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Synthesis of cyclodextrin derivatives suitable for binding to solid supports

Syntéza derivátů cyklodextrinů vhodných pro vazbu na pevné nosiče

Doctoral thesis

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PROHLÁŠENÍ

Prohlašuji, že jsem tuto práci vypracoval samostatně pod vedením školitele doc. RNDr. Jindřicha Jindřicha, CSc., a řádně ocitoval všechny použité prameny. Jsem si vědom, že použití výsledků této práce mimo Univerzitu Karlovu je možné pouze po předchozím souhlasu univerzity. Dále prohlašuji, že jsem tuto práci ani její část nepředložil k získání stejného nebo jiného akademického titulu.

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Petr Kasal

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ABSTRACT

Synthesis of cyclodextrin derivatives suitable for binding to solid supports

This presented doctoral thesis studies the preparation of new cyclodextrin (CD) derivatives suitable for binding to solid supports. This work aims to develop synthetic protocols for monosubstituted and selectively persubstituted CD derivatives possessing permanent positive charges. These compounds have the potential to be electrostatically bound to negatively charged supports, including silica gel, alumina, Nafion®, cation exchange resins, etc. Compared to the covalent bond, the advantages of this electrostatic binding are mainly the easiness of modification and maintenance. Dipping solid support for a defined time into a solution of charged CD derivatives should ensure the bond between positively and negatively charged partners.

Thus, this thesis is divided into several parts. The first part covers the preparation of neopentyl skeleton compounds (*anchors*) bearing positive charges suitable for ionic bonding with negatively charged solid supports, and a reactive functional group suitable for a reaction with CD derivatives. The first partially successful synthetic tries are described, together with various leaving groups kinetic studies performed by NMR spectroscopy. The final synthesis of anchors developed with potential for industrial scale-up is also reported.

The second part describes the synthesis of charged fluorescent CD and non-CD derivatives and their electrostatic binding strength test with solid supports and studies their pH and thermal stability. This part is ended with the preparation of monosubstituted charged CD derivatives and the study of their potential to work as selectors in chiral membrane separation systems after they are electrostatically bound.

The third part includes the synthesis of multiply charged CD derivatives utilizing selectively persubstituted CD precursors, their electrostatic bond formation with silica gel, and utilization of these modified solids in chiral and non-chiral TLC and HPLC separations.

Keywords: cyclodextrins, NMR, kinetic studies, neopentyl skeleton, ionic binding, separation, membranes, silica gel

ABSTRAKT

Syntéza derivátů cyklodextrinů vhodných pro vazbu na pevné nosiče

Tato disertační práce studuje přípravu nových derivátů cyklodextrinu (CD) vhodných pro vazbu na pevné nosiče. Tato práce si klade za cíl vyvinout syntetické protokoly pro monosubstituované a selektivně persubstituované CD deriváty s permanentními kladnými náboji. Tyto sloučeniny mají potenciál být elektrostaticky vázány na negativně nabité nosiče, včetně silikagelu, oxidu hlinitého, Nafionu®, katexových pryskyřic atd. Oproti kovalentní vazbě je výhoda této elektrostatické vazby především snadná příprava a údržba. Ponořením pevného nosiče na definovanou dobu do roztoku nabitých CD derivátů by mělo být zajištěno spojení mezi kladně a záporně nabitými partnery.

Tato práce je tedy rozdělena do několika částí. První část se zabývá přípravou sloučenin s neopentylovým skeletem (*kotev*) nesoucích kladné náboje vhodné pro iontovou vazbu se záporně nabitými pevnými nosiči a reaktivní funkční skupinu vhodnou pro reakci s deriváty CD. Jsou tu popsány první částečně úspěšné syntetické pokusy spolu s různými kinetickými studiemi odstupujících skupin provedenými NMR spektroskopií. Rovněž je popsána konečná syntéza kotev vyvinutých s potenciálem pro průmyslové využití.

