(2-(Di((Z)-dodec-6-en-1-yl)amino)ethyl)piperazin-1-yl)ethyl)-N^1,N^2,N^2-tridodecylethane-1,2-diamine

Step 1: 2-(Di((Z)-dodec-6-en-1-yl)amino)ethan-1-ol



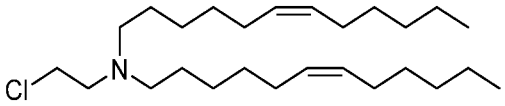
Chemical Formula: C26H51NO

Molecular Weight: 393.70

[00720] In the same manner as Step 1 for Compound 18, 2-(di((Z)-dodec-6-en-l-yl)amino)ethan-l-ol was synthesized from ethanolamine (60 mg, 1.0 mmol), (Z)-l-bromododec-6-ene (0.51 g, 2.1 mmol) and K2CO3 (0.14 g, 1.0 mmol) in 5 mL DCM. Yield (0.22 g, 0.56 mmol, 56%).

¾ NMR (400 MHz, CDCh) δ: ppm 5.34 (m, 4H); 3.59 (m, 2H); 2.65-2.53 (m, 6H); 2.00 (m, 9H); 1.49 (m, 4H); 1.23 (m, 20H); 0.86 (t, 6H).

Step 2: (Z)-*N*-(2-Chloroethy l)-*N*-((Z)-dodec-6-en- 1-yl)dodec-6-en- 1-amine



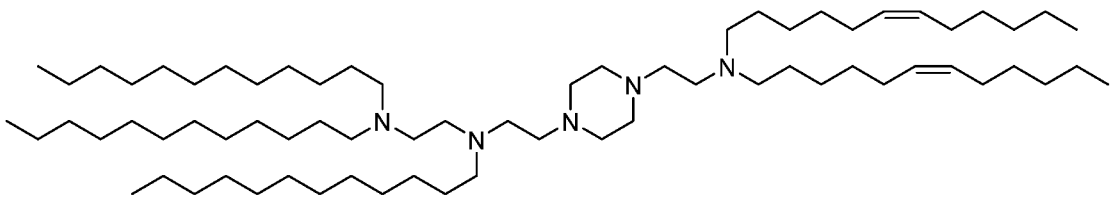
Chemical Formula: C26H50CIN

Molecular Weight: 412.14

[00721] In the same manner as Step 2 for Compound 18, (Z)-*N*-(2-chloroethyl)-*N*-((Z)-dodec-6-en-l-yl)dodec-6-en-l -amine was synthesized from2-(di((Z)-dodec-6-en-l-yl)amino)ethan-l-ol (0.22 g, 0.56 mmol), methanesulfonyl chloride (0.047 mL, 0.61 mmol) and triethylamine (0.12 mL, 0.84 mmol) in 3 mL DCM. (Yield (150 mg, 0.36 mmol, 65%).

¾ NMR (400 MHz, CDCh) δ: ppm 5.34 (m, 4H); 3.47 (t, 2H); 2.74 (t, 2H); 2.43 (t, 4H); 2.00 (m, 8H); 1.41-1.27 (m, 24H); 0.87 (m, 6H).

Step 3: Compound 25: N^1-(2-(4-(2-(Di((Z)-dodec-6-en-l-yl)amino)ethyl)piperazin-l-yl)ethyl)-N^1,N^2,N^2-tridodecylethane- 1,2-diamine



Chemical Formula: C70H141N5

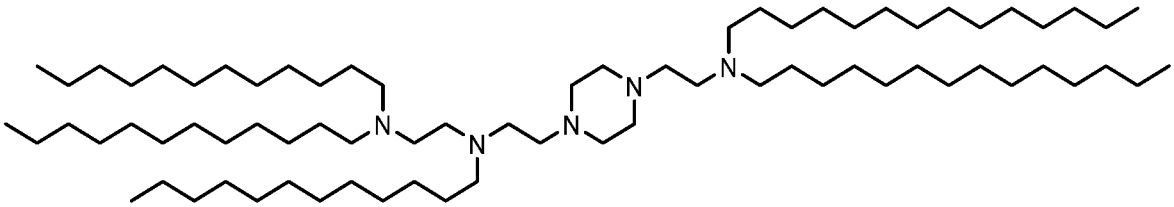
Molecular Weight: 1052.93

[00722] In the same manner as Step 6 for Compound 18, N^1-(2-(4-(2-(di((Z)-dodec-6-en-1-yl)amino)ethyl)piperazin-1-y^ethy ^-N^A^^-tridodecylethane-1,2-diamine was synthesized from (Z)-*N*-(2-chloroethyl)-*N*-((Z)-dodec-6-en-l-yl)dodec-6-en-l-amine (56 mg, 0.14 mmol), N^1,N^1,N^2-tridodecyl-N^2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine (84 mg, 0.12 mmol) and K2CO3 (17 mg, 0.12 mmol) in 1 mL MeCN. Yield (41.9 mg, 0.040 mmol, 33%).

UPLC: RT = 3.74 min. MS (ES): *m/z* (MH^+) 1053.564 for C70H141N5

¾ NMR (400 MHz, CDCh) δ: ppm 5.33 (m, 4H); 2.55-2.35 (br. m, 30H); 1.98 (m, 8H); 1.32-1.24 (m, 84H); 0.86 (m, 15H).

AA: Compound 26: N^1-(2-(4-(2-(Ditetradecylamino)ethyl)piperazin-l-yl)ethyl)-N^1,N^2,N^2-tridodecylethane-1,2-diamine



Chemical Formula: C74H153N5

Molecular Weight: 1113.07

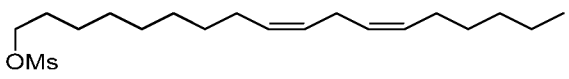
[00723] In the same manner as Step 6 for Compound 18, ^-(2-(4-(2-(ditetradecylamino)ethyl)piperazin-1-y^ethy ^-N^A^^-tridodecylethane-1,2-diamine was synthesized from N^1,N^1,N^2-tridodecyl-N^2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine (75 mg, 0.111 mmol), *N*-(2-chloroethyl)-*N*-tetradecyltetradecan-l-amine (58 mg, 0.122 mmol), K2CO3 (31 mg, 0.221 mmol), and KI (3 mg, 0.0181 mmol) in THF (4 mL). Yield (17 mg, 7%).

UPLC/ELSD: RT = 3.88 min. MS (ES): *m/z* (MH^+) 1113.59 for C74H153N5

¾-NMR (300 MHz, CDCh) δ: ppm 3.15-2.00 (br. m, 30H); 1.75-0.90 (br. m, 108H); 0.81 (t, 15H).

AB: Compound 27: N^1,N^1,N^2-Tridodecyl-N^2-(2-(4-(2-(dodecyl((9Z, 12Z)-octadeca-9, 12-dien-l-yl)amino)ethyl)piperazin-l-yl)ethyl)ethane-l,2-diamine

Step 1: (6Z,9Z)-1 8-(Methylsulfonyl)octadeca-6,9-diene



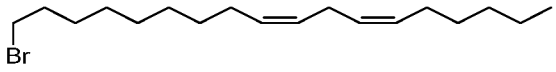
Chemical Formula: C19H36O3S

Molecular Weight: 344.55

[00724] To a 0 °C solution of linoleyl alcohol (10 mL, 31.2 mmol) and triethylamine (5.68 mL, 40.5 mmol)) in DCM (50 mL) was added dropwise a solution of methanesulfonyl chloride (2.66 mL, 34.3 mmol) in DCM (20 mL). The reaction was allowed to return to room temperature and let stir for 4 hours. The mixture was quenched by the addition of water and extracted with DCM. The organic layer was washed with saturated NaHCCb, brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-40% EtOAc/hexanes) provided (6Z,9Z)-1 8-(methylsulfonyl)octadeca-6,9-diene (10.0 g, 93%).

¾-NMR (300 MHz, CDCh) δ: ppm 5.35 (m, 4H); 4.22 (t, 2H); 2.99 (s, 3H); 2.77 (t, 2H); 2.04 (q, 4H); 1.74 (m, 2H); 1.30 (br. m, 16H); 0.89 (t, 3H).

Step 2: (6Z,9Z)-1 8-Bromooctadeca-6,9-diene



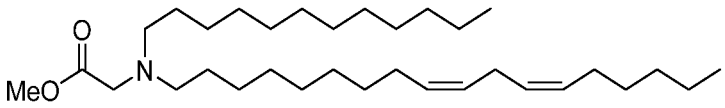
Chemical Formula: CisifeBr

Molecular Weight: 329.37

[00725] To a solution of (6Z,9Z)-1 8-(methylsulfonyl)octadeca-6,9-diene (10.0 g, 29.0 mmol) in diethyl ether (372 mL) was added magnesium bromide ethyl etherate (22.5 g, 87. 1 mmol). The reaction was let stir at room temperature for 16 hours. The mixture was quenched by the addition of water and extracted with diethyl ether. The combined organic layers were washed with 1% K2CO3, brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography provided (6Z,9Z)-1 8-bromooctadeca-6,9-diene (8.9 g, 93%).

¾-NMR (300 MHz, CDCh) δ: ppm 5.36 (m, 4H); 3.41 (t, 2H); 2.77 (t, 2H); 2.05 (q, 4H); 1.86 (m, 2H); 1.48-1 .22 (br. m, 16H); 0.89 (t, 3H).

Step 3: Methyl *N*-dodecyl-*N*-((9*Z*,12*Z*)-octadeca-9,12-dien-1-yl)glycinate



Chemical Formula: C33H63NO2

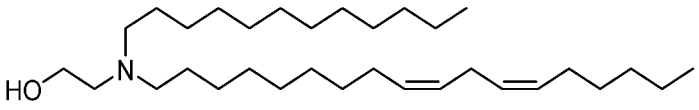
Molecular Weight: 505. 87

[00726] To a solution of methyl dodecylglycinate-HCl (1 g, 3.4 mmol) in 8.5 mL DMF, (Z)-l-bromooctadec-9-ene (1.68 g, 5.1 mmol) and K2CO3 (1.4 g, 10.2 mmol) were added. The

reaction was then allowed to stir at 85 °C for 12 h. After this time the reaction was allowed to cool to rt and was quenched by the addition of excess H2O. The mixture was extracted 3 times with EtOAc. The organics were pooled and washed with brine, dried over MgS04, filtered and concentrated. The crude material was purified by silica gel chromatography (0-10% EtOAc in hexanes) to afford the desired product (0.87 g, 1.72 mmol, 50%).

Ti-NMR (300 MHz, CDCh) δ: ppm 5.33 (m, 4H); 3.68 (s, 3H); 3.30 (s, 2H); 2.74 (t, 2H); 2.52 (m, 4H); 2.02 (m, 4H); 1.23 (m, 38H); 0.86 (m, 6H).

Step 4: 2-(Dodecy1((9Z, 12Z)-octadeca-9, 12-dien- 1-yl)amino)ethan- 1-ol



Chemical Formula: C32H63NO

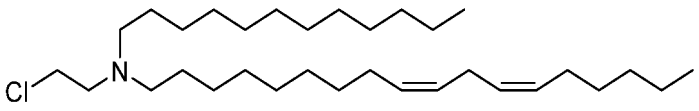
Molecular Weight: 477.86

[00727] To a 0 °C solution of methyl *N*-dodecyl-*N*-((9Z,12Z)-octadeca-9,l 2-dien- 1-yl)glycinate (980 mg, 1.94 mmol) in THF (10 mL) was added dropwise lithium aluminum hydride (184 mg, 4.85 mmol). The reaction was allowed to return to room temperature and let stir for 3 hours. The mixture was slowly quenched by the stepwise addition of water (0. 184 mL), 10% NaOH (0.552 mL) and water (0.184 mL). The reaction mixture was filtered, washed with THF and concentrated *in vacuo*. Purification by ISCO silica flash chromatography (0-100% [DCM, 20% MeOH, 1% NH_4OH]/MeOH) provided 2-(dodecyl((9Z,12Z)-octadeca-9,12-dien- 1-yl)amino)ethan- 1-ol (660 mg, 71%).

UPLC/ELSD: RT = 3.13 min. MS (ES): *m/z* (MH^+) 478.52 for C32H63NO

¾-NMR (300 MHz, CDCh) δ: ppm 5.39 (br. m, 4H); 3.56 (br. m, 2H); 2.80 (br. m, 2H); 2.61 (br. m, 2H); 2.48 (br. m, 4H); 2.09 (br. m, 4H); 1.57-1.17 (br. m, 38H); 0.91 (br. m, 6H).

Step 5: (9Z,12Z)-*N*-(2-Chloroethyl)-*N*-dodecyloctadeca-9,l 2-dien- 1-amine



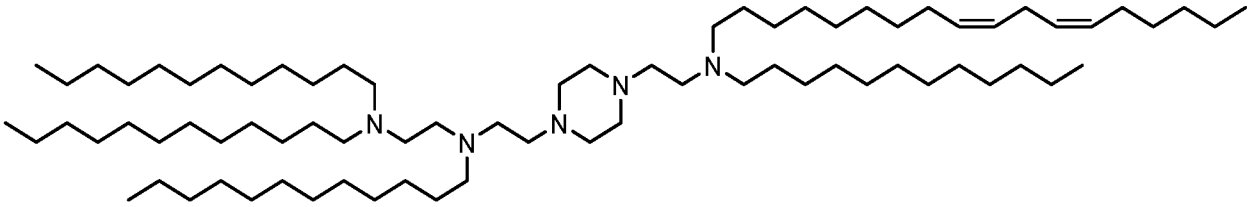
Chemical Formula: C32H62CIN

Molecular Weight: 496.31

[00728] In a same manner as Step 2 for Compound 18, (9Z, 12Z)-*N*-(2-chloroethyl)-*N*-dodecyloctadeca-9,1 2-dien- 1-amine was synthesized from 2-(dodecyl((9Z,12Z)-octadeca-9,12-

dien-l-yl)amino)ethan-l-ol (660 mg, 1.38 mmol), triethylamine (0.249 mL, 1.80 mmol), and methanesulfonyl chloride (0.172 mL, 1.73 mmol) in DCM (7 mL). Yield (123 mg, 18%).
UPLC/ELSD: RT = 3.23 min. MS (ES): *m/z* (MH^+) 496.72 for C32H62CIN
¾-NMR (300 MHz, CDCh) δ: ppm 5.29 (br. m, 4H); 3.43 (br. m, 2H); 2.71 (br. m, 4H); 2.38 (br. m, 4H); 1.98 (br. m, 4H); 1.45-1.07 (br. m, 38H); 0.82 (br. m, 6H).

Step 6: Compound 27: N^1,N^1,N^2-Tridodecyl -N^2-(2-(4-(2-(dodecyl((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)ethy l)piperazin- 1-yl)ethy l)ethane- 1,2-diamine

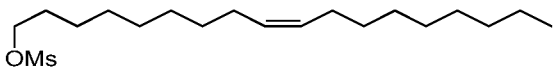


(Chemical Formula: C76H153N5
Molecular Weight: 1137. 10

[00729] In the same manner as Step 6 for Compound 18, N^1,N^1,N^2-tridodecyl -N^2-(2-(4-(2-(dodecy 1((9Z, 12Z)-octadeca-9, 12-dien- 1-yl)amino)ethy l)piperazin- 1-y l)ethy l)ethane- 1,2-diamine was synthesized from N^1,N^1,N^2-tridodecyl -N^2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine (67 mg, 0.099 mmol), (9Z,12Z)-*N*-(2-chloroethyl)-*N*-dodecyloctadeca-9,l 2-dien- 1-amine (64 mg, 0.129 mmol) K2CO3 (28 mg, 0.198 mmol), and KI (2 mg, 0.012 mmol) in THF (2 mL). Yield (48 mg, 43%).
UPLC/ELSD: RT = 3.90 min. MS (ES): *m/z* (MH^+) 1137.95 for C76H153N5
¾-NMR (300 MHz, CDCh) δ: ppm 5.48-5.29 (m, 4H); 3.15-2.15 (br. m, 32H); 2.07 (br. m, 4H); 1.83-1.00 (br. m, 98H); 0.91 (br. m, 15H).

AC: Compound 28: N^1-(2-(4-(2-(Di((Z)-octadec-9-en-l-yl)amino)ethyl)piperazin- 1-yl)ethyl)-N^1,N^2,N^2-tridodecy lethane- 1,2-diamine

Step 1: (Z)-l-(Methylsulfonyl)octadec-9-ene



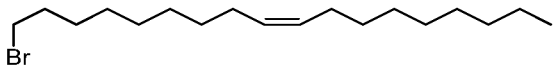
Chemical Formula: C19H38O3S
Molecular Weight: 346.57

[00730] In the same manner as Step 1 for Compound 27, (Z)-l-(methylsulfonyl)octadec-9-ene was synthesized from oleyl alcohol (10 mL, 31.7 mmol), triethylamine (5.74 mL, 41.2

mmol), and methanesulfonyl chloride (2.70 mL, 34.9 mmol) in DCM (50 mL). Yield (8.55 g, 78%).

¾-NMR (300 MHz, CDCh) δ: ppm 5.33 (m, 2H); 4.20 (t, 2H); 2.98 (s, 3H); 2.00 (m, 4H); 1.73 (m, 2H); 1.44-1.16 (br. m, 22H); 0.87 (t, 3H).

Step 2: (Z)-l -Bromooctadec-9-ene



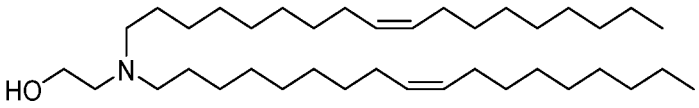
Chemical Formula: $C_{18}H_{35}Br$

Molecular Weight: 331.38

[00731] In the same manner as Step 2 for Compound 27, (Z)-l -bromooctadec-9-ene was synthesized from (Z)-l -(methylsulfonyl)octadec-9-ene (8.55 g, 24.7 mmol), and magnesium bromide ethyl etherate (19.1 g, 74.1 mmol) in diethyl ether (317 mL). Yield (7.42 g, 91%).

¾-NMR (300 MHz, CDCh) δ: ppm 5.35 (m, 2H); 3.41 (t, 2H); 2.01 (m, 4H); 1.85 (m, 2H); 1.48-1.14 (br. m, 22H); 0.88 (t, 3H).

Step 3: 2-(Di((Z)-octadec-9-en-1-yl)amino)ethan-1-ol



Chemical Formula: C38H75NO

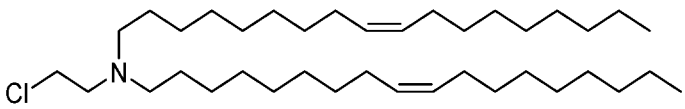
Molecular Weight: 562.02

[00732] In the same manner as Step 1 for Compound 18, 2-(di((Z)-octadec-9-en-l-yl)amino)ethan-l -ol was synthesized from (Z)-l-bromooctadec-9-ene (5 g, 15.1 mmol), ethanolamine, (0.414 mL, 6.86 mmol), K2CO3 (4.17 g, 30.2 mmol), and KI (114 mg, 0.686 mmol) in MeCN (32 mL). Yield (3.2 g, 83%).

UPLC/ELSD: RT = 7.325 min. MS (ES): *m/z* (MH^+) 562.60 for C38H75NO

¾-NMR (300 MHz, CDCh) δ: ppm 5.34 (m, 4H); 3.53 (t, 2H); 2.58 (t, 2H); 2.45 (t, 4H); 2.01 (m, 8H); 1.44 (m, 4H); 1.38-1.18 (br. m, 44H); 0.88 (t, 6H).

Step 4: (Z)-*N* -(2-Chloroethyl)-*N*-((*Z*)-octadec-9-en-1-yl)octadec-9-en-1-amine

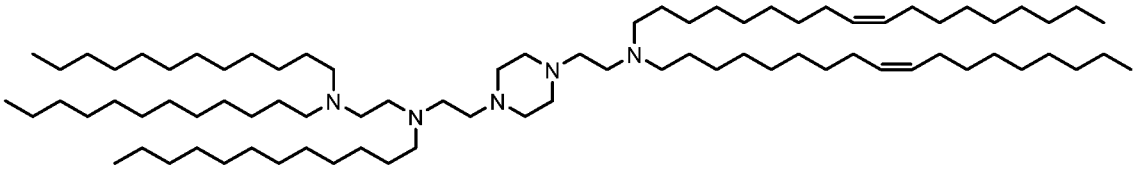


Chemical Formula: C38H74CIN

Molecular Weight: 580.47

[00733] In the same manner as Step 2 for Compound 18, (Z)-*N*-(2-chloroethyl)-*N*-((Z)-octadec-9-en-l-yl)octadec-9-en-l -amine was synthesized from 2-(di((Z)-octadec-9-en-l-yl)amino)ethan-l-ol (1.64 g, 2.92 mmol) triethylamine (0.529 mL, 3.79mmol), and methanesulfonyl chloride (0.282 mL, 3.65 mmol) in DCM (15 mL). Yield (1.47 g, 87%).
UPLC/ELSD: RT = 3.75 min. MS (ES): *m/z* (MH^+) 580.64 for C38H74CIN
¾-NMR (300 MHz, CDCh) δ: ppm 5.35 (m, 4H); 3.48 (br. m, 2H); 2.77 (br. m, 2H); 2.45 (br. m, 4H); 2.02 (br. m, 8H); 1.62-1.05 (br. m, 48H); 0.89 (t, 6H).

Step 5: Compound 28: N^1-(2-(4-(2-(Di((Z)-octadec-9-en-l-yl)amino)ethyl)piperazin-l-yl)ethyl)-N^1^^-tridodecy lethane- 1,2-diamine

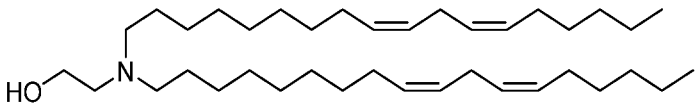


Chemical Formula: $C_{82}H_{165}N_5$
Molecular Weight: 1221.26

[00734] In the same manner as Step 6 for Compound 18, N^1-(2-(4-(2-(di((Z)-octadec-9-en-l-yl)amino)ethyl)piperazin-l-yl)ethyl)-N^1,N^2,N^2-tridodecy lethane- 1,2-diamine was synthesized from N^1,N^1,N^2-tridodecyl-N^2-(2-(piperazin-l-yl)ethyl)ethane- 1,2-diamine (75 mg, 0.111 mmol), (Z)-*N*-(2-chloroethyl)-*N*-((Z)-octadec-9-en-l-yl)octadec-9-en-l -amine (71 mg, 0.122 mmol) K2CO3 (31 mg, 0.222 mmol), and KI (3 mg, 0.018 mmol) in THF (1.5 mL). Yield (20 mg, 15%).
UPLC/ELSD: RT = 4.05 min. MS (ES): *m/z* (MH^+) 1221.72 for $C_{82}H_{165}N_5$
¾-NMR (300 MHz, CDCh) δ: ppm 5.29-5.14 (br. m, 4H); 2.95-2.00 (br. m, 30H); 1.96-1.77 (br. m, 8H); 1.60-0.85 (br. m, 108H); 0.76 (br. m, 15H).

AD: Compound 29: N^1-(2-(4-(2-(Di((9Z,12Z)-octadeca-9,12-dien-l-yl)amino)ethyl)piperazin-l-yl)ethyl)-N ^{1},N^2,N^2-tridodecylethane- 1,2-diamine

Step 1: 2-(Di((9Z,12Z)-octadeca-9,12-dien-l-yl)amino)ethan-l-ol



Chemical Formula: C38H71NO

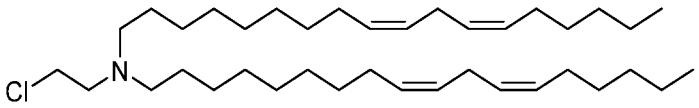
Molecular Weight: 557.99

[00735] In the same manner as Step 1 for Compound 18, 2-(di((9Z,12Z)-octadeca-9,12-dien-l-yl)amino)ethan-l-ol was synthesized from (6Z,9Z)-18-bromooctadeca-6,9-diene (4 g, 12.1 mmol), ethanolamine, (0.334 mL, 5.52 mmol), K2CO3 (3.36 g, 24.3 mmol), and KI (92 mg, 0.552 mmol) in MeCN (26 mL). Yield (1.9 g, 62%).

UPLC/ELSD: RT = 6.80 min. MS (ES): *m/z* (MH^+) 557.94 for **$C_{38}H_7$iNO**

¾-NMR (300 MHz, CDCh) δ: ppm 5.35 (m, 8H); 3.52 (t, 2H); 2.77 (t, 4H); 2.57 (t, 2H); 2.43 (t, 4H); 2.04 (q, 8H); 1.48-1.18 (br. m, 36H); 0.89 (t, 6H).

Step 2: (9Z,12Z*)-N*-(2-Chloroethyl *)-N*-((9Z,12Z)-octadeca-9,12-dien-l-yl)octadeca-9,12-dien-l-amine



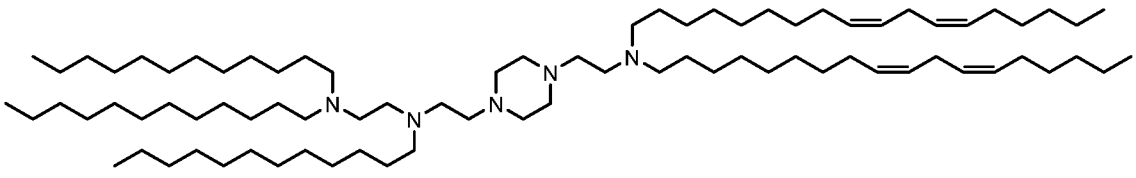
Chemical Formula: C38H70CIN

Molecular Weight: 576.44

[00736] In a same manner as Step 2 for Compound 18, (9Z, 12Z*)-N*-(2-chloroethyl *)-N*-((9Z,12Z)-octadeca-9,12-dien-l-yl)octadeca-9,12-dien-l -amine was synthesized from 2-(di((9Z,12Z)-octadeca-9,12-dien-l-yl)amino)ethan-l-ol (250 mg, 0.45 mmol), triethylamine (81 μL, 0.58 mmol), and methanesulfonyl chloride (38 μL, 0.49 mmol) in DCM (2 mL). Yield (134 mg, 52%).

¾-NMR (300 MHz, CDCh) δ: ppm 5.36 (m, 8H); 3.49 (t, 2H); 2.78 (m, 6H); 2.45 (t, 4H); 2.05 (q, 8H); 1.48-1.18 (br. m, 36H); 0.89 (t, 6H).

Step 3: Compound 29: N^1-(2-(4-(2-(Di((9Z,12Z)-octadeca-9,12-dien-l-yl)amino)ethyl)piperazin-l-yl)ethyl)-*N*^^-tridodecylethane-l^-diamine



Chemical Formula: C82H161N5

Molecular Weight: 1217.23

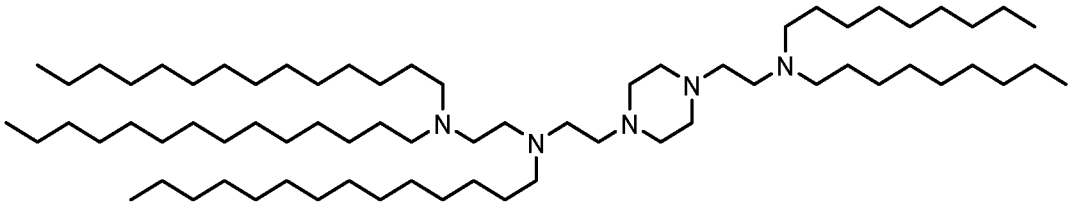
[00737] In the same manner as Step 6 for Compound 18, N^1-(2-(4-(2-(di((9Z, 12Z)-octadeca-9, 12-dien- 1-yl)amino)ethy l)piperazin- 1-yl)ethyl)-N^1,N^2,*N*Mridodecy lethane- 1,2-

diamine was synthesized from *N*[1],*N*[1],*N*[2]-tridodecyl -*N*[2]-(2-(piperazin-l -yl)ethyl)ethane-l ,2-diamine (75 mg, 0.111 mmol), (9Z, 12Z)-*N* -(2-chloroethyl)-*N* -((9Z, 12Z)-octadeca-9, 12-dien-l -yl)octadeca-9, 12-dien-l -amine (71 mg, 0.122) K2CO3 (31 mg, 0.222 mmol), and KI (3 mg, 0.018 mmol) in THF (3 mL). Yield (20 mg, 15%)

UPLC/ELSD: RT = 3.97 min. MS (ES): *m/z* (MH^+) 1217.95 for C82H161N5

¾-NMR (300 MHz, CDCh) δ: ppm 5.48-5.28 (m, 12H); 3.30-2.20 (br. m, 36H); 2.17-1.92 (br. m, 12H); 1.90-1.00 (br. m, 94H); 0.87 (br. m, 15H).

AE: Compound 30: *N*[1]-(2-(4-(2-(Dinonylamino)ethyl)piperazin-l-yl)ethyl)-*N*[1],*N*[2],*N*[2]-tritetradecylethane-l,2-diamine



Chemical Formula: C70H145N5

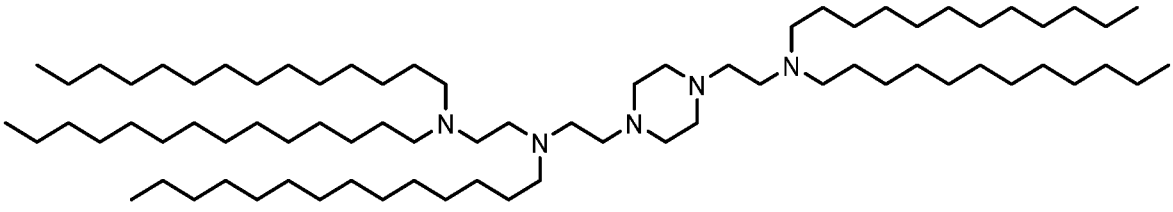
Molecular Weight: 1056.97

[00738] In the same manner as Step 6 for Compound 18, *N*[1]-(2-(4-(2-(dinonylamino)ethyl)piperazin-l -y^ethy ^-*N* ^A^^-tritetradecylethane-l ,2-diamine was synthesized from *N*[1]-(2-(piperazin-l -yl)ethyl)-*N*[1]^,*N*[2]-tritetradecylethane-l ,2-diamine (150 mg, 0.197 mmol), *N* -(2-chloroethyl)-*N* -nonylnonan-l -amine (79 mg, 0.236 mmol), K2CO3 (54 mg, 0.394 mmol), and KI (3 mg, 0.0134 mmol) in THF (4 mL). Yield (50 mg, 24%).

UPLC/ELSD: RT = 3.79 min. MS (ES): *m/z* (MH^+) 1057.74 for C70H145N5

¾-NMR (300 MHz, CDCh) δ: ppm 3.15-2.20 (br. m, 30H); 1.90-1.00 (br. m, 100H); 0.90 (t, 15H).

AF: Compound 31: *N*[1]-(2-(4-(2-(Didodecylamino)ethyl)piperazin-l-yl)ethyl)-N[1],N[2],N[2]-tritetradecy lethane- 1,2-diamine

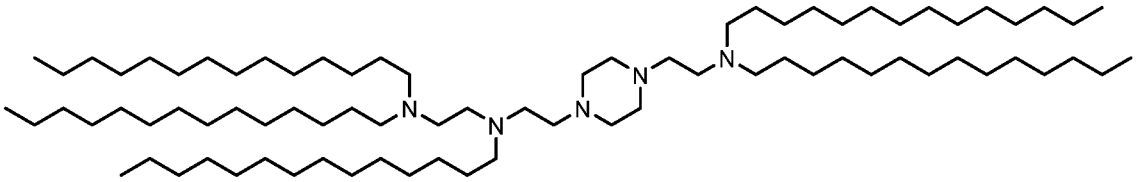


Chemical Formula: C76H157N5

Molecular Weight: 1141.13

[00739] In the same manner as Step 6 for Compound 18, N^1-(2-(4-(2-(Didodecylainino)ethyl)piperazin-l -yl)ethy l)-N^1,N^2,N^2-tritetradecylethane-1,2-diamine was synthesized from N^1-(2-(piperazin-l -yl)ethyl)-$N^1,N^2$4ritetradecylethane-l ,2-diamine (150 mg, 0.197 mmol), N-(2-chloroethyl)-N -dodecyldodecan-l -amine (98 mg, 0.236 mmol), K2CO3 (54 mg, 0.394 mmol), and KI (3 mg, 0.01 34 mmol) in THF (4 mL). Yield (42 mg, 19%). UPLC/ELSD: RT = 3.98 min. MS (ES): *m/z* (MH^+) 1142. 14 for C76H157N5 ¾-NMR (300 MHz, CDCh) δ: ppm 3.20-2.20 (br. m, 30H); 1.90-1 .00 (br. m, 112H); 0.90 (t, 15H).

AG: Compound 32: N^1-(2-(4-(2-(Ditetradecylamino)ethyl)piperazin-l -yl)ethyl)-N ^{1},N^2,N^2-tritetradecy lethane- 1,2-diamine



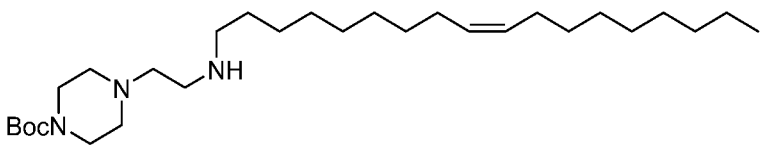
Chemical Formula: $C_{80}H_{165}N_5$

Molecular Weight: 1197.24

[00740] In the same manner as Step 6 for Compound 18, N^1-(2-(4-(2-(ditetradecylamino)ethyl)piperazin-l-yl)ethyl)-N^1,N^2,N^2-tritetradecy lethane- 1,2-diamine was synthesized from N^1-(2-(piperazin-l -yl)ethyl)-N^1,N^2-tritetradecylethane-l ,2-diamine (150 mg, 0.197 mmol), N-(2-chloroethyl)-N -tetradecyltetradecan-l -amine (130 mg, 0.276 mmol), K2CO3 (54 mg, 0.394 mmol), and KI (3 mg, 0.0134 mmol) in THF (4 mL). Yield (17 mg, 7%). UPLC/ELSD: RT = 4.11 min. MS (ES): *m/z* (MH^+) 1198.32 for $C_{80}H_{165}N_5$ ¾-NMR (300 MHz, CDCh) δ: ppm 3.20-2. 15 (br. m, 30H); 1.90-1 .00 (br. m, 120H); 0.90 (t, 15H).

AH: Compound 33: N^1-(2-(4-(2-(Didodecylamino)ethyl)piperazin-l -yl)ethyl)-N^1,N^2,N^2-tri((*Z*)-octadec-9-en- 1-yl)ethane- 1,2-diamine

Step 1: ɒ t -Butyl (Z)-4-(2-(octadec-9-en-l-ylamino)ethyl)piperazine-l-carboxylate

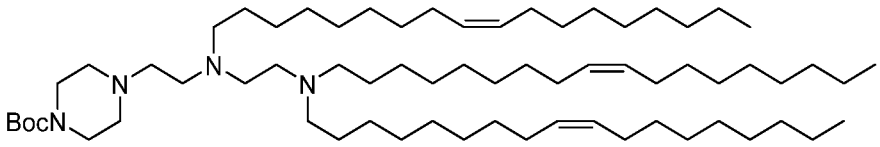


Chemical Formula: C29H57N3O2

Molecular Weight: 479.79

[00741] In the same manner as Step 3 for Compound 18, *tert*-butyl (Z)-4-(2-(octadec-9-en-l-ylamino)ethyl)piperazine-l-carboxylate was synthesized from (Z)-l-bromooctadec-9-ene (1.95 g, 11.8 mmol), 4-(2-aminoethyl)-l-boc-piperazine (1.35 g, 5.89 mmol), K2CO3 (1.60 g, 11.8 mmol), and KI (98 mg, 0.689 mmol) in MeCN (30 mL). Yield (790 mg, 28%).
¾-NMR (300 MHz, CDCh) δ: ppm 5.32 (m, 2H); 3.45 (br, 4H); 2.75 (t, 2H); 2.65 (t, 2H); 2.57 (t, 2H); 2.40 (br, 4H); 2.09 (br, 4H); 1.48 (br. m, 11H); 1.41-1.10 (br, 22H); 0.89 (t, 3H).

Step 2: *tert*-Butyl 4-(2-((2-(di((Z)-octadec-9-en-l-yl)amino)ethyl)((Z)-octadec-9-en-l-yl)amino)ethyl)piperazine-1-carboxylate

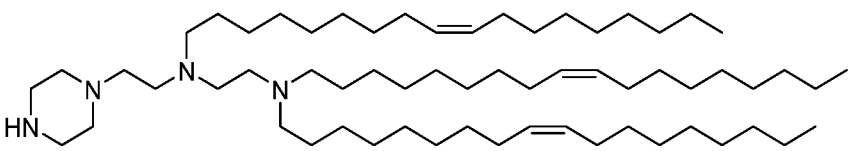


Chemical Formula: C67H130N4O2

Molecular Weight: 1023.80

[00742] In the same manner as Step 4 for Compound 18, *tert*-butyl 4-(2-((2-(di((Z)-octadec-9-en-1-yl)amino)ethyl)((Z)-octadec-9-en-1-yl)amino)ethyl)piperazine-1-carboxylate was synthesized from *tert*-butyl (Z)-4-(2-(octadec-9-en-l-ylamino)ethyl)piperazine-l-carboxylate (573 mg, 1.19 mmol), (Z)-*N*-(2-chloroethyl)-*N*-((Z)-octadec-9-en-l-yl)octadec-9-en-1-amine (693 mg, 1.19 mmol), K2CO3 (329 mg, 2.38 mmol), and KI (20 mg, 0.119 mmol) in THF (6 mL). Yield (918 mg, 75%).
¾-NMR (300 MHz, CDCh) δ: ppm 5.37 (m, 6H); 3.45 (br. m, 4H); 2.72-2.18 (br. m, 18H); 2.04 (br. m, 12H); 1.65-1.05 (br. m, 81H) 0.91 (br. m, 9H).

Step 3: N^1,N^1,N^2-Tri((Z)-octadec-9-en-1-yl)-N^2-(2-(piperazin-1-yl)ethyl)ethane-1,2-diamine



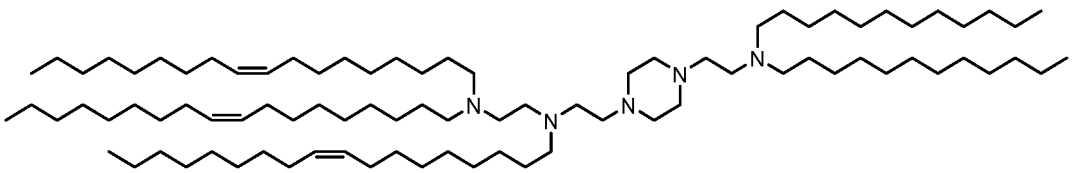
Chemical Formula: C62H122N4

Molecular Weight: 923.69

[00743] In the same manner as Step 5 for Compound 18, N^1,N^1,N^2-tri((Z)-octadec-9-en-l-yl)-N^2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine was synthesized from , *tert-butyl* 4-(2-((2-(di((Z)-octadec-9-en- 1-yl)amino)ethy l)((Z)-octadec-9-en- 1-yl)amino)ethy l)piperazine- 1-carboxylate (740 mg, 0.859 mmol), and TFA (3.3 mL, 42.9 mmol) in DCM (3.3 mL). Yield (115 mg, 14%).

¾-NMR (300 MHz, CDCh) δ: ppm 5.36 (m, 6H); 2.91 (br, 4H); 2.74-2.34 (br. m, 18H); 2.03 (br. m, 12H); 1.54-1.04 (br. m, 72H); 0.90 (br. m, 9H).

Step 4: Compound 33: N^1-(2-(4-(2-(Didodecylamino)ethyl)piperazin-l-yl)ethyl)-N^1,N^2,N^2-tri((Z)-octadec-9-en-l-yl)ethane-l,2-diamine



Chemical Formula: $C_{88}H_{175}N_5$

Molecular Weight: 1303.40

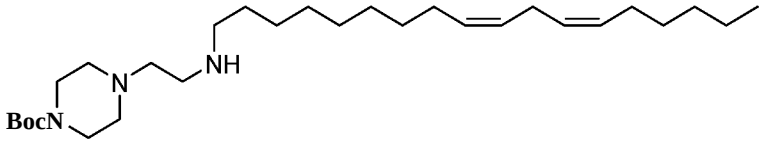
[00744] In the same manner as Step 6 for Compound 18, N^1-(2-(4-(2-(didodecy lamino)ethy l)piperazin- 1-yl)ethy l)-N^1,N^2,N^2-tri((Z)-octadec-9-en- 1-yl)ethane- 1,2-diamine was synthesized from N^1,N^1,N^2-tri((Z)-octadec-9-en-l-yl)-N^2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine (58 mg, 0.063 mmol), *N*-(2-chloroethyl)-*N*-dodecyldodecan-l-amine (31 mg, 0.075 mmol), K2CO3 (17 mg, 0.13 mmol), and KI (1 mg, 0.006 mmol) in THF (1.5 mL). Yield (30 mg, 37%).

UPLC/ELSD: RT = 4.16 min. MS (ES): *m/z* (MH^+) 1304.03 for CssHnsNs

¾-NMR (300 MHz, CDCh) δ: ppm 5.36-5.24 (br. m, 6H); 2.66-2.08 (br. m, 30H); 2.04-1.82 (br. m, 12H); 1.57-0.87 (br. m, 112H); 0.81 (br. m, 15H).

AI: Compound 34: N^1-(2-(4-(2-(Didodecylamino)ethyl)piperazin-l-yl)ethyl)-N ^{1},N^2,N^2-tri((9Z, 12Z)-octadeca-9, 12-dien-l -yl)ethane- 1,2-diamine

Step 1: *tert*-Butyl 4-(2-(((9Z,12Z)-octadeca-9,l 2-dien-l -yl)amino)ethyl)piperazine- 1-carboxylate



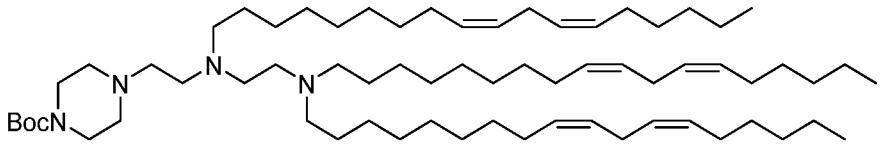
Chemical Formula: C29H55N3O2

Molecular Weight: 477.78

[00745] In the same manner as Step 3 for Compound 18, *tert*-butyl 4-(2-(((9Z,12Z)-octadeca-9, 12-dien-l -yl)amino)ethyl)piperazine-l -carboxylate was synthesized from (6Z,9Z)-18-bromooctadeca-6,9-diene (3.0 g, 9.11 mmol), 4-(2-aminoethyl)-l-boc-piperazine (2.09 g, 9.11 mmol), K2CO3 (2.52 g, 18.22 mmol), and KI (151 mg, 0.911 mmol) in MeCN (44 mL). Yield (1.20 g, 27%).

¾-NMR (300 MHz, CDCh) δ: ppm 5.35 (m, 4H); 3.42 (t, 4H); 2.77 (t, 2H); 2.73 (t, 2H); 2.62 (t, 2H); 2.51 (t, 2H); 2.38 (t, 4H); 2.04 (q, 4H); 1.60-1.20 (br. m, 27H); 0.89 (t, 3H).

Step 2: *tert*-Butyl 4-(2-((2-(di((9Z,12Z)-octadeca-9, l2-dien- 1-yl)amino)ethy 1)((9Z, 12Z)-octadeca-9, 12-dien- 1-yl)amino)ethy l)piperazine- 1-carboxy late



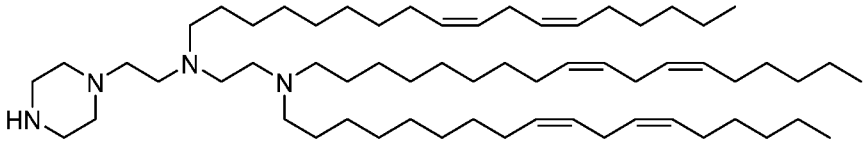
Chemical Formula: C67H124N4O2

Molecular Weight: 1017.76

[00746] In the same manner as Step 4 for Compound 18, *tert*-butyl 4-(2-((2-(di((9Z,12Z)-octadeca-9, 12-dien- l -yl)amino)ethyl)((9Z,12Z)-octadeca-9, l2-dien- 1-yl)amino)ethyl)piperazine-l -carboxy late was synthesized from *tert-b*utyl 4-(2-(((9Z, 12Z)-octadeca-9, 12-dien-l -yl)amino)ethyl)piperazine-l -carboxy late (600 mg, 1.26 mmol), (9Z, 12Z)-*N*-(2-chloroethy 1)-*N*-((9Z, 12Z)-octadeca-9, 12-dien- 1-yl)octadeca-9, 12-dien- 1-amine (796 mg, 1.38 mmol), K2CO3 (347 mg, 2.51 mmol), and KI (21 mg, 0.126 mmol) in THF (8 mL). Yield (793 mg, 62%).

UPLC/ELSD: RT = 3.95 min. MS (ES): *m/z* (MH^+) 1018.19 for C67H124N4O2

Step 3: *N*^*N* ^-Tri^Z, 12Z)-octadeca-9, 12-dien- 1-yl)-N^2-(2-(piperazin- 1-yl)ethy l)ethane- 1,2-diamine



Chemical Formula: C62H116N4

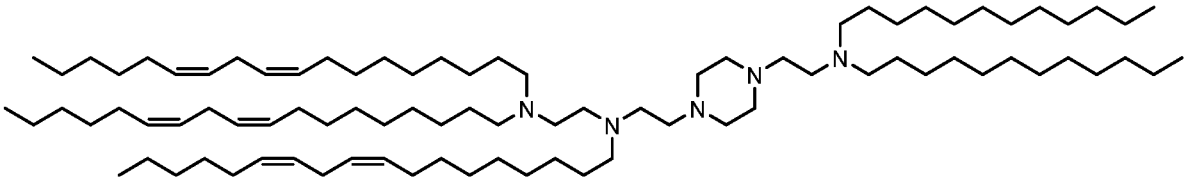
Molecular Weight: 917.64

[00747] In the same manner as Step 5 for Compound 18, *N*[1],*N*[1],*N*[2]-tri((9Z,12Z)-octadeca-9,12-dien-1-yl)-*N*[2]-(2-(piperazin-1-yl)ethyl)ethane-1,2-diamine was synthesized from *tert*-butyl 4-(2-((2-(di((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)ethyl)((9Z,12Z)-octadeca-9,12-dien-1-yl)amino)ethyl)piperazine-1-carboxylate (793 mg, 0.779 mmol), and TFA (3.0 mL, 39.0 mmol) in DCM (3.0 mL). Yield (374 mg, 52%).

UPLC/ELSD: RT = 3.68 min. MS (ES): *m/z* (MH^+) 918.84 for C62H116N4

¾-NMR (300 MHz, CDCh) δ: ppm 5.36 (m, 12H); 4.12 (m, 6H); 3.30-2.55 (22H); 2.04 (q, 12H); 1.80-1.00 (br. m, 54H); 0.89 (t, 9H).

Step 4: *N*[1]-(2-(4-(2-(Didodecylamino)ethyl)piperazin-1-yl)ethyl)-*N*^*N*^-tri^Z,12Z)-octadeca-9,12-dien-1-yl)ethane-1,2-diamine



Chemical Formula: C88H169N5

Molecular Weight: 1297.36

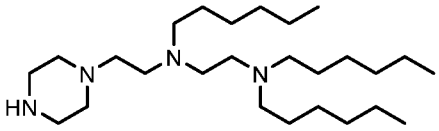
[00748] In the same manner as Step 6 for Compound 18, *N*[1]-(2-(4-(2-(didodecylamino)ethyl)piperazin-1-yl)ethyl)-*N*[1],*N*[2],*N*[2]-tri((9*Z*,12*Z*)-octadeca-9,12-dien-1-yl)ethane-1,2-diamine was synthesized from *N*[1],*N*[1],*N*[2]-tri((9Z,12Z)-octadeca-9,12-dien-1-yl)-*N*[2]-(2-(piperazin-1-yl)ethyl)ethane-1,2-diamine (75 mg, 0.082 mmol), *N*-(2-chloroethyl)-*N*-dodecyldodecan-1-amine (37 mg, 0.090) K2CO3 (23 mg, 0.163 mmol), and KI (2 mg, 0.012 mmol) in THF (3 mL). Yield (20 mg, 15%).

UPLC/ELSD: RT = 4.00 min. MS (ES): *m/z* (MH^+) 1297.88 for **$C_{88}Hi_{6}gN_5$**

¾-NMR (300 MHz, CDCh) δ: ppm 5.48-5.28 (Br, 12H); 3.30-2.20 (br. m, 36H); 2.17-1.92 (br. m, 12H); 1.90-1.00 (br. m, 94H); 0.87 (br. m, 15H).

AJ: Compound 35: *N*[1],*N*[1],*N*[2]-Trihexyl-*N*[2]-(2-(piperazin-1-yl)ethyl)ethane-1,2-diamine

Compound 35 was synthesized according to Steps 1-5 for Compound 17.

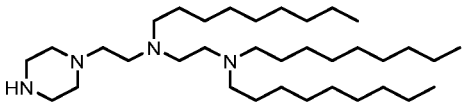


Chemical Formula: $C_{26}H_{56}N_4$

Molecular Weight: 424.76

AK: Compound 36: N^1,N^1,N^2-Trinonyl-N^2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine

Compound 36 was synthesized according to Steps 1-5 outlined for Compound 18.

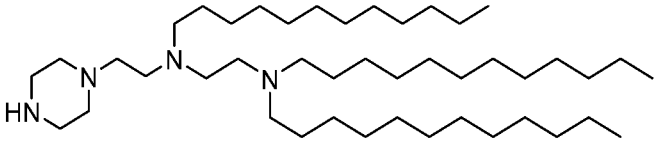


Chemical Formula: C35H74N4

Molecular Weight: 551.01

AL: Compound 37: N^1,N^1,N^2-Tridodecyl-N^2-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine

Compound 37 was synthesized according to Steps 1 and 2 for Compound 3.

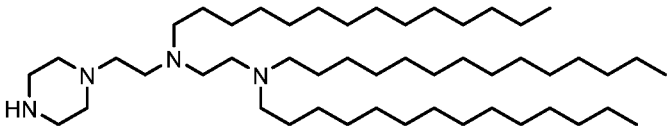


Chemical Formula: C44H92N4

Molecular Weight: 677.25

AM: Compound 38: N^1-(2-(Piperazin-l-yl)ethyl)-N^1,N^2,N^2-tritetradecylethane-l,2-diamine

[00749] Compound 38 was synthesized according to Steps 1-5 for Compound 13.

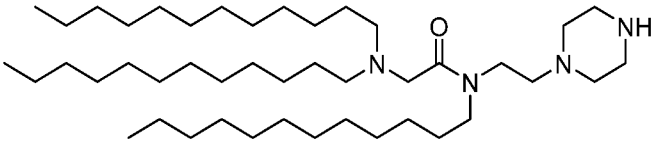


Chemical Formula: C50H104N4

Molecular Weight: 761.41

AN: Compound 39: 2-(Didodecylamino)-*N*-dodecyl-*N*-(2-(piperazin-l-yl)ethyl)acetamide

[00750] Compound 39 was synthesized according to Steps 1-3 for Compound 1.

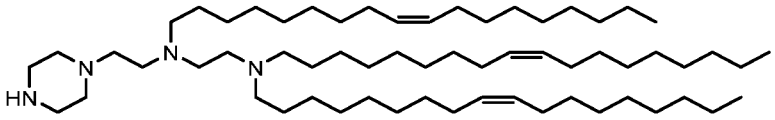


Chemical Formula: C44H90N4O

Molecular Weight: 691.23

AO: Compound 40: *N*[1],*N*[1],*N*[2]-Tri((Z)-octadec-9-en-l-yl)-*N*[2]-(2-(piperazin-l-yl)ethyl)ethane-l,2-diamine

[00751] Compound 40 was synthesized according to Steps 1-3 for Compound 33.

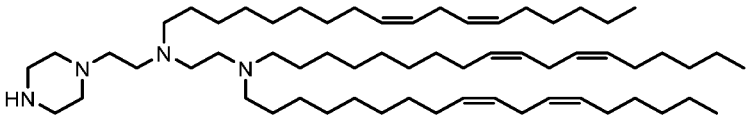


Chemical Formula: C62H122N4

Molecular Weight: 923.69

AP: Compound 41: *N*[1],*N*[1],*N*[2]-Tri((9Z,12Z)-octadeca-9,12-dien-l-yl)-*N*[2]-(2-(piperazin-l-yl)ethyl)ethane- 1,2-diamine

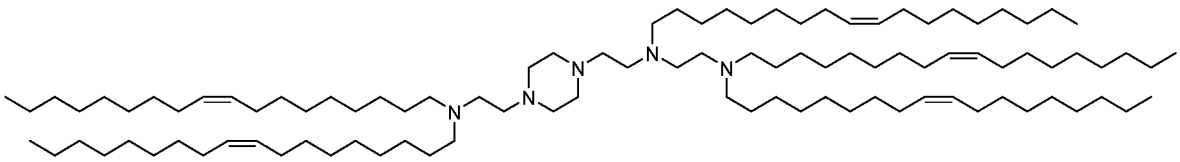
[00752] Compound 41 was synthesized according to Steps 1-3 for Compound 34.



Chemical Formula:

Molecular Weight:

AQ: Compound according to formula (IV): *N*[1]-(2-(4-(2-(Di((Z)-octadec-9-en-l-yl)amino)ethyl)piperazin-l-yl)ethyl)-*N*[1],*N*[2],*N*[2]-tri((Z)-octadec-9-en-l-yl)ethane-l,2-di amine



Chemical Formula: C100H195N5

Molecular Weight: 1467.70

[00753] In the same manner as Step 6 for Compound 18, *N*[1]-(2-(4-(2-(di((Z)-octadec-9-en-l-yl)amino)ethyl)piperazin-l-yl)ethyl)-*N*[1],*N*[2]^-tri((Z)-octadec-9-en-l-yl)ethane-l,2-diamine was synthesized from *N*[1],*N*[1],*N*[2]-tri((Z)-octadec-9-en-l-yl)-*N*[2]-(2-(piperazin-l-

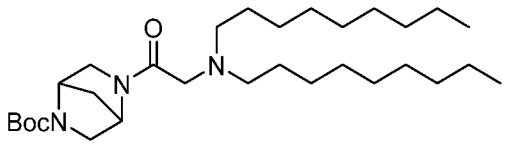
yl)ethyl)ethane-l,2-diamine (75 mg, 0.0812 mmol), (Z)-*N*-(2-chloroethyl)-*N*-((Z)-octadec-9-en-l-yl)octadec-9-en-l-amine (57 mg, 0.0974 mmol) K2CO3 (22 mg, 0.162 mmol), and KI (2 mg, 0.012 mmol) in THF (1.5 mL). Yield (30 mg, 25%)

UPLC/ELSD: RT = 4.41 min. MS (ES): *m/z* (MH^+) 1469.08 for C100H195N5

¾-NMR (300 MHz, CDCh) δ: ppm 5.40-5.27 (br. m, 10H); 3.18-2.22 (br. m, 30H); 2.06-1.89 (m, 20H); 1.80-0.97 (br. m, 120H); 0.88 (t, 15H).

AR:Compound 42: 2-(Dinonylamino)-l-(5-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)ethan-l-one

Step 1: tot-Butyl 5-(dinonylglycyl)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate



Chemical Formula: C30H57N3O3

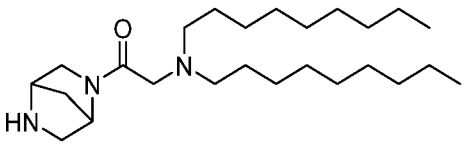
Molecular Weight: 507.80

[00754] In the same manner as Step 3 for Compound 11, tert-butyl 5-(dinonylglycyl)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate was synthesized from lithium dinonylglycinate (500 mg, 1.50 mmol), tert-butyl 2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (357 mg, 1.80 mmol), iPr_2EtN (628 μL, 3.60 mmol), and T3P (50% EtOAc solution, 2.68 mL, 4.50 mmol) in THF (15 mL). Yield (710 mg, 78%).

UPLC/ELSD: RT = 0.87 min. MS (ES): *m/z* (MH^+) 508.44 for C30H57N3O3

¾-NMR (300 MHz, CDCh) δ: ppm 4.98-4.46 (br. m, 2H); 4.30-3.15 (br. m, 10H); 2.14-1.60 (br. m, 6H); 1.49 (s, 9H); 1.40-1.00 (br. m, 24H); 0.89 (t, 6H).

Step 2: l-(2,5-Diazabicyclo[2.2.1]heptan-2-yl)-2-(dinonylamino)ethan-l-one



Chemical Formula: C25H49N3O

Molecular Weight: 407.69

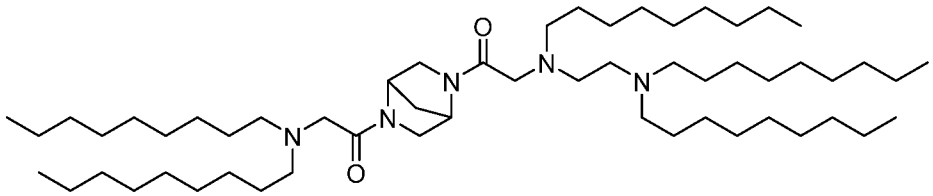
[00755] In the same manner as Step 4 for Compound 11, l-(2,5-diazabicyclo[2.2.1]heptan-2-yl)-2-(dinonylamino)ethan-l-one was synthesized from tot-butyl

5-(dinonylglycyl)-2,5-diazabicyclo[2.2. 1]heptane-2-carboxylate (710 mg, 1.40 mmol) and TFA (5.4 mL, 70 mmol) in DCM (5 mL). Yield (446 mg, 78%)

UPLC/ELSD: RT = 0.67 min. MS (ES): *m/z* (MH^+) 408.64 for C25H49N3O

¾-NMR (300 MHz, CDCh) δ: ppm 4.90-3.00 (br. m, 8H); 2.49 (br. m, 4H); 1.79 (br. m, 2H); 1.58-1.08 (br. m, 28H); 0.90 (t, 6H).

Step 3: 2-(Dinonylamino)-l -(5-*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)ethan-1-one



Chemical Formula: C56H111N5O2

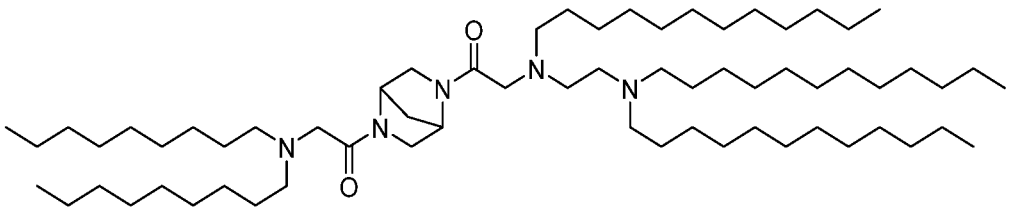
Molecular Weight: 886.54

[00756] In the same manner as Step 11 for Compound 11, 2-(dinonylamino)-l-(5-*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)-2,5-diazabicyclo[2.2. 1]heptan-2-yl)ethan-l-one was synthesized from l-(2,5-diazabicyclo[2.2. 1]heptan-2-yl)-2-(dinonylamino)ethan-l-one (100 mg, 0.25 mmol), *N*-(2-(dinonylamino)ethyl *)-N*-nonylglycine (134 mg, 0.27 mmol), iPr_2EtN (94 μL, 0.54 mmol), and T3P (50% EtOAc solution, 438 μL, 0.74 mmol) in THF (20 mL). Yield (50 mg, 23%).

UPLC/ELSD: RT = 3.60 min. MS (ES): *m/z* (MH^+) 887.12 for **C56H111N5O2**

^{1}H-NMR (300 MHz, CDCh) δ: ppm 5.15-2.28 (br. m, 26H); 2.16-1.00 (br. m, 70H); 0.90 (t, 15H).

AS: Compound 43: 2-((2-(Didodecylamino)ethyl)(dodecyl)amino)-l -(5-(dinonylglycyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)ethan-1-one 3



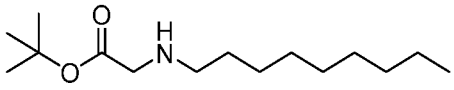
Chemical Formula: $C_{65}H_{129}N_5O_2$

Molecular Weight: 1012.78

[00757] In the same manner as Step 11 for Compound 11, 2-((2-(didodecylamino)ethyl)(dodecyl)amino)-l-(5-(dinonylglycyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)ethan-l-one was synthesized from l-(2,5-diazabicyclo[2.2.1]heptan-2-yl)-2-(dinonylamino)ethan-l-one (100 mg, 0.25 mmol), N-(2-(didodecylamino)ethyl)-N-dodecylglycine (168 mg, 0.27 mmol), iPr_2EtN (94 μL, 0.54 mmol), and T3P (50% EtOAc solution, 438 μL, 0.74 mmol) in THF (20 mL). Yield (150 mg, 60%).
UPLC/ELSD: RT = 3.60 min. MS (ES): *m/z* (MH^+) 1013.24 for C65H129N5O2
¾-NMR (300 MHz, CDCh) δ: ppm 5.15-2.26 (br. m, 24H); 2.13-1.09 (br. m, 90H); 0.90 (t, 15H).

AT: Compound 44: Methyl 8-((2-(4-*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)octanoate

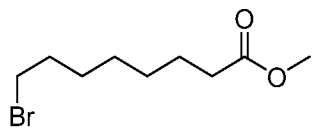
Step l :*tert*-Butyl nonylglycinate



Chemical Formula: C15H31NO2
Molecular Weight: 257.42

[00758] To a mixture of *tert-b*uty l glycine (3.0 g, 23 mmol) and 1-bromononane (2.4 g, 11.5 mmol) in MeCN (100 mL) was added K2CO3 (3.2 g, 23 mmol) and KI (190 mg, 1.1 mmol) and the mixture was allowed to stir at 82 °C for 24 hours. The suspension was cooled to RT and filtered through a celite plug, rinsing with hexanes. The MeCN was extracted 3x with hexanes, and the combined extracts were concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-15% MeOH/DCM) provided tert-butyl nonylglycinate as a clear colorless oil (848 mg, 29%).
^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.31 (s, 2H); 2.60 (t, 2H); 1.82-1.63 (br, 1H); 1.56-1.20 (br. m, 23H); 0.90 (t, 3H).

Step 2: Methyl 8-bromooctanoate



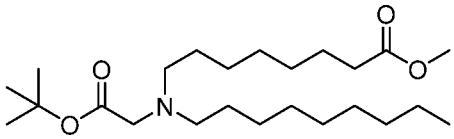
Chemical Formula: $C_9H_{17}BrO_2$

Molecular Weight: 237.14

[00759] In the same manner as Step 1 for Compound 15, methyl 8-bromooctanoate was synthesized from 8-bromooctanoic acid (5.0 g, 22 mmol), methanol (20 mL, 450 mmol), and H_2SO_4 (1.2 mL, 22 mmol) in THF (20mL). Yield (5.0 g, 95%).

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.42 (t, 2H); 2.33 (t, 2H); 1.88 (quint, 2H); 1.65 (quint, 2H); 1.54-1.27 (br. m, 6H).

Step 3: Methyl 8-((2-(teri-butoxy)-2-oxoethyl)(nonyl)amino)octanoate



Chemical Formula: $C_{24}H_{47}NO_4$

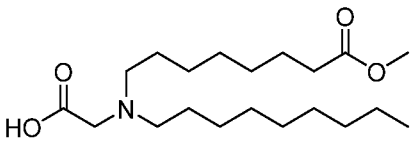
Molecular Weight: 413.64

[00760] To a mixture of *tert-b*uty*l* nonylglycinate (300 mg, 1.17 mmol) and methyl 8-bromooctanoate (290 mg, 1.22 mmol) in MeCN (12mL) was added K_2CO_3 (341 mg, 2.45 mmol) and KI (19 mg, 0.12 mmol) and the mixture was allowed to stir at 82 °C for 12 hours. The suspension was cooled to RT and filtered through a celite plug, rinsing with hexanes. The MeCN was extracted 3x with hexanes, and the combined extracts were concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-10% EtOAc/Hexanes) provided methyl 8-((2-(teri-butoxy)-2-oxoethyl)(nonyl)amino)octanoate (353 mg, 73%).

UPLC/ELSD: RT = 1.60 min. MS (ES): *m/z* (MH^+) 414.51 for $C_{24}H_{47}NO_4$

^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.23 (s, 2H); 2.57 (t, 4H); 2.32 (t, 2H); 1.70-1.18 (br. m, 33H); 0.90 (t, 3H).

Step 4: *N*-(8-Methoxy-8-oxooctyl)-*N*-nonylglycine



Chemical Formula: $C_{20}H_{39}NO_4$

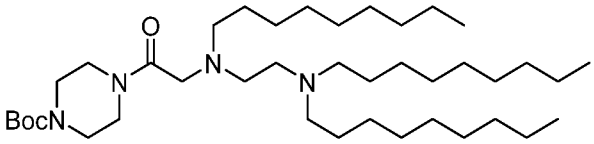
Molecular Weight: 357.54

[00761] To a solution of methyl 8-((2-(fer^butoxy)-2-oxoethyl)(nonyl)amino)octanoate (353 mg, 0.85 mmol) in DCM (4mL) was added TFA (3.3 mL, 43 mmol) and the solution was allowed to stir at RT for 4 hours. The solution was concentrated *in vacuo,* taken up in DCM,

and washed with 5% Na2CCb and brine, dried over Na2S04, filtered, and concentrated *in vacuo* to provide *N*-(8-methoxy-8-oxooctyl)-*N*-nonylglycine (305 mg, 99%).

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.49 (s, 2H); 3.06 (t, 4H); 2.32 (t, 2H); 1.79-1.14 (br. m, 24H); 0.90 (t, 3H).

Step 5: *tert*-Butyl 4-*(N*-(2-(dinonylamino)ethyl*)-N*-nonylglycyl)piperazine-l-carboxylate



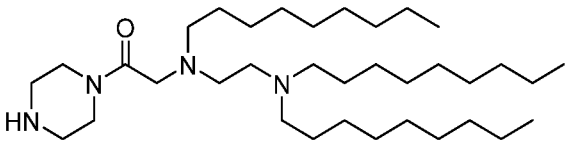
Chemical Formula: C40H80N4O3

Molecular Weight: 665.11

[00762] To a solution of *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (1.0 g, 2.0 mmol) and 1-boc-piperazine (412 mg, 2.2 mmol) in THF (20 mL) was added iPr_2EtN (773 μL, 4.4 mmol) and T3P (50% EtOAc solution, 3.6 mL, 6.0 mmol) and the solution was allowed to stir at RT for 12 hours. The reaction was quenched with water and extracted 3x with EtOAc. The combined organics were washed with brine, dried over Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-20% MeOH/DCM) provided *tert*-butyl 4-*(N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazine-l-carboxylate (961 mg, 72%). UPLC/ELSD: RT = 3.27 min. MS (ES): *m/z* (MH^+) 665.79 for C40H80N4O3

¾-NMR (300 MHz, CDCh) δ: ppm 4.43-2.90 (br. m, 20H); 2.04-0.99 (br. m, 51H); 0.90 (t, 9H).

Step 6: 2-((2-(Dinony lamino)ethyl)(nonyl)amino)-1-(piperazin-1-yl)ethan-1-one



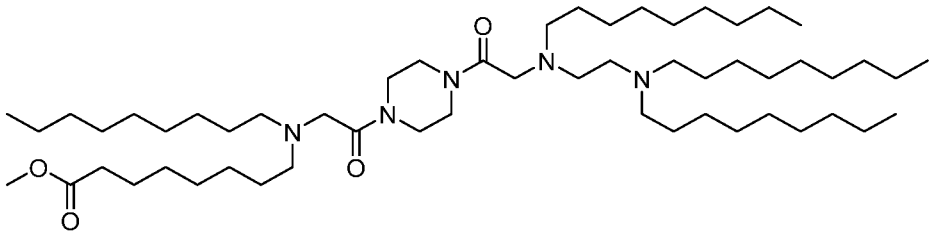
Chemical Formula: C35H72N4O

Molecular Weight: 564.99

[00763] To a solution of *tert*-butyl 4-*(N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazine-l-carboxylate (961 mg, 1.44 mmol) in DCM (6 mL) was added TFA (5.5 mL, 72 mmol) and the solution was allowed to stir for 4 hours. The solution was concentrated *in vacuo,* taken up in DCM, and washed with 5% Na2C03, brine, dried over

Na2S04, filtered, and concentrated *in vacuo* to provide 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l-(piperazin-l-yl)ethan-l-one (743 mg, 91%).
UPLC/ELSD: RT = 2.14 min. MS (ES): *m/z* (MH^+) 565.82 for C35H72N4O
¾-NMR (300 MHz, CDCh) δ: ppm 3.59 (br, 4H); 3.36 (br, 2H); 2.99-2.03 (br. m, 14H); 1.74-1.01 (br. m, 42H); 0.90 (t, 9H).

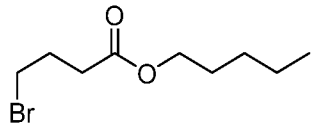
Step 7: Methyl 8-((2-(4-*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)octanoate



Chemical Formula: C55H109N5O4
Molecular Weight: 904.51

[00764] To a solution of *N*-(8-methoxy-8-oxooctyl *)-N*-nonylglycine (100 mg, 0.28 mmol) and 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l-(piperazin-l-yl)ethan-l-one (174 mg, 0.31 mmol) in THF (25 mL) was added), iPr_2EtN (107 μL, 0.62 mmol), and T3P (50% EtOAc solution, 0.50 mL, 0.84 mmol) and the reaction was allowed to stir at room temperature overnight. The reaction mixture was diluted with water and extracted with EtOAc. The organics were dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-100% DCM/[DCM 20% MeOH 1% NH_4OH]) provided methyl 8-((2-(4-*(N*-(2-(dinonylamino)ethyl *)-N*-nonylgly cyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)octanoate (121 mg, 48%).
UPLC/ELSD: RT = 2.88 min. MS (ES): *m/z* (MH^+) 905 for C55H109N5O4
¾-NMR (300 MHz, CDCh) δ: ppm 3.64-3.36 (br. m, 11H); 3.18 (s, 2H); 3.13 (s, 2H); 2.54-2.09 (br. m, 16H); 1.55-0.88 (br. m, 66H); 0.76 (t, 12H).

AU: Compound 45: Pentyl 4-((2-(4-*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)butanoate
Step 1: Pentyl 4-bromobutanoate



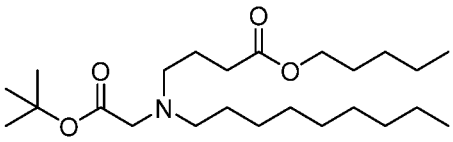
Chemical Formula: $C_9H_{17}BrO_2$

Molecular Weight: 237.14

[00765] In the same manner as Step 1 for Compound 15, pentyl 4-bromobutanoate was synthesized from 4-bromobutanoic acid (2.0 g, 12 mmol), pentanol (1.7 mL, 15.6 mmol), and H2SO4 (0.65 mL, 12 mmol) in THF (20mL). Yield (1.26 g, 44%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.10 (t, 2H); 3.49 (t, 2H); 2.52 (t, 2H); 2.20 (quint, 2H); 1.65 (quint, 2H); 1.35 (m, 4H); 0.93 (t, 3H).

Step 2: Pentyl 4-((2-(teri-butoxy)-2-oxoethyl)(nonyl)amino)butanoate



Chemical Formula: C24H47NO4

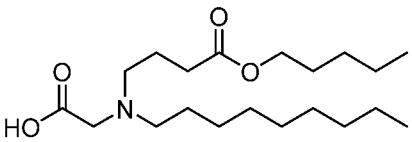
Molecular Weight: 413.64

[00766] In the same manner as Step 3 for Compound 44, pentyl 4-((2-(fer/-butoxy)-2-oxoethyl)(nonyl)amino)butanoate was synthesized from *tert-b*utyl nonylglycinate (300 mg, 1.17 mmol) and pentyl 4-bromobutanoate (290 mg, 1.22 mmol) in MeCN (12 mL) was added K2CO3 (341 mg, 2.45 mmol) and KI (19 mg, 0.12 mmol). Yield (343 mg, 71%).

UPLC/ELSD: RT = 1.81 min. MS (ES): *m/z* (MH^+) 415 for C24H47NO4

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.23 (s, 2H); 2.60 (br. m, 4H); 2.37 (t, 2H); 1.78 (m, 2H); 1.64 (m, 2H); 1.52-1.20 (br. m, 27H); 0.90 (m, 6H).

Step 3: *N*-Nonyl-*N*-(4-oxo-4-(pentyloxy)butyl)glycine



Chemical Formula: C20H39NO4

Molecular Weight: 357.54

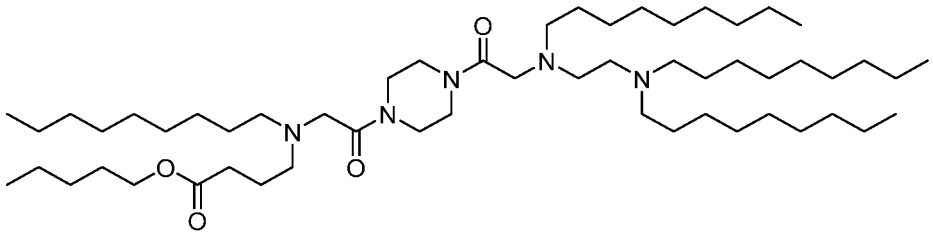
[00767] In the same manner as Step 4 for Compound 44, N-nonyl-*N*-(4-oxo-4-(pentyloxy)butyl)glycine was synthesized from pentyl 4-((2-(te/t-butoxy)-2-

oxoethyl)(nonyl)amino)butanoate (343 mg, 0.83 mmol) and TFA (3.17 mL, 41.5 mmol) in DCM (4 mL). Yield (296 mg, 99%).

UPLC/ELSD: RT = 1.29 min. MS (ES): *m/z* (MH^+) 358 for $C_{20}H_{39}NO_4$

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.43 (br, 2H); 2.94 (br, 4H); 2.41 (t, 2H); 1.98 (br. m, 2H); 1.74-1.54 (br. m, 4H); 1.40-1.16 (br. m, 16H); 0.91 (m, 6H).

Step 4: Pentyl 4-((2-(4-*(N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)butanoate



Chemical Formula: $C_{55}H_{109}N_5O_4$

Molecular Weight: 904.51

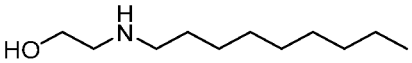
[00768] In the same manner as Step 7 for Compound 44, pentyl 4-((2-(4-*(N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)butanoate was synthesized from *N*-nonyl-N-(4-oxo-4-(pentyloxy)butyl)glycine (100 mg, 0.28 mmol), 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l-(piperazin-l-yl)ethan-l-one (174 mg, 0.31 mmol), iPr_2EtN (107 μL, 0.62 mmol), and T3P (50% EtOAc solution, 0.50 mL, 0.84 mmol) in THF (25 mL). Yield (121 mg, 48%).

UPLC/ELSD: RT = 3.01 min. MS (ES): *m/z* (MH^+) 905 for $C_{55}H_{109}N_5O_4$

¾-NMR (300 MHz, CDCh) δ: ppm 3.93 (t, 2H); 3.61-3.31 (br. m, 8H); 3.17 (m, 4H); 2.55-2.08 (br. m, 16H); 1.71-0.90 (br. m, 64H); 0.75 (m, 15H).

AV: Compound 46: Methyl 8-((2-((2-(4-(dinonylglycyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)ethyl)(nonyl)amino)octanoate

Step 1: 2-(Nonylamino)ethan-l-ol



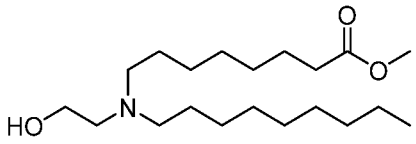
Chemical Formula: $C_{11}H_{25}NO$

Molecular Weight: 187.33

[00769] To a mixture of ethanolamine (4.4 mL, 72 mmol) and 1-bromononane (3.0g, 14.5 mmol) in MeCN (150 mL) was added K2CO3 (4.0 g, 29 mmol) and KI (240 mg, 1.5 mmol) and the mixture was allowed to stir at 82 °C for 12 hours. The suspension was cooled to RT and filtered over a pad of celite, rinsing with hexanes. The MeCN was extracted with hexanes 3x, and the combined hexanes were concentrated. Purification by ISCO silica flash chromatography (0-100% DCM/[DCM 20% MeOH 1% NH40H]) provided 2-(nonylamino)ethan-l-ol (1.0 g, 38%)

Ti-NMR (300 MHz, CDCh) δ: ppm 3.66 (t, 2H); 2.80 (t, 2H); 2.62 (t, 2H); 1.96 (br. m, 2H); 1.50 (br. m, 2H); 1.28 (br. m, 12H); 0.90 (t, 3H).

Step 2: Methyl 8-((2-hydroxyethyl)(nonyl)amino)octanoate



Chemical Formula: C20H41NO3

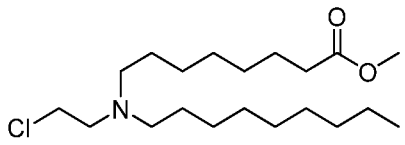
Molecular Weight: 343.55

[00770] In the same manner as Step 1 for Compound 18, methyl 8-((2-hydroxy ethyl)(nonyl)amino)octanoate was synthesized from 2-(nonylamino)ethan-l-ol (500 mg, 2.67 mmol), methyl 8-bromooctanoate (665 mg, 2.8 mmol), K2CO3 (780 mg, 5.6 mmol), and KI (44 mg, 0.27 mmol) in MeCN (30 mL). Yield (578 mg, 63%).

UPLC/ELSD: RT = 1.01 min. MS (ES): *m/z* (MH^+) 344.31 for C20H41NO3

^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.59 (t, 2H), 2.65 (br, 2H); 2.51 (t, 4H); 2.32 (t, 2H); 1.65 (br. m, 2H); 1.49 (br. m, 4H); 1.30 (br. m, 18H); 0.90 (t, 3H).

Step 3: Methyl 8-((2-chloroethyl)(nonyl)amino)octanoate



Chemical Formula: C20H40ClNO2

Molecular Weight: 362.00

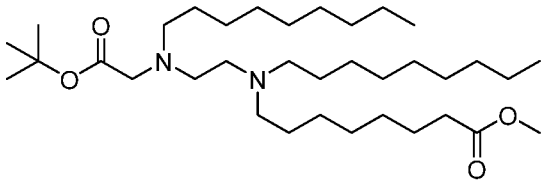
[00771] In the same manner as Step 2 for Compound 18, methyl 8-((2-chloroethyl)(nonyl)amino)octanoate was synthesized from methyl 8-((2-

hydroxyethyl)(nonyl)amino)octanoate (578 mg, 1.68 mmol), methanesulfonyl chloride (163 μL, 2.10 mmol) and trimethylamine (305 μL, 2.20 mmol) in DCM (10 mL). Yield (418 mg, 69%).

UPLC/ELSD: RT = 1.21 min. MS (ES): *m/z* (MH^+) 363 for C20H40CINO2

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (br, 3H); 3.51 (br, 2H), 2.78 (br. m, 2H); 2.47 (br. m, 4H); 2.33 (t, 2H); 1.72-1.20 (br. m, 24H); 0.91 (t, 3H).

Step 4: Methyl 8-((2-((2-(*tert*-butoxy)-2-oxoethyl)(nonyl)amino)ethyl)(nonyl)amino)octanoate



Chemical Formula: C35H70N2O4

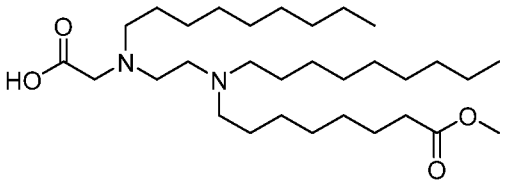
Molecular Weight: 582.96

[00772] To a mixture of *tert-b*utyl nonylglycinate (218 mg, 0.85 mmol) and methyl 8-((2-chloroethyl)(nonyl)amino)octanoate (337 mg, 0.93 mmol) in MeCN (10 mL) was added K2CO3 (236 mg, 1.69 mmol) and KI (14 mg, 0.08 mmol) and the mixture was allowed to stir at 82 °C for 12 hours. The suspension was cooled to RT and filtered through a pad of celite, rinsing with hexanes. The mixture was extracted with hexanes 3x and the combined hexanes were concentrated. Purification by ISCO silica flash chromatography (0-10% MeOH/DCM) provided methyl 8-((2-((2-(/er^butoxy)-2-oxoethyl)(nonyl)amino)ethyl)(nonyl)amino)octanoate (283 mg, 57%).

UPLC/ELSD: RT = 2.92 min. MS (ES): *m/z* (MH^+) 584 for C35H70N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.28 (s, 2H); 2.80-2.20 (br. m, 12H); 1.85-1.10 (br. m, 47H); 0.91 (t, 6H).

Step 5: *N*-(2-((8-Methoxy-8-oxooctyl)(nonyl)amino)ethyl)-*N*-nonylglycine

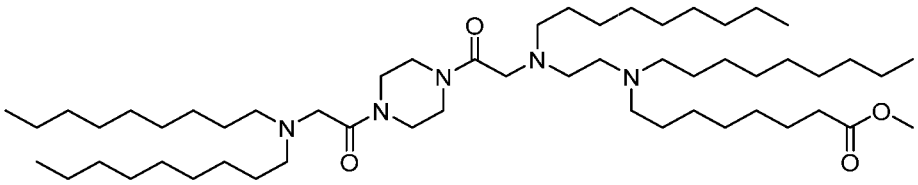


Chemical Formula: C31H62N2O4

Molecular Weight: 526.85

[00773] In the same manner as Step 4 for Compound 44, *N*-(2-((8-methoxy-8-oxooctyl)(nonyl)amino)ethyl)-*N*-nonylglycine was synthesized from methyl 8-((2-((2-(fer/-butoxy)-2-oxoethyl)(nonyl)amino)ethyl)(nonyl)amino)octanoate (283 mg, 0.49 mmol) and TFA (1.86 mL, 24.3 mmol) in DCM (2 mL). Yield (255 mg, 99%).
UPLC/ELSD: RT = 2.18 min. MS (ES): *m/z* (MH^+) 528 for C31H62N2O4
¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H), 3.26 (s, 2H), 2.79 (br. m, 8H), 2.59 (t, 2H), 2.33 (t, 2H), 1.76-1.08 (br. m, 38H); 0.90 (t, 6H).

Step 6: Methyl 8-((2-((2-(4-(dinonylglycyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)ethyl)(nonyl)amino)octanoate

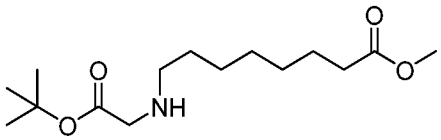


Chemical Formula: C55H109N5O4
Molecular Weight: 904.51

[00774] In the same manner as Step 11 for Compound 11, methyl 8-((2-((2-(4-(dinonylglycyl)piperazin-1-yl)-2-oxoethyl)(nonyl)amino)ethyl)(nonyl)amino)octanoate was synthesized from 2-(dinonylamino)-l-(piperazin-l-yl)ethan-l-one (75 mg, 0.20 mmol), *N*-(2-((8-methoxy-8-oxooctyl)(nonyl)amino)ethyl)-*N*-nonylglycine (150 mg, 0.28 mmol), iPnEtN (88 µL, 0.50 mmol) and T3P (50% EtOAc solution, 409 µL, 0.69 mmol). Yield (57 mg, 32%).
UPLC/ELSD: RT = 2.88 min. MS (ES): *m/z* (MH^+) 905 for C55H109N5O4
¾-NMR (300 MHz, CDCh) δ: ppm 3.82-2.87 (br. m, 27H); 2.64 (m, 2H); 2.33 (t, 2H); 1.80-1.15 (br. m, 66H); 0.90 (t, 12H).

AW: Compound 47: Methyl 8-((2-(dinonylamino)ethyl)(2-(4-(dinonylglycyl)piperazin-l-yl)-2-oxoethyl)amino)octanoate

Step 1: Methyl 8-((2-(/er/-butoxy)-2-oxoethyl)amino)octanoate



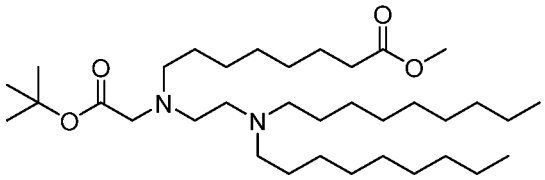
Chemical Formula: C15H29NO4

Molecular Weight: 287.40

[00775] In the same manner as Step 1 for Compound 44, methyl 8-((2-(*ter*/-butoxy)-2-oxoethyl)amino)octanoate was synthesized from *tert-b*utyl glycine (2.0 g, 12 mmol), methyl 8-bromooctanoate (2.8 g, 12 mmol), K2CO3 (3.3 g, 24 mmol), and KI (198 mg, 1.2 mmol) in MeCN (100 mL). Yield (1.16 g, 34%).

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.33 (s, 2H); 2.62 (t, 2H); 2.32 (s, 2H); 2.16-1.80 (br, 1H); 1.72-1.42 (br. m, 13H); 1.34 (br. m, 6H).

Step 2: Methyl 8-((2-(ter^butoxy)-2-oxoethyl)(2-(dinonylamino)ethyl)amino)octanoate



Chemical Formula: C35H70N2O4

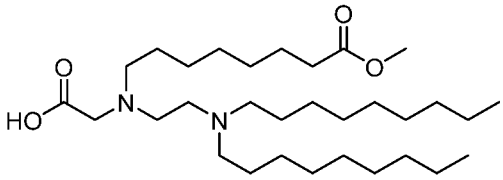
Molecular Weight: 582.96

[00776] In the same manner as Step 4 for Compound 46, methyl 8-((2-(fer/-butoxy)-2-oxoethyl)(2-(dinonylamino)ethyl)amino)octanoate was synthesized from methyl *8-((2-(tert-*butoxy)-2-oxoethyl)amino)octanoate (300 mg, 1.0 mmol), *N*-(2-chloroethyl *)-N*-nonylnonan-l-amine (381 mg, 1.15 mmol), K2CO3 (320 mg, 2.3 mmol), and KI (17 mg, 0.10 mmol) in MeCN (10 mL). Yield (285 mg, 47%).

UPLC/ELSD: RT = 2.89 min. MS (ES): *m/z* (MH^+) 584 for C35H70N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.29 (s, 2H); 3.10 (br. m, 8H); 2.59 (t, 2H); 2.32 (t, 2H); 1.82 (br. m, 4H); 1.74-1.16 (br. m, 43H); 0.91 (t, 6H).

Step 3: *N*-(2-(dinonylamino)ethyl *)-N*-(8-methoxy-8-oxooctyl)glycine



Chemical Formula: C31H62N2O4

Molecular Weight: 526.85

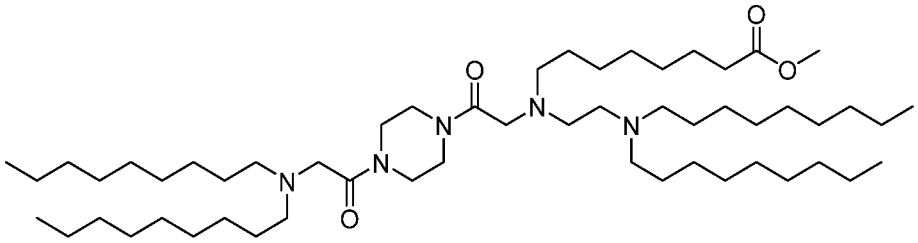
[00777] In the same manner as Step 5 for Compound 46, *N*-(2-(dinonylamino)ethyl *)-N*-(8-methoxy-8-oxooctyl)glycine was synthesized from methyl 8-((2-(fer/-butoxy)-2-oxoethyl)(2-

(dinonylamino)ethyl)amino)octanoate (285 mg, 0.50 mmol), and TFA (1.87 mL, 24.4 mmol) in DCM (2 mL). Yield (254 mg, 98%).

UPLC/ELSD: RT = 2.16 min. MS (ES): *m/z* (MH^+) 528 for $C_{31}H_{62}N_2O_4$

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.25 (s, 2H); 2.90-2.72 (br. m, 8H); 2.59 (t, 2H); 2.32 (t, 2H); 1.66 (br. m, 6H); 1.48 (br. m, 2H); 1.40-1.20 (br. m, 30H); 0.91 (t, 6H).

Step 4: Methyl 8-((2-(dinonylamino)ethyl)(2-(4-(dinonylglycyl)piperazin-l -yl)-2-oxoethyl)amino)octanoate



Chemical Formula: $C_{55}H_{109}N_5O_4$

Molecular Weight: 904.51

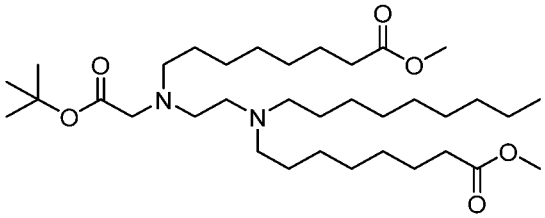
[00778] In the same manner as Step 11 for Compound 11, methyl 8-((2-(dinonylamino)ethyl)(2-(4-(dinonylgly cyl)piperazin- 1-yl)-2-oxoethyl)amino)octanoate was synthesized from 2-(dinonylamino)-l-(piperazin-l-yl)ethan-l-one (75 mg, 0.20 mmol), *N*-(2-(dinonylamino)ethyl *)-N*-(8-methoxy-8-oxooctyl)glycine (150 mg, 0.28 mmol), iPr_2EtN (88 μL, 0.50 mmol) and T3P (50% EtOAc solution, 409 μL, 0.69 mmol). Yield (80 mg, 39%).

UPLC/ELSD: RT = 2.87 min. MS (ES): *m/z* (MH^+) 905 for $C_{55}H_{109}N_5O_4$

^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.81-2.21 (br. m, 31H); 1.89-1.05 (br. m, 66H); 0.90 (t, 12H).

AX: Compound 48: Methyl 8-((2-(4-(dinonylglycyl)piperazin-l-yl)-2-oxoethyl)(2-((8-methoxy-8-oxooctyl)(nonyl)amino)ethyl)amino)octanoate

Step 1: Methyl 8-((2-(ter/-butoxy)-2-oxoethyl)(2-((8-methoxy-8-oxooctyl)(nonyl)amino)ethyl)amino)octanoate



Chemical Formula: C35H68N2O6

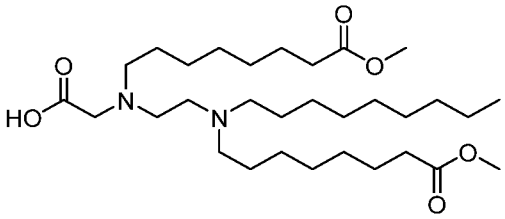
Molecular Weight: 612.94

[00779] In the same manner as Step 4 for Compound 46, methyl 8-((2-(Yer/-butoxy)-2-oxoethyl)(2-((8-methoxy-8-oxooctyl)(nonyl)amino)ethyl)amino)octanoate was synthesized from methyl 8-((2-(teri-butoxy)-2-oxoethyl)amino)octanoate (65 mg, 0.23 mmol), methyl 8-((2-chloroethyl)(nonyl)amino)octanoate (86 mg, 0.24 mmol), K2CO3 (69 mg, 0.50 mmol), and KI (4 mg, 0.02 mmol) in MeCN (4 mL). Yield (60 mg, 43%).

UPLC/ELSD: RT = 2.42 min. MS (ES): *m/z* (MH^+) 614 for $C_{35}H_{68}N_2O_6$

¾-NMR (300 MHz, CDCh) δ: ppm 3.66 (s, 6H); 3.25 (s, 2H); 2.66 (m, 2H); 2.54 (m, 4H); 2.38 (m, 4H); 2.28 (t, 4H); 1.61 (m, 4H); 1.54-1.10 (br. m, 39H); 0.87 (t, 3H).

Step 2: *N*-(8-methoxy-8-oxooctyl)-*N*-(2-((8-methoxy-8-oxooctyl)(nonyl)amino)ethyl)glycine



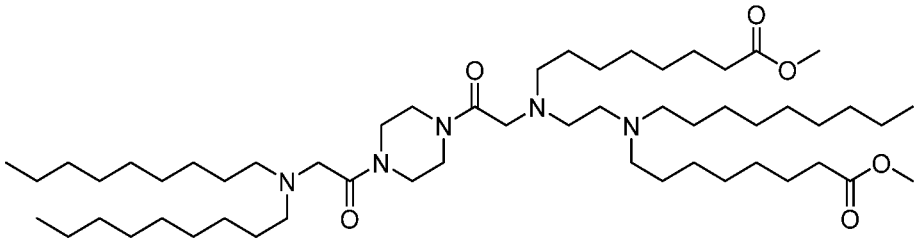
Chemical Formula: C31H60N2O6

Molecular Weight: 556.83

[00780] In the same manner as Step 5 for Compound 46, *N*-(8-methoxy-8-oxooctyl)-*N*-(2-((8-methoxy-8-oxooctyl)(nonyl)amino)ethyl)glycine was synthesized from methyl 8-((2-(*tert*-butoxy)-2-oxoethyl)(2-((8-methoxy-8-oxooctyl)(nonyl)amino)ethyl)amino)octanoate (60 mg, 0.10 mmol), and TFA (0.37 mL, 4.9 mmol) in DCM (1 mL). Yield (54 mg, 99%).

¾-NMR (300 MHz, CDCh) δ: ppm 3.60 (s, 6H); 3.15 (s, 2H); 2.72 (br. m, 8H); 2.50 (t, 2H); 2.22 (t, 4H); 1.70-1.05 (br. m, 34H); 0.81 (t, 3H).

Step 3: Methyl 8-((2-(4-(dinonylglycyl)piperazin-l-yl)-2-oxoethyl)(2-((8-methoxy-8-oxooctyl)(nonyl)amino)ethyl)amino)octanoate



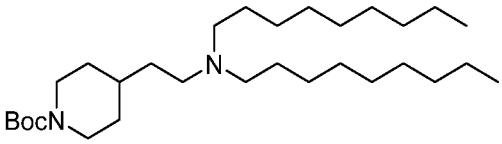
Chemical Formula: C55H107N5O6

Molecular Weight: 934.49

[00781] In the same manner as Step 11 for Compound 11, methyl 8-((2-(4-(dinonylglycyl)piperazin-l-yl)-2-oxoethyl)(2-((8-methoxy-8-oxooctyl)(nonyl)amino)ethyl)amino)octanoate was synthesized from 2-(dinonylamino)-l-(piperazin-l-yl)ethan-l-one (26 mg, 0.07 mmol), *N*-(8-methoxy-8-oxooctyl)-*N*-(2-((8-methoxy-8-oxooctyl)(nonyl)amino)ethyl)glycine (54 mg, 0.10 mmol), iPnEtN (30 μL, 0.17 mmol) and T3P (50% EtOAc solution, 140 μL, 0.24 mmol). Yield (20 mg, 27%).
UPLC/ELSD: RT = 2.56 min. MS (ES): *m/z* (MH^+) 935 for C55H107N5O6
¾-NMR (300 MHz, CDCh) δ: ppm 3.70-3.00 (br. m, 18H); 2.55-2.05 (br. m, 18H); 1.70-0.95 (br. m, 62H); 0.76 (t, 9H).

AY: Compound 49: 2-((2-(Dinonylamino)ethyl)(nonyl)amino)-l-(4-(2-(dinonylamino)ethyl)piperidin-l-yl)ethan-l-one

Step 1: *tert*-Butyl 4-(2-(dinonylamino)ethyl)piperi dine- 1-carboxy late



Chemical Formula: C30H60N2O2

Molecular Weight: 480.82

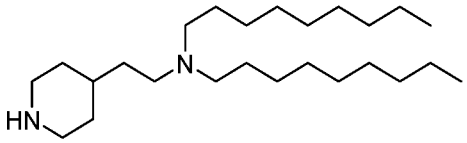
To a mixture of *tert*-butyl 4-(2-aminoethyl)piperi dine- 1-carboxy late (1.50 g, 6.6 mmol) and 1-bromononane (1.36 g, 6.57 mmol) in MeCN (100 mL) was added K2CO3 (1.83 g, 13.1 mmol) and KI (109 mg, 0.66 mmol) and the mixture was allowed to stir at 82 °C for 12 hours. The suspension was cooled to RT, filtered over a pad of celite rinsing with hexanes, and concentrated *in vacuo*. Purification by ISCO silica flash chromatography (0-100% DCM/[DCM 20% MeOH 1% NH4OH]) provided *tert*-butyl 4-(2-(dinonylamino)ethyl)piperi dine- 1-carboxy late (602 mg,

19%).

UPLC/ELSD: RT = 2.41 min. MS (ES): *m/z* (MH^+) 482 for C30H60N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.20-2.26 (br. m, 10H); 1.77-1.10 (br. m, 44H); 0.91 (t, 6H).

Step 2: *N*-Nonyl-*N*-(2-(piperidin-4-yl)ethyl)nonan-1-amine



Chemical Formula: C25H52N2

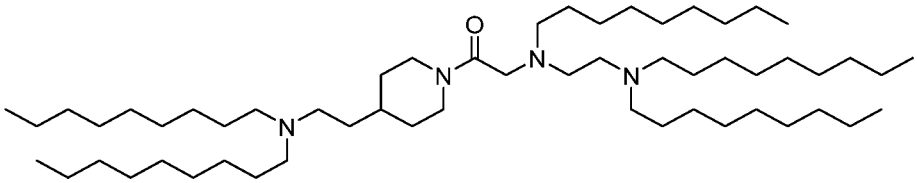
Molecular Weight: 380.71

In the same manner as Step 4 for Compound 11, *N*-nonyl-*N*-(2-(piperidin-4-yl)ethyl)nonan-l-amine was synthesized from *ter*/-butyl 4-(2-(dinony lamino)ethyl)piperi dine- 1-carboxy late (602 mg, 1.25 mmol) and TFA (4.8 mL, 63 mmol) in DCM (5 mL). Yield (406 mg, 85%).

UPLC/ELSD: RT = 1.27 min. MS (ES): *m/z* (MH^+) 382 for C25H52N2

¾-NMR (300 MHz, CDCh) δ: ppm 3.15 (br. m, 2H); 2.65 (br. m, 2H); 2.42 (br. m, 6H); 1.83-1.04 (br. m, 35H); 0.90 (t, 6H).

Step 3: 2-((2-(Dinonylamino)ethyl)(nonyl)amino)-l-(4-(2-(dinonylamino)ethyl)piperidm-1-yl)ethan-l-one



Chemical Formula: **C56H114N4O**

Molecular Weight: 859.56

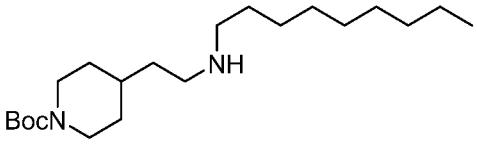
[00782] In the same manner as Step 11 for Compound 11, 2-((2-(dinony lamino)ethy l)(nony l)amino)- 1-(4-(2-(dinony lamino)ethy l)piperidin- 1-y l)ethan- 1-one was synthesized from *N*-nonyl-*N*-(2-(piperidin-4-yl)ethyl)nonan-l -amine (200 mg, 0.53 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (287 mg, 0.58 mmol), iPnEtN (201 μL, 1.2 mmol), and T3P (50% EtOAc solution, 938 μL, 1.6 mmol) in THF (10 mL). Yield (90 mg, 20%).

UPLC/ELSD: RT = 3.26 min. MS (ES): *m/z* (MH^+) 860 for **C56H114N4O**

¾-NMR (300 MHz, CDCh) δ: ppm 4.62-4.09 (br. m, 2H); 3.55-2.21 (br. m, 20H); 1.94-1.00 (br. m, 77H); 0.91 (t, 15H).

AZ: Compound 50: 2-(Dinonylamino)-l-(4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)ethyl)piperidin-1-yl)ethan-1-one

Step 1: *tert*-Butyl 4-(2-(nonylamino)ethyl)piperidine-1-carboxylate



Chemical Formula: C21H42N2O2

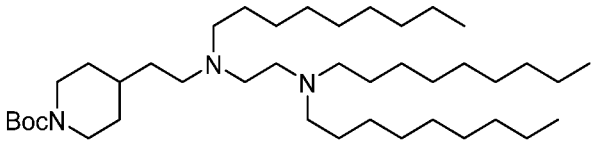
Molecular Weight: 354.58

[00783] In the same manner as Step 1 for Compound 49, *tert*-butyl 4-(2-(nonylamino)ethyl)piperidine-1-carboxylate was synthesized from *tert*-butyl 4-(2-aminoethyl)piperidine-1-carboxylate (1.50 g, 6.6 mmol), 1-bromononane (1.36 g, 6.57 mmol), K2CO3 (1.83 g, 13.1 mmol), and KI (109 mg, 0.66 mmol) in MeCN (100 mL). Yield (288 mg, 13%).

UPLC/ELSD: RT = 1.23 min. MS (ES): *m/z* (MH^+) 356 for C21H42N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (br. m, 2H); 2.67 (br. m, 6H); 1.80-0.98 (br. m, 30H); 0.90 (t, 3H).

Step 2: *tert*-Butyl 4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)ethyl)piperidine-l -carboxylate



Chemical Formula: C41H83N3O2

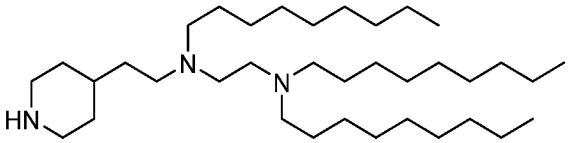
Molecular Weight: 650.13

To a mixture of *tert*-butyl 4-(2-(nonylamino)ethyl)piperidine-l -carboxylate (288 mg, 0.81 mmol) and *N*-(2-chloroethyl)-*N*-nonylnonan-l -amine (297 mg, 0.89 mmol) in MeCN (20 mL) was added K2CO3 (249 mg, 1.79 mmol), and KI (13 mg, 0.08 mmol) and the mixture was allowed to stir at 82 °C for 12 hours. The suspension was cooled to RT and filtered over a pad of celite rinsing with hexanes, and concentrated. Purification by ISCO silica flash chromatography (0-100% DCM/[DCM 20% MeOH 1% NH_4OH]) provided tert-butyl 4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)ethyl)piperidine-1-carboxylate (216 mg, 41%)

UPLC/ELSD: RT = 2.72 min. MS (ES): *m/z* (MH^+) 651 for C41H83N3O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (br, 2H); 2.83-2.29 (br. m, 14H); 1.75-1.00 (br. m, 58H); 0.90 (t, 9H).

Step 3: *N^N* ^-Trinony l-*N*^{2}-(2-(piperidin-4-y l)ethy l)ethane- 1,2-diamine



Chemical Formula: C36H75N3

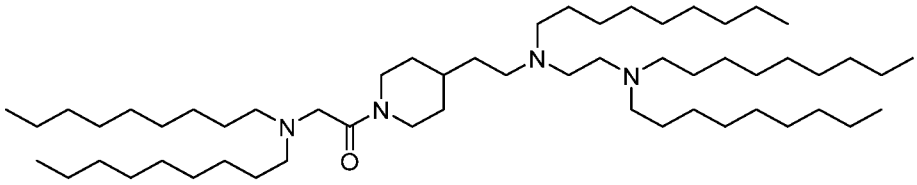
Molecular Weight: 550.02

[00784] In the same manner as Step 6 for Compound 44, *N* ^{1},*N* ^{1},*N*^{2}-trinonyl -*N*^{2}-(2-(piperidin-4-yl)ethyl)ethane-l ,2-diamine was synthesized from /*er*/-butyl 4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)ethyl)piperidine-l-carboxylate (216 mg, 0.33 mmol) and TFA (1.27 mL, 16.6 mmol) in DCM (2 mL). Yield (178 mg, 97%).

UPLC/ELSD: RT = 1.84 min. MS (ES): *m/z* (MH^+) 551 for C36H75N3

¾-NMR (300 MHz, CDCh) δ: ppm 3.08 (br. m, 2H); 2.70-2.25 (br. m, 14H); 2.0 (br, 1H); 1.80-1.02 (br. m, 49H); 0.90 (t, 9H).

Step 4: 2-(Dinonylamino)-l -(4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)ethyl)piperidm-1-yl)ethan-1-one



Chemical Formula: C56H114N4O

Molecular Weight: 859.56

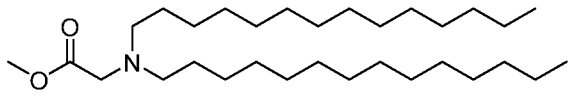
[00785] In the same manner as Step 7 for Compound 44, 2-(dinonylamino)-l -(4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)ethyl)piperidin-l-yl)ethan-l-one was synthesized from dinonylglycine (96 mg, 0.29 mmol), *N* ^{1},*N*^{1},*N*^{2}-trinonyl -*N*^{2}-(2-(piperidin-4-yl)ethyl)ethane-l,2-diamine (178 mg, 0.32 mmol), iPr_2EtN (112 μL, 0.65 mmol), and T3P (50% EtOAc solution, 525 μL, 0.88 mmol) in THF (6 mL). Yield (121 mg, 48%).

UPLC/ELSD: RT = 2.96 min. MS (ES): *m/z* (MH^+) 860 for C56H114N4O

¾-NMR (300 MHz, CDCh) δ: ppm 4.63-4.08 (br. m, 4H); 3.34-2.25 (br. m, 18H); 1.90-1.01 (br. m, 77H); 0.91 (t, 15H).

BA: Compound 51: 2-((2-(Dinonylamino)ethyl)(nonyl)amino)-l -(4-(ditetradecylgly cyl)piperazin- 1-yl)ethan- 1-one

Step 1: Methyl ditetradecylglycinate



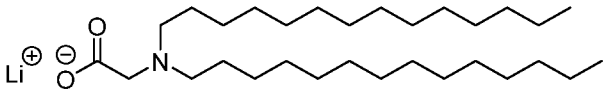
Chemical Formula: C31H63NO2

Molecular Weight: 481.85

[00786] In the same manner as Step 1 from Compound 11, methyl 3-(ditetradecylamino)propanoate was synthesized from glycine methyl ester hydrochloride (564 mg, 4.49 mmol), tetradecanal (2.1 g, 9.89 mmol), sodium triacetoxyborohydride (2.1 g, 9.89 mmol), acetic acid (0.6 mL, 9.89 mmol), trimethylamine (0.93 mL, 6.74 mmol), in DCE (22 mL). Yield (1.93 g, 89%).

¾-NMR (300 MHz, CDCh) δ: ppm 3.72 (s, 3H); 3.34 (s, 2H); 1.56 (t, 4H); 1.60-1.03 (br. m, 48H); 0.91 (t, 6H).

Step 2: Lithium ditetradecylglycinate



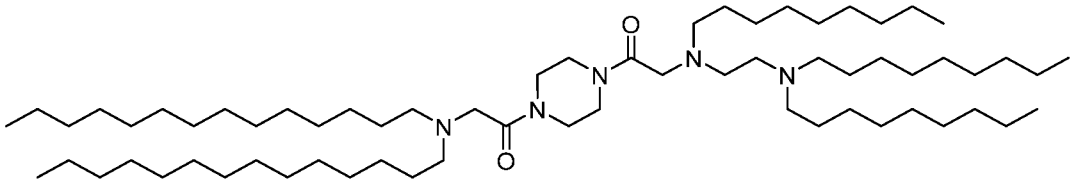
Chemical Formula: C30H60L1NO2

Molecular Weight: 473.76

[00787] In the same manner as Step 2 from Compound 11, lithium ditetradecylglycinate was synthesized from methyl ditetradecylglycinate (1.93 g, 4.0 mmol) and 1M LiOH (20 mL, 20 mmol) in THF (20 mL). Yield (1.81 g, 97%).

¾-NMR (300 MHz, CDCh) δ: ppm 3.17 (s, 2H); 2.64 (t, 4H); 1.52 (br. m, 4H); 1.31 (br. m, 44H); 0.93 (t, 6H).

Step 3: 2-((2-(Dinonylamino)ethyl)(nonyl)amino)-l -(4-(ditetradecylglycyl)piperazin-l-yl)ethan-1-one



Chemical Formula: C65H131N5O2

Molecular Weight: 1014.80

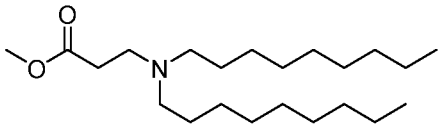
[00788] In the same manner as Step 7 for Compound 44, 2-((2-(dinonylamino)ethyl)(nonyl)amino)- 1-(4-(ditetradecylgly cyl)piperazin- 1-yl)ethan- 1-one was synthesized from lithium ditetradecylglycinate (126 mg, 0.26 mmol), 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l-(piperazin-l-yl)ethan-l-one (134 mg, 0.24 mmol), iPr_2EtN (91 μL, 0.52 mmol), and T3P (50% EtOAc solution, 424 μL, 0.71 mmol) in THF (4 mL)

UPLC/ELSD: RT = 3.64 min. MS (ES): *m/z* (MH^+) 1016 for $C_{65}H_{131}N_5O_2$

¾-NMR (300 MHz, CDCh) δ: ppm 3.84-2.34 (br. m, 26H); 1.88-0.99 (br. m, 90H); 0.90 (t, 15H).

BB: Compound 52: 3-(Dinonylamino)-l-(4-(3-((2-(dinony lamino)ethy l)(nony l)amino)propanoy l)piperazin- 1-y l)propan- 1-one

Step 1: Methyl 3-(dinonylamino)propanoate



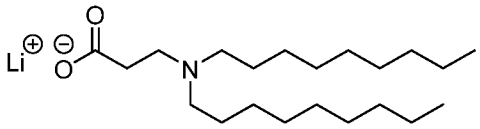
Chemical Formula: C22H45NO2

Molecular Weight: 355.61

[00789] To a mixture of methyl 3-aminopropanoate hydrochloride (2.0 g, 14 mmol) and 1-bromononane (2.7 mL, 14 mmol) in MeCN (100 mL) was added K2CO3 (4.0 g, 29 mmol) and KI (238 mg, 1.4 mmol) and the mixture was allowed to stir at 82 °C for 12 hours. The suspension was cooled to RT and filtered over a pad of celite washing with hexanes, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-10% MeOH/DCM) provided methyl 3-(dinonylamino)propanoate (663 mg, 13%).

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 2.80 (t, 2H); 2.41 (br. m, 6H); 1.70-1.10 (br. m, 28H); 0.90 (t, 6H).

Step 2: Lithium 3-(dinonylamino)propanoate



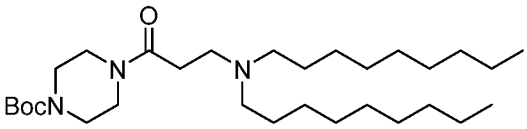
Chemical Formula: C21H42L1NO2

Molecular Weight: 347.51

[00790] In the same manner as Step 2 from Compound 11, lithium 3-(dinonylamino)propanoate was synthesized from methyl 3-(dinonylamino)propanoate (663 mg, 1.86 mmol) and 1M LiOH (9.32 mL, 9.32 mmol) in THF (10 mL). Yield (636 mg, 99%).

¾-NMR (300 MHz, CDCh) δ: ppm 2.94-1.65 (br. m, 8H); 1.65-1.04 (br. m, 28H); 0.90 (t, 6H).

tert-Butyl 4-(3-(dinonylamino)propanoyl)piperazine- 1-carboxylate



Chemical Formula: C30H59N3O3

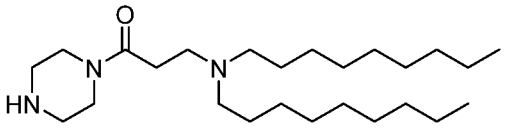
Molecular Weight: 509.82

[00791] In the same manner as Step 3 for Compound 11, *tert*-butyl 4-(3-(dinonylamino)propanoyl)piperazine-l -carboxylate was synthesized from lithium 3-(dinonylamino)propanoate (636 mg, 1.83 mmol), 1-boc-piperazine (388 mg, 2.08 mmol), iPr_2EtN (726 μL, 4.17 mmol), and T3P (50% EtOAc solution, 3.4 mL, 5.68 mmol) in THF (20 mL). Yield (839 mg, 87%).

UPLC/ELSD: RT = 2.26 min. MS (ES): *m/z* (MH^+) 511 for C30H59N3O3

¾-NMR (300 MHz, CDCh) δ: ppm 3.71-2.21 (br. m, 16H); 1.92-0.98 (br. m, 37H); 0.90 (t, 6H).

Step 3: 3-(Dinonylamino)-l-(piperazin-l-yl)propan-l-one



Chemical Formula: C25H51N3O

Molecular Weight: 409.70

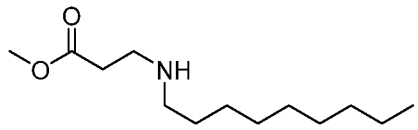
[00792] In the same manner as Step 4 for Compound 11, 3-(dinonylamino)-l-(piperazin-l-yl)propan-l-one was synthesized from *tert*-butyl 4-(3-(dinonylamino)propanoyl)piperazine-l-

carboxylate (839 mg, 1.65 mmol), and TFA (6.3 mL, 83 mmol) in DCM (7 mL). Yield (501 mg, 74%).

UPLC/ELSD: RT = 1.19 min. MS (ES): *m/z* (MH^+) 411 for C25H51N3O

¾-NMR (300 MHz, CDCh) δ: ppm 3.61 (t, 2H); 3.47 (t, 2H); 2.86 (br. m, 6H); 2.46 (br. m, 6H); 1.80 (br, 1H); 1.56-1.08 (br. m, 28H); 0.90 (t, 6H).

Step 4: Methyl 3-(nonylamino)propanoate



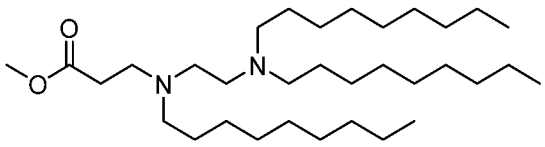
Chemical Formula: C13H27NO2

Molecular Weight: 229.36

[00793] In the same manner as Step 1 for Compound 52, methyl **3-**(nonylamino)propanoate was synthesized from methyl 3-aminopropanoate hydrochloride (2.0 g, 14 mmol), 1-bromononane (2.7 mL, 14 mmol), K2CO3 (4.0 g, 29 mmol) and KI (238 mg, 1.4 mmol) in MeCN (100 mL). Yield (300 mg, 9%)

¾-NMR (300 MHz, CDCh) δ: ppm 3.70 (s, 3H); 2.91 (t, 2H); 2.60 (br. m, 4H); 1.90 (br, 1H); 1.58-1.02 (br. m, 14H); 0.90 (t, 3H).

Step 5: Methyl 3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoate



Chemical Formula: C33H68N2O2

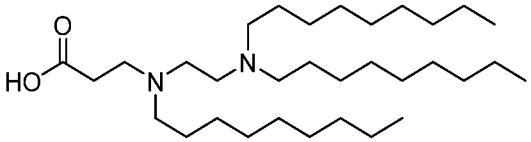
Molecular Weight: 524.92

[00794] In the same manner as Step 9 from Compound 10, methyl 3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoate was synthesized from methyl 3-(nonylamino)propanoate (300 mg, 1.31 mmol), *N*-(2-chloroethyl)-*N*-nonylnonan-1-amine (478 mg, 1.44 mmol), K2CO3 (400 mg, 2.88 mmol), and KI (22 mg, 0.13 mmol) in MeCN (20 mL). Yield (348 mg, 51%).

UPLC/ELSD: RT = 2.66 min. MS (ES): *m/z* (MH^+) 526 for C33H68N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 2.82 (t, 2H); 2.44 (br. m, 12H); 1.85-1.05 (br. m, 42H); 0.90 (t, 9H).

Step 6: 3-((2-(Dinonylamino)ethyl)(nonyl)amino)propanoic acid



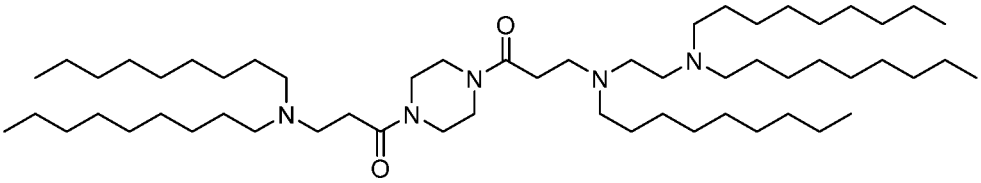
Chemical Formula: C32H66N2O2

Molecular Weight: 510.89

[00795] In the same manner as Step 10 from Compound 10, 3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoic acid was synthesized from methyl 3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoate (348 mg, 0.66 mmol) and 1M LiOH (3.3 mL, 3.3 mmol) in THF (3.3 mL). Yield (338 mg, 99%).

UPLC/ELSD: RT = 2.29 min. MS (ES): *m/z* (MH^+) 512 for C32H66N2O2

Step 7: 3-(Dinonylamino)-1-(4-(3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoyl)piperazin-1-yl)propan-1-one



Chemical Formula: C57H115N5O2

Molecular Weight: 902.58

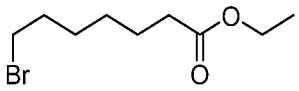
[00796] In the same manner as Step 11 for Compound 11, 3-(dinonylamino)-1-(4-(3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoyl)piperazin-1-yl)propan-1-one was synthesized from 3-(dinonylamino)-1-(piperazin-1-yl)propan-1-one (298 mg, 0.73 mmol), 3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoic acid (338 mg, 0.68 mmol), iPnEtN (254 μL, 1.46 mmol), and T3P (50% EtOAc solution, 1.18 mL, 1.98 mmol) in THF (10 mL). Yield (218 mg, 37%).

UPLC/ELSD: RT = 2.89 min. MS (ES): *m/z* (MH^+) 903 for C57H115N5O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.65 (br. m, 4H); 3.50 (br. m, 4H); 2.82 (br. m, 4H); 2.66-2.30 (br. m, 18H); 1.61-1.02 (br. m, 70H); 0.90 (t, 15H).

BC: Compound 53: Ethyl 7-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(nonyl)amino)heptanoate

Step 1: Ethyl 7-bromoheptanoate



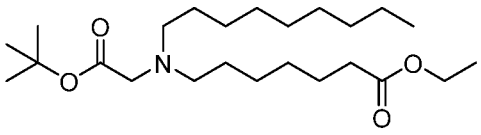
Chemical Formula: $C_9H_{17}BrO_2$

Molecular Weight: 237.14

[00797] In the same manner as Step 1 for Compound 15, ethyl 7-bromoheptanoate was synthesized from 7-bromoheptanoic acid (1.0 g, 4.8 mmol), ethanol (5.6 mL, 96 mmol), and H_2SO_4 (0.25 mL, 4.8 mmol) in THF (6 mL). Yield (91 1 mg, 80%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.15 (q, 2H); 3.42 (t, 2H); 2.33 (t, 2H); 1.87 (m, 2H); 1.66 (m, 2H); 1.57-1.14 (br. m, 7H).

Step 2: Ethyl 7-((2-(teri-butoxy)-2-oxoethyl)(nonyl)amino)heptanoate



Chemical Formula: $C_{24}H_{47}NO_4$

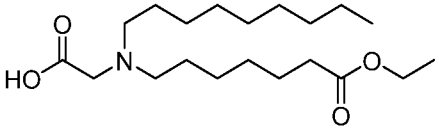
Molecular Weight: 413.64

[00798] In the same manner as Step 3 for Compound 44, ethyl 7-((2-(fer/-butoxy)-2-oxoethyl)(nonyl)amino)heptanoate was synthesized from *tert*-butyl nonylglycinate (250 mg, 0.97 mmol), ethyl 7-bromoheptanoate (253 mg, 1.07 mmol), K_2CO_3 (270 mg, 1.94 mmol), and KI (16 mg, 0.10 mmol) in MeCN (10 mL). Yield (298 mg, 74%).

UPLC/ELSD: RT = 1.60 min. MS (ES): *m/z* (MH^+) 414.68 for $C_{24}H_{47}NO_4$

¾-NMR (300 MHz, CDCh) δ: ppm 4.14 (q, 2H); 3.23 (s, 2H); 2.57 (t, 4H); 2.31 (t, 2H); 1.74-1.12 (br. m, 34H); 0.90 (t, 3H).

Step 3: *N*-(7-Ethoxy-7-oxoheptyl)-*N*-nonylglycine



Chemical Formula: $C_{20}H_{39}NO_4$

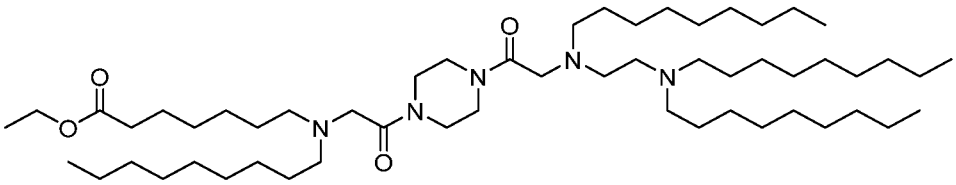
Molecular Weight: 357.54

[00799] In the same manner as Step 4 for Compound 44, *N*-(7-ethoxy-7-oxoheptyl)-*N*-nonylglycine was synthesized from ethyl 7-((2-(fer/-butoxy)-2-oxoethyl)(nonyl)amino)heptanoate (298 mg, 0.72 mmol), and TFA (2.8 mL, 36 mmol) in DCM (3 mL). Yield (244 mg, 95%).

UPLC/ELSD: RT = 1.07 min. MS (ES): *m/z* (MH^+) 358.50 for C20H39NO4

¾-NMR (300 MHz, CDCh) δ: ppm 4.15 (q, 2H); 3.46 (br, 2H); 3.01 (br, 4H); 2.31 (t, 2H); 1.86-1.10 (br. m, 25H); 0.91 (t, 3H).

Step 4: Ethyl 7-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)heptanoate



Chemical Formula: C55H109N5O4

Molecular Weight: 904.51

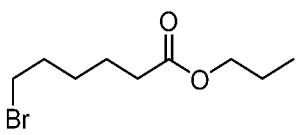
[00800] In the same manner as Step 7 for Compound 44, ethyl 7-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylgly cyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)heptanoate was synthesized from *N*-(7-ethoxy-7-oxoheptyl)-*N*-nonylgly cine (111 mg, 0.31 mmol), 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l-(piperazin-l-yl)ethan-l-one (160 mg, 0.28 mmol), iPr_2EtN (109 µL, 0.62 mmol), and T3P (50% EtOAc solution, 0.51 mL, 0.81 mmol) in THF (10 mL). Yield (70 mg, 27%).

UPLC/ELSD: RT = 2.88 min. MS (ES): *m/z* (MH^+) 905.33 for C55H109N5O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.00 (q, 2H); 3.65-3.34 (br. m, 8H); 3.19 (s, 2H); 3.13 (s, 2H); 2.50-2.10 (br. m, 16H); 1.65-0.90 (br. m, 67H); 0.75 (t, 12H).

BD: Compound 54: Propyl 6-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)hexanoate

Step 1: Propyl 6-bromohexanoate



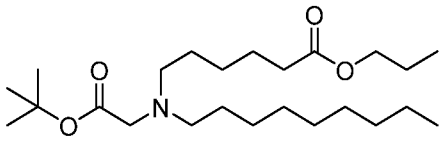
Chemical Formula: $C_9H_{17}BrO_2$

Molecular Weight: 237.14

[00801] In the same manner as Step 1 for Compound 15, propyl 6-bromohexanoate was synthesized from 6-bromohexanoic acid (1.0 g, 5.1 mmol), 1-propanol (1.5 g, 26 mmol), and H_2SO_4 (0.27 mL, 5.1 mmol) in THF (5 mL). Yield (1.14 g, 94%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.05 (t, 2H); 3.43 (t, 2H); 2.34 (t, 2H); 1.90 (m, 2H); 1.68 (m, 4H); 1.50 (m, 2H); 0.96 (t, 3H).

Step 2: Propyl 6-((2-(teri-butoxy)-2-oxoethyl)(nonyl)amino)hexanoate



Chemical Formula: $C_{24}H_{47}NO_4$

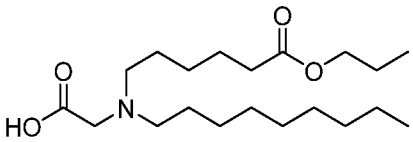
Molecular Weight: 413.64

[00802] In the same manner as Step 3 for Compound 44, propyl 6-((2-(/er/-butoxy)-2-oxoethyl)(nonyl)amino)hexanoate was synthesized from *tert-b*utyl nonylglycinate (250 mg, 0.97 mmol), propyl 6-bromohexanoate (253 mg, 1.07 mmol), K_2CO_3 (270 mg, 1.94 mmol), and KI (16 mg, 0.10 mmol) in MeCN (10 mL). Yield (258 mg, 64%).

UPLC/ELSD: RT = 1.62 min. MS (ES): *m/z* (MH^+) 414.59 for $C_{24}H_{47}NO_4$

¾-NMR (300 MHz, CDCh) δ: ppm 4.05 (t, 2H); 3.23 (s, 2H); 2.58 (br. m, 4H); 2.33 (t, 2H); 1.75-1.15 (br. m, 31H); 0.91 (m, 6H).

Step 3: *N*-Nonyl-*N*-(6-oxo-6-propoxyhexyl)glycine



Chemical Formula: $C_{20}H_{39}NO_4$

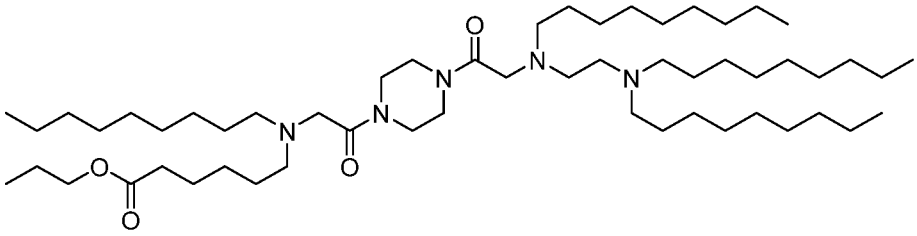
Molecular Weight: 357.54

[00803] In the same manner as Step 4 for Compound 44, *N*-nonyl-*N*-(6-oxo-6-propoxyhexyl)glycine was synthesized from propyl 6-((2-(/er/-butoxy)-2-oxoethyl)(nonyl)amino)hexanoate (258 mg, 0.62 mmol), and TFA (2.4 mL, 31 mmol) in DCM (3 mL). Yield (223 mg, 99%).

UPLC/ELSD: RT = 1.13 min. MS (ES): *m/z* (MH^+) 358.50 for $C_{20}H_{39}NO_4$

¾-NMR (300 MHz, CDCh) δ: ppm 4.09 (t, 2H); 3.34 (s, 2H); 2.87 (br. m, 4H); 2.36 (t, 2H); 1.77-1.10 (br. m, 22H); 0.92 (m, 6H).

Step 4: Propyl 6-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)hexanoate



Chemical Formula: C55H109N5O4

Molecular Weight: 904.51

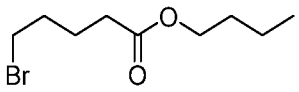
[00804] In the same manner as Step 7 for Compound 44, propyl 6-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(nonyl)amino)hexanoate was synthesized from *N*-nonyl-*N*-(6-oxo-6-propoxyhexyl)glycine (111 mg, 0.31 mmol), 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l-(piperazin-l-yl)ethan-l-one (160 mg, 0.28 mmol), iPnEtN (109 µL, 0.62 mmol), and T3P (50% EtOAc solution, 0.51 mL, 0.81 mmol) in THF (10 mL). Yield (72 mg, 28%).

UPLC/ELSD: RT = 2.91 min. MS (ES): *m/z* (MH^+) 905.33 for C55H109N5O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.09 (t, 2H); 3.78-3.46 (br. m, 8H); 3.34 (s, 2H); 3.28 (s, 2H); 2.68-2.24 (br. m, 16H); 1.85-1.10 (br. m, 64H); 0.92 (m, 15H).

BE: Compound 55: Butyl 5-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)pentanoate

Step 1: Butyl 5-bromopentanoate



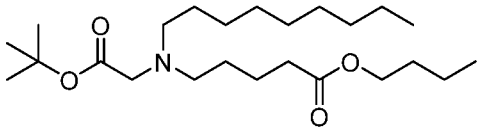
Chemical Formula: $C_9H_{17}BrO_2$

Molecular Weight: 237.14

[00805] In the same manner as Step 1 for Compound 15, butyl 5-bromopentanoate was synthesized from 5-bromopentanoic acid (1.47 g, 8.1 mmol), 1-butanol (0.50 g, 6.8 mmol), and H2SO4 (0.36 mL, 6.8 mmol) in THF (7 mL). Yield (1.42 g, 0.89%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.10 (t, 2H); 4.34 (t, 2H); 2.36 (t, 2H); 1.93 (m, 2H); 1.80 (m, 2H); 1.62 (m, 2H); 1.40 (m, 2H); 0.96 (t, 3H).

Step 2: Butyl 5-((2-(teri-butoxy)-2-oxoethyl)(nonyl)amino)pentanoate



Chemical Formula: C24H47NO4

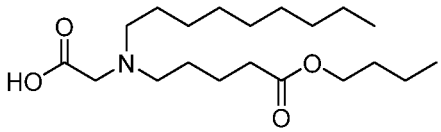
Molecular Weight: 413.64

[00806] In the same manner as Step 3 for Compound 44, butyl 5-((2-(/er/-butoxy)-2-oxoethyl)(nonyl)amino)pentanoate was synthesized from *tert*-butyl nonylglycinate (250 mg, 0.97 mmol), butyl 5-bromopentanoate (253 mg, 1.07 mmol), K2CO3 (270 mg, 1.94 mmol), and KI (16 mg, 0.10 mmol) in MeCN (10 mL). Yield (284 mg, 71%).

UPLC/ELSD: RT = 1.67 min. MS (ES): *m/z* (MH^+) 414.59 for C24H47NO4

¾-NMR (300 MHz, CDCh) δ: ppm 4.09 (t, 2H); 3.23 (s, 2H); 2.58 (m, 4H); 2.34 (t, 2H); 1.74-1.20 (br. m, 31H); 0.93 (m, 6H).

Step 3: *N*-(5-butoxy-5-oxopentyl)-*N*-nonylglycine



Chemical Formula: C20H39NO4

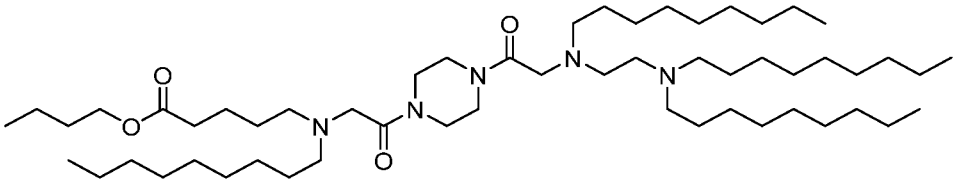
Molecular Weight: 357.54

[00807] In the same manner as Step 4 for Compound 44, *N*-(5-butoxy-5-oxopentyl)-*N*-nonylglycine was synthesized from butyl 5-((2-(/er/-butoxy)-2-oxoethyl)(nonyl)amino)pentanoate (284 mg, 0.69 mmol), and TFA (2.6 mL, 34 mmol) in DCM (3 mL). Yield (245 mg, 99%).

UPLC/ELSD: RT = 1.09 min. MS (ES): *m/z* (MH^+) 358.50 for C20H39NO4

¾-NMR (300 MHz, CDCh) δ: ppm 4.05 (t, 2H); 3.48 (s, 2H); 3.03 (br. m, 4H); 2.34 (t, 2H); 1.85-1.15 (br. m, 22H); 0.93 (m, 6H).

Step 4: Butyl 5-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)pentanoate



Chemical Formula: C55H109N5O4

Molecular Weight: 904.5 1

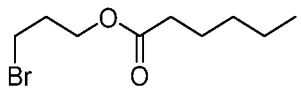
[00808] In the same manner as Step 7 for Compound 44, butyl 5-((2-(4 -(*N* -(2-(dinonylamino)ethyl)-*N* -nonylgly cyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)pentanoate was synthesized from *N* -(5-butoxy-5-oxopentyl)-*N* -nonylglycine (111 mg, 0.3 1 mmol), 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l -(piperazin-l-yl)ethan-l-one (160 mg, 0.28 mmol), iPr_2EtN (109 μL, 0.62 mmol), and T3P (50% EtOAc solution, 0.5 1 mL, 0.81 mmol) in THF (10 mL). Yield (92 mg, 36%).

UPLC/ELSD: RT = 2.88 min. MS (ES): *m/z* (MH^+) 905.33 for C55H109N5O4

¾-NMR (300 MHz, CDCh) δ: ppm 3.90 (t, 2H); 3.65-3.35 (br. m, 8H); 3.19 (s, 2H); 3.13 (t, 2H); 2.52-2.06 (br. m, 16H); 1.65-0.95 (br. m, 64H); 0.77 (m, 15H).

BF: Compound 56: 3-((2-(4 -(*N* -(2-(Dinonylamino)ethyl)-*N* -nonylglycyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)propyl hexanoate

Step 1: 3-Bromopropyl hexanoate



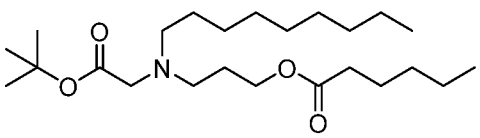
Chemical Formula: $C_9H_{17}BrO_2$

Molecular Weight: 237. 14

[00809] In the same manner as Step 1 for Compound 15, 3-bromopropyl hexanoate was synthesized from 3-bromopropan-l -ol (0. 87 mL, 9.6 mmol), hexanoic acid (1.0 mL, 8.0 mmol), and H2SO4 (1.0 mL, 8.0 mmol) in THF (10 mL). Yield (823 mg, 44%).

Ṫi-NMR (300 MHz, CDCh) δ: ppm 4.23 (t, 2H); 3.49 (t, 2H), 2.33 (t, 2H); 2.20 (m, 2H); 1.65 (m, 2H); 1.34 (m, 4H), 0.91 (t, 3H).

Step 2: 3-((2-(teri-Butoxy)-2-oxoethyl)(nonyl)amino)propyl hexanoate



Chemical Formula: C24H47NO4

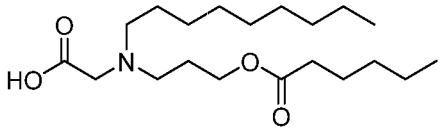
Molecular Weight: 413.64

[00810] In the same manner as Step 3 for Compound 44, 3-((2-(Yer/-butoxy)-2-oxoethyl)(nonyl)amino)propyl hexanoate was synthesized from *tert*-butyl nonylglycinate (250 mg, 0.97 mmol), 3-bromopropyl hexanoate (253 mg, 1.07 mmol), K2CO3 (270 mg, 1.94 mmol), and KI (16 mg, 0.10 mmol) in MeCN (10 mL). Yield (335 mg, 83%).

UPLC/ELSD: RT = 1.78 min. MS (ES): *m/z* (MH^+) 414.59 for C24H47NO4

¾-NMR (300 MHz, CDCh) δ: ppm 4.14 (t, 2H); 3.23 (s, 2H); 2.68 (t, 2H); 2.58 (t, 2H); 2.30 (t, 2H); 1.79 (m, 2H); 1.64 (m, 2H); 1.55-1.20 (br. m, 27H); 0.91 (m, 6H).

Step 3: *N*-(3-(Hexanoyloxy)propyl)-*N*-nonylglycine



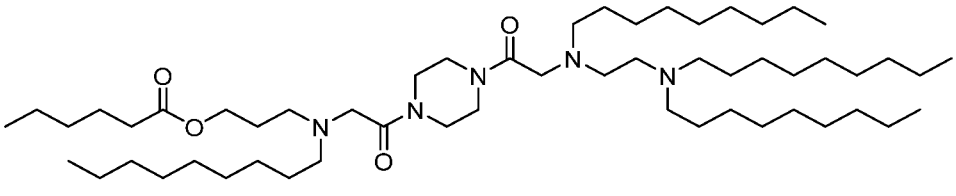
Chemical Formula: C20H39NO4

Molecular Weight: 357.54

[00811] In the same manner as Step 4 for Compound 44, *N*-(3-(hexanoyloxy)propyl)-*N*-nonylglycine was synthesized from 3-((2-(teri-butoxy)-2-oxoethyl)(nonyl)amino)propyl hexanoate (335 mg, 0.81 mmol), and TFA (3.1 mL, 40 mmol) in DCM (4 mL). Yield (284 mg, 98%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.11 (t, 2H); 3.17 (s, 2H); 2.68 (br. m, 4H); 2.30 (t, 2H); 1.87 (m, 2H); 1.63 (m, 2H); 1.48 (m, 2H); 1.28 (br. m, 16H); 0.91 (m, 6H).

Step 4: 3-((2-(4-(*N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(nonyl)amino)propyl hexanoate



Chemical Formula: C55H109N5O4

Molecular Weight: 904.51

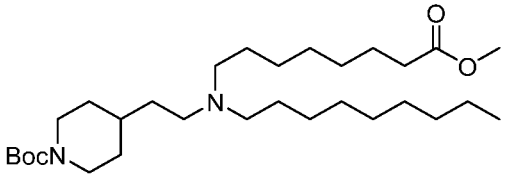
[00812] In the same manner as Step 7 for Compound 44, 3-((2-(4-*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(nonyl)amino)propyl hexanoate was synthesized from *N*-(3-(hexanoyloxy)propyl)-N-nonylglycine (111 mg, 0.31 mmol), 2-((2-(dinonylamino)ethyl)(nonyl)amino)-1-(piperazin-1-yl)ethan-1-one (160 mg, 0.28 mmol), iPr_2EtN (109 μL, 0.62 mmol), and T3P (50% EtOAc solution, 0.51 mL, 0.81 mmol) in THF (10 mL). Yield (55 mg, 21%).

UPLC/ELSD: RT = 2.94 min. MS (ES): *m/z* (MH^+) 905.25 for $C_{55}H_{109}N_5O_4$

¾-NMR (300 MHz, CDCh) δ: ppm 4.01 (t, 2H); 3.77-3.43 (br. m, 8H), 3.23 (m, 4H); 2.64-2.15 (m, 16H); 1.70 (m, 2H); 1.54 (m, 2H); 1.50-0.96 (br. m, 60H); 0.81 (m, 15H).

BG: Compound 57: Methyl 8-((2-(1-*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)octanoate

Step 1: tot-Butyl 4-(2-((8-methoxy-8-oxooctyl)(nonyl)amino)ethyl)piperidine-1-carboxylate



Chemical Formula: $C_{30}H_{58}N_2O_4$

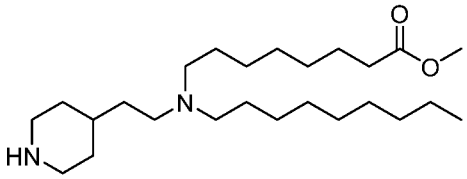
Molecular Weight: 510.80

[00813] To a mixture of tot-butyl 4-(2-(nonylamino)ethyl)piperidine-1-carboxylate (239 mg, 0.67 mmol) and methyl 8-bromooctanoate (192 mg, 0.81 mmol) in MeCN (10 mL) was added K_2CO_3 (188 mg, 1.35 mmol) and KI (11 mg, 0.07 mmol) and the mixture was allowed to stir at 82 °C for 12 hours. The suspension was cooled to RT and filtered over a pad of celite rinsing with EtOAc, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-80% EtOAc/hexanes) provided tot-butyl 4-(2-((8-methoxy-8-oxooctyl)(nonyl)amino)ethyl)piperidine-1-carboxylate (241 mg, 70%).

UPLC/ELSD: RT = 1.87 min. MS (ES): *m/z* (MH^+) 512.76 for $C_{30}H_{58}N_2O_4$

¾-NMR (300 MHz, CDCh) δ: ppm 4.07 (br. m, 2H); 3.69 (s, 3H); 2.70 (m, 2H); 2.37 (br. m, 8H); 1.75-1.00 (br. m, 40H); 0.90 (t, 3H).

Step 2: Methyl 8-(nonyl(2-(piperidin-4-yl)ethyl)amino)octanoate



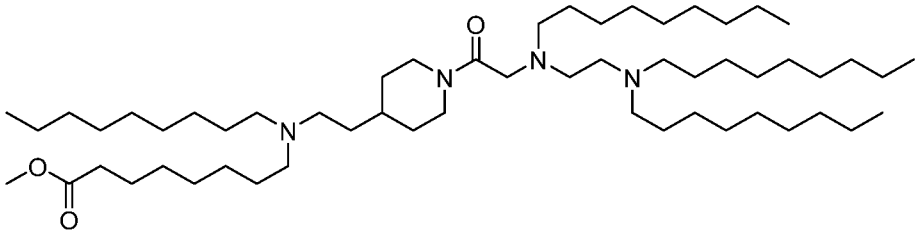
Chemical Formula: C25H50N2O2

Molecular Weight: 410.69

[00814] In the same manner as Step 4 for Compound 11, methyl 8-(nonyl(2-(piperidin-4-yl)ethyl)amino)octanoate was synthesized from *tert*-butyl 4-(2-((8-methoxy-8-oxooctyl)(nonyl)amino)ethyl)piperidine-1-carboxylate (241 mg, 0.47 mmol), and TFA (1.8 mL, 24 mmol) in DCM (2 mL). Yield (193 mg, 99%).

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.08 (m, 2H); 2.60 (m, 2H); 2.49-2.24 (br. m, 8H); 2.06 (br, 1H); 1.78-1.02 (br. m, 31H); 0.90 (t, 3H).

Step 3: Methyl 8-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)octanoate



Chemical Formula: C56H112N4O3

Molecular Weight: 889.54

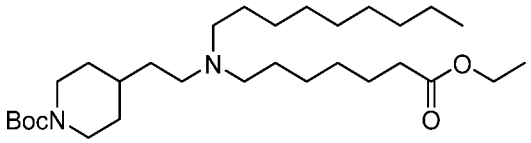
[00815] In the same manner as Step 11 for Compound 11, methyl 8-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)octanoate was synthesized from methyl 8-(nonyl(2-(piperidin-4-yl)ethyl)amino)octanoate (141 mg, 0.34 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (188 mg, 0.38 mmol), iPr_2EtN (132 μL, 0.76 mmol), and T3P (50% EtOAc solution, 614 μL, 1.03 mmol) in THF (10 mL). Yield (70 mg, 23%).

UPLC/ELSD: RT = 2.97 min. MS (ES): *m/z* (MH^+) 890.24 for C56H112N4O3

¾-NMR (300 MHz, CDCh) δ: ppm 4.64-4.09 (br. m, 2H); 3.69 (s, 3H); 3.42-2.83 (br. m, 3H); 2.69-2.24 (br. m, 19H); 1.81-0.99 (br. m, 73H); 0.90 (t, 12H).

BH: Compound 58: Ethyl 7-((2-(l -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)heptanoate

Step 1: *tert*-Butyl 4-(2-((7-ethoxy-7-oxoheptyl)(nonyl)amino)ethyl)piperidine-l -carboxylate



Chemical Formula: C30H58N2O4

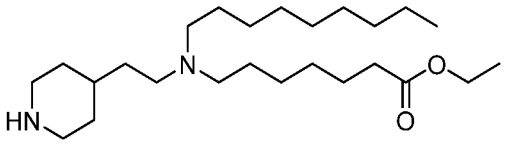
Molecular Weight: 510.80

[00816] In the same manner as Step 1 for Compound 57, *tert-butyl* 4-(2-((7-ethoxy-7-oxoheptyl)(nonyl)amino)ethyl)piperi dine- 1-carboxy late was synthesized from *tert-butyl* 4-(2-(nonylamino)ethyl)piperi dine- 1-carboxy late (239 mg, 0.67 mmol), ethyl 7-bromoheptanoate (192 mg, 0.81 mmol), K2CO3 (188 mg, 1.35 mmol) and KI (11 mg, 0.07 mmol) in MeCN (10 mL). Yield (247 mg, 72%).

UPLC/ELSD: RT = 1.91 min. MS (ES): *m/z* (MH^+) 511.62 for C30H58N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.12 (br. m, 4H); 2.80-2.15 (br. m, 10H); 1.75-1.00 (br. m, 41H); 0.90 (t, 3H).

Step 2: Ethyl 7-(nonyl(2-(piperidin-4-yl)ethyl)amino)heptanoate



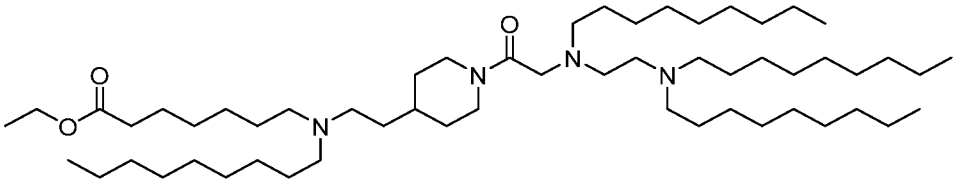
Chemical Formula: C25H50N2O2

Molecular Weight: 410.69

[00817] In the same manner as Step 4 for Compound 11, ethyl 7-(nonyl(2-(piperidin-4-yl)ethyl)amino)heptanoate was synthesized from *tert-butyl* 4-(2-((7-ethoxy-7-oxoheptyl)(nonyl)amino)ethyl)piperi dine- 1-carboxy late (247 mg, 0.48 mmol), and TFA (1.9 mL, 24 mmol) in DCM (2 mL). Yield (194 mg, 98%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.14 (t, 2H); 3.08 (m, 2H); 2.60 (m, 2H); 2.52-2.24 (br. m, 8H); 2.12 (br, 1H); 1.77-1.05 (br. m, 32H); 0.90 (t, 3H).

Ethyl 7-((2-(l -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)heptanoate



Chemical Formula: C56H112N4O3

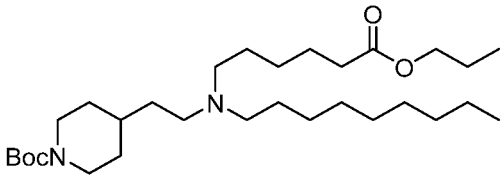
Molecular Weight: 889.54

[00818] In the same manner as Step 11 for Compound 11, ethyl 7-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)heptanoate was synthesized from ethyl 7-(nonyl(2-(piperidin-4-yl)ethyl)amino)heptanoate (141 mg, 0.34 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (188 mg, 0.38 mmol), iPnEtN (132 μL, 0.76 mmol), and T3P (50% EtOAc solution, 614 μL, 1.03 mmol) in THF (10 mL). Yield (42 mg, 14%). UPLC/ELSD: RT = 3.00 min. MS (ES): *m/z* (MH^+) 890.32 for C56H112N4O3

¾-NMR (300 MHz, CDCh) δ: ppm 4.55-3.95 (br. m, 4H); 3.38-2.72 (br. m, 4H); 2.66-2. 10 (br. m, 18H); 1.72-0.91 (br. m, 74H); 0.8 1 (t, 12H).

BI: Compound 59: Propyl 6-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)hexanoate

Step 1: *tert*-Butyl 4-(2-(nonyl(6-oxo-6-propoxyhexyl)amino)ethyl)piperi dine- 1-carboxy late



Chemical Formula: C30H58N2O4

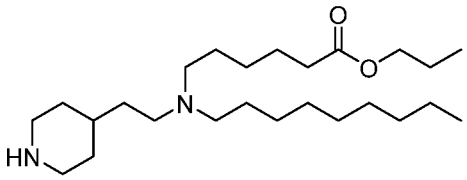
Molecular Weight: 510.80

[00819] In the same manner as Step 1 for Compound 57, *tert-butyl* 4-(2-(nonyl(6-oxo-6-propoxyhexyl)amino)ethyl)piperi dine- 1-carboxy late was synthesized from *tert-butyl* 4-(2-(nonylamino)ethyl)piperi dine- 1-carboxy late (239 mg, 0.67 mmol), propyl 6-bromohexanoate (192 mg, 0.81 mmol), K2CO3 (188 mg, 1.35 mmol) and KI (11 mg, 0.07 mmol) in MeCN (10 mL). Yield (240 mg, 70%).

UPLC/ELSD: RT = 1.93 min. MS (ES): *m/z* (MH^+) 511.78 for C30H58N2O4

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.05 (br. m, 4H); 2.80-2.20 (br. m, 10H); 1.85-1.04 (br. m, 38H); 0.92 (t, 6H).

Step 2: Propyl 6-(nonyl(2-(piperidin-4-yl)ethyl)amino)hexanoate



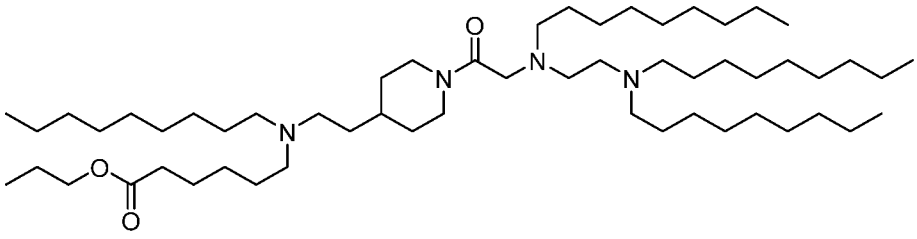
Chemical Formula: C25H50N2O2

Molecular Weight: 410.69

[00820] In the same manner as Step 4 for Compound 11, propyl 6-(nonyl(2-(piperidin-4-yl)ethyl)amino)hexanoate was synthesized from *tert*-butyl 4-(2-(nonyl(6-oxo-6-propoxyhexyl)amino)ethyl)piperidine-1-carboxylate (240 mg, 0.47 mmol), and TFA (1.8 mL, 23 mmol) in DCM (2 mL). Yield (183 mg, 95%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.04 (t, 2H); 3.08 (m, 2H); 2.60 (m, 2H); 2.35 (br. m, 8H); 1.95 (br, 1H); 1.75-1.00 (br. m, 29H); 0.92 (m, 6H).

Step 3: Propyl 6-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)hexanoate



Chemical Formula: C56H112N4O3

Molecular Weight: 889.54

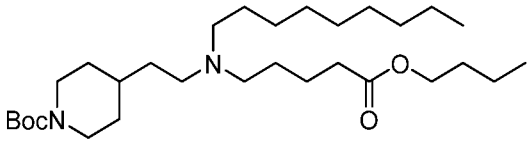
[00821] In the same manner as Step 11 for Compound 11, propyl 6-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)hexanoate was synthesized from propyl 6-(nonyl(2-(piperidin-4-yl)ethyl)amino)hexanoate (141 mg, 0.34 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (188 mg, 0.38 mmol), iPr_2EtN (132 μL, 0.76 mmol), and T3P (50% EtOAc solution, 614 μL, 1.03 mmol) in THF (10 mL). Yield (67 mg, 22%).

UPLC/ELSD: RT = 3.02 min. MS (ES): *m/z* (MH^+) 890.32 for C56H112N4O3

¾-NMR (300 MHz, CDCh) δ: ppm 4.65-4.13 (br. m, 2H); 4.05 (t, 2H); 3.50-2.81 (br. m, 4H); 2.69-2.18 (br. m, 18H); 1.98-1.02 (br. m, 71H); 0.92 (br. m, 15H).

BJ: Compound 60: Butyl 5-((2-(l -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)pentanoate

Step 1: *tert-Butyl* 4-(2-((5-butoxy-5-oxopentyl)(nonyl)amino)ethyl)piperidine-l-carboxylate



Chemical Formula: C30H58N2O4

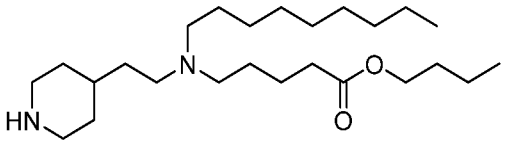
Molecular Weight: 510.80

[00822] In the same manner as Step 1 for Compound 57, *tert-b*utyl 4-(2-((5-butoxy-5-oxopenty l)(nonyl)amino)ethyl)piperi dine- 1-carboxy late was synthesized from *tert-butyl* 4-(2-(nonylamino)ethyl)piperi dine- 1-carboxy late (239 mg, 0.67 mmol), butyl 5-bromopentanoate (192 mg, 0.81 mmol), K2CO3 (188 mg, 1.35 mmol) and KI (11 mg, 0.07 mmol) in MeCN (10 mL). Yield (211 mg, 61%).

UPLC/ELSD: RT = 1.95 min. MS (ES): *m/z* (MH^+) 511.78 for C30H58N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.09 (br. m, 4H); 2.70 (br. m, 2H); 2.38 (br. m, 8H); 1.73-1.02 (br. m, 38H); 0.93 (br. m, 6H).

Step 2: Butyl 5-(nonyl(2-(piperidin-4-yl)ethyl)amino)pentanoate



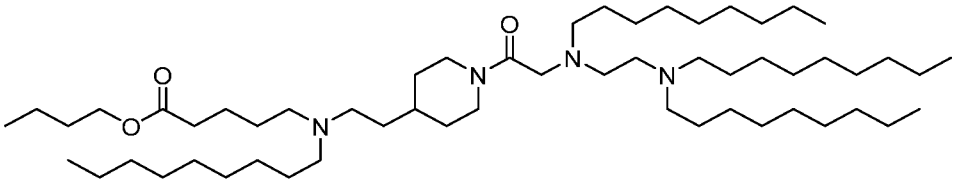
Chemical Formula: C25H50N2O2

Molecular Weight: 410.69

[00823] In the same manner as Step 4 for Compound 11, butyl 5-(nonyl(2-(piperidin-4-yl)ethyl)amino)pentanoate was synthesized from *tert-butyl* 4-(2-((5-butoxy-5-oxopentyl)(nonyl)amino)ethyl)piperi dine- 1-carboxy late (211 mg, 0.41 mmol), and TFA (1.6 mL, 21 mmol) in DCM (2 mL). Yield (169 mg, 99%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.09 (t, 2H); 3.07 (m, 2H); 2.60 (m, 2H); 2.37 (br. m, 8H); 1.83 (br, 1H); 1.76-1.04 (br. m, 29H); 0.93 (br. m, 6H).

Step 3: Butyl 5-((2-(l -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)pentanoate



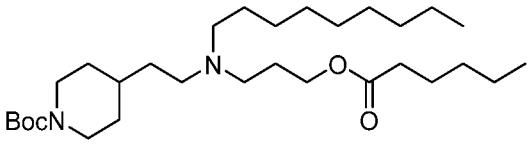
Chemical Formula: C56H112N4O3

Molecular Weight: 889.54

[00824] In the same manner as Step 11 for Compound 11, butyl 5-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)pentanoate was synthesized from butyl 5-(nonyl(2-(piperidin-4-yl)ethyl)amino)pentanoate (141 mg, 0.34 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (188 mg, 0.38 mmol), iPr_2EtN (132 μL, 0.76 mmol), and T3P (50% EtOAc solution, 614 μL, 1.03 mmol) in THF (10 mL). Yield (46 mg, 15%).
UPLC/ELSD: RT = 3.03 min. MS (ES): *m/z* (MH^+) 890.32 for C56H112N4O3
¾-NMR (300 MHz, CDCh) δ: ppm 4.62-4.13 (br. m, 2H); 4.09 (t, 2H); 3.41-2.84 (br. m, 4H); 2.72-2.25 (br. m, 18H); 1.82-1.02 (br. m, 71H); 0.91 (br. m, 15H).

BK: Compound 61: 3-((2-(1-(*N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)propyl hexanoate

Step 1: *tert*-Butyl 4-(2-((3-(hexanoyloxy)propyl)(nonyl)amino)ethyl)piperidine-1-carboxylate

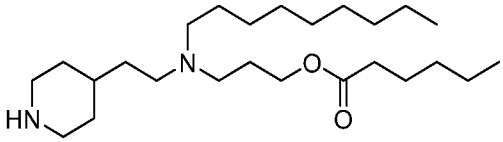


Chemical Formula: C30H58N2O4

Molecular Weight: 510.80

[00825] In the same manner as Step 1 for Compound 57, *tert*-butyl 4-(2-((3-(hexanoyloxy)propyl)(nonyl)amino)ethyl)piperidine-1-carboxylate was synthesized from *tert*-butyl 4-(2-(nonylamino)ethyl)piperidine-1-carboxylate (239 mg, 0.67 mmol), 3-bromopropyl hexanoate (192 mg, 0.81 mmol), K2CO3 (188 mg, 1.35 mmol) and KI (11 mg, 0.07 mmol) in MeCN (lO mL). Yield (195 mg, 57%).
UPLC/ELSD: RT = 1.97 min. MS (ES): *m/z* (MH^+) 511.86 for C30H58N2O4
^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.11 (br. m, 4H); 2.69 (m, 2H); 2.56-2.22 (br. m, 8H); 1.76 (m, 2H); 1.65 (m, 4H); 1.55-1.05 (br. m, 32H); 0.91 (m, 6H).

Step 2: 3-(Nonyl(2-(piperidin-4-yl)ethyl)amino)propyl hexanoate



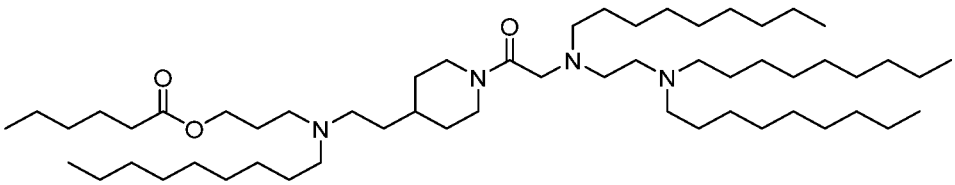
Chemical Formula: C25H50N2O2

Molecular Weight: 410.69

[00826] In the same manner as Step 4 for Compound 11, 3-(nonyl(2-(piperidin-4-yl)ethyl)amino)propyl hexanoate was synthesized from *tert*-butyl 4-(2-((3-(hexanoyloxy)propyl)(nonyl)amino)ethyl)piperidine-l-carboxylate (195 mg, 0.38 mmol), and TFA (1.5 mL, 19 mmol) in DCM (2 mL). Yield (149 mg, 95%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.12 (t, 2H); 3.08 (m, 2H); 2.70-2.22 (br. m, 10H); 2.07 (br, 1H); 1.70 (br. m, 6H); 1.48-1.00 (br. m, 23H); 0.91 (m, 6H).

Step 3: 3-((2-(l-(*N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)propyl hexanoate



Chemical Formula: C56H112N4O3

Molecular Weight: 889.54

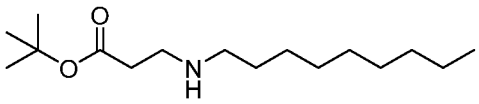
[00827] In the same manner as Step 11 for Compound 11, 3-((2-(l-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)propyl hexanoatewas synthesized from 3-(nonyl(2-(piperidin-4-yl)ethyl)amino)propyl hexanoate (141 mg, 0.34 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (188 mg, 0.38 mmol), iPr_2EtN (132 μL, 0.76 mmol), and T3P (50% EtOAc solution, 614 μL, 1.03 mmol) in THF (10 mL). Yield (64 mg, 21%).

UPLC/ELSD: RT = 3.02 min. MS (ES): *m/z* (MH^+) 890.41 for C56H112N4O3

¾-NMR (300 MHz, CDCh) δ: ppm 4.67 (br. m, 4H); 3.42-2.81 (br. m, 3H); 2.73-2.23 (br. m, 19H); 1.87-1.00 (br. m, 71H); 0.90 (t, 15H).

BL: Compound 62: Pentyl 4-((3-(4-(3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoyl)piperazin-l-yl)-3-oxopropyl)(nonyl)amino)butanoate

Step 1: *tert*-Butyl 3-(nonylamino)propanoate



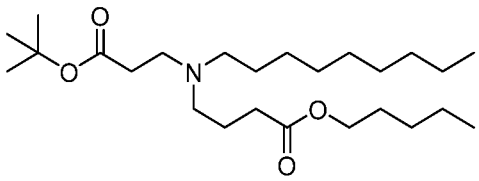
Chemical Formula: C16H33NO2

Molecular Weight: 271.45

[00828] In the same manner as Step 1 for Compound 44, *tert*-butyl 3-(nonylamino)propanoate was synthesized from *tert*-butyl 3-aminopropanoate hydrochloride (2.8 g, 15 mmol), 1-bromononane (3.2 g, 15 mmol), K2CO3 (4.3 g, 31 mmol), and KI (256 mg, 1.54 mmol) in MeCN (200 mL). Yield (1.74 g, 42%).

¾-NMR (300 MHz, CDCh) δ: ppm 2.86 (t, 2H); 2.63 (t, 2H); 2.46 (t, 2H); 1.65 (br, 1H); 1.47 (br. m, 11H); 1.29 (br. m, 12H); 0.90 (t, 3H).

Step 2: Pentyl 4-((3-(teri-butoxy)-3-oxopropyl)(nonyl)amino)butanoate



Chemical Formula: C25H49NO4

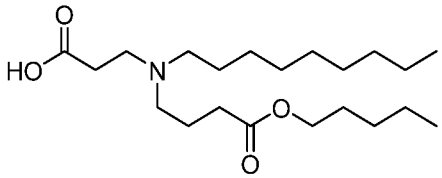
Molecular Weight: 427.67

[00829] In the same manner as Step 3 for Compound 44, pentyl 4-((3-(fer/-butoxy)-3-oxopropyl)(nonyl)amino)butanoate was synthesized from *tert*-butyl 3-(nonylamino)propanoate (750 mg, 2.76 mmol), pentyl 4-bromobutanoate (786 mg, 3.31 mmol), K2CO3 (764 mg, 5.52 mmol), and KI (46 mg, 0.28 mmol) in MeCN (30 mL). Yield (934 mg, 79%).

UPLC/ELSD: RT = 1.82 min. MS (ES): *m/z* (MH^+) 428.62 for C25H49NO4

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 2.74 (t, 2H); 2.50-2.28 (br. m, 8H); 1.76 (m, 2H); 1.64 (m, 2H); 1.50-1.14 (br. m, 27H); 0.91 (m, 6H).

Step 3: 3-(Nonyl(4-oxo-4-(pentyloxy)butyl)amino)propanoic acid



Chemical Formula: C21H41NO4

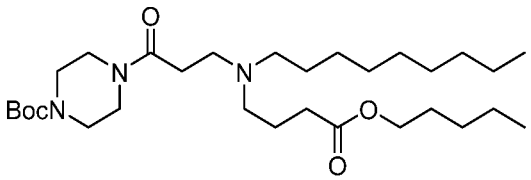
Molecular Weight: 371.56

[00830] In the same manner as Step 4 for Compound 44, 3-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)propanoic acid was synthesized from pentyl 4-((3-(/er/-butoxy)-3-oxopropyl)(nonyl)amino)butanoate (934 mg, 2.18 mmol), and TFA (8.4 mL, 109 mmol) in DCM (10 mL). Yield (793 mg, 98%).

UPLC/ELSD: RT = 1.23 min. MS (ES): *m/z* (MH^+) 372.52 for C21H41NO4

¾-NMR (300 MHz, $CDCl_3$) δ: ppm 4.10 (t, 2H); 2.88 (t, 2H); 2.70 (br. m, 4H); 2.52 (t, 2H); 2.38 (t, 2H); 1.90 (m, 2H); 1.73-1.49 (br. m, 4H); 1.47-1.17 (br. m, 16H); 0.92 (m, 6H).

Step 4: *tert*-Butyl 4-(3-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)propanoyl)piperazine-l-carboxylate



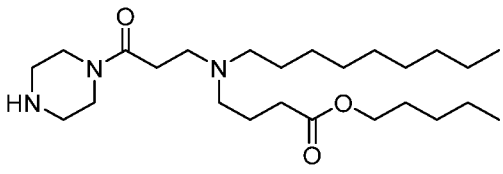
Chemical Formula: C30H57N3O5

Molecular Weight: 539.80

[00831] In the same manner as Step 3 for Compound 11, *tert-b*uty*l* 4-(3-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)propanoyl)piperazine-l-carboxylate was synthesized from 3-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)propanoic acid (793 mg, 2.13 mmol), 1-boc-piperazine (477 mg, 2.56 mmol), iPr_2EtN (0.82 mL, 4.7 mmol), and T3P (50% EtOAc solution, 3.8 mL, 6.4 mmol). Yield (1.15 g, 99%).

UPLC/ELSD: RT = 1.86 min. MS (ES): *m/z* (MH^+) 540.65 for C30H57N3O5

Step 5: Pentyl 4-(nonyl(3-oxo-3-(piperazin-l-yl)propyl)amino)butanoate



Chemical Formula: C25H49N3O3

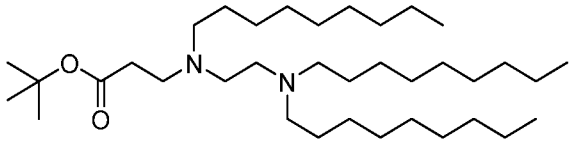
Molecular Weight: 439.69

[00832] In the same manner as Step 4 for Compound 44, pentyl 4-(nonyl(3-oxo-3-(piperazin-l-yl)propyl)amino)butanoate was synthesized from *tert*-butyl 4-(3-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)propanoyl)piperazine-l-carboxylate (1.15 g, 2.13 mmol), and TFA (8.2 mL, 106 mmol) in DCM (10 mL). Yield (901 mg, 96%).

UPLC/ELSD: RT = 0.75 min. MS (ES): *m/z* (MH^+) 440.47 for C25H49N3O3

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.70-3.40 (br. m, 4H); 2.88 (br. m, 6H); 2.57 (br. m, 6H); 2.36 (t, 2H); 1.83 (m, 2H); 1.64 (m, 2H); 1.49 (m, 2H); 1.41-1.18 (br. m, 17H); 0.91 (m, 6H).

Step 6: *tert*-Butyl 3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoate



Chemical Formula: C36H74N2O2

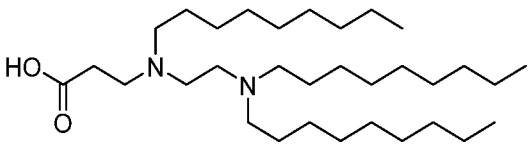
Molecular Weight: 567.00

[00833] In the same manner as Step 4 for Compound 46, *tert*-butyl 3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoate was synthesized from *tert*-butyl 3-(nonylamino)propanoate (1.13 mg, 4.14 mol), *N*-(2-chloroethyl)-*N*-nonylnonan-l-amine (1.65 g, 4.97 mmol), K2CO3 (1.15 g, 8.33 mmol), and KI (138 mg, 0.83 mmol) in MeCN (100 mL). Yield (1.41 g, 60%).

UPLC/ELSD: RT = 2.90 min. MS (ES): *m/z* (MH^+) 567.79 for C36H74N2O2

^{1}H-NMR (300 MHz, CDCh) δ: ppm 2.78 (t, 2H); 2.69-2.29 (br. m, 12H); 1.55-1.15 (br. m, 51H); 0.90 (t, 9H).

Step 7: 3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoic acid



Chemical Formula: C32H66N2O2

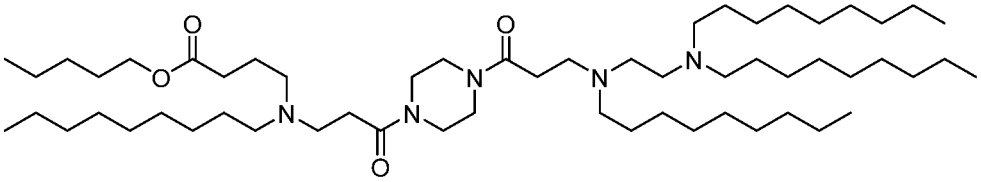
Molecular Weight: 510.89

[00834] In the same manner as Step 4 for Compound 44, 3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoic acid was synthesized from to/-butyl 3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoate (1.41 g, 2.49 mmol), and TFA (9.6 mL, 124 mmol) in DCM (10 mL). Yield (924 mg, 73%).

UPLC/ELSD: RT = 2.26 min. MS (ES): *m/z* (MH^+) 511.78 for C32H66N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 2.76 (br. m, 6H); 2.61 (br. m, 6H); 2.47 (t, 2H); 1.52 (br. m, 6H); 1.40-1.10 (br. m, 36H); 0.90 (t, 9H).

Step 8: Pentyl 4-((3-(4-(3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoyl)piperazin-l-yl)-3-oxopropyl)(nonyl)amino)butanoate



Chemical Formula: C57H113N5O4

Molecular Weight: 932.56

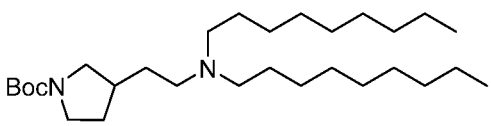
[00835] In the same manner as Step 11 for Compound 11, pentyl 4-((3-(4-(3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoyl)piperazin-l-yl)-3-oxopropyl)(nonyl)amino)butanoate was synthesized from pentyl 4-(nonyl(3-oxo-3-(piperazin-l-yl)propyl)amino)butanoate (268 mg, 0.61 mmol), 3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoic acid (343 mg, 0.67 mmol), iPr_2EtN (234 μL, 1.34 mmol), and T3P (50% EtOAc solution, 1.09 mL, 1.83 mmol) in THF (20 mL). Yield (243 mg, 43%).

UPLC/ELSD: RT = 2.26 min. MS (ES): *m/z* (MH^+) 933.10 for C57H113N5O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.07 (t, 2H); 3.72-3.40 (br. m, 8H); 2.81 (m, 4H); 2.66-2.28 (br. m, 20H); 1.77 (m, 2H); 1.64 (m, 2H); 1.54-1.08 (br. m, 60H); 0.90 (t, 15H).

BM: Compound 69: 2-((2-(Dinonylamino)ethyl)(nonyl)amino)-l-(3-(2-(dinonylamino)ethyl)pyrrolidin-1-yl)ethan-1-one

Step 1: tot-Butyl 3-(2-(dinonylamino)ethyl)pyrrolidine-l-carboxylate



Chemical Formula: C29H58N2O2

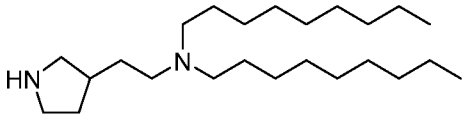
Molecular Weight: 466.80

[00836] In the same manner as Step 1 for Compound 49, *tert-b*utyl 3-(2-(dinonylamino)ethyl)pyrrolidine-l-carboxylate was synthesized from *tert-butyl* 3-(2-aminoethyl)pyrrolidine-l-carboxylate (1.25 g, 5.47 mmol), 1-bromononane (1.13 g, 5.47 mmol), K2CO3 (757 mg, 5.47 mmol), and KI (91 mg, 0.55 mmol) in MeCN (100 mL). Yield (710 mg, 28%).

UPLC/ELSD: RT = 2.23 min. MS (ES): *m/z* (MH^+) 467.74 for C29H58N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.67-3.34 (br. m, 2H); 3.34-2.75 (br. m 2H); 2.52-1.89 (br. m, 8H); 1.70-1.03 (br. m, 40H); 0.90 (t, 6H).

Step 2: *N*-Nonyl-*N*-(2-(pyrrolidin-3-yl)ethyl)nonan-1-amine



Chemical Formula: C24H50N2

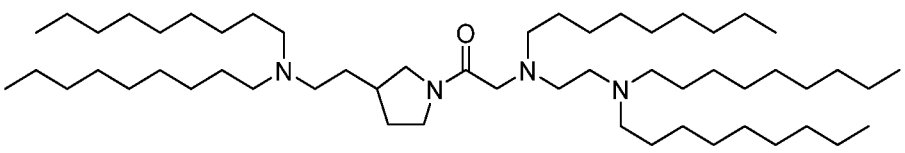
Molecular Weight: 366.68

[00837] In the same manner as Step 4 for Compound 11, *N*-nonyl-*N*-(2-(pyrrolidin-3-yl)ethyl)nonan-l -amine was synthesized from *tert-butyl* 3-(2-(dinonylamino)ethyl)pyrrolidine-1-carboxylate (710 mg, 1.52 mmol), and TFA (5.8 mL, 76 mmol) in DCM (6 mL). Yield (541 mg, 97%).

UPLC/ELSD: RT = 1.23 min. MS (ES): *m/z* (MH^+) 367.70 for C24H50N2

¾-NMR (300 MHz, CDCh) δ: ppm 3.32-1.90 (br. m, 11H); 1.66-1.14 (br. m, 33H); 0.90 (t, 6H).

Step 3: 2-((2-(Dinonylamino)ethyl)(nonyl)amino)-l-(3-(2-(dinonylamino)ethyl)pyrrolidm-1-yl)ethan-l-one



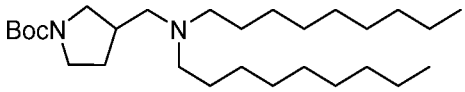
Chemical Formula: C55H112N4O

Molecular Weight: 845.53

[00838] In the same manner as Step 11 for Compound 11, 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l-(3-(2-(dinonylamino)ethyl)pyrrolidin- 1-yl)ethan-1-one was synthesized from *N*-nonyl-*N*-(2-(pyrrolidin-3-yl)ethyl)nonan-l -amine (250 mg, 0.68 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (308 mg, 0.62 mmol), iPr_2EtN (0.24 mL, 1.4 mmol), and T3P (50% EtOAc solution, 1.1 mL, 1.9 mmol) in THF (10 mL). Yield (100 mg, 19%).
UPLC/ELSD: RT = 3.17 min. MS (ES): *m/z* (MH^+) 846.20for C55H112N4O
¾-NMR (300 MHz, CDCh) δ: ppm 3.82-2.90 (br. m, 6H); 2.74-1.94 (br. m, 16H); 1.83-1.00 (br. m, 75H); 0.90 (t, 15H).

BN: Compound 70: 2-((2-(Dinonylamino)ethyl)(nonyl)amino)-l-(3-((dinony lamino)methy l)pyrrolidin- 1-y l)ethan- 1-one

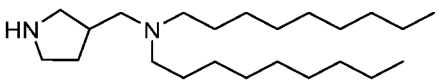
Step 1: tot-Butyl 3-((dinonylamino)methyl)pyrrolidine-l-carboxylate



Chemical Formula: C28H56N2O2
Molecular Weight: 452.77

[00839] In the same manner as Step 1 for Compound 49, tot-butyl 3-((dinonylamino)methyl)pyrrolidine-l-carboxylate was synthesized from tot-butyl 3-(aminomethyl)pyrrolidine-l-carboxylate (2.0 g, 10.0 mmol), 1-bromononane (2.07 g, 10.0 mmol), K2CO3 (1.39 g, 10.0 mmol), and KI (166 mg, 1.00 mmol) in MeCN (100 mL). Yield (1.16 g, 26%).
UPLC/ELSD: RT = 2.17 min. MS (ES): *m/z* (MH^+) 453.72 for C29H58N2O2
^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.72-2.90 (br. m, 4H); 2.36 (br. m, 6H); 2.04-1.04 (br. m, 40H); 0.90 (t, 6H).

Step 2: *N*-Nonyl-*N*-(pyrrolidin-3-ylmethyl)nonan-l -amine



Chemical Formula: C23H48N2
Molecular Weight: 352.65

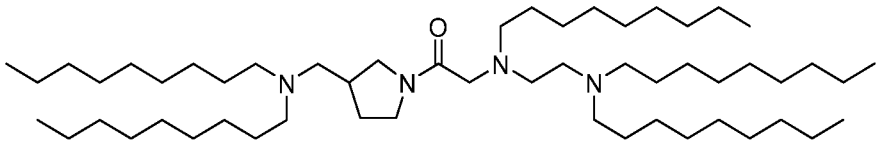
[00840] In the same manner as Step 4 for Compound 11, *N*-nonyl-*N*-(pyrrolidin-3-ylmethyl)nonan-l -amine was synthesized from tot-butyl 3-((dinonylamino)methyl)pyrrolidine-

1-carboxylate (1.16 g, 2.56 mmol), and TFA (9.8 mL, 128 mmol) in DCM (10 mL). Yield (900 mg, 99%).

UPLC/ELSD: RT = 1.17 min. MS (ES): *m/z* (MH^+) 353.66 for C23H48N2

¾-NMR (300 MHz, CDCh) δ: ppm 3.33-2.23 (br. m, 10H); 1.99 (br, 1H); 1.65-1.00 (br. m, 31H); 0.90 (t, 6H).

Step 3: 2-((2-(Dinonylamino)ethyl)(nonyl)amino)-l -(3-((dinonylamino)methyl)pyrrolidin-1-yl)ethan-l-one



Chemical Formula: C54H110N4O

Molecular Weight: 831.50

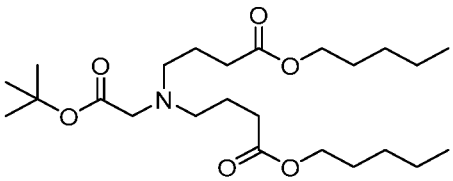
[00841] In the same manner as Step 11 for Compound 11, 2-((2-(dinonylamino)ethyl)(nonyl)amino)- 1-(3-((dinonylamino)methyl)pyrrolidin- 1-yl)ethan- 1-one was synthesized from *N*-nonyl-*N*-(pyrrolidin-3-ylmethyl)nonan-l -amine (200 mg, 0.57 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (256 mg, 0.52 mmol), iPr_2EtN (0.198 mL, 1.14 mmol), and T3P (50% EtOAc solution, 0.92 mL, 1.56 mmol). Yield (114 mg, 27%).

UPLC/ELSD: RT = 3.22 min. MS (ES): *m/z* (MH^+) 832.26 for C54H110N4O

^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.79-2.96 (br. m, 6H); 2.75-2.18 (br. m, 16H); 2.12-1.01 (br. m, 73H); 0.90 (t, 15H).

BO: Compound 72: Dipentyl 4,4'-((2-(4-(*N*-(2-(dinonylarnino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)azanediyl)dibutyrate

Step 1: Dipentyl 4,4'-((2-(/er/-butoxy)-2-oxoethyl)azanediyl)dibutyrate



Chemical Formula: C24H45NO6

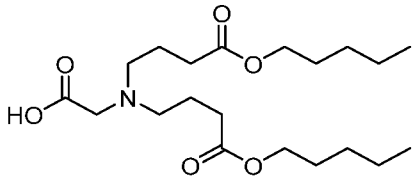
Molecular Weight: 443.63

[00842] To a mixture of *tert-b*utyl glycine (200 mg, 1.52 mmol) and pentyl 4-bromobutanoate (759 mg, 3.2 mmol) in MeCN (30 mL) was added K2CO3 (637 mg, 4.6 mmol) and KI (51 mg, 0.30 mmol) and the mixture was allowed to stir at 82 °C for 12 hours. The suspension was cooled to RT, filtered over a pad of celite rinsing with EtOAc, and concentrated *in vacuo*. Purification by ISCO silica flash chromatography (0-20% EtOAc/hexanes) provided dipentyl 4,4'-((2-(teri-butoxy)-2-oxoethyl)azanediyl)dibutyrate (230 mg, 34%).

UPLC/ELSD: RT = 1.54 min. MS (ES): *m/z* (MH^+) 444.61 for C24H45NO6

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.07 (t, 4H); 3.22 (s, 2H); 2.63 (t, 4H); 2.36 (t, 4H); 1.77 (m, 4H); 1.64 (m, 4H); 1.47 (s, 9H); 1.35 (br. m, 8H); 0.93 (t, 6H).

Step 2: Bis(4-oxo-4-(pentyloxy)bu†yl)glycine



Chemical Formula: C20H37NO6

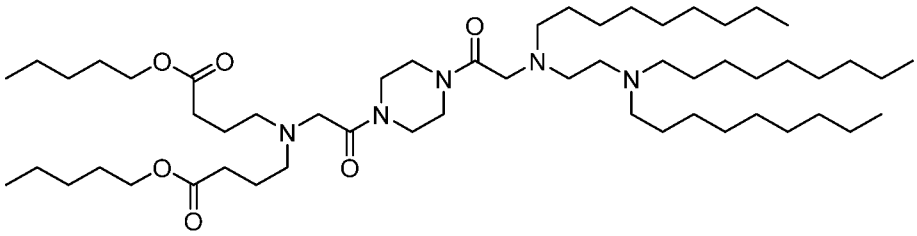
Molecular Weight: 387.52

[00843] In the same manner as Step 4 for Compound 44, bis(4-oxo-4-(pentyloxy)butyl)glycine was synthesized from dipentyl 4,4'-((2-(/er/-butoxy)-2-oxoethyl)azanediyl)dibutyrate (230 mg, 0.52 mmol), and TFA (2 mL, 26 mmol) in DCM (2 mL). Yield (200 mg, 99%).

UPLC/ELSD: RT = 0.80 min. MS (ES): *m/z* (MH^+) 388.5 1 for C20H37NO6

¾-NMR (300 MHz, CDCh) δ: ppm 4.05 (t, 4H); 3.10 (s, 2H); 2.58 (m, 4H); 2.32 (t, 4H); 1.80 (br. m, 4H); 1.63 (br. m, 4H); 1.32 (br. m, 8H); 0.92 (t, 6H).

Step 3: Dipentyl 4,4'-((2-(4 -*(N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l -yl)-2-oxoethyl)azanediyl)dibutyrate



Chemical Formula: C55H107N5O6

Molecular Weight: 934.49

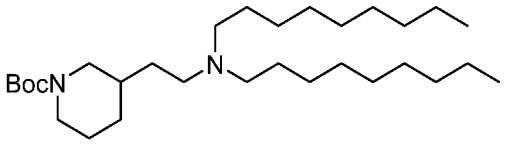
[00844] In the same manner as Step 7 for Compound 44, dipentyl 4,4'-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)azanediyl)dibutyrate was synthesized from bis(4-oxo-4-(pentyloxy)butyl)glycine (200 mg, 0.52 mmol), 2-((2-(dinonylamino)ethyl)(nonyl)amino)-1-(piperazin-1-yl)ethan-1-one (265 mg, 0.47 mmol), iPr_2EtN (180 μL, 1.03 mmol) and T3P (50% EtOAc solution, 838 μL, 1.41 mmol) in THF (10 mL). Yield (250 mg, 57%).

UPLC/ELSD: RT = 2.85 min. MS (ES): *m/z* (MH^+) 935.26 for $C_{55}H_{107}N_5O_6$

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 4H); 3.78-3.46 (br. m, 8H); 3.34 (br. m, 4H); 2.72-2.24 (br. m, 18H); 1.78 (m, 4H); 1.64 (m, 4H); 1.50-1.16 (br. m, 50H); 0.91 (m, 15H).

BP: Compound 73: 2-((2-(Dinonylamino)ethyl)(nonyl)amino)-1-(3-(2-(dinonylamino)ethyl)piperidin-1-yl)ethan-1-one

Step 1: *tert*-Butyl 3-(2-(dinonylamino)ethyl)piperidine-1-carboxylate

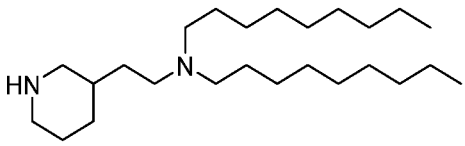


Chemical Formula: $C_{30}H_{60}N_2O_2$

Molecular Weight: 480.82

[00845] In the same manner as Step 1 for Compound 49, *tert-butyl* 3-(2-(dinonylamino)ethyl)piperidine-1-carboxylate was synthesized from *tert-butyl* 3-(2-aminoethyl)piperidine-1-carboxylate (1.00 g, 4.38 mmol), 1-bromononane (907 mg, 4.38 mmol), K_2CO_3 (610 mg, 4.38 mmol), and KI (73 mg, 0.44 mmol) in MeCN (50 mL). Yield (514 mg, 24%). ¾-NMR (300 MHz, CDCh) δ: ppm 4.12-2.24 (br. m, 10H); 1.92-1.00 (br. m, 44H); 0.90 (t, 6H).

N-Nonyl-*N*-(2-(piperidin-3-yl)ethyl)nonan-1-amine



Chemical Formula: $C_{25}H_{52}N_2$

Molecular Weight: 380.71

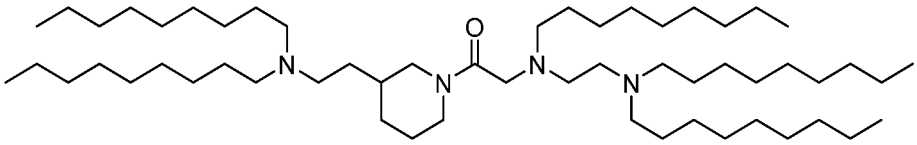
[00846] In the same manner as Step 4 for Compound 11, *N*-nonyl-*N*-(2-(piperidin-3-yl)ethyl)nonan-1-amine was synthesized from *tert-butyl* 3-(2-(dinonylamino)ethyl)piperidine-1-

carboxylate (514 mg, 1.07 mmol), and TFA (4.1 mL, 53mmol) in DCM (4 mL). Yield (378 mg, 93%).

UPLC/ELSD: RT = 1.27 min. MS (ES): *m/z* (MH^+) 381.62 for C25H52N2

¾-NMR (300 MHz, CDCh) δ: ppm 3.12-1.95 (br. m, 11H); 1.93-0.98 (br. m, 35H); 0.90 (t, 6H).

2-((2-(Dinonylamino)ethyl)(nonyl)amino)-1-(3-(2-(dinonylamino)ethyl)piperidin-1-yl)ethan-1-one



Chemical Formula: C56H114N4O

Molecular Weight: 859.56

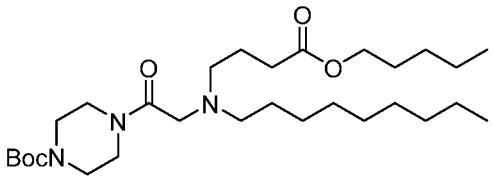
[00847] In the same manner as Step 11 for Compound 11, 2-((2-(dinonylamino)ethyl)(nonyl)amino)-1-(3-(2-(dinonylamino)ethyl)piperidin-1-yl)ethan-1-one was synthesized from *N*-nonyl-*N*-(2-(piperidin-3-yl)ethyl)nonan-l-amine (250 mg, 0.66 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (297 mg, 0.60 mmol), iPr_2EtN (0.23 mL, 1.3 mmol), and T3P (50% EtOAc solution, 1.06 mL, 1.8 mmol) in THF (10 mL). Yield (136 mg, 27%).

UPLC/ELSD: RT = 3.22 min. MS (ES): *m/z* (MH^+) 860.39 for **C56H114N4O**

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.56-4.01 (br. m, 2H); 3.48-2.20 (br. m, 20H), 1.99-1.00 (br. m, 77H); 0.90 (t. 15H).

BQ: Compound 71: Pentyl 4-(nonyl(2-(4-(*N*-nonyl-*N*-(2-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)ethyl)glycyl)piperazin-l-yl)-2-oxoethyl)amino)butanoate

Step 1: tot-Butyl 4-(*N*-nonyl-*N*-(4-oxo-4-(pentyloxy)butyl)glycyl)piperazine-l-carboxylate



Chemical Formula: C29H55N3O5

Molecular Weight: 525.78

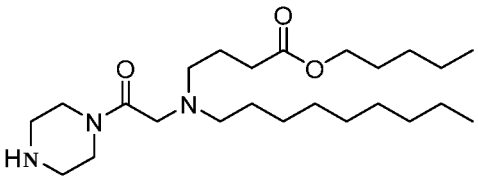
[00848] In the same manner as Step 3 for Compound 11, tot-butyl 4-(*N*-nonyl-*N*-(4-oxo-4-(pentyloxy)butyl)glycyl)piperazine-l-carboxylate was synthesized from *N*-nonyl-*N*-(4-oxo-4-(pentyloxy)butyl)glycine (480 mg, 1.34 mmol), 1-boc-piperazine (275 mg, 1.48 mmol), iPnEtN

(5.14 μL, 2.95 mmol) and T3P (50% EtOAc solution, 2.40 mL, 4.03 mmol) in THF (15 mL). Yield (700 mg, 99%).

UPLC/ELSD: RT = 1.90 min. MS (ES): *m/z* (MH^+) 526.79 for C29H55N3O5

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.70-3.10 (br. m, 14H); 2.45 (t, 2H); 2.13 (br. m, 2H); 2.00-1.00 (br. m, 29H); 0.91 (br. m, 6H).

Step 2: Pentyl 4-(nonyl(2-oxo-2-(piperazin-l-yl)ethyl)amino)butanoate



Chemical Formula: C24H47N3O3

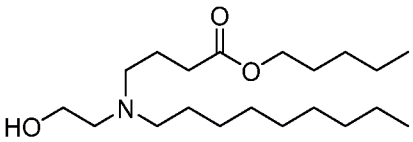
Molecular Weight: 425.66

[00849] In the same manner as Step 4 for Compound 11, pentyl 4-(nonyl(2-oxo-2-(piperazin-l-yl)ethyl)amino)butanoate was synthesized from *tert-b*utyl 4-*(N*-nonyl -*N*-(4-oxo-4-(pentyloxy)butyl)glycyl)piperazine-l -carboxylate (700 mg, 1.33 mmol), and TFA (5.1 mL, 66.6 mmol) in DCM (5 mL). Yield (560 mg, 99%).

UPLC/ELSD: RT = 0.77 min. MS (ES): *m/z* (MH^+) 426.65 for C24H47N3O3

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.07 (t, 2H); 3.59 (br. m, 4H); 3.28 (s, 2H); 2.86 (br. m, 4H); 2.50 (br. m, 4H); 2.33 (t, 2H); 2.05 (br, 1H); 1.77 (m, 2H); 1.63 (m, 2H); 1.30 (br. m, 18H); 0.91 (m, 6H).

Step 3: Pentyl 4-((2-hydroxyethyl)(nonyl)amino)butanoate



Chemical Formula: C20H41NO3

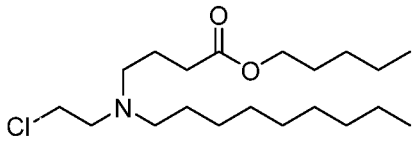
Molecular Weight: 343.55

[00850] In the same manner as Step 1 for Compound 18, pentyl 4-((2-hydroxy ethyl)(nonyl)amino)butanoate was synthesized from 2-(nonylamino)ethan-l -ol (350 mg, 1.87 mmol), pentyl 4-bromobutanoate (487 mg, 2.06 mmol), K2CO3 (572 mg, 4.11 mmol), and KI (31 mg, 0.19 mmol) in MeCN (40 mL). Yield (427 mg, 66%).

UPLC/ELSD: RT = 1.25 min. MS (ES): *m/z* (MH^+) 344.55 for C20H41NO3

¾-NMR (300 MHz, CDCh) δ: ppm 4.09 (t, 2H); 3.61 (t, 2H); 2.67 (t, 2H); 2.56 (m, 4H); 2.36 (t, 2H); 1.85 (m, 2H); 1.65 (m, 2H); 1.49 (m, 2H); 1.42-1.18 (br. m, 16H); 0.91 (m, 6H).

Step 4: Pentyl 4-((2-chloroethyl)(nonyl)amino)butanoate



Chemical Formula: C20H40CINO2

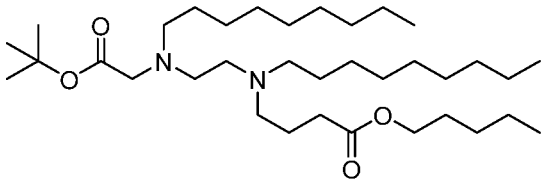
Molecular Weight: 362.00

[00851] In the same manner as Step 2 for Compound 18, pentyl 4-((2-chloroethyl)(nonyl)amino)butanoate was synthesized from pentyl 4-((2-hydroxyethyl)(nonyl)amino)butanoate (427 mg, 1.27 mmol), methanesulfonyl chloride (120 μL, 1.55 mmol), and triethylamine (225 μL, 1.62 mmol) in DCM (8 mL). Yield (448 mg, 99%).

UPLC/ELSD: RT = 1.52 min. MS (ES): *m/z* (MH^+) 362.51 for C20H40CINO2

¾-NMR (300 MHz, CDCh) δ: ppm 4.07-3.71 (br. m, 4H); 3.45-2.76 (br. m, 6H); 2.30 (br. m, 2H); 2.24-1.05 (br. m, 22H); 0.82 (br. m, 6H).

Step 5: Pentyl 4-((2-((2-(fe*r*^butoxy)-2-oxoethyl)(nonyl)amino)ethyl)(nonyl)amino)butanoate



Chemical Formula: C35H70N2O4

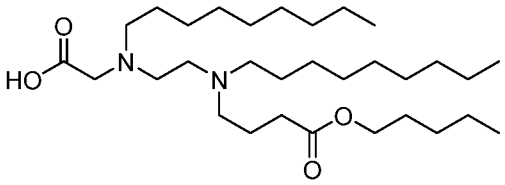
Molecular Weight: 582.96

[00852] In the same manner as Step 4 for Compound 46, pentyl 4-((2-((2-(/er/-butoxy)-2-oxoethyl)(nonyl)amino)ethyl)(nonyl)amino)butanoate was synthesized from *tert-b*utyl nonylglycinate (338 mg, 1.31 mmol), pentyl 4-((2-chloroethyl)(nonyl)amino)butanoate (527 mg, 1.46 mmol), K2CO3 (402 mg, 2.89 mmol), and KI (22 mg, 0.13 mmol) in MeCN (30 mL). Yield (200 mg, 26%).

UPLC/ELSD: RT = 3.03 min. MS (ES): *m/z* (MH^+) 583.95 for C35H70N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.07 (t, 2H); 3.27 (s, 2H); 2.76-2.24 (br. m, 12H); 1.85-1.10 (br. m, 45H); 0.90 (m, 9H).