Druhá část popisuje syntézu nabitých fluorescenčních CD a necyklodextrinových derivátů a test jejich elektrostatické vazebné síly s pevnými nosiči a studuje jejich pH a tepelnou stabilitu. Tato část je zakončena přípravou monosubstituovaných nabitých CD derivátů a studiem jejich potenciálu působit jako selektory v chirálních membránových separačních systémech poté, co jsou elektrostaticky navázány.

Třetí část zahrnuje syntézu vícenásobně nabitých CD derivátů využívajících selektivně persubstituované CD prekurzory, tvorbu jejich elektrostatické vazby se silikagelem a využití těchto modifikovaných pevných látek při chirálních a nechirálních TLC a HPLC separacích.

Klíčová slova: cyklodextriny, NMR, kinetické studie, neopentylový skelet, iontová vazba, separace, membrany, silikagel

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Ac	acetyl
AFM	atomic force microscopy
AIBN	azobisisobutyronitrile
ATR	attenuated total reflectance
BLM	bulk liquid membrane
*t*Bu	*tert*-butyl
Boc	*tert*-butyloxycarbonyl
CD	cyclodextrin
CAM	cerium ammonium molybdate stain
CCSP	chiral-coated stationary phase
CMC	critical micelle concentration
CMPA	chiral mobile phase additive
CS	chiral selector
CSP	chiral stationary phase
CuAAC	copper-catalyzed azide–alkyne cycloaddition
DBTDL	dibutyltin dilaurate
DCC	*N*,*N*′-dicyclohexylcarbodiimide
DEG	diethylene glycol
DIBAL	diisobutylaluminium hydride
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DNBPG	*N*-3,5-dinitrobenzoyl-phenylglycine
DNS	dansyl
DRIFT	diffuse reflectance infrared fourier transform spectroscopy
ELM	emulsion liquid membrane
ESI MS	electrospray ionization mass spectrometry

Et	ethyl
FTIR	Fourier-transform infrared spectroscopy
HDA	hexandiamine
HILIC	hydrophilic interaction chromatography
HPLC	high performance liquid chromatography
HRMS	high-resolution mass spectrometry
Im	imidazole
IR	infrared spectroscopy
ITC	isothiocyanate
Me	methyl
MIM	methylimidazolium
Ms	methanesulfonyl (mesyl)
MW	microwave irradiation
NA	naphthalic anhydride
NBP	4-(4-nitrobenzyl) pyridine
NBS	*N*-bromosuccinimide
NHS	*N*-hydroxysuccinimide
NMR	nuclear magnetic resonance
Np	neopentyl
NP	normal phase
NPNI	*N*-propylnaphthalimide
OEG	octaethylene glycol
PEMPDA	permethylated propylenediamine
Ph	phenyl
Pr	prop-1-yl
i-Pr	isopropyl (prop-2-yl)
Prg	propargyl
PYR	pyridinium
RP	reverse-phase
SA	selectand

SEM	scanning electron microscope
SFC	supercritical fluid chromatography
SLM	supported liquid membrane
S_N1	nucleophilic substitution reaction of the 1st order
S_N2	nucleophilic substitution reaction of the 2nd order
TBABr	tetra-*n*-butylammonium bromide
TBAI	tetra-*n*-butylammonium iodide
TBDMS/TBS	*tert*-butyldimethylsilyl
TBME	*tert*-butyl methyl ether
TEA	triethylamine
TEG	tetraethylene glycol
Tf	trifluoromethanesulfonyl (triflyl)
TFA	trifluoroacetic acid
TFC	thin film composite
THF	tetrahydrofuran
TLC	thin layer chromatography
TMA	trimethylammonium
TrEG	triethylene glycol
Ts	*p*-toluenesulfonyl (tosyl)
TsOH	*p*-toluenesulfonic acid monohydrate
TU	thiourea
UV-VIS	ultraviolet-visible spectroscopy

1 INTRODUCTION

Since their discovery about 140 years ago, cyclodextrins (CDs) have become widely used in industry and academic research. Their main interesting structural feature, a hydrophobic cavity, determines their utilization in cosmetics, food, and pharmaceutical industries. Since the 80s, many scientists have put their effort into synthesizing new CD derivatives to enhance some properties of native CDs and subsequently apply them in industry and novel academic fields, such as chiral separations.