Step 6: *N*-Nonyl-*N*-(2-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)ethyl)glycine



Chemical Formula: **C31H62N2O4**

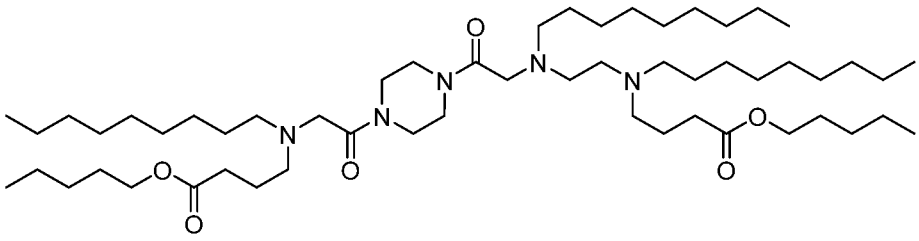
Molecular Weight: 526.85

[00853] In the same manner as Step 5 for Compound 46, *N*-nonyl-*N*-(2-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)ethyl)glycine was synthesized from pentyl 4-((2-((2-(*tert*-butoxy)-2-oxoethyl)(nonyl)amino)ethyl)(nonyl)amino)butanoate (200 mg, 0.34 mmol), and TFA (1.31 mL, 17.2 mmol), in DCM (2 mL). Yield (160 mg, 89%).

UPLC/ELSD: RT = 2.39 min. MS (ES): *m/z* (MH^+) 527.77 for C31H62N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.09 (t, 2H); 3.27 (s, 2H); 2.94-2.74 (br. m, 6H); 2.61 (t, 2H); 2.37 (m, 2H); 2.15-1.90 (br. m, 2H); 1.80-1.05 (br. m, 36H); 0.90 (m, 9H).

Step 7: Pentyl 4-(nonyl(2-(4-(*N*-nonyl-*N*-(2-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)ethyl)glycyl)piperazin-l-yl)-2-oxoethyl)amino)butanoate



Chemical Formula: **C55H107N5O6**

Molecular Weight: 934.49

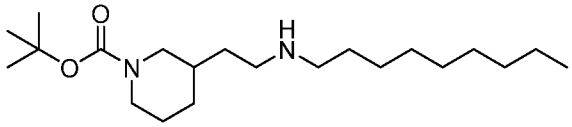
[00854] In the same manner as Step 11 for Compound 11, pentyl 4-(nonyl(2-(4-(*N*-nonyl-*N*-(2-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)ethyl)glycyl)piperazin-l-yl)-2-oxoethyl)amino)butanoate was synthesized from pentyl 4-(nonyl(2-oxo-2-(piperazin-l-yl)ethyl)amino)butanoate (142 mg, 0.33 mmol), *N*-nonyl-*N*-(2-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)ethyl)glycine (160 mg, 0.30 mmol), iPnEtN (116 μL, 0.67 mmol), and T3P (50% EtOAc solution, 542 μL, 0.91 mmol). Yield (53 mg, 19%).

UPLC/ELSD: RT = 2.79 min. MS (ES): *m/z* (MH^+) 935.34 for **C55H107N5O6**

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 4H); 3.78-3.53 (br. m, 8H); 3.32 (br. m, 4H); 2.76-2.24 (br. m, 18H); 1.87-1.10 (br. m, 58H); 0.91 (br. m, 15H).

BR: Compound 80: Pentyl 4-((2-(l -(*N* -(2-(dinonylamino)ethyl)-*N* -nonylglycyl)piperidin-3-yl)ethyl)(nonyl)amino)butanoate

Step 1: *tert*-butyl 3-(2-(nonylamino)ethyl)piperidine-l-carboxylate



Chemical Formula: C21H42N2O2

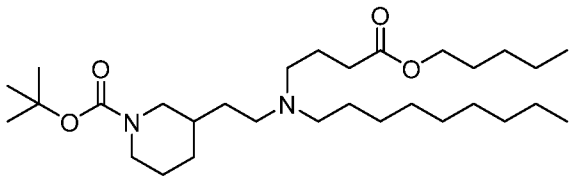
Molecular Weight: 354.58

[00855] In the same manner as Step 1 for Compound 49, *tert*-butyl 3-(2-(nonylamino)ethyl)piperi dine- 1-carboxy late was synthesized from *tert*-butyl 3-(2-aminoethyl)piperi dine- 1-carboxy late (1.00 g, 4.38 mmol), 1-bromononane (907 mg, 4.38 mmol), K2CO3 (610 mg, 4.38 mmol), and KI (73 mg, 0.44 mmol) in MeCN (50 mL). Yield (474 mg, 31%).

UPLC/ELSD: RT = 1.23 min. MS (ES): *m/z* (MH^+) 355.58 for C21H42N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.88 (br, 2H); 3.00-2.43 (br. m, 6H); 1.92-0.97 (br. m, 30H), 0.90 (t, 3H).

Step 2: *tert*-Butyl 3-(2-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)ethyl)piperi dine- 1-carboxy late



Chemical Formula: C30H58N2O4

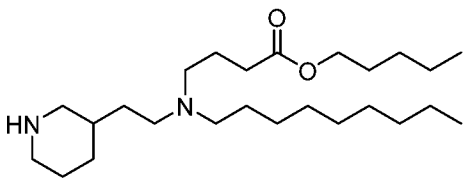
Molecular Weight: 510.80

[00856] In the same manner as Step 1 for Compound 57, *tert*-butyl 3-(2-(nonyl(4-oxo-4-(penty loxy)butyl)amino)ethyl)piperi dine- 1-carboxy late was synthesized from tert-butyl 3-(2-(nonylamino)ethyl)piperi dine- 1-carboxy late (474 mg, 1.34 mmol), pentyl 4-bromobutanoate (380 mg, 1.6 mmol), K2CO3 (223 mg, 1.60 mmol) and KI (44 mg, 0.27 mmol) in MeCN (15 mL). Yield (492 mg, 72%).

UPLC/ELSD: RT = 2.09 min. MS (ES): *m/z* (MH^+) 511.70 for $C_{30}H_{58}N_2O_4$

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.90 (br. m, 2H); 2.87-2.22 (br. m, 10H); 1.91-1.00 (br. m, 38H); 0.91 (m, 6H).

Step 3: Pentyl 4-(nonyl(2-(piperidin-3-yl)ethyl)amino)butanoate



Chemical Formula: $C_{25}H_{50}N_2O_2$

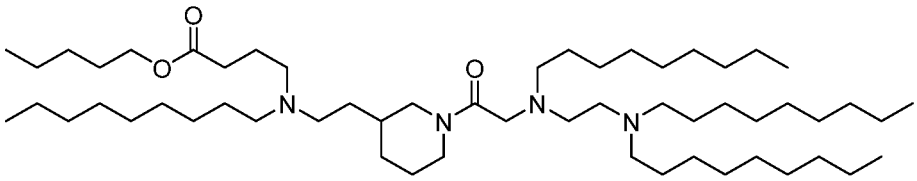
Molecular Weight: 410.69

[00857] In the same manner as Step 4 for Compound 11, pentyl 4-(nonyl(2-(piperidin-3-yl)ethyl)amino)butanoate was synthesized from *ter*/-butyl 3-(2-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)ethyl)piperi dine- 1-carboxy late (492 mg, 0.96 mmol), and TFA (3.7 mL, 48 mmol) in DCM (4 mL). Yield (390 mg, 99%).

UPLC/ELSD: RT = 0.85 min. MS (ES): *m/z* (MH^+) 411.72 for $C_{25}H_{50}N_2O_2$

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.03 (br. m, 2H); 2.66-2. 18 (br. m, 10H); 2.18-0.98 (br. m, 30H); 0.91 (m, 6H).

Step 4: Pentyl 4-((2-(l *-(N* -(2-(dinonylamino)ethyl *)-N* -nonylglycyl)piperidin-3-yl)ethyl)(nonyl)amino)butanoate



Chemical Formula: $C_{56}H_{112}N_4O_3$

Molecular Weight: 889.54

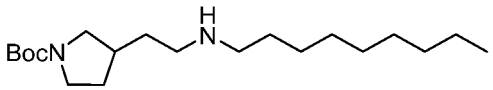
[00858] In the same manner as Step 11 for Compound 11, pentyl 4-((2-(l *-(N* -(2-(dinonylamino)ethyl *)-N* -nonylglycyl)piperidin-3-yl)ethyl)(nonyl)amino)butanoate was synthesized from pentyl 4-(nonyl(2-(piperidin-3-yl)ethyl)amino)butanoate (250 mg, 0.61 mmol), *N* -(2-(dinonylamino)ethyl *)-N* -nonylglycine (275 mg, 0.55 mmol), iPr_2EtN (0.21 mL, 1.2 mmol), and T3P (50% EtOAc solution, 0.98 mL, 1.7 mmol). Yield (96 mg, 20%).

UPLC/ELSD: RT = 3.08 min. MS (ES): *m/z* (MH^+) 890.32 for $C_{56}H_{112}N_4O_3$

^{1}H-NMR ^OO MHz, CDCh) δ: ppm 4.55-4.01 (br. m, 4H); 3.48-2.21 (br. m, 22H); 1.95-1 .00 (br. m, 71H); 0.90 (m, 15H).

BS: Compound 81: Pentyl 4-((2-(l -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)pyrrolidin-3-yl)ethyl)(nonyl)amino)butanoate

Step 1: *tert-B*utyl 3-(2-(nonylamino)ethyl)pyrrolidine-l-carboxylate



Chemical Formula: C20H40N2O2

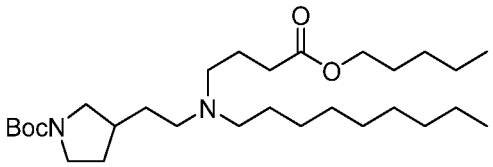
Molecular Weight: 340.55

[00859] In the same manner as Step 1 for Compound 49, *tert-butyl* 3-(2-(nonylamino)ethyl)pyrrolidine-l-carboxylate was synthesized from *tert-butyl* 3-(2-aminoethyl)pyrrolidine-l -carboxylate (1.25 g, 5.47 mmol), 1-bromononane (1.13 g, 5.47 mmol), K2CO3 (757 mg, 5.47 mmol), and KI (91 mg, 0.55 mmol) in MeCN (100 mL). Yield (420 mg, 23%).

UPLC/ELSD: RT = 1.08 min. MS (ES): *m/z* (MH^+) 341 .52 for C20H40N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.68-1 .91 (br. m, 9H); 1.71 - 1.12 (br. m, 28H); 0.90 (t, 3H).

Step 2: *tert-Butyl* 3-(2-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)ethyl)pyrrolidine-l-carboxylate



Chemical Formula: C29H56N2O4

Molecular Weight: 496.78

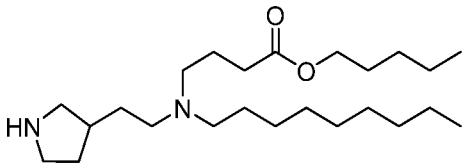
[00860] In the same manner as Step 1 for Compound 57, *tert-butyl* 3-(2-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)ethyl)pyrrolidine-l-carboxylate was synthesized from *tert-butyl* 3-(2-(nonylamino)ethyl)pyrrolidine-l-carboxylate (420 mg, 1.23 mmol), pentyl 4-bromobutanoate (321 mg, 1.36 mmol), K2CO3 (187 mg, 1.36 mmol), and KI (41 mg, 0.25 mmol). Yield (390 mg, 64%).

UPLC/ELSD: RT = 1.98 min. MS (ES): *m/z* (MH^+) 497.67 for C29H56N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.50 (br. m, 2H); 3.34-2.76 (br. m, 2H); 2.52-

1.87 (br. m, 10H); 1.87-1.02 (br. m, 34H); 0.91 (t, 6H).

Step 3: Pentyl 4-(nonyl(2-(pyrrolidin-3-yl)ethyl)amino)butanoate



Chemical Formula: C24H48N2O2

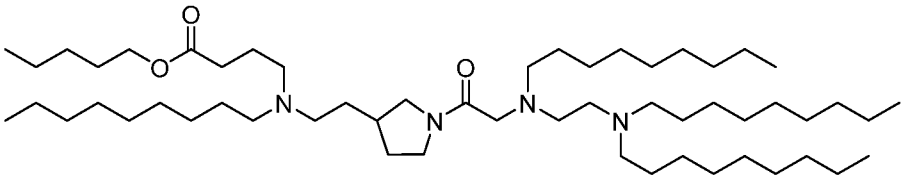
Molecular Weight: 396.66

[00861] In the same manner as Step 4 for Compound 11, pentyl 4-(nonyl(2-(pyrrolidin-3-yl)ethyl)amino)butanoate was synthesized from *ter*-butyl 3-(2-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)ethyl)pyrrolidine-1-carboxylate (390 mg, 0.79 mmol), and TFA (3.0 mL, 40 mmol) in DCM (3 mL). Yield (298 mg, 96%).

UPLC/ELSD: RT = 0.81 min. MS (ES): *m/z* (MH^+) 397.62 for C24H48N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.20-2.82 (br. m, 4H); 2.58-2.24 (br. m, 8H); 2.11-1.11 (br. m, 28H); 0.91 (m, 6H).

Step 4: Pentyl 4-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)pyrrolidin-3-yl)ethyl)(nonyl)amino)butanoate



Chemical Formula: C55H110N4O3

Molecular Weight: 875.51

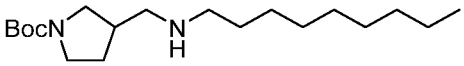
[00862] In the same manner as Step 11 for Compound 11, pentyl 4-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)pyrrolidin-3-yl)ethyl)(nonyl)amino)butanoate was synthesized from pentyl 4-(nonyl(2-(pyrrolidin-3-yl)ethyl)amino)butanoate (202 mg, 0.51 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (230 mg, 0.46 mmol), iPr_2EtN (0.177 mL, 1.0 mmol), and T3P (50% EtOAc solution, 0.82 mL, 1.4 mmol) in THF (10 mL). Yield (109 mg, 27%).

UPLC/ELSD: RT = 3.06 min. MS (ES): *m/z* (MH^+) 876.30 for C55H110N4O3

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.83-2.85 (br. m, 7H); 2.78-1.88 (br. m, 19H); 1.83-1.14 (br. m, 67H); 0.90 (m, 15H).

BT: Compound 82: Pentyl 4-(((l -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)pyrrolidin-3-yl)methyl)(nonyl)amino)butanoate

Step 1: *tert*-Butyl 3-((nonylamino)methyl)pyrrolidine- 1-carboxylate



Chemical Formula: C19H38N2O2

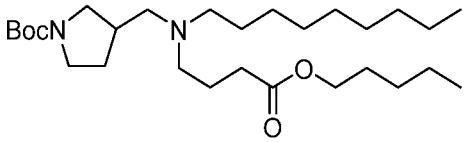
Molecular Weight: 326.53

In the same manner as Step 1 for Compound 49, *tert-b*utyl 3-((nonylamino)methyl)pyrrolidine-1-carboxylate was synthesized from *tert-butyl* 3-(aminomethyl)pyrrolidine- 1-carboxylate (2.0 g, 10.0 mmol), 1-bromononane (2.07 g, 10.0 mmol), K2CO3 (1.39 g, 10.0 mmol), and KI (166 mg, 1.00 mmol) in MeCN (100 mL). Yield (1.53 g, 47%).

UPLC/ELSD: RT = 0.92 min. MS (ES): *m/z* (MH^+) 327.54 for C19H38N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.69-1.79 (br. m, 9H); 1.74-1.13 (br. m, 26H); 0.89 (t, 3H).

Step 2: *tert-Butyl* 3-((nonyl(4-oxo-4-(pentyloxy)butyl)amino)methyl)pyrrolidine-1-carboxylate



Chemical Formula: C28H54N2O4

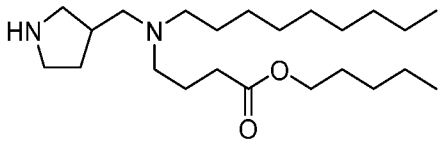
Molecular Weight: 482.75

[00863] In the same manner as Step 1 for Compound 57, /er/-butyl 3-((nonyl(4-oxo-4-(pentyloxy)butyl)amino)methyl)pyrrolidine- 1-carboxylate was synthesized from *tert-butyl* 3-((nonylamino)methyl)pyrrolidine- 1-carboxylate (500 mg, 1.53 mmol), pentyl 4-bromobutanoate (400 mg, 1.68 mmol), K2CO3 (423 mg, 3.07 mmol), and KI (51 mg, 0.31 mmol) in MeCN (100 mL). Yield (233 mg, 32%).

UPLC/ELSD: RT = 1.85 min. MS (ES): *m/z* (MH^+) 483.65 for C28H54N2O4

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.59-2.91 (br. m, 4H); 2.49-1.83 (br. m, 10H); 1.83-1.13 (br. m, 32H); 0.91 (m, 6H).

Step 3: Pentyl 4-(nonyl(pyrrolidin-3-ylmethyl)amino)butanoate



Chemical Formula: C23H46N2O2

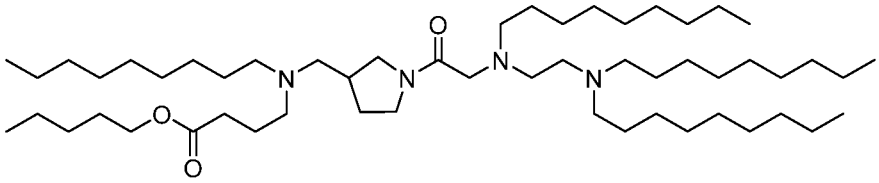
Molecular Weight: 382.63

[00864] In the same manner as Step 4 for Compound 11, pentyl 4-(nonyl(pyrrolidin-3-ylmethyl)amino)butanoate was synthesized from *tert*-butyl 3-((nonyl(4-oxo-4-(pentyloxy)butyl)amino)methyl)pyrrolidine-l -carboxylate (233 mg, 0.48 mmol), and TFA (1.84 mL, 24 mmol) in DCM (2 mL). Yield (179 mg, 97%).

UPLC/ELSD: RT = 0.70 min. MS (ES): *m/z* (MH^+) 383.5 1 for C23H46N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.11-2.81 (br. m, 3H); 2.67-1 .51 (br. m, 16H); 1.51-1.03 (br. m, 19H); 0.91 (m, 6H).

Step 4: Pentyl 4-(((l *-(N* -(2-(dinonylamino)ethyl *)-N* -nonylglycyl)pyrrolidin-3-yl)methyl)(nonyl)amino)butanoate



Chemical Formula: C54H108N4O3

Molecular Weight: 861.48

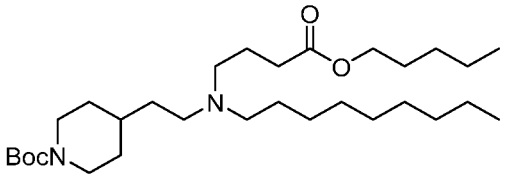
[00865] In the same manner as Step 11 for Compound 11, pentyl 4-(((l *-(N* -(2-(dinonylamino)ethyl *)-N* -nonylglycyl)pyrrolidin-3-yl)methyl)(nonyl)amino)butanoate was synthesized from pentyl 4-(nonyl(pyrrolidin-3-ylmethyl)amino)butanoate (179 mg, 0.47 mmol), *N* -(2-(dinonylamino)ethyl *)-N* -nonylglycine (211 mg, 0.43 mmol), iPr_2EtN (163 µL, 0.95 mmol), and T3P (50% EtOAc solution, 0.76 mL, 1.1 mmol). Yield (88 mg, 24%).

UPLC/ELSD: RT = 3.05 min. MS (ES): *m/z* (MH^+) 862.28 for C54H108N4O3

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.76-2. 17 (br. m, 24H); 2.12-1.05 (br. m, 67H); 0.90 (m, 15H).

BU: Compound 83: Pentyl 4-((2-(l *-(N* -(2-(dinonylamino)ethyl *)-N* -nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)butanoate

Step 1: *tert*-Butyl 4-(2-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)ethyl)piperidine-1-carboxylate



Chemical Formula: C30H58N2O4

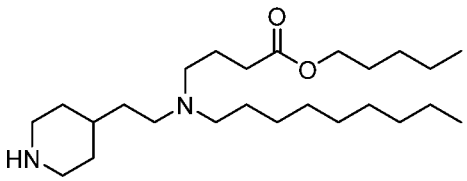
Molecular Weight: 510.80

[00866] In the same manner as Step 1 for Compound 57, *tert-butyl* 4-(2-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)ethyl)piperidine-1-carboxylate was synthesized from tert-butyl 4-(2-(nonylamino)ethyl)piperidine-1-carboxylate (500 mg, 1.41 mmol), pentyl 4-bromobutanoate (368 mg, 1.55 mmol), K_2CO_3 (390 mg, 2.82 mmol), and KI (23 mg, 0.14 mmol) in MeCN (100 mL). Yield (487 mg, 68%).

UPLC/ELSD: RT = 2.03 min. MS (ES): *m/z* (MH^+) 511.57 for $C_{30}H_{58}N_2O_4$

¾-NMR (300 MHz, $CDCl_3$) δ: ppm 4.08 (m, 4H); 2.69 (m, 2H); 2.51-2.25 (br. m, 8H); 1.83-1.55 (br. m, 6H); 1.53-1.02 (br. m, 32H); 0.91 (m, 6H).

Step 2: Pentyl 4-(nonyl(2-(piperidin-4-yl)ethyl)amino)butanoate



Chemical Formula: C25H50N2O2

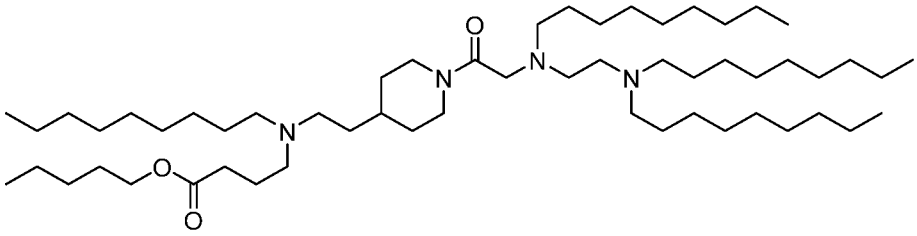
Molecular Weight: 410.69

[00867] In the same manner as Step 4 for Compound 11, pentyl 4-(nonyl(2-(piperidin-4-yl)ethyl)amino)butanoate was synthesized from *tert-butyl* 4-(2-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)ethyl)piperidine-1-carboxylate (487 mg, 0.953 mmol), and TFA (3.6 mL, 48 mmol) in DCM (4 mL). Yield (386 mg, 98%).

UPLC/ELSD: RT = 0.87 min. MS (ES): *m/z* (MH^+) 411.43 for $C_{25}H_{50}N_2O_2$

^{1}H-NMR (300 MHz, $CDCl_3$) δ: ppm 4.08 (t, 2H); 3.07 (m, 2H); 2.60 (m, 2H); 2.50-2.28 (br. m, 8H); 2.03 (br, 1H); 1.86-1.55 (br. m, 6H); 1.52-1.02 (br. m, 23H); 0.91 (m, 6H).

Step 3: Pentyl 4-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)butanoate



Chemical Formula: C56H112N4O3

Molecular Weight: 889.54

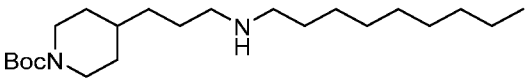
[00868] In the same manner as Step 11 for Compound 11, pentyl 4-((2-(l -(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)butanoate was synthesized from pentyl 4-(nonyl(2-(piperidin-4-yl)ethyl)amino)butanoate (35 1 mg, 0.855 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (467 mg, 0.941 mmol), iPr_2EtN (328 μL, 1.88 mmol), and T3P (50% EtOAc solution, 1.53 mL, 2.56 mmol) in THF (15 mL). Yield (192 mg, 25%).

UPLC/ELSD: RT = 3.00 min. MS (ES): *m/z* (MH^+) 890. 13 for C56H112N4O3

¾-NMR (300 MHz, CDCh) δ: ppm 4.61-4. 14 (br. m, 2H); 4.08 (t, 2H); 3.40-2.24 (br. m, 22H); 1.86-0.99 (br. m, 71H); 0.90 (m, 15H).

BV: Compound 84: Pentyl 4-((3-(l-(3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoyl)piperidin-4-yl)propyl)(nonyl)amino)buta^ oate

Step 1: *tert*-Butyl 4-(3-(nonylamino)propyl)piperi dine- 1-carboxy late



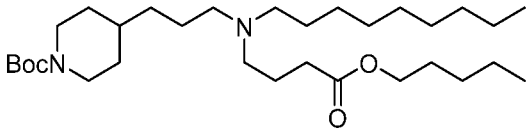
Chemical Formula: C22H44N2O2

Molecular Weight: 368.61

[00869] In the same manner as Step 1 for Compound 49, *tert-b*utyl 4-(3-(nonylamino)propyl)piperi dine- 1-carboxy late was synthesized from *tert-butyl* 4-(3-aminopropyl)piperi dine- 1-carboxy late (2.50 g, 10.3 mmol), 1-bromononane (2. 14 g, 10.3 mmol), K2CO3 (2. 85 g, 20.6 mmol), and KI (17 1 mg, 0.10 mmol) in MeCN (200 mL). Yield (1.27 g, 33%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (m, 2H), 2.69 (br. m, 6H); 1.79-0.98 (br. m, 32H); 0.89 (t, 3H).

Step 2: *tert*-Butyl 4-(3-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)propyl)piperidine-1-carboxylate



Chemical Formula: C31H60N2O4

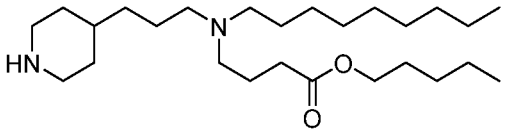
Molecular Weight: 524.83

[00870] In the same manner as Step 1 for Compound 57, *tert-butyl* 4-(3-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)propyl)piperidine-l-carboxylate was synthesized from *tert-butyl* 4-(3-(nonylamino)propyl)piperidine-1-carboxylate (500 mg, 1.36 mmol), pentyl 4-bromobutanoate (354 mg, 1.49 mmol), K2CO3 (375 mg, 2.71 mmol), and KI (23 mg, 0.14 mmol) in MeCN (20 mL). Yield (624 mg, 88%).

UPLC/ELSD: RT = 2.12 min. MS (ES): *m/z* (MH^+) 525.60 for C31H60N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (br. m, 4H); 2.69 (m, 2H); 2.38 (br. m, 8H); 1.85-1.55 (br. m, 6H); 1.54-1.00 (br. m, 34H); 0.91 (m, 6H).

Step 3: Pentyl 4-(nonyl(3-(piperidin-4-yl)propyl)amino)butanoate



Chemical Formula: C26H52N2O2

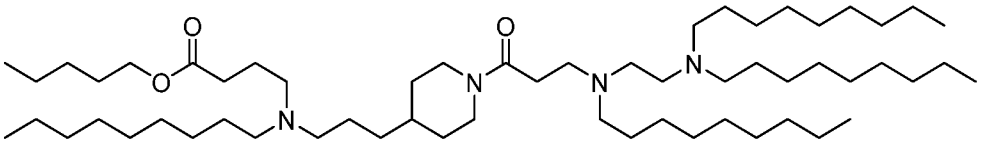
Molecular Weight: 424.71

[00871] In the same manner as Step 4 for Compound 44, pentyl 4-(nonyl(3-(piperidin-4-yl)propyl)amino)butanoate was synthesized from *tert-butyl* 4-(3-(nonyl(4-oxo-4-(pentyloxy)butyl)amino)propyl)piperidine-l-carboxylate (624 mg, 1.19 mmol), and TFA (4.5 mL, 60 mmol) in DCM (5 mL). Yield (467 mg, 92%).

UPLC/ELSD: RT = 0.94 min. MS (ES): *m/z* (MH^+) 424.62 for C26H52N2O2

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.16 (m, 2H); 2.65 (m, 2H); 2.39 (br. m, 8H); 1.84-1.57 (br. m, 6H); 1.52-1.04 (br. m, 26H); 0.91 (m, 6H).

Step 4: Pentyl 4-((3-(l-(3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoyl)piperidin-4-yl)propyl)(nonyl)amino)butanoate



Chemical Formula: C58H116N4O3

Molecular Weight: 917.59

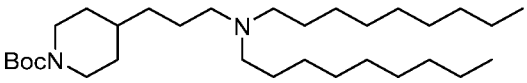
[00872] In the same manner as Step 11 for Compound 11, pentyl 4-((3-(l-(3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoyl)piperidin-4-yl)propyl)(nonyl)amino)buta^ oate was synthesized from pentyl 4-(nonyl(3-(piperidin-4-yl)propyl)amino)butanoate (259 mg, 0.61 mmol), 3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoic acid (343 mg, 0.67 mmol), iPnEtN (234 μL, 1.34 mmol), and T3P (50% EtOAc solution, 1.09 mL, 1.83 mmol) in THF (20 mL). Yield (270 mg, 48%).

UPLC/ELSD: RT = 2.85 min. MS (ES): *m/z* (MH^+) 918.18 for C58H116N4O3

¾-NMR (300 MHz, CDCh) δ: ppm 4.61 (m, 1H); 4.08 (t, 2H); 4.08 (m, 1H); 3.08-2.72 (br. m, 4H); 2.63-2.26 (br. m, 20H); 1.87-1.57 (br. m, 6H); 1.54-1.00 (br. m, 67H); 0.90 (m, 15H).

BW: Compound 85: 3-((2-(Dinonylamino)ethyl)(nonyl)amino)-l-(4-(3-(dinony lamino)propy l)piperidin- 1-yl)propan- 1-one

Step 1: *tert*-Butyl 4-(3-(dinonylamino)propyl)piperidine-1-carboxylate



Chemical Formula: C31H62N2O2

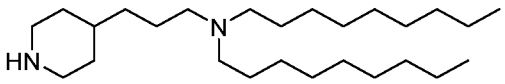
Molecular Weight: 494.85

[00873] In the same manner as Step 1 for Compound 49 *tert-butyl* 4-(3-(dinonylamino)propyl)piperi dine- 1-carboxy late was synthesized from *tert-butyl* 4-(3-aminopropyl)piperi dine- 1-carboxy late (2.50 g, 10.3 mmol), 1-bromononane (2.14 g, 10.3 mmol), K2CO3 (2.85 g, 20.6 mmol), and KI (171 mg, 0.10 mmol) in MeCN (200 mL). Yield (1.03 g, 20%).

UPLC/ELSD: RT = 2.46 min. MS (ES): *m/z* (MH^+) 495.66 for C31H62N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.09 (br. m, 2H); 2.69 (br. m, 2H); 2.39 (br. m, 6H); 1.75-1.00 (br. m, 46H); 0.90 (t, 6H).

Step 2: *N*-Nonyl-*N*-(3-(piperidin-4-yl)propyl)nonan-l -amine



Chemical Formula: C26H54N2

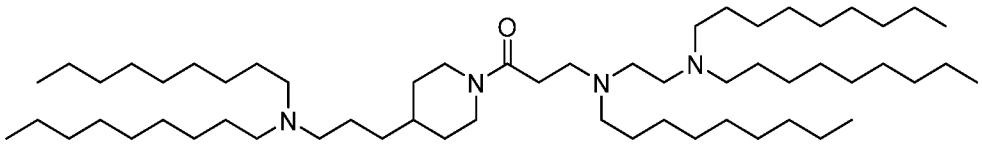
Molecular Weight: 394.73

[00874] In the same manner as Step 4 for Compound 11, *N*-nonyl-*N*-(3-(piperidin-4-yl)propyl)nonan-l -amine was synthesized from *tert-b*utyl 4-(3-(dinonylamino)propyl)piperidine-1-carboxylate (1.03 g, 2.08 mmol), and TFA (8.0 mL, 104 mmol) in DCM (10 mL). Yield (778 mg, 95%).

UPLC/ELSD: RT = 1.31 min. MS (ES): *m/z* (MH^+) 395.61 for C26H54N2

¾-NMR (300 MHz, CDCh) δ: ppm 3.18-2.50 (br. m, 4H); 2.40 (br. m, 6H); 1.70 (m, 2H); 1.58-1.03 (br. m, 36H); 0.90 (t, 6H).

Step 3: 3-((2-(Dinonylamino)ethyl)(nonyl)amino)-l-(4-(3-(dinonylamino)propyl)piperidm -1-yl)propan-l-one



Chemical Formula: C58H118N4O

Molecular Weight: 887.61

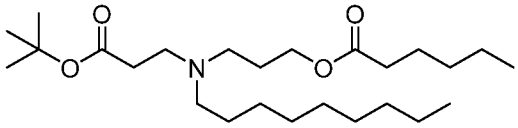
[00875] In the same manner as Step 11 for Compound 11, 3-((2-(dinonylamino)ethyl)(nonyl)amino)-l-(4-(3-(dinonylamino)propyl)piperidin-l-yl)propan-l-one was synthesized from *N*-nonyl-*N*-(3-(piperidin-4-yl)propyl)nonan-l -amine (247 mg, 0.63 mmol), 3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoic acid (352 mg, 0.69 mmol), iPr_2EtN (240 μL, 1.4 mmol), and T3P (50% EtOAc solution, 1.1 mL, 1.9 mmol) in THF (20 mL). Yield (293 mg, 53%).

UPLC/ELSD: RT = 3.01 min. MS (ES): *m/z* (MH^+) 888.08 for C58H118N4O

¾-NMR (300 MHz, CDCh) δ: ppm 4.61 (m, 1H); 3.86 (m, 1H); 2.99 (m, 1H); 2.82 (m, 2H); 2.61-2.29 (br. m, 19H); 1.75 (m, 2H); 1.60-1.00 (br. m, 77H); 0.89 (t, 15H).

BX: Compound 86: 3-((3-(4-(3-((2-(Dinonylamino)ethyl)(nonyl)amino)propanoyl)piperazin-l-yl)-3-oxopropyl)(nonyl)amino)propyl hexanoate

Step 1: 3-((3-(teri-Butoxy)-3-oxopropyl)(nonyl)amino)propyl hexanoate



Chemical Formula: C25H49NO4

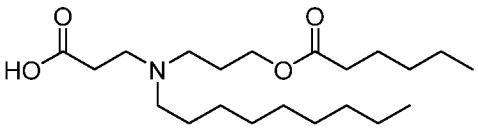
Molecular Weight: 427.67

In the same manner as Step 3 for Compound 44, 2-((3-(fer/-butoxy)-3-oxopropyl)(nonyl)amino)ethyl heptanoate was synthesized from *tert-b*utyl 3-(nonylamino)propanoate (750 mg, 2.76 mmol), 3-bromopropyl hexanoate (786 mg, 3.32 mmol), K2CO3 (764 mg, 5.53 mmol), and KI (46 mg, 0.28 mmol) in MeCN (100 mL). Yield (661 mg, 56%).

UPLC/ELSD: RT = 1.80 min. MS (ES): *m/z* (MH^+) 428.49 for C25H49NO4

¾-NMR (300 MHz, CDCh) δ: ppm 4.12 (t, 2H); 2.73 (t, 2H); 2.56-2.24 (br. m, 8H); 1.77 (m, 2H); 1.64 (m, 2H); 1.55-1.10 (br. m, 27H); 0.91 (m, 6H).

Step 2: 3-((3-(Hexanoyloxy)propyl)(nonyl)amino)propanoic acid



Chemical Formula: C21H41NO4

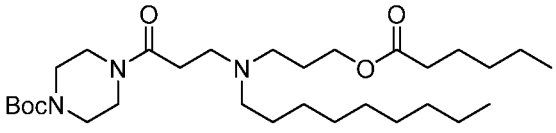
Molecular Weight: 371.56

In the same manner as Step 4 for Compound 44, 3-((3-(hexanoyloxy)propyl)(nonyl)amino)propanoic acid was synthesized from 3-((3-(/er/-butoxy)-3-oxopropyl)(nonyl)amino)propyl hexanoate (661 mg, 1.55 mmol), and TFA (5.9 mL, 77 mmol) in DCM (6 mL). Yield (556 mg, 97%).

UPLC/ELSD: RT = 1.14 min. MS (ES): *m/z* (MH^+) 372.31 for C21H41NO4

¾-NMR (300 MHz, CDCh) δ: ppm 4.13 (t, 2H); 2.84 (t, 2H); 2.72 (t, 2H); 2.62 (t, 2H); 2.46 (t, 2H); 2.31 (t, 2H); 1.90 (m, 2H); 1.72-1.10 (br. m, 20H); 0.90 (M, 6H).

Step 3: /er/-Butyl 4-(3-((3-(hexanoyloxy)propyl)(nonyl)amino)propanoyl)piperazine-l-carboxylate



Chemical Formula: C30H57N3O5

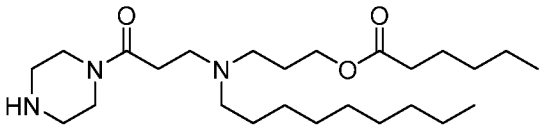
Molecular Weight: 539.80

[00876] In the same manner as Step 3 for Compound 11, *tert*-butyl 4-(3-((3-(hexanoyloxy)propyl)(nonyl)amino)propanoyl)piperazine-l-carboxylate was synthesized from 3-((3-(hexanoyloxy)propyl)(nonyl)amino)propanoic acid (570 mg, 1.49 mmol), 1-boc-piperazine (334 mg, 1.80 mmol), iPr_2EtN (573 µï¸, 3.29 mmol), and T3P (50% EtOAc solution, 2.67 mL, 4.49 mmol) in THF (20 mL). Yield (635 mg, 79%).

UPLC/ELSD: RT = 1.85 min. MS (ES): *m/z* (MH^+) 540.52 for C30H57N3O5

¾-NMR (300 MHz, CDCh) δ: ppm 4.12 (t, 2H); 3.60 (m, 2H); 3.46 (br. m, 6H); 2.80 (m, 2H); 2.58-2.37 (br. m, 6H); 2.30 (t, 2H); 1.78 (m, 2H); 1.63 (m, 2H); 1.54-1.10 (br. m, 27H); 0.90 (m, 6H) .

Step 4: 3-(Nonyl(3-oxo-3-(piperazin-l-yl)propyl)amino)propyl hexanoate



Chemical Formula: C25H49N3O3

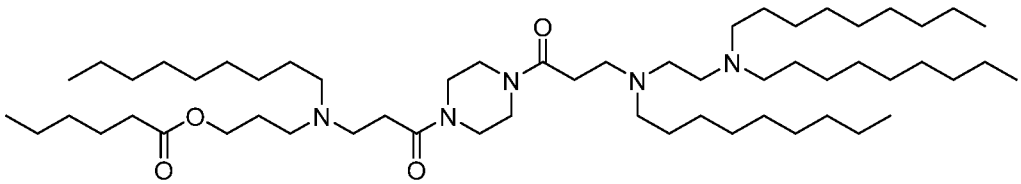
Molecular Weight: 439.69

In the same manner as Step 4 for Compound 11, 3-(nonyl(3-oxo-3-(piperazin-l-yl)propyl)amino)propyl hexanoate was synthesized from *tert*-butyl 4-(3-((3-(hexanoyloxy)propyl)(nonyl)amino)propanoyl)piperazine-l-carboxylate (635 mg, 1.18 mmol), and TFA (4.5 mL, 59 mmol) in DCM (5 mL). Yield (510 mg, 99%).

UPLC/ELSD: RT = 0.72 min. MS (ES): *m/z* (MH^+) 440.47 for C25H49N3O3

¹H-NMR (300 MHz, CDCh) δ: ppm 4.12 (t, 2H); 3.60 (m, 2H) 3.46 (m, 2H); 2.85 (br. m, 6H); 2.49 (br. m, 6H); 2.30 (t, 2H); 1.80 (m, 2H); 1.64 (m, 2H); 1.52-1.10 (br. m, 19H); 0.91 (m, 6H).

Step 5: 3-((3-(4-(3-((2-(Dinonylamino)ethyl)(nonyl)amino)propanoyl)piperazin-l-yl)-3-oxopropyl)(nonyl)amino)propyl hexanoate



Chemical Formula: C57H113N5O4

Molecular Weight: 932.56

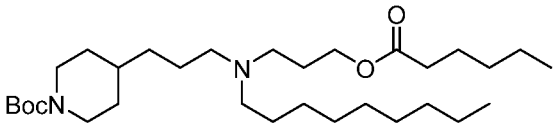
[00877] In the same manner as Step 11 for Compound 11, 3-((3-(4-(3-((2-(dinonylainino)ethyl)(nonyl)ainin^)propanoyl)piperazin-1-yl)-3-oxopropyl)(nonyl)amino)propyl hexanoate was synthesized from 3-(nonyl(3-oxo-3-(piperazin-l-yl)propyl)amino)propyl hexanoate (154 mg, 0.351 mmol), 3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoic acid (197 mg, 0.386 mmol), iPr_2EtN (134 μL, 0.77 mmol), and T3P (50% EtOAc solution, 616 μL, 1.05 mmol) in THF (10 mL). Yield (69 mg, 21%).

UPLC/ELSD: RT = 2.70 min. MS (ES): *m/z* (MH^+) 933.10 for C57H113N5O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.12 (t, 2H); 3.72-3.40 (br. m, 8H); 2.81 (br. m, 4H); 2.61-2.36 (br. m, 18H); 2.30 (t, 2H); 1.78 (m, 2H); 1.64 (m, 2H); 1.54-1.06 (br. m, 60H); 0.90 (m, 15H).

BY: Compound 87: 3-((3-(l-(3-((2-(Dinonylamino)ethyl)(nonyl)amino)propanoyl)piperidin-4-yl)propyl)(nonyl)amino)propyl hexanoate

Step 1: *tert*-Butyl 4-(3-((3-(hexanoyloxy)propyl)(nonyl)amino)propyl)piperidine-l-carboxylate



Chemical Formula: C31H60N2O4

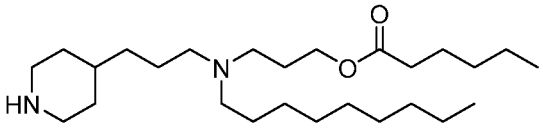
Molecular Weight: 524.83

[00878] In the same manner as Step 1 for Compound 57, *tert-b*utyl 4-(3-((3-(hexanoy loxy)propyl)(nonyl)amino)propyl)piperi dine- 1-carboxy late was synthesized from tert-butyl 4-(3-(nonylamino)propyl)piperidine-l-carboxylate (500 mg, 1.36 mmol), 3-bromopropyl hexanoate (386 mg, 1.63 mmol), K2CO3 (375 mg, 2.71 mmol), and KI (45 mg, 0.27 mmol) in MeCN (100 mL). Yield (322 mg, 45%).

UPLC/ELSD: RT = 2.09 min. MS (ES): *m/z* (MH^+) 525.60 for C31H60N2O4

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.12 (br. m, 4H); 2.67 (m, 2H); 2.56-2.24 (br. m, 8H); 1.90-1.00 (br. m, 40H); 0.91 (m, 6H).

Step 2: 3-(nonyl(3-(piperidin-4-yl)propyl)amino)propyl hexanoate



Chemical Formula: C26H52N2O2

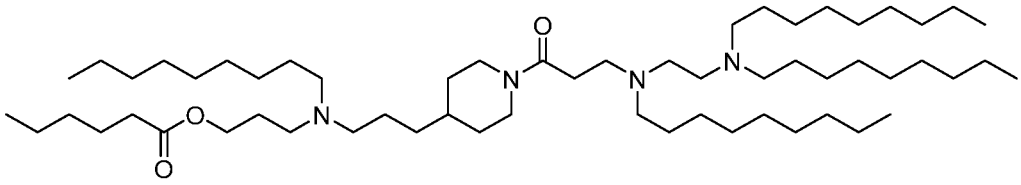
Molecular Weight: 424.7 1

[00879] In the same manner as Step 4 for Compound 11, 3-(nonyl(3-(piperidin-4-yl)propyl)amino)propyl hexanoate was synthesized from *tert-b*utyl 4-(3-((3-(hexanoyloxy)propyl)(nonyl)amino)propyl)piperi dine- 1-carboxy late (322 mg, 0.614 mmol), and TFA (2.3 mL, 31 mmol) in DCM (2.5 mL). Yield (260 mg, 99%).

UPLC/ELSD: RT = 0.89 min. MS (ES): *m/z* (MH^+) 425.54 for C26H52N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.12 (t, 2H); 3.12 (m, 2H); 2.75-2.24 (br. m, 10H); 1.84-1.54 (br. m, 6H); 1.54-1.02 (br. m, 26H); 0.90 (m, 6H).

Step 3: 3-((3-(l-(3-((2-(Dinonylamino)ethyl)(nonyl)amino)propanoyl)piperidin-4-yl)propyl)(nonyl)amino)propyl hexanoate



Chemical Formula: C58H116N4O3

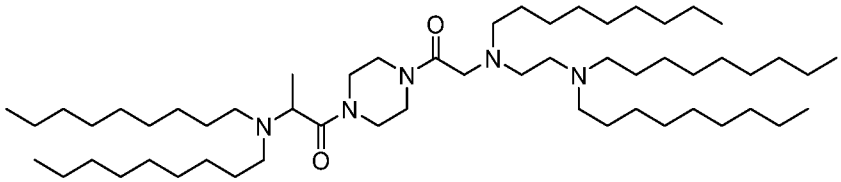
Molecular Weight: 917.59

[00880] In the same manner as Step 11 for Compound 11, 3-((3-(l -(3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoyl)piperidin-4-yl)propyl)(nonyl)amino)propyl hexanoate was synthesized from 3-(nonyl(3-(piperidin-4-yl)propyl)amino)propyl hexanoate (149 mg, 0.351 mmol), 3-((2-(dinonylamino)ethyl)(nonyl)amino)propanoic acid (197 mg, 0.386 mmol), iPr_2EtN (134 μL, 0.77 mmol), and T3P (50% EtOAc solution, 616 μL, 1.05 mmol) in THF (10 mL). Yield (39 mg, 12%).

UPLC/ELSD: RT = 2.83 min. MS (ES): *m/z* (MH^+) 918.01 for C58H116N4O3

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.61 (m, 1H); 4.12 (t, 2H); 3.87 (m, 1H); 3.08-2.74 (br. m, 4H); 2.70-2.23 (br. m, 20H); 1.82-1.56 (br. m, 6H); 1.56-1.00 (br. m, 67H); 0.90 (m, 15H).

BZ. Compound 119: 2-(Dinonylamino)-l -(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l -yl)propan-l -one



Chemical Formula: C56H113N5O2

Molecular Weight: 888.55

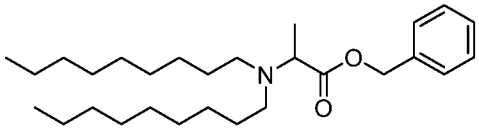
[00881] In the same manner as Step 11 for Compound 11, 2-(dinonylamino)-l-(4-(N-(2-(dinonylamino)ethyl)-N-nonylglycyl)piperazin-1-yl)propan-1-one was synthesized from 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l-(piperazin-l-yl)ethan-l-one (331 mg, 0.586 mmol), dinonylalanine (200 mg, 0.586 mmol), i-PnEtN (224 μL, 1.29 mmol), and propylphosphonic acid anhydride (50% EtOAc solution, 1.05 mL, 1.76 mmol). Yield (139 mg, 28%).

UPLC/ELSD: RT = 3.07 min. MS (ES): *m/z* (MH^+) 888.90 for C56H113N5O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.06-2.07 (br. m, 25H); 2.05-1.14 (br. m, 73H); 0.90 (m, 15H).

CA. Compound 111: Pentyl 4-((l-(4-*(N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-l-oxopropan-2-yl)(nonyl)amino)butanoate

Step 1: Benzyl dinonylalaninate



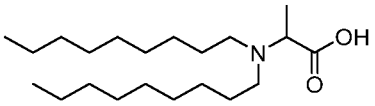
Chemical Formula: C28H49NO2

Molecular Weight: 431.71

[00882] In the same manner as Step 3 for Compound 44, benzyl dinonylalaninate was synthesized from l-(benzyloxy)-l-oxopropan-2-aminium 4-methylbenzenesulfonate (11.6 g, 33.0 mmol), 1-bromononane (10.61, 51.2 mmol), potassium carbonate (9.43 g, 68 mmol), and KI (0.283 g, 0.002 mmol) in acetonitrile (50 mL). Yield (2.2 g, 10%).

¾-NMR (300 MHz, CDCh) δ: ppm 7.37 (m, 5H); 5.15 (s, 2H); 3.59 (m, 1H); 2.51 (m, 4H); 1.56 (s, 3H); 1.27 (br. m, 28H); 0.90 (t, 6H).

Step 2: Dinonylalanine



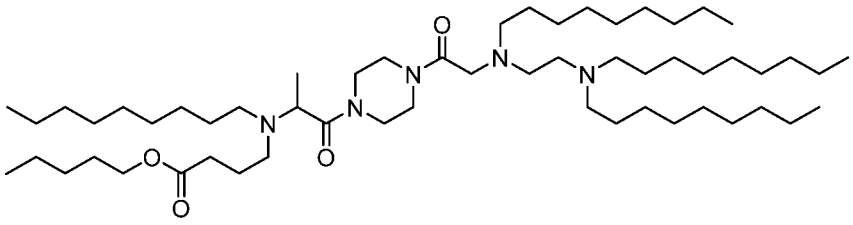
Chemical Formula: C21H43NO2

Molecular Weight: 341.58

[00883] To a flask under N_2 added benzyl dinonylalaninate (2.2 g, 5.10 mmol), $Pd(OH)_2$ (220 mg) followed by EtOH. The reaction was purged with H2 and was kept under H2 (balloon) with stirring for 16h at rt. The reaction was purged with N_2. The reaction was filtered through a plug of Celite followed by EtOAc (200 mL). The filtrate was evaporated under vacuum. The residue was purified by ISCO with EtOAc in hexanes (0-100%) to obtain dinonylalanine (1.29 g, 3.78 mmol, 74%).

¾-NMR (300 MHz, CDCh) δ: ppm 3.68 (m, 1H); 2.99 (m, 4H); 1.71 (m, 4H); 1.50 (d, 3H); 1.28 (m, 24H); 0.90 (t, 6H).

Step 3: Pentyl 4-((l-(4 -*(N* -(2-(dinonylamino)ethyl *)-N* -nonylglycyl)piperazin-l-yl)-l -oxopropan-2-yl)(nonyl)amino)butanoate



Chemical Formula: **C56H111N5O4**

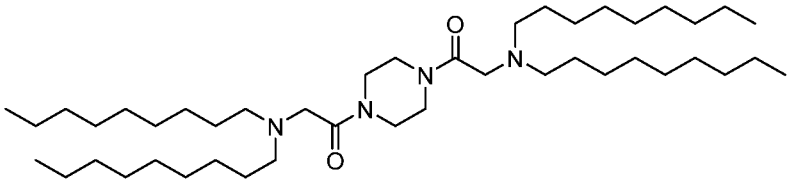
Molecular Weight: 918.54

[00884] In the same manner as Step 11 for Compound 11, pentyl 4-((l -(4-(N-(2-(dinonylamino)ethyl)-N-nonylgly cyl)piperazin- 1-yl)- 1-oxopropan-2-yl)(nonyl)amino)butanoate was synthesized from 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l -(piperazin-l-yl)ethan-l-one (304 mg, 0.538 mmol), N-nonyl-N-(4-oxo-4-(pentyloxy)butyl)alanine (200 mg, 0.538 mmol), *i*-Pr_2EtN (206 μL, 1.18 mmol), and propylphosphonic acid anhydride (50% EtOAc solution, 0.96 mL, 1.61 mmol). Yield (109 mg, 22%).

UPLC/ELSD: RT = 2.92 min. MS (ES): *m/z* (MH^+) 918.90 for **C56H111N5O4**

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.94-2.98 (br. m, 12H); 2.70-2.22 (br. m, 15H); 1.88-1.11 (br. m, 67H); 0.90 (m, 15H).

CB. Compound 112: l,r-(piperazine-l ,4-diyl)bis(2-(dinonylamino)ethan-l -one)



Chemical Formula: $C_{44}H_{88}N_4O_2$

Molecular Weight: 705.21

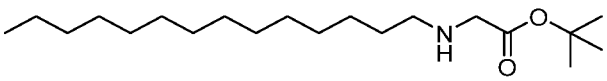
[00885] In the same manner as Step 11 for Compound 11, 1,1'-(piperazine-1,4-diyl)bis(2-(dinonylamino)ethan-1-one) was synthesized from 2-(dinonylamino)-1-(piperazin-1-yl)ethan-1-one (242 mg, 0.611 mmol), dinonylglycine (200 mg, 0.611 mmol), i-Pr_2EtN (234 μL, 1.34 mmol), and propylphosphonic acid anhydride (50% EtOAc solution, 1.09 mL, 1.83 mmol). Yield (49 mg, 11%).

UPLC/ELSD: RT = 2.45 min. MS (ES): *m/z* (MH^+) 705.69 for $\mathbf{C_{44}H_{88}N_4O_2}$

¾-NMR (300 MHz, CDCh) δ: ppm 3.64 (m, 8H); 3.27 (m, 4H); 2.46 (br. m, 8H); 1.53-1.20 (br. m, 56H); 0.90 (m, 12H).

CC. Compound 91: Heptyl 6-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(tetradecyl)amino)hexanoate

Step 1: *tert*-Butyl tetradecylglycinate



Chemical Formula: C20H41NO2

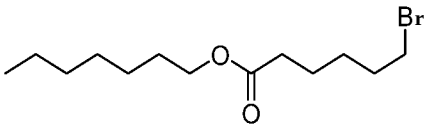
Molecular Weight: 327.55

[00886] In the same manner as Step 1 for Compound 44, *tert*-butyl tetradecylglycinate was synthesized from *tert*-butyl glycinate hydrochloride (2.5 g, 15 mmol), 1-bromotetradecane (4.1 g, 15 mmol), K_2CO_3 (4.1 g, 30 mmol), and KI (250 mg, 1.5 mmol) in MeCN (30 mL). Yield (1.57 g, 32%).

UPLC/ELSD: RT = 1.78 min. MS (ES): *m/z* (MH^+) 328.35 for C20H41NO2

¾-NMR (300 MHz, CDCh) δ: ppm 3.31 (s, 2H); 2.59 (t, 2H); 1.70-1.05 (br. m, 34H); 0.90 (t, 3H).

Step 2: Heptyl 6-bromohexanoate



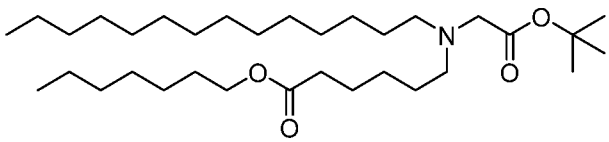
Chemical Formula: Ci3H25Br02

Molecular Weight: 293.25

[00887] In the same manner as Step 1 for Compound 15, heptyl 6-bromohexanoate was synthesized from 6-bromohexanoic acid (3.0 g, 15 mmol), 1-heptanol (2.3 g, 20 mmol), and sulfuric acid (0.82 mL, 15 mmol) in THF (20 mL). Yield (3.4 g, 75%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.09 (t, 2H); 3.42 (t, 2H); 2.34 (t, 2H); 1.90 (quint, 2H); 1.80-1.10 (br. m, 14H); 0.91 (t, 3H).

Step 3: Heptyl 6-((2-(teri-butoxy)-2-oxoethyl)(tetradecyl)amino)hexanoate



Chemical Formula: $C_{33}H_{65}NO_4$

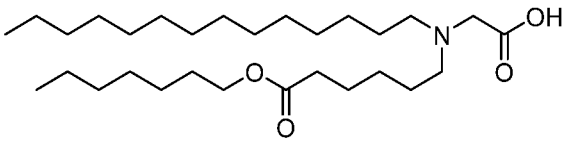
Molecular Weight: 539.89

[00888] In the same manner as Step 3 for Compound 44, heptyl 6-((2-(/er/-butoxy)-2-oxoethyl)(tetradecyl)amino)hexanoate was synthesized from *tert-b*utyl tetradecylglycinate (370 mg, 1.13 mmol), heptyl 6-bromohexanoate (364 mg, 1.24 mmol), K2CO3 (312 mg, 2.26 mmol), and KI (19 mg, 0.11 mmol) in MeCN (20 mL). Yield (493 mg, 81%).

UPLC/ELSD: RT = 2.87 min. MS (ES): *m/z* (MH^+) 540.52 for C33H65NO4

¾-NMR (300 MHz, CDCh) δ: ppm 4.07 (t, 2H); 3.22 (s, 2H); 2.57 (br. m, 4H); 2.32 (t, 2H); 1.65 (m, 4H); 1.55-1.08 (br. m, 45H); 0.91 (t, 6H).

N-(6-(Heptyloxy)-6-oxohexyl)-*N*-tetradecylglycine



Chemical Formula: C29H57NO4

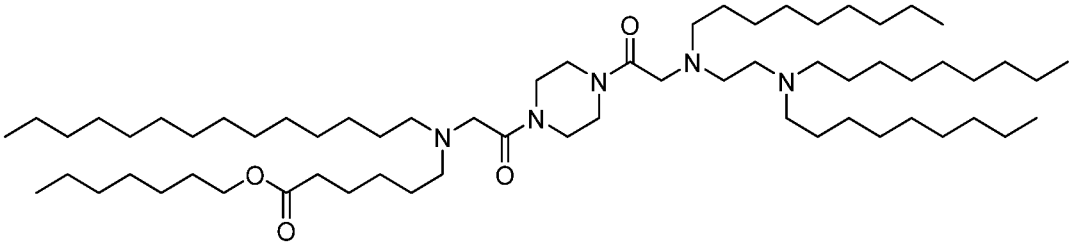
Molecular Weight: 483.78

[00889] In the same manner as Step 4 for Compound 44, *N*-(6-(heptyloxy)-6-oxohexyl)-*N*-tetradecylglycine was synthesized from heptyl 6-((2-(/er/-butoxy)-2-oxoethyl)(tetradecyl)amino)hexanoate (493 mg, 0.913 mmol) and TFA (3.5 mL, 46 mmol) in DCM (3.5 mL). Yield (413 mg, 93%).

UPLC/ELSD: RT = 2.55 min. MS (ES): *m/z* (MH^+) 484.51 for C29H57NO4

¾-NMR (300 MHz, CDCh) δ: ppm 4.07 (t, 2H); 3.42 (s, 2H); 2.96 (br. m, 4H); 2.33 (t, 2H); 1.66 (br. m, 6H); 1.50-1.10 (br. m, 34H); 0.91 (t, 6H).

Step 4: Heptyl 6-((2-(4 -(*N* -(2-(dinonylamino)ethyl)-*N* -nonylglycyl)piperazin- 1-yl)-2-oxoethyl)(tetradecyl)amino)hexanoate



Chemical Formula: C64H127N5O4

Molecular Weight: 1030.75

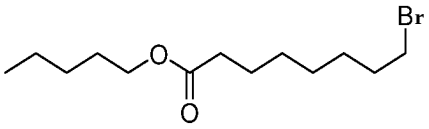
[00890] In the same manner as Step 7 for Compound 44, heptyl 6-((2-(4 -(*N* -(2-(dinonylamino)ethyl)-*N* -nonylglycyl)piperazin-l-yl)-2-oxoethyl)(tetradecyl)amino)hexanoate was synthesized from *N* -(6-(heptyloxy)-6-oxohexyl)-*N* -tetradecylgly cine (413 mg, 0.854 mmol),), 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l-(piperazin-l -yl)ethan-l -one (402 mg, 0.7 11 mmol), iPr_2EtN (0.273 mL, 1.57 mmol) and T3P (50% EtOAc solution, 1.27 mL, 2.13 mmol). Yield (183 mg, 25%).

UPLC/ELSD: RT = 3.35 min. MS (ES): *m/z* (MH^+) 103 1.11 for C64H127N5O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.07 (t, 2H); 3.80-3.50 (br. m, 8H); 3.36-3.23 (br. m, 4H); 2.65-2.25 (br. m, 16H); 1.75-1 .05 (br. m, 4H); 1.75-1 .05 (br. m, 78H); 0.90 (t, 15H).

CD: Compound 90: Pentyl 8-((2-(4 -(*N* -(2-(dinonylamino)ethyl)-*N* -nonylglycyl)piperazin- 1-yl)-2-oxoethyl)(tetradecyl)amino)octanoate

Step 1: Pentyl 8-bromooctanoate



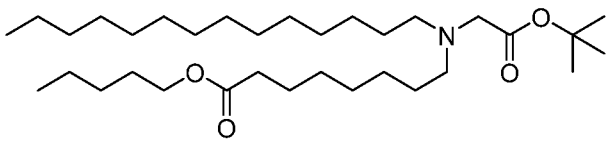
Chemical Formula: $Ci_3H_{25}BrO_2$

Molecular Weight: 293.25

[00891] In the same manner as Step 1 for Compound 15, pentyl 8-bromooctanoate was synthesized from 8-bromooctanoic acid (3.0 g, 13 mmol), 1-pentanol (1.5 g, 17 mmol), and sulfuric acid (0.72 mL, 13 mmol) in THF (20 mL). Yield (2.7 g, 69%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.42 (t, 2H); 2.3 1 (t, 2H); 1.87 (quint, 2H); 1.78-1 .10 (br. m, 14H); 0.93 (t, 3H).

Step 2: Pentyl 8-((2-(*tert*-butoxy)-2-oxoethyl)(tetradecyl)amino)octanoate



Chemical Formula: $C_{33}H_{65}NO_4$

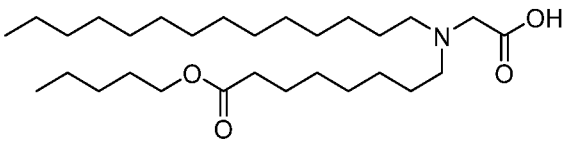
Molecular Weight: 539.89

[00892] In the same manner as Step 3 for Compound 44, pentyl 8-((2-(*tert*-butoxy)-2-oxoethyl)(tetradecyl)amino)octanoate was synthesized from *tert*-butyl tetradecylglycinate (350 mg, 1.07 mmol), pentyl 8-bromooctanoate (345 mg, 1.18 mmol), K_2CO_3 (295 mg, 2.14 mmol), and KI (18 mg, 0.11 mmol) in MeCN (15 mL). Yield (431 mg, 75%).

UPLC/ELSD: RT = 2.77 min. MS (ES): *m/z* (MH^+) 540.44 for $C_{33}H_{65}NO_4$

¾-NMR (300 MHz, $CDCl_3$) δ: ppm 4.08 (t, 2H); 3.23 (s, 2H); 2.56 (t, 4H); 2.31 (t, 2H); 1.72-1.01 (br. m, 49H); 0.91 (m, 6H).

Step 3: *N*-(8-Oxo-8-(pentyloxy)octyl)-*N*-tetradecylglycine



Chemical Formula: **$C_{29}H_{57}NO_4$**

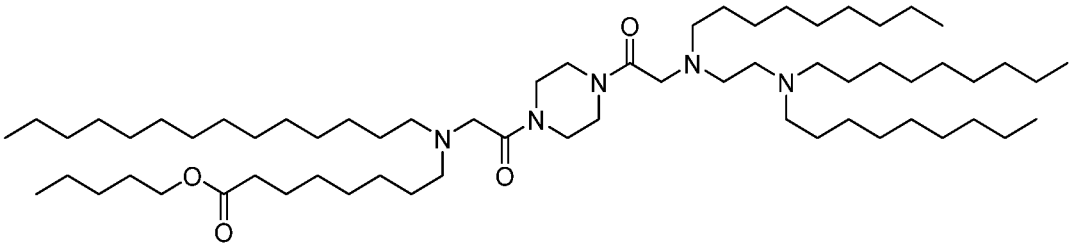
Molecular Weight: 483.78

[00893] In the same manner as Step 4 for Compound 44, *N*-(8-oxo-8-(pentyloxy)octyl)-*N*-tetradecylglycine was synthesized from pentyl 8-((2-(*tert*-butoxy)-2-oxoethyl)(tetradecyl)amino)octanoate (431 mg, 0.798 mmol), and TFA (3.0 mL, 40 mmol) in DCM (3 mL). Yield (375 mg, 97%).

UPLC/ELSD: RT = 2.49 min. MS (ES): *m/z* (MH^+) 484.26 for **$C_{29}H_{57}NO_4$**

¾-NMR (300 MHz, $CDCl_3$) δ: ppm 4.08 (t, 2H); 3.45 (s, 2H); 3.02 (br. m, 4H); 2.31 (t, 2H); 1.65 (br. m, 8H); 1.50-1.10 (br. m, 32H); 0.91 (m, 6H).

Step 4: Pentyl 8-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(tetradecyl)amino)octanoate



Chemical Formula: C64H127N5O4

Molecular Weight: 1030.75

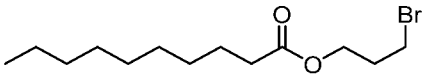
[00894] In the same manner as Step 7 for Compound 44, pentyl 8-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(tetradecyl)amino)octanoate was synthesized from *N*-(8-oxo-8-(pentyloxy)octyl)-*N*-tetradecylglycine (300 mg, 0.620 mmol), 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l-(piperazin-l-yl)ethan-l-one (385 mg, 0.682 mmol), iPrNEt (0.237 mL, 1.36 mmol) and T3P (50% EtOAc solution, 1.10 mL, 1.86 mmol). Yield (196 mg, 31%).

UPLC/ELSD: RT = 3.32 min. MS (ES): *m/z* (MH^+) 1031.02 for C64H127N5O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.80-3.48 (br. m, 8H); 3.38-3.23 (br. m, 4H); 2.66-2.24 (br. m, 16H); 1.75-1.05 (br. m, 82H); 0.90 (m, 15H).

CE: Compound 89: 3-((2-(4-(*N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(tetradecyl)amino)propyl decanoate

Step 1: 3-Bromopropyl decanoate



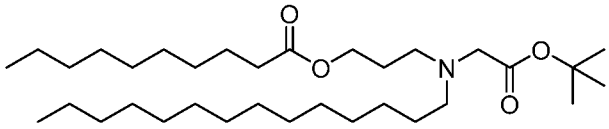
Chemical Formula: $C_{13}H_{25}BrO_2$

Molecular Weight: 293.25

[00895] In the same manner as Step 1 for Compound 15, 3-bromopropyl decanoate was synthesized from 3-bromopropan-l-ol (2.0 g, 14 mmol), decanoic acid (3.2 g, 19 mmol), and sulfuric acid (0.77 mL, 14 mmol) in THF (20 mL). Yield (2.98 g, 71%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.23 (t, 2H); 3.48 (t, 2H); 2.33 (t, 2H); 2.20 (quint, 2H); 1.64 (quint, 2H); 1.46-1.10 (br. m, 12H); 0.90 (t, 3H).

Step 2: 3-((2-(teri-Butoxy)-2-oxoethyl)(tetradecyl)amino)propyl decanoate



Chemical Formula: $C_{33}H_{65}NO_4$

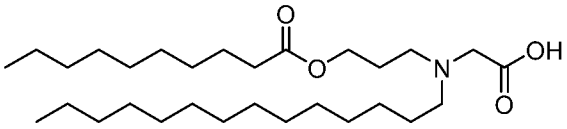
Molecular Weight: 539.89

[00896] In the same manner as Step 3 for Compound 44, 3-((2-(*tert*-butoxy)-2-oxoethyl)(tetradecyl)amino)propyl decanoate was synthesized from *tert*-butyl tetradecylglycinate (370 mg, 1.13 mmol), 3-bromopropyl decanoate (364 mg, 1.24 mmol), K_2CO_3 (312 mg, 2.26 mmol), and KI (19 mg, 0.11 mmol) in MeCN (20 mL). Yield (481 mg, 79%).

UPLC/ELSD: RT = 2.92 min. MS (ES): *m/z* (MH^+) 540.52 for $C_{33}H_{65}NO_4$

¾-NMR (300 MHz, $CDCl_3$) δ: ppm 4.14 (t, 2H); 3.23 (s, 2H); 2.68 (t, 2H); 2.58 (t, 2H); 2.30 (t, 2H); 1.79 (quint, 2H); 1.63 (m, 2H); 1.56-1.10 (br. m, 45H); 0.90 (t, 6H).

Step3: *N*-(3-(Decanoyloxy)propyl)-*N*-tetradecylglycine



Chemical Formula: $C_{29}H_{57}NO_4$

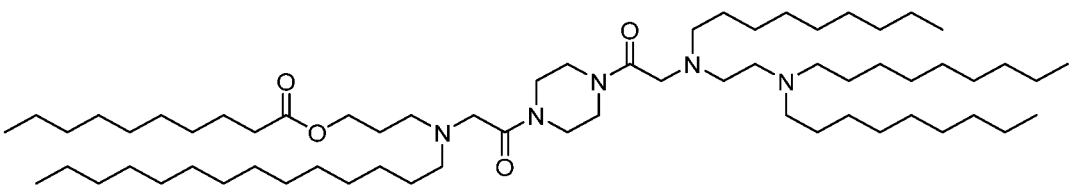
Molecular Weight: 483.78

[00897] In the same manner as Step 4 for Compound 44, *N*-(3-(decanoyloxy)propyl)-*N*-tetradecylglycine was synthesized from 3-((2-(*tert*-butoxy)-2-oxoethyl)(tetradecyl)amino)propyl decanoate (481 mg, 0.891 mmol) and TFA (3.4 mL, 44 mmol) in DCM (3.4 mL). Yield (346 mg, 80%).

UPLC/ELSD: RT = 2.67 min. MS (ES): *m/z* (MH^+) 484.26 for $C_{29}H_{57}NO_4$

¾-NMR (300 MHz, $CDCl_3$) δ: ppm 4.15 (t, 2H); 3.27 (s, 2H); 2.90-2.60 (br. m, 4H); 2.32 (t, 2H); 1.92 (m, 2H); 1.71-1.05 (br. m, 38H); 0.90 (t, 6H).

Step 4: 3-((2-(4-(*N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(tetradecyl)amino)propyl decanoate



Chemical Formula: C64H127N5O4

Molecular Weight: 1030.75

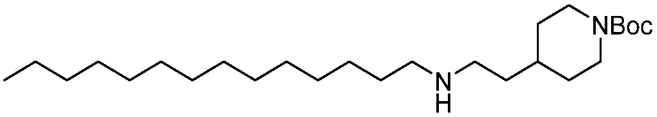
[00898] In the same manner as Step 7 for Compound 44, 3-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(tetradecyl)amino)propyl decanoate was synthesized from *N*-(3-(decanoyloxy)propyl)-*N*-tetradecylglycine (346 mg, 0.715 mmol), 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l-(piperazin-l-yl)ethan-l-one (444 mg, 0.787 mmol), iPr_2EtN (0.274 mL, 1.57 mmol), and T3P (50% EtOAc solution, 1.3 mL, 2.1 mmol). Yield (110 mg, 15%).

UPLC/ELSD: RT = 3.40 min. MS (ES): *m/z* (MH^+) 1031.11 for C64H127N5O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.10 (t, 2H); 3.78-3.50 (br. m, 8H); 3.37-3.26 (br. m, 4H); 2.71-2.22 (br. m, 16H); 1.79 (m, 2H); 1.62 (m, 2H); 1.52-1.04 (br. m, 78H); 0.90 (t, 15H).

CF: Compound 94: Heptyl 6-((2-(l-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(tetradecyl)amino)hexanoate

Step 1: *tert*-Butyl 4-(2-(tetradecylamino)ethyl)piperidine-1-carboxylate



Chemical Formula: C26H52N2O2

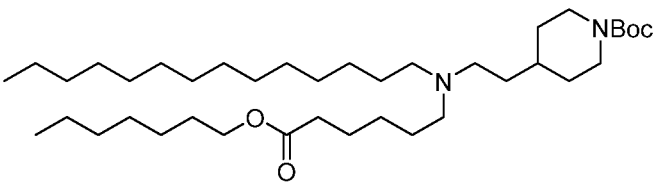
Molecular Weight: 424.71

[00899] In the same manner as Step 1 for Compound 49, *tert*-butyl 4-(2-(tetradecylamino)ethyl)piperidine-1-carboxylate was synthesized from /*er*/-butyl 4-(2-aminoethyl)piperidine-1-carboxylate (2.0 g, 8.8 mmol), 1-bromotetradecane (2.4 g, 8.8 mmol), K2CO3 (2.4 g, 18 mmol), and KI (145 mg, 0.876 mmol) in MeCN (20 mL). Yield (1.7 g, 46%).

UPLC/ELSD: RT = 2.07 min. MS (ES): *m/z* (MH^+) 425.46 for C26H52N2O2

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.08 (br. m, 2H); 2.72 (br. m, 6H); 1.76-1.03 (br. m, 40H); 0.90 (t, 3H).

Step 2: /er/-Butyl 4-(2-((6-(heptyloxy)-6-oxohexyl)(tetradecyl)amino)ethyl)piperidine-l-carboxylate



Chemical Formula: C39H76N2O4

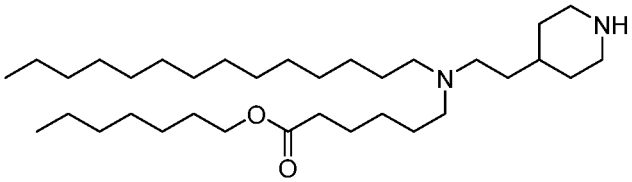
Molecular Weight: 637.05

[00900] In the same manner as Step 1 for Compound 57, *tert*-butyl 4-(2-((6-(heptyloxy)-6-oxohexyl)(tetradecyl)amino)ethyl)piperi dine-1-carboxylate was synthesized from *tert*-butyl 4-(2-(tetradecylamino)ethyl)piperi dine-1-carboxylate (400 mg, 0.942 mmol), heptyl 6-bromohexanoate (331 mg, 1.13 mmol), K2CO3 (260 mg, 1.88 mmol), and KI (16 mg, 0.094 mmol) in MeCN (20 mL). Yield (445 mg, 74%).

UPLC/ELSD: RT = 2.99 min. MS (ES): *m/z* (MH^+) 637.62 for C39H76N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (br. m, 4H); 3.40-2.15 (br. m, 10H); 2.05-1.00 (br. m, 56H); 0.91 (t, 6H).

Step 3: Heptyl 6-((2-(piperidin-4-yl)ethyl)(tetradecyl)amino)hexanoate



Chemical Formula: C34H68N2O2

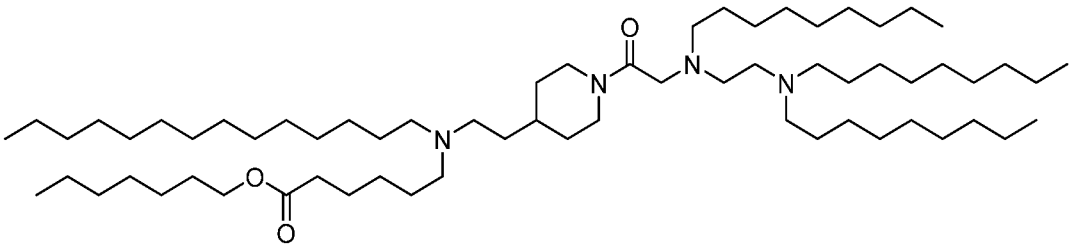
Molecular Weight: 536.93

[00901] In the same manner as Step 4 for Compound 11, heptyl 6-((2-(piperidin-4-yl)ethyl)(tetradecyl)amino)hexanoate was synthesized from *tert*-butyl 4-(2-((6-(heptyloxy)-6-oxohexyl)(tetradecyl)amino)ethyl)piperi dine-1-carboxylate (445 mg, 0.699 mmol), and TFA (2.7 mL, 35 mmol) in DCM (2.7 mL). Yield (326 mg, 87%).

UPLC/ELSD: RT = 2.05 min. MS (ES): *m/z* (MH^+) 537.65 for C34H68N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.13 (br. m, 2H); 2.75-2.20 (br. m, 10H); 1.SO-LOS (br. m, 47H); 0.90 (t, 6H).

Step 4: Heptyl 6-((2-(1 -(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(tetradecyl)amino)hexanoate



Chemical Formula: C65H130N4O3

Molecular Weight: 1015.78

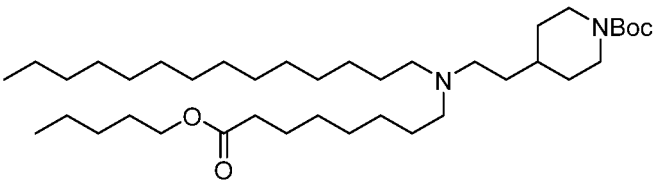
[00902] In the same manner as Step 11 for Compound 11, heptyl 6-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(tetradecyl)amino)hexanoate was synthesized from heptyl 6-((2-(piperidin-4-yl)ethyl)(tetradecyl)amino)hexanoate (326 mg, 0.607 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (274 mg, 0.552 mmol), iPnEtN (0.212 mL, 1.21 mmol), and T3P (50% EtOAc solution, 1.0 mL, 1.66 mmol) in THF (10 mL). Yield (126 mg, 22%).

UPLC/ELSD: RT = 3.48 min. MS (ES): *m/z* (MH^+) 1016.10 for C34H68N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.65-2.22 (br. m, 26H); 1.94-1.00 (br. m, 89H); 0.90 (t, 15H).

CG: Compound 93: Pentyl 8-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(tetradecyl)amino)octanoate

Step 1: *tert*-Butyl 4-(2-((8-oxo-8-(pentyloxy)octyl)(tetradecyl)arnino)ethyl)piperidine-l-carboxylate



Chemical Formula: C39H76N2O4

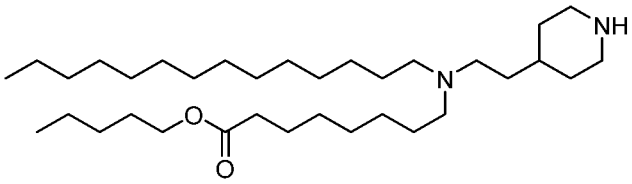
Molecular Weight: 637.05

[00903] In the same manner as Step 1 for Compound 57, *tert-butyl* 4-(2-((8-oxo-8-(pentyloxy)octyl)(tetradecyl)arnino)ethyl)piperidine-l-carboxylate was synthesized from tert-butyl 4-(2-(tetradecylamino)ethyl)piperidine-1-carboxylate (400 mg, 0.942 mmol), pentyl 8-bromooctanoate (331 mg, 1.13 mmol), K2CO3 (260 mg, 1.88 mmol), and KI (16 mg, 0.094 mmol) in MeCN (20 mL). Yield (411 mg, 69%).

UPLC/ELSD: RT = 2.95 min. MS (ES): *m/z* (MH^+) 637.62 for C39H76N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (br. m, 4H); 3.15-2.15 (br. m, 10H); 1.90-1.03 (br. m, 56H); 0.92 (m, 6H).

Step 2: Pentyl 8-((2-(piperidin-4-yl)ethyl)(tetradecyl)amino)octanoate



Chemical Formula: C34H68N2O2

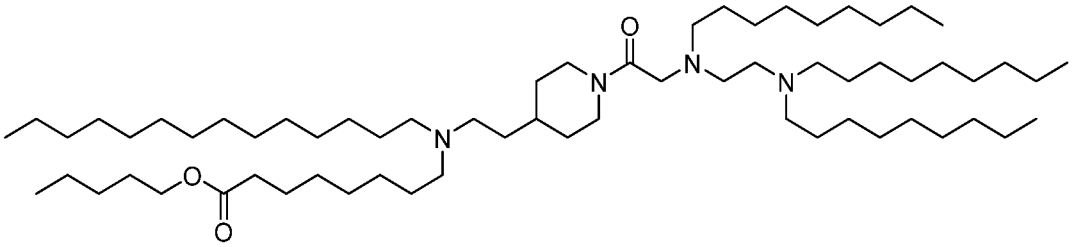
Molecular Weight: 536.93

[00904] In the same manner as Step 4 for Compound 11, pentyl 8-((2-(piperidin-4-yl)ethyl)(tetradecyl)amino)octanoate was synthesized from *tert-b*utyl 4-(2-((8-oxo-8-(pentyloxy)octyl)(tetradecyl)amino)ethyl)piperidine-l-carboxylate (41 1 mg, 0.645 mmol), and TFA (2.5 mL, 32 mmol) in DCM (2.5 mL). Yield (309 mg, 89%).

UPLC/ELSD: RT = 1.97 min. MS (ES): *m/z* (MH^+) 537.65 for C34H68N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.10 (br. m, 2H); 2.71-2.10 (br. m, 10H); 1.80-1.05 (br. m, 47H); 0.92 (m, 6H).

Step 3: Pentyl 8-((2-(l -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperidin-4-yl)ethyl)(tetradecyl)amino)octanoate



Chemical Formula: C65H130N4O3

Molecular Weight: 1015.78

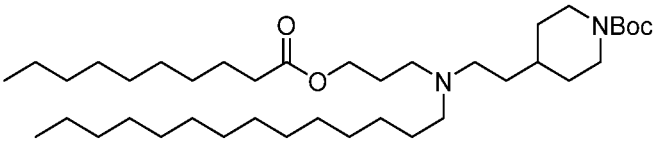
[00905] In the same manner as Step 11 for Compound 11, pentyl 8-((2-(l -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperidin-4-yl)ethyl)(tetradecyl)amino)octanoate was synthesized from pentyl 8-((2-(piperidin-4-yl)ethyl)(tetradecyl)amino)octanoate (309 mg, 0.575 mmol), *N*-(2-(dinonylamino)ethyl)-N-nonylglycine (260 mg, 0.523 mmol), iPr_2EtN (0.200 mL, 1.15 mmol), and T3P (50% EtOAc solution, 0.94 mL, 1.57 mmol). Yield (157 mg, 30%).

UPLC/ELSD: RT = 3.43 min. MS (ES): *m/z* (MH^+) 1016.02 for C65H130N4O3

¾-NMR (300 MHz, CDCh) δ: ppm 4.65-2.22 (br. m, 26H); 1.94-1.00 (br. m, 89H); 0.90 (m, 15H).

CH: Compound 92: 3-((2-(l -*(N*-(2-(Dinonylamino)ethyl *)-N*-nonylglycyl)piperidin-4-yl)ethyl)(tetradecyl)amino)propyl decanoate

Step 1: *tert*-Butyl 4-(2-((3-(decanoyloxy)propyl)(tetradecyl)amino)ethyl)piperidine-1-carboxylate



Chemical Formula: C39H76N2O4

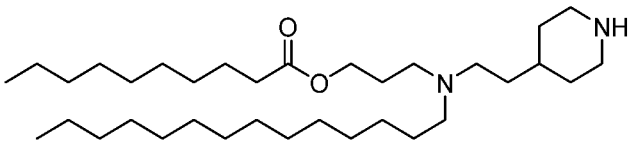
Molecular Weight: 637.05

[00906] In the same manner as Step 1 for Compound 57, tert-butyl 4-(2-((3-(decanoyloxy)propyl)(tetradecyl)amino)ethyl)piperi dine-1-carboxylate was synthesized from tert-butyl 4-(2-(tetradecylamino)ethyl)piperi dine-1-carboxylate (400 mg, 0.942 mmol), 3-bromopropyl decanoate (331 mg, 1.13 mmol), K2CO3 (260 mg, 1.88 mmol), and KI (16 mg, 0.094 mmol) in MeCN (20 mL). Yield (458 mg, 76%).

UPLC/ELSD: RT = 3.08 min. MS (ES): *m/z* (MH^+) 637.62 for C39H76N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.13 (br. m, 4H); 3.05-2.20 (br. m, 10H); 2.00-1.00 (br. m, 56H); 0.90 (t, 6H).

Step 2: 3-((2-(Piperidin-4-yl)ethyl)(tetradecyl)amino)propyl decanoate



Chemical Formula: C34H68N2O2

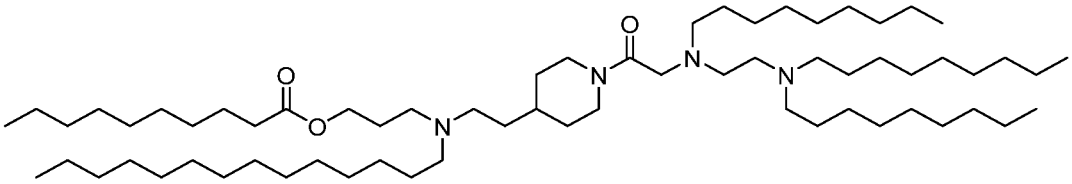
Molecular Weight: 536.93

[00907] In the same manner as Step 4 for Compound 11, 3-((2-(piperidin-4-yl)ethyl)(tetradecyl)amino)propyl decanoate was synthesized from tert-butyl 4-(2-((3-(decanoyloxy)propyl)(tetradecyl)amino)ethyl)piperi dine-1-carboxylate (458 mg, 0.719 mmol), and TFA (2.8 mL, 36 mmol) in DCM (2.8 mL). Yield (384 mg, 99%).

UPLC/ELSD: RT = 2.13 min. MS (ES): *m/z* (MH^+) 537.57 for C34H68N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.12 (t, 2H); 3.12 (br. m, 2H); 2.71-2.24 (br. m, 10H); 1.85-1.03 (br. m, 47H); 0.90 (t, 6H).

Step 3: 3-((2-(l -(*N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(tetradecyl)amino)propyl decanoate



Chemical Formula: C65H130N4O3

Molecular Weight: 1015.78

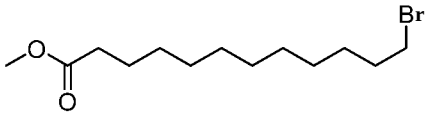
[00908] In the same manner as Step 11 for Compound 11, 3-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(tetradecyl)amino)propyl decanoate was synthesized from 3-((2-(piperidin-4-yl)ethyl)(tetradecyl)amino)propyl decanoate (384 mg, 0.715 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (323 mg, 0.650 mmol), iPr_2EtN (0.249 mL, 1.43 mmol), and T3P (50% EtOAc solution, 1.16 mL, 1.95 mmol) in THF (10 mL). Yield (226 mg, 34%).

UPLC/ELSD: RT = 3.53 min. MS (ES): *m/z* (MH^+) 1016.10 for C65H130N4O3

¾-NMR (300 MHz, CDCh) δ: ppm 4.65-2.22 (br. m, 26H); 1.94-1.00 (br. m, 89H); 0.90 (t, 15H).

CI: Compound 116: Methyl 12-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(tetradecyl)amino)dodecanoate

Step 1: Methyl 12-bromododecanoate



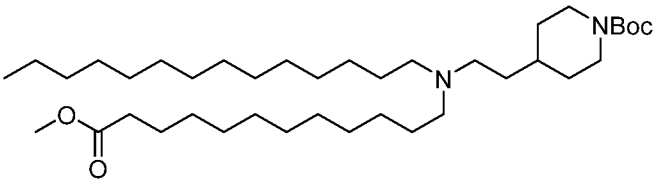
Chemical Formula: Ci3H25Br02

Molecular Weight: 293.25

[00909] In the same manner as Step 1 for Compound 15, methyl 12-bromododecanoate was synthesized from 12-bromododecanoic acid (3.0 g, 11 mmol), methanol (8.7 mL, 210 mmol), and sulfuric acid (0.57 mL, 11 mmol) in THF (10 mL). Yield (2.82 g, 90%).

ʿTi-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.43 (t, 2H); 2.32 (t, 2H); 1.87 (quint, 2H); 1.63 (m, 2H); 1.52-1.15 (br. m, 14H).

Step 2: *tert*-Butyl 4-(2-((12-methoxy-12-oxododecyl)(tetradecyl)amino)ethyl)piperidine-l-carboxylate



Chemical Formula: C39H76N2O4

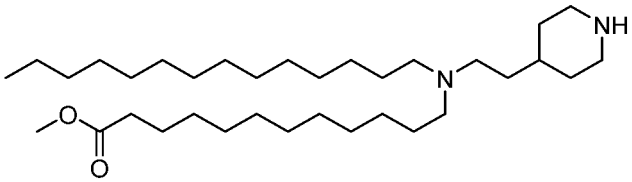
Molecular Weight: 637.05

[00910] In the same manner as Step 1 for Compound 57, *tert-b*utyl 4-(2-((12-methoxy-12-oxododecyl)(tetradecyl)amino)ethyl)piperidine-1-carboxylate was synthesized from *tert-butyl* 4-(2-(tetradecylamino)ethyl)piperidine-1-carboxylate (442 mg, 1.04 mmol), methyl 12-bromododecanoate (366 mg, 1.25 mmol), K2CO3 (288 mg, 2.08 mmol), and KI (17 mg, 0.10 mmol) in MeCN (20 mL). Yield (410 mg, 62%).

UPLC/ELSD: RT = 2.87 min. MS (ES): *m/z* (MH^+) 637.62 for C39H76N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.07 (br. m, 2H); 3.69 (s, 3H); 2.70 (br. m, 2H); 2.52-2.20 (br. m, 8H); 1.72-1.00 (br. m, 58H); 0.90 (t, 3H).

Step 3: Methyl 12-((2-(piperidin-4-yl)ethyl)(tetradecyl)amino)dodecanoate



Chemical Formula: C34H68N2O2

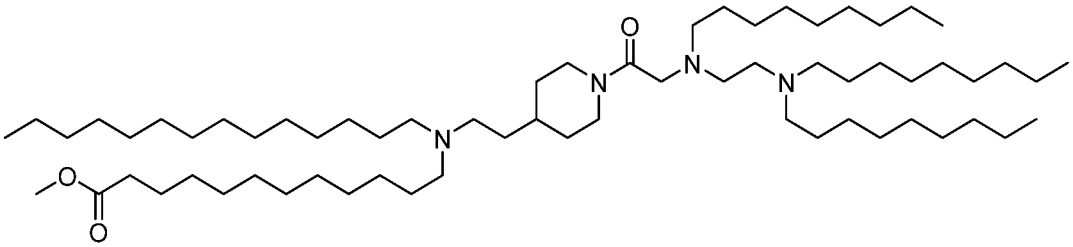
Molecular Weight: 536.93

[00911] In the same manner as Step 4 for Compound 11, methyl 12-((2-(piperidin-4-yl)ethyl)(tetradecyl)amino)dodecanoate was synthesized from *tert-butyl* 4-(2-((12-methoxy-12-oxododecyl)(tetradecyl)amino)ethyl)piperidine-1-carboxylate (410 mg, 0.644 mmol), and TFA (2.5 mL, 32 mmol) in DCM (2.5 mL). Yield (339 mg, 98%).

UPLC/ELSD: RT = 1.96 min. MS (ES): *m/z* (MH^+) 537.57 for C34H68N2O2

^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.09 (br. m, 2H); 2.70-2.24 (br. m, 10H); 1.76-1.05 (br. m, 49H); 0.90 (t, 3H).

Step 4: Methyl 12-((2-(l-*(N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(tetradecyl)amino)dodecanoate



Chemical Formula: C65H130N4O3

Molecular Weight: 1015.78

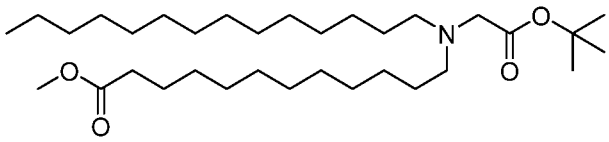
[00912] In the same manner as Step 11 for Compound 11, methyl 12-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl)(tetradecyl)amino)dodecanoate was synthesized from methyl 12-((2-(piperidin-4-yl)ethyl)(tetradecyl)amino)dodecanoate (339 mg, 0.631 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (376 mg, 0.758 mmol), iPrNEt (0.242 mL, 1.39 mmol), and T3P (50% EtOAc solution, 1.12 mL, 1.89 mmol) in THF (10 mL). Yield (81 mg, 13%).

UPLC/ELSD: RT = 3.41 min. MS (ES): *m/z* (MH^+) 1016.10 for C65H130N4O3

¾-NMR (300 MHz, CDCh) δ: ppm 4.62-4.12 (br. m, 2H); 3.69 (s, 3H); 3.41-2.20 (br. m, 22H); 1.82-1.00 (br. m, 91H); 0.90 (t, 12H).

CJ: Compound 117: Methyl 12-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(tetradecyl)amino)dodecanoate

Step 1: Methyl 12-((2-(*tert*-butoxy)-2-oxoethyl)(tetradecyl)amino)dodecanoate



Chemical Formula: C33H65NO4

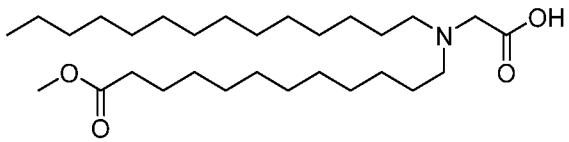
Molecular Weight: 539.89

[00913] In the same manner as Step 3 for Compound 44, methyl 12-((2-(/er/-butoxy)-2-oxoethyl)(tetradecyl)amino)dodecanoate was synthesized from *tert*-butyl tetradecylglycinate (500 mg, 1.53 mmol), methyl 12-bromododecanoate (537 mg, 1.83 mmol), K2CO3 (422 mg, 3.05 mmol), and KI (25 mg, 0.15 mmol) in MeCN (20 mL). Yield (562 mg, 68%).

UPLC/ELSD: RT = 2.78 min. MS (ES): *m/z* (MH^+) 540.52 for C33H65NO4

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.23 (s, 2H); 2.57 (t, 4H); 2.32 (t, 2H); 1.70-1.02 (br. m, 51H); 0.90 (t, 3H).

Step 2: *N*-(12-methoxy-12-oxododecyl)-*N*-tetradecylglycine



Chemical Formula: C29H57NO4

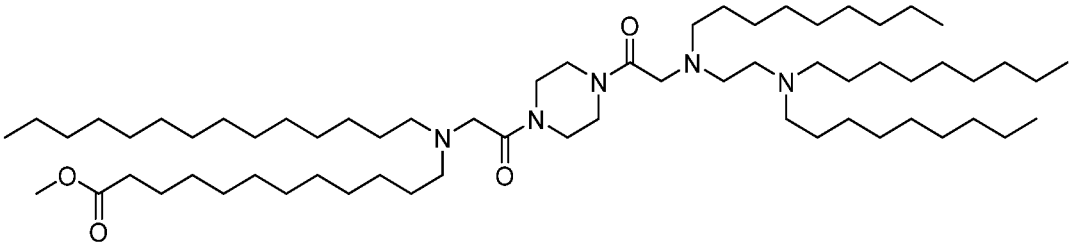
Molecular Weight: 483.78

[00914] In the same manner as Step 4 for Compound 44, *N*-(12-methoxy-12-oxododecyl)-*N*-tetradecylglycine was synthesized from methyl 12-((2-(*tert*-butoxy)-2-oxoethyl)(tetradecyl)amino)dodecanoate (562 mg, 1.04 mmol), and TFA (4.0 mL, 52 mmol) in DCM (4 mL). Yield (486 mg, 97%).

UPLC/ELSD: RT = 2.43 min. MS (ES): *m/z* (MH^+) 484.43 for C29H57NO4

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 2.50-2.45 (br. m, 6H); 2.32 (t, 2H); 1.80-1.02 (br. m, 42H); 0.90 (t, 3H).

Step 3: Methyl 12-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(tetradecyl)amino)dodecanoate



Chemical Formula: **C64H127N5O4**

Molecular Weight: 1030.75

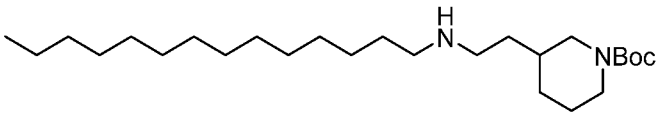
[00915] In the same manner as Step 7 for Compound 44, methyl 12-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(tetradecyl)amino)dodecanoate was synthesized from *N*-(12-methoxy-12-oxododecyl)-*N*-tetradecylglycine (248 mg, 0.513 mmol), 2-((2-(dinonylamino)ethyl)(nonyl)amino)-1-(piperazin-1-yl)ethan-1-one (348 mg, 0.615 mmol), iPr_2EtN (0.196 mL, 1.13 mmol), and T3P (50% EtOAc solution, 0.916 mL, 1.54 mmol). Yield (277 mg, 52%).

UPLC/ELSD: RT = 3.28 min. MS (ES): *m/z* (MH^+) 1031.02 for **C64H127N5O4**

^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.75-3.52 (br. m, 11H); 3.36-3.21 (br. m, 4H); 2.65-2.25 (br. m, 16H); 1.70-1.05 (br. m, 84H); 0.90 (t, 12H).

CK: Compound 120: Pentyl 8-((2-(l -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperidin-3-yl)ethyl)(tetradecyl)amino)octanoate

Step 1: *tert*-Butyl 3-(2-(tetradecylamino)ethyl)piperi dine- 1-carboxy late

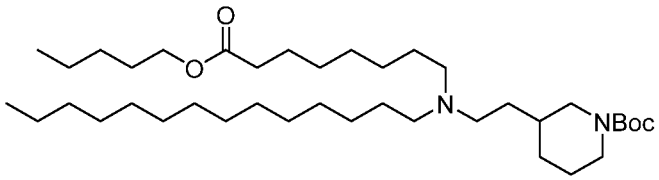


Chemical Formula: C26H52N2O2

Molecular Weight: 424.7 1

[00916] In the same manner as Step 1 for Compound 49, *tert-butyl* 3-(2-(tetradecylamino)ethyl)piperi dine- 1-carboxy late was synthesized from *tert-butyl* 3-(2-aminoethyl)piperi dine- 1-carboxy late (3.0 g, 13 mmol), 1-bromotetradecane (3.6 g, 13 mmol), K2CO3 (3.6 g, 26 mmol), and KI (218 mg, 1.31 mmol) in MeCN (100 mL). Yield (2.2 g, 39%).
UPLC/ELSD: RT = 2.09 min. MS (ES): *m/z* (MH^+) 425.46 for C26H52N2O2
¾-NMR (300 MHz, CDCh) δ: ppm 4.03-2.50 (br. m, 8H); 1.92-1.00 (br. m, 41H); 0.90 (t, 3H).

Step 2: *tert-Butyl* 3-(2-((8-oxo-8-(pentyloxy)octyl)(tetradecyl)amino)ethyl)piperidine-l-carboxylate

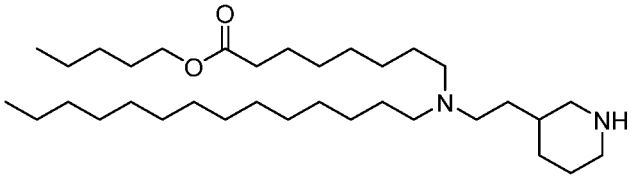


Chemical Formula: C39H76N2O4

Molecular Weight: 637.05

[00917] In the same manner as Step 1 for Compound 57, *tert-butyl* 3-(2-((8-oxo-8-(pentyloxy)octyl)(tetradecyl)arnino)ethyl)piperidine-l -carboxylate was synthesized from *tert-butyl* 3-(2-(tetradecylamino)ethyl)piperi dine- 1-carboxy late (500 mg, 1.18 mmol), pentyl 8-bromooctanoate (449 mg, 1.53 mmol), K2CO3 (325 mg, 2.36 mmol), and KI (20 mg, 0.12 mmol) in MeCN (20 mL). Yield (474 mg, 63%).
UPLC/ELSD: RT = 2.96 min. MS (ES): *m/z* (MH^+) 637.62 for C39H76N2O4
¾-NMR (300 MHz, CDCh) δ: ppm 4.17-3.80 (br. m, 4H); 2.90-2. 15 (br. m, 10H); 1.90-1.05 (br. m, 56H); 0.92 (m, 6H).

Step 3: Pentyl 8-((2-(piperidin-3-yl)ethyl)(tetradecyl)amino)octanoate



Chemical Formula: C34H68N2O2

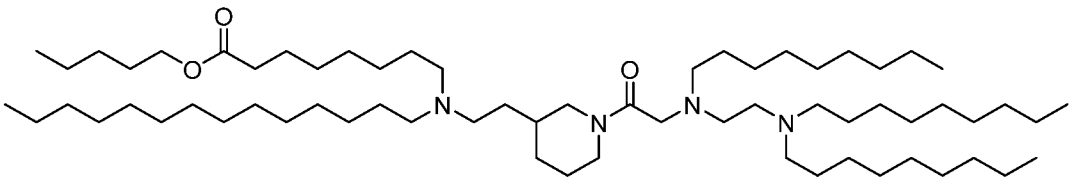
Molecular Weight: 536.93

[00918] In the same manner as Step 4 for Compound 11, pentyl 8-((2-(piperidin-3-yl)ethyl)(tetradecyl)amino)octanoate was synthesized from *tert-b*utyl 3-(2-((8-oxo-8-(pentyloxy)octyl)(tetradecyl)amino)ethyl)piperi dine- 1-carboxy late (474 mg, 0.744 mmol), and TFA (2.8 mL, 37 mmol) in DCM (2.8 mL). Yield (383 mg, 96%).

UPLC/ELSD: RT = 2.01 min. MS (ES): *m/z* (MH^+) 537.57 for C34H68N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.03 (br. m, 2H); 2.67-0.98 (br. m, 58H); 0.91 (m, 6H).

Step 4: Pentyl 8-((2-(l -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperidin-3-yl)ethyl)(tetradecyl)amino)octanoate



Chemical Formula: C65H130N4O3

Molecular Weight: 1015.78

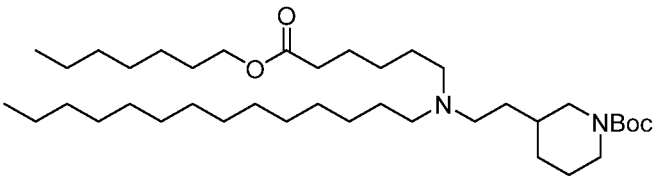
[00919] In the same manner as Step 11 for Compound 11, pentyl 8-((2-(l -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperidin-3-yl)ethyl)(tetradecyl)amino)octanoate was synthesized from pentyl 8-((2-(piperidin-3-yl)ethyl)(tetradecyl)amino)octanoate (383 mg, 0.713 mmol), *N*-(2-(dinonylamino)ethyl)-N-nonylglycine (425 mg, 0.856 mmol), iPr_2EtN (0.273 mL, 1.57 mmol), and T3P (50% EtOAc solution, 1.27 mL, 2.14 mmol) in THF (lOmL). Yield (243 mg, 34%).

UPLC/ELSD: RT = 3.44 min. MS (ES): *m/z* (MH^+) 1016.02 for C65H130N4O3

¾-NMR (300 MHz, CDCh) δ: ppm 4.50-3.96 (br. m, 4H); 3.46-2.86 (br. m, 4H); 2.72-2.20 (br. m, 18H); 2.00-1.05 (br. m, 89H); 0.91 (m, 15H).

CL: Compound 121: Heptyl 6-((2-(l -*(N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-3-yl)ethyl)(tetradecyl)amino)hexanoate

Step 1: *tert*-Butyl 3-(2-((6-(heptyloxy)-6-oxohexyl)(tetradecyl)amino)ethyl)piperidine-l-carboxylate



Chemical Formula: C39H76N2O4

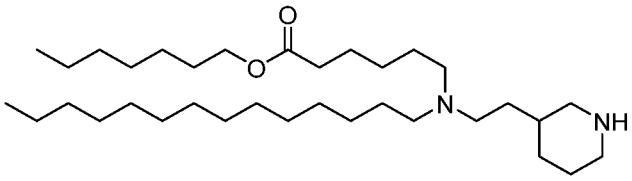
Molecular Weight: 637.05

[00920] In the same manner as Step 1 for Compound 57, *tert*-butyl 3-(2-((6-(heptyloxy)-6-oxohexyl)(tetradecyl)amino)ethyl)piperidine-1-carboxylate was synthesized from *tert*-butyl 3-(2-(tetradecylamino)ethyl)piperidine-l -carboxylate (500 mg, 1.18 mmol), heptyl 6-bromohexanoate (449 mg, 1.53 mmol), K2CO3 (325 mg, 2.36 mmol), and KI (20 mg, 0.12 mmol) in MeCN (20 mL). Yield (520 mg, 69%).

UPLC/ELSD: RT = 3.02 min. MS (ES): *m/z* (MH^+) 637.71 for C39H76N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.20-3.80 (br. m, 4H); 2.90-2.20 (br. m, 10H); 1.90-1.00 (br. m, 56H); 0.90 (t, 6H).

Step 2: Heptyl 6-((2-(piperidin-3-yl)ethyl)(tetradecyl)amino)hexanoate



Chemical Formula: C34H68N2O2

Molecular Weight: 536.93

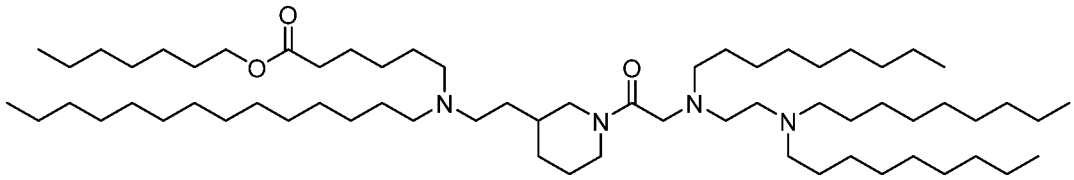
[00921] In the same manner as Step 4 for Compound 11, heptyl 6-((2-(piperidin-3-yl)ethyl)(tetradecyl)amino)hexanoate was synthesized from tot-butyl 3-(2-((6-(heptyloxy)-6-oxohexyl)(tetradecyl)amino)ethyl)piperidine-l -carboxylate (520 mg, 0.816 mmol), and TFA (3.1 mL, 41 mmol) in DCM (3.1 mL). Yield (407 mg, 93%).

UPLC/ELSD: RT = 2.06 min. MS (ES): *m/z* (MH^+) 537.57 for C34H68N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.03 (br. m, 2H); 2.70-1.00 (br. m, 58H); 0.90

(t, 6H).

Step 3: Heptyl 6-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-3-yl)ethyl)(tetradecyl)amino)hexanoate



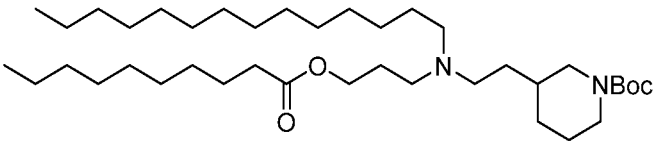
Chemical Formula: C65H130N4O3

Molecular Weight: 1015.78

[00922] In the same manner as Step 11 for Compound 11, heptyl 6-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-3-yl)ethyl)(tetradecyl)amino)hexanoate was synthesized from heptyl 6-((2-(piperidin-3-yl)ethyl)(tetradecyl)amino)hexanoate (407 mg, 0.758 mmol), N-(2-(dinonylamino)ethyl)-N-nonylglycine (452 mg, 0.910 mmol), iPr_2EtN (0.290 mL, 1.67 mmol), and T3P (50% EtOAc solution, 1.35 mL, 2.27 mmol). Yield (218 mg, 28.3%). UPLC/ELSD: RT = 3.45 min. MS (ES): *m/z* (MH^+) 1016.10 for C65H130N4O3 ¾-NMR (300 MHz, CDCh) δ: ppm 4.50-4.00 (br. m, 4H); 3.44-2.84 (br. m, 4H); 2.68-2.24 (br. m, 18H); 1.95-1.05 (br. m, 89H); 0.90 (t, 15H).

CM: Compound 122: 3-((2-(1-(*N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-3-yl)ethyl)(tetradecyl)amino)propyl decanoate

Step 1: *tert*-Butyl 3-(2-((3-(decanoyloxy)propyl)(tetradecyl)amino)ethyl)piperidine-1-carboxylate



Chemical Formula: C39H76N2O4

Molecular Weight: 637.05

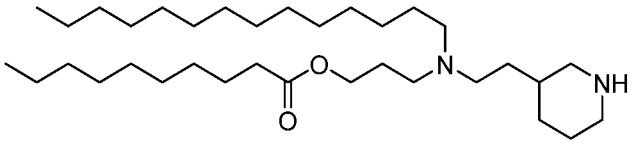
[00923] In the same manner as Step 1 for Compound 57, tert-butyl 3-(2-((3-(decanoyloxy)propyl)(tetradecyl)amino)ethyl)piperidine-1-carboxylate was synthesized from tert-butyl 3-(2-(tetradecylamino)ethyl)piperidine-1-carboxylate (550 mg, 1.30 mmol), 3-

bromopropyl decanoate (494 mg, 1.68 mmol), K2CO3 (358 mg, 2.59 mmol), and KI (21 mg, 0.13 mmol) in MeCN (20 mL). Yield (560 mg, 68%).

UPLC/ELSD: RT = 3.12 min. MS (ES): *m/z* (MH^+) 637.62 for C39H76N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.16-3.79 (br. m, 4H); 2.95-2.20 (br. m, 10H); 1.90-1.00 (br. m, 56H); 0.90 (t, 6H).

3-((2-(Piperidin-3-yl)ethyl)(tetradecyl)amino)propyl decanoate



Chemical Formula: C34H68N2O2

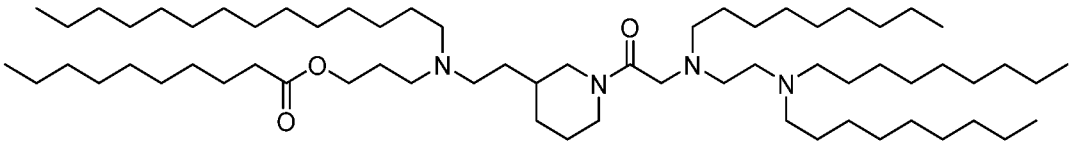
Molecular Weight: 536.93

[00924] In the same manner as Step 4 for Compound 11, 3-((2-(piperidin-3-yl)ethyl)(tetradecyl)amino)propyl decanoate was synthesized from *tert-b*utyl 3-(2-((3-(decanoyloxy)propyl)(tetradecyl)amino)ethyl)piperidine-l -carboxylate (560 mg, 0.879 mmol), and TFA (3.4 mL, 44 mmol) in DCM (3.4 mL). Yield (444 mg, 94%).

UPLC/ELSD: RT = 2.16 min. MS (ES): *m/z* (MH^+) 537.49 for C34H68N2O2

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.12 (t, 2H); 3.02 (m, 2H); 2.62-1.00 (br. m, 58H); 0.90 (t, 6H).

3-((2-(l -*(N*-(2-(Dinonylamino)ethyl *)-N*-nonylglycyl)piperidin-3-yl)ethyl)(tetradecyl)amino)propyl decanoate



Chemical Formula: C65H130N4O3

Molecular Weight: 1015.78

[00925] In the same manner as Step 11 for Compound 11, 3-((2-(l -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperidin-3-yl)ethyl)(tetradecyl)amino)propyl decanoate was synthesized from 3-((2-(piperidin-3-yl)ethyl)(tetradecyl)amino)propyl decanoate (444 mg, 0.827 mmol), *N*-(2-(dinonylamino)ethyl *)-N*-nonylgly cine (493 mg, 0.992 mmol), iPr_2EtN (0.317

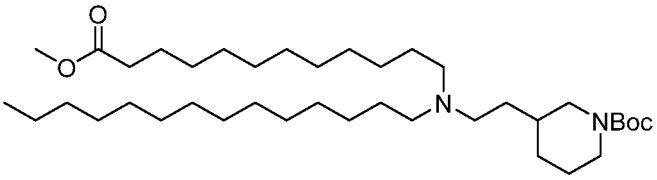
mL, 1.82 mmol), and T3P (50% EtOAc solution, 1.46 mL, 2.48 mmol) in THF (10 mL). Yield (271 mg, 32%).

UPLC/ELSD: RT = 3.49 min. MS (ES): *m/z* (MH^+) 1016.10 for $C_{65}H_{130}N_4O_3$

¾-NMR (300 MHz, CDCh) δ: ppm 4.56-3.96 (br. m, 4H); 3.42-2. 85 (br. m, 4H); 2.74-2. 18 (br. m, 18H); 1.95-1 .00 (br. m, 89H); 0.90 (t, 15H).

CN: Compound 123: Methyl 12-((2-(l -*(N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-3-yl)ethyl)(tetradecyl)amino)dodecanoate

tert-Butyl 3-(2-((12-methoxy-12-oxododecyl)(tetradecyl)amino)ethyl)piperidine-l-carboxylate



Chemical Formula: C39H76N2O4

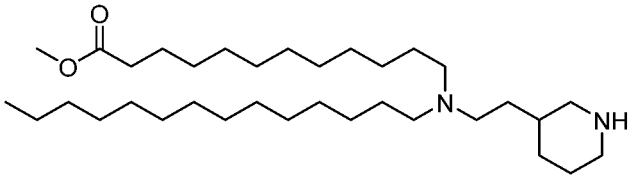
Molecular Weight: 637.05

[00926] In the same manner as Step 1 for Compound 57, *tert-butyl* 3-(2-((12-methoxy-12-oxododecyl)(tetradecyl)amino)ethyl)piperidine-1-carboxylate was synthesized from *tert-butyl* 3-(2-(tetradecylamino)ethyl)piperidine-1-carboxylate (550 mg, 1.30 mmol), methyl 12-bromododecanoate (494 mg, 1.68 mmol), K2CO3 (358 mg, 2.59 mmol), and KI (21 mg, 0.13 mmol) in MeCN (20 mL). Yield (544 mg, 66%).

UPLC/ELSD: RT = 2.92 min. MS (ES): *m/z* (MH^+) 637.54 for C39H76N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 3.91 (m, 2H); 3.69 (s, 3H); 2.86-2.25 (br. m, 10H); 1.90-1.00 (br. m, 58H); 0.90 (t, 3H).

Methyl 12-((2-(piperidin-3-yl)ethyl)(tetradecyl)amino)dodecanoate

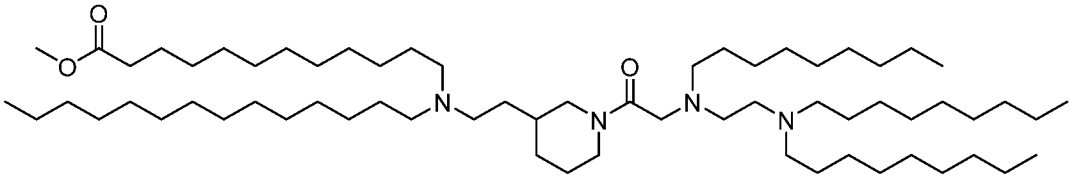


Chemical Formula: C34H68N2O2

Molecular Weight: 536.93

[00927] In the same manner as Step 4 for Compound 11, methyl 12-((2-(piperidin-3-yl)ethyl)(tetradecyl)amino)dodecanoate was synthesized from *tert*-butyl 3-(2-((12-methoxy-12-oxododecyl)(tetradecyl)amino)ethyl)piperidine-1-carboxylate (544 mg, 0.854 mmol), and TFA (3.3 mL, 43 mmol) in DCM (3.3 mL). Yield (440 mg, 96%)
UPLC/ELSD: RT = 1.94 min. MS (ES): *m/z* (MH^+) 537.32 for $C_{34}H_{68}N_2O_2$
^{1}H-NMR (300 MHz, $CDCl_3$) δ: ppm 3.69 (s, 3H); 3.03 (m, 2H); 2.66-2.20 (br. m, 10H); 1.91-1.00 (br. m, 50H); 0.90 (t, 3H).

Methyl 12-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-3-yl)ethyl)(tetradecyl)amino)dodecanoate

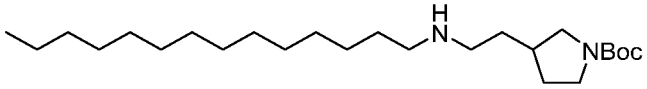


Chemical Formula: $C_{65}H_{130}N_4O_3$
Molecular Weight: 1015.78

[00928] In the same manner as Step 11 for Compound 11, methyl 12-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-3-yl)ethyl)(tetradecyl)amino)dodecanoate was synthesized from methyl 12-((2-(piperidin-3-yl)ethyl)(tetradecyl)amino)dodecanoate (440 mg, 0.819 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (489 mg, 0.983 mmol), iPr_2EtN (0.314 mL, 1.80 mmol), and T3P (50% EtOAc solution, 1.46 mL, 2.46 mmol) in THF (10 mL)
UPLC/ELSD: RT = 3.39 min. MS (ES): *m/z* (MH^+) 1016.10 for $C_{65}H_{130}N_4O_3$
^{1}H-NMR (300 MHz, $CDCl_3$) δ: ppm 4.50-4.00 (br. m, 2H); 3.69 (s, 3H); 3.32 (br. m, 2H); 3.00-2.20 (br. m, 20H); 1.95-1.05 (br. m, 91H); 0.90 (t, 12H).

CO: Compound 124: Pentyl 8-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)pyrrolidin-3-yl)ethyl)(tetradecyl)amino)octanoate

/er/-Butyl 3-(2-(tetradecylamino)ethyl)pyrrolidine-1-carboxylate



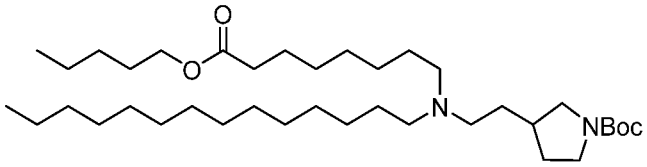
Chemical Formula: $C_{25}H_{50}N_2O_2$
Molecular Weight: 410.69

[00929] In the same manner as Step 1 for Compound 49, *tert-butyl* 3-(2-(tetradecylamino)ethyl)pyrrolidine-1-carboxylate was synthesized from *tert-b*utyl 3-(2-aminoethyl)pyrrolidine-l-carboxylate (1.0 g, 4.7 mmol), 1-bromotetradecane (1.3 g, 4.7 mmol), K_2CO_3 (1.3 g, 9.3 mmol), and KI (77 mg, 0.47 mmol) in MeCN (50 mL). Yield (784 mg, 41%).

UPLC/ELSD: RT = 2.00 min. MS (ES): *m/z* (MH^+) 411.43 for $C_{25}H_{50}N_2O_2$

¾-NMR (300 MHz, CDCh) δ: ppm 3.70-1.90 (br. m, 8H); 1.85-1.05 (br. m, 39H); 0.90 (t, 3H).

tert-Butyl 3-(2-((8-oxo-8-(pentyloxy)octyl)(tetradecyl)amino)ethyl)pyrrolidine-l-carboxylate



Chemical Formula: $C_{38}H_{74}N_2O_4$

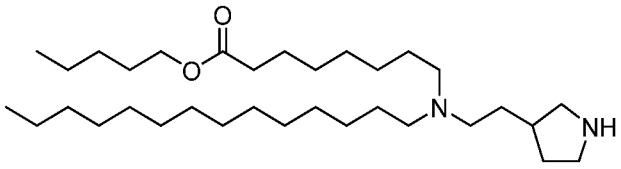
Molecular Weight: 623.02

[00930] In the same manner as Step 1 for Compound 57, *tert-butyl* 3-(2-((8-oxo-8-(pentyloxy)octyl)(tetradecyl)arnino)ethyl)pyrrolidine-1-carboxylate was synthesized from tert-butyl 3-(2-aminoethyl)pyrrolidine-1-carboxylate (196 mg, 0.477 mmol), pentyl 8-bromooctanoate (168 mg, 0.573 mmol), K_2CO_3 (132 mg, 0.954 mmol), KI (8 mg, 0.05 mmol). Yield (119 mg, 40%).

UPLC/ELSD: RT = 2.82 min. MS (ES): *m/z* (MH^+) 623.60 for $C_{38}H_{74}N_2O_4$

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.65-2.78 (br. m, 4H); 2.50-1.88 (br. m, 8H); 1.76-1.05 (br. m, 54H); 0.91 (m, 6H).

Pentyl 8-((2-(pyrrolidin-3-yl)ethyl)(tetradecyl)amino)octanoate



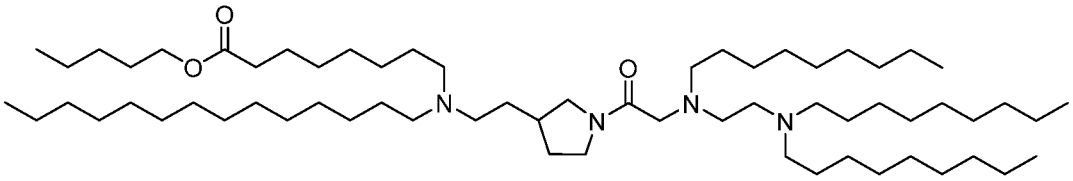
Chemical Formula: $C_{33}H_{66}N_2O_2$

Molecular Weight: 522.90

[00931] In the same manner as Step 4 for Compound 11, pentyl 8-((2-(pyrrolidin-3-yl)ethyl)(tetradecyl)amino)octanoate was synthesized from *tert-butyl* 3-(2-((8-oxo-8-(pentyloxy)octyl)(tetradecyl)amino)ethyl)pyrrolidine-l-carboxylate (119 mg, 0.191 mmol), and TFA (0.73 mL, 9.6 mmol) in DCM (1 mL). Yield (97 mg, 97%).

UPLC/ELSD: RT = 1.97 min. MS (ES): *m/z* (MH^+) 523.55 for $C_{33}H_{66}N_2O_2$

Pentyl 8-((2-(l -(N -(2-(dinonylamino)ethyl)-N -nonylglycyl)pyrrolidin-3-yl)ethyl)(tetradecyl)amino)octanoate



Chemical Formula: C64H128N4O3

Molecular Weight: 1001.75

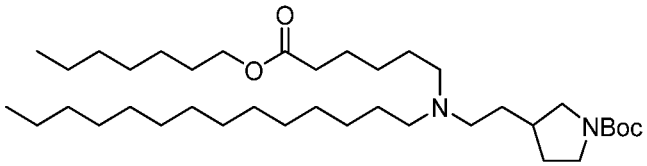
[00932] In the same manner as Step 11 for Compound 11, pentyl 8-((2-(l-(N-(2-(dinonylamino)ethyl)-N-nonylglycyl)pyrrolidin-3-yl)ethyl)(tetradecyl)amino)octanoate was synthesized from pentyl 8-((2-(pyrrolidin-3-yl)ethyl)(tetradecyl)amino)octanoate (97 mg, 0.19 mmol), N-(2-(dinonylamino)ethyl)-N-nonylglycine (111 mg, 0.22 mmol), iPnEtN (71 μL, 0.41 mmol), and T3P (50% EtOAc solution, 0.33 mL, 0.56 mmol). Yield (82 mg, 44%).

UPLC/ELSD: RT = 3.41 min. MS (ES): *m/z* (MH^+) 1001.99 for $C_{64}H_{128}N_4O_3$

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.85-1.90 (br. m, 24H); 1.80-1.05 (br. m, 87H); 0.90 (m, 15H).

CP: Compound 125: Heptyl 6-((2-(l -(N -(2-(dinonylamino)ethyl)-N -nonylglycyl)pyrrolidin-3-yl)ethyl)(tetradecyl)amino)hexanoate

tot-Butyl 3-(2-((6-(heptyloxy)-6-oxohexyl)(tetradecyl)amino)ethyl)pyrrolidine- 1-carboxylate



Chemical Formula: C38H74N2O4

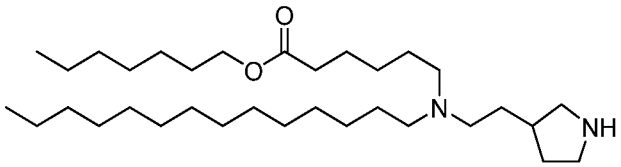
Molecular Weight: 623.02

[00933] In the same manner as Step 1 for Compound 57, tot-butyl 3-(2-((6-(heptyloxy)-6-oxohexyl)(tetradecyl)amino)ethyl)pyrrolidine- 1-carboxylate was synthesized from tot-butyl 3-(2-aminoethyl)pyrrolidine-l -carboxylate (196 mg, 0.477 mmol), heptyl 6-bromohexanoate (168 mg, 0.573 mmol), K2CO3 (132 mg, 0.954 mmol), KI (8 mg, 0.05 mmol). Yield (121 mg, 41%).

UPLC/ELSD: RT = 2.90 min. MS (ES): *m/z* (MH^+) 623.60 for C38H74N2O4

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.65-2.75 (br. m, 4H); 2.55-1.90 (br. m, 8H); 1.75-1.05 (br. m, 54H); 0.91 (t, 6H).

Heptyl 6-((2-(pyrrolidin-3-yl)ethyl)(tetradecyl)amino)hexanoate



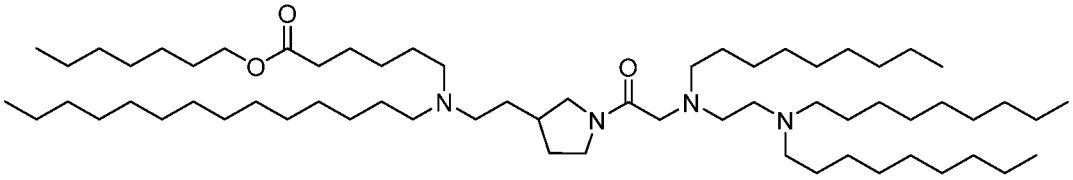
Chemical Formula: C33H66N2O2

Molecular Weight: 522.90

[00934] In the same manner as Step 4 for Compound 11, heptyl 6-((2-(pyrrolidin-3-yl)ethyl)(tetradecyl)amino)hexanoate was synthesized from *tert*-butyl 3-(2-((6-(heptyloxy)-6-oxohexyl)(tetradecyl)amino)ethyl)pyrrolidine-l-carboxylate (121 mg, 0.194 mmol), and TFA (0.74 mL, 9.7 mmol) in DCM (1 mL). Yield (97 mg, 96%).

UPLC/ELSD: RT = 2.06 min. MS (ES): *m/z* (MH^+) 523.46 for C33H66N2O2

Heptyl 6-((2-(l -*(N* -(2-(dinonylamino)ethyl *)-N* -nonylglycyl)pyrrolidin-3-yl)ethyl)(tetradecyl)amino)hexanoate



Chemical Formula: C64H128N4O3

Molecular Weight: 1001.75

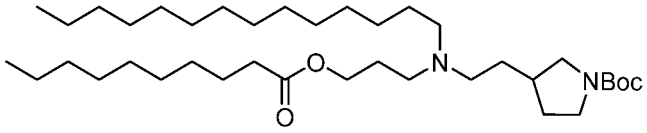
[00935] In the same manner as Step 11 for Compound 11, heptyl 6-((2-(l -*(N* -(2-(dinonylamino)ethyl *)-N* -nonylglycyl)pyrrolidin-3-yl)ethyl)(tetradecyl)amino)hexanoate was synthesized from heptyl 6-((2-(pyrrolidin-3-yl)ethyl)(tetradecyl)amino)hexanoate (97 mg, 0.19 mmol), *N* -(2-(dinonylamino)ethyl *)-N* -nonylglycine (111 mg, 0.22 mmol), iPr_2EtN (71 μL, 0.41 mmol), and T3P (50% EtOAc solution, 0.33 mL, 0.56 mmol). Yield (75 mg, 40%).

UPLC/ELSD: RT = 3.42 min. MS (ES): *m/z* (MH^+) 1002.07 for C64H128N4O3

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.70-1.90 (br. m, 24H); 1.75-1.05 (87H); 0.90 (t, 15H).

CQ: Compound 126: 3-((2-(l -*(N* -(2-(Dinonylamino)ethyl *)-N* -nonylglycyl)pyrrolidin-3-yl)ethyl)(tetradecyl)amino)propyl decanoate

ter/-Butyl 3-(2-((3-(decanoyloxy)propyl)(tetradecyl)amino)ethyl)pyrrolidine-l-carboxylate



Chemical Formula: C38H74N2O4

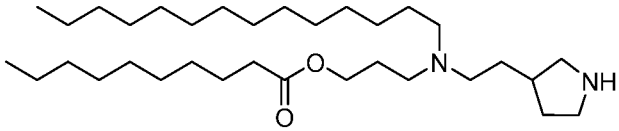
Molecular Weight: 623.02

[00936] In the same manner as Step 1 for Compound 57, *tert-b*utyl 3-(2-((3-(decanoyloxy)propyl)(tetradecyl)amino)ethyl)pyrrolidine-l-carboxylate was synthesized from *tert-butyl* 3-(2-aminoethyl)pyrrolidine-l-carboxylate (196 mg, 0.477 mmol), 3-bromopropyl decanoate (168 mg, 0.573 mmol), K2CO3 (132 mg, 0.954 mmol), KI (8 mg, 0.05 mmol). Yield (132 mg, 44%).

UPLC/ELSD: RT = 2.97 min. MS (ES): *m/z* (MH^+) 623.60 for C38H74N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.12 (t, 2H); 3.70-2.75 (br. m, 4H); 2.55-1.90 (br. m, 8H); 1.85-1.05 (br. m, 54H); 0.90 (t, 6H).

3-((2-(Pyrrolidin-3-yl)ethyl)(tetradecyl)amino)propyl decanoate



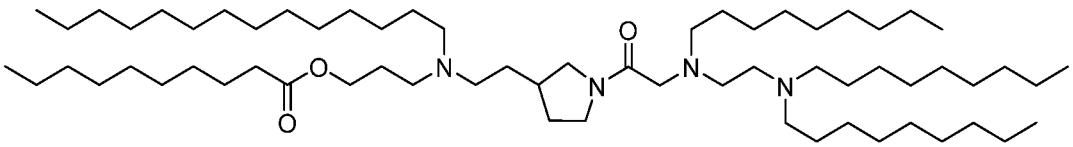
Chemical Formula: C33H66N2O2

Molecular Weight: 522.90

[00937] In the same manner as Step 4 for Compound 11, 3-((2-(pyrrolidin-3-yl)ethyl)(tetradecyl)amino)propyl decanoate was synthesized from *tert-butyl* 3-(2-((3-(decanoyloxy)propyl)(tetradecyl)amino)ethyl)pyrrolidine-l-carboxylate (132 mg, 0.212 mmol), and TFA (0.81 mL, 11 mmol) in DCM (1 mL). Yield (106 mg, 96%).

UPLC/ELSD: RT = 2.15 min. MS (ES): *m/z* (MH^+) 523.63 for C33H66N2O2

3-((2-(l -*(N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)pyrrolidin-3-yl)ethyl)(tetradecyl)amino)propyl decanoate



Chemical Formula: C64H128N4O3

Molecular Weight: 1001.75

[00938] In the same manner as Step 11 for Compound 11, 3-((2-(l -*(N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)pyrrolidin-3-yl)ethyl)(tetradecyl)amino)propyl decanoate

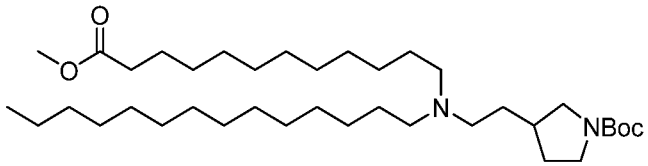
was synthesized from 3-((2-(pyrrolidin-3-yl)ethyl)(tetradecyl)amino)propyl decanoate (106 mg, 0.203 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (131 mg, 0.264 mmol), iPr_2EtN (78 µL, 0.45 mmol) and T3P (50% EtOAc solution, 0.36 mL, 0.61 mmol) in THF (5 mL). Yield (75 mg, 37%).

UPLC/ELSD: RT = 3.51 min. MS (ES): *m/z* (MH^+) 1001.99 for $C_{64}H_{128}N_4O_3$

¾-NMR (300 MHz, CDCh) δ: ppm 4.12 (t, 2H); 3.80-2.00 (br. m, 24H); 1.85-1.10 (87H); 0.90 (t, 15H).

CR: Compound 127: Methyl 12-((2-(1-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)pyrrolidin-3-yl)ethyl)(tetradecyl)amino)dodecanoate

tert-Butyl 3-(2-((12-methoxy-12-oxododecyl)(tetradecyl)amino)ethyl)pyrrolidine-1-carboxylate



Chemical Formula: C38H74N2O4

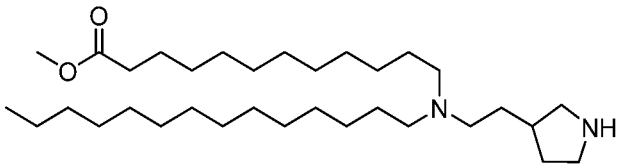
Molecular Weight: 623.02

[00939] In the same manner as Step 1 for Compound 57, *tert*-butyl 3-(2-((12-methoxy-12-oxododecyl)(tetradecyl)amino)ethyl)pyrrolidine-1-carboxylate was synthesized from from tert-butyl 3-(2-aminoethyl)pyrrolidine-1-carboxylate (196 mg, 0.477 mmol), methyl 12-bromododecanoate (168 mg, 0.573 mmol), K2CO3 (132 mg, 0.954 mmol), KI (8 mg, 0.05 mmol). Yield (134 mg, 45%).

UPLC/ELSD: RT = 2.83 min. MS (ES): *m/z* (MH^+) 623.52 for C38H74N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.65-2.75 (br. m, 4H); 2.50-1.90 (8H); 1.75-1.05 (br. m, 56H); 0.90 (t, 3H).

Methyl 12-((2-(pyrrolidin-3-yl)ethyl)(tetradecyl)amino)dodecanoate



Chemical Formula: C33H66N2O2

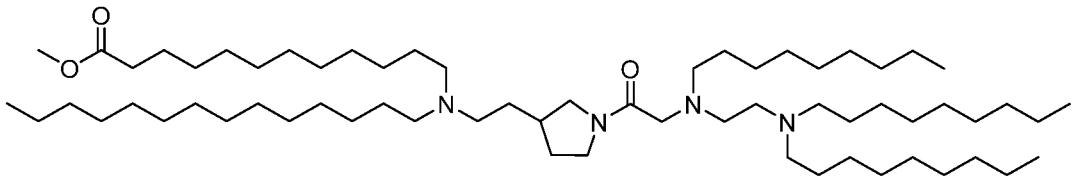
Molecular Weight: 522.90

[00940] In the same manner as Step 4 for Compound 11, methyl 12-((2-(pyrrolidin-3-yl)ethyl)(tetradecyl)amino)dodecanoate was synthesized from /er/-butyl 3-(2-((12-methoxy-12-

oxododecyl)(tetradecyl)amino)ethyl)pyrrolidine-l-carboxylate (134 mg, 0.215 mmol), and TFA (0.82 mL, 11 mmol) in DCM (1 mL). Yield (106 mg, 94%).

UPLC/ELSD: RT = 1.89 min. MS (ES): *m/z* (MH^+) 523.46 for C33H66N2O2

Methyl 12-((2-(l -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)pyrrolidin-3-yl)ethyl)(tetradecyl)amino)dodecanoate



Chemical Formula: C64H128N4O3

Molecular Weight: 1001.75

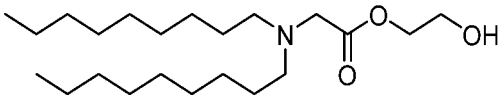
[00941] In the same manner as Step 11 for Compound 11, methyl 12-((2-(l -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)pyrrolidin-3-yl)ethyl)(tetradecyl)amino)dodecanoate was synthesized from methyl 12-((2-(pyrrolidin-3-yl)ethyl)(tetradecyl)amino)dodecanoate (106 mg, 0.203 mmol), *N*-(2-(dinonylamino)ethyl *)-N*-nonylglycine (121 mg, 0.243 mmol), iPr_2EtN (78 μL, 0.45 mmol), and T3P (50% EtOAc solution, 0.36 mL, 0.61 mmol) in THF (5 mL). Yield (42 mg, 21%).

UPLC/ELSD: RT = 3.38 min. MS (ES): *m/z* (MH^+) 1002.07 for C64H128N4O3

^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.80-2.90 (br. m, 7H); 2.75-1.95 (br. m, 20H); 1.80-1.05 (br. m, 89H); 0.90 (t, 12H).

CS: Compound 150: 2-*((N*-(2-(Dinonylamino)ethyl *)-N*-nonylglycyl)oxy)ethyl dinonylglycinate

2-Hydroxyethyl dinonylglycinate



Chemical Formula: C22H45NO3

Molecular Weight: 371.61

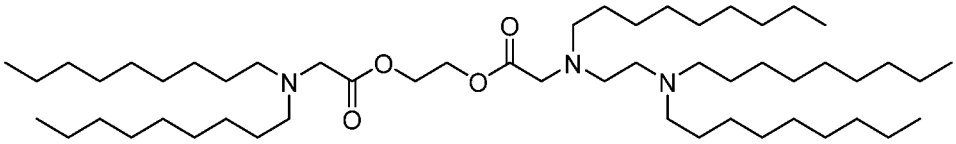
[00942] To a solution of dinonylglycine (500 mg, 1.53 mmol) in 10 mL THF was added ethylene glycol (284 mg, 4.58 mmol), DMAP (75 mg, 0.61 mmol), EDC (474 mg, 3.05 mmol) and iPr_2EtN (0.532 mL, 3.05 mmol), and the reaction was allowed to stir at RT for 12 hours. The reaction was diluted with DCM and washed with water. The organics were dried over anhydrous MgS04, filtered, and concentrated *in vacuo*. Purification by ISCO silica flash

chromatography (0-60% EtOAc/hexanes) provided 2-hydroxy ethyl dinonylglycinate (282 mg, 50%).

UPLC/ELSD: RT = 1.59 min. MS (ES): *m/z* (MH^+) 372.40 for $C_{22}H_{45}NO_3$

¾-NMR (300 MHz, CDCh) δ: ppm 4.27 (t, 2H); 3.86 (t, 2H); 3.39 (s, 2H); 2.58 (t, 4H); 2.12 (br, 1H); 1.58-1.06 (br. m, 28H); 0.90 (t, 6H).

2-*((N*-(2-(Dinonylamino)ethyl *)-N*-nonylglycyl)oxy)ethyl dinonylglycinate



Chemical Formula: $C_{53}H_{107}N_3O_4$

Molecular Weight: 850.46

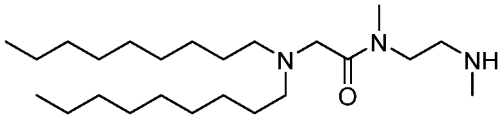
[00943] To a solution of 2-hydroxy ethyl dinonylglycinate (282 mg, 0.759 mmol) in THF (7 mL) was added *N*-(2-(dinonylamino)ethyl *)-N*-nonylgly cine (452 mg, 0.91 1 mmol), DMAP (37 mg, 0.30 mmol), EDC (236 mg, 1.52 mmol), and iPr_2EtN (0.264 mL, 1.52 mmol), and the reaction was allowed to stir at RT for 12 hours. The reaction was diluted with DCM and washed with water. The organics were dried over anhydrous Na2S04, filtered and concentrated *in vacuo.* Purification by ISCO C18 flash chromatography (10-100% water 0.1% TFA/MeCN 0.1% TFA) provided 2-*((N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)oxy)ethyl dinonylglycinate as a clear colorless oil (343 mg, 53%).

UPLC/ELSD: RT = 3.10 min. MS (ES): *m/z* (MH^+) 850.85 for $C_{53}H_{107}N_3O_4$

¾-NMR (300 MHz, CDCh) δ: ppm 4.33 (s, 4H); 3.43 (s, 2H); 3.37 (s, 2H); 2.70 (m, 2H); 2.57 (m, 8H); 2.41 (t, 4H); 1.55-1.05 (br. m, 70H); 0.90 (t, 15H).

CT: Compound 151: 2-(Dinonylamino *)-N*-(2-(2-((2-(dinonylamino)ethyl)(nonyl)amino *)-N*-methylacetamido)ethyl *)-N*-methylacetamide

2-(Dinonylamino *)-N*-methyl -*N*-(2-(methylamino)ethyl)acetamide



Chemical Formula: $C_{24}H_{51}N_3O$

Molecular Weight: 398.56

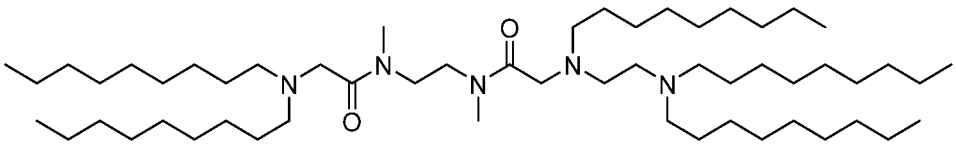
[00944] To a solution of dinonylglycine (500 mg, 1.53 mmol) in THF (15 mL) was added 1-2, dimethylethylenediamine (0.493 mL, 4.57 mmol), iPr_2EtN (0.585 mL, 3.36 mmol), and T3P

(50% EtOAc solution, 2.72 mL, 4.58 mmol), and the reaction was allowed to stir at RT for 12 hours, at which time the reaction had turned to a slushy mixture. The reaction was quenched with water and extracted with EtOAc. The combined organics were dried over anhydrous Na2S04, filtered, and concentrated *in vacuo*. Purification by ISCO silica flash chromatography (0-100% DCM/[DCM 20% MeOH 1% NH_4OH]) provided 2-(dinonylamino)-*N*-methyl-*N*-(2-(methylamino)ethyl)acetamide (261 mg, 43%).

UPLC/ELSD: RT = 1.09 min. MS (ES): *m/z* (MH^+) 398.56 for C24H51N3O

^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.65-2.35 (br. m, 16H); 1.56-1.04 (br. m, 29H); 0.90 (t, 6H)

2-(Dinonylamino)-*N*-(2-(2-((2-(dinonylamino)ethyl)(nonyl)amino)-*N*-methylacetamido)ethyl)-*N*-methylacetamide



Chemical Formula: C55H113N5O2

Molecular Weight: 876.54

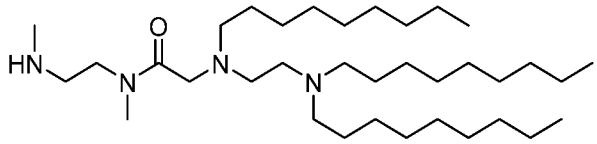
[00945] To a 0 °C solution of 2-(dinonylamino)-*N*-methyl-*N*-(2-(methylamino)ethyl)acetamide (261 mg, 0.656 mmol) and *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (391 mg, 0.788 mmol) in THF (10 mL) was added iPr_2EtN (0.251 mL, 1.44 mmol) and T3P (50% EtOAc solution, 0.586 mL, 1.97 mmol), and the reaction was allowed to stir for 1 hour at 0 °C. The reaction was quenched with water and extracted with EtOAc. The organics were dried over anhydrous Na2S04, filtered, and concentrated *in vacuo*. Purification by ISCO C18 flash chromatography (10-100% water 0.1% TFA/MeCN 0.1% TFA) provided 2-(dinonylamino)-*N*-(2-(2-((2-(dinonylamino)ethyl)(nonyl)amino)-*N*-methylacetand do)ethyl)-*N*-methylacetamide (117 mg, 20%).

UPLC/ELSD: RT = 3.03 min. MS (ES): *m/z* (MH^+) 876.93 for C55H113N5O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.73-2.25 (br. m, 28H); 1.60-0.99 (br. m, 70H); 0.90 (t, 15H).

CU: Compound 152: Pentyl 8,1 l-dimethyl-5,14,17-trinonyl-7,12-dioxo-5,8,l 1,14,17-pentaazahexacosanoate

2-((2-(Dinonylamino)ethyl)(nonyl)aniino)-*N*-methyl-*N*-(2-(methylandno)ethyl)acetami de



Chemical Formula: C35H74N4O

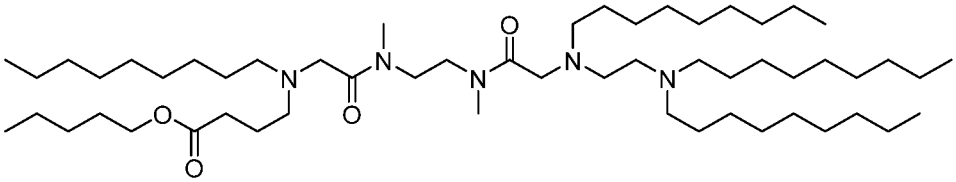
Molecular Weight: 567.00

[00946] To a 0 °C solution of *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (750 mg, 1.51 mmol) in THF (20 mL) was added 1-2, dimethylethylenediamine (0.650 mL, 6.04 mmol), iPr_2EtN (0.578 mL, 3.32 mmol), and T3P (50% EtOAc solution, 2.6 mL, 4.5 mmol), and the reaction was allowed to stir at 0 °C for 1 hour. The reaction was quenched with water and extracted with EtOAc. The organics were dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-100% DCM/[DCM 20% MeOH 1% NH_4OH]) provided 2-((2-(dinonylamino)ethyl)(nonyl)amino)-*N*-methyl-*N*-(2-(methylamino)ethyl)acetamide (480 mg, 56%).

UPLC/ELSD: RT = 2.04 min. MS (ES): *m/z* (MH^+) 567.59 for C35H74N4O

Ṫi-NMR (300 MHz, CDCh) δ: ppm 3.65-2.26 (br. m, 22H); 1.71-1.01 (br. m, 43H); 0.90 (t, 9H).

Pentyl 8,11-dimethyl-5,14,17-trinonyl-7,12-dioxo-5, 8,1 1,14,17-pentaazahexacosanoate



Chemical Formula: C55H111N5O4

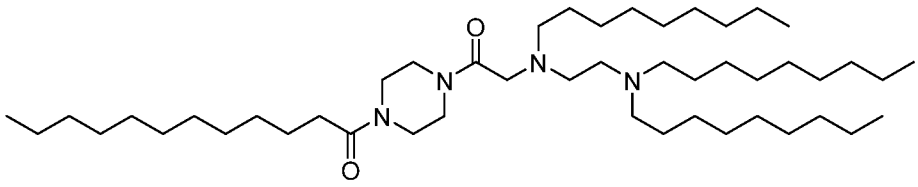
Molecular Weight: 906.52

[00947] To a 0 °C solution of *N*-nonyl-*N*-(4-oxo-4-(pentyloxy)butyl)glycine (291 mg, 0.814 mmol) and 2-((2-(dinony lamino)ethy l)(nony l)amino)-*N*-methyl-*N*-(2-(methylamino)ethyl)acetamide (508 mg, 0.895 mmol) in THF (10 mL) was added iPnEtN (0.312 mL, 1.79 mmol) and T3P (50% EtOAc solution, 1.5 mL, 2.4 mmol), and the reaction was allowed to stir at 0 °C for 1 hour. The reaction was quenched with water and extracted with EtOAc. The organics were dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO C18 flash chromatography (10-100% water 0.1% TFA/MeCN 0.1% TFA) provided pentyl 8,1 l-dimethyl-5,14,17-trinonyl-7,12-dioxo-5,8,l 1,14,17-pentaazahexacosanoate (154 mg, 21%).

UPLC/ELSD: RT = 2.90 min. MS (ES): *m/z* (MH^+) 906.94 for C55H111N5O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.07 (t, 2H); 3.72-2.24 (br. m, 30H); 1.87-1.00 (br. m, 64H); 0.90 (m, 15H).

CV: Compound 143: 1-(4-*(N*-(2-(Dinonylamino)ethyl *)-N*-nonylglycyl)piperazin-l-yl)dodecan-1-one



Chemical Formula: C47H94N4O2

Molecular Weight: 747.30

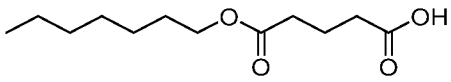
[00948] In the same manner as Step 7 for Compound 44, l-(4-(N-(2-(dinonylamino)ethyl)-N-nonylgly cyl)piperazin- 1-yl)dodecan- 1-one was synthesized from 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l-(piperazin-l-yl)ethan-l-one (400 mg, 0.708 mmol), dodecanoic acid (170 mg, 0.850 mmol), iPr_2EtN (0.271 mL, 1.56 mmol), and T3P (50% EtOAc solution, 1.26 mL, 2.12 mmol) in THF (10 mL). Yield (117 mg, 22%).

UPLC/ELSD: RT = 3.45 min. MS (ES): *m/z* (MH^+) 747.93 for C47H94N4O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.82-3.24 (br. m, 10H); 2.66-2.26 (br. m, 12H); 1.75-1.00 (br. m, 60H); 0.90 (t, 12H)

CW: Compound 144: Heptyl 5-(4-*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperazin-l-yl)-5-oxopentanoate

5-(Heptyloxy)-5-oxopentanoic acid



Chemical Formula: C12H22O4

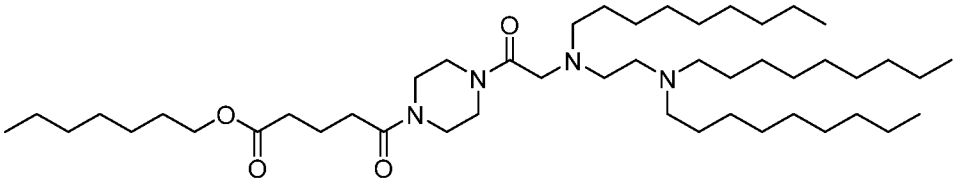
Molecular Weight: 230.30

[00949] To a solution of glutaric acid (5.7 g, 43 mmol) and 1-heptanol (1.0 g, 8.6 mmol) in THF (40 mL) was added sulfuric acid (0.46 mL, 8.6 mmol), and the reaction was allowed to stir at 65 °C for 12 hours. The reaction was cooled to RT and concentrated *in vacuo.* The crude material was taken up in EtOAc and washed 2x with saturated NaHCCb, brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica flash

chromatography (0-10% DCM/MeOH) provided 5-(heptyloxy)-5-oxopentanoic acid as a clear colorless oil (827 mg, 42%).

¾-NMR (300 MHz, CDCh) δ: ppm 10.90 (br, 1H); 4.09 (t, 2H); 2.44 (m, 4H); 1.98 (quint, 2H); 1.64 (quint, 2H); 1.31 (br. m, 8H); 0.90 (t, 3H).

Heptyl 5-(4-*(N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-5-oxopentanoate



Chemical Formula: C47H92N4O4

Molecular Weight: 777.28

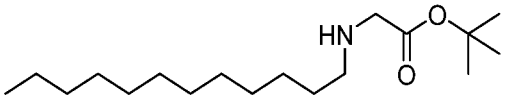
[00950] In the same manner as Step 7 for Compound 44, heptyl 5-(4-*(N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-5-oxopentanoate was synthesized from 5-(heptyloxy)-5-oxopentanoic acid (196 mg, 0.850 mmol), 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l-(piperazin-l-yl)ethan-l-one (400 mg, 0.708 mmol), iPr_2EtN (0.271 mL, 1.56 mmol), and T3P (50% EtOAc solution, 1.26 mL, 2.12 mmol) in THF (10 mL). Yield (93 mg, 17%).

UPLC/ELSD: RT = 3.22 min. MS (ES): *m/z* (MH^+) 777.88 for C47H92N4O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.09 (t, 2H); 3.85-3.15 (br. m, 10H); 2.70-2.30 (br. m, 14H); 1.99 (m, 2H); 1.75-1.00 (br. m, 52H); 0.90 (t, 12H).

CX: Compound 135: 3-((2-(4-*(N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(dodecyl)amino)propyl octanoate

tot-Butyl dodecylglycinate



Chemical Formula: C18H37NO2

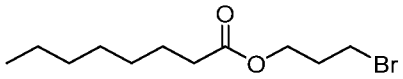
Molecular Weight: 299.50

[00951] In the same manner as Step 1 for Compound 44, tot-butyl dodecylglycinate was synthesized from tot-butyl glycinate hydrochloride (2.0 g, 12 mmol), 1-bromododecane (1.5 g, 6.0 mmol), K2CO3 (1.6 g, 12 mmol), and KI (99 mg, 0.60 mmol) in MeCN (100 mL). Yield (448 mg, 25%).

UPLC/ELSD: RT = 1.36 min. MS (ES): *m/z* (MH^+) 300.42 for C18H37NO2

¾-NMR (300 MHz, CDCh) δ: ppm 3.31 (s, 2H); 2.60 (t, 2H); 1.66 (br, 1H); 1.60-1.12 (br. m, 29H); 0.90 (t, 3H).

3-Bromopropyl octanoate



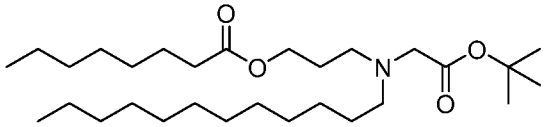
Chemical Formula: **CnH2iBr02**

Molecular Weight: 265.19

[00952] In the same manner as Step 1 for Compound 15, **3**-bromopropyl octanoate was synthesized from octanoic acid (2.0 g, 14 mmol), **3**-bromopropan-l-ol (1.4 mL, 15 mmol), and sulfuric acid (0.74 mL, 14 mmol) in THF (100 mL). Yield (718 mg, 20%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.23 (t, 2H); **3**.48 (t, 2H); **2.33** (t, 2H); 2.19 (quint, 2H); 1.64 (m, 2H); 1.41-1.21 (br. m, **8H);** 0.90 (t, **3H).**

3-((2-(teri-Butoxy)-2-oxoethyl)(dodecyl)amino)propyl octanoate



Chemical Formula: **C29H57NO4**

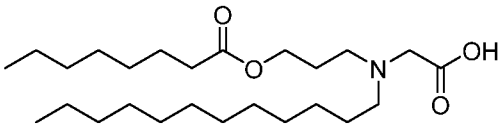
Molecular Weight: 483.78

[00953] In the same manner as Step 3 for Compound 44, 3-((2-(fer/-butoxy)-2-oxoethyl)(dodecyl)amino)propyl octanoate was synthesized from *tert*-butyl dodecylglycinate (448 mg, 1.50 mmol), 3-bromopropyl octanoate (436 mg, 1.65 mmol), K2CO3 (207 mg, 1.50 mmol), and KI (25 mg, 0.15 mmol). Yield (525 mg, 73%).

UPLC/ELSD: RT = 2.50 min. MS (ES): *m/z* (MH^+) 484.57 for **C29H57NO4**

¾-NMR (300 MHz, CDCh) δ: ppm 4.14 (t, 2H); 3.23 (s, 2H); 2.68 (t, 2H); 2.58 (t, 2H); 2.31 (t, 2H); 1.79 (m, 2H); 1.63 (m, 2H); 1.54-1.06 (37H); 0.90 (t, 6H).

N-Dodecyl-*N*-(3-(octanoyloxy)propyl)glycine



Chemical Formula: **C25H49NO4**

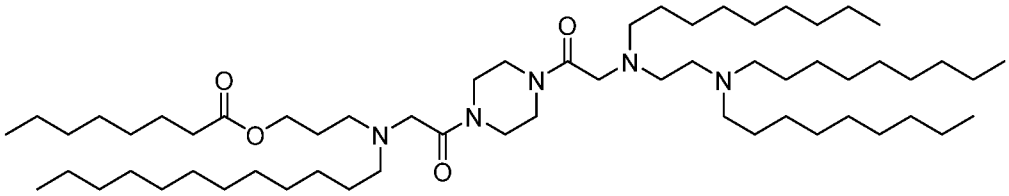
Molecular Weight: 427.67

[00954] In the same manner as Step 4 for Compound 44, *N*-dodecyl-*N*-(3-(octanoyloxy)propyl)glycine was synthesized from 3-((2-(tot-butoxy)-2-

oxoethyl)(dodecyl)amino)propyl octanoate (525 mg, 1.09 mmol), and TFA (4.2 mL, 54 mmol) in DCM (4.2 mL). Yield (411 mg, 89%).

UPLC/ELSD: RT = 2.11 min. MS (ES): *m/z* (MH^+) 428.57 for C25H49NO4

3-((2-(4-(*N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(dodecyl)amino)propyl octanoate



Chemical Formula: C60H119N5O4

Molecular Weight: 974.64

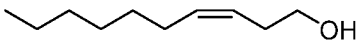
[00955] In the same manner as Step 7 for Compound 44, 3-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin- 1-yl)-2-oxoethyl)(dodecyl)amino)propyl octanoate was synthesized from *N*-dodecyl-*N*-(3-(octanoyloxy)propyl)glycine (248 mg, 0.581 mmol), 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l-(piperazin-l-yl)ethan-l-one (361 mg, 0.639 mmol), iPr_2EtN (0.223 mL, 1.28 mmol), and T3P (50% EtOAc solution, 1.0 mL, 1.7 mmol) in THF (10 mL). Yield (178 mg, 31%).

UPLC/ELSD: RT = 3.20 min. MS (ES): *m/z* (MH^+) 975.19 for C60H119N5O4

ŧTi-NMR (300 MHz, CDCh) δ: ppm 4.07 (t, 2H); 3.80-3.00 (br. m, 12H); 2.72-2.18 (br. m, 16H); 1.85-1.00 (br. m, 74H); 0.88 (t, 15H).

CY: Compound 137: 3-((2-(4-(*N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(tetradecyl)amino)propyl (Z)-dec-3-enoate

(Z)-Dec-3-en-l-ol



Chemical Formula: **C10H20O**

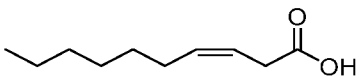
Molecular Weight: 156.27

[00956] To a round bottom flask containing Lindlar's catalyst (5% loading, 276 mg, 0.130 mmol) under N_2 was added EtOH (10 mL) and dec-3-yn-l-ol (2.0 g, 13 mmol). The flask was evacuated and backfilled with N_2 three times, followed by H_2 three times. The reaction mixture was allowed to stir vigorously for 12 hours under 1 atm H_2. The flask was degassed and filtered

over a pad of celite, rinsing with EtOAc. The filtrate was concentrated to provide (Z)-dec-3-en-l-ol (1.72 g, 85%).

Ti-NMR (300 MHz, CDCh) δ: ppm 5.65 (m, 1H); 5.36 (m, 1H); 3.64 (t, 2H); 2.33 (q, 2H); 2.06 (q, 2H); 1.52-1.06 (br. m, 8H); 0.88 (t, 3H).

(Z)-Dec-3-enoic acid



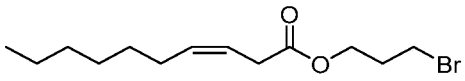
Chemical Formula: C10H18O2

Molecular Weight: 170.25

[00957] To a 0 °C solution of nitric acid (70%, 49 μL, 1.1 mmol) and potassium dichromate (32 mg, 0.11 mmol) in water (16 mL) was added sodium periodate (5.18 g, 24.2 mmol) and a solution of (Z)-dec-3-en-l-ol (1.72 g, 11.0 mmol) in MeCN (32 mL). The reaction was allowed to stir at 0 °C for 4 hours, and then allowed to stir at RT for 8 hours. The reaction mixture was filtered and the filtrate was extracted with diethyl ether. The combined organic extracts were dried over anhydrous Na2S04, filtered, and concentrated. Purification by ISCO silica flash chromatography provided (Z)-dec-3-enoic acid (699 mg, 37%).

1H-NMR (300 MHz, CDCh) δ: ppm 11.10 (br, 1H); 5.68-5.45 (br. m, 2H); 3.14 (d, 2H); 2.04 (q, 2H); 1.47-1.07 (br. m, 8H); 0.88 (t, 3H).

3-Bromopropyl (Z)-dec-3-enoate



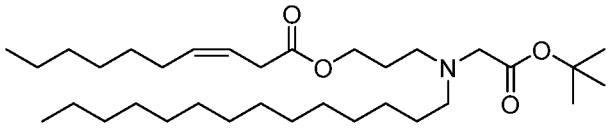
Chemical Formula: Ci3H23Br02

Molecular Weight: 291.23

[00958] To a solution of (Z)-dec-3-enoic acid (699 mg, 4.11 mmol) and 3-bromopropan-l-ol (685 mg, 4.93 mmol) in DCM (20 mL) was added EDC (765 mg, 4.93 mmol) and DMAP (100 mg, 0.821 mmol) and the reaction was allowed to stir at RT for 12 hours. The reaction was filtered and concentrated *in vacuo*. Purification by ISCO silica flash chromatography (0-20% hexanes/EtOAc) provided 3-bromopropyl (Z)-dec-3-enoate (730 mg, 61%).

¾-NMR (300 MHz, CDCh) δ: ppm 5.68-5.48 (br. m, 2H); 4.25 (t, 2H); 3.48 (t, 2H); 3.12 (d, 2H); 2.20 (quint, 2H); 2.05 (q, 2H); 1.50-1.15 (br. m, 8H); 0.90 (t, 3H).

3-((2-(teri-Butoxy)-2-oxoethyl)(tetradecyl)amino)propyl (Z)-dec-3-enoate



Chemical Formula: C33H63NO4

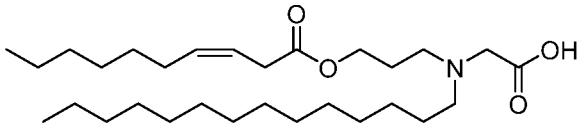
Molecular Weight: 537.87

[00959] In the same manner as Step 3 for Compound 44, 3-((2-**(/e**r/-butoxy)-2-oxoethyl)(tetradecyl)amino)propyl **(Z)**-dec-3-enoate was synthesized from */er*/-butyl tetradecylglycinate (350 mg, 1.07 mmol), 3-bromopropyl **(Z)**-dec-3-enoate (342 mg, 1.18 mmol), K2CO3 (295 mg, 2.14 mmol), and KI (18 mg, 0.11 mmol) in MeCN (10 mL). Yield (473 mg, 82%).

UPLC/ELSD: RT = 2.82 min. MS (ES): *m/z* (MH^+) 538.69 for C33H63NO4

¾-NMR (300 MHz, CDCh) δ: ppm 5.68-5.46 (br. m, 2H); 4.16 (t, 2H); 3.23 (s, 2H); 3.10 (d, 2H); 2.68 (t, 2H); 2.57 (t, 2H); 2.06 (q, 2H); 1.80 (quint, 2H); 1.53-1.00 (br. m, 41H); 0.90 (t, 6H).

(Z)-*N* -(3-(Dec-3-enoyloxy)propyl)-*N* -tetradecylglycine



Chemical Formula: C29H55NO4

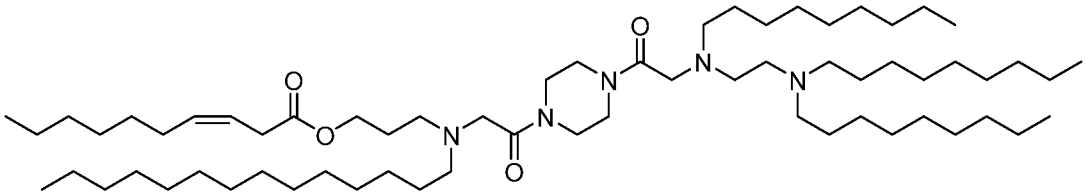
Molecular Weight: 481.76

[00960] In the same manner as Step 4 for Compound 44, **(Z)**-*N* -(3-(dec-3-enoyloxy)propyl)-*N* -tetradecylglycine was synthesized from 3-((2-**(/er**/-butoxy)-2-oxoethyl)(tetradecyl)amino)propyl **(Z)**-dec-3-enoate (473 mg, 0.879 mmol), and TFA (3.4 mL, 44 mmol) in DCM (3.4 mL). Yield (346 mg, 82%).

UPLC/ELSD: RT = 2.49 min. MS (ES): *m/z* (MH^+) 482.60 for C29H55NO4

¾-NMR (300 MHz, CDCh) δ: ppm 5.65-5.48 (br. m, 2H); 4.14 (t, 2H); 3.22 (s, 2H); 3.10 (d, 2H); 2.76 (br. m, 4H); 2.10-1.83 (br. m, 4H); 1.65-1.05 (32H); 0.90 (t, 6H).

3-((2-(4 -(*N* -(2-(Dinonylamino)ethyl)-*N* -nonylgly cyl)piperazin- 1-yl)-2-oxoethyl)(tetradecyl)amino)propyl **(Z)**-dec-3-enoate



Chemical Formula: C64H125N5O4

Molecular Weight: 1028.74

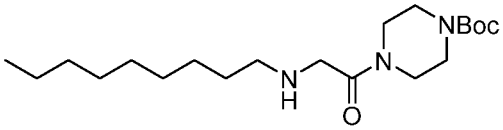
[00961] In the same manner as Step 7 for Compound 44, 3-((2-(4-*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(tetradecyl)amino)propyl (Z)-dec-3-enoate was synthesized from (Z)-*N*-(3-(dec-3-enoyloxy)propyl *)-N*-tetradecylglycine (346 mg, 0.718 mmol), 2-((2-(dinonylamino)ethyl)(nonyl)amino)-1-(piperazin-1-yl)ethan-1-one (446 mg, 0.790 mmol), iPr_2EtN (0.276 mL, 1.58 mmol), and T3P (50% EtOAc solution, 1.28 mL, 2.16 mmol). Yield (353 mg, 48%).

UPLC/ELSD: RT = 3.34 min. MS (ES): *m/z* (MH^+) 1029.31 for C64H125N5O4

¾-NMR (300 MHz, CDCh) δ: ppm 5.57 (m, 2H); 4.12 (t, 2H); 3.76-3.53 (br. m, 8H); 3.32 (br. m, 4H); 3.10 (d, 2H); 2.57-2.33 (br. m, 14H); 2.06 (q, 2H); 1.80 (quint, 2H); 1.55-1.05 (br. m, 74H); 0.90 (t, 15H).

CZ: Compound 139: Methyl 8-((2-(4-*(N*-(2-(Di((Z)-non-3-en-1-yl)amino)ethyl *)-N*-((Z)-non-3-en-1-yl)glycyl)piperazin-1-yl)-2-oxoethyl)(nonyl)amino)octanoate

tert-Butyl 4-(nonylglycyl)piperazine-1-carboxylate



Chemical Formula: C20H39N3O3

Molecular Weight: 369.55

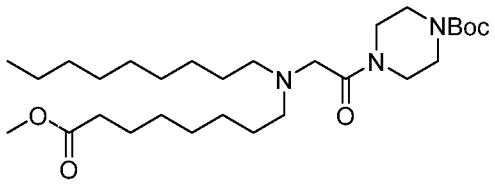
[00962] To a solution of *tert-b*utyl 4-glycylpiperazine-1-carboxylate (2.0 g, 7.1 mmol) in MeCN (70 mL) was added 1-bromononane (1.5 g, 7.1 mmol), K2CO3 (2.0 g, 14 mmol), and KI (120 mg, 0.71 mmol), and the reaction was allowed to stir at 82 °C for 12 hours. The reaction was cooled to RT, filtered over a pad of celite, rinsing with EtOAc, and concentrated *in vacuo.* The crude mixture was taken up in EtOAc and washed with 10% citric acid, brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica flash

chromatography (0-20% DCM/MeOH) provided *tert*-butyl 4-(nonylglycyl)piperazine-l-carboxylate (583 mg, 22%).

UPLC/ELSD: RT = 0.77 min. MS (ES): *m/z* (MH^+) 370.52 for $C_{20}H_{39}N_3O_3$

¾-NMR (300 MHz, CDCh) δ: ppm 3.70-3.28 (br. m, 10H); 2.62 (t, 2H); 2.02 (br, 1H); 1.60-1.18 (br. m, 23H); 0.90 (t, 3H).

tert-Butyl 4-(*N*-(8-methoxy-8-oxooctyl)-*N*-nonylglycyl)piperazine-1-carboxylate



Chemical Formula: $C_{29}H_{55}N_3O_5$

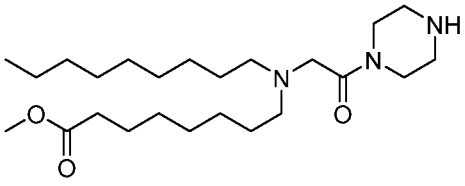
Molecular Weight: 525.78

[00963] To a solution of *tert*-butyl 4-(nonylglycyl)piperazine-l-carboxylate (583 mg, 1.58 mmol) in MeCN (15 mL) was added methyl 8-bromooctanoate (449 mg, 1.89 mmol), K_2CO_3 (436 mg, 3.16 mmol), and KI (26 mg, 0.16 mmol), and the reaction was allowed to stir at 82 °C for 12 hours. The reaction was cooled to RT, filtered over a pad of celite, rinsing with EtOAc, and concentrated *in vacuo*. Purification by ISCO silica flash chromatography (0-100% hexanes/EtOAc) provided tot-butyl 4-*(N*-(8-methoxy-8-oxooctyl *)-N*-nonylglycyl)piperazine-l-carboxylate (646 mg, 78%).

UPLC/ELSD: RT = 1.57 min. MS (ES): *m/z* (MH^+) 526.55 for $C_{20}H_{39}N_3O_3$

^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.67-3.30 (br. m, 8H); 3.26 (s, 2H); 2.45 (t, 4H); 2.32 (t, 2H); 1.72-1.10 (br. m, 33H); 0.90 (t, 3H).

Methyl 8-(nonyl(2-oxo-2-(piperazin-l-yl)ethyl)amino)octanoate



Chemical Formula: $C_{24}H_{47}N_3O_3$

Molecular Weight: 425.66

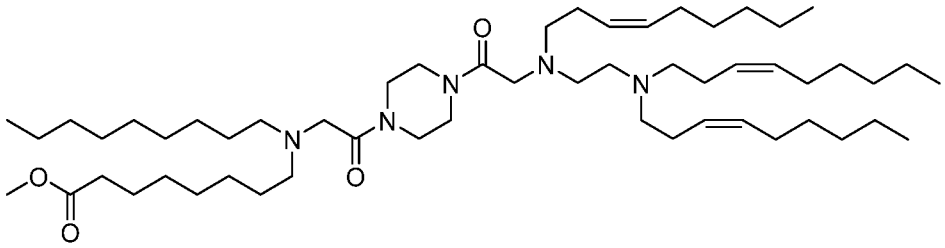
[00964] In the same manner as Step 4 for Compound 11, methyl 8-(nonyl(2-oxo-2-(piperazin-l-yl)ethyl)amino)octanoate was synthesized from tert-butyl 4-(N-(8-methoxy-8-

oxooctyl)-N-nonylglycyl)piperazine-l -carboxylate (646 mg, 1.23 mmol), and TFA (4.7 mL, 61 mmol) in DCM (4.7 mL). Yield (487 mg, 93%).

UPLC/ELSD: RT = 0.37 min. MS (ES): *m/z* (MH^+) 426.52 for $C_{24}H_{47}N_3O_3$

¾-NMR (300 MHz, CDCh) δ: ppm 3.69 (s, 3H); 3.61 (br. m, 4H); 3.27 (s, 2H); 2.87 (m, 4H); 2.47 (t, 4H); 2.32 (t, 2H); 2.06 (br, 1H); 1.63 (m, 2H); 1.53-1.02 (br. m, 22H); 0.90 (t, 3H).

Methyl 8-((2-(4 -*(N* -(2-(Di((Z)-non-3-en-l -yl)amino)ethyl *)-N* -((Z)-non-3-en-l -yl)gly cyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)octanoate



Chemical Formula: $C_{55}H_{103}N_5O_4$

Molecular Weight: 898.46

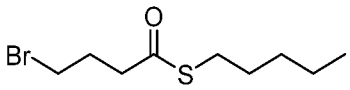
[00965] In the same manner as Step 11 for Compound 11, methyl 8-((2-(4 -*(N* -(2-(di((Z)-non-3-en- 1-yl)amino)ethyl *)-N* -((Z)-non-3-en- 1-yl)gly cyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)octanoate was synthesized from methyl 8-(nonyl(2-oxo-2-(piperazin-l-yl)ethyl)amino)octanoate (240 mg, 0.564 mmol), *N* -(2-(di((Z)-non-3-en-l -yl)amino)ethyl *)-N*-((Z)-non-3-en-l-yl)glycine (231 mg, 0.47 mmol), iPr_2EtN (0.181 mL, 1.03 mmol), and T3P (50% EtOAc solution, 0.84 mL, 1.4 mmol) in THF (8 mL). Yield (161 mg, 38%).

UPLC/ELSD: RT = 2.60 min. MS (ES): *m/z* (MH^+) 899.08 for $C_{55}H_{103}N_5O_4$

^{1}H-NMR (300 MHz, CDCh) δ: ppm 5.51-5.23 (br. m, 6H); 3.77-3.47 (br. m, 11H); 3.37 (s, 2H); 3.27 (s, 2H); 2.85-1.90 (br. m, 28H); 1.75-1.05 (br. m, 42H); 0.91 (m, 12H).

DA: Compound 134: S-Pentyl 4-((2-(4 -*(N* -(2-(dinonylamino)ethyl *)-N* -nonylglycyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)butanethioate

S-Pentyl 4-bromobutanethioate



Chemical Formula: $C_9H_{17}BrOs$

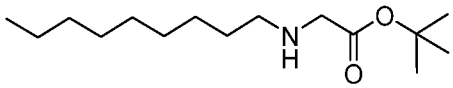
Molecular Weight: 253.20

[00966] DMAP (0.439 g, 3.60 mmol) was placed into a flask and dissolved in DCM (36 mL) under nitrogen. Triethylamine (5.1 mL, 36 mmol) and 1-pentanethiol (2.2 mL,

18 mmol) were then added. 4-Bromobutanoyl chloride (3.1 mL, 27 mmol) was added rapidly drop-wise. The cloudy yellow solution was allowed to stir overnight at room temperature. The reaction was quenched with 50 mL of water. The organic layer was collected and the aqueous layer washed with DCM 2x. The combined organic layers were dried over anhydrous sodium sulfate and then concentrated *in vacuo* to afford an orange oil. The crude was purified by ISCO silica flash chromatography (Hexanes: Ethyl Acetate 0-5%) to obtain S-pentyl 4-bromobutanethioate. Yield (4.0 g, 88%).

Ti-NMR (400 Hz, CDCb) δ: ppm 3.44 (t, 2H), 2.88 (t, 2H), δ.74 (t, 2H), 2.21 (m, 2H), 1.57 (m, 2H), 1.33 (m, 4H), 0.89 (m, 3H).

tert-Butyl nonylglycinate



Chemical Formula: C15H31NO2

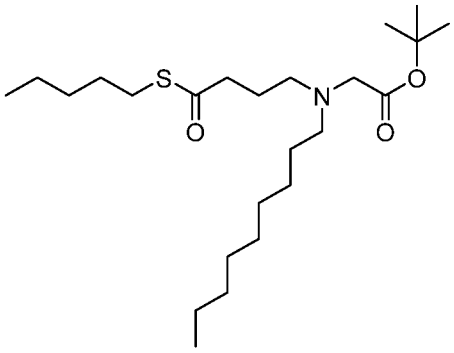
Molecular Weight: 257.42

Potassium carbonate (20.0 g, 145 mmol) and potassium iodide (0.80 g, 4.8 mmol) were placed into a flask and suspended in MeCN (120 mL). 1-Bromo-*N*-nonane (5.0 g, 24 mmol) and *tert*-butyl 2-aminoacetate-HCl (16.2 g, 96.5 mmol) were then added at once and the solution was heated to 75 °C and allowed to stir overnight. The reaction mixture was diluted with water, and extracted with DCM 3x. The combined organic layers were dried over anhydrous sodium sulfate and then concentrated *in vacuo* to yield an off-white oil. The crude material was purified by ISCO silica flash chromatography (Hexanes: Ethyl Acetate 0-30%) to obtain *tert*-Butyl nonylglycinate. Yield (2.15 g, 35%).

UPLC/ELSD: RT = 0.64 min. MS (ES): *m/z* (M-56) 201.964 for C15H31NO2

¾-NMR (400 Hz, CDCb) δ: ppm 3.32 (s, 2H), 2.61 (t, 2H), 1.49 (s, 11H), 1.28 (s, 12H), 0.90 (t, 3H).

tert-Butyl *N*-nonyl-*N*-(4-oxo-4-(pentylthio)butyl)glycinate



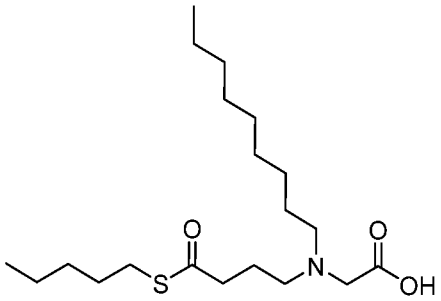
Chemical Formula: C24H47NO3S

Molecular Weight: 429.70

[00967] Potassium carbonate (3.2 g, 23 mmol) and potassium iodide (0.26 g, 1.6 mmol) were placed in a flask and suspended in MeCN (44 mL). 4-Bromo-l -(pentylsulfanyl)butan-l-one (2.36 g, 9.32 mmol) and *tert*-Butyl nonylglycinate (2.0 g, 7.8 mmol) were then added. The solution was heated to 75 °C and allowed to stir overnight. The reaction mixture was diluted with water and then extracted with DCM 3x. The combined organic layers were dried over anhydrous sodium sulfate and then concentrated *in vacuo* to afford a yellow oil. The crude material was purified by ISCO silica flash chromatography (Hexanes: Ethyl Acetate 0-10%) to obtain *tert*-butyl *N*-nonyl-*N*-(4-oxo-4-(pentylthio)butyl)glycinate (1.7 g, 51%). UPLC/ELSD: RT = 1.79 min. MS (ES): *m/z* (MH^+) 430.461 for C24H47NO3S

^{1}H-NMR (400 MHz, CDCh) δ: ppm 3.22 (s, 2H), 2.88 (t, 2H), 2.62 (m, 6H), 1.81 (m, 2H), 1.48 (s, 9H), 1.34 (m, 5H), 1.29 (s, 13H), 0.9 (m, 8H).

N-Nonyl-*N*-(4-oxo-4-(pentylthio)butyl)glycine



Chemical Formula: C20H39NO3S

Molecular Weight: 373.60

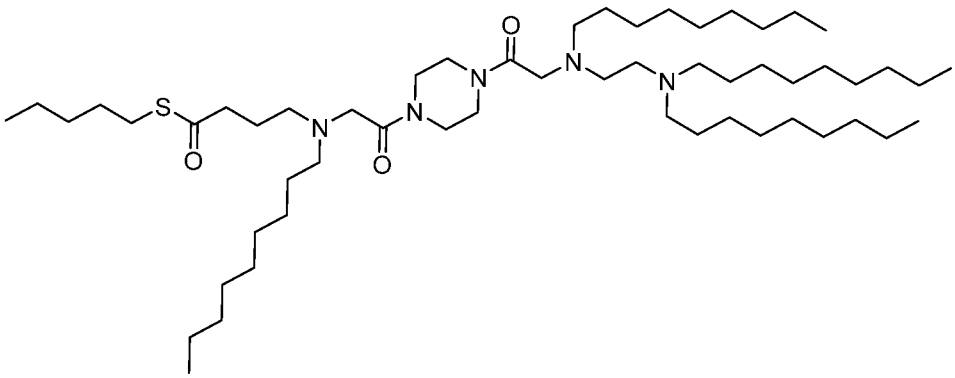
[00968] *tert*-Butyl *N*-nonyl-*N*-(4-oxo-4-(pentylthio)butyl)glycinate (1.78 g, 4.14 mmol) was dissolved in DCM (21 mL) and then trifluoroacetic acid (13 mL, 170 mmol) was added at ambient temperature. The reaction mixture was allowed to stir overnight. The reaction was

diluted with DCM, concentrated *in vacuo*, and then reconstituted in DCM. The pH was adjusted to pH of 8, and then the organic layer was dried over anhydrous sodium sulfate. The organic layer was concentrated *in vacuo* to afford *N*-nonyl-*N*-(4-oxo-4-(pentylthio)butyl)glycine. Yield (1.0 g, 64%).

UPLC/ELSD: RT = 1.45 min. MS (ES): *m/z* (MH^+) 373.953 for C20H39NO3S

^{1}Ti-NMR (400 MHz, CDCh) δ: ppm 3.44 (s, 2H), 2.98 (m, 4H), 2.87 (t, 2H), 2.64 (t, 2H), 2.01 (m, 2H), 1.59 (m, 4H), 1.31 (m, 16H), 0.87 (m, 6H).

S-Pentyl 4-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(nonyl)amino)butanethioate



Chemical Formula: C55H109N5O3S

Molecular Weight: 920.57

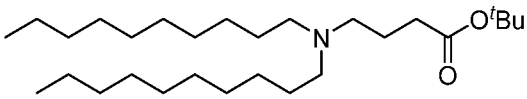
[00969] To a solution of 2-((2-(dinonylamino)ethyl)(nonyl)amino)-1-(piperazin-1-yl)ethan-1-one (0.40 g, 0.71 mmol) and *N*-nonyl-*N*-(4-oxo-4-(pentylthio)butyl)glycine (0.27 g, 0.71 mmol) in THF was added iPr_2EtN (0.20 g, 0.27 mL, 1.6 mmol) and T3P (50% EtOAc solution, 1.4 mL, 2.1 mmol). The solution was allowed to stir for 1 hour at 0 °C. The reaction was quenched with water and extracted 3x with EtOAc. The combined organics were dried over Na2S04, filtered, and concentrated *in vacuo*. The crude material was purified by ISCO silica flash chromatography (0-20% MeOH/DCM with 1% NH_4OH) to obtain S-pentyl 4-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(nonyl)amino)butanethioate. Yield (140 mg, 21%).

UPLC/ELSD: RT = 2.79 min. MS (ES): *m/z* (MH^+) 921.787 for C55H109N5O3S

^{1}H-NMR (400 MHz, CDCh) δ: 3.6 (m, 7H), 3.28 (s, 3H), 3.02 (s, 2H), 2.86 (t, 2H), 2.55 (m, 10H), 1.56 (m, 10H), 1.43 (s, 5H), 1.21 (s, 52H), 1.00 (m, 3H), 0.87 (t, 15H).

DB: Compound 146 1,1-(Piperazine-1,4-diyl)bis(4-(didecylamino)butan-1-one)

tert-Butyl 4-(didecylamino)butanoate



Chemical Formula: C28H57NO2

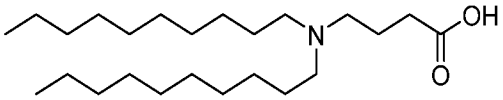
Molecular Weight: 439.77

[00970] To a solution of *tert*-butyl 4-aminobutanoate hydrochloride (2.45 g, 12.5 mmol) in cyclopentylmethyl ether (20 mL) and acetonitrile (20 mL) was added 1-bromodecane (5.45 mL, 26 mmol) followed by potassium carbonate (6.99 g, 5 mmol) and potassium iodide (2.29 g, 13.8 mmol). The reaction mixture was heated to 85 °C for 18 h. The reaction mixture was cooled to room temperature and filtered through celite, and then the celite bed was washed with ethyl acetate. The filtrate was concentrated and the residue was purified by flash chromatography (Column: silica gel 80 g; eluent: ethylacetate/hexane 0 to 60%) to provide *tert*-butyl 4-(didecylamino)butanoate (4.12 g, 75%) as an oil.

HPLC/ELSD: MS (ES): *m/z* (MH^+) 440.4 for C28H57NO2.

¾ NMR (300 MHz, CDCh): δ 2.41-2.33 (m, 6H), 2.21 (t, 2H, *J* = 7.3 Hz), 1.73-1.64 (m, 2H), 1.43 (s, 9H), 1.31-1.23 (m, 32H), 0.87 (t, 6H, *J* = 6.6 Hz).

4-(Didecylamino)butanoic acid



Chemical Formula: C24H49NO2

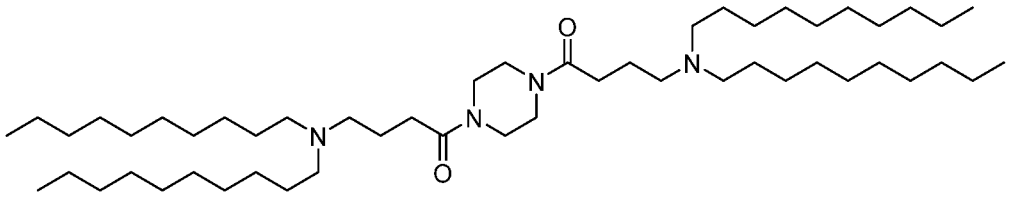
Molecular Weight: 383.66

[00971] At 0 °C, to a solution of tot-butyl 4-(didecylamino)butanoate (4.12 g, 9.4 mmol) in dichloromethane (15 mL) was added trifluoroacetic acid (10 mL) dropwise, and then the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was cooled to 0 °C and saturated sodium bicarbonate solution (10 mL) was added. The organic layer was separated, washed with saturated sodium bicarbonate (2 X 10 mL) and brine (2 X 10 mL), dried with anhydrous sodium sulfate and the solvent removed to provide 4-(didecylamino)butanoic acid (3.46 g, 96%) as white solid.

HPLC/ELSD: MS (ES): *m/z* (MH^+) 384.4 for C24H49NO2.

¾ NMR (300 MHz, CDCh): δ 2.86 (t, 2H, *J* = 5.7 Hz), 2.79-2.73 (m, 4H), 2.59-2.55 (m, 2H), 1.91-1.84 (m, 2H), 1.64-1.63 (m, 2H), 1.31-1.23 (m, 30H), 0.87 (t, 6H, *J* = 6.6 Hz).

1,l-(Piperazine- 1,4-diy l)bis(4-(didecy lamino)butan- 1-one)



Chemical Formula: C52H104N4O2

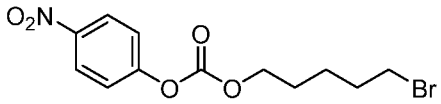
Molecular Weight: 817.43

[00972] At 0 °C, to a solution of 4-(didecylamino)butanoic acid (1.0 g, 2.6 mmol), piperazine (0.107 g, 1.24 mmol), and *N,N* -diisopropylethyl amine (1.08 mL, 6.2 mmol) in tetrahydrofuran (20 mL), propylphisphonic anhydride (50 wt% in EtOAc, 4.74 mL, 6.2 mmol) was added slowly and then the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was quenched with ice and the organic layer separated. The organic layer was washed with saturated sodium bicarbonate solution (2 X 10 ml) and brine (2 X 10 ml), dried with anhydrous sodium sulfate and the solvent removed to get the crude product which was purified by flash chromatography (Column: silica gel 40 g; eluent: methanol/dichloromethane 0 to 20%) to get l,l'-(piperazine-l ,4-diyl)bis(4-(didecylamino)butan-l -one) (330 mg, 33%) as yellow oil. HPLC/ELSD: RT = 9.96 min. MS (ES): *m/z* (MH^+) 817.7 for C52H104N4O2.

¾ NMR (300 MHz, CD3OD): δ 3.61 (bs, 8H), 2.98-2.94 (bm, 12H), 2.58 (t, 4H, *J* = 6.4 Hz), 1.94 (t, 4H, *J* = 6.4 Hz), 1.65 (bm, 8H), 1.35-1.29 (m, 56H), 0.89 (t, 12H, *J* = 6.7 Hz).

DC: Compound 109. 5-((2-(4 *-(N* -(2-(Dinonylamino)ethyl *)-N* -nonylglycyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)pentyl methyl carbonate

5-Bromopentyl (4-nitrophenyl) carbonate



Chemical Formula: $C_{12}H_{14}BrNO_5$

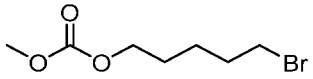
Molecular Weight: 332.15

[00973] 4-Nitrophenyl chloroformate (7.24 g, 35.9 mmol, 1.2 equiv.) was added in one portion to a solution of 5-bromopentan-l -ol (5 g, 29.9 mmol, 1 equiv.) in DCM (100 mL) in a round bottom flask charged with a magnetic stir bar at RT. The reaction was kept under N_2 and pyridine was added (3.55 g, 3.63 mL, 44.8 mmol, 1.5 equiv.) dropwise over 10 min. The

reaction was allowed to stir at RT for 16 h. The reaction was diluted with water and DCM. The organic layer was separated and the aqueous layer was washed with DCM. The combined organic layer was washed with brine dried with Na2S04 and evaporated under vacuum. The residue was purified by flash chromatography (ISCO) by 0-100% ethyl acetate in hexanes to obtain the desired product.

¾ NMR (300 MHz, CDCh) δ: ppm 8.30 (d, 2H); 7.40 (d, 2H); 4.33 (t, 2H); 3.46 (d, 2H); 2.03 - 1.75 (m, 4H), 1.63 (m, 2H).

5-Bromopentyl methyl carbonate



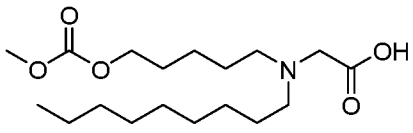
Chemical Formula: $C_7H_{13}BrO_3$

Molecular Weight: 225.08

[00974] Methanol (0.91 g, 1.15 mL, 28.4 mmol, 4 equiv.) was added dropwise to a solution of 5-bromopentyl 4-nitrophenyl carbonate (2.36 g, 7.1 mmol, 1 equiv.) in DCM (35.5 mL) in a round bottom flask charged with a magnetic stir bar at rt under N_2. The reaction was kept under N_2 and pyridine (0.70 g, 0.71 mL, 8.8 mmol, 1.25 equiv.) was added dropwise over 10 min. followed by 4-dimethylaminopyridine (0.17 g, 1.4 mmol, 0.2 equiv.). The reaction was allowed to stir at RT for 16 h. The reaction was diluted with water and DCM. The organic layer was separated and the aqueous layer was washed with DCM. The combined organic layer was washed with brine, dried with Na2S04 and evaporated under vacuum. The residue was purified by flash chromatography (ISCO) by 0-100% ethyl acetate in hexanes to obtain the desired product.

¾ NMR (300 MHz, CDCh) δ: ppm 4.17 (t, 2H); 3.80 (s, 3H); 3.43 (t, 2H); 1.92 (m, 2H); 1.73 (m, 2H), 1.56 (m, 2H).

N-(5-((Methoxycarbonyl)oxy)pentyl)-*N*-nonylglycine



Chemical Formula: **C18H35NO5**

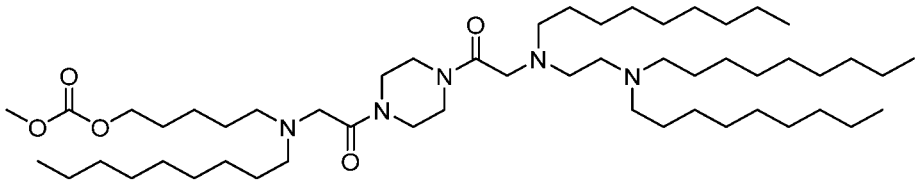
Molecular Weight: 345.48

[00975] *N*-(5-((Methoxycarbonyl)oxy)pentyl)-*N*-nonylglycine was synthesized In the same manner as Step 4 for Compound 44.

¾ NMR (300 MHz, CDCh) δ: ppm 4.16 (t, 2H); 3.80 (s, 3H); 3.47 (t, 2H); 3.02 (m, 4H); 1.73

(m, 6H), 1.53-1.22 (m, 15H); 0.90 (m, 3H).

5-((2-(4-(*N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)pentyl methyl carbonate



Chemical Formula: C53H105N5O5

Molecular Weight: 892.453

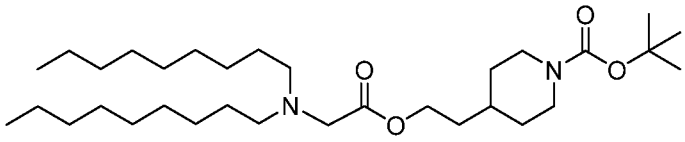
[00976] 5-((2-(4-(*N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)pentyl methyl carbonate was synthesized in the same manner as Step 7 for Compound 44.

UPLC/ELSD: RT = 2.66 min. MS (ES): *m/z* (MH^+) 892.8 for C53H105N5O5

¾ NMR (300 MHz, CDCh) δ: ppm 4.15 (t, 2H); 3.80 (s, 3H); 3.53 (m, 8H); 3.31 (m, 4H); 2.66-2.31 (m, 13H), 1.71 (m, 3H); 1.55-1.18 (m, 60H); 0.90 (m, 12H).

DD: Compound 114 2-(l-(*N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)piperidin-4-yl)ethyl dinonylglycinate

tert-Butyl 4-(2-((dinonylglycyl)oxy)ethyl)piperidine-1-carboxylate



Chemical Formula: C32H62N2O4

Molecular Weight: 538.86

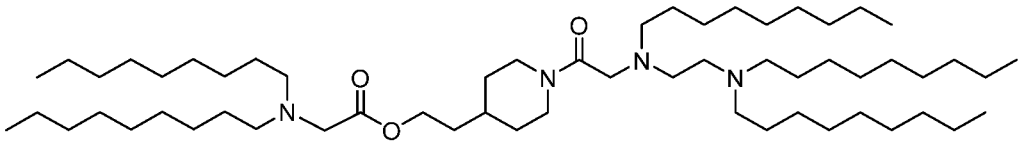
[00977] To a solution of 4-dimethylaminopyridine (0.14 g, 0.01 mol, 0.4 equiv.), *tert*-butyl 4-(2-hydroxyethyl)piperidine-l-carboxylate (0.7 g, 3.05 mmol, 1 equiv.), (dinonylamino)acetic acid (1 g, 3.1 mmol, 1 equiv.) in DCM (15 mL) was added disopropylethylamine (0.79 g, 1.1 mL, 6.1 mmol, 2 equiv.) and ({[3-(dimethylamino)propyl]imino}methylidene)(ethyl)amine hydrochloride (0.59 g, 3.1 mmol, 1 equiv.). The reaction was allowed to stir at RT for 16 h. The reaction was diluted with DCM and extracted with saturated NaHCCb, followed by brine. The organic layer was separated,

dried with MgS04, filtered, and evaporated under vacuum. The residue was purified by flash chromatography (0-30%) EtOAc in hexanes.

UPLC/ELSD: RT = 2.31 min. MS (ES): *m/z* (MH^+) 539.2 for C32H62N2O4

¾ NMR (300 MHz, CDCh) δ: ppm 4.25-4.00 (m, 4H); 3.33 (s, 2H); 2.79-2.48 (m, 6H); 1.74-1.41 (m, 18H); 1.38-1.07 (m, 26H), 0.90 (m, 6H).

2-(l -*(N*-(2-(Dinonylamino)ethyl *)-N* -nonylglycyl)piperidin-4-yl)ethyl dinonylglycinate



Chemical Formula: **C58H116N4O3**

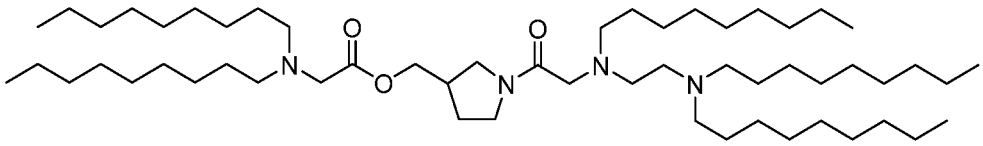
Molecular Weight: 917.591

[00978] 2-(l -*(N* -(2-(Dinonylamino)ethyl *)-N* -nonylglycyl)piperidin-4-yl)ethyl dinonylglycinate was synthesized in the same manner as methyl 8-((2-(4 -*(N* -(2-(dinonylamino)ethyl *)-N* -nonylgly cyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)octanoate using 2-(piperidin-4-yl)ethyl dinonylglycinate and *N* -(2-(dinonylamino)ethyl *)-N* -nonylglycine.

UPLC/ELSD: RT = 3.12 min. MS (ES): *m/z* (MH^+) 917.9 for **C58H116N4O3**

¾ NMR (300 MHz, CDCh) δ: ppm 4.56 (m, 1H); 4.33-4.09 (m, 3H); 3.50-2.81 (m, 8H); 2.81-2.68 (m, 12H); 1.86-1.54 (m, 6H), 1.53-1.04 (m, 71H); 0.90 (m, 15H).

DE: Compound 102: (l -*(N* -(2-(Dinonylamino)ethyl *)-N* -nonylglycyl)pyrrolidin-3-yl)methyl dinonylglycinate



Chemical Formula: C56H112N4O3

Molecular Weight: 889.5

[00979] (l -*(N* -(2-(Dinonylamino)ethyl *)-N* -nonylglycyl)pyrrolidin-3-yl)methyl dinonylglycinate was synthesized in the same manner as methyl 8-((2-(4 -*(N* -(2-(dinonylamino)ethyl *)-N* -nonylgly cyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)octanoate using pyrrolidin-3-ylmethyl dinonylglycinate and *N* -(2-(dinonylamino)ethyl *)-N* -nonylglycine.

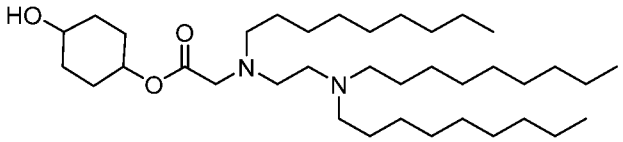
UPLC/ELSD: RT = 2.95 min. MS (ES): *m/z* (MH^+) 890.9 for C56H112N4O3

¾ NMR (300 MHz, CDCh) δ: ppm 4.21-3.96 (m, 2H); 3.87-3.55 (m, 3H); 3.50-3.25 (m, 4H);

2.83-2.36 (m, 14H); 2.28-1 .57 (m, 5H), 1.54-1 .19 (m, 69H); 0.90 (m, 15H).

DF: Compound 115: 4-*((N*-(2-(Dinonylamino)ethyl *)-N*-nonylglycyl)oxy)cyclohexyl dinonylglycinate

4-Hydroxycyclohexyl *N*-(2-(dinonylamino)ethyl *)-N*-nonylglycinate



Chemical Formula: C37H74N2O3

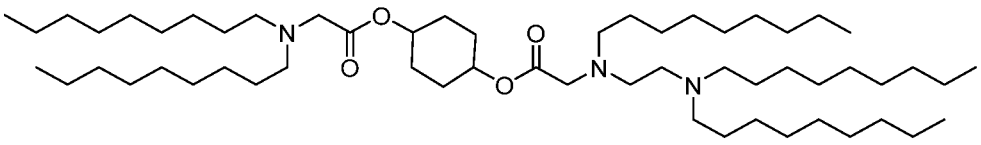
Molecular Weight: 595.010

[00980] 4-hydroxycyclohexyl *N*-(2-(dinonylamino)ethyl *)-N*-nonylglycinate was synthesized in the same manner as *tert-Butyi* 4-(2-((dinonylglycyl)oxy)ethyl)piperidine-l -carboxylate using cyclohexane-l,4-diol and *N*-(2-(dinonylamino)ethyl *)-N*-nonylgly cine.

UPLC/ELSD: RT = 2.82 min. MS (ES): *m/z* (MH^+) 595.6 for C37H74N2O3

¾ NMR (300 MHz, CDCh) δ: ppm 4.89 (m, 1H); 3.78 (m, 1H); 3.38 (m, 2H); 3.10 (m, 1H); 2.80-2.48 (m, 5H), 2.41 (m, 3H); 2.12-1.18 (m, 51H); 0.90 (m, 9H).

4-*((N*-(2-(Dinonylamino)ethyl *)-N*-nonylglycyl)oxy)cyclohexyl dinonylglycinate



Chemical Formula: C57H113N3O4

Molecular Weight: 904.548

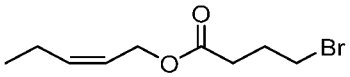
[00981] 4-*((N*-(2-(Dinonylamino)ethyl *)-N*-nonylglycyl)oxy)cyclohexyl dinonylglycinate was synthesized in the same manner as *tert-Butyi* 4-(2-((dinonylglycyl)oxy)ethyl)piperidine-l -carboxylate using 4-hydroxycyclohexyl *N*-(2-(dinonylamino)ethyl *)-N*-nonylglycinate and dinonylglycine.

UPLC/ELSD: RT = 3.15 min. MS (ES): *m/z* (MH^+) 904. 8 for C57H113N3O4

¾ NMR (300 MHz, CDCh) δ: ppm 4.90 (m, 2H); 3.63-0.97 (m, 96H); 0.90 (m, 15H).

DG: Compound 107: (Z)-Pent-2-en-l -yl 4-((2-(4 -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperazin-l -yl)-2-oxoethyl)(nonyl)amino)butanoate

(Z)-Pent-2-en-l -yl 4-bromobutanoate



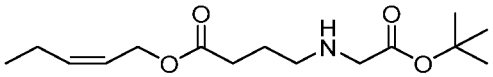
Chemical Formula: $C_9H_{15}BrO_2$

Molecular Weight: 235.12

[00982] In a 250 mL round-bottom flask, 4-bromobutanoic acid (5 g, 29.94 mmol) *NN*-dimethylpyridin-4-amine (0.73 g, 5.988 mmol) were dissolved in DCM at room temperature. (Z)-pent-2-en-l-ol (6.09 mL, 59.88 mmol) was then added at once, followed by the addition of 3-(((ethylimino)methylene)amino)-*N*,*N*-dimethylpropan-l -amine (4.65 g, 29.94 mmol) . The solution was allowed to stir at room temperature for 3 hours. The reaction was diluted with DCM and then quenched with saturated sodium bicarbonate. The organic layer was washed with brine, dried over anhydrous MgS04, and then concentrated under reduced pressure. The residue was purified by flash chromatography (0-10%) EtOAc in hexanes to obtain (Z)-pent-2-en-l-yl 4-bromobutanoate (3 g. 42.6%)

¾ NMR (300 MHz, CDCb) δ: ppm 5.7(m, 1H); 5.47(m, 1H); 4.65(d, 2H); 3.47(t, 2H); 2.5 l(t, 2H); 2.18(m, 4H); 1.00 (t, 3H).

(Z)-Pent-2-en-l -yl 4-((2-(teri-butoxy)-2-oxoethyl)amino)butanoate



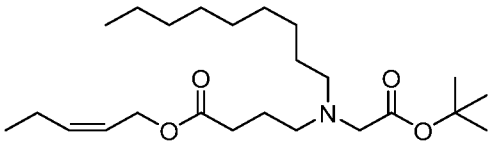
Chemical Formula: $C_{15}H_{27}NO_4$

Molecular Weight: 285.38

[00983] (Z)-Pent-2-en-l-yl 4-bromobutanoate (6.67 g, 28.368 mmol), potassium carbonate (23.525 g, 170.211 mmol,) and potassium iodide (0.94 g, 5.674 mmol) were placed in a flask and dissolved in acetonitrile (100 mL). *Tert-b*utyl glycinate hydrochloride (19.02 g, 113.474 mmol) was added at once and the solution was heated to 75 °C and stirred overnight. The reaction was diluted with water and then extracted with DCM. The organic layer was washed with brine, dried over anhydrous Na2S04, and then concentrated under reduced pressure. The residue was purified by flash chromatography (0-20%) EtOAc in hexanes to obtain (Z)-pent-2-en-l-yl 4-((2-(ter^-butoxy)-2-oxoethyl)amino)butanoate (2.42 g , 29.9%)

¾ NMR (300 MHz, CDCb) δ: ppm 5.64 (m, 1H); 5.53 (m, 1H); 4.65 (d, 2H); 3.31(s, 2H); 2.67(t, 2H); 2.41(t, 2H); 2.13 (q, 2H); 1.87 (m, 2H); 1.49(s, 9H); 1.01 (t, 3H).

(Z)-Pent-2-en-l -yl 4-((2-(teri-butoxy)-2-oxoethyl)(nonyl)amino)butanoate



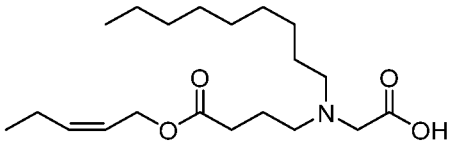
Chemical Formula: C24H45NO4

Molecular Weight: 411.63

[00984] Potassium iodide (0.349 g, 2.102 mmol) and potassium carbonate (4.36 g, 31.536 mmol) were placed into a flask under nitrogen. A solution of (Z)-pent-2-en-l -yl *4-((2-(tert-*butoxy)-2-oxoethyl)amino)butanoate (3 g, 10.512 mmol) in acetonitrile (60 mL) was added at once, followed by the addition of 1-bromononane (2.613 g, 12.615 mmol). The solution was then heated to 75 °C and stirred for 16 h. The reaction was diluted with water then extracted with DCM 3x. The organic layer was dried over anhydrous sodium sulfate. The residue was purified by flash chromatography (0-30%) EtOAc in hexanes to obtain (Z)-pent-2-en-l -yl 4-((2-(teri-butoxy)-2-oxoethyl)(nonyl)amino)butanoate (2.2 g, 50.8%).

¾ NMR (300 MHz, CDCh) δ: ppm 5.61 (m, 1H); 5.50 (m, 1H); 4.63 (d, 2H); 3.2(s, 2H); 2.62(m, 4H); 2.36(t, 2H); 2.12 (m, 2H); 1.76 (m, 2H); 1.45 (s, 10H); 1.26(s, 13H); 0.99 (t, 3H); 0.88(t, 3H).

(Z*)-N*-Nonyl-*N*-(4-oxo-4-(pent-2-en-1-yloxy)butyl)gly cine



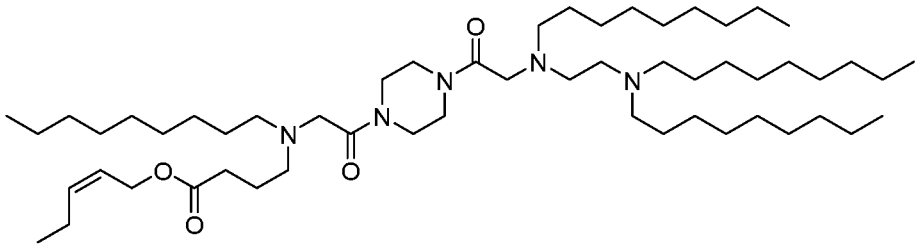
Chemical Formula: C20H37NO4

Molecular Weight: 355.52

[00985] (Z)-Pent-2-en-l-yl 4-((2-(ter^-butoxy)-2-oxoethyl)(nonyl)amino)butanoate (2.2 g, 5.345 mmol) was dissolved in DCM. Trifluoroacetic acid (16.4 mL, 214 mmol) was added at ambient temperature and stirred overnight. The reaction was diluted with DCM and then concentrated under reduced pressure. The red oil was then reconstituted in DCM, and then quenched with 5% sodium bicarbonate to achieve a pH of 7-8. The organic layer was dried over anhydrous sodium sulfate and then concentrated under reduced pressure. The crude was carried on to the next step without further purification.

¾ NMR (300 MHz, CDCh) δ: ppm 5.63 (m, 1H); 5.48 (m, 1H); 4.63 (d, 2H); 3.55(s, 2H); 3.13(m, 4H); 2.40(t, 2H); 2.11 (m, 4H); 1.6 (s, 2H); 1.27 (s, 12H); 1.01(t, 3H); 0.89(t, 3H).

(Z)-Pent-2-en-l -yl 4-((2-(4 -*(N* -(2-(dinonylamino)ethyl *)-N* -nonylglycyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)butanoate



Chemical Formula: **C55H107N5O4**

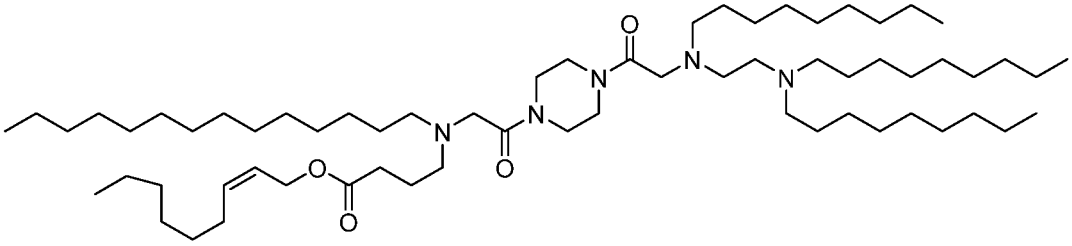
Molecular Weight: 902.5

[00986] (Z)-Pent-2-en- 1-yl 4-((2-(4 -*(N* -(2-(dinonylamino)ethyl *)-N* -nonylgly cyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)butanoate was synthesized in the same manner as methyl 8-((2-(4-*(N* -(2-(dinonylamino)ethyl *)-N* -nonylgly cyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)octanoate.

UPLC/ELSD: RT = 2.8 1 min. MS (ES): *m/z* (MH^+) 903.9 for **C55H107N5O4**

¾ NMR (300 MHz, CDCh) δ: ppm 5.73-5.42 (m, 2H); 4.64 (m, 2H); 3.78-3.52 (m, 8H); 3.32 (m, 4H); 2.65-2.29 (m, 14H); 2.14 (m, 2H), 1.79 (m, 2H); 1.56-1.17 (m, 58H); 1.02 (t, 3H); 0.90 (m, 12H).

DH: Compound 118: (Z)-Non-2-en-l -yl 4-((2-(4 -*(N* -(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperazin-l -yl)-2-oxoethyl)(tetradecyl)amino)butanoate



Chemical Formula: C64H125N5O4

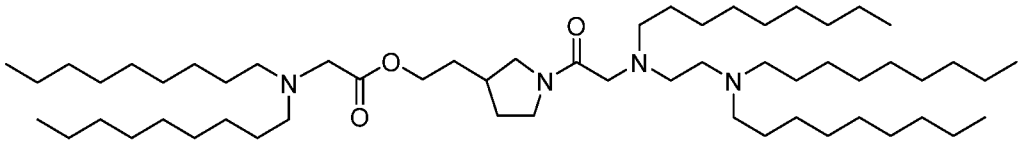
Molecular Weight: 1028.7

[00987] (Z)-Non-2-en- 1-yl 4-((2-(4 -*(N* -(2-(dinonylamino)ethyl *)-N* -nonylgly cyl)piperazin-l-yl)-2-oxoethyl)(tetradecyl)amino)butanoatewas synthesized in the same manner as (Z)-Pent-2-en-l -yl 4-((2-(4 -*(N* -(2-(dinonylamino)ethyl *)-N* -nonylglycyl)piperazin-l -yl)-2-oxoethyl)(nonyl)amino)butanoate.

UPLC/ELSD: RT = 3.36 min. MS (ES): *m/z* (MH^+) 1029.0 for C64H125N5O4

¾ NMR (300 MHz, CDCh) δ: ppm 5.75-5.45 (m, 2H); 4.65 (m, 2H); 3.78-2.95 (m, 20H); 2.675-2.42 (m, 6H); 2.34 (m, 2H), 2.12 (m, 2H); 1.91-1.60 (m, 6H); 1.52-1.19 (m, 70H); 0.90 (m, 15H).

DI: Compound 103: 2-(l -*(N*-(2-(Dinonylamino)ethyl *)-N*-nonylglycyl)pyrrolidin-3-yl)ethyl dinonylglycinate



Chemical Formula: C57H114N4O3

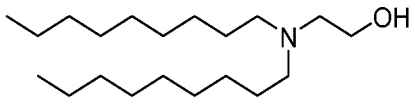
Molecular Weight: 903.6

[00988] 2-(l -*(N*-(2-(Dinonylamino)ethyl *)-N*-nonylglycyl)pyrrolidin-3-yl)ethyl dinonylglycinate was synthesized under same reaction conditions as methyl 8-((2-(4-*(N*-(2-(dinonylamino)ethyl *)-N*-nonylgly cyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)octanoate using 2-(pyrrolidin-3-yl)ethyl dinonylglycinate and *N*-(2-(dinonylamino)ethyl *)-N*-nonylglycine. UPLC/ELSD: RT = 4.15 min. MS (ES): *m/z* (MH^+) 904.9 for C57H114N4O3

¾ NMR (300 MHz, CDCh) δ: ppm 4.17(m, 2H); 3.88-3.38 (m, 3H); 3.20-2.95 (m, 2H); 2.73-2.48 (m, 10H), 2.41 (m, 4H); 2.32-1.95 (m, 3H); 1.86-1.57 (m, 4H); 1.54-1.18 (m, 71H); 0.90 (m, 15H).

DJ: Compound 128: l-(2-(Dinonylamino)ethyl) 4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)ethyl) cy clohexane- 1,4-dicarboxylate

2-(Dinony lamino)ethan- 1-ol



Chemical Formula: C20H43NO

Molecular Weight: 313.57

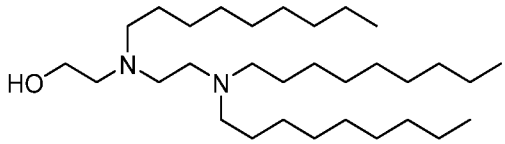
[00989] A solution of (dinonylamino)acetic acid (1 g, 3.05 mmol, 1 equiv.) in dry THF (20 mL) was slowly added to a stirred solution of lithium aluminium hydride (0.232 g, 6.1 mL, 6.12 mmol, 2 equiv.) in dry THF (10 mL) at 0 °C. The reaction was allowed to warm to rt and stirred at rt for 12 h. 10 mL of saturated Na2SO4.10H2O was added to the solution slowly. White solid precipitated out. Added solid Na2S04. IOH2O and filtered through a plug of

celite and washed with ethyl acetate. Mixture was evaporated under vacuum. The residue was purified by flash chromatography (ISCO) by 0-100% ethyl acetate in hexanes to obtain 2-(dinonylamino)ethan-l-ol (0.76 g, 79%).

UPLC/ELSD: RT = 1.49 min. MS (ES): *m/z* (MH^+) 313.8 for C20H43NO

¾ NMR (300 MHz, CDCh) δ: ppm 3.57 (m, 2H); 2.63 (m, 2H); 2.50 (m, 4H); 1.47 (m, 4H); 1.29 (m, 25H); 0.90 (m, 6H).

2-((2-(Dinonylamino)ethyl)(nonyl)amino)ethan-1-ol



Chemical Formula: C31H66N2O

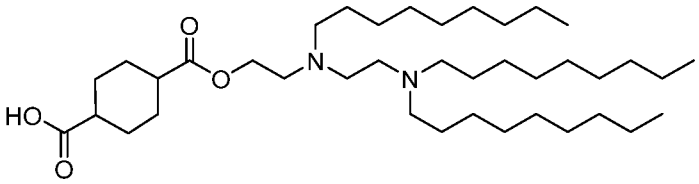
Molecular Weight: 482.88

[00990] 2-((2-(Dinonylamino)ethyl)(nonyl)amino)ethan-l-ol was synthesized in the same manner as 2-(Dinonylamino)ethan-l-ol.

UPLC/ELSD: RT = 1.92 min. MS (ES): *m/z* (MH^+) 484.38 for C31H66N2O

¾ NMR (300 MHz, CDCh) δ: ppm 3.55 (m, 2H); 2.66-2.35 (m, 12H); 1.47 (m, 6H); 1.29 (m, 37H); 0.90 (m, 9H).

4-((2-((2-(Dinonylamino)ethyl)(nonyl)amino)ethoxy)carbonyl)cyclohexane-1-carboxylic acid



Chemical Formula: C39H76N2O4

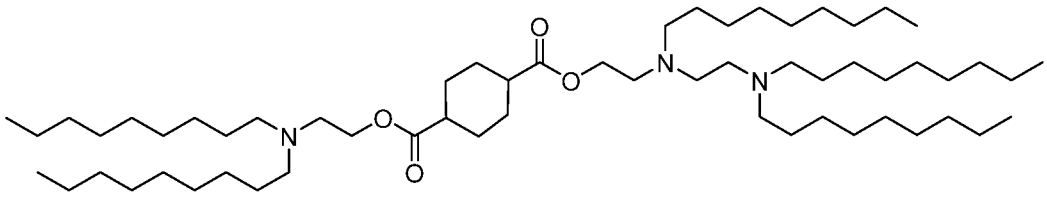
Molecular Weight: 637.05

[00991] 2-(1-(*N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)pyrrolidin-3-yl)ethyl dinonylglycinate was synthesized under same reaction conditions as *tert*-Butyl 4-(2-((dinonylglycyl)oxy)ethyl)piperidine-1-carboxylate with cyclohexane-1,4-dicarboxylic acid and 2-((2-(dinonylamino)ethyl)(nonyl)amino)ethan-l-ol.

UPLC/ELSD: RT = 2.38 min. MS (ES): *m/z* (MH+) 637.5 for C39H76N2O4

¾ NMR (300 MHz, CDCh) δ: ppm 4.12 (m, 2H); 3.08-2.29 (m, 14H); 2.25-1.83 (m, 4H); 1.79-1.18 (m, 46H); 0.90 (m, 9H).

1-(2-(Dinonylamino)ethyl) 4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)ethyl) cyclohexane- 1,4-dicarboxylate



Chemical Formula: C59H117N3O4

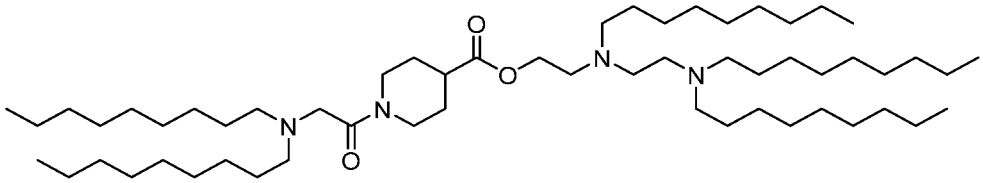
Molecular Weight: 932.60

[00992] 1-(2-(Dinony lamino)ethy 1) 4-(2-((2-(dinony lamino)ethy l)(nony l)amino)ethy 1) cyclohexane-l,4-dicarboxylate was synthesized under same reactions conditions as *tert*-Butyl 4-(2-((dinonylglycyl)oxy)ethyl)piperidine-l-carboxylate using 4-((2-((2-(dinonylamino)ethyl)(nonyl)amino)ethoxy)carbonyl)cyclohexane-l-carboxylic acid and 2-(dinony lamino)ethan- 1-ol.

UPLC/ELSD: RT = 2.89 min. MS (ES): *m/z* (MH^+) 933.6 for C59H117N3O4

¾NMR (300 MHz, CDCh) δ: ppm 4.14 (m, 4H); 2.71 (m, 4H), 2.63-2.34 (m, 15H); 2.13-1.84 (m, 5H); 1.69 (m, 4H); 1.53-1.18 (m, 70H); 0.90 (m, 15H).

DK: Compound 129: 2-((2-(Dinonylamino)ethyl)(nonyl)amino)ethyl 1-(dinonylglycyl)piperidine-4-carboxylate



Chemical Formula: C57H114N4O3

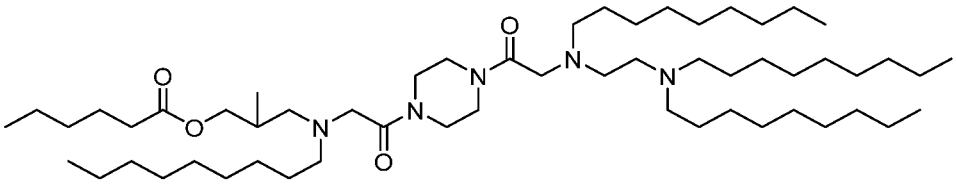
Molecular Weight: 903.56

[00993] 2-((2-(Dinonylamino)ethyl)(nonyl)amino)ethyl l-(dinonylglycyl)piperidine-4-carboxylate was synthesized under same reaction conditions as *tert*-Butyl 4-(2-((dinonylgly cyl)oxy)ethyl)piperidine- 1-carboxylate with 1-(dinonylgly cyl)piperidine-4-carboxylic acid and 2-((2-(dinonylamino)ethyl)(nonyl)amino)ethan-l-ol.

UPLC/ELSD: RT = 2.75 min. MS (ES): *m/z* (MH+) 903.9 for C57H114N4O3

¾ NMR (300 MHz, CDCh) δ: ppm 4.39 (m, 1H); 4.17 (m, 3H); 3.22-2.68 (m, 6H); 2.65-2.34 (m, 14H), 1.94(m, 2H); 1.85-1.56 (m, 4H); 1.53-1.18 (m, 69H); 0.90 (m, 15H).

DL: Compound 130: 3-((2-(4 -(*N* -(2-(Dinonylamino)ethyl)-*N* -nonylglycyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)-2-methylpropyl hexanoate



Chemical Formula: C56H111N5O4

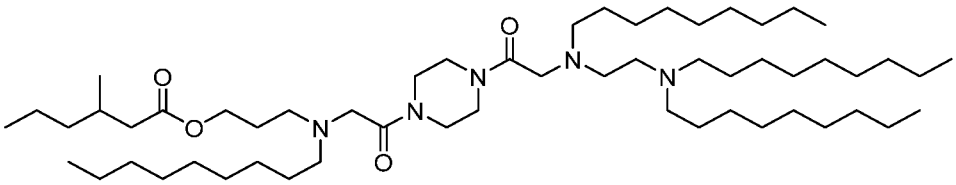
Molecular Weight: 918.54

[00994] 3-((2-(4-(N-(2-(dinonylamino)ethyl)-N-nonylglycyl)piperazin-l -yl)-2-oxoethyl)(nonyl)amino)-2-methylpropyl hexanoate was synthesized in the same manner as methyl 8-((2-(4-(N-(2-(dinonylamino)ethyl)-N-nonylgly cyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)octanoate.

UPLC/ELSD: RT = 2.87 min. MS (ES): *m/z* (MH+) 919.1 for **C56H111N5O4**

¾ NMR (300 MHz, CDCh) δ: ppm 4.08 (m, 1H); 3.86 (m, 1H); 3.79-3.52 (m, 8H); 3.24 (m, 4H); 2.99 (m, 1H); 2.68-2.25 (m, 15H), 2.08-1 .73 (m, 2H); 1.64 (m, 2H); 1.53-1 .18 (m, 59H); 0.90 (m, 18H).

DM: Compound 131: 3-((2-(4 -(*N* -(2-(Dinonylamino)ethyl)-*N* -nonylglycyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)propyl 3-methylhexanoate



Chemical Formula: **C56H111N5O4**

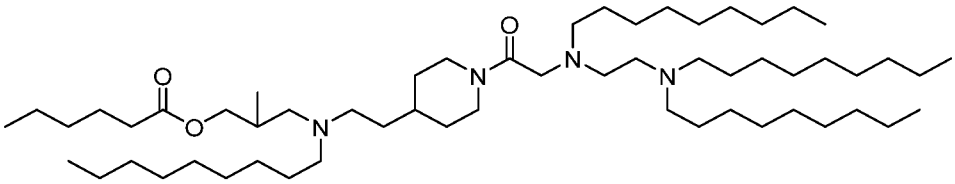
Molecular Weight: 918.54

[00995] 3-((2-(4 -(*N* -(2-(Dinonylamino)ethyl)-*N* -nonylgly cyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)propyl 3-methylhexanoate was synthesized in the same manner as methyl 8-((2-(4 -(*N* -(2-(dinonylamino)ethyl)-*N* -nonylgly cyl)piperazin- 1-yl)-2-oxoethyl)(nonyl)amino)octanoate.

UPLC/ELSD: RT = 2.81 min. MS (ES): *m/z* (MH+) 919.09 for **C56H111N5O4**

¾ NMR (300 MHz, CDCh) δ: ppm 4.10 (m, 1H); 3.85 (m, 1H); 3.75-3.56 (m, 8H); 3.31 (m, 4H); 2.69-2.37 (m, 13H); 2.17-1.90 (m, 2H); 1.79 (m, 2H); 1.53-1 .17 (m, 62H); 0.90 (m, 18H).

DN: Compound 132: 3-((2-(l -(N -(2-(Dinonylamino)ethyl)-N -nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)-2-methylpropyl hexanoate



Chemical Formula: C57H114N4O3

Molecular Weight: 903.56

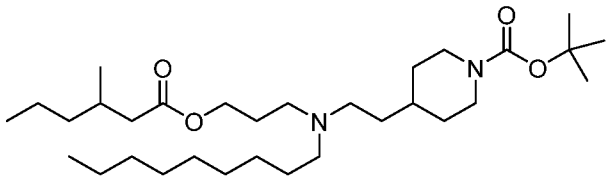
[00996] 3-((2-(l -(N -(2-(Dinonylamino)ethyl)-N -nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)-2-methylpropyl hexanoate was synthesized in the same manner as 3-((2-(l -(N -(2-(dinonylamino)ethyl)-N -nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)propyl 3-methylhexanoate.

UPLC/ELSD: RT = 2.97 min. MS (ES): m/z (MH+) 904.09 for **C57H114N4O3**

¾ NMR (300 MHz, CDCh) δ: ppm 4.54 (m, 1H); 4.28-4.06 (m, 2H); 3.92 (m, 1H); 3.56-2. 80 (m, 5H); 2.7 1-2.26 (m, 15H); 2.16 (m, 1H); 1.94 (m, 1H); 1.85-1 .20 (m, 68H); 1.11 (m, 2H); 0.90 (m, 18H).

DO: Compound 133: 3-((2-(l -(N -(2-(Dinonylamino)ethyl)-N -nonylglycyl)piperidin-^ l-yl)ethyl)(nonyl)amino)propyl 3-methylhexanoate

tert-Butyl 4-(2-((3-((3-methylhexanoyl)oxy)propyl)(nonyl)amino)ethyl)piperidine-l- carboxylate



Chemical Formula: C31H60N2O4

Molecular Weight: 524. 83

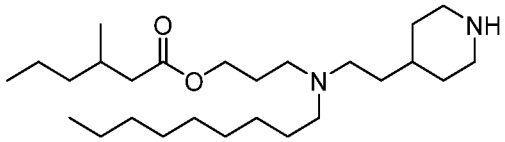
[00997] *tert*-Butyl 4-(2-((3-((3-methylhexanoyl)oxy)propyl)(nonyl)amino)ethyl)piperidine-l -carboxylate was synthesized in the same manner as methyl 8-((2-(teri-butoxy)-2-oxoethyl)(nonyl)amino)octanoate.

UPLC/ELSD: RT = 2.00 min. MS (ES): m/z (MH+) 525.4 for C31H60N2O4

¾ NMR (300 MHz, CDCh) δ: ppm 4.12 (m, 4H); 2.69 (m, 2H); 2.54-2.25 (m, 6H); 2.17-1.90

(m, 2H); 1.84-1 .56 (m, 5H); 1.54-1 .03 (m, 32H); 0.94 (m, 9H).

3-(Nonyl(2-(piperidin-4-yl)ethyl)amino)propyl 3-methylhexanoate



Chemical Formula: C26H52N2O2

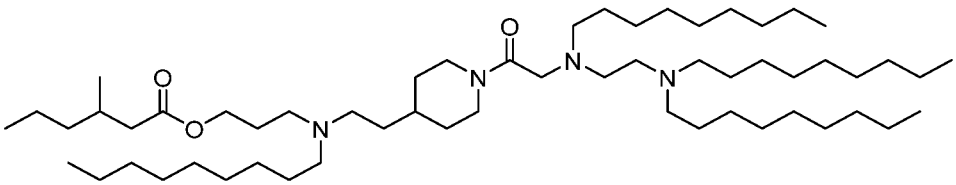
Molecular Weight: 424.7 14

[00998] 3-(Nonyl(2-(piperidin-4-yl)ethyl)amino)propyl 3-methylhexanoate was synthesized in the same manner as 2-((2-(dinonylamino)ethyl)(nonyl)amino)-l -(piperazin-l -yl)ethan-l-one.

UPLC/ELSD: RT = 0.94 min. MS (ES): *m/z* (MH+) 425.4 for C26H52N2O2

¾ NMR (300 MHz, CDCh) δ: ppm 4.13 (t, 2H); 3.10 (m, 2H), 2.60 (t, 2H); 2.52-2.26 (m, 7H); 2.20-1 .91 (m, 3H); 1.87-1 .59 (m, 4H); 1.55-1 .04 (m, 23H); 0.94 (m, 9H).

3-((2-(l -*(N* -(2-(Dinonylamino)ethyl *)-N* -nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)propy^ 3-methylhexanoate



Chemical Formula: **C57H114N4O3**

Molecular Weight: 903.56

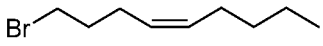
[00999] 3-((2-(l -*(N* -(2-(Dinonylamino)ethyl *)-N* -nonylglycyl)piperidin-4-yl)ethyl)(nonyl)amino)propyl 3-methylhexanoate was synthesized in the same manner as methyl 8-((2-(4 -*(N* -(2-(dinonylamino)ethyl *)-N* -nonylglycyl)piperazin-l-yl)-2-oxoethyl)(nonyl)amino)octanoate.

UPLC/ELSD: RT = 2.97 min. MS (ES): *m/z* (MH+) 904.09 for **C57H114N4O3**

¾ NMR (300 MHz, CDCh) δ: ppm 4.56 (m, 1H); 4.28-4.06 (m, 3H), 3.30 (m, 2H); 2.93 (m, 1H); 2.67-2.26 (m, 17H); 2.19-1 .89 (m, 2H); 1.74 (m, 4H); 1.62-1 .03 (m, 66H); 0.90 (m, 18H).

DP: Compound 136: 2-((2-(Di((Z)-non-3-en-l -yl)amino)ethyl)((Z)-non-3-en-l-yl)amino)-l -(4-(dinonylgly cyl)piperazin- 1-yl)ethan- 1-one

(Z)- 1-Bromonon-4-ene



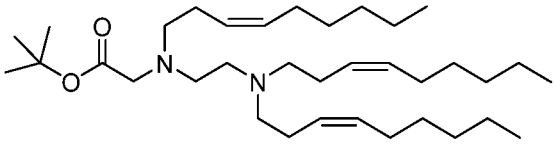
Chemical Formula: C9H17O

Molecular Weight: 205. 14

[001000] To a solution of (3Z)-non-3-en-l-ol (5 g, 0.035 mol) and carbon tetrabromide (11.66 g, 35. 15 1 mmol) in DCM (175 mL) at 0 °C added triphenylphosphine (9.22 g, 0.035 mol) portion wise. The reaction was allowed to stir at 0 °C for lh and at rt for 2h. The reaction was evaporated under vacuum and the residue was suspended in hexanes and filtered. The filtrate was evaporated and the residue was purified by flash chromatography with 0-1 .5% diethyl ether in hexanes to obtain (Z)-l -bromonon-4-ene (5.3 g, 73.5%).

¾ NMR (300 MHz, CDCh) δ: ppm 5.54 (m, 1H), 5.40 (m, 1H), 3.39 (t, 2H), 2.65 (q, 2H), 2.05 (m, 2H), 1.32 (m, 6H), 0.91 (t, 3H)

tert-butyl *N*-(2-(Di((Z)-non-3-en-1-yl)amino)ethyl)-*N*-((Z)-non-3-en- 1-yl)gly cinate



Chemical Formula: C35H66N2O2

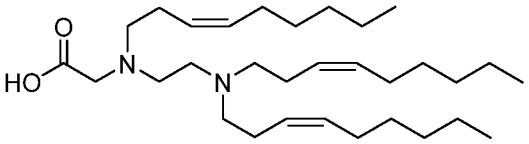
Molecular Weight: 546.93

[001001] A mixture of (2-ammonioethyl)[2-(teri-butoxy)-2-oxoethyl]azanium oxalate (2. 15 g, 8.125 mmol,) in CPME (20 mL) was treated with potassium carbonate (6.74 g, 0.049 mol) and KI (4.05 g, 0.024 mol). The mixture was heated at 81 °C for 18 h. The reaction mixture was allowed to cool to 30 °C, filtered through a pad of Celite which was rinsed with DCM (3 X 50 mL) and concentrated *in vacuo* at 40 °C to give pale brown oil. The pale brown oil was diluted in ^-heptane (260 mL) and MeCN (260 mL), shaken well and phase split. The heptane layer was separated from MeCN layer followed by washed with water (260 mL). The heptane layer was separated from aqueous layer, washed with MeCN (260 mL) and concentrated in vacuum at 40 °C to provide crude product. The crude was purified by flash chromatography by 0-100% (a solution of 20% MeOH, 80% DCM, 1% NH_4OH) in DCM to obtain *tert*-butyl *N*-(2-(di((Z)-non-3-en-l-yl)amino)ethyl)-*N*-((Z)-non-3-en-l -yl)gly cinate (1.89 g, 42.5%).

UPLC/ELSD: RT = 2.94 min. MS (ES): *m/z* (MH+) 547.46 for C35H66N2O2

¾ NMR (300 MHz, CDCh) δ: ppm 5.39 (m, 6H), 3.32 (s, 2H), 2.75 (m, 2H), 2.64 (m, 4H); 2.52 (m, 4H); 2.22 (m, 6H); 2.04 (m, 6H), 1.48 (s, 9H); 1.32 (m, 18H), 0.91 (m, 9H).

N-(2-(Di((Z)-non-3 -en- 1-yl)amino)ethy l)-*N*-((Z)-non-3-en- 1-yl)gly cine



Chemical Formula: C31H58N2O2

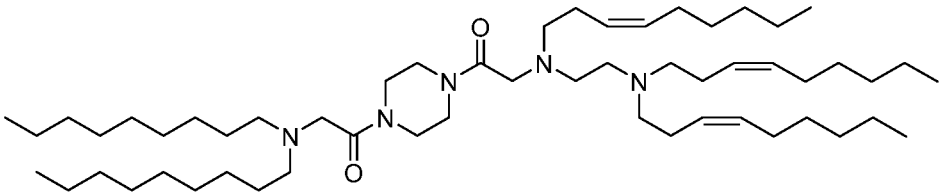
Molecular Weight: 490.82

[001002] *N*-(2-(Di((Z)-non-3-en-l-yl)amino)ethyl)-*N*-((Z)-non-3-en-l-yl)glycine was synthesized in the same manner as methyl 8-((2-(tert-butoxy)-2-oxoethyl)(nonyl)amino)octanoate.

UPLC/ELSD: RT = 2.30 min. MS (ES): *m/z* (MH+) 492.52 for C31H58N2O2

¾ NMR (300 MHz, CDCh) δ: ppm 5.61-5.19 (m, 6H); 3.32 (s, 2H); 2.78 (m, 8H); 2.66 (m, 2H); 2.42 (m, 4H); 2.25 (m, 2H); 2.04 (m, 6H), 1.31 (m, 18H), 0.91 (m, 9H).

2-((2-(Di((Z)-non-3-en-1-yl)amino)ethyl)((Z)-non-3-en-1-yl)amino)-1-(4-(dinonylglycyl)piperazin-1-yl)ethan-1-one



Chemical Formula: C55H105N5O2

Molecular Weight: 868.49

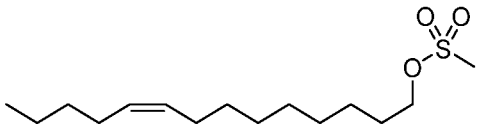
[001003] 2-((2-(Di((Z)-non-3-en-l-yl)amino)ethyl)((Z)-non-3-en-l-yl)amino)-l-(4-(dinonylglycyl)piperazin-l-yl)ethan-l-one was synthesized in the same manner as methyl 8-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(nonyl)amino)octanoate.

UPLC/ELSD: RT = 2.85 min. MS (ES): *m/z* (MH+) 869.07 for C55H105N5O2

¾ NMR (300 MHz, CDCh) δ: ppm 5.50-5.20 (m, 6H); 3.71-3.49 (m, 8H), 3.35 (s, 2H); 3.25 (s, 2H); 2.71-2.36 (m, 14H); 2.17 (m, 6H); 2.02 (m, 6H); 1.51-1.29 (m, 46H); 0.89 (m, 15H).

DQ: Compound 140: (Z)-3-((2-(4-(*N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(tetradec-9-en-1-yl)amino)propy 1decanoate

(Z)-Tetradec-9-en-l-yl methanesulfonate



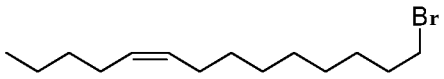
Chemical Formula: C15H30O3S

Molecular Weight: 290.46

[001004] To a 0 °C solution of (Z)-tetradec-9-en-l-ol (500 mg, 2.4 mmol) and triethylamine (430 μL, 3.1 mmol) in DCM (4 mL) was added a solution of methanesulfonyl chloride (200 μL, 2.6 mmol) in DCM (1 mL), and the reaction was allowed to return to RT and stir for 4 hours. The reaction was quenched with water and extracted with DCM. The combined organics were washed with saturated NaHCCb, brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-30% hexanes/EtOAc) provided (Z)-tetradec-9-en-l-yl methanesulfonate (574 mg, 84%).

¾-NMR (300 MHz, CDCh) δ: ppm 5.37 (m, 2H); 4.25 (t, 2H); 3.03 (s, 3H); 2.05 (m, 4H); 1.77 (quint, 2H); 1.65-1.15 (br. m, 14H); 0.92 (t, 3H).

(Z)-14-Bromotetradec-5-ene



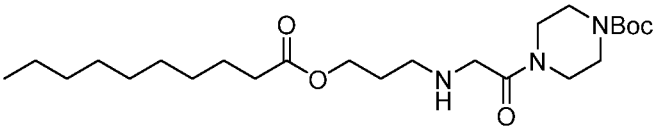
Chemical Formula: Ci4H27Br

Molecular Weight: 275.27

[001005] To a solution of (Z)-tetradec-9-en-l-yl methanesulfonate (574 mg, 1.98 mmol) in diethyl ether (20 mL) was added magnesium bromide ethyl etherate (1.5 g, 5.9 mmol), and the reaction was allowed to stir at RT overnight. The reaction was quenched with water and extracted with diethyl ether. The combined organic layers were washed with 1% K2CO3, brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-10% hexanes/EtOAc) provided (Z)-14-bromotetradec-5-ene (444 mg, 82%).

¾-NMR (300 MHz, CDCh) δ: ppm 5.37 (m, 2H); 3.43 (t, 2H); 2.05 (m, 4H); 1.87 (quint, 2H); 1.65-1.05 (br. m, 14H); 0.92 (t, 3H).

tert-Butyl 4-((3-(decanoyloxy)propyl)glycyl)piperazine-l-carboxylate



Chemical Formula: C24H45N3O5

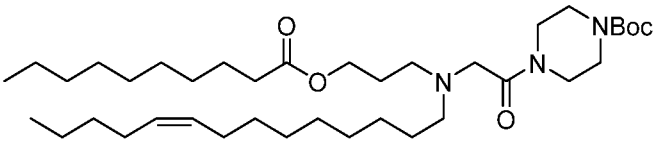
Molecular Weight: 455.64

[001006] In the same manner as Step 1 for methyl 8-((2-(4-(*N*-(2-(Di((Z)-non-3-en-1-yl)amino)ethyl)-*N*-((Z)-non-3-en-1-yl)glycyl)piperazin-1-yl)-2-oxoethyl)(nonyl)amino)octanoate, 3-((2-oxo-2-(piperazin-1-yl)ethyl)amino)propyl decanoate, *tert*-butyl 4-((3-(decanoyloxy)propyl)glycyl)piperazine-1-carboxylate was synthesized from *tert*-butyl 4-glycylpiperazine-1-carboxylate (3.3 g, 14 mmol), 3-bromopropyl decanoate (2.0 g, 6.8 mmol), K_2CO_3 (1.9 g, 14 mmol), and KI (230 mg, 1.4 mmol) in MeCN (70 mL). Yield (1.64 g, 53%).

UPLC/ELSD: RT = 1.26 min. MS (ES): m/z (MH^+) 456.61 for $C_{24}H_{45}N_3O_5$

¾-NMR (300 MHz, $CDCl_3$) δ: ppm 4.30-2.50 (br. m, 14H); 2.44-2.02 (br. m, 4H); 1.85-1.05 (br. m, 23H); 0.90 (t, 3H).

tert-Butyl (Z)-4-(*N*-(3-(decanoyloxy)propyl)-*N*-(tetradec-9-en-1-yl)glycyl)piperazine-1-carboxylate



Chemical Formula: $C_{38}H_{71}N_3O_5$

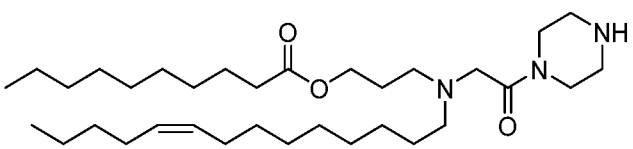
Molecular Weight: 650.00

[001007] In the same manner as Step 2 for methyl 8-((2-(4-(*N*-(2-(Di((Z)-non-3-en-1-yl)amino)ethyl)-*N*-((Z)-non-3-en-1-yl)glycyl)piperazin-1-yl)-2-oxoethyl)(nonyl)amino)octanoate, *tert-butyl* (Z)-4-(*N*-(3-(decanoyloxy)propyl)-*N*-(tetradec-9-en-1-yl)glycyl)piperazine-1-carboxylate was synthesized from *tert-butyl* 4-((3-(decanoyloxy)propyl)glycyl)piperazine-1-carboxylate (670 mg, 1.5 mmol), (Z)-14-bromotetradec-5-ene (444 mg, 1.61 mmol), K_2CO_3 (400 mg, 2.9 mmol), and KI (24 mg, 0.15 mmol) in MeCN (15 mL). Yield (444 mg, 47%).

UPLC/ELSD: RT = 2.69 min. MS (ES): m/z (MH^+) 650.86 for $C_{38}H_{71}N_3O_5$

¾-NMR (300 MHz, $CDCl_3$) δ: ppm 5.37 (m, 2H); 4.10 (t, 2H); 3.59 (m, 4H); 3.44 (m, 4H); 3.29 (s, 2H); 2.64-2.41 (br. m, 4H); 2.30 (t, 2H); 2.04 m, 4H); 1.86-1.10 (br. m, 41H); 0.91 (m, 6H).

(Z)-3-((2-Oxo-2-(piperazin-1-yl)ethyl)(tetradec-9-en-1-yl)amino)propyl decanoate



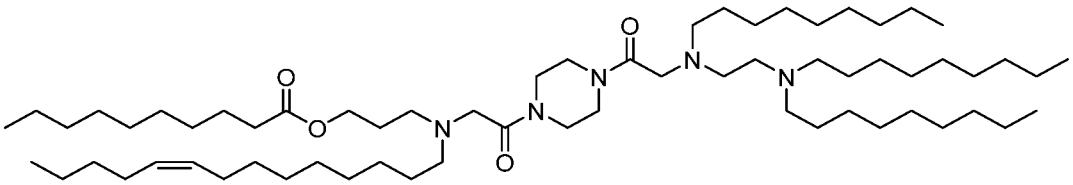
Chemical Formula: C33H63N3O3

Molecular Weight: 549.89

[001008] In the same manner as Step 4 for Compound 11, (Z)-3-((2-oxo-2-(piperazin-l-yl)ethyl)(tetradec-9-en-l-yl)amino)propyl decanoate was synthesized from *tert*-butyl (Z)-4-*(N*-(3-(decanoyloxy)propyl *)-N*-(tetradec-9-en- 1-yl)glycyl)piperazine- 1-carboxylate (444 mg, 0.683 mmol) and TFA (2.6 mL, 34 mmol) in DCM (3 mL). Yield (361 mg, 96%). UPLC/ELSD: RT = 1.91 min. MS (ES): m/z (MH^+) 550.74 for **C33H63N3O35**

¾-NMR (300 MHz, CDCh) δ: ppm 5.37 (m, 2H); 4.10 (t, 2H); 3.59 (br. 4H); 3.28 (s, 2H); 2.87 (m, 4H); 2.59 (t, 2H); 2.49 (t, 2H); 2.30 (t, 2H); 2.15-1.05 (br. m, 37H); 0.91 (m, 6H).

(Z)-3-((2<4 -(*N*-(2-(Dinonylamino)ethyl *)-N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(tetradec-9-en-l-yl)amino)propyl decanoate



Chemical Formula: C64H125N5O4

Molecular Weight: 1028.74

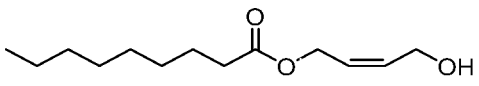
[001009] In the same manner as Step 11 for Compound 11, (Z)-3-((2-(4 -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperazin- 1-yl)-2-oxoethyl)(tetradec-9-en- 1-yl)amino)propyl decanoate was synthesized from (Z)-3-((2-oxo-2-(piperazin-l-yl)ethyl)(tetradec-9-en-l-yl)amino)propyl decanoate (361 mg, 0.657 mmol), *N*-(2-(dinonylamino)ethyl *)-N*-nonylglycine (360 mg, 0.72 mmol), iPnEtN (250 µL, 1.4 mmol), and T3P (50% EtOAc solution, 1.2 mL, 2.0 mmol) in THF (7 mL). Yield (172 mg, 25%)

UPLC/ELSD: RT = 3.30 min. MS (ES): m/z (MH^+) 1029.31 for C64H125N5O4

¾-NMR (300 MHz, CDCh) δ: ppm 5.37 (m, 2H); 4.10 (t, 2H); 3.80-2.20 (br. m, 28H); 2.03 (m, 4H); 1.85-1.05 (br. m, 74H); 0.90 (m, 15H).

DR: Compound 141: (Z)-4-((2-(4 -*(N*-(2-(dinonylamino)ethyl *)-N*-nonylglycyl)piperazin-l-yl)-2-oxoethyl)(tetradecyl)amino)but-2-en- 1-y 1nonanoate

(Z)-4-Hydroxybut-2-en-l -yl nonanoate



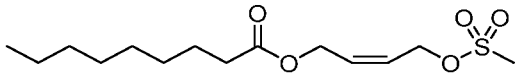
Chemical Formula: C13H24O3

Molecular Weight: 228.33

[001010] In the same manner as Step 3 for 3-((2-(4 -(N -(2-(Dinonylamino)ethyl)-N-nonylglycyl)piperazin-l -yl)-2-oxoethyl)(tetradecyl)amino)propyl (Z)-dec-3-enoate, (Z)-4-hydroxybut-2-en-l -yl nonanoate was synthesized from nonanoic acid (1.4 g, 8.5 mmol), (Z)-but-2-ene-l ,4-diol (3.0 g, 34 mmol), EDC (1.6 g, 10 mmol), and DMAP (210 mg, 1.7 mmol) in DCM (20 mL). Yield (1.45 g, 75%).

¾-NMR (300 MHz, CDCh) δ: ppm 5.87 (m, 1H); 5.65 (m, 1H); 4.70 (d, 2H); 4.28 (d, 2H); 2.32 (t, 2H), 1.92 (s, 1H); 1.63 (m, 2H); 1.30 (br. m, 10H); 0.90 (t, 3H)

(Z)-4-((methy lsulfony l)oxy)but-2-en-1-yl nonanoate



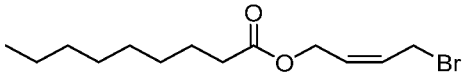
Chemical Formula: C14H26O5S

Molecular Weight: 306.42

[001011] In the same manner as Step 1 for (Z)-3-((2-(4 -(N -(2-(dinonylamino)ethyl)-N-nonylglycyl)piperazin-l -yl)-2-oxoethyl)(tetradec-9-en-l -yl)amino)propyl decanoate, (Z)-4-((methylsulfonyl)oxy)but-2-en-l-yl nonanoate was synthesized from (Z)-4-hydroxybut-2-en-l-yl nonanoate (1.45 g, 6.35 mmol), methanesulfonyl chloride (540 μL, 7.0 mmol), and triethylamine (1.2 mL, 8.3 mmol) in DCM (12 mL). Yield (959 mg, 49%).

¾-NMR (300 MHz, CDCh) δ: ppm 5.86 (m, 2H); 4.89 (d, 2H); 4.70 (d, 2H); 3.06 (s, 3H); 2.34 (t, 2H); 1.63 (m, 2H); 1.30 (br. m, 10H); 0.90 (t, 3H).

(Z)-4-Bromobut-2-en-l -yl nonanoate



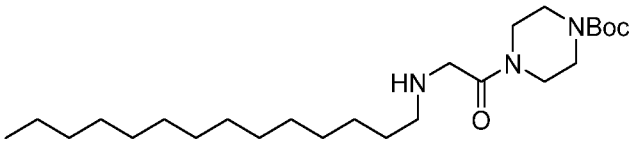
Chemical Formula: Ci3H23Br02

Molecular Weight: 291 .23

[001012] In the same manner as Step 2 for (Z)-3-((2-(4 -(N -(2-(dinonylamino)ethyl)-N -nonylgly cyl)piperazin- 1-yl)-2-oxoethyl)(tetradec-9-en- 1-yl)amino)propyl decanoate, (Z)-4-bromobut-2-en-l -yl nonanoate was synthesized from (Z)-4-((methylsulfonyl)oxy)but-2-en-l-yl nonanoate (959 mg, 3.13 mmol) and magnesium bromide ethyl etherate (2.4 g, 9.4 mmol) in diethyl ether (30 mL). Yield (797 mg, 87%).

¾-NMR (300 MHz, $CDCl_3$) δ: ppm 5.96 (m, 1H); 5.72 (m, 1H); 4.72 (d, 2H); 4.05 (d, 2H); 2.34 (t, 2H); 1.72-1.05 (br. m, 12H); 0.90 (t, 3H).

*tert-B*utyl 4-(tetradecylglycyl)piperazine-l -carboxylate



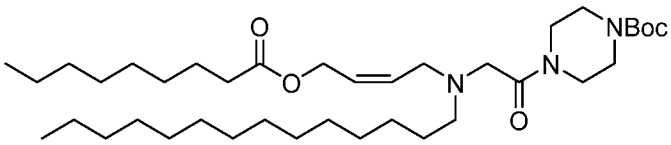
Chemical Formula: C25H49N3O3

Molecular Weight: 439.69

[001013] In the same manner as Step 1 for methyl 8-((2-(4-*(N*-(2-(Di((Z)-non-3-en-l-yl)amino)ethy l*)-N*-((Z)-non-3-en-1-yl)gly cyl)piperazin-1-yl)-2-oxoethyl)(nonyl)amino)octanoate, *tert-butyl* 4-(tetradecylglycyl)piperazine-l -carboxylate was synthesized from *tert-butyl* 4-glycylpiperazine-l -carboxylate (5.3 g, 22 mmol), 1-bromotetradecane (3.0 g, 11 mmol), K2CO3 (3.0 g, mmol), and KI (180 mg, 1.1 mmol) in MeCN (100 mL). Yield (1.62 g, 34%).

UPLC/ELSD: RT = 1.81 min. MS (ES): m/z (MH^+) 440.62 for C25H49N3O3

tert-Butyl (Z)-4-*(N*-(4-(nonanoyloxy)but-2-en-1-yl*)-N*-tetradecylgly cyl)piperazine-1-carboxylate



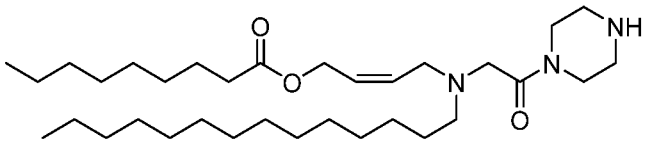
Chemical Formula: C38H71N3O5

Molecular Weight: 650.00

[001014] In the same manner as Step 1 for methyl 8-((2-(4-*(N*-(2-(Di((Z)-non-3-en-l-yl)amino)ethy l*)-N*-((Z)-non-3-en-1-yl)gly cyl)piperazin-1-yl)-2-oxoethy l)(nony l)amino)octanoate, *tert-butyl* (Z)-4-*(N*-(4-(nonanoy loxy)but-2-en-1-y l*)-N*-tetradecylglycyl)piperazine-l -carboxylate was synthesized from *tert-butyl* 4-(tetradecylglycyl)piperazine-l -carboxylate (300 mg, 0.68 mmol), (Z)-4-bromobut-2-en-l-yl nonanoate (220 mg, 0.75 mmol), K2CO3 (190 mg, 1.4 mmol), and KI (23 mg, 0.14 mmol) in MeCN (7 mL). Yield (185 mg, 42%).

UPLC/ELSD: RT = 2.85 min. MS (ES): m/z (MH^+) 650.78 for C38H71N3O5

(Z)-4-((2-Oxo-2-(piperazin-l -yl)ethyl)(tetradecyl)amino)but-2-en-l-yl nonanoate

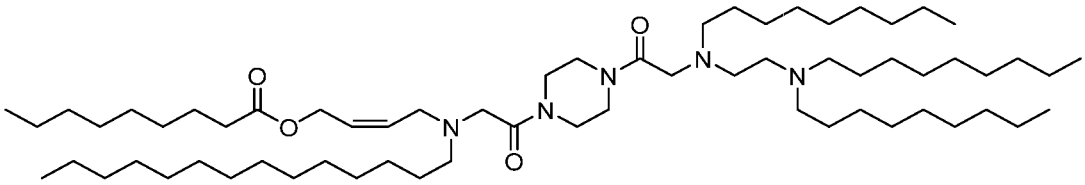


Chemical Formula: C33H63N3O3

Molecular Weight: 549.89

[001015] In the same manner as Step 4 for Compound 11, (Z)-4-((2-oxo-2-(piperazin-1-yl)ethyl)(tetradecyl)amino)but-2-en-1-yl nonanoate was synthesized from *tert*-butyl (Z)-4-(*N*-(4-(nonanoyloxy)but-2-en-1-yl)-*N*-tetradecylglycyl)piperazine-1-carboxylate (185 mg, 0.285 mmol) and TFA (1.1 mL, 14 mmol) in DCM (1.5 mL). Yield (128 mg, 82%).
UPLC/ELSD: RT = 2.00 min. MS (ES): m/z (MH^+) 550.74 for C33H63N3O3

(Z)-4-((2-(4-(*N*-(2-(Dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(tetradecyl)amino)but-2-en-1-yl nonanoate



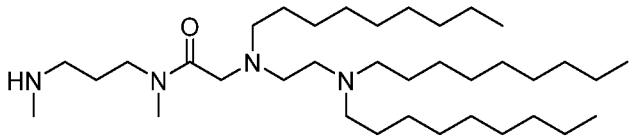
Chemical Formula: C64H125N5O4

Molecular Weight: 1028.74

[001016] In the same manner as Step 11 for Compound 11, (Z)-4-((2-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin-1-yl)-2-oxoethyl)(tetradecyl)amino)but-2-en-1-yl nonanoate was synthesized from (Z)-4-((2-oxo-2-(piperazin-1-yl)ethyl)(tetradecyl)amino)but-2-en-1-yl nonanoate (128 mg, 0.233 mmol), *N*-(2-(dinonylamino)ethyl)-*N*-nonylglycine (127 mg, 0.256 mmol), iPr_2EtN (89 μL, 0.51 mmol), and T3P (50% EtOAc solution, 420 μL, 0.70 mmol) in THF (3 mL). Yield (81 mg, 34%).
UPLC/ELSD: RT = 3.33 min. MS (ES): m/z (MH^+) 1029.39 for C64H125N5O4
¾-NMR (300 MHz, CDCh) δ: ppm 5.71 (m, 2H); 4.65 (d, 2H); 3.80-2.25 (br. m, 28H); 1.85-1.00 (br. m, 78H); 0.90 (t, 15H).

DS: Compound 155: 2-(dinonylamino)-N-(3-(2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-methylacetamido)propyl)-N-methylacetamide

Step 1: 2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-methyl-N-(3-(methylamino)propyl)acetamide



Chemical Formula: C36H76N4O

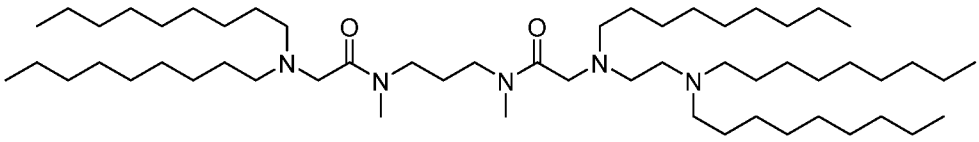
Molecular Weight: 581.03

[001017] In the same manner as Step 5 for Compound 44, 2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-methyl-N-(3-(methylamino)propyl)acetamide was synthesized from N-(2-(dinonylamino)ethyl)-N-nonylglycine (750 mg, 1.51 mmol), N^1,N^3-dimethylpropane-l,3-diamine (755 μL, 1.51 mmol), DIPEA (580 μL, 3.32 mmol), and T3P (50% EtOAc solution, 2.70 mL, 4.53 mmol) in THF (20 mL). Yield (793 mg, 90.4%).

UPLC/ELSD: RT = 2.03 min. MS (ES): *m/z* (MH^+) 581.82 for C36H76N4O

¾-NMR (300 MHz, CDCh) δ: ppm 3.60-2.20 (br. m, 22H); 2.00-1.00 (br. m, 44H); 0.90 (t, 9H)

Step 2: 2-(dinonylamino)-N-(3-(2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-methylacetamido)propyl)-N-methylacetamide (Compound 155)



Chemical Formula: C56H115N5O2

Molecular Weight: 890.57

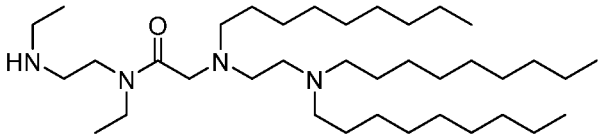
[001018] In the same manner as Step 7 for Compound 44, 2-(dinonylamino)-N-(3-(2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-methylacetamido)propyl)-N-methylacetamide was synthesized from 2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-methyl-N-(3-(methylamino)propyl)acetamide (357 mg, 0.614 mmol), dinonylglycine (221 mg, 0.676 mmol), DIPEA (236 μL, 1.35 mmol), and T3P (50% EtOAc solution, 1.10 mL, 1.84 mmol) in THF (10 mL). Yield (314 mg, 57.4%).

UPLC/ELSD: RT = 2.97 min. MS (ES): *m/z* (MH^+) 891.29 for C56H115N5O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.50-2.30 (br. m, 28H); 1.95-1.10 (br. m, 72H); 0.90 (t, 15H)

DT: Compound 157: 2-(dinonylamino)-N-(2-(2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-ethylacetamido)ethyl)-N-ethylacetamide

Step 1: 2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-ethyl-N-(2-(ethylamino)ethyl)acetamide



Chemical Formula: C37H78N4O

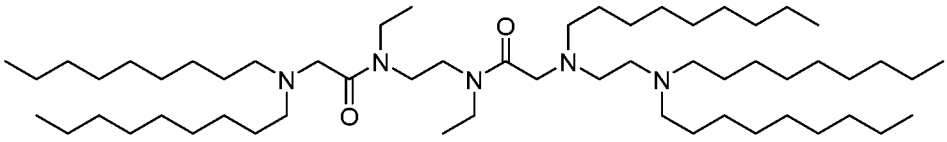
Molecular Weight: 595.06

[001019] In the same manner as Step 5 for Compound 44, 2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-ethyl-N-(2-(ethylamino)ethyl)acetamide was synthesized from N-(2-(dinonylamino)ethyl)-N-nonylglycine (750 mg, 1.51 mmol), N^1,N^2-diethylethane-1,2-diamine (865 μL, 6.04 mmol), DIPEA (580 μL, 3.32 mmol), and T3P (50% EtOAc solution, 2.70 mL, 4.53 mmol) in THF (20 mL). Yield (725 mg, 80.7%).

UPLC/ELSD: RT = 2.09 min. MS (ES): *m/z* (MH^+) 595.92 for C37H78N4O

¾-NMR (300 MHz, CDCh) δ: ppm 3.70 -2.20 (br. m, 20H); 1.70-1.05 (br. m, 48H); 0.90 (t, 9H)

Step 2: 2-(dinonylamino)-N-(2-(2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-ethylacetamido)ethyl)-N-ethylacetamide (Compound 157)



Chemical Formula: C57H117N5O2

Molecular Weight: 904.60

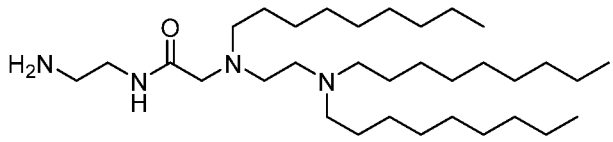
[001020] In the same manner as Step 7 for Compound 44, 2-(dinonylamino)-N-(2-(2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-ethylacetamido)ethyl)-N-ethylacetamide was synthesized from 2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-ethyl-N-(2-(ethylamino)ethyl)acetamide (471 mg, 0.792 mmol), dinonylglycine (285 mg, 0.871 mmol), DIPEA (304 uL, 1.74 mmol), and T3P (50% EtOAc solution, 1.41 mL, 2.38 mmol) in THF (10 mL). Yield (447 mg, 62.4%).

UPLC/ELSD: RT = 3.00 min. MS (ES): *m/z* (MH^+) 905.32 for C57H117N5O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.70-2.20 (br. m, 26H); 1.54-1.04 (br. m, 76H); 0.90 (t, 15H)

DU: Compound 153: 2-(dinonylamino)-N-(2-(2-((2-(dinonylamino)ethyl)(nonyl)amino)acetamido)ethyl)acetamide

Step 1: N-(2-aminoethyl)-2-((2-(dinonylamino)ethyl)(nonyl)amino)acetamide



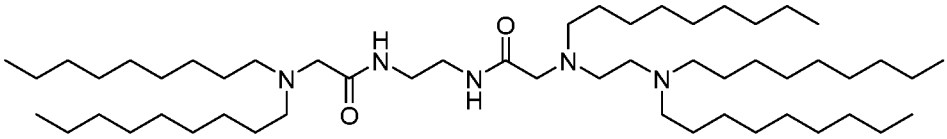
Chemical Formula: C33H70N4O

Molecular Weight: 538.95

[001021] In the same manner as Step 5 for Compound 44, N-(2-aminoethyl)-2-((2-(dinonylamino)ethyl)(nonyl)amino)acetamide was synthesized from N-(2-(dinonylamino)ethyl)-N-nonylglycine (750 mg, 1.51 mmol), ethane-1,2-diamine (403 μL, 6.04 mmol), DIPEA (580 μL, 3.32 mmol), and T3P (50% EtOAc solution, 2.70 mL, 4.53 mmol) in THF (20 mL). Yield (563 mg, 69.2%).

¾-NMR (300 MHz, CDCh) δ: ppm 3.45-2.30 (br. m, 16H); 1.80-1.00 (br. m, 44H); 0.90 (t, 9H)

Step 2: 2-(dinonylamino)-N-(2-(2-((2-(dinonylamino)ethyl)(nonyl)amino)acetamido)ethyl)acetamide (Compound 153)



Chemical Formula: C53H109N5O2

Molecular Weight: 848.49

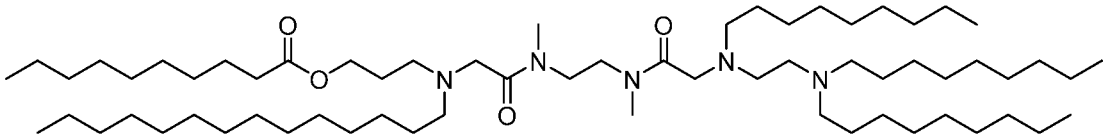
[001022] In the same manner as Step 7 for Compound 44, 2-(dinonylamino)-N-(2-(2-((2-(dinonylamino)ethyl)(nonyl)amino)acetamido)ethyl)acetamide was synthesized from N-(2-aminoethyl)-2-((2-(dinonylamino)ethyl)(nonyl)amino)acetamide (339 mg, 0.629 mmol), dinonylglycine (227 mg, 0.692 mmol), DIPEA (242 μL, 1.38 mmol), and T3P (50% EtOAc solution, 1.12 mL, 1.89 mmol) in THF (10 mL). Yield (439 mg, 82.3%).

UPLC/ELSD: RT = 2.99 min. MS (ES): *m/z* (MH^+) 849.39 for C53H109N5O2

¾-NMR (300 MHz, CDCh) δ: ppm 8.09 (m, 1H); 7.63 (m, 1H); 3.50-2.25 (br. m, 22H); 1.90-1.05 (br. m, 70H); 0.90 (t, 15H)

DV: Compound 158: 7,10-dimethyl-13,16-dinonyl-6,ll-dioxo-4-tetradecyl-4,7,10,13,16-pentaazapentacosyl decanoate

Step 1: 7,10-dimethyl-13,16-dinonyl-6,l l-dioxo-4-tetradecyl-4,7, 10,13, 16-pentaazapentacosyl decanoate (Compound 158)



Chemical Formula: C64H129N5O4

Molecular Weight: 1032.77

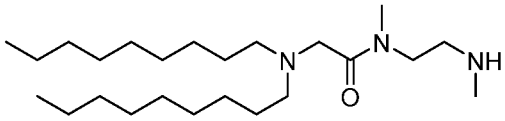
[001023] In the same manner as Step 7 for Compound 44, 7,10-dimethyl-13,16-dinonyl-6,1 l-dioxo-4-tetradecyl-4,7,l 0,1 3,1 6-pentaazapentacosyl decanoate was synthesized from 2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-methyl-N-(2-(methylamino)ethyl)acetamide (227 mg, 0.400 mmol), N-(3-(decanoyloxy)propyl)-N-tetradecylglycine (213 mg, 0.440 mmol), DIPEA (154 μL, 0.881 mmol), and T3P (50% EtOAc solution, 714 μL, 1.20 mmol) in THF (5 mL). Yield (138 mg, 33.4%).

UPLC/ELSD: RT = 3.31 min. MS (ES): *m/z* (MH^+) 1033.49 for C64H129N5O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.10 (t, 2H); 3.70-2.20 (br. m, 32H); 1.85-1.00 (br. m, 80H); 0.90 (t, 15H)

DW: Compound 154: 2-((2-(di((Z)-non-3-en-l-yl)amino)ethyl)((Z)-non-3-en-l-yl)amino)-N-(2-(2-(dinonylamino)-N-methylacetamido)ethyl)-N-methylacetamide

Step 1: 2-(dinonylamino)-N-methyl-N-(2-(methylamino)ethyl)acetamide



Chemical Formula: C24H51N3O

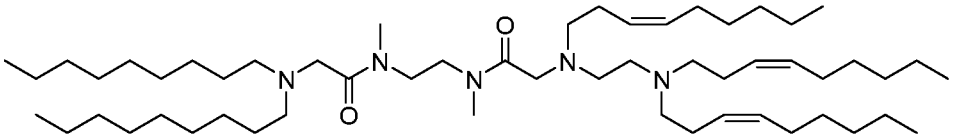
Molecular Weight: 397.69

[001024] In the same manner as Step 3 for Compound 11, 2-(dinonylamino)-N-methyl-N-(2-(methylamino)ethyl)acetamide was synthesized from dinonylglycine (300 mg, 0.916 mmol), N^N 2-dime%lethane-l,2-diamine (394 μL, 3.66 mmol), DIPEA (352 uL, 2.02 mmol), and T3P (50% EtOAc solution, 1.64 mL, 2.75 mmol) in THF (20 mL). Yield (343 mg, 94.2%).

UPLC/ELSD: RT = 1.07 min. MS (ES): *m/z* (MH^+) 398.39 for C24H51N3O

¾-NMR (300 MHz, CDCh) δ: ppm 3.65-2.35 (br. m, 16H); 1.85-1.05 (br. m, 29H); 0.90 (t, 6H)

Step 2: 2-((2-(di((Z)-non-3-en-l-yl)amino)ethyl)((Z)-non-3-en-l-yl)amino)-N-(2-(2-(dinonylamino)-N-methylacetamido)ethyl)-N-methylacetamide (Compound 154)



Chemical Formula: C55H107N5O2

Molecular Weight: 870.49

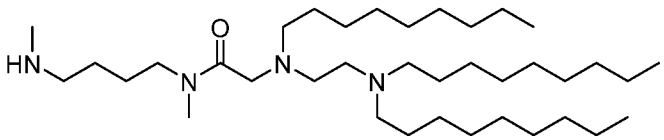
[001025] In the same manner as Step 11 for Compound 11, 2-((2-(di((Z)-non-3-en-l-yl)amino)ethyl)((Z)-non-3-en-l-yl)amino)-N-(2-(2-(dinonylamino)-N-methylacetamido)ethyl)-N-methylacetamide was synthesized from 2-(dinonylamino)-N-methyl-N-(2-(methylamino)ethyl)acetamide (150 mg, 0.377 mmol), N-(2-(di((Z)-non-3-en-l-yl)amino)ethyl)-N-((Z)-non-3-en-l-yl)glycine (204 mg, 0.415 mmol), DIPEA (145 μL, 0.830 mmol), and T3P (50% EtOAc solution, 674 μL, 1.13 mmol) in THF (5 mL). Yield (75 mg, 23%).

UPLC/ELSD: RT = 2.79 min. MS (ES): *m/z* (MH^+) 871.28 for C55H107N5O2

^{1}H-NMR (300 MHz, CDCh) δ: ppm 5.26 (m, 6H); 3.60-1.80 (br. m, 40H); 1.48-1.00 (br. m, 46H); 0.81 (m, 15H)

DX7: Compound 159: 2-(dinonylamino)-N-(4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-methylacetamido)butyl)-N-methylacetamide

Step 1: 2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-methyl-N-(4-(methylamino)butyl)acetamide



Chemical Formula: C37H78N4O

Molecular Weight: 595.06

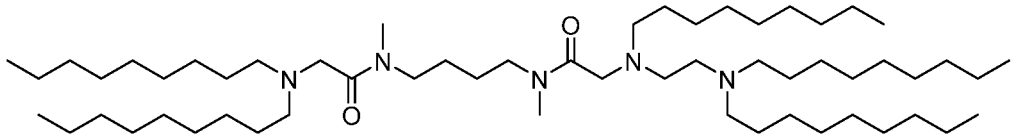
[001026] In the same manner as Step 5 for Compound 44, 2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-methyl-N-(4-(methylamino)butyl)acetamide was synthesized from N-(2-(dinonylamino)ethyl)-N-nonylgly cine (750 mg, 1.51 mmol), N^1,N^4-

dimethylbutane-l,4-diamine (702 mg, 6.04 mmol), DIPEA (580 μL, 3.32 mmol), and T3P (50% EtOAc solution, 2.70 mL, 4.53 mmol) in THF (20 mL). Yield (759 mg, 84.5%).

UPLC/ELSD: RT = 2.03 min. MS (ES): *m/z* (MH^+) 595.92 for $C_{37}H_{78}N_4O$

¾-NMR (300 MHz, CDCh) δ: ppm 3.50-2.30 (br. m, 22H); 1.75-1.10 (br. m, 47H); 0.90 (t, 9H)

Step 2: 2-(dinonylamino)-N-(4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-methylacetamido)butyl)-N-methylacetamide (Compound 159)



Chemical Formula: C57H117N5O2

Molecular Weight: 904.60

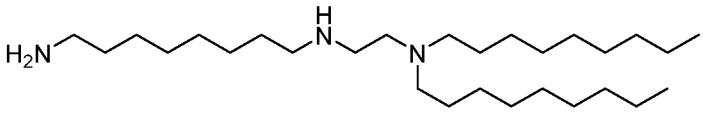
[001027] In the same manner as Step 7 for Compound 44, 2-(dinonylamino)-N-(4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-methylacetamido)butyl)-N-methylacetamide was synthesized from 2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-methyl-N-(4-(methylarnino)butyl)acetamide (92 mg, 0.16 mmol), dinonylglycine (56 mg, 0.17 mmol), DIPEA (59 μL, 0.34 mmol), and T3P (50% EtOAc solution, 276 μL, 0.464 mmol) in THF (3 mL). Yield (27 mg, 19%).

UPLC/ELSD: RT = 2.98 min. MS (ES): *m/z* (MH^+) 905.32 for C57H117N5O2

¾-NMR (300 MHz, CDCh) δ: ppm 3.60-2.20 (br. m, 28H); 1.80-1.10 (br. m, 74H); 0.90 (t, 15H)

DZ: Compound 160: N^1-(2-(dinonylamino)ethyl)-N\\N ^{8},N^8-trinonyloctane-l,8-diamine

Step 1: N^1-(2-(dinonylamino)ethyl)octane-l,8-diamine



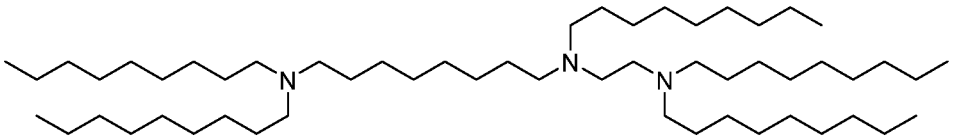
Chemical Formula: C28H61N3

Molecular Weight: 439.82

[001028] To a solution of N-(2-chloroethyl)-N-nonylnonan-l -amine (500 mg, 1.51 mmol) and octane- 1,8-diamine (869 mg, 6.02 mmol) in MeCN (15 mL) was added potassium carbonate (416 mg, 3.01 mmol) and potassium iodide (25 mg, 0.15 mmol) and the reaction was allowed to stir at 82°C for 12 hours. The mixture was allowed to cool to RT, filtered over celite, and

concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-20% MeOH in DCM) provided N^1-(2-(dinonylamino)ethyl)octane-l,8-diamine (454 mg, 68.5%).
UPLC/ELSD: RT = 0.96 min. MS (ES): *m/z* (MH^+) 440.54 for C28H61N3
¾-NMR (300 MHz, CDCh) δ: ppm 2.75 - 2.50 (br. m, 8H); 2.40 (t, 4H); 1.62-1.06 (br. m, 43H); 0.90 (t, 6H)

Step 2: N^1-(2-(dinonylamino)ethyl)-N^1,N^8,N^8-trinonyloctane-l,8-diamine (Compound 160)

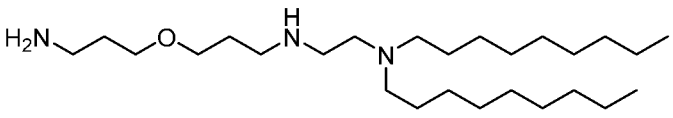


Chemical Formula: C55H115N3
Molecular Weight: 818.55

[001029] To a solution of N^1-(2-(dinonylamino)ethyl)octane-l,8-diamine (337 mg, 0.766 mmol) in DCE (3 mL) was added TEA (214 μL, 1.53 mmol) and the reaction was allowed to stir at RT for 15 minutes. A solution of nonanal (436 mg, 3.07 mmol) in DCE (2 mL) was added and the solution was cooled to 0°C before addition of sodium triacetoxyborohydride (646 mg, 3.07 mmol) and acetic acid (175 μL, 3.07 mmol). The reaction was allowed to return to RT and stir for 12 hours. The solution was quenched with saturated sodium bicarbonate and extracted three times with DCM. The organics were dried over anhydrous Na2S04, filtered, and concentrated in vacuo. Purification by ISCO C18 flash chromatography (10-100% MeCN in water with 0.1% TFA) provided N^1-(2-(dinonylamino)ethyl)-N^1,N^8,N^8-trinonyloctane-l, 8-diamine (266 mg, *42.4%).*
UPLC/ELSD: RT = 2.84 min. MS (ES): *m/z* (MH^+) 819.46 for C55H115N3
¾-NMR (300 MHz, CDCh) δ: ppm 2.68-2.26 (br. m, 18H); 1.65-1.05 (br. m, 82H); 0.90 (t, 15H)

EA: Compound 161: N^1-(3-(3-(dinonylamino)propoxy)propyl)-N^1,N^2,N^2-trinonylethane-l,2-diamine

Step 1: N^1-(3-(3-aminopropoxy)propyl)-N^2,N^2-dinonylethane-l,2-diamine



Chemical Formula: C26H57N3O

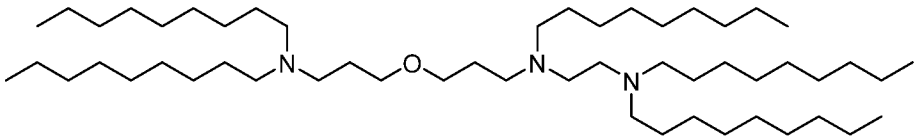
Molecular Weight: 427.76

[001030] In the same manner as Step 1 for Compound 160, N^1-(3-(3-aminopropoxy)propyl)-N^2,N^2-dinonylethane-l,2-diamine was synthesized fromN-(2-chloroethyl)-N-nonylnonan-l -amine (500 mg, 1.51 mmol), 3,3'-oxybis(propan-l-amine) (796 mg, 6.02 mmol), potassium carbonate (416 mg, 3.01 mmol) and potassium iodide (25 mg, 0.15 mmol) in MeCN (15 mL). Yield (297 mg, 46.1%).

UPLC/ELSD: RT = 0.86 min. MS (ES): *m/z* (MH^+) 428.65 for C26H57N3O

¾-NMR (300 MHz, CDCh) δ: ppm 3.54 (t, 4H); 3.05-2.40 (br. m, 15H); 1.96-1.68 (br. m, 4H); 1.52-1.00 (br. m, 28H); 0.90 (t, 6H)

Step 2: N^1-(3-(3-(dinonylamino)propoxy)propyl)-N\N ^{2},N^2-trinonylethane-l,2-diamine (Compound 161)



Chemical Formula: C53H111N3O

Molecular Weight: 806.49

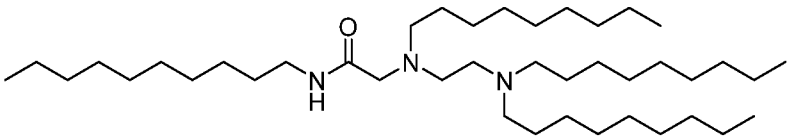
[001031] In the same manner as Step 2 for Compound 160, N^1-(3-(3-(dinonylamino)propoxy)propyl)-N\N ^{2},N^2-trinonylethane-l,2-diamine was synthesized fromN^1-(3-(3-aminopropoxy)propyl)-N^2,N^2-dinonylethane-l,2-diamine (297 mg, 0.694 mmol), TEA (194 μL, 1.39 mmol), nonanal (395 mg, 2.78 mmol), sodium triacetoxyborohydride (586 mg, 2.78 mmol), and acetic acid (159 μL, 2.78 mmol) in DCE (5 mL). Yield (304 mg, 54.3%).

UPLC/ELSD: RT = 2.86 min. MS (ES): *m/z* (MH^+) 807.48 for C53H111N3O

^{1}H-NMR (300 MHz, CDCh) δ: ppm 3.44 (t, 4H); 2.76-2.26 (br. m, 18H); 1.73 (br. m, 4H); 1.58-1.14 (br. m, 70H); 0.90 (t, 15H)

EB: Compound 162: N-decyl-2-((2-(dinonylamino)ethyl)(nonyl)amino)

Step 1: N-decyl-2-((2-(dinonylamino)ethyl)(nonyl)amino)acetamide (Compound 162)



Chemical Formula: C41H85N3O

Molecular Weight: 636.15

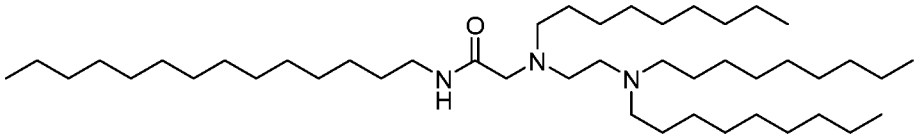
[001032] In the same manner as Step 5 for Compound 44, N-decyl-2-((2-(dinonylamino)ethyl)(nonyl)amino)acetamide was synthesized from N-(2-(dinonylamino)ethyl)-N-nonylglycine (300 mg, 0.604 mmol), decan-l-amine (133 uL, 0.664 mmol), DIPEA (232 μL, 1.33 mmol), and T3P (50% EtOAc solution, 1.08 mL, 1.81 mmol) in THF (7 mL). Yield (233 mg, 60.7%).

UPLC/ELSD: RT = 3.45 min. MS (ES): *m/z* (MH^+) 636.84 for **C41H85N3O**

¾-NMR (300 MHz, CDCh) δ: ppm 7.85 (br. m, 1H); 3.27 (m, 2H); 3.08 (s, 2H); 2.60-2.30 (br. m, 10H); 1.65-1.05 (br. m, 58H); 0.90 (t, 12H)

EC: Compound 163: 2-((2-(dinonylamino)ethyl)(nonyl)armno)-N-tetradecylacetamide

Step 1: 2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-tetradecylacetarnide (Compound 163)



Chemical Formula: **C45H93N3O**

Molecular Weight: 692.26

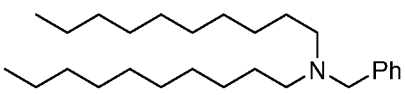
[001033] In the same manner as Step 5 for Compound 44, 2-((2-(dinonylamino)ethyl)(nonyl)amino)-N-tetradecylacetarnide was synthesized from N-(2-(dinonylamino)ethyl)-N-nonylglycine (300 mg, 0.604 mmol), tetradecan-1 -amine (142 mg, 0.664 mmol), DIPEA (232 μL, 1.33 mmol), and T3P (50% EtOAc solution, 1.08 mL, 1.81 mmol) in THF (7 mL). Yield (183 mg, 43.8%).

UPLC/ELSD: RT = 3.70 min. MS (ES): *m/z* (MH^+) 693.01 for **C45H93N3O**

¾-NMR (300 MHz, CDCh) δ: ppm 7.84 (br. m, 1H); 3.26 (m, 2H); 3.08 (s, 2H); 2.60-2.30 (br. m, 10H); 1.80-1.05 (br. m, 66H); 0.90 (t, 12H)

ED: Compound 164: l,l'-(Piperazine-l,4-diyl)bis(5-(didecylamino)pentan-l-one

Step 1: *N*-benzyl-*N*-decyldecan-1-amine

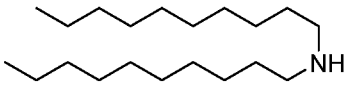


Chemical Formula: **C27H49N**

Molecular Weight: 387.70

[001034] To a solution of benzyl amine hydrochloride (2.0 g, 13.9 mmol) in cyclpentylmethyl ether (30 mL) was added potassium carbonate (7.70 g, 55.7 mmol), potassium iodide (4.62 g, 27.8 mmol) and a solution of 1-bromodecane (6.35 mL, 30.6 mmol) in acetonitrile and the reaction mixture was stirred at 80 °C for 18 hours. The reaction mixture was then filtered through Celite, washed with ethyl acetate. The solvent was removed, and the crude was purified by flash chromatography (SiO_2: ethyl acetae/hexane 0 to 40%) to get *N*-benzyl-*N*-decyldecan-l-amine (4.37 g, 81%) as a colorless oil. MS (APCI): *m/z* (MH^+) 388.3 for C27H49N. ¾ NMR (300 MHz, CDCh): δ 7.30-7.23 (m, 5H); 3.52 (s, 2H); 2.37 (t, 4H, *J* = 7.3 Hz); 1.SO-1.3S (m, 4H); 1.34-1.20 (m, 28H); 0.87 (t, 6H, *J* = 6.6 Hz).

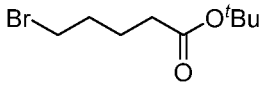
Step 2: Didecylamine



Chemical Formula: C20H43N
Molecular Weight: 297.57

[001035] A mixture of *N*-benzyl-*N*-decyldecan-l-amine (2.4 g, 5.16 mmol) and palladium on charcoal (200 mg) in ethanol (50 mL) was stirred under hydrogen balloon overnight. It was filtered through Celite, washed with ethyl acetate (2 x 50 mL), and the solvent removed to get pure didecylamine (1.75 g, 96%) as a white solid. MS (APCI): *m/z* (MH^+) 298.3 for C20H43N. ¾ NMR (300 MHz, CDCh): δ 2.57 (t, 4H, *J* = 7.3Hz); 1.59-1.44 (m, 4H); 1.31-1.20 (m, 28H); 0.87 (t, 6H, *J* = 6.6 Hz).

Step 3: *tert*-Butyl 5-bromopentanoate

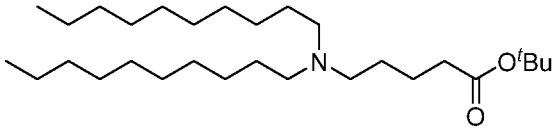


Chemical Formula: $C_9H_{17}BrO2$
Molecular Weight: 237.14

[001036] To a solution of 5-bromovaleric acid (2.5 g, 14.0 mmol) in dichloromethane (10 mL) was added oxalyl chloride (1.15 mL, 13.2 mmol) and the reaction mixture stirred at room temperature for 4 hours. The solvent and excess oxalyl chloride were removed under vacuum. The crude acid chloride was dissolved in dichloromethane (10 mL) and cooled to 0°C. Then *tert*-butanol (10 mL) was added and the reaction mixture stirred at room temperature overnight. The solvent was removed, and the crude was purified by flash chromatography (SiCh: ethyl acetate/hexane 0 to 50%) to get *tert*-butyl 5-bromopentanoate (3.02 g, 92%) as a colorless oil.

^{1}H NMR (300 MHz, CDCh): δ 3.40 (t, 2H, *J* = 6.6 Hz); 2.24 (t, 2H, *J* = 7.3 Hz); 1.90-1.81 (m, 2H); 1.75-1.69 (m, 2H); 1.43 (s, 9H).

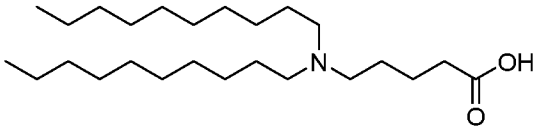
Step 4: *tert*-Butyl 5-(didecylamino)pentanoate



Chemical Formula: $C_{29}H_{59}NO_2$
Molecular Weight: 453.80

[001037] To a solution of didecylamine (1.75 g, 5.88 mmol) in cyclpentyl methyl ether (40 mL) was added potassium carbonate (3.28 g, 23.5 mmol), potassium iodide (1.07 g, 6.47 mmol) and a solution of *tert*-butyl 5-bromopentanoate (1.46 g, 6.18 mmol) in acetonitrile and the reaction mixture was stirred at 85°C for 18 hours. The reaction mixture was then filtered through Celite and washed with ethyl acetate. The solvent was removed, and the residue was purified by flash chromatography (SiO_2: ethyl acetate/hexane 0 to 50%) to get *tert*-butyl 5-(didecylamino)pentanoate (2.1 g, 78%) as a colorless oil. MS (APCI): *m/z* (MH^+) 454.4 for C29H59NO2. ¾ NMR (300 MHz, CDCh): δ 2.37 (dd, 6H, *J* = 8.2 Hz, 15.0 Hz); 2.21 (t, 2H, *J* = 7.4 hz); 1.62-1.50 (m, 2H); 1.42 (s, 9H); 1.48-1.32 (m, 6H); 1.31-1.15 (m, 28H); 0.86 (t, 6H, *J* = 6.6 Hz).

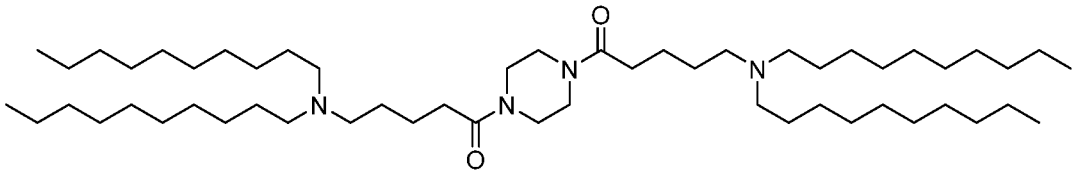
Step 5: 5-(Didecylamino)pentanoic acid



Chemical Formula: $C_{25}H_{51}NO_2$
Molecular Weight: 397.69

[001038] To a solution of *tert*-butyl 5-(didecylamino)pentanoate (2.1 g, 4.6 mmol) in dichloromethane (20 mL) at 0°C was added trifluoroacetic acid (10 mL) slowly and then the reaction mixture was stirred at room temperature for 18 hours. The reaction mixture was quenched at 0°C with saturated sodium bicarbonate solution. The organic layer was washed with saturated sodium bicarbonate solution, brine, dried with anhydrous sodium sulfate. The solvent was removed to get pure 5-(didecylamino)pentanoic acid (1.82 g, 98%) as colorless oil. MS (APCI): *m/z* (MH^+) 398.4 for C25H51NO2. ¾ NMR (300 MHz, CDCh): δ 2.91-2.82 (m, 6H); 2.28-2.24 (m, 2H); 1.75-1.55 (m, 8H); 1.28-1.20 (m, 28H); 0.86 (t, 6H, *J* = 6.6 Hz).

Step 6: l,l'-(Piperazine-l,4-diyl)bis(5-(didecylamino)pentan-l-one) (Compound 164)

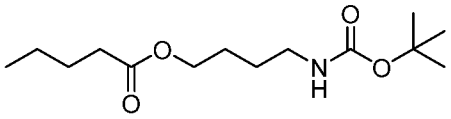


Chemical Formula: $C_{54}H_{108}N_4O_2$
Molecular Weight: 845.48

[001039] To a solution of 4-(didecylamino)butanoic acid (1.0 g, 2.5 mmol), piperazine (103 mg, 1.19 mmol), and *N,N* -diisopropylethyl amine (1.04 mL, 5.99 mmol) in tetrahydrofuran (20 mL) at 0°C was added propylphosphonic anhydride (50 wt% in EtOAc, 4.6 mL, 7.12 mmol) slowly and then the reaction mixture was stirred at room temperature for 18 hours. The reaction mixture was quenched with ice, and the organic layer was washed with saturated sodium bicarbonate solution (2 × 10 mL), brine (2 × 10 mL), and then dried with anhydrous sodium sulfate. The solvent was removed to get the crude product which was purified by flash chromatography (SiCh: methanol/dichloromethane 0 to 20%) to get l,l '-(piperazine-l,4-diyl)bis(4-(didecylamino)butan-l-one) (150 mg, 15%) as yellow oil, NMR showed it was carbonate salt. The compound was taken in dichloromethane and washed with 2 *N* sodium hydroxide, brine, dried with anhydrous sodium sulfate. After concentration, the residue was purified by flash chromatography (SiCh: methanol/dichloromethane 0 to 20%) to get pure 1,1-(piperazine-l,4-diyl)bis(4-(didecylamino)pentan-l-one) (88 mg, 9%) as colorless oil. HPLC/UV (210 nm): RT = 7.30 min. MS (APCI): *m/z* (MH^+) 845.7 for C54H108N4O2. ¾ NMR (300 MHz, CDCh): δ 3.66-3.57 (m, 4H); 3.48-3.40 (m, 4H); 2.45-2.32 (m, 16H); 1.69-1.57 (m, 4H); 1.52-1.35 (m, 12H); 1.30-1.18 (m, 56H); 0.86 (t, 12H, *J* = 6.6 Hz).

EF: Compound 165: 4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)acetamido)butyl pentanoate

Step 1: 4-((tert-butoxycarbonyl)amino)butyl pentanoate

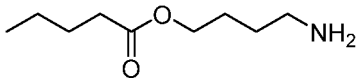


Chemical Formula: C14H27NO4
Molecular Weight: 273.37

[001040] To a solution of pentanoic acid (237 mg, 2.33 mmol) and tert-butyl (4-hydroxybutyl)carbamate (400 mg, 2.11 mmol) in DCM (20 mL) was added EDC HCL HCl (394 mg, 2.54 mmol) and DMAP (52 mg, 0.42 mmol) and the reaction was allowed to stir at RT for 12 hours. The solution was concentrated *in vacuo*, taken up in EtOAc, and washed with 10% citric acid, brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-20% MeOH in DCM) provided 4-((tert-butoxycarbonyl)amino)butyl pentanoate (570 mg, 98.7%).

Ti-NMR (300 MHz, CDCh) δ: ppm 4.58 (br, 1H); 4.10 (t, 2H); 3.17 (br, 2H); 2.32 (t, 2H); 1.76-1.24 (br. m, 17H); 0.94 (t, 3H)

Step 2: 4-aminobutyl pentanoate



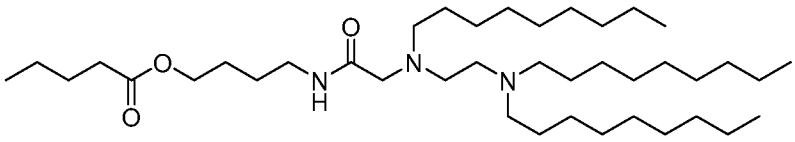
Chemical Formula: C9H19NO2

Molecular Weight: 173.26

[001041] To a solution of 4-((tert-butoxycarbonyl)amino)butyl pentanoate (570 mg, 2.09 mmol) in DCM (8 mL) was added TFA (8.0 mL, 100 mmol) and the reaction was allowed to stir at RT for 12 hours. The solution was concentrated *in vacuo*, taken up in EtOAc, and washed with 5% Na2CCb, brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Yield (345 mg, 95.5%).

¾-NMR (300 MHz, CDCh) δ: ppm 7.36 (br, 2H); 4.15 (t, 2H); 3.16 (m, 2H); 2.35 (t, 2H); 1.95-1.20 (br. m, 8H); 0.93 (t, 3H)

Step 3: 4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)acetamido)butyl pentanoate (Compound 165)



Chemical Formula: C40H81N3O3

Molecular Weight: 652.11

[001042] In the same manner as Step 5 for Compound 44, 4-(2-((2-(dinonylamino)ethyl)(nonyl)amino)acetamido)butyl pentanoate was synthesized from N-(2-(dinonylamino)ethyl)-N-nonylglycine (391 mg, 0.787 mmol), 4-aminobutyl pentanoate (150 mg,

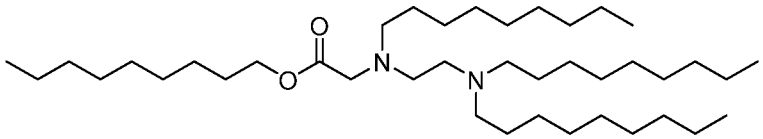
0.866 mmol), DIPEA (550 µï, 3.15 mmol), and T3P (50% EtOAc solution (1.40 mL, 2.36 mmol) in THF (10 mL). Yield (62 mg, 12%).

UPLC/ELSD: RT = 3.03 min. MS (ES): *m/z* (MH^+) 653.07 for C40H81N3O3

¾-NMR (300 MHz, CDCh) δ: ppm 7.94 (m, 1H); 4.10 (t, 2H); 3.40-2.25 (br. m, 16H); 1.90-1.10 (br. m, 50H); 0.91 (m, 12H)

EG: Compound 166: Nonyl N-(2-(dinonylamino)ethyl)-N-nonylglycinate

Step 1: nonyl N-(2-(dinonylamino)ethyl)-N-nonylglycinate (Compound 166)



Chemical Formula: C40H82N2O2

Molecular Weight: 623.11

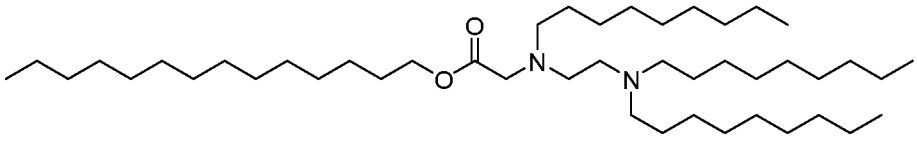
[001043] In the same manner as Step 5 for Compound 44, nonyl N-(2-(dinonylamino)ethyl)-N-nonylglycinate was synthesized from N-(2-(dinonylamino)ethyl)-N-nonylglycine (300 mg, 0.604 mmol), 1-nonanol (96 mg, 0.66 mmol), DIPEA (232 uL, 1.33 mmol), and T3P (50% EtOAc solution (1.08 mL, 1.81 mmol) in THF (7 mL). Yield (90 mg, 24%).

UPLC/ELSD: RT = 3.50 min. MS (ES): *m/z* (MH^+) 623.96 for C40H82N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.11 (t, 2H); 3.40 (s, 2H); 3.20-2.30 (br. m, 10H); 1.90-1.00 (br. m, 56H); 0.90 (t, 12H)

EH: Compound 167: Tetradecyl N-(2-(dinonylamino)ethyl)-N-nonylglycinate

Step 1: tetradecyl N-(2-(dinonylamino)ethyl)-N-nonylglycinate (Compound 167)



Chemical Formula: C45H92N2O2

Molecular Weight: 693.24

[001044] In the same manner as Step 5 for Compound 44, tetradecyl N-(2-(dinonylamino)ethyl)-N-nonylglycinate was synthesized from N-(2-(dinonylamino)ethyl)-N-

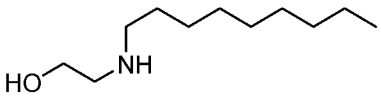
nonylglycine (300 mg, 0.604 mmol), tetradecan-l-ol (142 mg, 0.664 mmol), DIPEA (232 μL, 1.33 mmol), and T3P (50% EtOAc solution (1.08 mL, 1.81 mmol) in THF (7 mL). Yield (184 mg, 44.0%)

UPLC/ELSD: RT = 3.79 min. MS (ES): *m/z* (MH^+) 694.07 for $C_{45}H_{92}N_2O_2$

¾-NMR (300 MHz, CDCh) δ: ppm 4.11 (t, 2H); 3.40 (s, 2H); 3.20-2.30 (br. m, 10H); 1.90-1.00 (br. m, 66H); 0.90 (t, 12H)

EI: Compound 168: 2-((2-(Dinonylamino)ethyl)(nonyl)amino)ethyl nonanoate

Step 1: 2-(nonylamino)ethan-l-ol



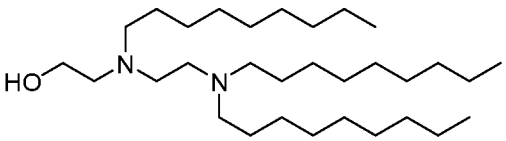
Chemical Formula: $C_{11}H_{25}NO$

Molecular Weight: 187.33

[001045] In the same manner as Step 2 for Compound 12, 2-(nonylamino)ethan-l-ol was synthesized from ethanolamine (2.33 mL, 38.6 mmol), 1-bromononane (2.00 g, 9.66 mmol), potassium carbonate (2.67 g, 19.3 mmol), and potassium iodide (160 mg, 0.965 mmol) in MeCN (100 mL). Yield (1.23 g, 68.0%).

¾-NMR (300 MHz, CDCh) δ: ppm 3.57 (t, 2H); 2.72 (t, 2H); 2.55 (t, 2H); 1.42 (m, 2H); 1.33-1.10 (br. m, 12H); 0.81 (t, 3H)

Step 2: 2-((2-(dinonylamino)ethyl)(nonyl)amino)ethan-l-ol



Chemical Formula: $C_{31}H_{66}N_2O$

Molecular Weight: 482.88

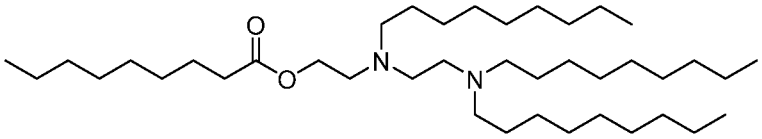
[001046] To a solution of 2-(nonylamino)ethan-l-ol (500 mg, 2.67 mmol) and N-(2-chloroethyl)-N-nonylnonan-l -amine (975 mg, 2.94 mmol) in MeCN (30 mL) was added potassium carbonate (738 mg, 5.34 mmol) and potassium iodide (44 mg, 0.27 mmol) and the suspension was allowed to stir at 82°C for 12 hours. The reaction mixture was allowed to cool to RT, filtered over celite, and concentrated *in vacuo.* Purification by ISCO silica flash

chromatography (0-20% MeOH in DCM) provided 2-((2-(dinonylamino)ethyl)(nonyl)amino)ethan-l-ol (490 mg, 38.0%).

UPLC/ELSD: RT = 1.96 min. MS (ES): *m/z* (MH^+) 483.75 for C31H66N2O

¾-NMR (300 MHz, CDCh) δ: ppm 3.90-2.50 (br. m, 14H); 1.90-1.10 (br. m, 42H); 0.90 (t, 9H)

Step 3: 2-((2-(dinonylamino)ethyl)(nonyl)amino)ethyl nonanoate (Compound 168)



Chemical Formula: C40H82N2O2

Molecular Weight: 623.11

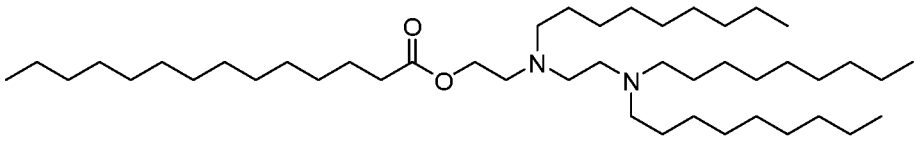
[001047] To a solution of 2-((2-(dinonylamino)ethyl)(nonyl)amino)ethan-l-ol (245 mg, 0.507 mmol) and nonanoic acid (80 mg, 0.51 mmol) in DCM (5 mL) was added EDC HCL (158 mg, 1.02 mmol) and DMAP (12 mg, 0.10 mmol) and the reaction was allowed to stir at RT for 4 hours. The reaction was concentrated in vacuo to a crude oil. Purification by ISCO C18 flash chromatography (10-100% MeCN in water with 0.1% TFA) provided 2-((2-(dinonylamino)ethyl)(nonyl)amino)ethyl nonanoate (114 mg, 36. 1%).

UPLC/ELSD: RT = 3.08 min. MS (ES): *m/z* (MH^+) 623.96 for C40H82N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.14 (t, 2H); 2.85-2.20 (14H); 1.80-1.05 (br. m, 54H); 0.90 (t, 12H)

EJ: Compound 169: 2-((2-(Dinonylamino)ethyl)(nonyl)amino)ethyl tetradecanoate

Step 1: 2-((2-(dinonylamino)ethyl)(nonyl)amino)ethyl tetradecanoate (Compound 169)



Chemical Formula: C45H92N2O2

Molecular Weight: 693.24

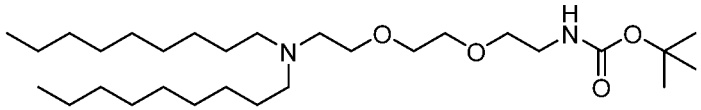
[001048] In the same manner as Step 3 for Compound 168, 2-((2-(dinonylamino)ethyl)(nonyl)amino)ethyl tetradecanoate was synthesized from 2-((2-(dinonylamino)ethyl)(nonyl)amino)ethan-l-ol (245 mg, 0.507 mmol), tetradecanoic acid (139 mg, 0.609 mmol), EDC HCL (158 mg, 1.02 mmol) and DMAP (12 mg, 0.10 mmol) in DCM (5 mL). Yield (134 mg, 38.1%).

UPLC/ELSD: RT = 3.46 min. MS (ES): *m/z* (MH^+) 694.07 for C45H92N2O2

¾-NMR (300 MHz, CDCh) δ: ppm 4.14 (t, 2H); 2.85-2.20 (14H); 1.80-1.05 (br. m, 64H); 0.90 (t, 12H)

EK: Compound 173: Nonyl 18-nonyl-9-(8-(nonyloxy)-8-oxooctyl)-12,15-dioxa-9,18-diazaheptacosanoate

Step 1: tert-butyl (2-(2-(2-(dinonylamino)ethoxy)ethoxy)ethyl)carbamate



Chemical Formula: C29H60N2O4

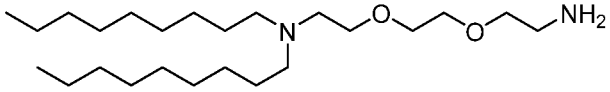
Molecular Weight: 500.81

[001049] To a solution of tert-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (2.00 g, 8.05 mmol) and 1-bromononane (3.67 g, 17.7 mmol) in MeCN (100 mL) was added potassium carbonate (2.23 g, 16.1 mmol) and potassium iodide (267 mg, 1.61 mmol) and the suspension was allowed to stir at 82°C for 12 hours. The reaction mixture was allowed to cool to RT, filtered over celite, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-10% MeOH in DCM) provided tert-butyl (2-(2-(2-(dinonylamino)ethoxy)ethoxy)ethyl)carbamate (2.8 g, 69%)

UPLC/ELSD: RT = 2.01 min. MS (ES): *m/z* (MH^+) 501.62 for C29H60N2O4

^{1}H-NMR (300 MHz, CDCh) δ: ppm 5.04 (br, 1H); 3.76-3.24 (br. m, 10H); 2.90-2.45 (br. m, 6H); 1.65-1.05 (br. m, 37H); 0.90 (t, 6H)

Step 2: N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-N-nonylnonan-l -amine



Chemical Formula: C24H52N2O2

Molecular Weight: 400.69

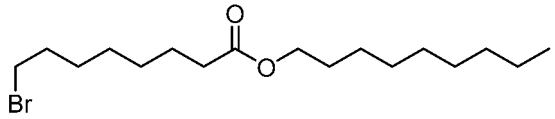
[001050] To a solution of tert-butyl (2-(2-(2-(dinonylamino)ethoxy)ethoxy)ethyl)carbamate (2.8 g, 5.59 mmol) in DCM (21 mL) was added TFA (21 mL, 279 mmol) and the reaction was allowed to stir at RT overnight. The solution was concentrated *in vacuo* and taken up EtOAc, washed with 5% Na2CCb, brine, dried over

anhydrous Na2S04, filtered, and concentrated *in vacuo* to provide N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-N-nonylnonan-l -amine (2.0 g, 89%).

UPLC/ELSD: RT = 1.03 min. MS (ES): *m/z* (MH^+) 401.59 for $C_{24}H_{52}N_2O_2$

¾-NMR (300 MHz, CDCh) δ: ppm 3.70-3.45 (br. m, 8H); 2.89 (t, 2H); 2.71 (t, 2H); 2.50 (t, 4H); 1.83 (br, 2H); 1.58-1.04 (br. m, 28H); 0.91 (t, 6H)

Step 3: nonyl 8-bromooctanoate



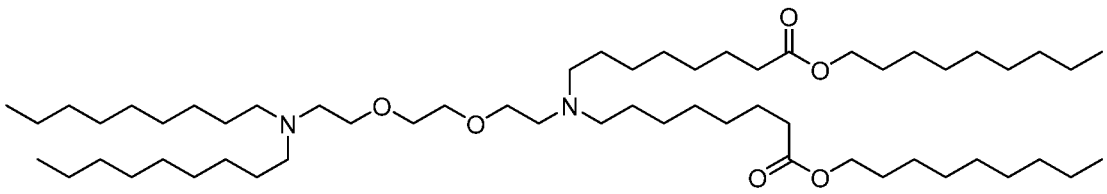
Chemical Formula: $C_{17}H_{33}BrO_2$

Molecular Weight: 349.35

[001051] In the same manner as Step 1 for Compound 12, nonyl 8-bromooctanoate was synthesized from 8-bromooctanoic acid (5.0 g, 22 mmol), 1-nonanol (3.4 g, 24 mmol), EDC HCL (5.2 g, 34 mmol), and DMAP (550 mg, 4.9 mmol) in DCM (50 mL). Yield (4.2 g, 54%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 2H); 3.42 (t, 2H); 2.32 (t, 2H); 1.88 (quint, 2H); 1.72-1.05 (br. m, 22H); 0.90 (t, 3H)

Step 4: Nonyl 18-nonyl-9-(8-(nonyloxy)-8-oxooctyl)-12,15-dioxa-9,18-diazaheptacosanoate (Compound 173)



Chemical Formula: $C_{58}H_{116}N_2O_6$

Molecular Weight: 937.57

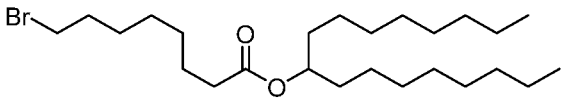
[001052] To a solution of N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-N-nonylnonan-l -amine (2.0 g, 5.0 mmol) and nonyl 8-bromooctanoate (1.7 g, 5.0 mmol) in MeCN (100 mL) was added potassium carbonate (1.4 g, 10.0 mmol) and potassium iodide (170 mg, 1.0 mmol) and the suspension was allowed to stir at 82°C for 12 hours. The reaction mixture was allowed to cool to RT, filtered over celite, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-20% MeOH in DCM) provided nonyl 18-nonyl-9-(8-(nonyloxy)-8-oxooctyl)-12,15-dioxa-9,18-diazaheptacosanoate (529 mg, 11%)

UPLC/ELSD: RT = 3.10 min. MS (ES): *m/z* (MH^+) 938.45 for $C_{58}H_{116}N_2O_6$

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 4H); 3.58 (br. m, 8H); 2.78-2.22 (br. m, 16H); 1.80-1.10 (br. m, 76H); 0.90 (t, 12H)

EL: Compound 174: Heptadecan-9-yl 18-nonyl-9-(8-(nonyloxy)-8-oxooctyl)-12,15-dioxa-9,18-diazaheptacosanoate

Step 1: heptadecan-9-yl 8-bromooctanoate



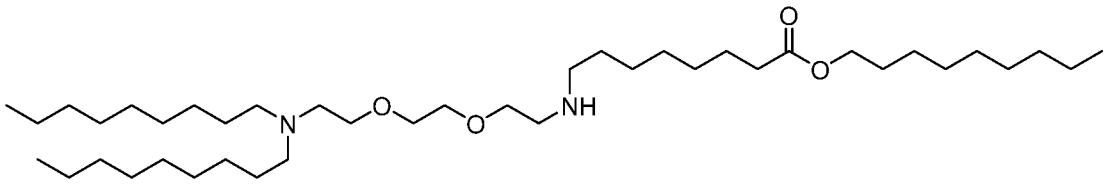
Chemical Formula: $C_{25}H_{49}Br0_2$

Molecular Weight: 461.57

[001053] In the same manner as Step 1 for Compound 12, heptadecan-9-yl 8-bromooctanoate was synthesized from 8-bromooctanoic acid (5.3 g, 24 mmol), 9-heptadecanol (5.1 g, 20 mmol), EDC HCL (4.6 g, 30 mmol), and DMAP (490 mg, 4.0 mmol) in DCM (30 mL). Yield (8.0 g, 87%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.80 (quint, 1H); 3.33 (t, 2H); 2.21 (t, 2H); 1.78 (quint, 2H); 1.65-1.00 (br. m, 36H); 0.91 (t, 6H)

Step 2: nonyl 18-nonyl-12,15-dioxa-9,18-diazaheptacosanoate



Chemical Formula: $C_{41}H_{84}N_2O_4$

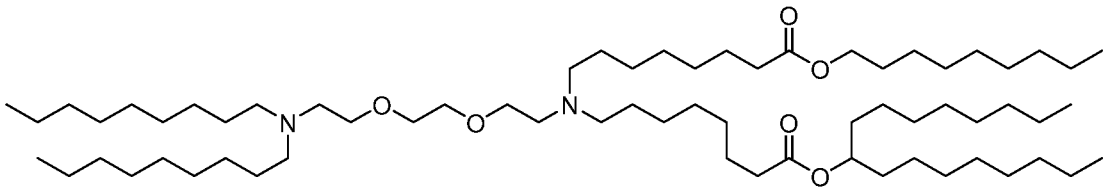
Molecular Weight: 669.13

[001054] To a solution of N-(2-(2-(2-aminoethoxy)ethoxy)ethyl)-N-nonylnonan-l -amine (2.0 g, 5.0 mmol) and nonyl 8-bromooctanoate (1.7 g, 5.0 mmol) in MeCN (100 mL) was added potassium carbonate (1.4 g, 10.0 mmol) and potassium iodide (170 mg, 1.0 mmol) and the suspension was allowed to stir at 82°C for 12 hours. The reaction mixture was allowed to cool to RT, filtered over celite, and concentrated *in vacuo.* Purification by ISCO silica flash chromatography (0-20% MeOH in DCM) provided nonyl 18-nonyl-12,15-dioxa-9,18-diazaheptacosanoate (791 mg, 23.7%).

UPLC/ELSD: RT = 2.31 min. MS (ES): *m/z* (MH^+) 669.97 for $C_{41}H_{84}N_2O_4$

¾-NMR (300 MHz, CDCh) δ: ppm 4.07 (t, 2H); 3.70-3.50 (br. m, 8H); 2.85-2.25 (br. m, 12H); 1.85-1.00 (br. m, 52H); 0.90 (t, 9H)

Step 3: heptadecan-9-yl 18-nonyl-9-(8-(nonyloxy)-8-oxooctyl)-12,15-dioxa-9,18-diazaheptacosanoate (Compound 174)



Chemical Formula: $C_{66}H_{132}N_2O_6$

Molecular Weight: 1049.79

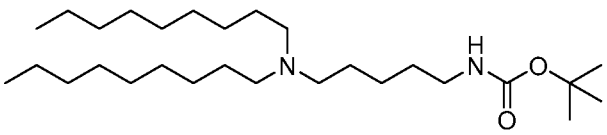
[001055] To a solution of nonyl 18-nonyl-12,15-dioxa-9,18-diazaheptacosanoate (791 mg, 1.18 mmol) and heptadecan-9-yl 8-bromooctanoate (600 mg, 1.30 mmol) in MeCN (20 mL) was added potassium carbonate (327 mg, 2.36 mmol) and potassium iodide (39 mg, 0.24 mmol) and the suspension was allowed to stir at 82°C for 12 hours. The reaction mixture was allowed to cool to RT, filtered over celite, and concentrated *in vacuo.* Purification by ISCO CI8 flash chromatography (10-100% MeCN in water with 0.1% TFA) provided heptadecan-9-yl 18-nonyl-9-(8-(nonyloxy)-8-oxooctyl)-12,15-dioxa-9,18-diazaheptacosanoate (354 mg, 28.5%).

UPLC/ELSD: RT = 3.52 min. MS (ES): *m/z* (MH^+) 1050.72 for $C_{66}H_{132}N_2O_6$

Ṫi-NMR (300 MHz, CDCh) δ: ppm 4.74 (quint, 1H); 3.93 (t, 2H); 3.50-3.35 (br. m, 8H); 2.60-2.00 (br. m, 16H); 1.60-0.90 (br. m, 90H); 0.76 (t, 15H)

EM: Compound 177: Dinonyl 8,8'-((5-(dinonylamino)pentyl)azanediyl)dioctanoate

Step 1: tert-butyl (5-(dinonylamino)pentyl)carbamate



Chemical Formula: $C_{28}H_{58}N_2O_2$

Molecular Weight: 454.78

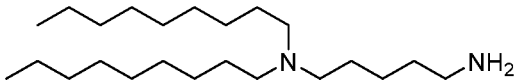
[001056] In the same manner as Step 1 for Compound 170, tert-butyl (5-(dinonylamino)pentyl)carbamate was synthesized from tert-butyl (5-aminopentyl)carbamate (2.0

g, 10 mmol), 1-bromononane (4.5 g, 22 mmol), potassium carbonate (2.7 g, 20 mmol), and potassium iodide (330 mg, 2.0 mmol) in MeCN (100 mL). Yield (3.5 g, 78%).

UPLC/ELSD: RT = 2.05 min. MS (ES): *m/z* (MH^+) 455.54 for $C_{28}H_{58}N_2O_2$

¾-NMR (300 MHz, CDCh) δ: ppm 4.57 (br, 1H); 3.13 (m, 2H); 2.51 (br, 6H); 1.65-1.05 (br. m, 43H); 0.90 (t, 6H)

Step 2: N^N^dinonylpentane-l^-diamine



Chemical Formula: $C_{23}H_{50}N_2$

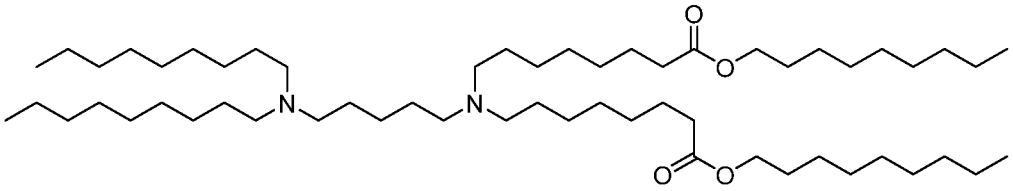
Molecular Weight: 354.67

[001057] In the same manner as Step 2 for Compound 170, N^N^dinonylpentane-l^-diamine was synthesized from tert-butyl (5-(dinonylamino)pentyl)carbamate (3.5 g, 8.0 mmol) and TFA (29 mL, 380 mmol) in DCM (30 mL). Yield (2.38 g, 87%).

UPLC/ELSD: RT = 1.08 min. MS (ES): *m/z* (MH^+) 355.51 for $C_{28}H_{58}N_2O_2$

¾-NMR (300 MHz, CDCh) δ: ppm 5.24 (br, 2H); 2.94-2.52 (br. m, 8H); 1.75-1.00 (br. m, 34H); 0.90 (t, 6H)

Step 3: Dinonyl 8,8'-((5-(dinonylamino)pentyl)azanediyl)dioctanoate (Compound 177)



Chemical Formula: $C_{57}H_{114}N_2O_4$

Molecular Weight: 891.55

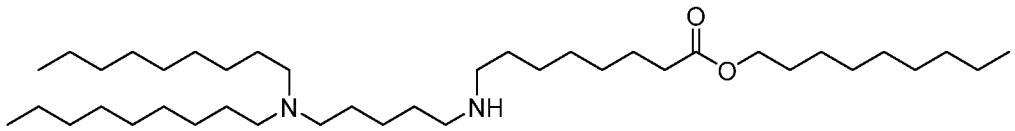
[001058] In the same manner as Step 4 for Compound 170, nonyl 8-((5-(dinonylamino)pentyl)amino)octanoate was synthesized fromN^N^dinonylpentane-l^-diamine (2.38 g, 6.71 mmol), nonyl 8-bromooctanoate (2.34 g, 6.71 mmol), potassium carbonate (1.86 mmol, 13.4 mmol), and potassium iodide (223 mg, 1.34 mmol) in MeCN (60 mL). Yield (135 mg, 3.4%).

UPLC/ELSD: RT = 3.10 min. MS (ES): *m/z* (MH^+) 892.36 for $C_{57}H_{114}N_2O_4$

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 4H); 2.66-2.22 (br. m, 16H); 1.80-1.05 (br. m, 82H); 0.90 (t, 12H)

EN: Compound 178: Heptadecan-9-yl 8-((5-(dinonylamino)pentyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate

Step 1: nonyl 8-((5-(dinonylamino)pentyl)amino)octanoate



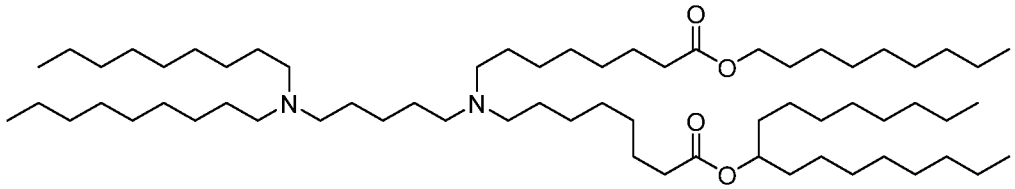
Chemical Formula: C40H82N2O2

Molecular Weight: 623.11

[001059] In the same manner as Step 2 for Compound 174, nonyl 8-((5-(dinonylamino)pentyl)amino)octanoate was synthesized from N^N^dinonylpentane-l^-diamine (2.38 g, 6.71 mmol), nonyl 8-bromooctanoate (2.34 g, 6.71 mmol), potassium carbonate (1.86 mmol, 13.4 mmol), and potassium iodide (223 mg, 1.34 mmol) in MeCN (60 mL). Yield (2.1 g, 50%).

¾-NMR (300 MHz, CDCh) δ: ppm 4.07 (t, 2H); 3.30-2.80 (br. m, 10H); 2.29 (t, 2H); 2.15-1.05 (br. m, 58H); 0.90 (t, 9H)

Step 2: Heptadecan-9-yl 8-((5-(dinonylamino)pentyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate (Compound 178)



Chemical Formula: C65H130N2O4

Molecular Weight: 1003.77

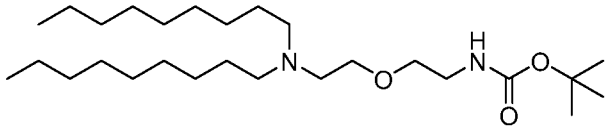
[001060] In the same manner as Step 3 for Compound 174, heptadecan-9-yl 8-((5-(dinonylamino)pentyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate was synthesized from nonyl 8-((5-(dinonylamino)pentyl)amino)octanoate (915 mg, 1.47 mmol), heptadecan-9-yl 8-bromooctanoate (746 mg, 1.62 mmol), potassium carbonate (406 mg, 2.94 mmol), and potassium iodide (49 mg, 0.29 mmol) in MeCN (30 mL). Yield (467 mg, 31.7%).

UPLC/ELSD: RT = 3.57 min. MS (ES): *m/z* (MH^+) 1004.46 for C65H130N2O4

¾-NMR (300 MHz, CDCh) δ: ppm 4.89 (quint, 1H); 4.08 (t, 2H); 2.80-2.20 (br. m, 16H); 1.80-1.10 (br. m, 96H); 0.90 (t, 15H)

EO: Compound 179: Dinonyl 8,8'-((2-(2-(dinonylamino)ethoxy)ethyl)azanediyl)dioctanoate

Step 1: tert-butyl (2-(2-(dinonylamino)ethoxy)ethyl)carbamate



Chemical Formula: C27H56N2O3

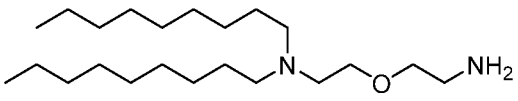
Molecular Weight: 456.76

[001061] In the same manner as Step 1 for Compound 170, tert-butyl (2-(2-(dinonylamino)ethoxy)ethyl)carbamate was synthesized from tert-butyl (2-(2-aminoethoxy)ethyl)carbamate (2.0 g, 9.8 mmol), 1-bromononane (4.5 g, 4.2 mmol), potassium carbonate (2.7 g, 20 mmol), and potassium iodide (330 mg, 2.0 mmol) in MeCN (100 mL). Yield (3.7 g, 83%)

UPLC/ELSD: RT = 1.99 min. MS (ES): *m/z* (MH^+) 457.67 for C27H56N2O3

¾-NMR (300 MHz, CDCh) δ: ppm 5.25 (br, 1H); 3.78-3.22 (br. m, 6H); 2.95-2.30 (br. m, 6H); 1.65-1.10 (br. m, 37H); 0.90 (t, 6H)

Step 2: N-(2-(2-aminoethoxy)ethyl)-N-nonylnonan-l -amine



Chemical Formula: C22H48N2O

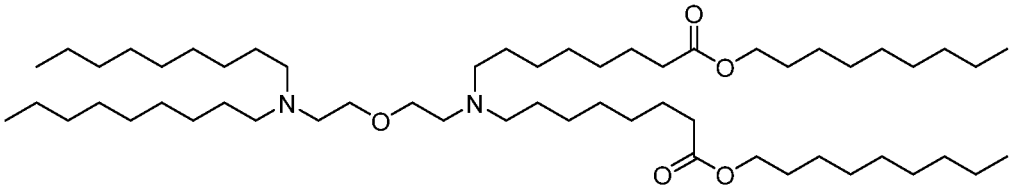
Molecular Weight: 356.64

[001062] In the same manner as Step 2 for Compound 170, N-(2-(2-aminoethoxy)ethyl)-N-nonylnonan-1 -amine was synthesized from tert-butyl (2-(2-(dinonylamino)ethoxy)ethyl)carbamate (3.7 g, 8.1 mmol) and TFA (31 mL) in DCM (30 mL). Yield (2.8 g, 97%).

UPLC/ELSD: RT = 1.00 min. MS (ES): *m/z* (MH^+) 357.56 for C22H48N2O

¾-NMR (300 MHz, CDCh) δ: ppm 6.55 (br, 2H); 3.72 (m, 4H); 3.03 (m, 4H); 2.86 (t, 4H); 1.70-1.05 (br. m, 28H); 0.90 (t, 6H)

Step 3: dinonyl 8,8'-((2-(2-(dinonylamino)ethoxy)ethyl)azanediyl)dioctanoate



Chemical Formula: C56H112N2O5

Molecular Weight: 893.52

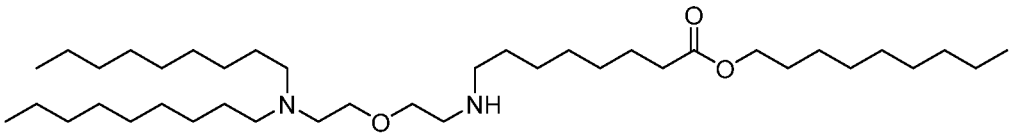
[001063] In the same manner as Step 4 for Compound 170, dinonyl 8,8'-((2-(2-(dinonylamino)ethoxy)ethyl)azanediyl)dioctanoate was synthesized from N-(2-(2-aminoethoxy)ethyl)-N-nonylnonan-l -amine (2.0 g, 5.6 mmol), nonyl 8-bromooctanoate (2.0 g, 5.6 mmol), potassium carbonate (1.6 g, 11 mmol), and potassium iodide (190 mg, 1.1 mmol) in MeCN (60 mL). Yield (676 mg, 13.5%).

UPLC/ELSD: RT = 3.19 min. MS (ES): *m/z* (MH^+) 894.25 for C56H112N2O5

¾-NMR (300 MHz, CDCh) δ: ppm 4.08 (t, 4H); 3.51 (t, 4H); 2.74-2.22 (br. m, 16H); 1.75-1.05 (br. m, 76H); 0.90 (t, 12H)

EP: Compound 180: Heptadecan-9-yl 8-((2-(2-(dinonylamino)ethoxy)ethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate

Step 1: nonyl 8-((2-(2-(dinonylamino)ethoxy)ethyl)amino)octanoate



Chemical Formula: C39H80N2O3

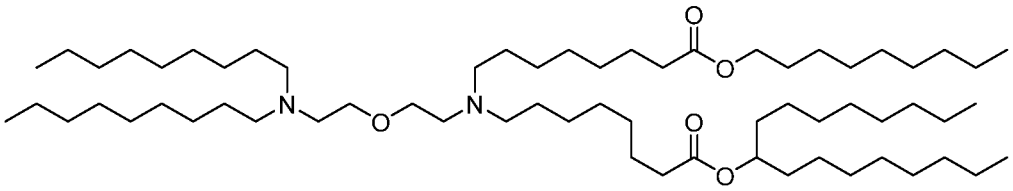
Molecular Weight: 625.08

[001064] In the same manner as Step 2 for Compound 174, nonyl 8-((2-(2-(dinonylamino)ethoxy)ethyl)amino)octanoate was synthesized from N-(2-(2-aminoethoxy)ethyl)-N-nonylnonan-l -amine (2.0 g, 5.6 mmol), nonyl 8-bromooctanoate (2.0 g, 5.6 mmol), potassium carbonate (1.6 g, 11 mmol), and potassium iodide (190 mg, 1.1 mmol) in MeCN (60 mL). Yield (395 mg, 11.3 %).

UPLC/ELSD: RT = 2.37 min. MS (ES): *m/z* (MH^+) 625.85 for C39H80N2O3

¾-NMR (300 MHz, CDCh) δ: ppm 4.07 (t, 2H); 3.56 (m, 4H); 2.80 (t, 2H); 2.65 (m, 4H); 2.46 (t, 4H); 2.30 (t, 2H); 1.80-1.05 (br. m, 52H); 0.90 (t, 9H)

Step 2: heptadecan-9-yl 8-((2-(2-(dinonylamino)ethoxy)ethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate (Compound 180)



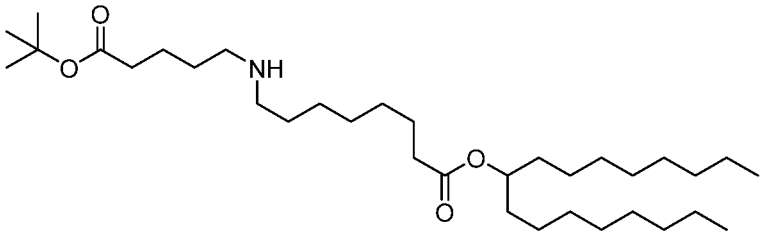
Chemical Formula: C64H128N2O5

Molecular Weight: 1005.74

[001065] In the same manner as Step 3 for Compound 174, heptadecan-9-yl 8-((2-(2-(dinonylamino)ethoxy)ethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate was synthesized from nonyl 8-((2-(2-(dinonylamino)ethoxy)ethyl)amino)octanoate (395 mg, 0.632 mmol), heptadecan-9-yl 8-bromooctanoate (321 mg, 0.695 mmol), potassium carbonate (175 mg, 1.26 mmol), and potassium iodide (21 mg, 0.13 mmol) in MeCN (10 mL). Yield (UPLC/ELSD: RT = 3.66 min. MS (ES): *m/z* (MH^+) 1006.51 for C64H128N2O5 ¾-NMR (300 MHz, CDCh) δ: ppm 4.89 (quint, 1H); 4.08 (t, 2H); 3.51 (t, 4H); 2.85-2.20 (br. m, 16H); 1.80-1.05 (br. m, 90H); 0.90 (t, 15H)

EQ: Compound 181: Di(heptadecan-9-yl) 15,18-diethyl-9,24-bis(8-(nonyloxy)-8-oxooctyl)-14,19-dioxo-9,15,18,24-tetraazadotriacontanedioate

Step 1: heptadecan-9-yl 8-((5-(tert-butoxy)-5-oxopentyl)amino)octanoate



Chemical Formula: C34H67NO4

Molecular Weight: 553.91

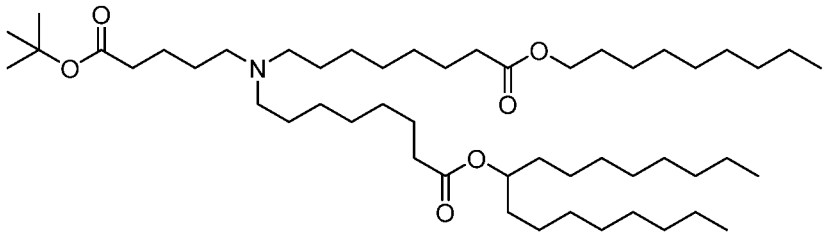
[001066] To a solution of tert-butyl 5-aminopentanoate (2.81 g, 16.2 mmol) and heptadecan-9-yl 8-bromooctanoate (5.00 g, 10.8 mmol) in MeCN (100 mL) was added potassium carbonate (3.00 g, 21.6 mmol) and potassium iodide (359 mg, 2.16 mmol) and the suspension was allowed to stir at 82°C for 12 hours. The reaction mixture was allowed to cool

to RT, filtered over celite, and concentrated *in vacuo*. Purification by ISCO silica flash chromatography 0-10% MeOH in DCM provided heptadecan-9-yl 8-((5-(tert-butoxy)-5-oxopentyl)amino)octanoate (3.14 g, 52.4%).

UPLC/ELSD: RT = 2.68 min. MS (ES): *m/z* (MH^+) 554.59 for $C_{34}H_{67}NO_4$

¾-NMR (300 MHz, CDCh) δ: ppm 4.88 (quint, 1H); 3.35 (br, 1H); 2.64 (m, 4H); 2.28 (m, 4H); 1.90-1.10 (br. m, 51H); 0.90 (t, 6H)

Step 2: heptadecan-9-yl 8-((5-(tert-butoxy)-5-oxopentyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate



Chemical Formula: C51H99NO6

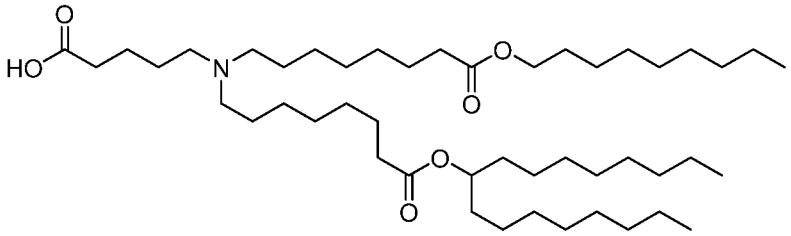
Molecular Weight: 822.35

[001067] To a solution of heptadecan-9-yl 8-((5-(tert-butoxy)-5-oxopentyl)amino)octanoate (3.14 g, 5.67 mmol) and nonyl 8-bromooctanoate (2.08 g, 5.95 mmol) in MeCN (60 mL) was added potassium carbonate (1.57 g, 11.3 mmol) and potassium iodide (188 mg, 1.13 mmol) and the suspension was allowed to stir at 82°C for 12 hours. The reaction mixture was allowed to cool to RT, filtered over celite, and concentrated *in vacuo*. Purification by ISCO silica flash chromatography (0-40% EtOAc in hexanes) provided heptadecan-9-yl 8-((5-(tert-butoxy)-5-oxopentyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate (3.31 g, 71.1%).

UPLC/ELSD: RT = 3.79 min. MS (ES): *m/z* (MH^+) 823.89 for C51H99NO6

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.89 (quint, 1H); 4.07 (t, 2H); 2.50-2.16 (br. m, 12H); 1.75-1.05 (br. m, 75H); 0.90 (t, 9H)

Step 3: 5-((8-(heptadecan-9-yloxy)-8-oxooctyl)(8-(nonyloxy)-8-oxooctyl)amino)pentanoic acid (Compound 181)



Chemical Formula: C47H91NO6

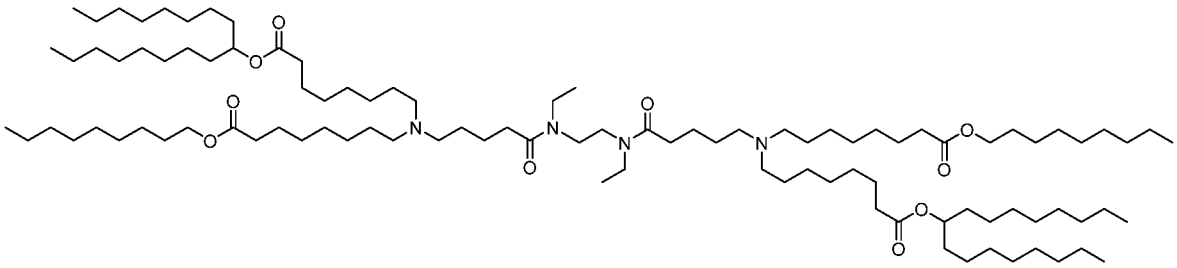
Molecular Weight: 766.25

[001068] To a solution of heptadecan-9-yl 8-((5-(tert-butoxy)-5-oxopentyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate (3.31 g, 4.03 mmol) in DCM (16 mL) was added TFA (15 mL, 200 mmol) and the reaction was allowed to stir at RT for 12 hours. The solution was concentrated *in vacuo,* taken up in EtOAc, washed with 5% Na2CCb, brine, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo* to provide 5-((8-(heptadecan-9-yloxy)-8-oxooctyl)(8-(nonyloxy)-8-oxooctyl)amino)pentanoic acid (3.0 g, 97%).

UPLC/ELSD: RT = 3.58 min. MS (ES): *m/z* (MH^+) 766.81 for C47H91NO6

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.89 (quint, 1H); 4.08 (t, 2H); 2.82 (m, 6H); 2.31 (m, 6H); 1.90-1.10 (br. m, 66H); 0.90 (t, 9H)

Step 4: di(heptadecan-9-yl) 15,18-diethyl-9,24-bis(8-(nonyloxy)-8-oxooctyl)-14,19-dioxo-9,15,18,24-tetraazadotriacontanedioate



Chemical Formula: C100H194N4O10

Molecular Weight: 1612.67

[001069] To a 0 °C solution of 5-((8-(heptadecan-9-yloxy)-8-oxooctyl)(8-(nonyloxy)-8-oxooctyl)amino)pentanoic acid (500 mg, 0.653 mmol) and N^1,N^2-diethylethane-l,2-diamine (44 μL, 0.311 mmol) in THF (7 mL) was added DIPEA (119 μL, 0.684 mmol) and T3P (50% EtOAc solution, 740 μL, 1.24 mmol) and the reaction was allowed to stir at 0 °C for 2 hours. The reaction was quenched with water and extracted twice with EtOAc. The organics were dried over anhydrous Na2S04, filtered, and concentrated *in vacuo.* Purification by ISCO C18 (10-100% MeCN in water with 0.1% TFA) provided di(heptadecan-9-yl) 15,18-diethyl-9,24-

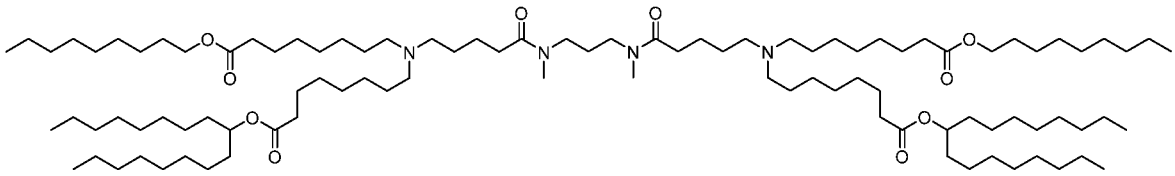
bis(8-(nonyloxy)-8-oxooctyl)-14, 19-dioxo-9, 15, 18,24-tetraazadotriacontanedioate (261 mg, 52.1%).

UPLC/ELSD: RT = 4.11 min. MS (ES): *m/z* (MH^+) 1613.79 for $C_{100}H_{194}N_4O_{10}$

¾-NMR (300 MHz, CDCh) δ: ppm 4.74 (quint, 2H); 3.93 (t, 4H); 3.25 (m, 8H); 2.48-2.04 (br. m, 24H); 1.70-0.90 (br. m, 138H); 0.75 (t, 18H)

ER: Compound 182: Di(heptadecan-9-yl) 15,19-dimethyl-9,25-bis(8-(nonyloxy)-8-oxooctyl)-14,20-dioxo-9, 15, 19,25-tetraazatritriacontanedioate

Step 1: di(heptadecan-9-yl) 15,19-dimethyl-9,25-bis(8-(nonyloxy)-8-oxooctyl)-14,20-dioxo-9,15,19,25-tetraazatritr acontanedioate (Compound 182)



Chemical Formula: $C_{99}H_{192}N_4O_{10}$

Molecular Weight: 1598.64

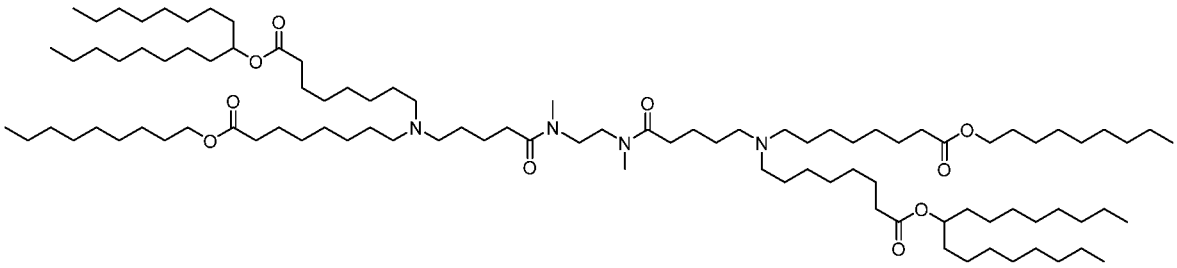
[001070] In the same manner as Step 4 for Compound 181, di(heptadecan-9-yl) 15,19-dimethyl-9,25-bis(8-(nonyloxy)-8-oxooctyl)-14,20-dioxo-9,15,19,25-tetraazatritriacontanedioate was synthesized from 5-((8-(heptadecan-9-yloxy)-8-oxooctyl)(8-(nonyloxy)-8-oxooctyl)amino)pentanoic acid (500 mg, 0.653 mmol), N\NMimethylpropane-l,3-diamine (39 μL, 0.311 mmol), DIPEA (119 μL, 0.684 mmol) and T3P (50% EtOAc solution, 740 μL, 1.24 mmol) in THF (7 mL). Yield (215 mg, 43.4%).

UPLC/ELSD: RT = 4.09 min. MS (ES): *m/z* (MH^+) 1599.69 for $C_{99}H_{192}N_4O_{10}$

^{1}H-NMR (300 MHz, CDCh) δ: ppm 4.89 (quint, 2H); 4.08 (t, 4H); 3.36 (m, 4H); 3.08-2.86 (br. m, 6H); 2.52-2.22 (br. m, 24H); 1.90-1.10 (br. m, 134H); 0.90 (t, 18H)

ES: Compound 183: Di(heptadecan-9-yl) 15,18-dimethyl-9,24-bis(8-(nonyloxy)-8-oxooctyl)-14, 19-dioxo-9, 15,1 8,24-tetraazadotriacontanedioate

Step 1: di(heptadecan-9-yl) 15,18-dimethyl-9,24-bis(8-(nonyloxy)-8-oxooctyl)-14,19-dioxo-9,15,18,24-tetraazadotriacontanedioate (Compound 183)



Chemical Formula: C98H190N4O10

Molecular Weight: 1584.62

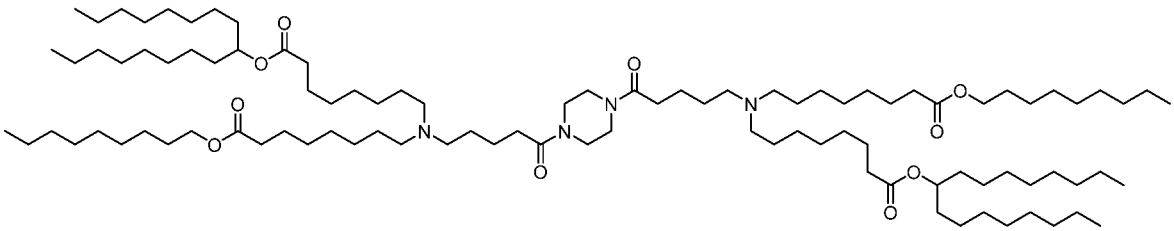
[001071] In the same manner as Step 4 for Compound 181, di(heptadecan-9-yl) 15,18-dimethyl-9,24-bis(8-(nonyloxy)-8-oxooctyl)-14,19-dioxo-9,15,18,24-tetraazadotriacontanedioate was synthesized from 5-((8-(heptadecan-9-yloxy)-8-oxooctyl)(8-(nonyloxy)-8-oxooctyl)amino)pentanoic acid (500 mg, 0.653 mmol), N^1,N^2-dimethylethane-1,2-diamine (32 μL, 0.297 mmol), DIPEA (155 μL, 0.890 mmol) and T3P (50% EtOAc solution, 706 μL, 1.19 mmol) in THF (7 mL). Yield (177 mg, 37.7%).

UPLC/ELSD: RT = 4.10 min. MS (ES): *m/z* (MH^+) 1585.58 for C98H190N4O10

¾-NMR (300 MHz, CDCh) δ: ppm 4.80 (quint, 2H); 3.99 (t, 4H); 3.50-3.34 (br. m, 4H); 3.02-2.86 (br. m, 6H); 2.66-2.12 (br. m, 24H); 1.70-1.00 (br. m, 132H); 0.82 (t, 18H)

ET: Compound 184: Di(heptadecan-9-yl) 8,8'-((piperazine-1,4-diylbis(5-oxopentane-5,1-diyl))bis((8-(nonyloxy)-8-oxooctyl)azanediyl))dioctanoate

Step 1: di(heptadecan-9-yl) 8,8'-((piperazine-1,4-diylbis(5-oxopentane-5,1-diyl))bis((8-(nonyloxy)-8-oxooctyl)azanediyl))dioctanoate (Compound 184)



Chemical Formula: C98H188N4O10

Molecular Weight: 1582.60

[001072] In the same manner as Step 4 for Compound 181, di(heptadecan-9-yl) 8,8'-((piperazine-1,4-diylbis(5-oxopentane-5,1-diyl))bis((8-(nonyloxy)-8-oxooctyl)azanediyl))dioctanoate was synthesized from 5-((8-(heptadecan-9-yloxy)-8-oxooctyl)(8-(nonyloxy)-8-oxooctyl)amino)pentanoic acid (593 mg, 0.774 mmol), piperazine (32

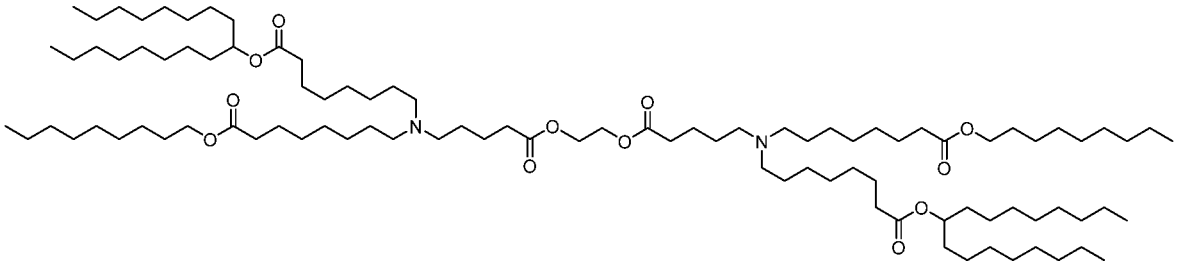
mg, 0.37 mmol), DIPEA (193 μL, 1.11 mmol), and T3P (50% EtOAc solution, 878 μL, 1.47 mmol) in THF (10 mL). Yield (172 mg, 29.5%).

UPLC/ELSD: RT = 4.07 min. MS (ES): *m/z* (MH^+) 1583.70 for $C_{98}H_{190}N_4O_{10}$

¾-NMR (300 MHz, CDCh) δ: ppm 4.88 (quint, 2H); 4.08 (t, 4H); 3.72-3.38 (br. m, 8H); 2.62-2.18 (br. m, 24H); 1.90-1.00 (br. m, 132H); 0.90 (t, 18H)

EU: Compound 185: Di(heptadecan-9-yl) 8,8'-(ll,24,29,42-tetraoxo-10,25,28,43-tetraoxa-19,34-diazadopentacontane-19,34-diyl)dioctanoate

Step 1: di(heptadecan-9-yl) 8,8'-(ll,24,29,42-tetraoxo-10,25,28,43-tetraoxa-19,34-diazadopentacontane-19,34-diyl)dioctanoate (Compound 185)



Chemical Formula: $C_{96}H_{184}N_2O_{12}$

Molecular Weight: 1558.53

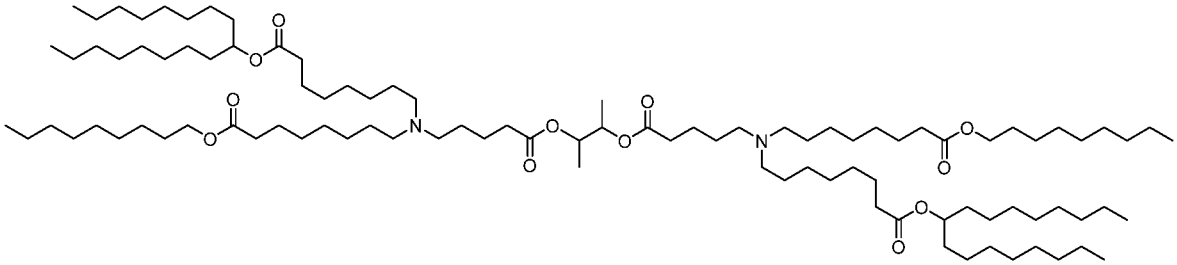
[001073] To a solution of 5-((8-(heptadecan-9-yloxy)-8-oxooctyl)(8-(nonyloxy)-8-oxooctyl)amino)pentanoic acid (500 mg, 0.653 mmol) and ethylene glycol (17 μL, 0.311 mmol) in DCM (10 mL) was added EDC HCL (145 mg, 0.932 mmol) and DMAP (8 mg, 0.06 mmol) and the solution was allowed to stir at RT for 1 hour. The reaction was diluted with chloroform and washed with 10% citric acid, dried over anhydrous Na2S04, filtered, and concentrated *in vacuo*. Purification by ISCO C18 flash chromatography (10-100% MeCN in water with 0.1% TFA) provided di(heptadecan-9-yl) 8,8'-(ll,24,29,42-tetraoxo-10,25,28,43-tetraoxa-19,34-diazadopentacontane-19,34-diyl)dioctanoate (305 mg, 63.0%).

UPLC/ELSD: RT = 4.11 min. MS (ES): *m/z* (MH^+) 1559.91 for $C_{96}H_{184}N_2O_{12}$

¾-NMR (300 MHz, CDCh) δ: ppm 4.74 (quint, 2H); 4.14 (s, 4H); 3.92 (t, 4H); 2.60-2.08 (br. m, 24H); 1.65-0.95 (br. m, 132H); 0.75 (t, 18H)

EV: Compound 186: Di(heptadecan-9-yl) 8,8'-(26,27-dimethyl-ll,24,29,42-tetraoxo-10,25,28,43-tetraoxa-19,34-diazadopentacontane-19,34-diyl)dioctanoate

Step 1: di(heptadecan-9-yl) 8,8'-(26,27-dimethyl-11,24,29,42-tetraoxo-10,25,28,43-tetraoxa-19,34-diazadopentacontane-19,34-diyl)dioctanoate (Compound 186)



Chemical Formula: C98H188N2O12

Molecular Weight: 1586.58

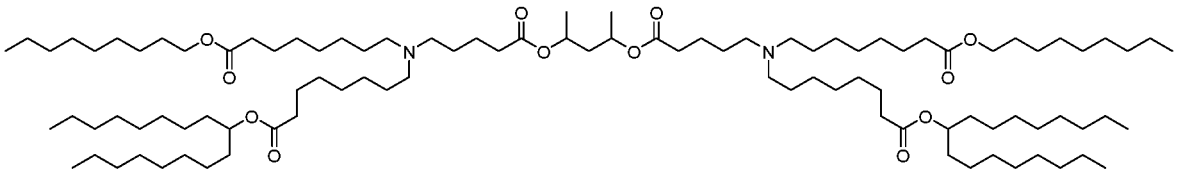
[001074] In the same manner as Step 1 for Compound 185, di(heptadecan-9-yl) 8,8'-(26,27-dimethyl-11,24,29,42-tetraoxo-10,25,28,43-tetraoxa-19,34-diazadopentacontane-19,34-diyl)dioctanoate was synthesized from 5-((8-(heptadecan-9-yloxy)-8-oxooctyl)(8-(nonyloxy)-8-oxooctyl)amino)pentanoic acid (500 mg, 0.653 mmol), butane-2,3-diol (28 μL, 0.311 mmol), EDC HCL (145 mg, 0.932 mmol) and DMAP (8 mg, 0.06 mmol) in DCM (10 mL). Yield (248 mg, 50.3%).

UPLC/ELSD: RT = 4.14 min. MS (ES): *m/z* (MH^+) 1587.14 for C98H188N2O12

¾-NMR (300 MHz, CDCh) δ: ppm 5.10-4.75 (br. m, 4H); 4.07 (t, 4H); 2.75-2.15 (br. m, 24H); 1.90-1.00 (br. m, 138H); 0.90 (t, 18H)

EW: Compound 187: Di(heptadecan-9-yl) 8,8'-(26,28-dimethyl-11,24,30,43-tetraoxo-10,25,29,44-tetraoxa-19,35-diazatripentacontane-19,35-diyl)dioctanoate

Step 1: di(heptadecan-9-yl) 8,8'-(26,28-dimethyl-11,24,30,43-tetraoxo-10,25,29,44-tetraoxa-19,35-diazatripentacontane-19,35-diyl)dioctanoate (Compound 187)



Chemical Formula: C99H190N2O12

Molecular Weight: 1600.61

[001075] In the same manner as Step 1 for Compound 185, di(heptadecan-9-yl) 8,8'-(26,28-dimethyl-11,24,30,43-tetraoxo-10,25,29,44-tetraoxa-19,35-diazatripentacontane-19,35-diyl)dioctanoate was synthesized from 5-((8-(heptadecan-9-yloxy)-8-oxooctyl)(8-(nonyloxy)-8-

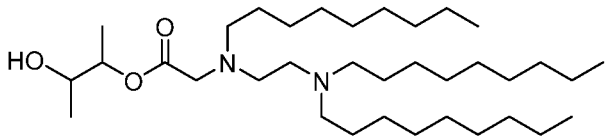
oxooctyl)amino)pentanoic acid (500 mg, 0.653 mmol), pentane-2,4-diol (34 μL, 0.311 mmol), EDC HCL (145 mg, 0.932 mmol) and DMAP (8 mg, 0.06 mmol) in DCM (10 mL). Yield (248 mg, 50.3%).

UPLC/ELSD: RT = 4.16 min. MS (ES): *m/z* (MH^+) 1601.74 for $C_{99}H_{190}N_2O_{12}$

¾-NMR (300 MHz, CDCh) δ: ppm 5.06-4.80 (br. m, 4H); 4.07 (t, 4H); 2.70-2.16 (br. m, 24H); 1.85-1.00 (br. m, 140H); 0.90 (t, 18H)

EX: Compound 188: 3-Hydroxybutan-2-yl N-(2-(dinonylamino)ethyl)-N-nonyl glycinate

Step 1: 3-hydroxybutan-2-yl N-(2-(dinonylamino)ethyl)-N-nonylglycinate



Chemical Formula: $C_{35}H_{72}N_2O_3$

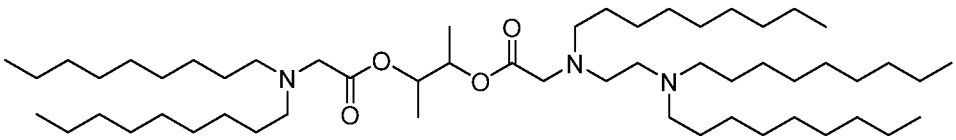
Molecular Weight: 568.97

[001076] In the same manner as Step 5 for Compound 44, 3-hydroxybutan-2-yl N-(2-(dinonylamino)ethyl)-N-nonylglycinate was synthesized from N-(2-(dinonylamino)ethyl)-N-nonylglycine (400 mg, 0.805 mmol), butane-2,3-diol (1.45 g, 16.1 mmol), EDC HCL (500 mg, 3.22 mmol), and DMAP (98 mg, 0.81 mmol) in DCM (10 mL). Yield (420 mg, 91.7%).

UPLC/ELSD: RT = 2.99 min. MS (ES): *m/z* (MH^+) 569.43 for $C_{35}H_{72}N_2O_3$

¾-NMR (300 MHz, CDCh) δ: ppm 5.00-4.77 (br. m, 1H); 4.10-2.50 (br. m, 13H); 1.90-1.00 (br. m, 48H); 0.90 (t, 9H)

Step 2: 3-((N-(2-(dinonylamino)ethyl)-N-nonylglycyl)oxy)butan-2-yl dinonylglycinate (Compound 188)



Chemical Formula: $C_{55}H_{111}N_3O_4$

Molecular Weight: 878.51

[001077] In the same manner as Step 7 for Compound 44, 3-((N-(2-(dinonylamino)ethyl)-N-nonylglycyl)oxy)butan-2-yl dinonylglycinate was synthesized from 3-hydroxybutan-2-yl N-(2-(dinonylamino)ethyl)-N-nonylglycinate (352 mg, 0.619 mmol), dinonylglycine (223 mg,

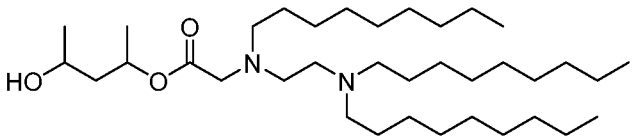
0.681 mmol), EDC HCL (192 mg, 1.24 mmol), and DMAP (38 mg, 0.31 mmol) in DCM (10 mL). Yield (119 mg, 21.9%).

UPLC/ELSD: RT = 3.26 min. MS (ES): *m/z* (MH^+) 879.16 for $C_{55}H_{111}N_3O_4$

¾-NMR (300 MHz, CDCh) δ: ppm 5.05 (m, 2H); 3.50-2.20 (br. m, 18H); 2.00-1.00 (br. m, 76H); 0.91 (t, 15H)

EY: Compound 189: 4-((N-(2-(Dinonylamino)ethyl)-N-nonylglycyl)oxy)pentan-2-yl dinonylglycinate

Step 1: 4-hydroxypentan-2-yl N-(2-(dinonylamino)ethyl)-N-nonylglycinate



Chemical Formula: $C_{36}H_{74}N_2O_3$

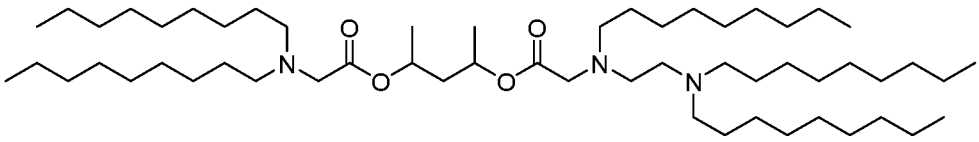
Molecular Weight: 583.00

[001078] In the same manner as Step 5 for Compound 44, 3-hydroxybutan-2-yl N-(2-(dinonylamino)ethyl)-N-nonylglycinate was synthesized from N-(2-(dinonylamino)ethyl)-N-nonylglycine (400 mg, 0.805 mmol), pentane-2,4-diol (1.68 g, 16.1 mmol), EDC HCL (500 mg, 3.22 mmol), and DMAP (20 mg, 0.16 mmol) in DCM (10 mL). Yield (427 mg, 91.0%).

UPLC/ELSD: RT = 3.04 min. MS (ES): *m/z* (MH^+) 583.54 for $C_{36}H_{74}N_2O_3$

¾-NMR (300 MHz, CDCh) δ: ppm 5.26 (m, 1H); 4.25-2.55 (br. m, 13H); 2.05-1.00 (br. m, 50H); 0.90 (t, 9H)

Step 2: 4-((N-(2-(dinonylamino)ethyl)-N-nonylglycyl)oxy)pentan-2-yl dinonylglycinate (Compound 189)



Chemical Formula: $C_{56}H_{113}N_3O_4$

Molecular Weight: 892.54

[001079] In the same manner as Step 7 for Compound 44, 4-((N-(2-(dinonylamino)ethyl)-N-nonylglycyl)oxy)pentan-2-yl dinonylglycinate was synthesized from 4-hydroxypentan-2-yl N-(2-(dinonylamino)ethyl)-N-nonylglycinate (427 mg, 0.732 mmol), dinonylglycine (264 mg,

0.806 mmol), EDC HCL (455 mg, 2.93 mmol), and DMAP (89 mg, 0.73 mmol) in DCM (10 mL). Yield (208 mg, 31.8%).

UPLC/ELSD: RT = 3.27 min. MS (ES): *m/z* (MH^+) 893.02 for $C_{56}H_{113}N_3O_4$

¾-NMR (300 MHz, CDCh) δ: ppm 5.02 (m, 2H); 3.34 (m, 4H); 2.75-2.35 (br. m, 14H); 1.85-1.00 (br. m, 78H); 0.91 (t, 15H)

Example 2: Production of nanoparticle compositions

Production of nanoparticle compositions

[001080] In order to investigate safe and efficacious nanoparticle compositions for use in the delivery of therapeutic and/or prophylactic agents to cells, a range of formulations are prepared and tested. Specifically, the particular elements and ratios thereof in the lipid component of nanoparticle compositions are optimized.

[001081] Nanoparticles can be made with mixing processes such as microfluidics and T-junction mixing of two fluid streams, one of which contains the therapeutic and/or prophylactic agent and the other has the lipid components.

[001082] Lipid compositions are prepared by combining a lipid according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), a phospholipid (such as DOPE or DSPC, obtainable from Avanti Polar Lipids, Alabaster, AL), a PEG lipid (such as 1,2-dimyristoyl-OT-glycerol methoxypoly ethylene glycol, also known as PEG-DMG, obtainable from Avanti Polar Lipids, Alabaster, AL, or a PEG lipid according to one of formulae (VI), (Vl-a) or (VI-b)), and a structural lipid (such as cholesterol, obtainable from Sigma-Aldrich, Taufkirchen, Germany) at concentrations of about 50 mM in ethanol. Solutions should be refrigeration for storage at, for example, -20° C. Lipids are combined to yield desired molar ratios (see, for example, Table 1) and diluted with water and ethanol to a final lipid concentration of between about 5.5 mM and about 25 mM.

[001083] Nanoparticle compositions including a therapeutic and/or prophylactic agent and a lipid component are prepared by combining the lipid solution with a solution including the therapeutic and/or prophylactic agent at lipid component to therapeutic and/or prophylactic agent wt:wt ratios between about 5:1 and about 50:1. The lipid solution is rapidly injected using a NanoAssemblr microfluidic based system at flow rates between about 10 ml/min and about 18 ml/min into the therapeutic and/or prophylactic agent solution to produce a suspension with a water to ethanol ratio between about 1:1 and about 4:1.

[001084] For nanoparticle compositions including an RNA, solutions of the RNA at concentrations of 0.1 mg/ml in deionized water are diluted in 50 mM sodium citrate buffer at a pH between 3 and 4 to form a stock solution.

[001085] Nanoparticle compositions can be processed by dialysis to remove ethanol and achieve buffer exchange. Formulations are dialyzed twice against phosphate buffered saline (PBS), pH 7.4, at volumes 200 times that of the primary product using Slide-A-Lyzer cassettes (Thermo Fisher Scientific Inc., Rockford, IL) with a molecular weight cutoff of 10 kD. The first dialysis is carried out at room temperature for 3 hours. The formulations are then dialyzed overnight at 4° C. The resulting nanoparticle suspension is filtered through 0.2 μιτι sterile filters (Sarstedt, Numbrecht, Germany) into glass vials and sealed with crimp closures. Nanoparticle composition solutions of 0.01 mg/ml to 0.10 mg/ml are generally obtained.

[001086] The method described above induces nano-precipitation and particle formation. Alternative processes including, but not limited to, T-junction and direct injection, may be used to achieve the same nano-precipitation.

Characterization of nanoparticle compositions

[001087] A Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, Worcestershire, UK) can be used to determine the particle size, the polydispersity index (PDI) and the zeta potential of the nanoparticle compositions in 1*PBS in determining particle size and 15 mM PBS in determining zeta potential.

[001088] Ultraviolet-visible spectroscopy can be used to determine the concentration of a therapeutic and/or prophylactic agent (e.g., RNA) in nanoparticle compositions. 100 μL of the diluted formulation in 1**xPBS** is added to 900 μL of a 4:1 (v/v) mixture of methanol and chloroform. After mixing, the absorbance spectrum of the solution is recorded, for example, between 230 nm and 330 nm on a DU 800 spectrophotometer (Beckman Coulter, Beckman Coulter, Inc., Brea, CA). The concentration of therapeutic and/or prophylactic agent in the nanoparticle composition can be calculated based on the extinction coefficient of the therapeutic and/or prophylactic agent used in the composition and on the difference between the absorbance at a wavelength of, for example, 260 nm and the baseline value at a wavelength of, for example, 330 nm.

[001089] For nanoparticle compositions including an RNA, a QUANT-IT™ RIBOGREEN® RNA assay (Invitrogen Corporation Carlsbad, CA) can be used to evaluate the encapsulation of an RNA by the nanoparticle composition. The samples are diluted to a

concentration of approximately 5 μg/mL in a TE buffer solution (10 mM Tris-HCl, 1 mM EDTA, pH 7.5). 50 μL of the diluted samples are transferred to a polystyrene 96 well plate and either 50 μL of TE buffer or 50 μL of a 2% Triton X-100 solution is added to the wells. The plate is incubated at a temperature of 37° C for 15 minutes. The RIBOGREEN® reagent is diluted 1:100 in TE buffer, and 100 μL of this solution is added to each well. The fluorescence intensity can be measured using a fluorescence plate reader (Wallac Victor 1420 Multilablel Counter; Perkin Elmer, Waltham, MA) at an excitation wavelength of, for example, about 480 nm and an emission wavelength of, for example, about 520 nm. The fluorescence values of the reagent blank are subtracted from that of each of the samples and the percentage of free RNA is determined by dividing the fluorescence intensity of the intact sample (without addition of Triton X-100) by the fluorescence value of the disrupted sample (caused by the addition of Triton X-100).

In vivo formulation studies

[001090] In order to monitor how effectively various nanoparticle compositions deliver therapeutic and/or prophylactic agents to targeted cells, different nanoparticle compositions including a particular therapeutic and/or prophylactic agent (for example, a modified or naturally occurring RNA such as an mRNA) are prepared and administered to rodent populations. Mice are intravenously, intramuscularly, intraarterially, or intratumorally administered a single dose including a nanoparticle composition with a formulation such as those provided in Table 1. In some instances, mice may be made to inhale doses. Dose sizes may range from 0.001 mg/kg to 10 mg/kg, where 10 mg/kg describes a dose including 10 mg of a therapeutic and/or prophylactic agent in a nanoparticle composition for each 1 kg of body mass of the mouse. A control composition including PBS may also be employed.

[001091] Upon administration of nanoparticle compositions to mice, dose delivery profiles, dose responses, and toxicity of particular formulations and doses thereof can be measured by enzyme-linked immunosorbent assays (ELISA), bioluminescent imaging, or other methods. For nanoparticle compositions including mRNA, time courses of protein expression can also be evaluated. Samples collected from the rodents for evaluation may include blood, sera, and tissue (for example, muscle tissue from the site of an intramuscular injection and internal tissue); sample collection may involve sacrifice of the animals.

[001092] Nanoparticle compositions including mRNA are useful in the evaluation of the efficacy and usefulness of various formulations for the delivery of therapeutic and/or prophylactic agents. Higher levels of protein expression induced by administration of a

composition including an mRNA will be indicative of higher mRNA translation and/or nanoparticle composition mRNA delivery efficiencies. As the non-RNA components are not thought to affect translational machineries themselves, a higher level of protein expression is likely indicative of a higher efficiency of delivery of the therapeutic and/or prophylactic agent by a given nanoparticle composition relative to other nanoparticle compositions or the absence thereof.

Example 3: Sample formulations

[001093] Nanoparticle compositions including a therapeutic and/or prophylactic agent can be optimized according to the selection of a compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) , the selection of additional lipids, the amount of each lipid in the lipid component, and the wt:wt ratio of the lipid component to the therapeutic and/or prophylactic agent, as described herein.

Initial studies were performed to compare the delivery efficiency of nanoparticle compositions including various compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) . The cationic lipid MC3 is a current standard in the art. Also determined was the stability of nanoparticles of the disclosure, i.e., the purity after storage in EtOH for 96h at 25°C or 37°C (Table Id).

[001094] Accordingly, the standard MC3 formulation including about 50 mol % MC3, about 10 mol % DSPC, about 38.5 mol % cholesterol, and about 1.5 mol % PEG-DMG was used as a basis for this study. Nanoparticle compositions including DOPE or DSPC as a phospholipid, cholesterol as a structural lipid, PEG-DMG as a PEG lipid, an RNA, and a compound according to one of formulae disclosed herein, e.g., selected from compounds of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), were prepared according to Examples 1 and 2. The ratios of the lipids were 40:20:38.5:1.5 mol% for the lipid described herein: DOPE: cholesterol: PEG-DMG or 50:10:38.5:1.5 mol% for the lipid described herein:DSPC:cholesterol:PEG-DMG. The RNA used was an mRNA encoding G5 luciferase (Luc) or G5 hEPO. Tables 1, 1b and l c summarize the content and characteristics of the formulations.

[001095] As shown in Tables 1 and la, nanoparticle compositions including Compound 1 produced the largest particles amongst those of Tables 1 and la, while those including Compounds 34 and 50 produced the smallest particles amongst those of Tables 1 and Ida.

Encapsulation efficiencies amongst those of Tables 1 and 1a were highest for compositions including Compounds 36, 37, 40, and 41 and lowest for those including Compounds 1 and 24.

Table 1. Characteristics of nanoparticle compositions including compounds of one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

Compound No.	Size (nm)	PDI	EE (%)	pKa
1	203.2		46.2	5.95
2*	94.7	0.108	96	6.39
3	73.5	0.044	92.5	n.d.
4	89.7	0.120	96.78	7.12
5	85.1	0.140	98.1	7.01
6	81.4	0.160	98.9	6.62
7	85.1	0.130	99.1	6.94
8	83.3	0.110	98.5	6.87
9	78.0	0.170	99.5	6.76
10	81.3	0.130	99.2	6.58
11	87.4	0.099	99.5	6.54
12	87.8	0.096	96.9	5.44
13	97.7	0.080	64.2	6.30
14	88.7	0.008	96.6	6.31
15#	100.3	0.120	90.2	6.32
16#	77.4	0.140	98.2	6.28
17	82.3	0.180	96.6	6.67
18	76.7	0.120	98.4	6.17
19	76.1	0.100	97.2	6.29
20	106.4	0.150	84.2	6.12
21	98.3	0.239	98.6	6.29
22#	75.4	0.130	98.3	6.15
23	85.4	0.058	82.9	6.07
24*	110.4	0.131	36.4	6.01
25	90.0	0.186	97.0	6.20
26*	74.2	0.112	84.9	6.19

Compound No.	Size (nm)	PDI	EE (%)	pKa
27	86.4	0.211	97.9	6.14
28	87.4	0.099	80.2	6.04
29	105.3	0.060	48.8	5.97
30	95.0	0.110	74.3	6.09
31	87.9	0.130	77.5	6.31
32	79.3	0.160	83.6	6.28
33	79.7	0.138	98.1	6.06
34*	66.0	0.077	98.1	5.74
36*	100.8	0.110	100.2	7.81
37*	86.6	0.107	99.9	6.45
40*	78.9	0.210	100.0	6.78
41*	69.0	0.239	99.9	7.02
42	116.4	0.190	97.1	6.77
43	99.0	0.220	99.1	6.72
44	94.9	0.190	89.5	6.82
45	100.2	0.200	94.9	6.77
46	81.8	0.160	97.5	6.77
47	89.8	0.180	53.1	6.82
48	111.4	0.099	79.3	6.99
49	95.8	0.200	98.8	6.4
50	65.6	0.190	98.7	5.55
51	76.6	0.190	98.4	6.44
52	94.4	0.100	97.5	6.77
Formula IV	94.2		97.6	6.25
MC3	86.2	0.117	97.70	n.d.

n.d.=not determined

*==Formulated with lipid:DSPC:Chol:PEG-DMG 50:10:38.5:1.5

#=Formulated with hEPO mRNA

5

Table lb. Characteristics of nanoparticle compositions including compounds of one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

Compound No.	Size (nm)	PDI	EE (%)	pKa
53	103.0	0.23	82.8	6.91
54	93.0	0.23	96.9	6.88
55	119.5	0.23	95.2	6.96
56	117.4	0.24	99.0	6.94
57	101.9	0.23	98.8	6.73
58	112.8	0.23	98.9	6.7
59	104.7	0.23	98.6	6.68
60	105.7	0.23	98.8	6.66
61	86.8	0.23	99.1	6.61
62	61.7	0.18	97.73	7.56
69	74.4	0.24	99.31	7.01
70	79.8	0.24	99.14	6.75
71	99.9	0.18	91.0	6.44
72	102.5	0.22	92.7	7.19
73	84.3	0.25	98.85	6.65
80	65.8	0.2	98.93	6.78
81	65.3	0.17	99.27	7.22
82	76.1	0.24	99.23	7.05
83	73.2	0.22	99.12	6.72
84	68.6	0.19	98.48	7.01
85	69.9	0.24	99.18	6.74
86	53.6	0.14	97.42	7.58
87	80.9	0.21	98.67	6.79
MC3	74.7	0.17	97.3	

Table lc. Characteristics of nanoparticle compositions including compounds of one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

Compound No.	Size (nm)	PDI	EE (%)	pKa
110	78.7	0.22	97.6	6.68
111	86.8	0.21	96.7	6.82
112	73.5	0.22	97.1	6.14

91	82.9	0.21	97.6	6.74
90	81.5	0.19	98.4	6.83
89	80.1	0.20	97.6	6.94
94	74.6	0.20	98.4	6.65
93	75.6	0.17	97.7	6.6
92	73.8	0.17	97.1	6.53
109	75.0	0.16	95.0	7.27
114	77.2	0.23	98.1	6.64
102	71.5	0.23	97.6	5.85
115	66.0	0.16	97.0	5.01
107	80.6	0.19	96.6	6.99
116	77.1	0.19	98.9	6.55
117	84.6	0.20	95.2	6.71
118	82.3	0.20	97.8	6.7
103	76.9	0.17	99.0	7.25
120	78.7	0.21	99.0	6.49
121	75.7	0.22	98.6	6.50
122	77.1	0.23	99.1	6.44
123	85.1	0.21	98.7	6.51
124	92.7	0.21	99.2	6.94
125	85.7	0.24	99.0	6.89
126	87.0	0.24	98.7	6.76
127	79.8	0.20	99.5	6.81
150	70.1	0.22	96.9	5.10
128	68.0	0.20	97.1	4.93
151	77.4	0.18	98.8	7.46
152	79.1	0.16	98.5	7.48
143	93.7	0.21	97.4	7.26
144	83.3	0.19	97.2	7.59
129	70.3	0.24	96.9	5.17
130	84.9	0.23	98.0	6.90
131	85.4	0.17	98.5	7.02
132	83.1	0.23	98.6	6.49
133	88.2	0.24	98.6	6.58
134	82.1	0.21	96.7	6.85
135	81.1	0.22	98.6	6.90
136	72.1	0.23	97.7	6.13
137	78.5	0.22	98.8	6.93
139	83.8	0.23	94.8	6.15

Table Id. Characteristics of nanoparticle compositions including compounds of one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

Compound No.	Size (nm)	PDI	EE (%)
146*	97.5	0.15	97.6
140*	131.1	0.14	86.9
141*	131.5	0.15	97.6
155*	84.4	0.22	98.4
157*	89.8	0.11	98.3
153*	116.7	0.23	97.2
158*	124.2	0.20	97.8
154*	103.3	0.18	97.8
159*	76.5	0.12	92.9
160*	115.5	0.25	97.3
4**	101.5	0.23	99.1
MC3#	105.0	0.20	84.1

*=Formulated with lipid:Chol:DOPE:PEG 1 40:38.5:20:1.5

**=Formulated with lipid:Chol:DOPE:PEG 1 30:38.5:30:1.5

#=Formulated with lipid:Chol:PEG 1 50:47.5:2.5

Table Ie. Characteristics of nanoparticle compositions including compounds of one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

Compound No.	Size (nm)	PDI	EE (%)
161*	61.8	0.17	97.8
162*	73.1	0.23	98.4
163*	88.6	0.23	98.1
164*	74.4	0.15	97.7
165*	107.8	0.22	97.0
166*	59.4	0.24	97.1
167*	64.5	0.22	97.6
168*	60.0	0.17	97.3
169*	72.5	0.24	97.6
4**	99.2	0.19	98.6
MC3#	109.0	0.22	93.0

*=Formulated with lipid:Chol:DOPE:PEG 1 40:38.5:20:1.5

**=Formulated with lipid:Chol:DOPE:PEG 1 30:38.5:30:1.5

#=Formulated with lipid:Chol:PEG 1 50:47.5:2.5

Table If Characteristics of nanoparticle compositions including compounds of one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

Compound No.	Size (nm)	PDI	EE (%)
181*	129.9	0.25	97.7

182*	97.7	0.28	98.8
183*	128.9	Multimodal	99.1
184*	107.6	Multimodal	99.1
185*	98.4	0.27	98.6
186*	111.5	0.21	98.2
187*	126.0	0.20	98.5
188*	78.0	0.12	97.5
189*	70.1	0.16	98.3
4**	111.5	0.21	98.8
MC3#	129.7	0.26	92.5

*=Formulated with lipid:Chol:DOPE:PEG 1 40:38.5:20:1.5

**=Formulated with lipid:Chol:DOPE:PEG 1 30:38.5:30:1.5

#=Formulated with lipid:Chol:PEG 1 50:47.5:2.5

Table lg. Purity of nanoparticle compositions including compounds of one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) after storage in EtOH for 96h at different temperatures.

	Lipid % change purity in EtOH, 25°C, 96h	**Lipid % change purity in EtOH, 37°C, 96h**
86	8.1	20.8
111	6.3	6.5
91	1	2.3
90	0.1	9.6
89	2.3	3.9
94	5.9	8.3
93	0	2.2
92	4.5	9
109	0	4.1
114	2.6	9
102	14.8	31.5
115	43.1	59.5
107	4	3.6
116	2.2	2
117	2.9	4.7
120	0.8	2.9
121	0.3	1.5
122	1	4.1
123	1.6	3.1
103	1.5	5.7
124	-4.6	-3.5
125	0	0.8
126	0.6	2.2
127	2.7	2.9
150	25.9	52.5
128	6.9	12.2

151	-0.7	2.1
152	0	2
143	1.10	1.80
144	5.06	7.63
129	8.46	15.47
130	3.54	4.46
131	11.96	12.43
132	1.84	5.54
133	-0.29	1.68
134	1.60	11.81
135	2.26	4.09
136	-0.71	0.77
137	0.36	1.17
139	0.67	1.49

Table lh. Purity of nanoparticle compositions including compounds of one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(Idl O), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) at the beginning of the study, at 25°C, and after storage for 65h at 25°C or 37°C.

Purity %			
Compound	**25°C no shaking**		**37°C shaking**
	t=0h	**t=65h**	**t=65h**
4	98	99	99
44	90	92	91

Example 4: Expression of Luc induced by sample formulations

[001096] The efficacy of the nanoparticle compositions presented in Tables 1, la, 17-1, 19-1, 20-1 and 21-1 was evaluated with a bioluminescence study. Formulations were administered intravenously to mice (n = 6) at a dosage of 0.5 mg/kg (mpk) and bioluminescence measured at 3, 6, and 24 hour time points. The standard MC3 formulation and a PBS control were evaluated for comparison.

[001097] As is evident in Table 2, the total flux for the compositions presented therein was generally comparable at 3 and 6 hours. The total flux after 24 hours was generally lower than that at earlier time points. Amongst the compositions of Table 2, compositions including Compounds 18, 23, and 30 displayed the highest flux after 3 hours. Of the compositions of Table 2, compositions including Compounds 36 and 37 displayed the lowest flux after 24 hours. In general, these results suggest that the compounds described herein may be useful in transfection applications.

Table 2. Expression of luciferase induced by administration of nanoparticle compositions including compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

	Total Flux		
Compound No.	**3 hours**	**6 hours**	**24 hours**
2	6.01E+09	3.23E+09	3.23E+09
3	3.75E+08	1.12E+09	n.d.
11	1.23E+10	3.81E+09	8.20E+08
12	1.06E+10	1.38E+10	6.03E+08
13	3.59E+09	3.80E+09	3.11E+08
14	9.86E+08	1.56E+09	1.02E+08
17	7.55E+09	2.49E+09	7.61E+08
18	2.13E+10	1.76E+10	7.00E+08
19	1.06E+10	6.52E+09	2.65E+09
20	1.00E+11	1.11E+11	4.60E+09
21	1.13E+10	1.08E+10	1.18E+08
23	2.33E+10	3.40E+10	1.06E+09
25	1.06E+10	1.08E+10	2.72E+08
26	1.65E+07	1.04E+07	2.75E+06
27	4.56E+09	4.70E+09	1.36E+08
28	6.18E+09	7.28E+09	4.02E+08
29	1.22E+08	2.51E+08	2.69E+07
30	2.87E+10	1.59E+10	1.95E+09
31	1.43E+10	1.42E+10	3.07E+08
32	6.85E+08	5.88E+08	4.37E+07
33	1.64E+09	4.71E+09	1.54E+08
34	7.77E+06	1.88E+07	2.19E+06
36	6.90E+05	3.93E+05	1.68E+05
37	1.19E+07	6.66E+06	9.38E+05
40	1.24E+08	1.07E+07	5.62E+06
41	4.06E+07	2.04E+07	6.05E+07

	Total Flux		
Compound No.	**3 hours**	**6 hours**	**24 hours**
42	n.d	4.99E+10	n.d
43	n.d	4.54E+09	n.d
44	n.d	1.07E+10	n.d
45	n.d	7.86E+10	n.d
46	n.d	5.26E+09	n.d
47	n.d	2.64E+09	n.d
48	n.d	1.05E+08	n.d
49	n.d	5.67E+10	n.d
50	n.d	1.48E+08	n.d
51	n.d	6.70E+10	n.d
52	n.d	9.85E+10	n.d
MC3	1.63E+10	1.73E+10	1.16E+09

n.d.=not determined

Example 5: Expression of Luc induced by sample formulations in different organs

[001098] The efficacy of the nanoparticle compositions presented in Tables 1, 1a, 17-1, 19-1, 20-1 and 21-1 was further evaluated by measuring the expression of modified luciferase in the liver, lung, spleen, and femur upon administration of a given composition. Formulations were administered intravenously to mice (n = 3) at a dosage of 0.5 mpk and bioluminescence measured after 6 hours. The standard MC3 formulation and a PBS control were also tested.

[001099] As is evident in Table 3, expression was highest in the liver for all formulations of Table 3. Of the compositions of Table 3, the highest total flux was measured for compositions including Compound 20. Lung and spleen expression were generally comparable for compounds of Table 3, while expression in the femur, where measured, was somewhat lower.

Table 3. Expression of luciferase in various organs 6 hours after administration of nanoparticle compositions including compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(Idl O), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

	Total Flux			
Compound No.	**Liver**	**Lung**	**Spleen**	**Femur**
2	3.33E+08	4.76E+05	1.14E+07	n.d.

	Total Flux			
Compound No.	**Liver**	**Lung**	**Spleen**	**Femur**
3	1.36E+08	8.47E+05	9.51E+05	n.d.
11	1.67E+09	2.21E+06	7.21E+06	1.47E+06
12	1.05E+09	6.76E+06	1.11E+07	n.d.
13	6.10E+08	2.89E+06	3.63E+07	1.99E+06
14	2.62E+08	3.56E+06	1.46E+07	n.d.
17	4.26E+08	7.26E+05	3.20E+06	6.71E+05
18	3.91E+09	1.87E+07	1.60E+07	3.31E+06
19	1.89E+09	2.27E+06	8.28E+06	2.75E+06
20	1.42E+10	1.46E+08	6.11E+07	8.91E+06
21	1.24E+09	2.51E+06	1.17E+07	n.d.
23	4.94E+09	1.51E+07	2.95E+07	3.17E+06
25	2.68E+09	5.88E+06	6.00E+06	n.d.
26	2.35E+06	3.49E+04	2.30E+05	n.d.
27	7.84E+08	3.56E+06	3.34E+06	n.d.
28	8.10E+08	5.73E+06	5.67E+06	1.16E+06
29	2.27E+07	4.70E+05	2.97E+06	n.d.
30	2.42E+09	1.61E+07	7.18E+06	2.22E+06
31	1.54E+09	9.81E+06	1.28E+07	9.89E+05
32	8.36E+07	6.75E+05	9.38E+05	1.02E+05
33	6.15E+08	2.84E+06	4.82E+06	1.18E+06
34	2.79E+06	5.63E+04	1.22E+06	n.d.
36	5.85E+04	2.74E+04	1.24E+05	n.d.
37	1.92E+06	6.90E+05	9.75E+05	n.d.
40	1.33E+06	1.42E+05	5.68E+05	n.d.
41	3.00E+06	1.34E+05	2.13E+06	n.d.
42	5.53E+09	n.d	2.29E+08	n.d
43	2.60E+08	n.d	4.52E+07	n.d
44	1.11E+09	n.d	1.19E+08	n.d
45	7.87E+09	n.d	1.70E+08	n.d
46	3.84E+08	n.d	4.35E+07	n.d

	Total Flux			
Compound No.	**Liver**	**Lung**	**Spleen**	**Femur**
47	4.95E+08	n.d	1.42E+08	n.d
48	1.04E+07	n.d	1.50E+07	n.d
49	1.21E+10	n.d	6.65E+07	n.d
50	2.14E+07	n.d	1.94E+05	n.d
51	3.55E+09	n.d	2.24E+07	n.d
52	1.18E+10	n.d	8.74E+08	n.d
IV	9.15E+08	n.d	6.15E+08	n.d
MC3	2.31E+09	8.61E+06	1.95E+07	3.08E+06

n.d.=not determined

Example 6: Cytokine production induced by sample formulations

[001100] The introduction of foreign material into a mammalian body induces an innate immune response that promotes cytokine production. Such immune responses to, for example, nanoparticle compositions including therapeutic and/or prophylactic agents, are undesirable. The induction of certain cytokines is thus measured to evaluate the efficacy of nanoparticle compositions. The concentrations of various cytokines in mice upon intravenous administration of nanoparticle compositions presented in Tables 1, la, lb, and lcat a dosage of 0.5 mpk was measured at 6 hours. The standard MC3 formulation and a PBS control were also tested.

[001101] As is evident in Table 4, IP-10 expression was lower than IL-6 expression for compositions of Table 4. Of the compositions of Table 4, compositions including Compound 13 induced the highest expression of both IL-6 and IP-10, while compositions including Compound 3 induced the lowest IL-6 expression and those including Compound 36 induced the lowest IP-10 expression.

Table 4. Cytokine induction 6 hours after administration of nanoparticle compositions including compounds according to one of formulae (I), (Ial)-(Ial O), (lb), (Ic), (Id), (Idl)-(Idl O), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

Compound No.	**IL-6**	**IP-10**
2	n.d.	3365
3	7	341
11	250	3305

Compound No.	IL-6	IP-10
12	31	2382
13	301	7266
14	17	209
17	164	545
18	263	655
19	70	2326
20	127	2080
21	48	652
23	259	3702
25	131	1823
26	17	175
27	42	2564
28	73	5364
29	108	3454
30	300	4235
31	188	2513
32	174	727
33	37	1577
34	28	159
36	41	118
37	n.d.	198
40	134	919
41	116	350
MC3	92	438

n.d.=not determined

Example 7: Expression of hEPO induced by sample formulations

[001102] Formulations were prepared according to Table 5 and included mRNA encoding hEPO.

Table 5. Characteristics of nanoparticle compositions including compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

Compound No.	Composition (mol %)	Components	Size (nm)	PDI	EE (%)	pKa	Conc. (ug/ml)
12	40:20:38.5:1.5	Lipid:DOPE:Chol: PEG-DMG	87.5	0.13	93.79	5.444	320.14
14	40:20:38.5:1.5	Lipid:DOPE:Chol: PEG-DMG	76.8	0.14	98.91	6.308	603.76
15	40:20:38.5:1.5	Lipid:DOPE:Chol: PEG-DMG	100.3	0.12	90.15	6.323	713.00
16	40:20:38.5:1.5	Lipid:DOPE:Chol: PEG-DMG	77.4	0.14	98.22	6.282	665.11
20	40:20:38.5:1.5	Lipid:DOPE:Chol: PEG-DMG	114.5	0.14	94.39	n.d.	1264.28
22	40:20:38.5:1.5	Lipid:DOPE:Chol: PEG-DMG	75.4	0.13	98.29	6.153	564.97
23	40:20:38.5:1.5	Lipid:DOPE:Chol: PEG-DMG	98.5	0.16	77.19	6.070	438.20
23	50:10:38.5:1.5	Lipid:DSPC:Chol: PEG-DMG	95.2	0.11	51.46	6.164	454.58
MC3	50:10:38.5:1.5	Lipid:DSPC:Chol: PEG-DMG	76.5	0.11	97.37	n.d.	470.45

n.d.=not determined

[001103] Formulations were administered intravenously, subcutaneously or intramuscularly to rats (n=3 or 6) at a dosage of 0.2 mg/kg or 0.5 mg/kg (mpk) and hEPO levels measured at 3, 6, and 24 hour time points. After the 48 hour time point, livers and spleens were harvested and frozen. As is evident in Table 6, compositions including MC3 yielded the highest hEPO expression at each time point, while compositions including Compound 16 yielded the lowest hEPO expression at each time point.

Table 6. Expression of hEPO induced by intravenous administration of nanoparticle compositions including compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

	hEPO (pg/mL)		
Compound No.	**3 hours**	**6 hours**	**24 hours**
12	592260	424740	165404
14	280973	158520	58805
15	103773	125320	67965
16	35387	41720	17184
20	n.d.	227420	n.d.
22	181627	267680	75571
23 (DOPE)	249213	275440	120104
23 (DSPC)	86627	71360	29008
MC3	1407947	1036013	436243

n.d.=not determined

[001104] As shown in Table 7a, hEPO expression in mice was substantially higher for compositions including Compound 12 than those including MC3. In contrast, hEPO expression in rats induced by administration of a nanoparticle composition including a compound according to one of formulae (I), (Ial)-(Ia6), (lb), (II), (Ila), (III), and (Ilia) was substantially lower than that measured for MC3. Luc expression in mice was several fold higher for Compound 23 than for MC3 but significantly lower for Compounds 12 and 14. Table 7b shows the Luc expressions in CD-I mice vs. LDLr-/- mice. Tables 7c and 7d show additional protein expressions and clearance data from compositions with various compounds disclosed herein as compared to with MC3. Tables 7e, 7f and 7g show hEPO expession data in CD-I mice at a dose of 0.5 mpk. Similar results were achieved with different strains of mice, e.g. chimeric mice with humanized livers (PXB) or immunodefficient mice (SCID).

Table 7a. Comparison of expression induced by administration of nanoparticle compositions including MC3 or compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

Compound No.	Lipid/MC3 Luc CD-I (mice)	Lipid/MC3 hEPO CD-I (mice)	Lipid/MC3 hEPO S.D. (rats)	%Dose remaining 6 h CD-I mice	%Dose remaining 24 h CD-I mice
11	1.03	n.d.	n.d.		
12	0.21	2.3	0.32		
14	0.070	n.d.	0.12		
15	n.d.	n.d.	0.095		
16	n.d.	n.d.	0.031		
20	5.0	n.d.	n.d.		
22	n.d.	n.d.	0.20		
23 (DOPE)	5.2	2.4	0.21		
42	3.99	n.d.	n.d.	58	53
43	0.34			69	64
44	0.82	n.d.	n.d.	<1	<1
45	6.50	n.d.	n.d.	33	24
46	0.46			1	<1
47	0.22			1	<1
48	0.01			<1	<1
49	5.23	n.d.	n.d.	49	40
50	0.01			56	47
51	5.22	n.d.	n.d.	54	41
52	7.46	n.d.	n.d.	46	40
MC3	1	1	1	88	55

n.d.=not determined

Table 7b. Comparison of Luc expression induced by intravenous administration of nanoparticle compositions including MC3 or compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) in CD-I mice and LDLr-/- mice.

Compound No.	Lipid/MC3 AUC CD-I mice 0.5 mpk	Lipid/KL22 AUC LDLr-/- mice 0.5 mpk, LDLr	LDL levels lipid/untreated LDLr-/- mice 0.5 mpk, LDLr
4	3.70	2.03	0.54

5	2.62	1.86	0.47
6	1.72	0.37	0.91
7	1.51	2.46	0.63
8	2.33	3.74	0.66
9	0.73	0.58	0.87
10	1.14	0.71	0.98
MC3	1	0.15	0.55
KL22		1	0.51

Table 7c. Comparison of expression induced by intravenous administration of nanoparticle compositions including MC3 or compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

Compound No.	AUC (p/s*h)	Lipid/MC3 AUC	Liver/Spleen Ratio	% Dose Remaining 6hr	% Dose Remaining 24hr
53	9.22 E+09	0.07	17	1.35	0.77
54	1.89 E+10	0.13	32	5.18	4.77
55	2.00 E+10	0.14	9	2.58	1.60
56	1.77 E+11	1.25	29	1.21	0.16
57	1.21 E+11	0.85	15	0.88	0.24
58	1.38 E+11	0.97	11	1.37	0.61
59	1.19 E+11	0.84	5	6.99	5.03
60	2.84 E+11	2.00	15	21.18	15.98
61	4.65 E+11	3.27	30	1.31	0.13
71	1.77 E+11	1.25	25	12.39	9.25
72	6.53 E+10	0.46	6	7.06	6.40
MC3	1.42 E+11	-	55	55.70	55.43

Table 7d. Comparison of hEPO expression in S.D. rats induced by intravenous administration of nanoparticle compositions including MC3 or compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

Compound No	AUC (0.1 mpk)	AUC/MC3 0.1 mpk	AUC (1 mpk)	AUC/MC3 1 mpk	% dose remaining Rat liver (48 h)	% dose remaining Mouse liver (24 h)
4	2.12 E+06	1.3	6.79 E+07	0.58	37.82	N.A.
45	1.45 E+06	0.90	2.00 E+08	1.7	8.57	23.7
49	5.98 E +06	3.7	1.44 E+08	1.2	38.45	40.3
MC3	1.62 E +06	-	1.17 E+08	-	43.11	55

Table 7e. Comparison of hEPO expression in CD-I mice induced by intravenous administration of nanoparticle compositions including MC3 or compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) at a dose of 0.5 mpk.

Compound No.	AUC (p/s*h) CD-1 mice 0.5 mpk	Lipid/MC3 AUC
62	3.46 E+7	0.97
69	2.33 E+8	6.52
70	6.34 E+7	1.77
73	1.41 E+8	3.93
80	6.24 E+7	1.74
81	1.08 E+8	3.01
82	1.29 E+8	3.62
83	5.21 E+7	1.46
84	5.10 E+7	1.43
85	1.27 E+8	3.54
86	1.75 E+7	0.49
87	2.86 E+7	0.80
45	6.98E+07	2.02
49	1.59E+08	4.59
52	7.78E+07	2.25

56	4.52E+07	1.31
60	4.86E+07	1.40
61	6.29E+07	1.82
71	2.48E+07	0.72
110	1.30E+08	3.44
111	1.75E+08	4.64
112	1.78E+04	0.00
91	3.22E+07	0.85
90	2.08E+07	0.55
89	1.36E+08	3.62
94	2.39E+07	0.63
93	8.63E+07	2.29
92	8.25E+07	2.19
109	5.56E+07	1.48
114	7.86E+07	2.09
102	6.34E+06	0.17
115	2.31E+06	0.06
107	1.76E+08	4.66
116	1.01E+08	2.68
117	6.91E+07	1.83
118	1.64E+08	4.35
103	1.82E+08	4.84
4	2.26E+08	6.00
MC3	3.77E+07	
120	4.17E+07	0.99
121	2.84E+07	0.67
122	5.71E+07	1.35
123	5.84E+07	1.38
124	3.88E+07	0.92
125	7.89E+06	0.19
126	6.24E+07	1.48

127	3.45E+07	0.82
150	2.29E+05	0.01
128	3.04E+05	0.01
151	1.54E+08	3.64
152	1.09E+08	2.57
4	1.88E+08	4.46
MC3	4.23E+07	-

Table 7f. Comparison of hEPO expression in CD-I mice induced by intramuscular administration of nanoparticle compositions including MC3 or Compound 4 at a dose of 0.01 mg/kg hEPO

Compound No.	**AUC (p/s*h) CD-1 mice 0.5 mpk**	**Lipid/MC3 AUC**
MC3	27727	
4	366551	13.2

Table 7g. Comparison of hEPO expression in CD-I mice induced by subcutaneous administration of nanoparticle compositions including MC3 or Compound 4 at a dose of 0.5 mg/kg hEPO

Compound No.	**AUC (p/s*h) CD-1 mice 0.5 mpk**	**Lipid/MC3 AUC**
MC3	375994	
4	859874	2.29

[001105] The amount of lipid in the liver and spleen 48 hours after administration of a nanoparticle composition was also measured. As shown in Table 8, less than 6% of doses including Compounds 14, 15, and 16 remained in the liver after 48 hours. In contrast, approximately 60% of doses including MC3 or Compound 22 remained in the liver after 48 hours. Less than 3% of the dose remained in the spleen for each composition tested.

Table 8. Lipids levels in the liver and spleen following administration of nanoparticle compositions including MC3 or compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

Compound No.	**Lipid in liver (ng/g)**	**% remaining dose in liver**	**Lipid in spleen (ng/g)**	**% remaining dose in spleen**
4	21950	38	5345	0.61
12	16850	23.8	2325	0.22
14	3990	5.54	1620	0.15
15	3070	4.22	971	0.089
16	597	0.79	293	0.026
22	36800	58.7	3887	0.41
23 (DOPE)	32900	51.4	26100	2.72
MC3	21750	51	2785	0.44

Table 8a. Lipids levels in the liver following intravenous administration of nanoparticle compositions including Compounds of the disclosure (0.5 mg/kg dose, CD-I mouse).

Compound No.	% Dose Remaining 6 hr	% Dose Remaining 24 hr
4		60.36
62	16.99	14.36
69	59.21	52.03
70	56.30	57.20
73	68.53	64.75
80	23.78	25.25
81	15.28	19.27
82	37.85	38.66
83	31.32	30.54
84	21.23	20.86
85	52.40	53.65
86	0.16	0.12
87	0.07	0.05
110	N.T.	59.33
111	N.T.	59.89
112	N.T.	46.37

91	N.T.	9.19
90	N.T.	3.93
89	N.T.	0.05
94	N.T.	18.52
93	N.T.	10.37
92	N.T.	0.10
109	N.T.	0.54
114	N.T.	38.43
102	N.T.	-
115	N.T.	82.18
107	N.T.	38.15
116	N.T.	6.22
117	N.T.	2.03
118	N.T.	55.92
103	N.T.	71.45
120	N.T.	9.32
121	N.T.	13.97
122	N.T.	0.05
123	N.T.	3.75
124	N.T.	5.60
125	N.T.	2.74
126	N.T.	0.09
127	N.T.	0.86
150	N.T.	57.89
128	N.T.	52.19
151	N.T.	75.96
152	N.T.	34.01
143	N.T.	38.63
144	N.T.	3.25
129	N.T.	9.10
130	N.T.	2.33
131	N.T.	80.27

132	N.T.	0.81
133	N.T.	13.02
134	N.T.	33.71
135	N.T.	0.16
136	N.T.	56.18
137	N.T.	0.26
139	N.T.	0.29
MC3	N.T.	64.65

Table 8b. Lipids levels in the liver following administration of nanoparticle compositions including MC3 or compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (le), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), and mRNA expressing hEPO (miR126 + miR142).

Compound No.	AUC (pg/mL*h)	Lipid/MC3 AUC	% Dose Remaining (liver, 24h)
146	1.27E+07	0.71	81.04
140	4.55E+07	2.56	1.66
141	2.70E+07	1.52	1.41
155	8.89E+07	5.00	78.61
157	2.08E+08	11.71	42.48
153	5.62E+07	3.16	84.71
158	1.03E+07	0.58	0.30
154	1.37E+08	7.69	78.44
159	6.56E+07	3.69	NT
160	2.19E+05	0.01	60.54
4	6.70E+07	3.77	39.23

Table 8c. Lipids levels in the liver following administration of nanoparticle compositions including MC3 or compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (le), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), and mRNA expressing hEPO (miR126 + miR142).

Compound No.	AUC (pg/mL*h)	Lipid/MC3 AUC	% Dose Remaining (liver, 24h)
161	1.28E+05	0.01	113.64
162	2.30E+07	1.51	63.12
163	1.01E+07	0.67	65.12
164	4.09E+06	0.27	40.18
165	9.38E+07	6.17	0.13
166	O.OOE+00	0.00	75.26

167	O.OOE+00	0.00	89.60
168	O.OOE+00	0.00	2.74
169	O.OOE+00	0.00	0.30
4	8.47E+07	5.57	37.05
MC3	1.52E+07	1.00	58.41

Table 8d. Lipids levels in the liver following administration of nanoparticle compositions including MC3 or compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (le), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), and mRNA expressing hEPO (miR126 + miR142).

Compound No.	**AUC (pg/mL*h)**	**Lipid/MC3 AUC**	**% Dose Remaining (liver, 24h)**
181	1.69E+08	0.24	2.51
182	6.06E+07	0.09	1.64
183	6.53E+07	0.09	1.87
184	4.21E+08	0.61	2.23
185	2.23E+07	0.03	18.21
186	2.19E+07	0.03	49.08
187	5.22E+07	0.08	42.86
188	8.76E+06	0.01	117.31
189	1.15E+07	0.02	91.63
4	1.83E+09	2.63	43.02
MC3	6.95E+08	1.00	54.04

Table 8e. Lipids levels in the liver following administration of nanoparticle compositions including MC3 or compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (le), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), and mRNA expressing hEPO (miR126 + miR142). The nanopraticles were formulated with lipid:Chol:PEG 1 50:47.5:2.5.

Lipid	**AUC (mIU/mL*h)**	**Lipid/MC3 AUC**	**% Dose Remaining (liver, 24h)**
170	5.50E+07	2.45	13.50
171	1.11E+08	4.93	4.67
173	6.70E+06	0.30	8.55
174	5.03E+07	2.24	30.05
175	5.02E+07	2.24	13.66
177	6.84E+07	3.05	35.91
178	8.44E+07	3.76	44.93
179	7.79E+06	0.35	26.22
180	6.53E+07	2.91	50.47
4	2.76E+08	12.31	
MC3	2.24E+07	1.00	

Example 8: Optimization of lipid:therapeutic agent ratios

[001106] The relative amounts of lipid component and therapeutic and/or prophylactic agent in a nanoparticle composition can be optimized according to considerations of efficacy and tolerability. For compositions including an RNA as a therapeutic and/or prophylactic agent, the N:P ratio can serves as a useful metric.

[001107] As the N:P ratio of a nanoparticle composition controls both expression and tolerability, nanoparticle compositions with low N:P ratios and strong expression are desirable. N:P ratios vary according to the ratio of lipids to RNA in a nanoparticle composition. Thus, the wt/wt ratio of total lipid to RNA is varied between 10: 1, 15:1, 20: 1, 32: 1, 40: 1, 50: 1, and 60: 1 for a lipid formulation including about 50 mol % of a compound according to one of formulae (I), (Ial)-(Ial O), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia), about 10 mol % phospholipid (e.g., DOPE or DSPC), about 38.5 mol % structural lipid (e.g., cholesterol), and about 1.5 mol % PEG lipid (e.g., PEG-DMG). N:P ratios are calculated for each nanoparticle composition assuming a single protonated nitrogen atom. The encapsulation efficiency (EE), size, and polydispersity index of each composition are also measured.

[001108] Generally, compositions with higher total lipid:RNA ratios yield smaller particles with higher encapsulation efficiencies, both of which are desirable. However, the N:P ratio for such formulations generally exceeds 4. Current standards in the art such as the MC3 formulation described above have N:P ratios of 5.67. Thus, a balance between the N:P ratio, size, and encapsulation efficiency should be struck.

[001109] In order to explore the efficacy of nanoparticle compositions with different N:P ratios, the expression of luciferase (Luc) or human erythropoietin (hEPO) in mice after low (0.05 mg/kg) or high (0.5 mg/kg) doses of intravenously administered nanoparticle compositions is examined. The concentration of Luc or hEPO expressed is measured 3, 6, and/or 24 hours after administration.

Example 9: Optimization of content of a composition comprising a compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia)

[001110] As smaller particles with higher encapsulation efficiencies are generally desirable, the relative amounts of various elements in lipid components of nanoparticle compositions are optimized according to these parameters.

[001111] A compound according to one of formulae (I), (Ial)-(Ial O), (lb), (Ic), (Id), (Idl)-(Idl O), (le), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) is selected for optimization. The relative amount of the compound according to one of formulae (I), (Ial)-(Ial O), (lb), (Ic), (Id), (Idl)-(Idl O), (le), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) is varied between 30 mol % and 60 mol % in compositions including DOPE or DSPC as phospholipids to determine the optimal amount of the compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (le), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) in the formulations. Formulations are prepared using a standardized process with a water to ethanol ratio in the lipid-mRNA solution of 3:1 and a rate of injection of the lipid solution into the mRNA solution of 12 mL/min on a NanoAssemblr microfluidic based system. This method induces nano-precipitation and particle formation. Alternative processes including, but not limited to, T-junction or direct injection, may also be used to achieve the same nano-precipitation.

[001112] Formulations producing the smallest particles with the highest encapsulation efficiencies are generally preferred, however larger or smaller particle sizes may be desirable based on a given application (e.g., based on the fenestration size of a target organ). Compositions are also evaluated for their Luc or hEPO expression levels and cytokine profiles.

Example 10: Optimization of phospholipid

[001113] The relative amount of phospholipid in a lipid component of a nanoparticle composition is varied to further optimize the formulation. A compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(Idl O), (le), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) is selected for use in the nanoparticle composition and DOPE and DSPC are selected as phospholipids. Additional phospholipids can also be evaluated. Nanoparticle compositions are prepared with the relative phospholipid content varying between 0 mol % and 30 mol %. Compositions are evaluated for their size, encapsulation efficiency, Luc or hEPO expression levels, and cytokine profiles.

Example 11: Optimization of structural lipid

[001114] The relative amount of structural lipid in a lipid component of a nanoparticle composition is varied to further optimize the formulation. A compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(Idl O), (le), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) is selected for use in the nanoparticle composition and cholesterol is selected as a structural lipid. Additional structural lipids can also be evaluated. Nanoparticle compositions are

prepared with the relative structural lipid content varying between 18.5 mol % and 48.5 mol %. Compositions are evaluated for their size, encapsulation efficiency, Luc or hEPO expression levels, and cytokine profiles.

Example 12: Optimization of PEG lipid

[001115] The relative amount of PEG lipid in a lipid component of a nanoparticle composition is varied to further optimize the formulation. A compound according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) is selected for use in the nanoparticle composition and PEG-DMG is selected as a PEG lipid. Additional PEG lipids can also be evaluated. Nanoparticle compositions are prepared with the relative PEG lipid content varying between 0 mol % and 10 mol %. Compositions are evaluated for their size, encapsulation efficiency, Luc or hEPO expression levels, and cytokine profiles.

[001116] Exemplary formulations useful in the optimization of nanoparticle composition formulations are presented in Tables 9 and 9a.

Table 9. Exemplary formulations including compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

Composition (mol %)	**Components**
40:20:38.5:1.5	Compound:Phospholipid:Chol:PEG-DMG
45:15:38.5:1.5	Compound:Phospholipid:Chol:PEG-DMG
50:10:38.5:1.5	Compound:Phospholipid:Chol:PEG-DMG
55:5:38.5:1.5	Compound:Phospholipid:Chol:PEG-DMG
60:5:33.5:1.5	Compound:Phospholipid:Chol:PEG-DMG
45:20:33.5:1.5	Compound:Phospholipid:Chol:PEG-DMG
50:20:28.5:1.5	Compound:Phospholipid:Chol:PEG-DMG
55:20:23.5:1.5	Compound:Phospholipid:Chol:PEG-DMG
60:20:18.5:1.5	Compound:Phospholipid:Chol:PEG-DMG
40:15:43.5:1.5	Compound:Phospholipid:Chol:PEG-DMG
50:15:33.5:1.5	Compound:Phospholipid:Chol:PEG-DMG
55:15:28.5:1.5	Compound:Phospholipid:Chol:PEG-DMG
60:15:23.5:1.5	Compound:Phospholipid:Chol:PEG-DMG
40:10:48.5:1.5	Compound:Phospholipid:Chol:PEG-DMG
45:10:43.5:1.5	Compound:Phospholipid:Chol:PEG-DMG
55:10:33.5:1.5	Compound:Phospholipid:Chol:PEG-DMG

Composition (mol %)	Components
60:10:28.5:1.5	Compound: Phospholipid: Choi :PEG-DMG
40:5:53.5:1.5	Compound: Phospholipid: Choi :PEG-DMG
45:5:48.5:1.5	Compound: Phospholipid: Choi :PEG-DMG
50:5:43.5:1.5	Compound: Phospholipid: Choi :PEG-DMG
40:20:40:0	Compound: Phospholipid: Choi :PEG-DMG
45:20:35:0	Compound: Phospholipid: Choi :PEG-DMG
50:20:30:0	Compound: Phospholipid: Choi :PEG-DMG
55:20:25:0	Compound: Phospholipid: Choi :PEG-DMG
60:20:20:0	Compound: Phospholipid: Choi :PEG-DMG
40:15:45:0	Compound: Phospholipid: Choi :PEG-DMG
45:15:40:0	Compound: Phospholipid: Choi :PEG-DMG
50:15:35:0	Compound: Phospholipid: Choi :PEG-DMG
55:15:30:0	Compound: Phospholipid: Choi :PEG-DMG
60:15:25:0	Compound: Phospholipid: Choi :PEG-DMG
40:10:50:0	Compound: Phospholipid: Choi :PEG-DMG
45:10:45:0	Compound: Phospholipid: Choi :PEG-DMG
50:0:48.5:1.5	Compound: Phospholipid: Choi :PEG-DMG
50:10:40:0	Compound: Phospholipid: Choi :PEG-DMG
55:10:35:0	Compound: Phospholipid: Choi :PEG-DMG
60:10:30:0	Compound: Phospholipid: Choi :PEG-DMG

Table 9a. Exemplary formulations including compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia).

Composition (mol %)	Components
40:20:38.5:1.5	Compound:Phospholipid:Chol:PEG 1
45:15:38.5:1.5	Compound:Phospholipid:Chol:PEG 1
50:10:38.5:1.5	Compound:Phospholipid:Chol:PEG 1
55:5:38.5:1.5	Compound:Phospholipid:Chol:PEG 1
60:5:33.5:1.5	Compound:Phospholipid:Chol:PEG 1
45:20:33.5:1.5	Compound:Phospholipid:Chol:PEG 1
50:20:28.5:1.5	Compound:Phospholipid:Chol:PEG 1

Composition (mol %)	Components
55:20:23.5:1.5	Compound:Phospholipid:Chol:PEG 1
60:20:18.5:1.5	Compound:Phospholipid:Chol:PEG 1
40:15:43.5:1.5	Compound:Phospholipid:Chol:PEG 1
50:15:33.5:1.5	Compound:Phospholipid:Chol:PEG 1
55:15:28.5:1.5	Compound:Phospholipid:Chol:PEG 1
60:15:23.5:1.5	Compound:Phospholipid:Chol:PEG 1
40:10:48.5:1.5	Compound:Phospholipid:Chol:PEG 1
45:10:43.5:1.5	Compound:Phospholipid:Chol:PEG 1
55:10:33.5:1.5	Compound:Phospholipid:Chol:PEG 1
60:10:28.5:1.5	Compound:Phospholipid:Chol:PEG 1
40:5:53.5:1.5	Compound:Phospholipid:Chol:PEG 1
45:5:48.5:1.5	Compound:Phospholipid:Chol:PEG 1
50:5:43.5:1.5	Compound:Phospholipid:Chol:PEG 1
40:20:40:0	Compound:Phospholipid:Chol:PEG 1
45:20:35:0	Compound:Phospholipid:Chol:PEG 1
50:20:30:0	Compound:Phospholipid:Chol:PEG 1
55:20:25:0	Compound:Phospholipid:Chol:PEG 1
60:20:20:0	Compound:Phospholipid:Chol:PEG 1
40:15:45:0	Compound:Phospholipid:Chol:PEG 1
45:15:40:0	Compound:Phospholipid:Chol:PEG 1
50:15:35:0	Compound:Phospholipid:Chol:PEG 1
55:15:30:0	Compound:Phospholipid:Chol:PEG 1
60:15:25:0	Compound:Phospholipid:Chol:PEG 1
40:10:50:0	Compound:Phospholipid:Chol:PEG 1
45:10:45:0	Compound:Phospholipid:Chol:PEG 1
50:0:48.5:1.5	Compound:Phospholipid:Chol:PEG 1
50:10:40:0	Compound:Phospholipid:Chol:PEG 1
55:10:35:0	Compound:Phospholipid:Chol:PEG 1
60:10:30:0	Compound:Phospholipid:Chol:PEG 1
30:30:38.5:1.5	Compound:Phospholipid:Chol:PEG 1
50:37.5:2.5	Compound:Chol:PEG 1

Example 13: Optimization of particle sizes

[001117] The fenestration sizes for different bodily organs often vary; for example, the kidney is known to have a smaller fenestration size than the liver. Thus, targeting delivery of a therapeutic and/or prophylactic agent (e.g., specifically delivering) to a particular organ or group of organs may require the administration of nanoparticle compositions with different particle sizes. In order to investigate this effect, nanoparticle compositions with formulations such as those included in Table 9 or 9a are prepared with a variety of particle sizes using a Nanoassemblr instrument. Nanoparticle compositions include an RNA encoding Luc. Each differently sized nanoparticle composition is subsequently administered to mice to evaluate the effect of particle size on delivery selectivity. Luc expression in two or more organs or groups of organs can be measured using bioluminescence to evaluate the relative expression in each organ.

Example 14: Administration following pretreatment

[001118] Administration of nanoparticle compositions to subjects can result in inflammation, infusion related reactions, and other undesirable effects indicative of low tolerability. These effects can be attributed to undesirable immunoactivity.

[001119] In order to combat negative effects, nanoparticle compositions are co-administered with one or more substances (e.g., co-medications or additional therapeutic and/or prophylactic agents) to subjects. Potentially useful additional therapeutic and/or prophylactic agents include steroids (e.g., corticosteroids), anti-histamines, H1 receptor blockers, H2 receptor blockers, anti-inflammatory compounds, statins, BTK inhibitors, S1P1 agonists, glucocorticoid receptor modulators (GRMs), and estradiols. Non-human primates are pretreated with one or more additional therapeutic agents selected from dexamethasone and acetaminophen. The additional therapeutic agent is administered either 24 hours, 1 hour, or both 24 hours and 1 hour before administration of a nanoparticle composition. Sample protocol are summarized in Table 10. Cytokine profiles, inflammation, and other parameters are measured and compared to evaluate the effectiveness of pretreatment.

Table 10. Sample protocol for pretreatment study.

Group	**Pretreatment Time**	**Additional Therapeutic Agent(s) Administered**
1	None	None
2	24 hours	Dexamethasone

Group	Pretreatment Time	Additional Therapeutic Agent(s) Administered
3	24 hours	Acetaminophen
4	24 hours	Dexamethasone and Acetaminophen
5	1 hour	Dexamethasone
6	1 hour	Acetaminophen
7	1 hour	Dexamethasone and Acetaminophen
8	24 hours and 1 hour	Dexamethasone
9	24 hours and 1 hour	Acetaminophen
10	24 hours and 1 hour	Dexamethasone and Acetaminophen

[001120] For example, a useful therapeutic treatment course may involve administering an additional therapeutic and/or prophylactic agent both the day before and the day of (one hour prior) to administration of a nanoparticle composition at a dose level of 1.3 mpk. Additional therapeutic and/or prophylactic agents can be formulated for delivery by a variety of different routes. For example, dexamethasone may be delivered orally. In general, additional therapeutic and/or prophylactic agents are administered at clinically approved or typical dosage levels.

Example 15: Administration to non-human primates

[001121] The tolerability and efficacy of nanoparticle compositions to non-human primates is evaluated in Cynomolgus monkeys. Monkeys are administered an optimized nanoparticle composition including an mRNA encoding hEPO once weekly for four weeks. The levels of hEPO protein, mRNA, and cytokine profiles are measured using ELISA-based techniques before and 2, 6, 12, 24, 48, 72, and 120 hours after each administration.

[001122] The effects of pretreatment to non-human primates are evaluated using a standard MC3 formulation including an mRNA encoding hEPO. The study design is summarized in Table 11. Male monkeys are administered the nanoparticle composition once weekly for four weeks at a dose rate of 5 ml/kg/h and are pretreated with either methotrexate or dexamethasone.

Table 11. Protocol for pretreatment study in Cynomolgus monkeys.

Group	Test Material	Dose level (mg/kg)	Additional Therapeutic Agent Administered	Dose concentration (mg/ml)	Number of monkeys
1	MC3	0	None	0	3
2	hEPO mRNA in MC3	0.3	None	0.06	3
3	hEPO mRNA in MC3	0.3	Methotrexate	0.06	3
4	hEPO mRNA in MC3	0.3	Dexamethasone	0.06	3

Example 16: Methods of treating diseases and disorders

[001123] A nanoparticle composition formulation having high tolerability (e.g., provoking a low immune response) and efficacy (e.g., facilitating efficient and effective encapsulation of a therapeutic and/or prophylactic agent and delivery of the agent to a desired target) is selected for use. A therapeutic and/or prophylactic agent for formulation with the nanoparticle composition is selected for use based on the condition of a subject. For example, an mRNA encoding a vascular endothelial growth factor A (VEGF-A) may be selected to promote angiogenesis to treat atherosclerotic renovascular disease, while an siRNA capable of knocking down apolipoprotein B (apoB) may be selected to treat a metabolic disease or disorder such as dyslipidemia.

[001124] A subject in need of treatment is pretreated with a small dose of dexamethasone one or more hours prior to treatment with the nanoparticle composition. The nanoparticle composition is preferably administered to the subject intravenously, however intramuscular, intradermal, subcutaneous, intranasal, or inhalation administration routes are also acceptable. Treatment is provided in a dose of about 0.001 mg/kg to about 10 mg/kg of therapeutic and/or prophylactic agent and is repeated daily, weekly, biweekly, or monthly according to needs of the subject.

Example 17: hEPO expression in Cynomolgus monkeys

[001125] Nanoparticle compositions of the disclosure, including an mRNA encoding hEPO and comprising a miR binding site, were administered to Cynomolgus monkeys at a dose of

0.05mg/kg via a 60 min intravenous infusion. The results of the study are summarized in Table 12.

Table 12: hEPO expression in non-human primates after intravenous administration of nanoparticle compositions including compounds according to one of formulae (I), (Ial)-(IalO), (lb), (Ic), (Id), (Idl)-(IdlO), (Ie), (Iel)-(Ie6), (II), (Ila), (III), and (Ilia) .

LNP	hEPO mRNA	C_{max} (ng/mL)		AUC (h*ng/mL)	
		Mean	SD	Mean	SD
MC3/DSPC/PEG-DMG	miR126.3p + miR142.3p	39.9	12.8	1060	328
	+ miR122	5.87	3.99	52	34.9
Compound 4/DOPE/PEG-DMG	miR126.3p + miR142.3p	206	91.8	5630	2580
	+ miR122	41.9	20.1	509	224

[001126] The results summarized in Table 12 indicate that compositions comprising Compound 4 lead to improved levels of hEPO expressions when compared to MC3.

Example 18: Characteristics of nanoparticle compositions formulated with PEG 1.

[001127] Formulations were prepared according to Table 13 and included an mRNA encoding hEPO and the particle size, polydispersity index and encapsulation efficiency were recorded on day 1 (week 1), day 8 (week 2), day 15 (week 3), and day 22 (week 4), of the study.

Table 13. Characteristics of nanoparticle compositions including compounds according to one of formulae (I), (Ial)-(Ia8), (lb), (Ic), (II), (Ila), (III) and (Ilia), formulated with PEG-DMG or PEG 1.

Week	Cmpd. No.	Composition (mol %)	Components	Size (nm)	PDI	EE (%)
1	4	40:20:38.5:1.5	Lipid:DOPE:Chol:PEG-DMG	79.9	0.23	99
		40:20:38.5:1.5	Lipid:DOPE:Chol: PEG 1	101.5	0.22	98
2	4	40:20:38.5:1.5	Lipid:DOPE:Chol:PEG-DMG	79.9	0.21	99
		40:20:38.5:1.5	Lipid:DOPE:Chol: PEG 1	104.1	0.21	97
3	4	40:20:38.5:1.5	Lipid:DOPE:Chol:PEG-DMG	77.3	0.22	99
		40:20:38.5:1.5	Lipid:DOPE:Chol: PEG 1	100.4	0.2	97

4	4	40:20:38.5:1.5	Lipid:DOPE:Chol:PEG-DMG	73.6	0.21	99
		40:20:38.5:1.5	Lipid:DOPE:Chol: PEG 1	101.9	0.2	97

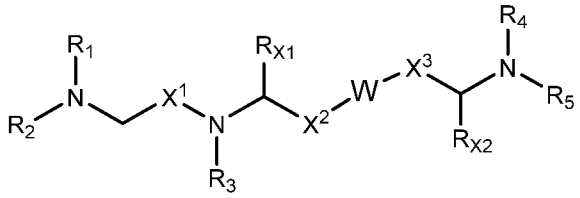
Example 19: hEPO expression and anti-PEG IgM response of nanoparticle compositions.

[001128] Formulations comprising Compound 4:DOPE:Chol:PEG-DMG or Compound 4:DOPE:Chol:PEG 1 at a ratio of 40:20:38.5:1.5 (mole %), and a mRNA encoding hEPO, were administered intravenously to CD-I mice (n=8) for 4 weeks at a once weekly dose of 0.5 mg/kg (mpk). hEPO levels measured at 6 hours after administration and anti-PEG IgM levels were determined after the second and the third dose, 96 hours after administration each time. After the 48 hour time point, livers and spleens were harvested and frozen. The results of this study are summarized in Figures 21 and 22.

It is to be understood that while the compounds and methods of the present disclosure have been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the present disclosure, which is defined by the scope of the appended claims. Other aspects, advantages, and alterations are within the scope of the following claims.

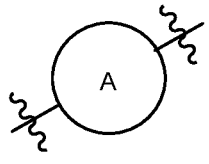
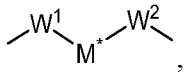
What is claimed is

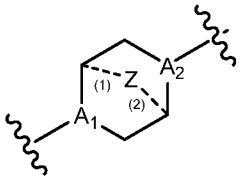
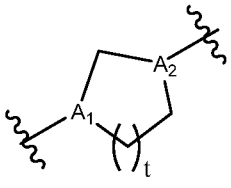
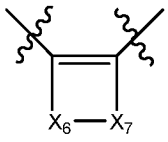
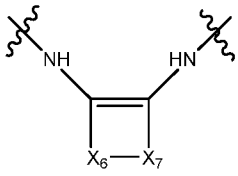
1. A compound having the formula (I):



(I),

or a salt or isomer thereof, wherein

W is  or  ,

ring A is  ,  ,  , or  ;

t is 1 or 2;

A_1 and A_2 are each independently selected from CH or N;

Z is CH_2 or absent wherein when Z is CH_2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

R_1, R_2, R_3, R_4, and R_5 are independently selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

R_{X1} and R_{X2} are each independently H or C1-3 alkyl;

each M is independently selected from the group consisting of -C(0)0-, -OC(O)-, -OC(0)0-, -C(0)N(R')-, -N(R')C(0)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0-, $-S(0)_2-$, -C(0)S-, -SC(O)-, an aryl group, and a heteroaryl group;

M* is -0-, or Ci-Ce alkyl,

W^1 and W^2 are each independently selected from the group consisting of a bond, -O- and $-N(R_6)-$;

each R_6 is independently selected from the group consisting of H and C1-5 alkyl;

X^1, X^2, and X^3 are independently selected from the group consisting of a bond, -CH2-, -(CH $_2$)$_2$-, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, -(CH $_2$)n-C(0)-, -C(0)-(CH $_2$)n-, -(CH$_2$)$_n$-C(0)0-, -OC(0)-(CH $_2$)$_n$-, -(CH$_2$)$_n$-OC(0)-, -C(0)0-(CH $_2$)$_n$-, -CH(OH)-, -C(S)-, and -CH(SH)-;

each $_Y$ is independently a $_{C3-6}$ carbocycle;

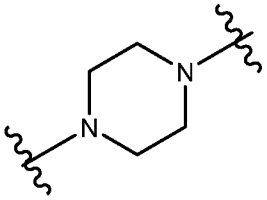
each $_{R*}$ is independently selected from the group consisting of $_{Ci-i2}$ alkyl and $_{C2-i2}$ alkenyl;

each $_R$ is independently selected from the group consisting of $_{C1-3}$ alkyl and a $_{C3-6}$ carbocycle;

each $_{R'}$ is independently selected from the group consisting of $_{C1-18}$ alkyl, $_{C2-i8}$ alkenyl, and H;

each $_{R''}$ is independently selected from the group consisting of $_{C3-i2}$ alkyl, *C3-12* alkenyl and -R*MR' ; and

n is an integer from 1-6;

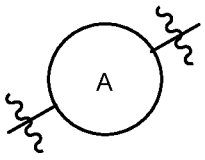


wherein when ring A is , then

i) at least one of X^1, X^2, and X^3 is not -CH_2-; and/or

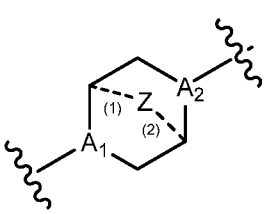
ii) at least one of $_{Ri}$, $_{R2}$, R3, R4, and $_{R5}$ is -R"MR' .

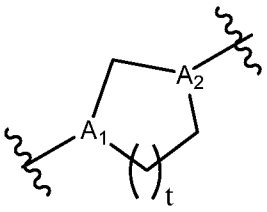
2. The compound of claim 1, wherein M* is $_{C1-C6}$ alkyl and W^1 and W^2 are each independently selected from the group consisting of -O- and -N(R6)-.

3. The compound of claim 1, wherein W is 

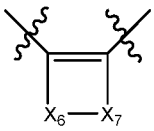
.

4. The compound of any one of the preceding claims, wherein ring A is

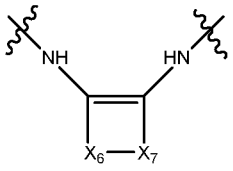


or


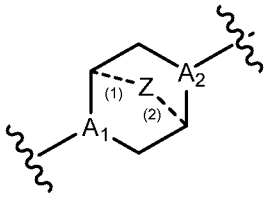
.

5. The compound of any one of the preceding claims, wherein ring A is 

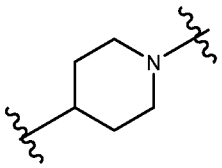
.

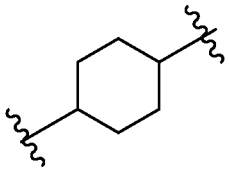
6. The compound of any one of the preceding claims, wherein ring A is 

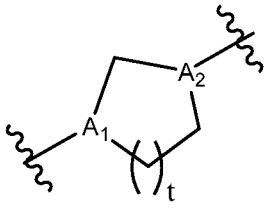
.

7. The compound of claim 3, wherein ring A is 

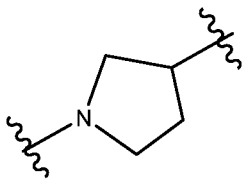
, preferably



or

.

8. The compound of claim 3, wherein ring A is 

, preferably



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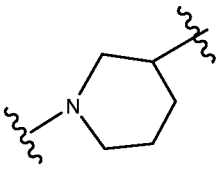
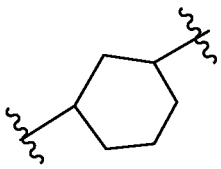
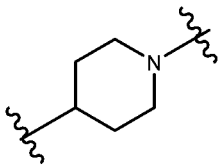
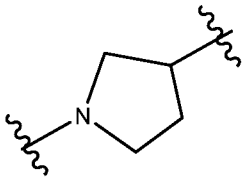
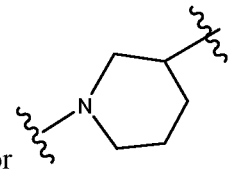


, or

.

9. The compound of claim 3, wherein ring A is  , in which the N atom is connected with X^2.

10. The compound of claim 3, wherein ring A is  or  , in which the N atom is connected with X^2.

11. The compound of any one of the preceding claims, wherein at least one of Ai and A^2 is N.

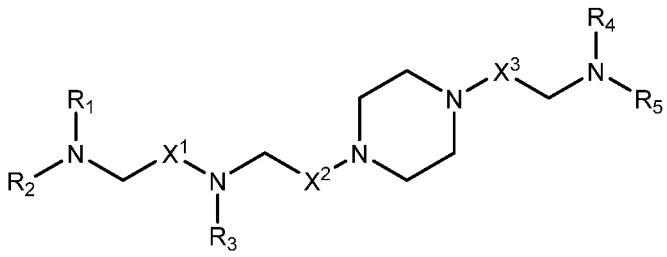
12. The compound of any one of the preceding claims, wherein Ai is N and A^2 is CH.

13. The compound of any one of the preceding claims, wherein Ai is CH and A^2 is N.

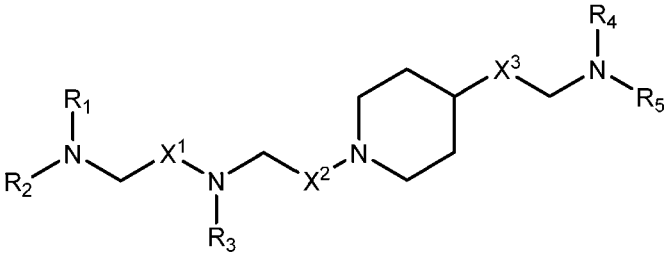
14. The compound of any one of the preceding claims, each of Ai and A^2 is N.

15. The compound of any one of the preceding claims, wherein each of Ai and A^2 is CH.

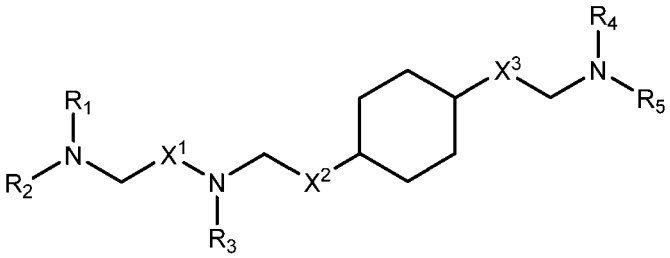
16. The compound of any one of the preceding claims, being of any of formulae (Ial)-(Ia7):



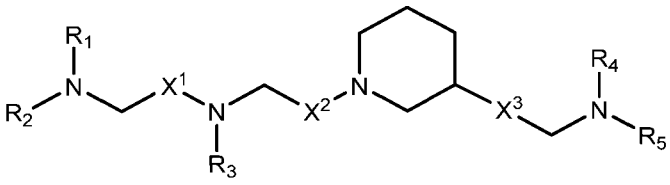
(Ial),



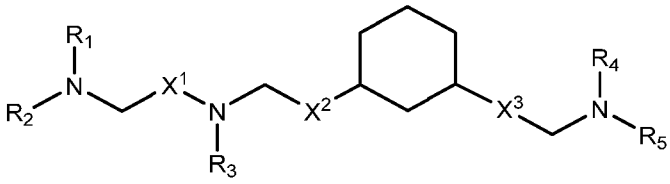
(Ia2),



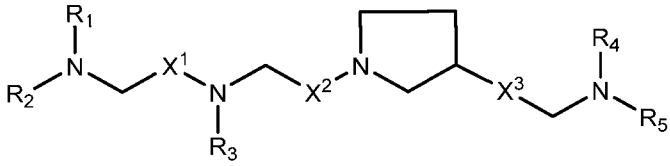
(Ia3),



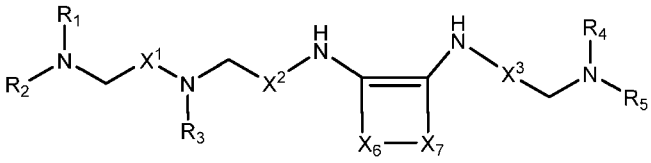
(Ia4),



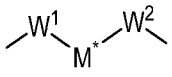
(Ia5),



(Ia6), or



(Ia7).

17. The compound of claim 1 or 2, wherein W is  .

18. The compound of any one of the preceding claims, wherein W^1 and W^2 are each a bond.

19. The compound of any one of the preceding claims, wherein at least one of W^1 and W^2 is -O-.

20. The compound of any one of the preceding claims, wherein W^1 and W^2 are each -0-.

21. The compound of any one of the preceding claims, wherein at least one of W^1 and W^2 is –-N(R^6)-.

22. The compound of any one of the preceding claims, wherein W^1 and W^2 are each –-N(R^6)-.

23. The compound of any one of the preceding claims, wherein at least one R^6 is H.

24. The compound of any one of the preceding claims, wherein each R^6 is H.

25. The compound of any one of the preceding claims, wherein at least one R^6 is Ci alkyl.

26. The compound of any one of the preceding claims, wherein each R^6 is Ci alkyl.

27. The compound of any one of the preceding claims, wherein at least one R^6 is C_2 alkyl.

28. The compound of any one of the preceding claims, wherein each R^6 is C_2 alkyl.

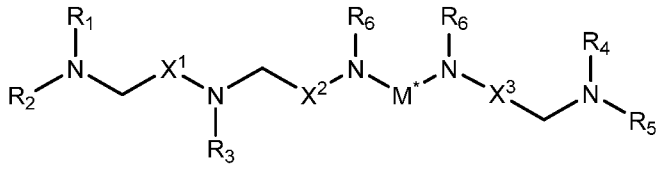
29. The compound of any one of the preceding claims, wherein M* is C_2 alkyl.

30. The compound of any one of the preceding claims, wherein M* is C_3 alkyl.

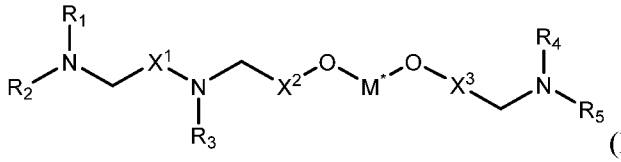
31. The compound of any one of the preceding claims, wherein M* is C_4 alkyl.

32. The compound of any one of the preceding claims, wherein M* is -0-.

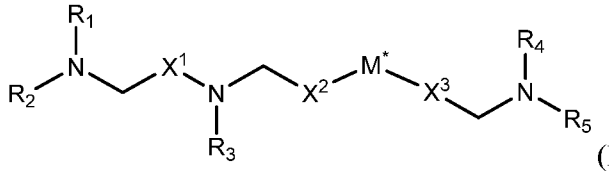
33. The compound of any one of the preceding claims, being of any of formulae (Ia8)-(Ial 0):



(Ia8), or



(Ia9), or



(Ia10).

34. The compound of any one of the preceding claims, wherein X^1, X^2, and X^3 are independently selected from the group consisting of -CH2-, $-(CH_2)_2$, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, -C(0)-CH_2-, -CH_2-C(0)-, -C(0)0-CH_2-, -OC(0)-CH_2-, -CH_2-C(0)0-, -CH_2-OC(0)-, -**(CH 2)n-OC(0)**-, -C(0)0-$(CH_2)_n$-, -CH(OH)-, -C(S)-, and -CH(SH)-.

35. The compound of any one of the preceding claims, wherein at least one of X^1, X^2, and X^3 is not -CH_2-.

36. The compound of any one of the preceding claims, wherein n is 1 or 2.

37. The compound of any one of the preceding claims, wherein X^1 is not -CH_2-.

38. The compound of any one of the preceding claims, wherein X^1 is -CH_2-.

39. The compound of any one of the preceding claims, wherein X^1 is -C(O)-.

40. The compound of any one of the preceding claims, wherein X^2 is not -CH_2-.

41. The compound of any one of the preceding claims, wherein X^2 is -CH_2-.

42. The compound of any one of the preceding claims, wherein X^2 is -$(CH_2)_2$-.

43. The compound of any one of the preceding claims, wherein X^2 is -C(O)-, -C(0)0-, -OC(O)-, -C(0)-CH_2-, -CH_2-C(0)-, -C(0)0-CH_2-, -OC(0)-CH_2-, -CH_2-C(0)0-, or -CH_2-OC(0)-.

44. The compound of any one of the preceding claims, wherein X^3 is not -CH_2-.

45. The compound of any one of the preceding claims, wherein X^3 is -CH_2-.

46. The compound of any one of the preceding claims, wherein X^3 is -$(CH_2)_2$-.

47. The compound of any one of the preceding claims, wherein X^3 is -C(O)-, -**C(0)0-,** -OC(O)-, -C**(0**)-CH_2-, -**CH_2-C(0)-,** -C**(0)0** -CH_2-, -OC**(0**)-CH_2-, -CH_2-**C(0)0-,** or -CH_2-OC**(0)-.**

48. The compound of any one of the preceding claims, wherein X^2 and X^3 are each -C(O)-.

49. The compound of any one of the preceding claims, wherein X^3 is a bond or -$(CH_2)_2$-.

50. The compound of any one of the preceding claims, wherein R_i and R_2 are the same.

51. The compound of any one of the preceding claims, wherein R_i, R_2, and R_3 are the same.

52. The compound of any one of the preceding claims, wherein R_4 and R_5 are the same.

53. The compound of any one of the preceding claims, wherein R_i, R_2, R_3, R_4, and R_5 are the same.

54. The compound of any one of the preceding claims, wherein at least one of R_i, R_2, R_3, R_4, and R_5 is -R"MR'.

55. The compound of any one of the preceding claims, wherein at least one of R_i, R_2, and R_3 is -R"MR'.

56. The compound of any one of the preceding claims, wherein R_i is -R"MR'.

57. The compound of any one of the preceding claims, wherein R_2 is -R"MR'.

58. The compound of any one of the preceding claims, wherein R_3 is -R"MR'.

59. The compound of any one of the preceding claims, wherein at least one of R_4 and R_5 is -R"MR'.

60. The compound any one of the preceding claims, wherein R_4 is -R"MR'.

61. The compound any one of the preceding claims, wherein R_5 is -R"MR'.

62. The compound of any one of the preceding claims, wherein at least one of R_i, R_2, and R_3 is R*YR".

63. The compound of any one of the preceding claims, wherein Y is a C_3 carbocycle.

64. The compound of any one of the preceding claims, wherein R" is -R*MR'.

65. The compound any one of the preceding claims, wherein Rxi and Rx2 are each independently Ci-3 alkyl.

66. The compound any one of the preceding claims, wherein Rxi and Rx2 are each H.

67. The compound any one of the preceding claims, wherein Rxi and Rx2 are each C i alkyl.

68. The compound any one of the preceding claims, wherein Rxi is H and Rx2 is C1-3 alkyl.

69. The compound any one of the preceding claims, wherein Rxi is H and Rx2 is C i alkyl.

70. The compound any one of the preceding claims, wherein Rxi is C1-3 alkyl and Rx2 is H.

71. The compound any one of the preceding claims, wherein Rxi is C i alkyl and Rx2 is H.

72. The compound of any one of the preceding claims, wherein at least one M is -C(0)O.

73. The compound of any one of the preceding claims, wherein each M is -C(0)O.

74. The compound of any one of the preceding claims, wherein at least one M is -OC(O)-.

75. The compound of any one of the preceding claims, wherein each M is - OC(O)-.

76. The compound of any one of the preceding claims, wherein at least one M is -0C(0)O.

77. The compound of any one of the preceding claims, wherein each M is -0C(0)O.

78. The compound of any one of the preceding claims, wherein at least one M is -C(0)S-.

79. The compound of any one of the preceding claims, wherein each M is -C(0)S-.

80. The compound of any one of the preceding claims, wherein at least one M is -SC(O)-.

81. The compound of any one of the preceding claims, wherein each M is -SC(O)-.

82. The compound of any one of the preceding claims, wherein at least one R" is C11 alkyl.

83. The compound of any one of the preceding claims, wherein at least one R" is C10 alkyl.

84. The compound of any one of the preceding claims, wherein at least one R" is $_{C3-7}$ alkyl.

85. The compound of any one of the preceding claims, wherein at least one R" is $_{C\ 3}$ alkyl.

86. The compound of any one of the preceding claims, wherein at least one R" is $_{C\ 5}$ alkyl.

87. The compound of any one of the preceding claims, wherein at least one R" is C_6 alkyl.

88. The compound of any one of the preceding claims, wherein at least one R" is $_{C\ 7}$ alkyl.

89. The compound of any one of the preceding claims, wherein at least one R" is branched $_{C\ 3-12}$ alkyl or $_{C\ 3-12}$ alkenyl.

90. The compound of any one of the preceding claims, wherein each R" independently is $_{C3-7}$ alkyl.

91. The compound of any one of the preceding claims, wherein each R" is $_{C\ 3}$ alkyl.

92. The compound of any one of the preceding claims, wherein each R" is $_{C\ 5}$ alkyl.

93. The compound of any one of the preceding claims, wherein each R" is C_6 alkyl.

94. The compound of any one of the preceding claims, wherein each R" is $_{C\ 7}$ alkyl.

95. The compound of any one of the preceding claims, wherein at least one R" is $_{C3-7}$ alkenyl.

96. The compound of any one of the preceding claims, wherein each R" is $_{C3-7}$ alkenyl.

97. The compound of any one of the preceding claims, wherein at least one R" is $_{C\ 4}$ alkenyl.

98. The compound of any one of the preceding claims, wherein each R" is $_{C\ 4}$ alkenyl.

99. The compound of any one of the preceding claims, wherein at least one R' is $_{C10-18}$ alkyl.

100. The compound of any one of the preceding claims, wherein at least one R' is $_{C1-9}$ alkyl.

101. The compound of any one of the preceding claims, wherein at least one R' is $_{C1-5}$ alkyl.

102. The compound of any one of the preceding claims, wherein at least one R' is C_i alkyl.

103. The compound of any one of the preceding claims, wherein at least one R' is C2 alkyl.

104. The compound of any one of the preceding claims, wherein at least one R' is C_5 alkyl.

105. The compound of any one of the preceding claims, wherein at least one R' is C_7 alkyl.

106. The compound of any one of the preceding claims, wherein at least one R' is C_9 alkyl.

107. The compound of any one of the preceding claims, wherein at least one R' is C_{4-10} alkenyl.

108. The compound of any one of the preceding claims, wherein at least one R' is C_5 alkenyl.

109. The compound of any one of the preceding claims, wherein at least one R' is C_9 alkenyl.

110. The compound of any one of the preceding claims, wherein at least one R' is branched C_{3-18} alkyl, C_{3-18} alkenyl.

111. The compound of any one of the preceding claims, wherein each R' independently is C1-7 alkyl.

112. The compound of any one of the preceding claims, wherein each R' is C_9 alkyl.

113. The compound of any one of the preceding claims, wherein each R' is C_7 alkyl.

114. The compound of any one of the preceding claims, wherein each R' is C1-5 alkyl.

115. The compound of any one of the preceding claims, wherein each R' is C_5 alkyl.

116. The compound of any one of the preceding claims, wherein each R' is C2 alkyl.

117. The compound of any one of the preceding claims, wherein each R' is Ci alkyl.

118. The compound of any one of the preceding claims, wherein each R' is C_{4-10} alkenyl.

119. The compound of any one of the preceding claims, wherein each R' is C_5 alkenyl.

120. The compound of any one of the preceding claims, wherein each R' is C_9 alkenyl.

121. The compound of any one of the preceding claims, wherein each R' is branched C_{3-18} alkyl, C_{3-18} alkenyl.

122. The compound of any one of the preceding claims, wherein each **R'** is branched ***Cn*** alkyl.

123. The compound of any one of the preceding claims, wherein each **R'** is branched $_{C\ 3\text{-}12}$ alkyl, $_{C\ 3\text{-}12}$ alkenyl.

124. The compound of any one of the preceding claims, wherein at least one of **Ri, R2, R3, R4,** and $_{R\ 5}$ is **C9, C12,** or **C14** alkyl.

125. The compound of any one of the preceding claims, wherein **Ri, R2, R3, R4,** and $_{R\ 5}$ are each independently selected from **-R"MR'** and $_{C\ 5\text{-}10}$ alkyl.

126. The compound of any one of the preceding claims, wherein **Ri, R2, R3,** and **R4** are each independently selected from **-R"MR'** and $_{C\ 9}$ alkyl.

127. The compound of any one of the preceding claims, wherein at least one of **R4** and $_{R\ 5}$ is $_{C\ 9}$ alkyl.

128. The compound of any one of the preceding claims, wherein at least one of **R4** and $_{R\ 5}$ is **C12** alkyl.

129. The compound of any one of the preceding claims, wherein at least one of **R4,** and $_{R\ 5}$ is **Ci4** alkyl.

130. The compound of any one of the preceding claims, wherein one of **R4** and $_{R\ 5}$ is **C14** alkyl.

131. The compound of any one of the preceding claims, wherein at least one of **R4** and $_{R\ 5}$ is **-R"MR'** .

132. The compound of any one of the preceding claims, wherein one of **R4** and $_{R\ 5}$ is **-R"MR'** .

133. The compound of any one of the preceding claims, wherein **Ri, R2, R3, R4,** and $_{R\ 5}$ each independently are **C9, C12,** or **C14** alkyl.

134. The compound of any one of the preceding claims, wherein **Ri, R2, R3, R4,** and $_{R\ 5}$ each independently are $_{C\ 5\text{-}10}$ alkyl.

135. The compound of any one of the preceding claims, wherein **Ri, R2, R3, R4,** and $_{R\ 5}$ each are $_{C\ 9}$ alkyl.

136. The compound of any one of the preceding claims, wherein only one of **Ri, R2, R3, R4,** and **R 5** is selected from **C6-20** alkenyl.

137. The compound of any one of the preceding claims, wherein **Ri, R2, R3, R4,** and **R 5** have the same number of carbon atoms.

138. The compound of any one of the preceding claims, wherein **R** 4 is selected from **C5-20** alkenyl.

139. The compound of any one of the preceding claims, wherein **R 4** is **C12** alkenyl.

140. The compound of any one of the preceding claims, wherein **R 4** is **Cis** alkenyl.

141. The compound of any one of the preceding claims, wherein **Ri, R2,** and **R 3** are selected from **C6-20** alkenyl, and **R 4** and **R 5** are selected from **C6-20** alkyl.

142. The compound of any one of the preceding claims, wherein **Ri, R2,** and **R 3** are selected from **C6-20** alkyl, and **R 4** and **R 5** are selected from **C6-20** alkenyl.

143. The compound of any one of the preceding claims, wherein **Ri, R2,** and **R 3** have the same number of carbon atoms.

144. The compound of any one of the preceding claims, wherein **Ri, R2,** and **R 3** have 6, 8, 9, 12, 14, or 18 carbon atoms.

145. The compound of any one of the preceding claims, wherein **R 4** and **R 5** have the same number of carbon atoms.

146. The compound of any one of the preceding claims, wherein **R 4** and **R 5** have 6, 8, 9, 12, 14, or 18 carbon atoms.

147. The compound of any one of the preceding claims, wherein **Ri, R2,** and **R 3** are **Cis** alkenyl.

148. The compound of any one of the preceding claims, wherein **Ri, R2,** and **R 3** are linoleyl.

149. The compound of any one of the preceding claims, wherein at least one of **Ri, R2,** and **R 3** is **C5-10** alkenyl.

150. The compound of any one of the preceding claims, wherein **Ri, R2,** and **R 3** each independently are **C5-10** alkenyl.

151. The compound of any one of the preceding claims, wherein at least one of **Ri, R2, R3, R4,** and **R 5** is **C5-10** alkenyl.

152. The compound of any one of the preceding claims, wherein at least one of **Ri, R2,** and **R 3** is **C 9** alkenyl.

153. The compound of any one of the preceding claims, wherein **Ri, R2,** and **R 3** each are **C 9** alkenyl.

154. The compound of any one of the preceding claims, wherein R 4 and R 5 are ***C*** 6 alkyl.

155. The compound of any one of the preceding claims, wherein **R** 4 and **R 5** are **Cs** alkyl.

156. The compound of any one of the preceding claims, wherein R 4 and R 5 are C 9 alkyl.

157. The compound of any one of the preceding claims, wherein **R 4** and **R 5** are **C12** alkyl.

158. The compound of any one of the preceding claims, wherein R 4 and R 5 are C14 alkyl.

159. The compound of any one of the preceding claims, wherein **Ri, R2,** and **R 3** are ***C*** 6 alkyl.

160. The compound of any one of the preceding claims, wherein **Ri, R2,** and **R 3** are **Cs** alkyl.

161. The compound of any one of the preceding claims, wherein **Ri, R2,** and **R 3** are **C 9** alkyl.

162. The compound of any one of the preceding claims, wherein **Ri, R2,** and **R 3** are **C12** alkyl.

163. The compound of any one of the preceding claims, wherein **Ri, R2,** and **R 3** are **C14** alkyl.

164. The compound of any one of the preceding claims, wherein **R 4** and **R 5** are **Cis** alkenyl.

165. The compound of any one of the preceding claims, wherein R 4 and R 5 are linoleyl.

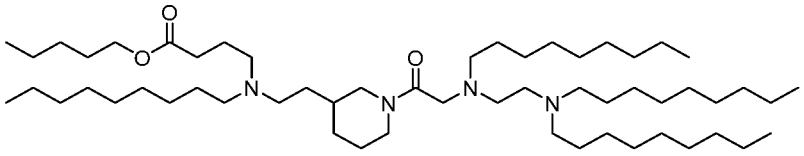
166. The compound of any one of the preceding claims, wherein the compound is selected from Compounds 1-116, 42-66, 68-76, and 80-109, and salts or isomers thereof.

167. The compound of any one of the preceding claims, wherein the compound is selected from Compounds 4, 56-58, 61-62, 71, 80-84, 87, and 89-109, and salts or isomers thereof.

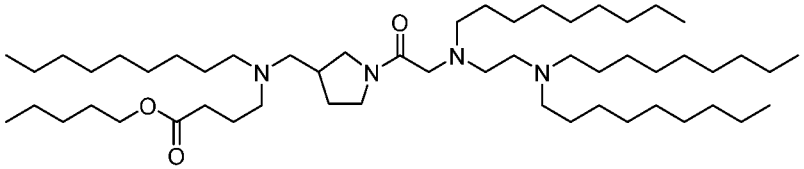
168. The compound of any one of the preceding claims, wherein the compound is selected from Compounds 109-142 and 150-156, and salts or isomers thereof.

169. The compound of any one of the preceding claims, wherein the compound is selected from Compounds 157-161, and salts or isomers thereof

170. The compound of any one of the preceding claims, wherein the compound is selected from:

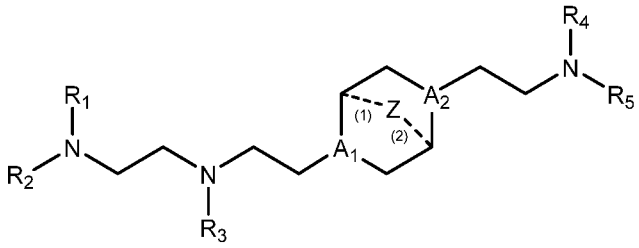


(Compound 80),



(Compound 82), and salts or isomers thereof.

171. **A** compound having the formula **(II):**



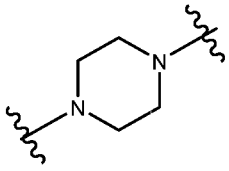
(II),

or a salt or isomer thereof, wherein

A i and **A 2** are each independently selected from CH or N and at least one of **A i** and **A 2** is N;

Z is **CH2** or absent wherein when Z is **CH2,** the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

Ri, R2, R3, R4, and **R 5** are independently selected from the group consisting of **C 6-20** alkyl and **C 6-20** alkenyl;

wherein when ring A is  , then

i) **Ri, R2, R3, R4,** and **R 5** are the same, wherein **R i** is not **C12** alkyl, **Cis** alkyl, or **Cis** alkenyl;

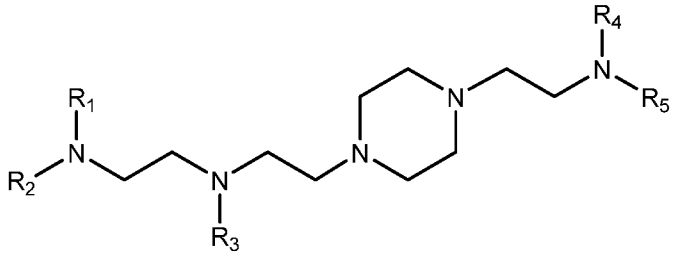
ii) only one of **Ri, R2, R3, R4,** and **R 5** is selected from **C6-20** alkenyl;

iii) at least one of **Ri, R2, R3, R4,** and **R 5** have a different number of carbon atoms than at least one other of **Ri, R2, R3, R4,** and **R5;**

iv) **Ri, R2,** and **R 3** are selected from **C6-20** alkenyl, and **R 4** and **R 5** are selected from **C6-20** alkyl; or

v) **Ri, R2,** and **R 3** are selected from **C6-20** alkyl, and **R 4** and **R 5** are selected from **C6-20** alkenyl.

172. The compound of any one of the preceding claims, being of formula (Ila)



(Ila).

173. The compound of any one of the preceding claims, wherein **Ri, R2, R3, R4,** and **R 5** are the same, wherein **R i** is not **C12** alkyl, **Cis** alkyl, or **Cis** alkenyl.

174. The compound of any one of the preceding claims, wherein **Ri, R2, R3, R4,** and **R 5** are **C 9** alkyl.

175. The compound of any one of the preceding claims, wherein **Ri, R2, R3, R4,** and **R 5** are **Ci4** alkyl.

176. The compound of any one of the preceding claims, wherein only one of **Ri, R2, R3, R4,** and **R 5** is selected from **C6-20** alkenyl.

177. The compound of any one of the preceding claims, wherein Ri, R2, R3, R4, and R_5 have the same number of carbon atoms.

178. The compound of any one of the preceding claims, wherein R_4 is selected from C_{5-20} alkenyl.

179. The compound of any one of the preceding claims, wherein R_4 is C12 alkenyl.

180. The compound of any one of the preceding claims, wherein R_4 is Cis alkenyl.

181. The compound of any one of the preceding claims, wherein at least one of Ri, R2, R3, R4, and R_5 have a different number of carbon atoms than at least one other of Ri, R2, R3, R4, and R_5.

182. The compound of any one of the preceding claims, wherein Ri, R2, and R_3 are selected from C_{6-20} alkenyl, and R_4 and R_5 are selected from C_{6-20} alkyl.

183. The compound of any one of the preceding claims, wherein Ri, R2, and R_3 are selected from C_{6-20} alkyl, and R_4 and R_5 are selected from C_{6-20} alkenyl.

184. The compound of any one of the preceding claims, wherein Ri, R2, and R_3 have the same number of carbon atoms.

185. The compound of any one of the preceding claims, wherein Ri, R2, and R_3 have 6, 8, 9, 12, 14, or 18 carbon atoms.

186. The compound of any one of the preceding claims, wherein R_4 and R_5 have the same number of carbon atoms.

187. The compound of any one of the preceding claims, wherein R_4 and R_5 have 6, 8, 9, 12, 14, or 18 carbon atoms.

188. The compound of any one of the preceding claims, wherein Ri, R2, and R_3 are Cis alkenyl.

189. The compound of any one of the preceding claims, wherein Ri, R2, and R_3 are linoleyl.

190. The compound of any one of the preceding claims, wherein R_4 and R_5 are C_6 alkyl.

191. The compound of any one of the preceding claims, wherein R_4 and R_5 are C_8 alkyl.

192. The compound of any one of the preceding claims, wherein R_4 and R_5 are C_9 alkyl.

193. The compound of any one of the preceding claims, wherein R_4 and R_5 are C12 alkyl.

194. The compound of any one of the preceding claims, wherein R_4 and R_5 are C14 alkyl.

195. The compound of any one of the preceding claims, wherein Ri, R2, and R_3 are C_6 alkyl.

196. The compound of any one of the preceding claims, wherein Ri, R2, and R_3 are C_8 alkyl.

197. The compound of any one of the preceding claims, wherein Ri, R2, and R_3 are C_9 alkyl.

198. The compound of any one of the preceding claims, wherein Ri, R2, and R_3 are C12 alkyl.

199. The compound of any one of the preceding claims, wherein Ri, R2, and R_3 are C14 alkyl.

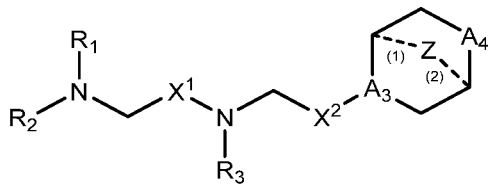
200. The compound of any one of the preceding claims, wherein R_4 and R_5 are Cis alkenyl.

201. The compound of any one of the preceding claims, wherein R_4 and R_5 are linoleyl.

202. The compound of any one of the preceding claims, wherein (i) R_i has a different number of carbon atoms than R2, R3, R4, and R_5, (ii) R_3 has a different number of carbon atoms than Ri, R2, R4, and R_5, or (iii) R_4 has a different number of carbon atoms than Ri, R2, R3, and R_5.

203. The compound of any one of the preceding claims, wherein the compound is selected from Compounds 17 to 34, and salts or isomers thereof.

204. A compound having the formula (III):



(HI),

or a salt or isomer thereof, wherein

A_3 is CH or N;

A_4 is CH2 or NH; and at least one of A_3 and A_4 is N or NH;

Z is CH2 or absent wherein when Z is CH2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

Ri, R2, and R 3 are independently selected from the group consisting of C_{5-20} alkyl, C_{5-20} alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

each M is independently selected from -C(0)0-, -OC(O)-, -C(0)N(R')-, -N(R')C(0)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0-, -$S(0)_2$-, an aryl group, and a heteroaryl group;X^1 and X^2 are independently selected from the group consisting of -CH2-, -$(CH_2)_2$-, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, -C(0)-CH_2-, -CH_2-C(0)-, -C(0)0-CH_2-, -OC(0)-CH_2-, -CH_2-C(0)0-, -CH_2-OC(0)-, -CH(OH)-, -C(S)-, and -CH(SH)-;

each Y is independently a C3-6 carbocycle;

each R* is independently selected from the group consisting of Ci-i_2 alkyl and C_2-i_2 alkenyl;

each R is independently selected from the group consisting of C1-3 alkyl and a C3-6 carbocycle;

each R' is independently selected from the group consisting of C1-18 alkyl, C_2-is alkenyl, and H; and

each R" is independently selected from the group consisting of C3-i_2 alkyl and *C3-12* alkenyl.

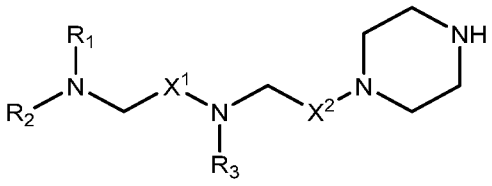
205. The compound of any one of the preceding claims, wherein at least one of A 3 and A_4 is N orNH.

206. The compound of any one of the preceding claims, wherein A 3 is N and A_4 is NH.

207. The compound of any one of the preceding claims, wherein A 3 is N and A 4 is CH_2.

208. The compound of any one of the preceding claims, wherein A 3 is CH and A 4 is NH.

209. The compound of any one of the preceding claims being of formula (Ilia)



(Ilia)

210. The compound of any one of the preceding claims, wherein Ri, R_2, and R 3 are independently selected from the group consisting of C_{5-20} alkyl and C_{5-20} alkenyl.

211. The compound of any one of the preceding claims, wherein **Ri, R2,** and R_3 are the same.

212. The compound of any one of the preceding claims, wherein **Ri, R2,** and R_3 are C_6 alkyl.

213. The compound of any one of the preceding claims, wherein **Ri, R2,** and R_3 are C_9 alkyl.

214. The compound of any one of the preceding claims, wherein **Ri, R2,** and R_3 are **C12** alkyl.

215. The compound of any one of the preceding claims, wherein **Ri, R2,** and R_3 are C_{14} alkyl.

216. The compound of any one of the preceding claims, wherein **Ri, R2,** and R_3 are **Cis** alkenyl.

217. The compound of any one of the preceding claims, wherein **Ri, R2,** and R_3 are linoleyl.

218. The compound of any one of the preceding claims, wherein at least one of X^1 and X^2 is not $-CH_2-$.

219. The compound of any one of the preceding claims, wherein X^1 is not **-CH2-.**

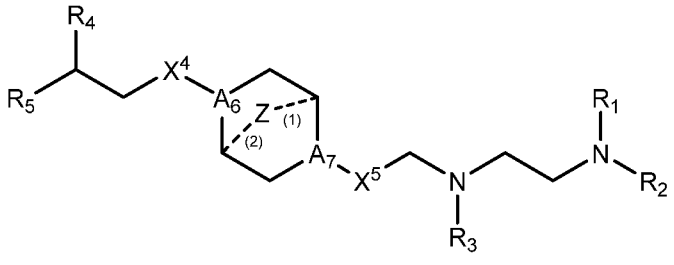
220. The compound of any one of the preceding claims, wherein X^1 is **-C(O)-.**

221. The compound of any one of the preceding claims, wherein X^2 is not **-CH2-.**

222. The compound of any one of the preceding claims, wherein X^2 is **-C(O)-.**

223. The compound of any one of the preceding claims, wherein the compound is selected from Compounds 35-41 and 77, and salts or isomers thereof.

224. A compound having the formula (lb):



(Ib),

or a salt or isomer thereof, wherein

A_6 and A_7 are each independently selected from CH or N, wherein at least one of A_6 and A_7 is N;

Z is CH2 or absent wherein when Z is CH2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

X^4 and X^5 are independently selected from the group consisting of -CH2-, $-(CH_2)_2-$, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, $-C(0)-CH_2-$, $-CH_2-C(0)-$, $-C(0)0-CH_2-$, $-OC(0)-CH_2-$, $-CH_2-C(0)0-$, $-CH_2-OC(0)-$, -CH(OH)-, -C(S)-, and -CH(SH)-;

Ri, R2, R3, R4, and R_5 each are independently selected from the group consisting of C_{5-20} alkyl and C_{5-20} alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

each M is independently selected from the group consisting of -C(0)0-, -OC(O)-, -OC(0)0-, -C(0)N(R')-, -N(R')C(0)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0-, $-S(0)_2-$, an aryl group, and a heteroaryl group;

each Y is independently a C_{3-6} carbocycle;

each R* is independently selected from the group consisting of Ci-12 alkyl and C2-12 alkenyl;

each R is independently selected from the group consisting of C1-3 alkyl and a C_{3-6} carbocycle;

each R' is independently selected from the group consisting of C1-18 alkyl, C2-18 alkenyl, and H; and

each R" is independently selected from the group consisting of C_{3-12} alkyl and C_{3-12} alkenyl.

225. The compound of any one of the preceding claims, wherein A_6 is N and A_7 is N, or A_6 is CH and Av is N.

226. The compound of any one of the preceding claims, wherein when A_6 is N and A_7 is N, then (i) at least one of X^4 and X^5 is not -CH2-, (ii) at least one of X^4 and X^5 is -C(O)-; and/or (iii) at least one of Ri, R2, R3, R4, and R_5 is -R"MR'.

227. The compound of any one of the preceding claims, wherein X^4 is -CH2- and X^5 is -C(O)-.

228. The compound of any one of the preceding claims, wherein X^4 and X^5 are each -C(O)-.

229. The compound of any one of the preceding claims, wherein R2, R3, R4, and R_5 are the same.

230. The compound of any one of the preceding claims, wherein R2, R3, R4, and R_5 are C_9 alkyl.

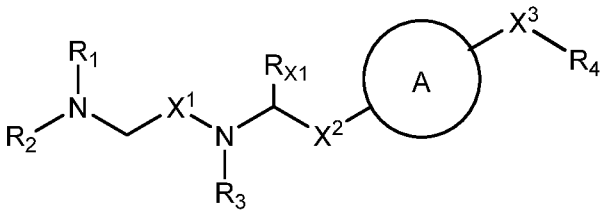
231. The compound of any one of the preceding claims, wherein R2, R3, R4, and R_5 are each Ci8 alkenyl.

232. The compound any one of the preceding claims wherein the compound is selected from Compound 67 and salts or isomers thereof.

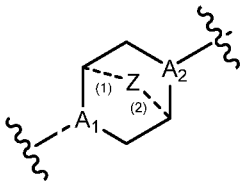
233. The compound of any one of the preceding claims, wherein Z is absent.

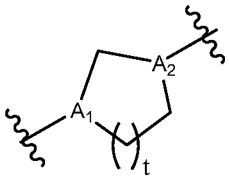
234. The compound of any one of the preceding claims, wherein Z is CH2.

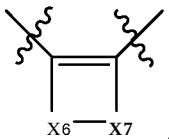
235. A compound having the formula (Ic):

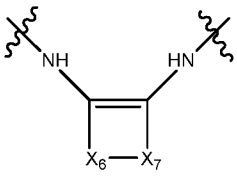


(Ic), or a salt or isomer thereof, wherein

ring A is 

, 

, 

, Qj- 

;

t is 1 or 2;

A i and A 2 are each independently selected from CH or N;

Z is CH2 or absent wherein when Z is CH2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

Ri, R2, R3, and R 4 are independently selected from the group consisting of $C_{5\text{-}20}$ alkyl, $C_{5\text{-}20}$ alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

Rxi is H or C1-3 alkyl;

each M is independently selected from the group consisting of -C(0)0-, -OC(O)-, -OC(0)0-, -C(0)N(R')-, -N(R')C(0)-, -C(O)-,

-C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0-, -S(0)$_2$-, -C(0)S-, -SC(O)-, an aryl group, and a heteroaryl group;

X^1, X^2, and X^3 are independently selected from the group consisting of a bond, **-CH2-,** -$(CH_2)_2$-, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, -$(CH_2)_n$-C(0)-, -C(0)-$(CH_2)_n$-,-$(CH_2)_n$-C(0)0-, -OC(0)-$(CH_2)_n$-, -$(CH_2)_n$-OC(0)-, -C(0)0-$(CH_2)_n$-, -CH(OH)-, -C(S)-, and -CH(SH)-;

each Y is independently a $_{C3-6}$ carbocycle;

each R* is independently selected from the group consisting of **Ci-i$_2$** alkyl and **C$_2$-i$_2$** alkenyl;

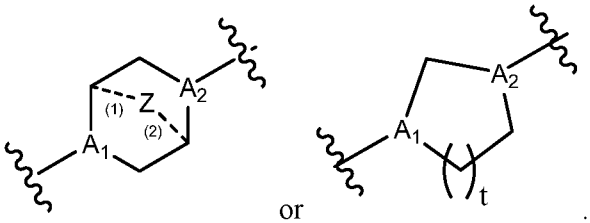
each R is independently selected from the group consisting of **C 1-3** alkyl and a **C 3-6** carbocycle;

each R' is independently selected from the group consisting of **C 1-18** alkyl, **C$_2$-is** alkenyl, and H;

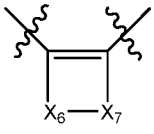
each R" is independently selected from the group consisting of **C 3-i$_2$** alkyl, ***C3-12*** alkenyl and -R*MR'; and

n is an integer from 1-6.

236. The compound of any one of the preceding claims, wherein ring A is



.

237. The compound of any one of the preceding claims, wherein ring A is 

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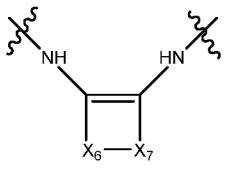
238. The compound of any one of the preceding claims, wherein ring A is 

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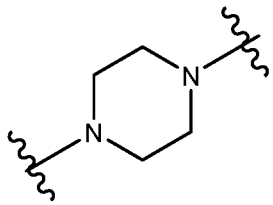
239. The compound of any one of the preceding claims, wherein when ring **A** is



, then

i) at least one of X^1, X^2, and X^3 is not **-CH2-;**

ii) at least one of X^2 and X^3 is -C(O)-; and/or

iii) at least one of **Ri, R2,** $_{R3}$, and $_{R4}$ is **-R"MR' .**

240. The compound of any one of the preceding claims, wherein at least one of **A i** and **A 2** is N.

241. The compound of any one of the preceding claims, wherein **A i** is N and **A 2** is CH.

242. The compound of any one of the preceding claims, wherein **A i** is CH and **A 2** is N.

243. The compound of any one of the preceding claims, wherein **A i** is N and **A 2** is N.

244. The compound of any one of the preceding claims, wherein X^2 and X^3 are independently selected -C(O)-, -C(0)0-, -OC(O)-, -C(0)-CH_2-, -CH_2-C(0)-, -C(0)0-CH_2-, -OC(O)-**CH2-,** -CH_2-C(0)0-, and -CH_2-OC(0)-.

245. The compound of any one of the preceding claims, wherein X^1 is not **-CH2-.**

246. The compound of any one of the preceding claims, wherein X^1 is **-CH2-.**

247. The compound of any one of the preceding claims, wherein at least one of X^2 and X^3 is -C(O)-.

248. The compound of any one of the preceding claims, wherein X^2 and X^3 are each -C(O)-.

249. The compound of any one of the preceding claims, wherein **Ri, R2,** and $_{R3}$ are the same.

250. The compound of any one of the preceding claims, wherein **Ri, R2,** and $_{R3}$ are each **C 9** alkyl.

251. The compound of any one of the preceding claims, wherein $_{R4}$ is **C 9-13** alkyl.

252. The compound of any one of the preceding claims, wherein R_4 is C_9 alkyl.

253. The compound of any one of the preceding claims, wherein R_4 is C10 alkyl.

254. The compound of any one of the preceding claims, wherein R_4 is C11 alkyl.

255. The compound of any one of the preceding claims, wherein R_4 is C12 alkyl.

256. The compound of any one of the preceding claims, wherein R_4 is C_{13} alkyl.

257. The compound of any one of the preceding claims, wherein at least one of **Ri,** R2, R_3, and R_4 is -R"MR' .

258. The compound of any one of the preceding claims, wherein at least one of **Ri,** R2, and R_3 is -R"MR' .

259. The compound of any one of the preceding claims, wherein R_4 is -R"MR'.

260. The compound of any one of the preceding claims, wherein at least one M is -C(**0**)O-.

261. The compound of any one of the preceding claims, wherein at least one R" is C2-5 alkyl.

262. The compound of any one of the preceding claims, wherein each R" is C2-5 alkyl.

263. The compound of any one of the preceding claims, wherein at least one R" is C_3 alkyl.

264. The compound of any one of the preceding claims, wherein each R" is C_3 alkyl.

265. The compound of any one of the preceding claims, wherein at least one R' is C1-9 alkyl.

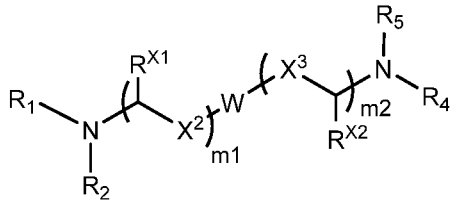
266. The compound of any one of the preceding claims, wherein each R' is C1-9 alkyl.

267. The compound of any one of the preceding claims, wherein at least one R' is C_7 alkyl.

268. The compound of any one of the preceding claims, wherein each R' is C_7 alkyl.

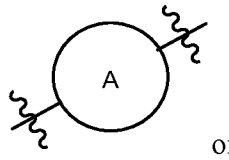
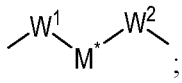
269. The compound of any one of the preceding claims, wherein the compound is selected from Compounds 143 and 144, and salts or isomers thereof.

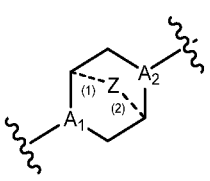
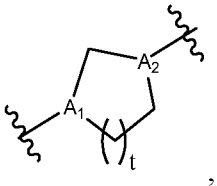
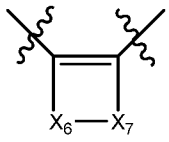
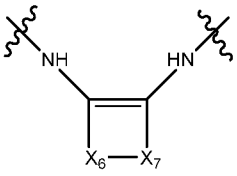
270. A compound having the formula (Id):



(Id),

or a salt or isomer thereof, wherein

W is  or  ;

ring A is  ,  ,  , or  ;

t is 1 or 2;

A i and A 2 are each independently selected from CH or N;

Z is CH2 or absent wherein when Z is CH2, the dashed lines (1) and (2) each represent a single bond; and when Z is absent, the dashed lines (1) and (2) are both absent;

Ri, R.2, R.4, and R 5 are independently selected from the group consisting of C 5-20 alkyl, C5-20 alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

Rxi and Rx2 are each independently H or C1-3 alkyl;

each M is independently selected from the group consisting of -C(0)0-, -OC(O)-, -OC(0)0-, -C(0)N(R')-, -N(R')C(0)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0-, -S(0)$_2$-, -C(0)S-, -SC(O)-, an aryl group, and a heteroaryl group;

M* is -0-, Ci-Ce alkyl, -((CH2)pCH(R7)(CH2) $_q$CH(R7)(CH $_2$)r)s-, or -((CH2)tC(=R8)(CH2)uC(=R8)(CH$_2$)v)s-;

W ^{1} and W ^{2} are each independently selected from the group consisting of a bond, -0-, and -N(R $_6$)-;

each R $_6$ is independently selected from the group consisting of H and C1-3 alkyl;

each R 7 is independently selected from the group consisting of C1-3 alkyl,(CH2)oCN, C1-3 hydroxy alkyl, and C1-C3 haloalkyl;

each R $_8$ is independently selected from the group consisting of CH(CH2)oCN, CH(CH$_2$)oCH$_3$, CH(CH$_2$)oOH, and CHX^{Hal};

X^{Hal} is C_{1-3} haloalkyl;

w is an integer from 1-3;

X^2 and X^3 are independently selected from the group consisting of a bond, -CH2-, $-(CH_2)_2-$, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, $-(CH_2)$n-C(0)-, -C(0)-(CH_2)n-, $-(CH_2)_n$-C(0)0-, -OC(0)-(CH_2)n-, $-(CH_2)_n$-OC(0)-, -C(0)0-$(CH_2)_n$-, -CH(OH)-, -C(S)-, and -CH(SH)-;

X^6 and X^7 are each independently selected from the group consisting of -C(O)-, -C(S)-, -C((CH2)wOH)-, and $-C(X^{Ha1})-$;

each Y is independently a C_{3-6} carbocycle;

each R^* is independently selected from the group consisting of **C_1-i_2** alkyl and **C_2-i_2** alkenyl;

each R is independently selected from the group consisting of C_{1-3} alkyl and a C_{3-6} carbocycle;

each R' is independently selected from the group consisting of C_{1-18} alkyl, **C_2-is** alkenyl, and H;

each R'' is independently selected from the group consisting of **C_3-i_2** alkyl, *C_{3-12}* alkenyl and $-R^*MR'$;

m1 and m2 are each independently 0 or 1;

n is an integer from 1-6;

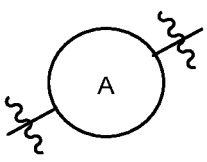
0 is an integer from 0-3;

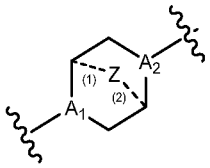
p, q, r, t, u, and v are each independently an integer from 0-3;

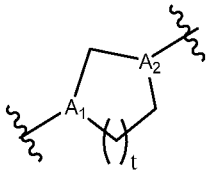
w is an integer from 0-3; and

s is an integer from 1-3.

271. The compound of claim 270, wherein

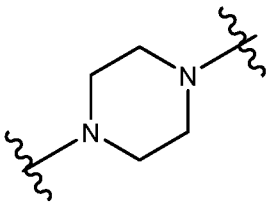
W is 

and ring A is 

or 

; and m1 and m2 are each 1.

272. The compound of any one of the preceding claims, wherein when ring A is

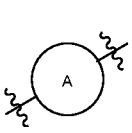
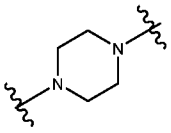


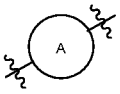
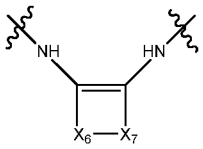
, then

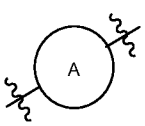
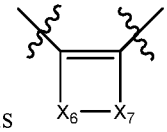
i) at least one of X^2 and X^3 is not -CH2-;

ii) at least one of X^2 and X^3 is -C(O)-; and/or

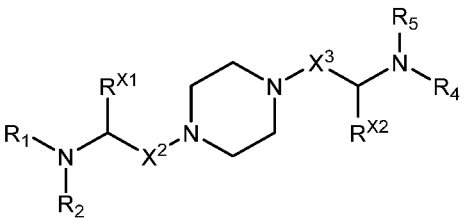
iii) at least one of Ri, R2, R4, and R_5 is -R"MR' .

273. The compound of any one of the preceding claims, wherein  is  .

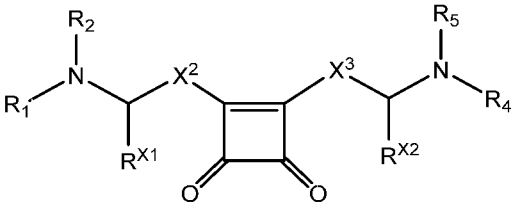
274. The compound of any one of the preceding claims, wherein  is  .

275. The compound of any one of the preceding claims, wherein  is  .

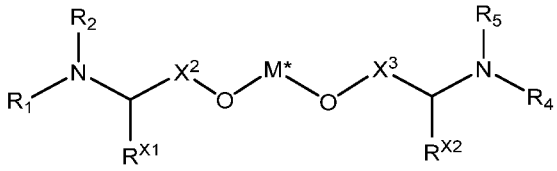
276. The compound of any one of the preceding claims, being of any of formulae (Idl)-(IdlO):

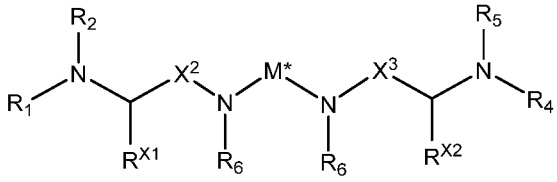


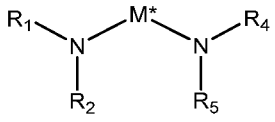
(Id1),

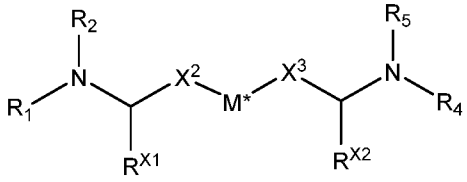


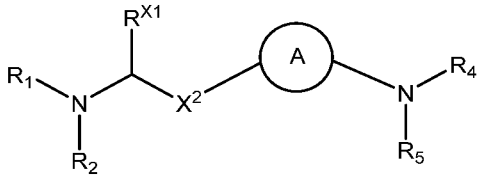
(Id2),

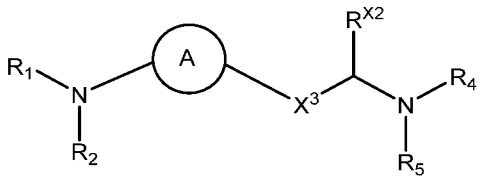

(Id3),

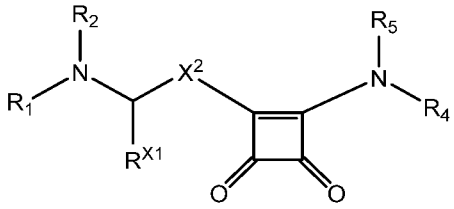

(Id4),

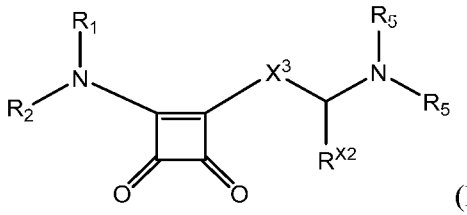

(Id5),


(Id6),


(Id7),


(Id8),


(Id9), or



(Id10).

277. The compound of any one of the preceding claims, wherein at least one of A i and A 2 is N .

278. The compound of any one of the preceding claims, wherein A i is N and A 2 is CH.

279. The compound of any one of the preceding claims, wherein A i is CH and A 2 is N .

280. The compound of any one of the preceding claims, wherein A i is N and A 2 is N .

281 . The compound of any one of the preceding claims, wherein X^2 and X^3 are independently selected from the group consisting of -C(O)-, C(0)-$(CH_2)_n$-, and -$(CH_2)_n$-C(0)-.

282. The compound of any one of the preceding claims, wherein X^2 is -CH2-.

283. The compound of any one of the preceding claims, wherein X^2 is -$(CH_2)_n$-.

284. The compound of any one of the preceding claims, wherein X^2 is a bond.

285. The compound of any one of the preceding claims, wherein X^2 is -C(O)-.

286. The compound of any one of the preceding claims, wherein X^2 is -(CH_2)nC(0)-.

287. The compound of any one of the preceding claims, wherein X^3 is -CH2-.

288. The compound of any one of the preceding claims, wherein X^3 is -$(CH_2)_n$-.

289. The compound of any one of the preceding claims, wherein X^3 is a bond.

290. The compound of any one of the preceding claims, wherein X^3 is -C(O)-.

291 . The compound of any one of the preceding claims, wherein X^3 is -$(CH_2)_n$C(0)-.

292. The compound of any one of the preceding claims, wherein n is 2 or 3.

293. The compound any one of the preceding claims, wherein **Rxi** and **Rx2** are each independently **C 1-3** alkyl.

294. The compound any one of the preceding claims, wherein **Rxi** and **Rx2** are each H.

295. The compound any one of the preceding claims, wherein **Rxi** and **Rx2** are each **C i** alkyl.

296. The compound any one of the preceding claims, wherein **Rxi** is H and **Rx2** is **C 1-3** alkyl.

297. The compound any one of the preceding claims, wherein **Rxi** is H and **Rx2** is **C i** alkyl.

298. The compound any one of the preceding claims, wherein **Rxi** is **C 1-3** alkyl and **Rx2** is H.

299. The compound any one of the preceding claims, wherein **Rxi** is **C i** alkyl and **Rx2** is H.

300. The compound of any one of the preceding claims, wherein **Ri, R2, R4,** and **R 5** are the same.

301. The compound of any one of the preceding claims, wherein at least one of **Ri, R2, R4,** and R_5 is -R"MR'.

302. The compound of any one of the preceding claims, wherein **Ri, R2, R4,** and **R 5** are selected from -R"MR' and **C 5-12** alkyl.

303. The compound of any one of the preceding claims, wherein **Ri, R2, R4,** and **R 5** are selected from -R"MR' and **C 9** alkyl.

304. The compound of any one of the preceding claims, wherein **Ri, R2, R4,** and **R 5** are selected from -R"MR' and **C10** alkyl.

305. The compound of any one of the preceding claims, wherein **Ri, R2, R4,** and **R 5** are each R'"MR'.

306. The compound of any one of the preceding claims, wherein R_4 and R_5 are each R"MR'.

307. The compound of any one of the preceding claims, wherein **R i** and **R 2** are each R"MR'.

308. The compound of any one of the preceding claims, wherein at least one M is -C(0)O.

309. The compound of any one of the preceding claims, wherein each M is -C(0)O.

310. The compound of any one of the preceding claims, wherein at least one M is -OC(O)- .

311. The compound of any one of the preceding claims, wherein each M is -OC(O)- .

312. The compound of any one of the preceding claims, wherein at least one **R"** is $_{C3-7}$ alkyl.

313. The compound of any one of the preceding claims, wherein each **R"** is $_{C3-7}$ alkyl.

314. The compound of any one of the preceding claims, wherein at least one **R"** is $_{C\ 3}$ alkyl.

315. The compound of any one of the preceding claims, wherein each **R"** is $_{C\ 3}$ alkyl.

316. The compound of any one of the preceding claims, wherein at least one **R"** is **C 7** alkyl.

317. The compound of any one of the preceding claims, wherein each **R"** is **C 7** alkyl.

318. The compound of any one of the preceding claims, wherein at least one **R'** is **C1-5** alkyl.

319. The compound of any one of the preceding claims, wherein at least one **R'** is $_{C\ 5}$ alkyl.

320. The compound of any one of the preceding claims, wherein at least one **R'** is $_{C5-10}$ alkyl.

321 . The compound of any one of the preceding claims, wherein at least one **R'** is $_{C\ 9}$ alkyl.

322. The compound of any one of the preceding claims, wherein each **R'** is $_{C5-10}$ alkyl.

323. The compound of any one of the preceding claims, wherein each **R'** is $_{C\ 9}$ alkyl.

324. The compound of any one of the preceding claims, wherein one **R'** is $_{C5-10}$ alkyl.

325. The compound of any one of the preceding claims, wherein two **R'** are $_{C5-10}$ alkyl.

326. The compound of any one of the preceding claims, wherein one **R'** is $_{C\ 9}$ alkyl.

327. The compound of any one of the preceding claims, wherein two **R'** are $_{C\ 9}$ alkyl.

328. The compound of any one of the preceding claims, wherein at least one **R'** is **C11-18** alkyl.

329. The compound of any one of the preceding claims, wherein at least one **R'** is $_{C17}$ alkyl.

330. The compound of any one of the preceding claims, wherein at least one R' is branched Civ alkyl.

331. The compound of any one of the preceding claims, wherein each R' is Cii-is alkyl.

332. The compound of any one of the preceding claims, wherein each R' is Cn alkyl.

333. The compound of any one of the preceding claims, wherein each R' is branched C_{17} alkyl.

334. The compound of any one of the preceding claims, wherein one R' is C_{17} alkyl.

335. The compound of any one of the preceding claims, wherein one R' is branched C_{17} alkyl.

336. The compound of any one of the preceding claims, wherein two R' are C_{17} alkyl.

337. The compound of any one of the preceding claims, wherein two R' are branched Cn alkyl.

338. The compound of any one of the preceding claims, wherein at least one of Ri, R2, R4, and R_5 is C_{5-10} alkyl.

339. The compound of any one of the preceding claims, wherein at least one of Ri, R2, R4, and R_5 is C10 alkyl.

340. The compound of any one of the preceding claims, wherein one of Ri and R2, and one of R4, and R_5 are C_{5-10} alkyl.

341. The compound of any one of the preceding claims, wherein one of Ri and R2, and one of R4 and R_5 are C10 alkyl.

342. The compound of any one of the preceding claims, wherein Ri and R2 are each C_{5-10} alkyl.

343. The compound of any one of the preceding claims, wherein Ri and R2 are each C_8 alkyl.

344. The compound of any one of the preceding claims, wherein Ri and R2 are each C_9 alkyl.

345. The compound of any one of the preceding claims, wherein R3 and R4 are each C5-10 alkyl.

346. The compound of any one of the preceding claims, wherein R3 and R4 are each **Cs** alkyl.

347. The compound of any one of the preceding claims, wherein R3 and R4 are each C9 alkyl.

348. The compound of any one of the preceding claims, wherein **Ri,** R2, R4, and R5 are each C5-10 alkyl.

349. The compound of any one of the preceding claims, wherein **Ri,** R2, R4, and R5 are each C9 alkyl.

350. The compound of any one of the preceding claims, wherein **Ri,** R2, R4, and R5 are each **Cio** alkyl.

351. The compound of any one of the preceding claims, wherein m1 is 1 and m2 is 0.

352. The compound of any one of the preceding claims, wherein m1 is 0 and m2 is 1.

353. The compound of any one of the preceding claims, wherein m1 is 0 and m2 are each 0.

354. The compound of any one of the preceding claims, wherein m1 and m2 are each 1.

355. The compound of any one of the preceding claims, wherein M* is -0-.

356. The compound of any one of the preceding claims, wherein M* is C1-C6 alkyl.

357. The compound of any one of the preceding claims, wherein M* is **Ci** alkyl.

358. The compound of any one of the preceding claims, wherein M* is C2 alkyl.

359. The compound of any one of the preceding claims, wherein M* is C3 alkyl.

360. The compound of any one of the preceding claims, wherein M* is -((CH2)pCH(R7)(CH2)qCH(R7)(CH2)r)s-.

361. The compound of any one of the preceding claims, wherein M* **is** -((CH2)tC**(=R8)**(CH2)uC**(=R8)**(CH2)v)s-.

362. The compound of any one of the preceding claims, wherein W^1 and W^2 are each a bond.

363. The compound of any one of the preceding claims, wherein W^1 and W^2 are each -0-.

364. The compound of any one of the preceding claims, wherein W^1 and W^2 are each -N(R.6)-.

365. The compound of any one of the preceding claims, wherein W^1 is a bond and W^2 is -N(R_6)-.

366. The compound of any one of the preceding claims, wherein W^1 is -N(R.6)- and W^2 is a bond.

367. The compound of any one of the preceding claims, wherein p is 0.

368. The compound of any one of the preceding claims, wherein q is 0.

369. The compound of any one of the preceding claims, wherein q is 1.

370. The compound of any one of the preceding claims, wherein r is 0.

371. The compound of any one of the preceding claims, wherein p, q, and r are each 0.

372. The compound of any one of the preceding claims, wherein p and r are each 0 and q is 1.

373. The compound of any one of the preceding claims, wherein t is 0.

374. The compound of any one of the preceding claims, wherein u is 0.

375. The compound of any one of the preceding claims, wherein v is 0.

376. The compound of any one of the preceding claims, wherein t, u, and v are each 0.

377. The compound of any one of the preceding claims, wherein s is 1.

378. The compound of any one of the preceding claims, wherein s is 1 and p, q, and r are each 0.

379. The compound of any one of the preceding claims, wherein s is 1, p and r are each 0, and q is 1.

380. The compound of any one of the preceding claims, wherein s is 1 and t, u, and v are each 0.

381. The compound of any one of the preceding claims, wherein each each R_7 is Ci-3 alkyl.

382. The compound of any one of the preceding claims, wherein R_7 is Ci alkyl.

383. The compound of any one of the preceding claims, wherein R_8 is CH(CH2)oCN.

384. The compound of any one of the preceding claims, wherein o is 0.

385. The compound of any one of the preceding claims, wherein at least one R_6 is H.

386. The compound of any one of the preceding claims, wherein each R_6 is H.

387. The compound of any one of the preceding claims, wherein at least one R_6 is Ci alkyl.

388. The compound of any one of the preceding claims, wherein each R_6 is Ci alkyl.

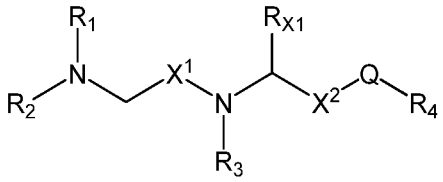
389. The compound of any one of the preceding claims, wherein at least one R_6 is C_2 alkyl.

390. The compound of any one of the preceding claims, wherein each R_6 is C_2 alkyl.

391. The compound of any one of the preceding claims, wherein the compound is selected from Compounds 145-156, and salts or isomers thereof.

392. The compound of any one of the preceding claims, wherein the compound is selected from Compounds 164 and 170-189, and salts or isomers thereof.

393. A compound having the formula:



(Ie), or a salt or isomer thereof, wherein

Ri, R2, R3, and R_4 are independently selected from the group consisting of C5-20 alkyl, C5-20 alkenyl, -R"MR', -R*YR", -YR", and -R*OR";

Rxi is H or C1-3 alkyl;

each M is independently selected from the group consisting of -C(0)0-, -OC(O)-, -OC(0)0-, -C(0)N(R')-, -N(R')C(0)-, -C(O)-, -C(S)-, -C(S)S-, -SC(S)-, -CH(OH)-, -P(0)(OR')0-, -S(0)$_2$-, -C(0)S-, -SC(O)-, an aryl group, and a heteroaryl group;

X^1, and X^2 are independently selected from the group consisting of a bond, **-CH2-,** -$(CH_2)_2$-, -CHR-, -CHY-, -C(O)-, -C(0)0-, -OC(O)-, -$(CH_2)_n$-C(0)-, -C(0)-$(CH_2)_n$-,-$(CH_2)_n$-C(0)0-, -OC(0)-$(CH_2)_n$-, -$(CH_2)_n$-OC(0)-, -C(0)0-$(CH_2)_n$-, -CH(OH)-, -C(S)-, and -CH(SH)-;

Q is **-NR9-,** or a bond;

R^9 is H or **Ci-3** alkyl;

each Y is independently a **C3-6** carbocycle;

each R* is independently selected from the group consisting of **Ci-i$_2$** alkyl and **C$_2$-i$_2$** alkenyl;

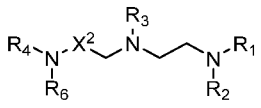
each R is independently selected from the group consisting of **C1-3** alkyl and a **C3-6** carbocycle;

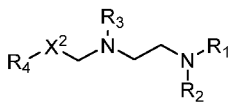
each R' is independently selected from the group consisting of **C1-18** alkyl, **C$_2$-is** alkenyl, and H;

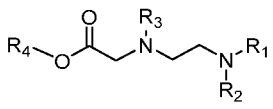
each R" is independently selected from the group consisting of **C 3-i$_2$** alkyl, ***C3-12*** alkenyl and -R*MR'; and

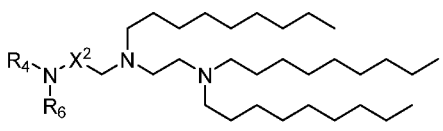
n is an integer from 1-6.

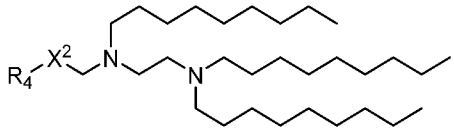
394. The compound of any one of the preceding claims, being of any of formulae (Iel)-(Ie6):


(Ie1),

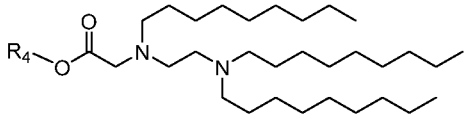

(Ie2),


(Ie3),


(Ie4),



(Ie5), or



(Ie6).

395. The compound of any one of the preceding claims, wherein X^1 is CH2.

396. The compound of any one of the preceding claims, wherein X^2 is -C(O)-.

397. The compound of any one of the preceding claims, wherein X^2 is -OC(O)-.

398. The compound of any one of the preceding claims, wherein X^2 is -C(0)O.

399. The compound of any one of the preceding claims, wherein X^2 is -CH20C(0)-.

400. The compound of any one of the preceding claims, wherein X^2 is - CH2C(0)O.

401 . The compound any one of the preceding claims, wherein Rxi is H .

402. The compound any one of the preceding claims, wherein Rxi is C1-3 alkyl.

403. The compound any one of the preceding claims, wherein Rxi is C i alkyl.

404. The compound of any one of the preceding claims, wherein Ri, R2, R 3 and R_4 are the same.

405. The compound of any one of the preceding claims, wherein R_4 is different from Ri, R2, and R3.

406. The compound of any one of the preceding claims, wherein R4is C_{5-10} alkyl.

407. The compound of any one of the preceding claims, wherein R4is C_8 alkyl.

408. The compound of any one of the preceding claims, wherein R4is C_9 alkyl.

409. The compound of any one of the preceding claims, wherein R4is C10 alkyl.

410. The compound of any one of the preceding claims, wherein R4is Cii-20 alkyl.

411. The compound of any one of the preceding claims, wherein R^4 is C_{13} alkyl.

412. The compound of any one of the preceding claims, wherein R^4 is C_{14} alkyl.

413. The compound of any one of the preceding claims, wherein **Ri** is **-R"MR'.**

414. The compound of any one of the preceding claims, wherein **R2** is **-R"MR'.**

415. The compound of any one of the preceding claims, wherein **R3** is **-R"MR'.**

416. The compound of any one of the preceding claims, wherein at least one of **Ri, R2** and **R3** is **-R"MR'.**

417. The compound of any one of the preceding claims, wherein **Ri, R2** and **R3** are each **-R"MR'.**

418. The compound of any one of the preceding claims, wherein **R4** is **-R"MR'.**

419. The compound of any one of the preceding claims, wherein M is -C(0)O.

420. The compound of any one of the preceding claims, wherein M is -OC(O)-.

421. The compound of any one of the preceding claims, wherein at least one **R"** is C_{3-7} alkyl.

422. The compound of any one of the preceding claims, wherein at least one **R"** is C_4 alkyl.

423. The compound of any one of the preceding claims, wherein at least one **R"** is C_{1-5} alkyl.

424. The compound of any one of the preceding claims, wherein at least one **R"** is C_4 alkyl.

425. The compound of any one of the preceding claims, wherein each **R"** is C_{3-7} alkyl.

426. The compound of any one of the preceding claims, wherein each **R"** is C_4 alkyl.

427. The compound of any one of the preceding claims, wherein at least one **R'** is C_{1-5} alkyl.

428. The compound of any one of the preceding claims, wherein each **R'** is C_{1-5} alkyl.

429. The compound of any one of the preceding claims, wherein at least one **R'** is C_4 alkyl.

430. The compound of any one of the preceding claims, wherein each **R'** is C_4 alkyl

431. The compound of any one of the preceding claims, wherein at least one of Ri, R2, and R3 is C_{5-10} alkyl.

432. The compound of any one of the preceding claims, wherein at least one of Ri, R2, and R3 is Cio alkyl.

433. The compound of any one of the preceding claims, wherein at least one of Ri, R2, and R3 is C_9 alkyl.

434. The compound of any one of the preceding claims, wherein at least one of Ri, R2, and R3 is C_8 alkyl.

435. The compound of any one of the preceding claims, wherein Ri, R2, and R3 are each C_{5-10} alkyl.

436. The compound of any one of the preceding claims, wherein Ri, R2, and R3 are each Cio alkyl.

437. The compound of any one of the preceding claims, wherein Ri, R2, and R3 are each C_9 alkyl.

438. The compound of any one of the preceding claims, wherein Ri, R2, and R3 are each C_8 alkyl.

439. The compound of any one of the preceding claims, wherein Q is NR9.

440. The compound of any one of the preceding claims, wherein R9 is H.

441. The compound of any one of the preceding claims, wherein Q is a bond.

442. The compound of any one of the preceding claims, wherein the compound is selected from Compounds 162, 163, and 165-169 and salts or isomers thereof.

443. The nanoparticle composition of any one of the preceding claims, wherein the phospholipid is selected from the group consisting of l,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), l,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), l,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC),

l,2-distearoyl-sn-glycero-3-phosphocholine (DSPC),
1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC),
l-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC),
l,2-di-0-octadecenyl-OT-glycero-3-phosphocholine (18:0 Diether PC),
l-oleoyl-2-cholesterylhemisuccinoyl-5 «-glycero-3-phosphocholine (OChemsPC),
l-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC),
l,2-dilinolenoyl-sn-glycero-3-phosphocholine,
l,2-diarachidonoyl-sn-glycero-3-phosphocholine,
l,2-didocosahexaenoyl-sn-glycero-3-phosphocholine,l,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), l,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE),
l,2-distearoyl-sn-glycero-3-phosphoethanolamine,
l,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine,
l,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine,
l,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine,
l,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine,
l,2-dioleoyl-sn-glycero-3-phospho-rac-(l-glycerol) sodium salt (DOPG), sphingomyelin, and mixtures thereof.

444. The nanoparticle composition of any one of the preceding claims, wherein the phospholipid is DOPE.

445. The nanoparticle composition of any one of the preceding claims, wherein the phospholipid is DSPC.

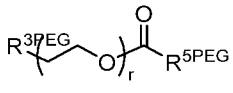
446. The nanoparticle composition of any one of the preceding claims, wherein the structural lipid is selected from the group consisting of cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, ursolic acid, alpha-tocopherol, and mixtures thereof.

447. The nanoparticle composition of any one of the preceding claims, wherein the structural lipid is cholesterol.

448. The nanoparticle composition of any one of the preceding claims, wherein the PEG lipid is selected from the group consisting of a PEG-modified phosphatidylethanolamine, a PEG-

modified phosphatidic acid, a PEG-modified ceramide, aPEG-modified dialkylaniine, aPEG-modified diacylglyceroi, a PEG-modified dialkylglycerol, and mixtures thereof.

449. The nanoparticle composition of any one of the preceding claims, wherein the PEG lipid is a compound of Formula (VI):


(VI), or a salt or isomer thereof, wherein:

R^{3PEG} is $-OR^{O}$;

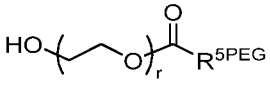
R^{O} is hydrogen, C_{1-5} alkyl or an oxygen protecting group;

r is an integer between 1 and 100;

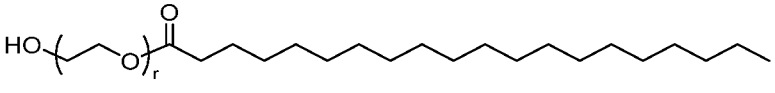
R^{5PEG} is C_{10-40} alkyl, C_{10-40} alkenyl, or C_{10-40} alkynyl; and optionally one or more methylene groups of R^{5PEG} are independently replaced with C_{3-10} carbocyclylene, 4 to 10 membered heterocyclylene, C_{6-10} arylene, 4 to 10 membered heteroarylene, $-N(R^{N})-$, -0-, -S-, -C(O)-, $-C(O)N(R^{N})-$, $-NR^{N}C(O)-$, $-NR^{N}C(O)N(R^{N})-$, -C(O)0-, -OC(O)-, -OC(O)0-, $-OC(O)N(R^{N})-$, $-NR^{N}C(O)0-$, -C(O)S-, -SC(O)-, $-C(=NR^{N})-$, $-C(=NR^{N})N(R^{N})-$, $-NR^{N}C(=NR^{N})-$, $-NR^{N}C(=NR^{N})N(R^{N})-$, -C(S)-, -C(S)N($R^{N}$)-, $-NR^{N}C(S)-$, $-NR^{N}C(S)N(R^{N})-$, -S(O)-, -OS(O)-, -S(0)0-, -OS(0)0-, $-OS(O)_2-$, $-S(O)_20-$, $-OS(O)_20-$, $-N(R^{N})S(0)-$, $-S(O)N(R^{N})-$, $-N(R^{N})S(O)N(R^{N})-$, $-OS(O)N(R^{N})-$, $-N(R^{N})S(0)0-$, $-S(O)_2-$, $-N(R^{N})S(O)_2-$, $-S(O)_2N(R^{N})-$, $-N(R^{N})S(O)_2N(R^{N})-$, $-OS(O)_2N(R^{N})-$, or $-N(R^{N})S(O)_20-$; and

each instance of R^{N} is independently hydrogen, C_{1-6} alkyl, or a nitrogen protecting group.

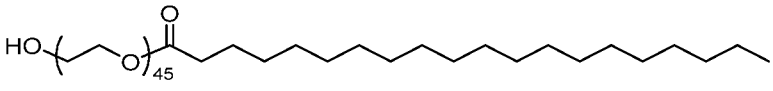
450. The nanoparticle composition of any one of the preceding claims, wherein the PEG lipid is a compound of Formula (Vl-a):


(IV-a), or a salt or isomer thereof.

451. The nanoparticle composition of any one of the preceding claims, wherein the PEG lipid is a compound of Formula (Vl-b):


, (Vl-b) or a salt or isomer thereof.

452. The nanoparticle composition of any one of the preceding claims, wherein the PEG lipid is a compound having the formula:



, or a salt or isomer thereof.

453. The nanoparticle composition of any one of the preceding claims, wherein the lipid component comprises about 30 mol % to about 60 mol % said compound, about 0 mol % to about 30 mol % phospholipid, about 18.5 mol % to about 48.5 mol % structural lipid, and about 0 mol % to about 10 mol % PEG lipid.

454. The nanoparticle composition of any one of the preceding claims, wherein the lipid component comprises about 50 mol % said compound, about 10 mol % phospholipid, about 38.5 mol % structural lipid, and about 1.5 mol % PEG lipid.

455. The nanoparticle composition of any one of the preceding claims, wherein the lipid component further comprises a cationic and/or ionizable lipid selected from the group consisting of 3-(didodecylamino)-Nl ,Nl ,4-tridodecyl-l-piperazineethanamine (KL1 0), N142-(didodecylamino)ethyl]-Nl ,N4,N4-tridodecyl-l,4-piperazinediethanamine (KL22), 14,25-ditridecyl-1 5, 18,21 ,24-tetraaza-octatriacontane (KL25), l,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[l,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,3 1-tetraen-1 9-yl 4-(dimethylamino)butanoate (DLin-MC3-DMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[l,3]-dioxolane (DLin-KC2-DMA), l,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), 2-({8-[(3P)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z, 12Z)-octadeca-9, 12-dien-l-yloxy]propan-l -amine (Octyl-CLinDMA), (2R)-2-({8-[(3P)-cholest-5-en-3-yloxy]octyl} oxy)-N,N-dimethyl-3-[(9Z, 12Z)-octadeca-9, 12-dien-l-yloxy]propan-l -amine (Octyl-CLinDMA (2R)), (2S)-2-({8-[(3P)-cholest-5-en-3-yloxy]octyl} oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9, 12-dien-l-yloxy]propan-l -amine (Octyl-CLinDMA (2S)), (12Z, 15Z)-N,N-dimethyl-2-nonylhenicosa-12, 15-dien-l -amine, and N,N-dimethyl-l- {(l S,2R)-2-octylcyclopropyl}heptadecan-8-amine.

456. The nanoparticle composition of any one of the preceding claims, further comprising a therapeutic and/or prophylactic agent.

457. The nanoparticle composition of any one of the preceding claims, wherein the therapeutic and/or prophylactic agent is a vaccine or a compound capable of eliciting an immune response.

458. The nanoparticle composition of any one of the preceding claims, wherein the therapeutic and/or prophylactic agent is a nucleic acid.

459. The nanoparticle composition of any one of the preceding claims, wherein the therapeutic and/or prophylactic agent is a ribonucleic acid (RNA).

460. The nanoparticle composition of any one of the preceding claims, wherein the RNA is selected from the group consisting of a small interfering RNA (siRNA), an asymmetrical interfering RNA (aiRNA), a microRNA (miRNA), a Dicer-substrate RNA (dsRNA), a small hairpin RNA (sliRNA), a messenger RNA (niRNA), and mixtures thereof.

461. The nanoparticle composition of any one of the preceding claims, wherein the RNA is an mRNA.

462. The nanoparticle composition of any one of the preceding claims, wherein the mRNA includes one or more of a stem loop, a chain terminating nucleoside, a polyA sequence, a polyadenylation signal, and/or a 5' cap structure.

463. The nanoparticle composition of any one of the preceding claims, wherein the encapsulation efficiency of the therapeutic and/or prophylactic agent is at least 50%.

464. The nanoparticle composition of any one of the preceding claims, wherein the encapsulation efficiency of the therapeutic and/or prophylactic agent is at least 80%.

465. The nanoparticle composition of any one of the preceding claims, wherein the encapsulation efficiency of the therapeutic and/or prophylactic agent is at least 90%.

466. The nanoparticle composition of any one of the preceding claims, wherein the wt/wt ratio of the lipid component to the therapeutic and/or prophylactic agent is from about 10: 1 to about 60:1.

467. The nanoparticle composition of any one of the preceding claims, wherein the wt/wt ratio of the lipid component to the therapeutic and/or prophylactic agent is about 20:1.

468. The nanoparticle composition of any one of the preceding claims, wherein the N:P ratio is from about 2:1 to about 30: 1.

469. The nanoparticle composition of any one of the preceding claims, wherein the N:P ratio is about 5.67: 1.

470. The nanoparticle composition of any one of the preceding claims, wherein the mean size of the nanoparticle composition is from about 70 nm to about 100 nm.

471. The nanoparticle composition of any one of the preceding claims, wherein the polydispersity index of the nanoparticle composition is from about 0.10 to about 0.20.

472. The nanoparticle composition of any one of the preceding claims, wherein the nanoparticle composition has a zeta potential of about -10 mV to about +20 mV.

473. The nanoparticle composition of any one of the preceding claims, wherein upon contacting the nanoparticle composition with a mammalian cell, the cell uptake of the nanoparticle composition is LDLR-independent.

474. The nanoparticle composition of any one of the preceding claims, wherein upon contacting the nanoparticle composition with a mammalian cell, the cell uptake of the nanoparticle composition is LDLR-dependent.

475. The nanoparticle composition of any one of the preceding claims, wherein upon contacting the nanoparticle composition with a mammalian cell, the cell uptake of the nanoparticle composition is apoE-independent.

476. The nanoparticle composition of any one of the preceding claims, wherein upon contacting the nanoparticle composition with a mammalian cell, the cell uptake of the nanoparticle composition is apoE-dependent.

477. The nanoparticle composition of any one of the preceding claims, wherein upon contacting the nanoparticle composition with a mammalian cell, the cell uptake of the nanoparticle composition is LDLR-apoE-interaction dependent.

478. The nanoparticle composition of any one of the preceding claims, wherein upon contacting the nanoparticle composition with a mammalian cell, the cell uptake of the nanoparticle composition is LDLR-apoE-interaction independent.

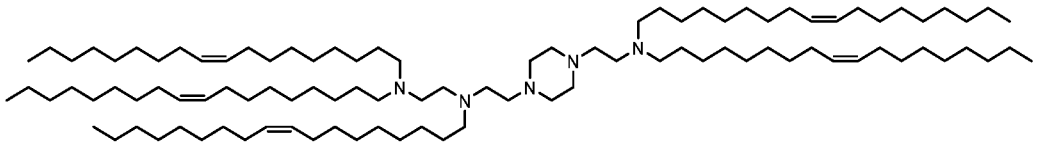
479. The nanoparticle composition of any one of the preceding claims, wherein upon contacting the nanoparticle composition with a mammalian cell to produce a polypeptide, the production of the polypeptide is higher in mammalian hepatocytes than cells from a different tissue.

480. The nanoparticle composition of any one of the preceding claims, wherein upon contacting the nanoparticle composition with a mammalian cell to produce a polypeptide, the production of the polypeptide occurs substantively in mammalian hepatocytes.

481. A pharmaceutical composition comprising the nanoparticle composition of any one of the preceding claims and a pharmaceutically acceptable carrier.

482. A method of delivering a therapeutic and/or prophylactic agent to a mammalian cell, the method comprising administering to a subject the nanoparticle composition of any one of the preceding claims, said administering comprising contacting the cell with the nanoparticle composition, whereby the therapeutic and/or prophylactic agent is delivered to the cell.

483. A method of delivering an mRNA to a mammalian cell, the method comprising administering to a subject a nanoparticle composition comprising (i) a lipid component comprising a phospholipid, a structural lipid, a PEG lipid, wherein the PEG lipid is a compound of any one of claims 449-452, and a compound of formula (IV):



(IV),

and (ii) an mRNA, said administering comprising contacting the cell with the nanoparticle composition, whereby the therapeutic and/or prophylactic agent is delivered to the cell.

484. The method of any one of the preceding claims, wherein the nanoparticle composition is administered intravenously, intramuscularly, intradermally, subcutaneously, intranasally, or by inhalation.

485. The method of any one of the preceding claims, wherein a dose of about 0.01 mg/kg to about 10 mg/kg of the therapeutic and/or prophylactic agent is administered to the mammal.

486. The method of any one of the preceding claims, wherein the delivery of the therapeutic and/or prophylactic agent to the mammalian cell is LDLR-independent.

487. The method of any one of the preceding claims, wherein the delivery of the therapeutic and/or prophylactic agent to the mammalian cell is LDLR-dependent.

488. The method of any one of the preceding claims, wherein the delivery of the therapeutic and/or prophylactic agent to the mammalian cell is apoE-independent.

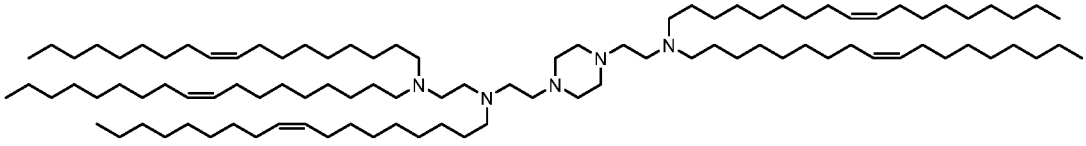
489. The method of any one of the preceding claims, wherein the delivery of the therapeutic and/or prophylactic agent to the mammalian cell is apoE-dependent.

490. The method of any one of the preceding claims, wherein the delivery of the therapeutic and/or prophylactic agent to the mammalian cell is LDLR-apoE-interaction independent.

491. The method of any one of the preceding claims, wherein the delivery of the therapeutic and/or prophylactic agent to the mammalian cell is LDLR-apoE-interaction dependent.

492. A method of producing a polypeptide of interest in a mammalian cell, the method comprising contacting the cell with the nanoparticle composition of any one of the preceding claims, wherein the therapeutic and/or prophylactic agent is an mRNA, and wherein the mRNA encodes the polypeptide of interest, whereby the mRNA is capable of being translated in the cell to produce the polypeptide of interest.

493. A method of producing a polypeptide of interest in a mammalian cell, the method comprising contacting the cell with a nanoparticle composition comprising (i) a lipid component comprising a phospholipid, a structural lipid, a PEG lipid, wherein the PEG lipid is a compound of any one of claims 449-452, and a compound of formula (IV)



(IV),

and (ii) an mRNA encoding the polypeptide of interest, whereby the mRNA is capable of being translated in the cell to produce the polypeptide of interest.

494. The method of any one of the preceding claims, wherein the mammalian cell is in a mammal.

495. The method of any one of the preceding claims, wherein the production of the polypeptide is higher in mammalian hepatocytes than cells from a different tissue.

496. The method of any one of the preceding claims, wherein the production of the polypeptide occurs substantively in mammalian hepatocytes.

497. The method of any one of the preceding claims, wherein the nanoparticle composition is administered intravenously, intramuscularly, intradermally, subcutaneously, intranasally, or by inhalation.

498. The method of any one of the preceding claims, wherein a dose of about 0.01 mg/kg to about 10 mg/kg of the mRNA is administered to the mammal.

499. The method of any one of the preceding claims, wherein the production of the polypeptide of interest in the mammalian cell is LDLR-independent.

500. The method of any one of the preceding claims, wherein the production of the polypeptide of interest in the mammalian cell is LDLR-dependent.

501. The method of any one of the preceding claims, wherein the production of the polypeptide of interest in the mammalian cell is apoE-independent.

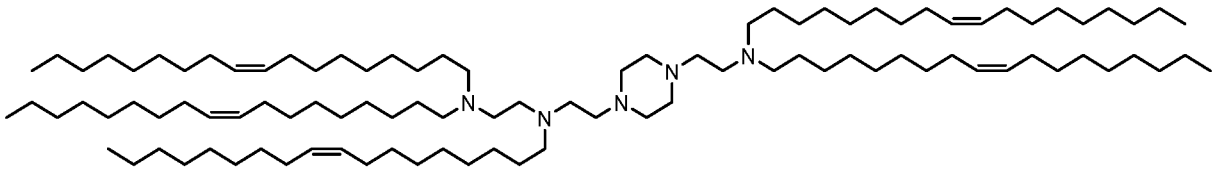
502. The method of any one of the preceding claims, wherein the production of the polypeptide of interest in the mammalian cell is apoE-dependent.

503. The method of any one of the preceding claims, wherein the production of the polypeptide of interest in the mammalian cell is LDLR-apoE-interaction independent.

504. The method of any one of the preceding claims, wherein the production of the polypeptide of interest in the mammalian cell is LDLR-apoE-interaction dependent.

505. A method of treating a disease or disorder in a mammal in need thereof, the method comprising administering to the mammal a therapeutically effective amount of the nanoparticle composition of any one of the preceding claims.

506. A method of treating a disease or disorder in a mammal in need thereof, the method comprising administering to the mammal a therapeutically effective amount of a nanoparticle composition comprising (i) a lipid component comprising a phospholipid, a structural lipid, a PEG lipid, wherein the PEG lipid is a compound of any one of claims 449-452, and a compound of the formula (IV)



(IV),

and (ii) an mRNA.

507. The method of any one of the preceding claims, wherein the disease or disorder is characterized by dysfunctional or aberrant protein or polypeptide activity.

508. The method of any one of the preceding claims, wherein the disease or disorder is selected from the group consisting of infectious diseases, cancer and proliferative diseases, genetic diseases, autoimmune diseases, diabetes, neurodegenerative diseases, cardio- and renovascular diseases, and metabolic diseases.

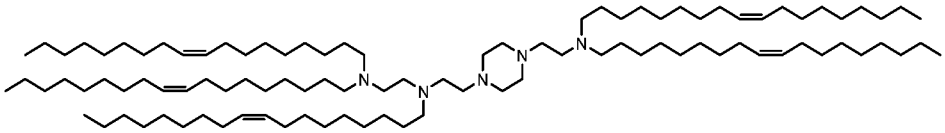
509. The method of any one of the preceding claims, wherein the nanoparticle composition is administered intravenously, intramuscularly, intradermally, subcutaneously, intranasally, or by inhalation.

510. The method of any one of the preceding claims, wherein a dose of about 0.01 mg/kg to about 10 mg/kg of the therapeutic and/or prophylactic agent is administered to the mammal.

511. A method of specifically delivering a therapeutic and/or prophylactic agent to a mammalian organ, the method comprising administering to a mammal the nanoparticle composition of any one of the preceding claims, said administering comprising contacting the mammalian organ with the nanoparticle composition, whereby the therapeutic and/or prophylactic agent is delivered to the organ.

512. A method of specifically delivering an mRNA to a mammalian organ, the method comprising administering to a mammal a nanoparticle composition comprising (i) a lipid

component comprising a phospholipid, a structural lipid, a PEG lipid, wherein the PEG lipid is a compound of any one of claims 449-452, and a compound of the formula (IV)



(IV),

and (ii) an mRNA, said administering comprising contacting the mammalian organ with the nanoparticle composition, whereby the therapeutic and/or prophylactic agent is delivered to the organ.

513. The method of any one of the preceding claims, wherein the delivery of said mRNA to liver is substantively higher than that to a different organ.

514. The method of any of the preceding claims, wherein the nanoparticle composition comprises a compound of formula (I).

515. The method of any of the preceding claims, wherein the nanoparticle composition comprises Compound 4.

516. The method of any one of the preceding claims, wherein the nanoparticle composition is administered intravenously, intramuscularly, intradermally, subcutaneously, intranasally, or by inhalation.

517. The method of any one of the preceding claims, wherein a dose of about 0.01 mg/kg to about 10 mg/kg of the therapeutic and/or prophylactic agent is administered to the mammal.

518. The method of any one of the preceding claims, further comprising, prior to the contacting or administering step, pretreating said mammal with one or more additional compounds, wherein pretreating comprises administering said one or more additional compounds to said mammal.

519. The method of any one of the preceding claims, wherein said mammal is pretreated 24 or fewer hours prior to the contacting or administering step.

520. The method of any one of the preceding claims, wherein said mammal is pretreated about one hour prior to the contacting or administering step.

521. The method of any one of the preceding claims, wherein said one or more additional compounds are selected from the group consisting of anti-inflammatory compounds, steroids, statins, estradiols, BTK inhibitors, S1P1 agonists, glucocorticoid receptor modulators (GRMs), and anti-histamines.

522. The method of any one of the preceding claims, wherein said one or more additional compounds are selected from the group consisting of dexamethasone, methotrexate, acetaminophen, an H1 receptor blocker, and an H2 receptor blocker.

523. The method of any one of the preceding claims, wherein the mammal is LDLR-deficient.

524. The method of any one of the preceding claims, wherein the mammal is not LDLR-deficient.

525. The method of any one of the preceding claims, wherein the mammal is apoE-deficient.

526. The method of any one of the preceding claims, wherein the mammal is not apoE-deficient.

527. The method of any one of the preceding claims, wherein the mammal has an abnormal LDLR-apoE interaction.

528. The method of any one of the preceding claims, wherein the mammal has a normal LDLR-apoE interaction.

529. The method of any of the preceding claims, wherein the mammal is a human.

FIGURE 1A

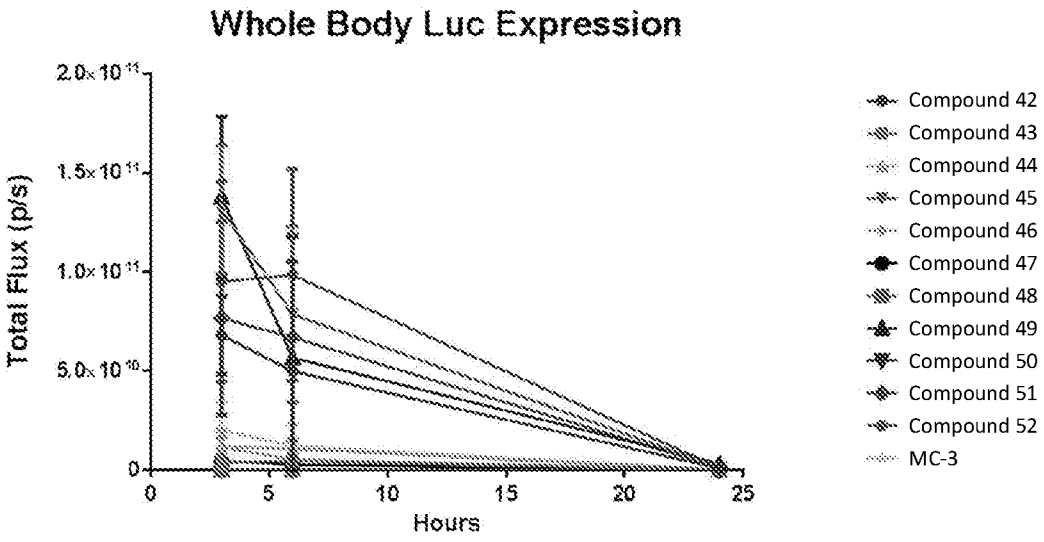


FIGURE 1B

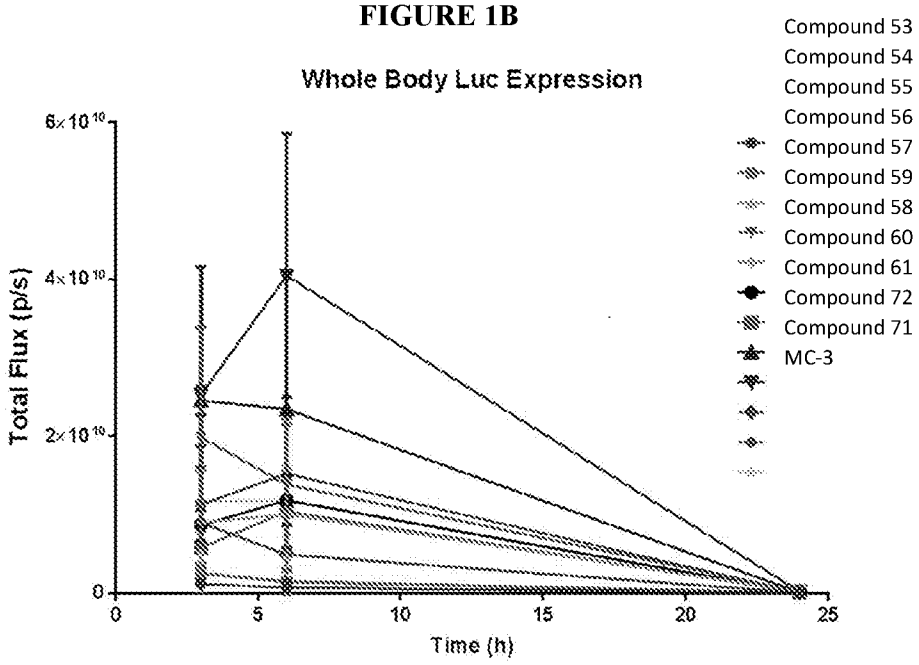


FIGURE 2

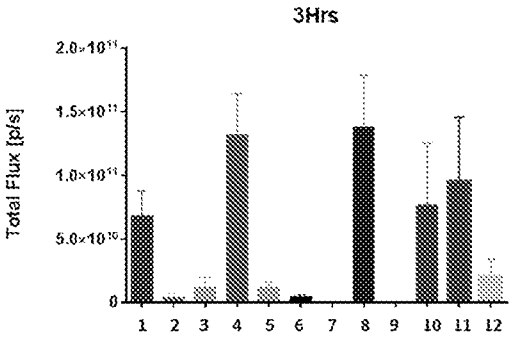


FIGURE 3

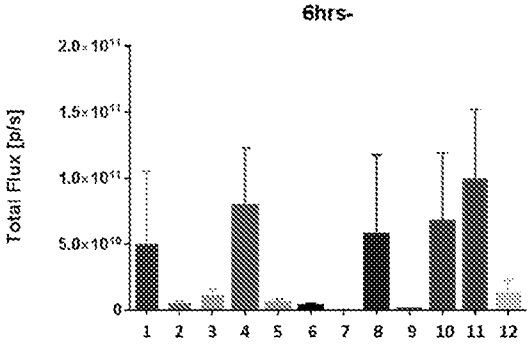


FIGURE 4

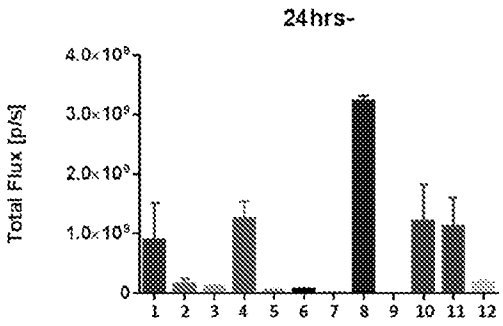


FIGURE 5

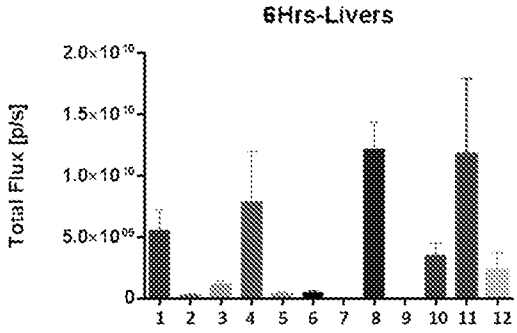


FIGURE 6

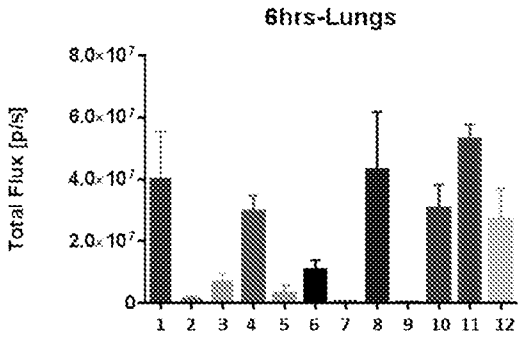


FIGURE 7

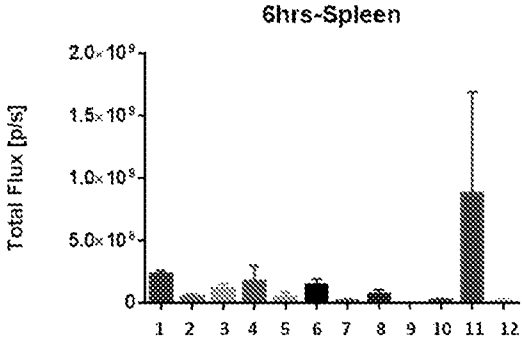


FIGURE 8A

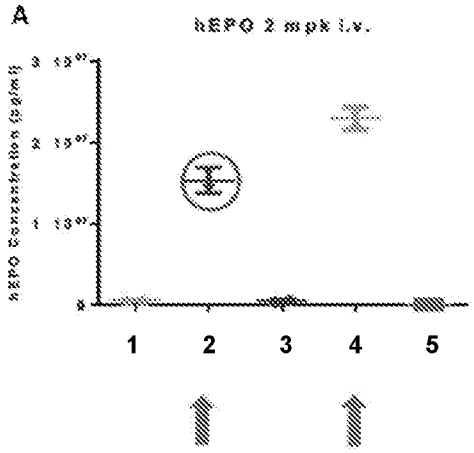


FIGURE 8B

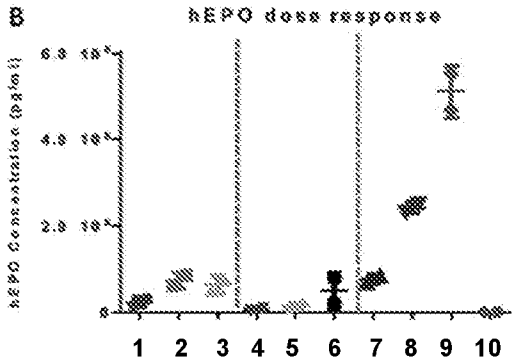


FIGURE 9A

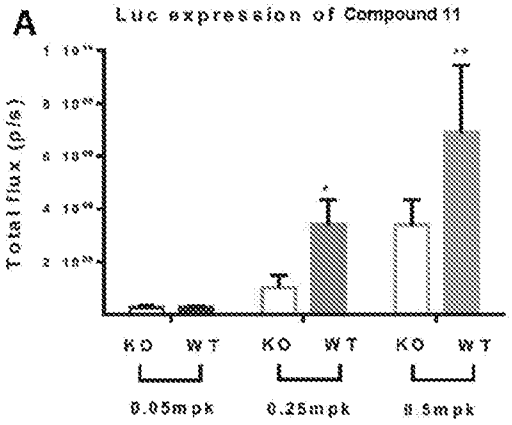


FIGURE 9B

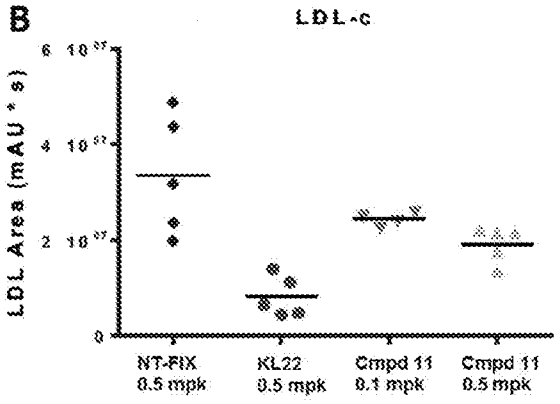


FIGURE 10

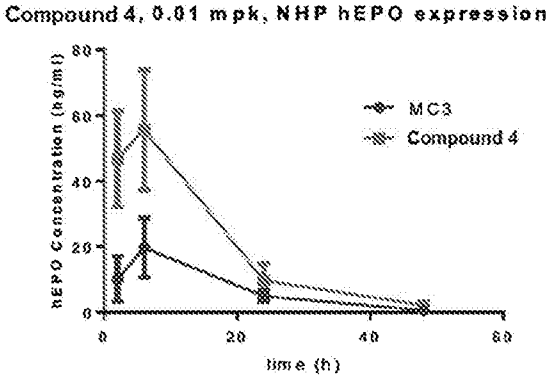


FIGURE 11A

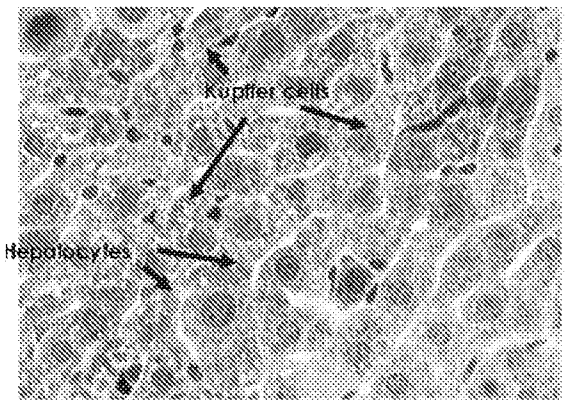


FIGURE 11B

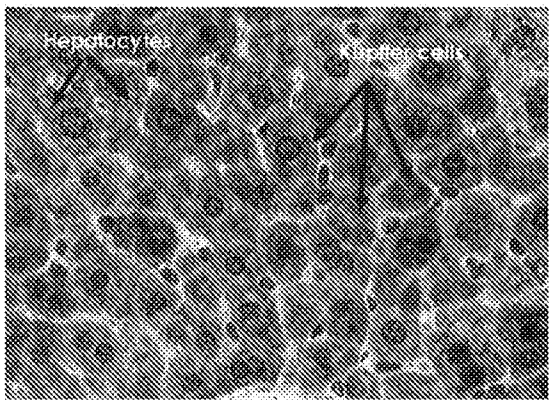


FIGURE 12A

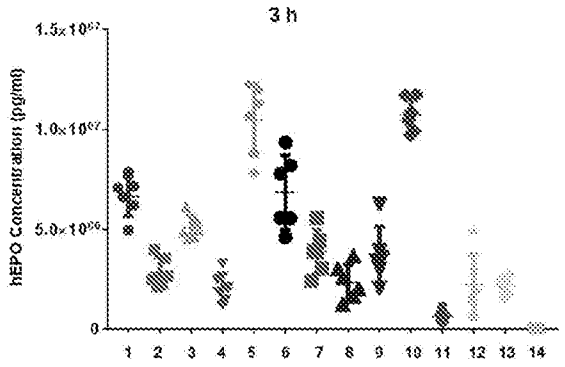


FIGURE 12B

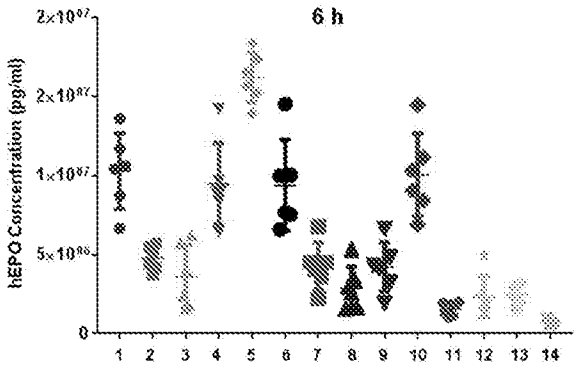


FIGURE 12C

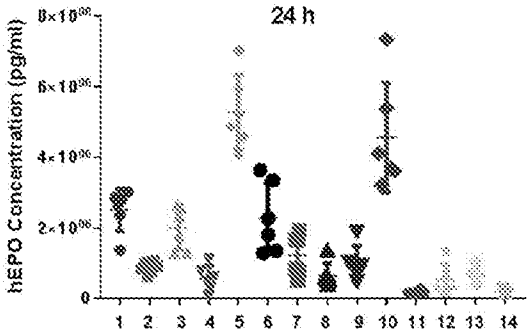


FIGURE 13

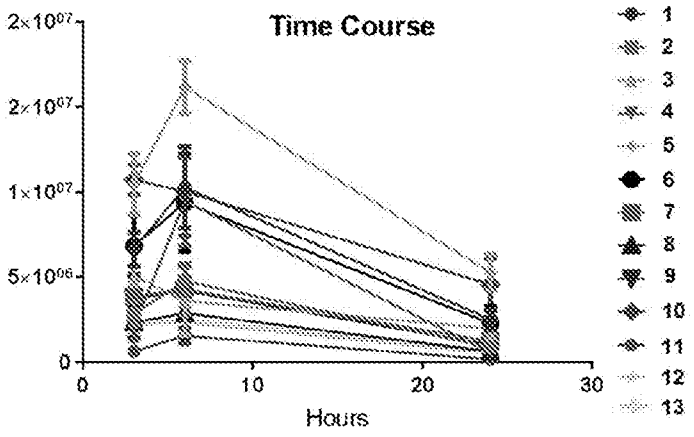


FIGURE 14A

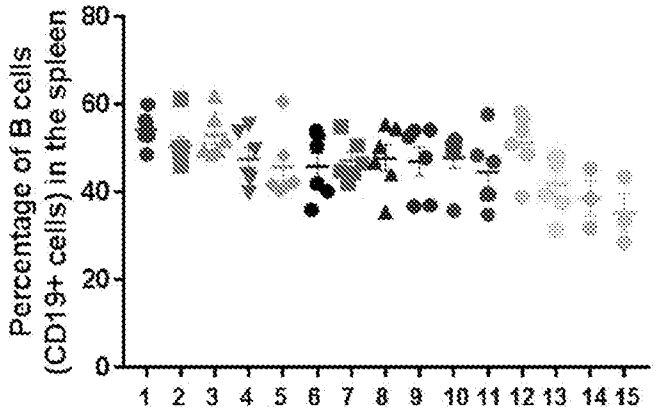


FIGURE 14B

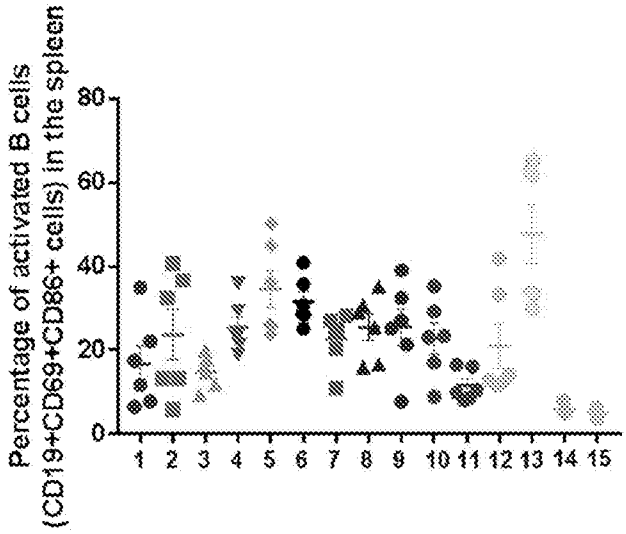


FIGURE 15

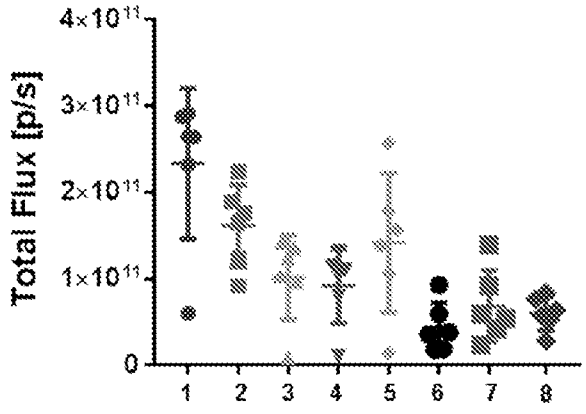


FIGURE 16

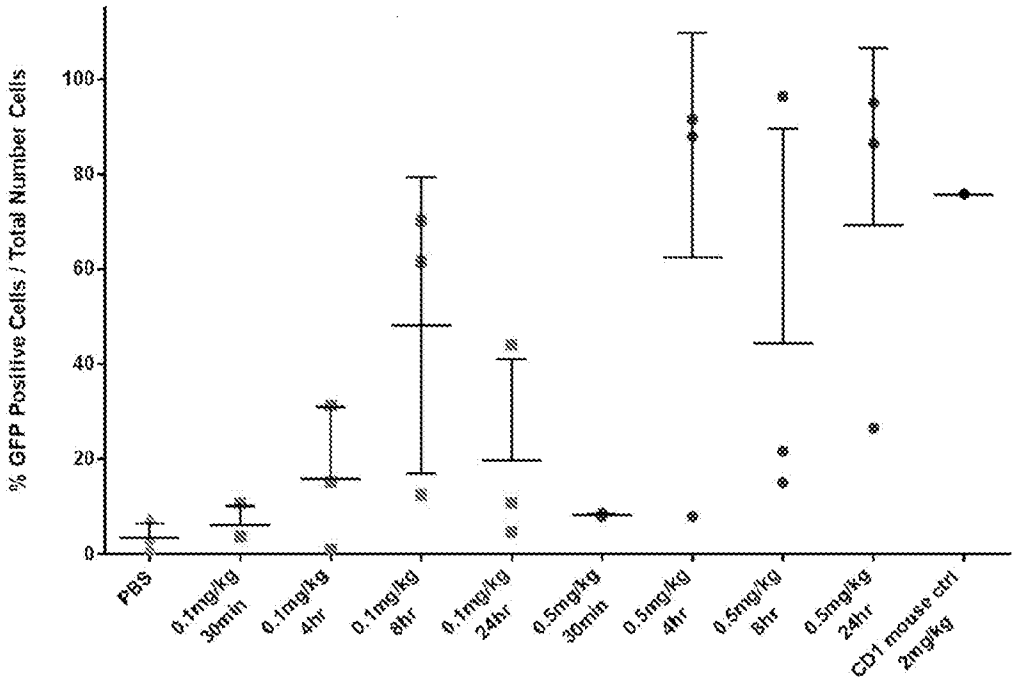


FIGURE 17A

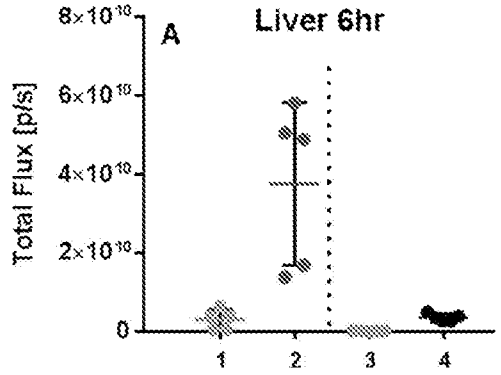


FIGURE 17B

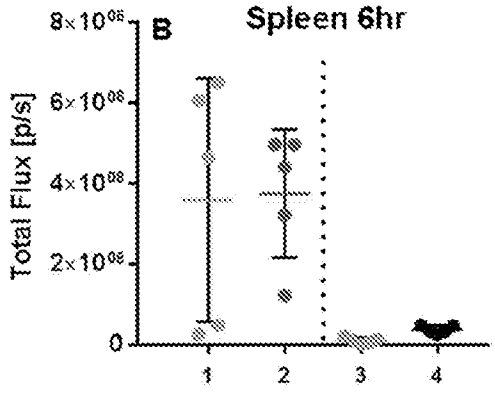


FIGURE 18A

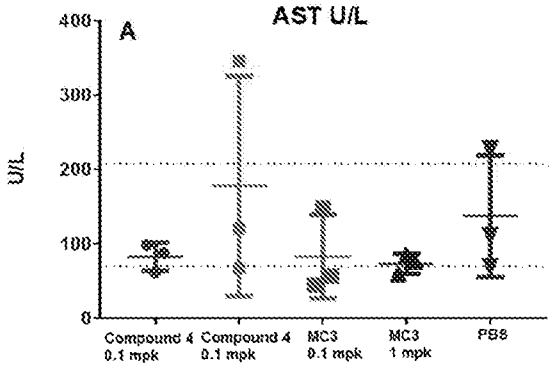


FIGURE 18B

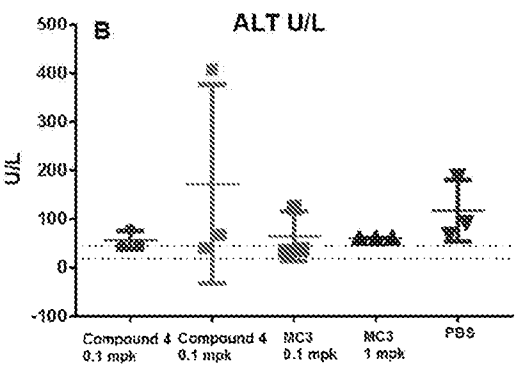


FIGURE 19A

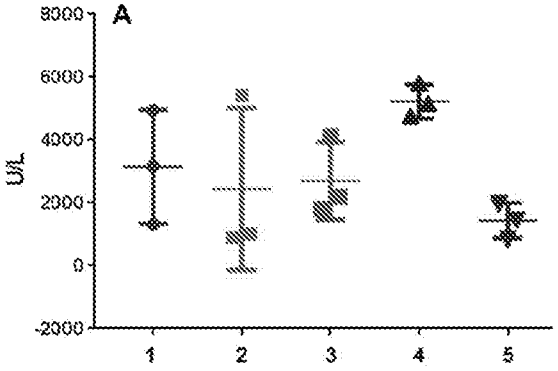


FIGURE 19B

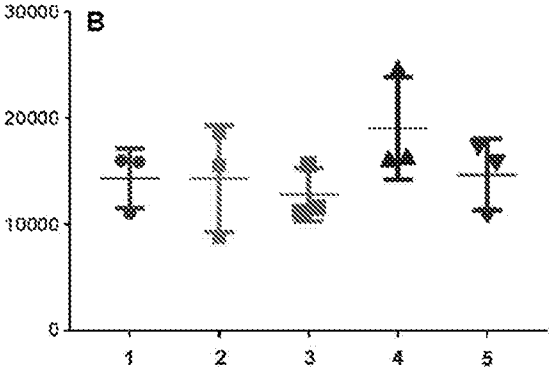


FIGURE 19C

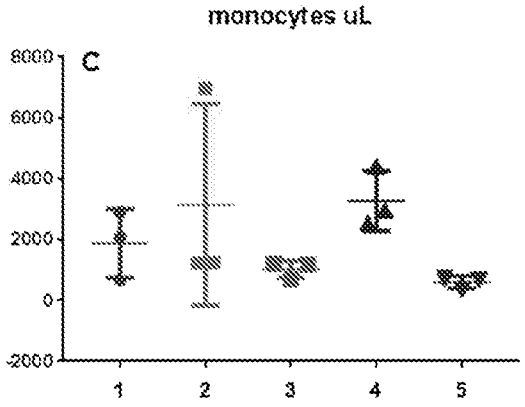


FIGURE 20

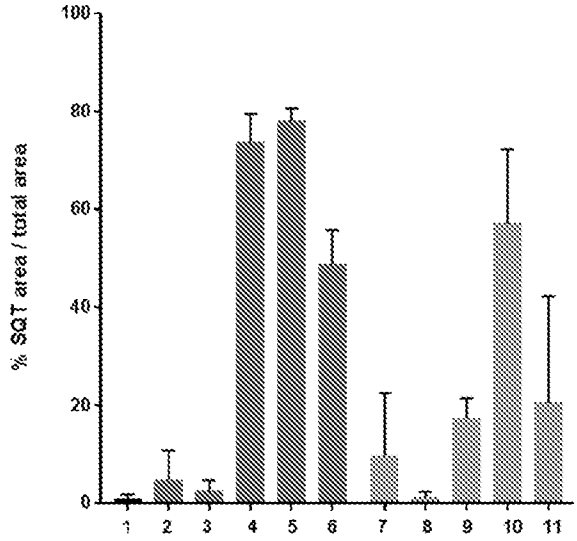


FIGURE 21

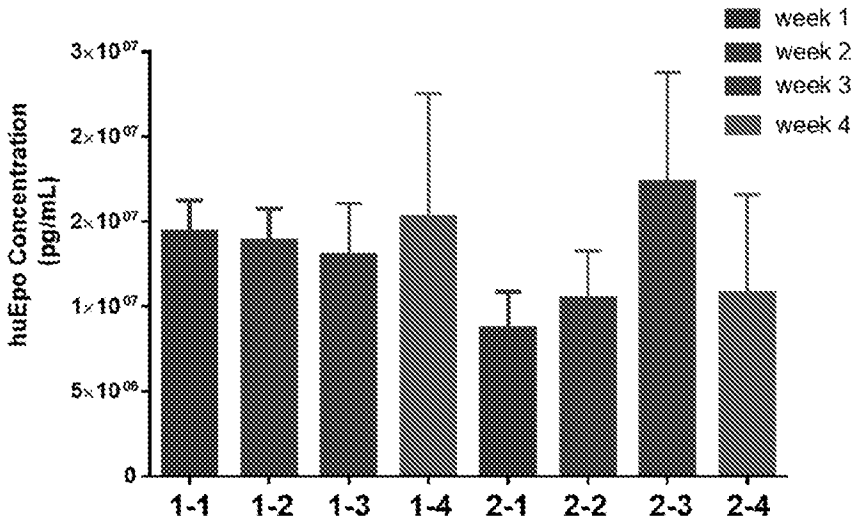


FIGURE 22A

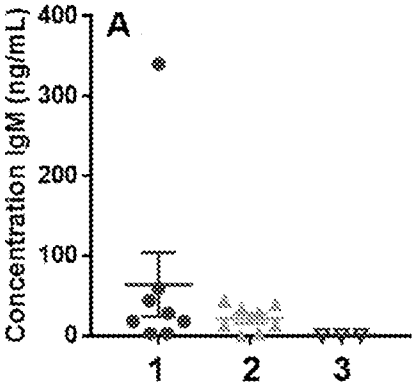


FIGURE 22B

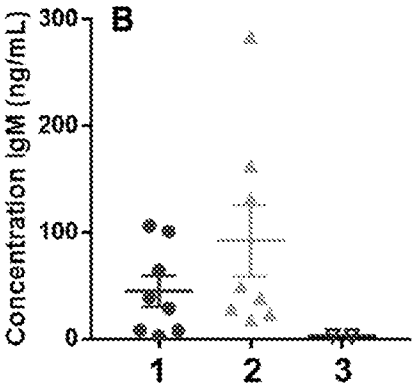


FIGURE 23A

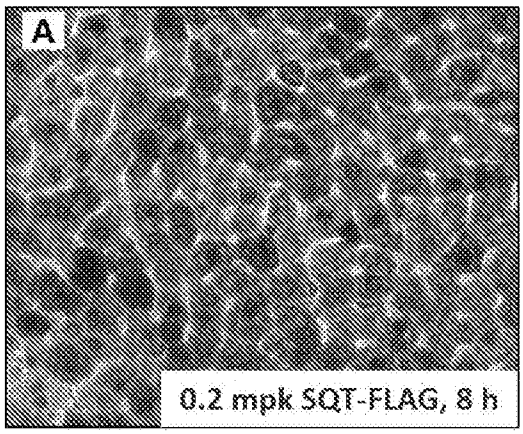


FIGURE 23B

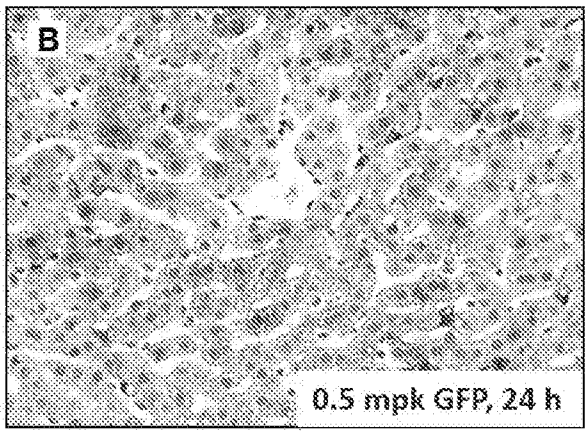


FIGURE 24

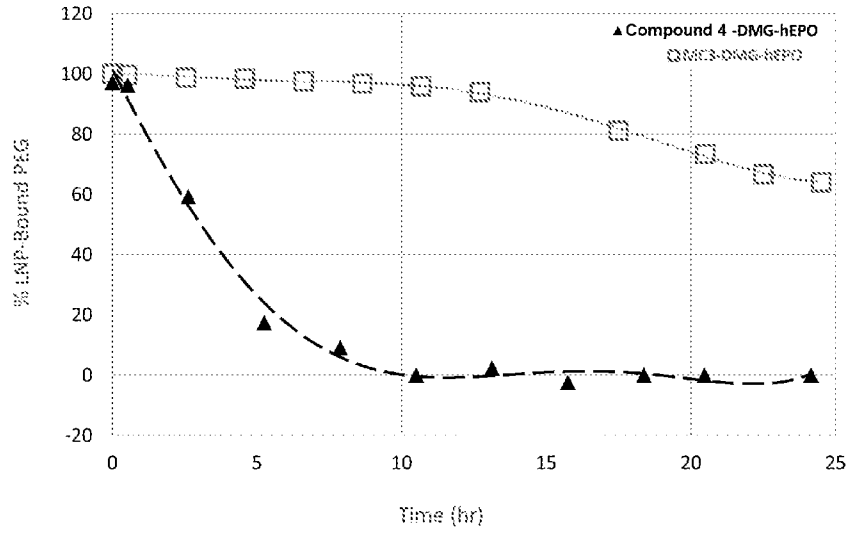


FIGURE 25

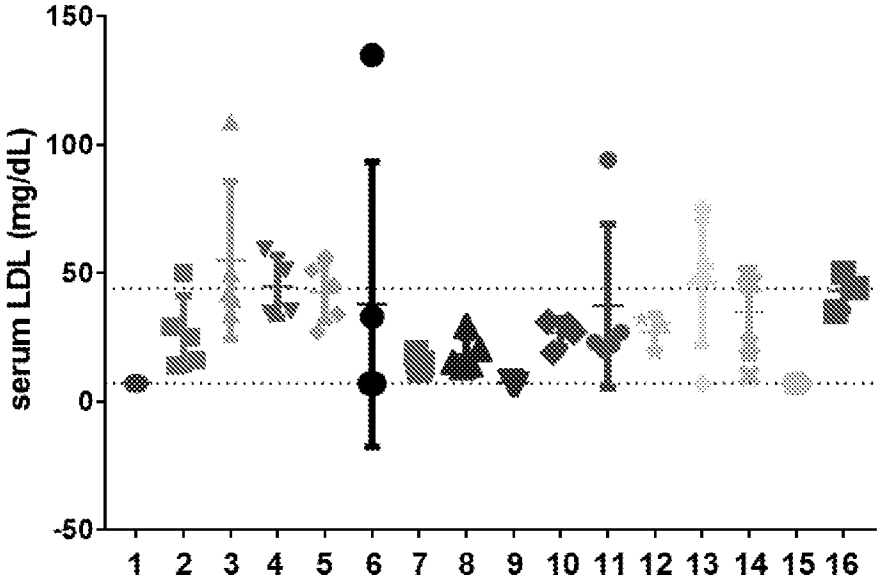


FIGURE 26

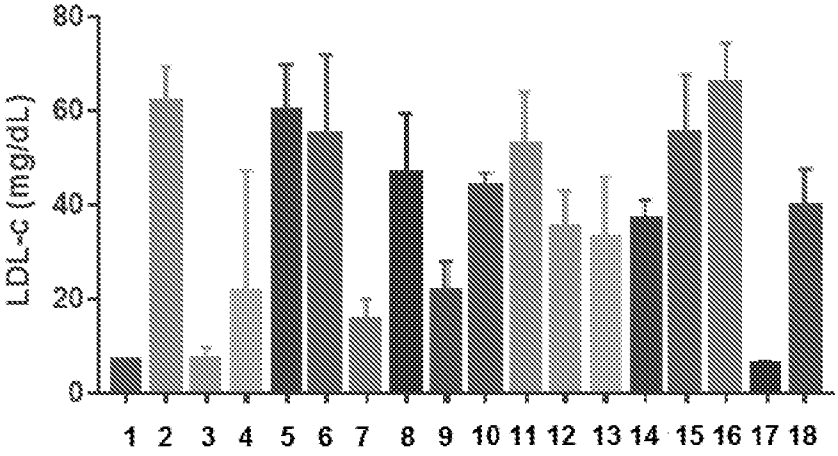


FIGURE 27A

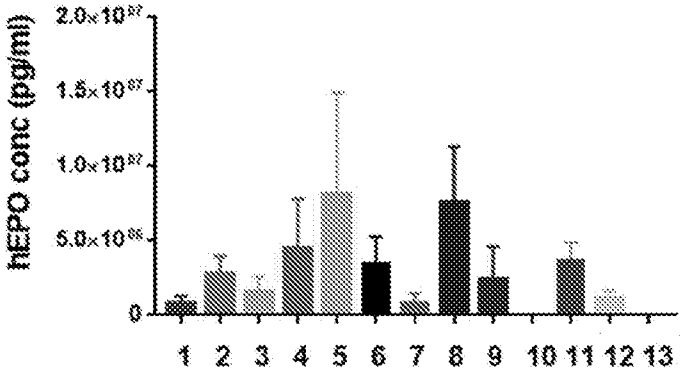


FIGURE 27B

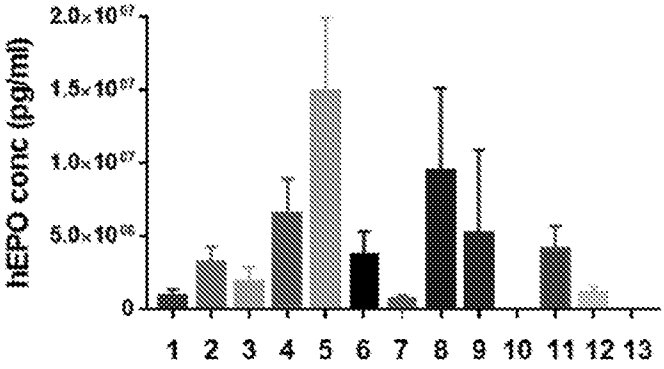


FIGURE 27C

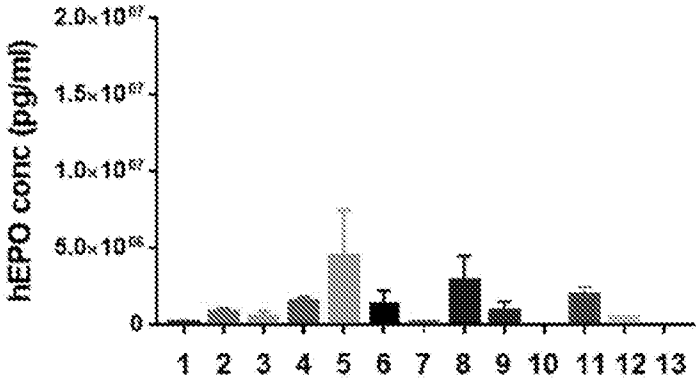


FIGURE 28

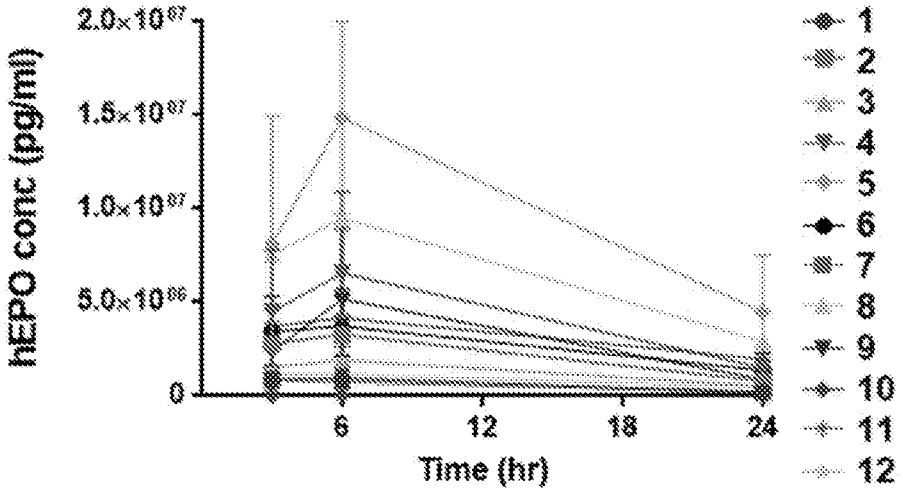


FIGURE 29A

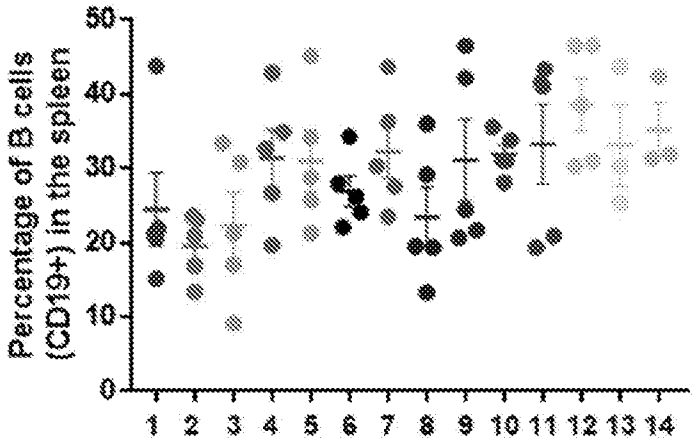


FIGURE 29B

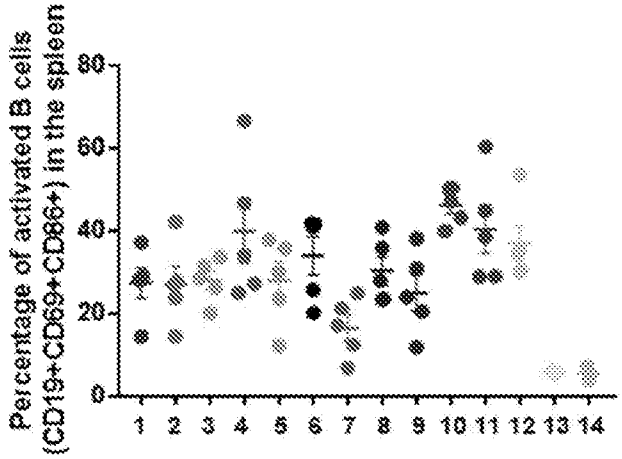


FIGURE 30

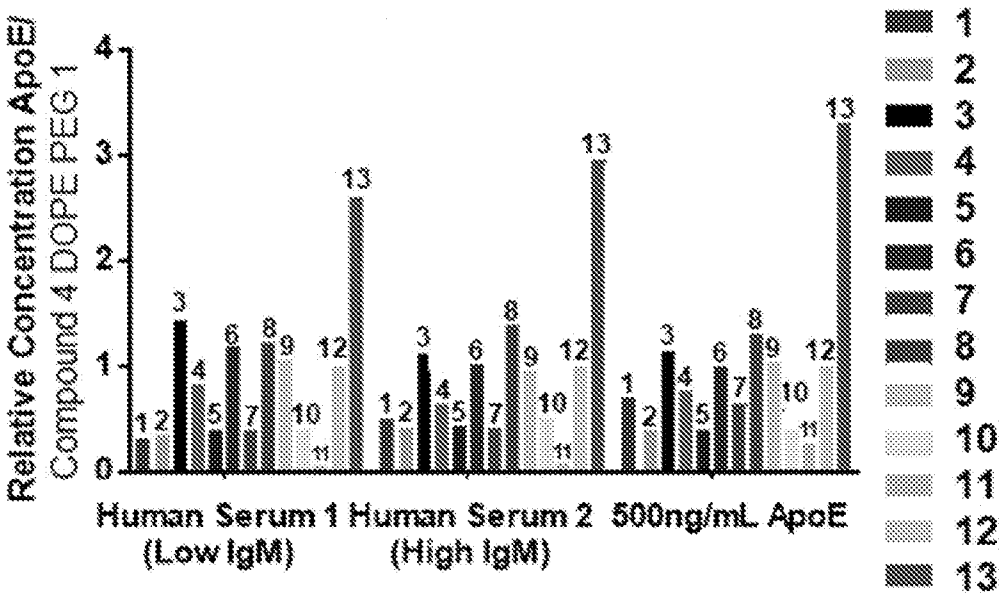


FIGURE 31

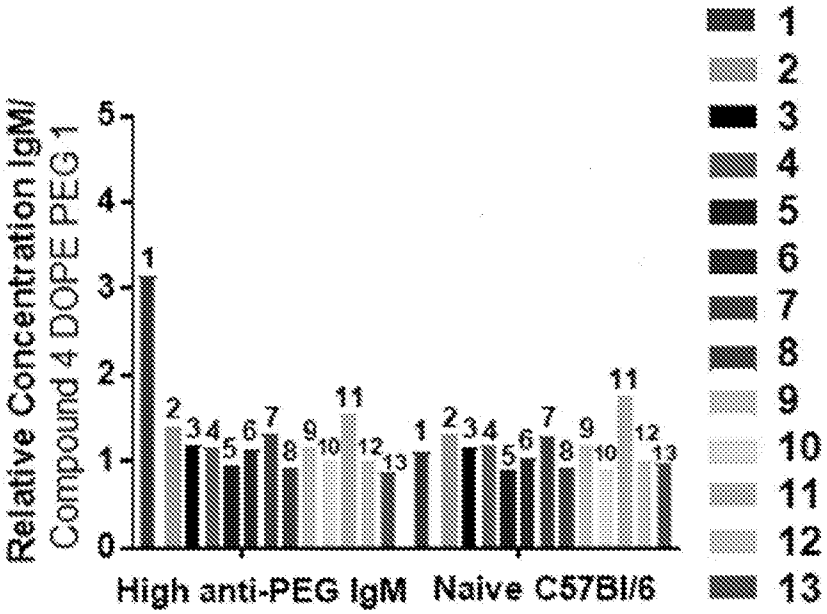


FIGURE 32A

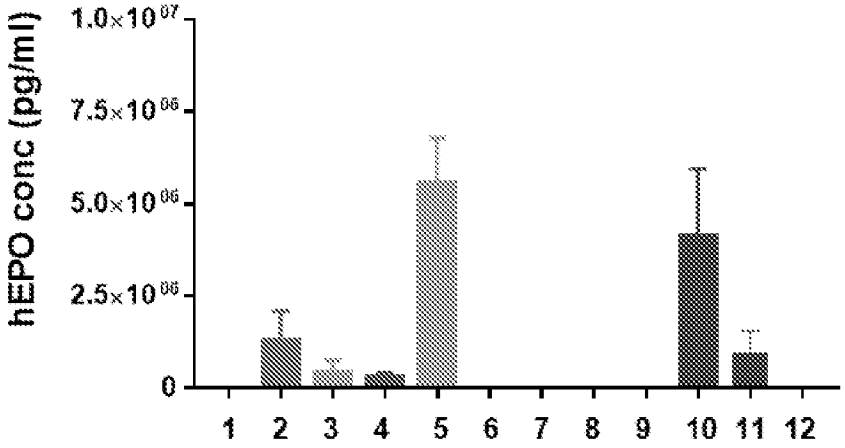


FIGURE 32B

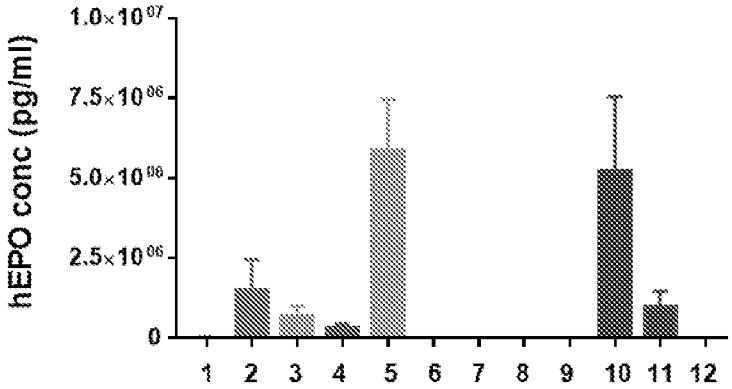


FIGURE 32C

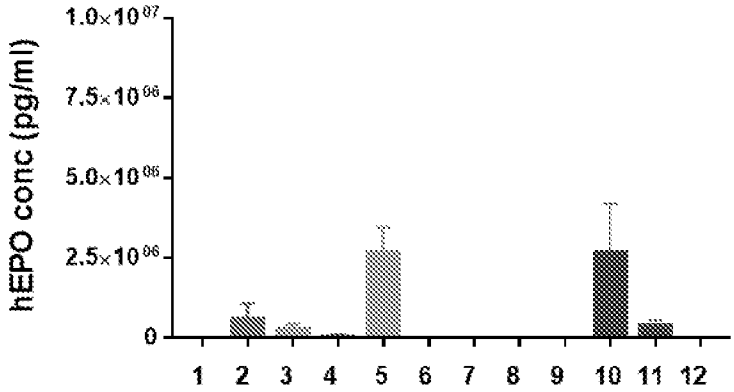


FIGURE 33

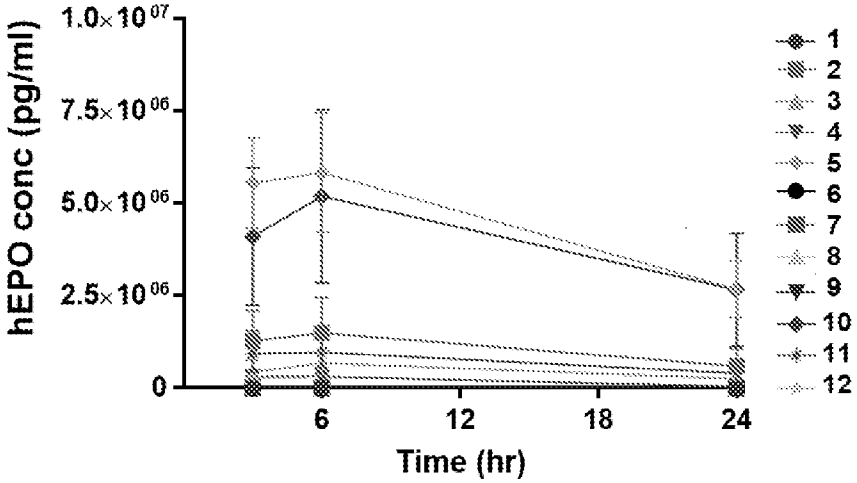


FIGURE 34A

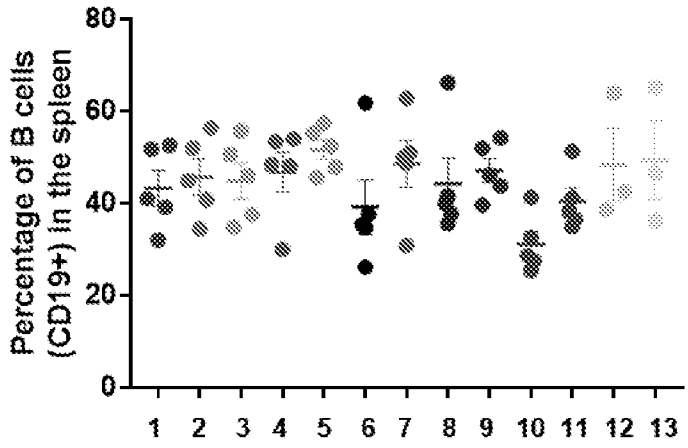


FIGURE 34B

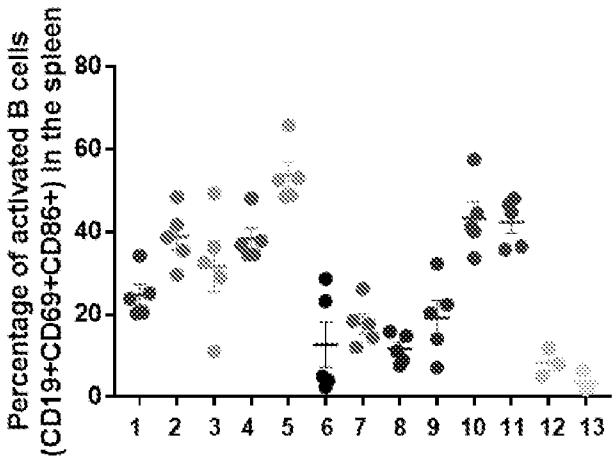


FIGURE 35

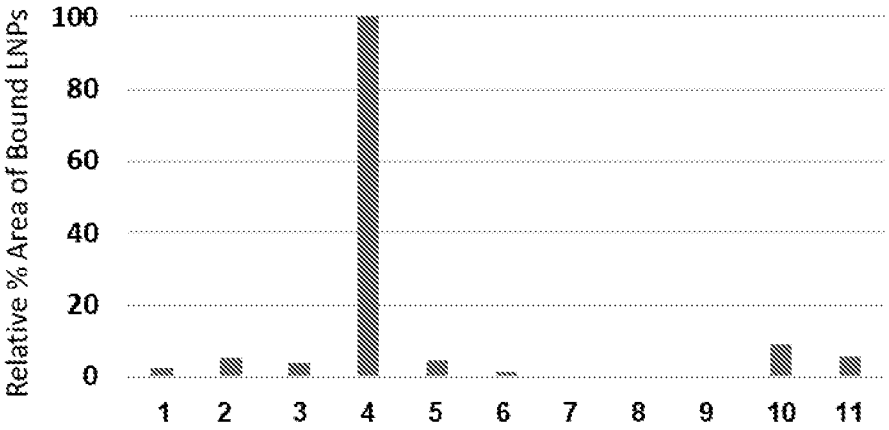


FIGURE 36

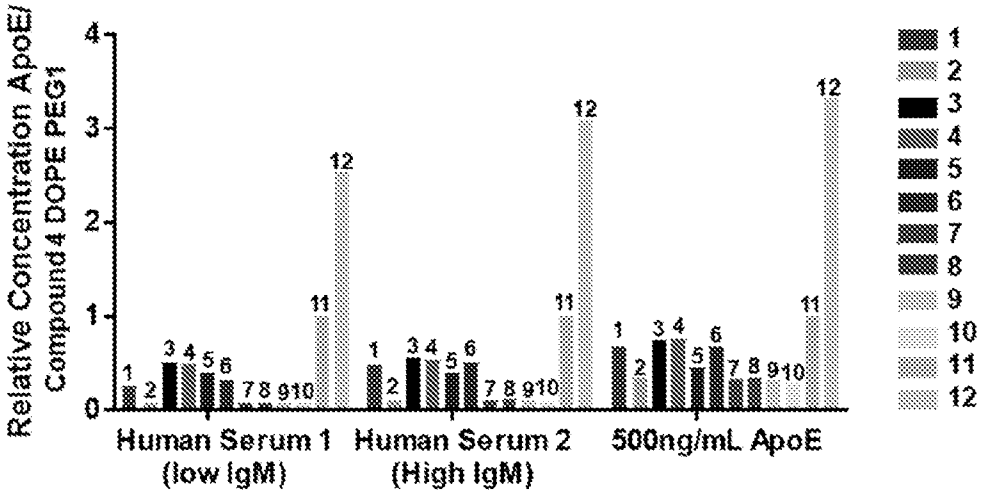


FIGURE 37

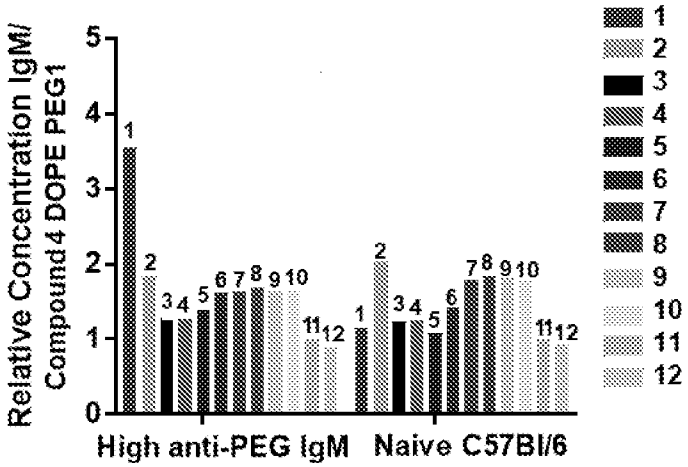


FIGURE 38A

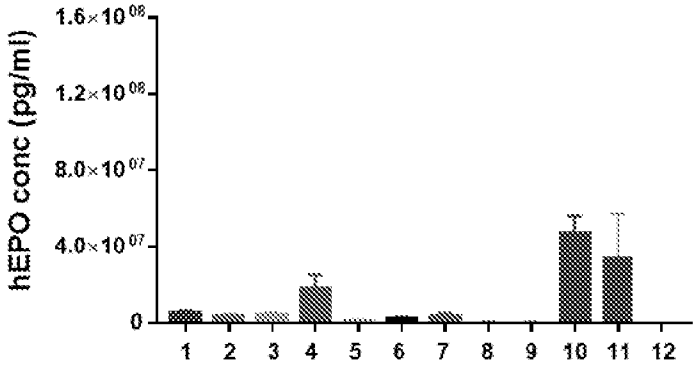


FIGURE 38B

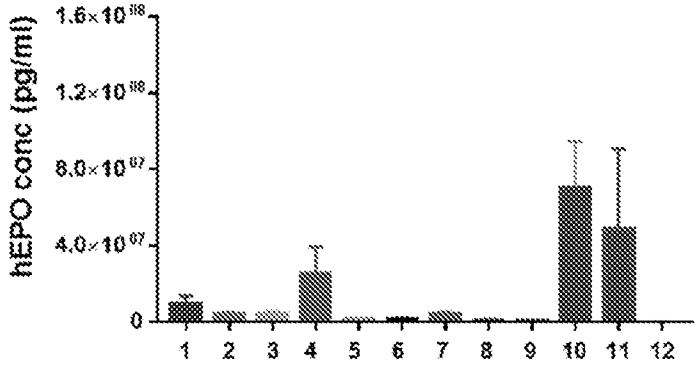


FIGURE 38C

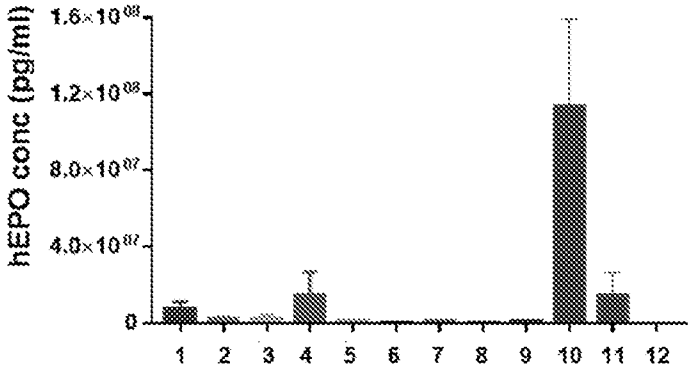


FIGURE 39

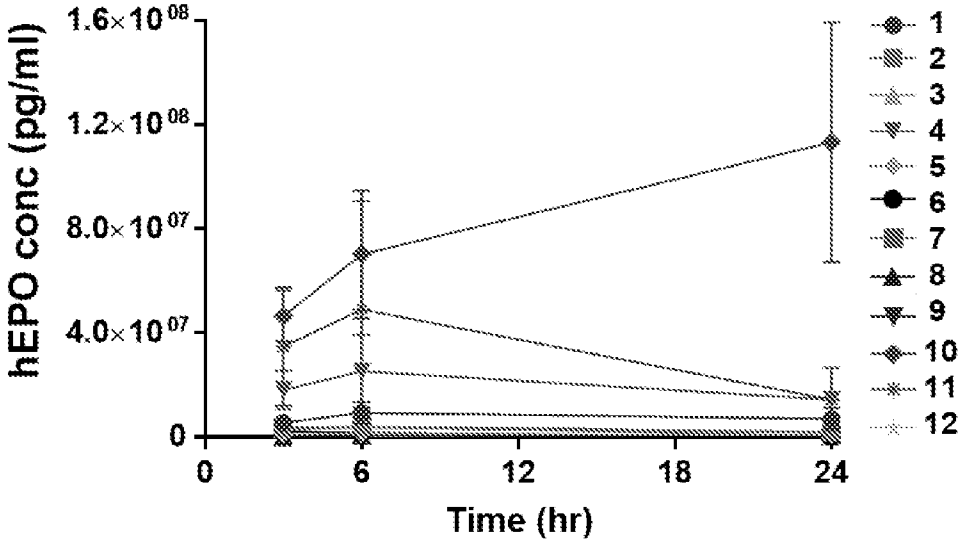


FIGURE 40A

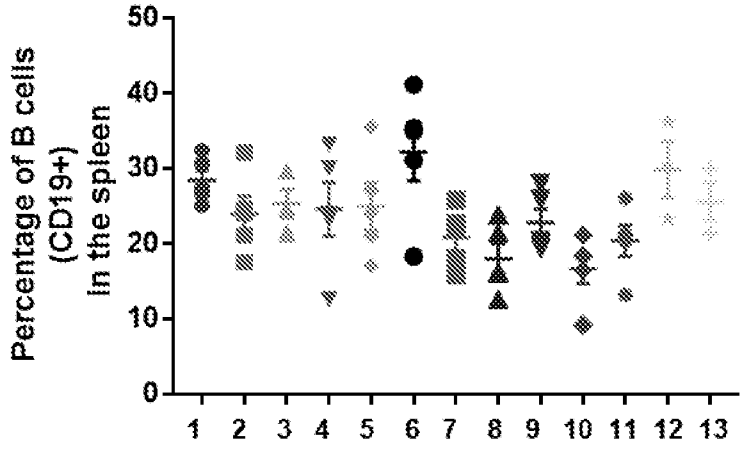
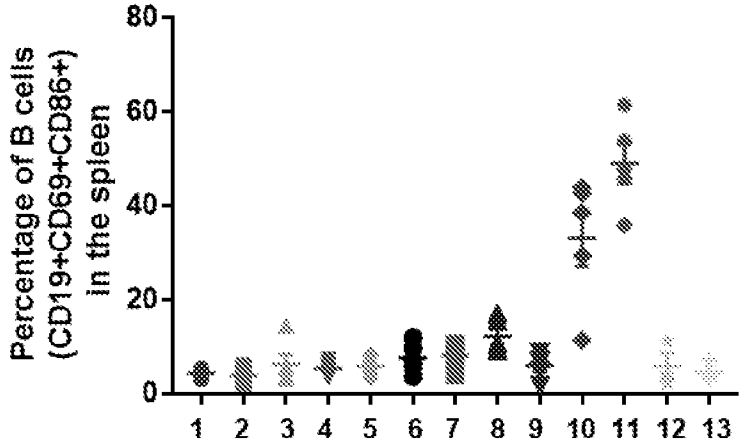


FIGURE 40B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/037541

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

2, 17-33, 169(completely); 1, 34-165, 168, 172-202, 362-366, 385-391
395-438, 443-482, 484-492, 494-505, 507-511, 513-529(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/037541

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D487/08 C07C69/013 C07D207/09 C07D211/26 C07D295/13
C07D295/185 C12N15/11

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D C07C C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal , WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with Indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2012/295832 A1 (CONSTIEN RAINER [DE] ET AL) 22 November 2012 (2012-11-22) J.KL22, S.KL33, T.KL34 and U.KL35 of FIG. 1; paragraph [0010]; claims 1,3 -----	1,2, 17-165, 168,169, 172-202, 362-366, 385-391, 395-438, 443-482, 484-492, 494-505, 507-511, 513-529

-/--

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the International filing date

"L" document which may throw doubts on priority claim(s) orwhich is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 August 2018	09/11/2018

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Grassi , Damian

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/037541

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/161830 A1 (ANDERSON DANIEL GRIFFITH [US] ET AL) 12 June 2014 (2014-06-12) compounds in the paragraphs bridging pages 27/28 or 35/36; the first and the seven last compounds in paragraph [186]; claim 21: formulas Ic to Ie; paragraph [0005] -----	1,2, 17-165, 168,169, 172-202, 362-366, 385-391, 395-438, 443-482, 484-492, 494-505, 507-511, 513-529
X,P	WO 2017/112865 A1 (MODERNATX INC [US]) 29 June 2017 (2017-06-29) paragraph [0185]; claims 1-150 -----	1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2018/037541

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2012295832 Al	22-11--2012	US 2012295832 Al	22-11-2012
		US 2014162934 Al	12-06-2014
		US 2014162962 Al	12-06-2014
		US 2014170175 Al	19-06-2014
		US 2016324781 Al	10-11-2016
US 2014161830 Al	12-06--2014	CA 2884870 Al	20-02-2014
		EP 2882706 Al	17-06-2015
		US 2014161830 Al	12-06-2014
		US 2016114042 Al	28-04-2016
		US 2017152213 Al	01-06-2017
		Wo 2014028487 Al	20-02-2014
Wo 2017112865 Al	29-06--2017	AU 2016377681 Al	14-06-2018
		CA 3007297 Al	29-06-2017
		EP 3394030 Al	31-10-2018
		WO 2017112865 Al	29-06-2017

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 2, 17-33, 169(completely) ; 1, 34-165, 168, 172-202, 362-366, 385-391, 395-438, 443-482, 484-492, 494-505, 507-511, 513-529 (partially)

Compounds according to claim 1 wherein W is -Wl-M*-W2- ("first invention" because it is referred to in dependent claim 2, see PCT-EPO Guidelines F-V.8.2; the group is still not unitary).

---

2. claims: 7, 9, 170, 203(completely) ; 1, 3, 4, 11-16, 34-168, 172-202, 272, 273, 277-361, 395-438, 443-482, 484-492, 494-505, 507-511, 513-529 (partially)

Compounds according to claim 1 wherein W is an optionally bridged 6-membered ring (i.e. the first mentioned ring; the group is still not unitary).

---

3. claims: 8, lO(completely) ; 1, 3, 4, 11-16, 34-168, 173-202, 277-361, 395-438, 443-482, 484-492, 494-505, 507-511, 513-529(partially)

Compounds according to claim 1 wherein W is the second mentioned ring (5- or 6-membered).

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4. claims: 5, 6(completely) ; 1, 16, 34-165, 173-202, 274, 275, 392, 395-438, 443-482, 484-492, 494-505, 507-511, 513-529(partially)

Compounds according to claim 1 wherein W is a 4-membered ring (X6 and X7 are not defined).

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5. claims: 171(completely) ; 173-202, 277-361, 395-438, 443-482, 484-492, 494-505, 507-511, 513-529(partially)

Compounds according to claim 171.

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6. claims: 204-223(completely) ; 395-438, 443-482, 484-492, 494-505, 507-511, 513-529 (partially)

Compounds according to claim 204.

---

7. claims: 224-234(completely) ; 395-438, 443-482, 484-492, 494-505, 507-511, 513-529 (partially)

Compounds according to claim 224.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

8. claims: 235-269(completely) ; 277-366, 395-438, 443-482, 484-492, 494-505, 507-511, 513-529(partial ly)

Compounds according to claim 235.

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9. claims: 270, 271, 276, 367-384(completely) ; 272-275, 277-366, 385-392, 395-438, 443-482, 484-492, 494-505, 507-511, 513-529(partially)

Compounds according to claim 270.

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10. claims: 393, 394, 439-442(completely) ; 395-438, 443-482, 484-492, 494-505, 507-511, 513-529 (partia l ly)

Compounds according to claim 393.

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11. claims: 483, 493, 506, 512(completely) ; 443-482, 484-492, 494-505, 507-511, 513-529 (partia l ly)

Method of claims 483, 493, 506 or 512.