CD derivatives have been used in chiral column separations since the 80s, and commercial suppliers offer some CD-based chiral columns. There is ongoing academic research in this area. However, it is almost impossible to find references in the literature concerning the physisorption of permanently charged CDs on silica gel through a stable electrostatic bond and utilization of these columns in chiral HPLC systems. Despite the undeniable ease of preparation of such columns precisely according to the user's current needs. All the user needs to do is to mix the suitable charged CD with silica gel in the commercial column by simply pumping the CD solution through the column. So basically, everyone with a standard silica gel column and appropriate charged chiral compounds can tailor their chiral columns in hours.

The same can be said about chiral thin-layer chromatography (TLC) systems. These systems are well established, and some non-CD chiral TLCs are offered commercially. Notwithstanding, it is impossible to find any publications dealing with chiral TLC prepared from permanently charged CDs and commercial non-chiral TLC plates.

There are a few examples utilizing CD as a chiral selector concerning chiral membrane separation systems. However, the system with electrostatically bonded CD has not been found.

In most cases, permanently charged CDs are utilized in capillary electrophoreses chiral and non-chiral separations. If permanently charged CDs are applied in chiral columns HPLC systems, they are covalently bound to a solid support, and charged groups interact with oppositely charged analytes.

Another group of charged CDs with charged groups distributed over the whole primary or secondary rim is studied as gene delivery systems due to their ability to form nanoparticles and vesicles. However, the author of this thesis has not found any article concerning the development of any chiral separation system, neither chiral columns nor chiral TLCs nor chiral membrane separation systems, utilizing these types of compounds.

2 OBJECTIVES

This work's primary goal was to prepare multiply charged cyclodextrins (CDs) and assess them as chiral selectors in chiral separations systems such as membrane filtration, HPLC, and TLC.

This general goal is subdivided into a series of partial goals:

1) to develop robust, high-yielding, and easy-to-scale-up syntheses of neopentyl skeleton substances containing one to three permanent positive charges (anchors)
2) to prepare the first series of CD derivatives and fluorescent compounds possessing a suitable functional group enabling an easy connection with anchors developed in 1)
3) to prepare the second series of CD derivatives with a completely modified primary rim by a suitable functional group allowing an easy connection with anchors developed in 1)
4) to prepare multiply charged CDs and fluorescent compounds from compounds prepared in 1), 2), and 3)
5) to test the bond strength of charged compounds synthesized in 4) with various kinds of solid supports, including silica gel, sulfonated silica gel, cation exchangers, Nafion®
6) to prepare multiply charged CDs with the potential to work as chiral selectors in chiral separation systems (columns, membranes, etc.)
7) to adsorb charged compounds synthesized in 6) on silica gel plates, columns from commercial suppliers, and Nafion® membranes
8) to test modified silica gel plates, columns, and membranes in chiral separations

3 STATE OF THE ART

3.1 Cyclodextrins

3.1.1 History

CDs were isolated in 1891 by Villiers in France[1], studying *Bacillus amylobacter's* action on 1000 g of potato starch. He observed the formation of 3 g of undesired crystals and determined their empirical formula as $(C_6H_{10}O_5)_2 \cdot 3H_2O$. He named them "*cellulosines*" because of their similar properties to cellulose, e.g., non-reductive properties and acidic hydrolysis stability. He did not proceed with further structure recognition.

The first proposal of the structure is attributed to Schardinger[2], an Austrian chemist and bacteriologist. In 1903 he isolated the microorganism capable of synthesizing the enzyme that catalyzed starch degradation into CDs and named it *Bacillus macerans*. During this degradation, he obtained two crystalline side-products, which were like Villiers's *cellulosines*. Later, in 1911, he purified them by precipitating them from the fermenting liquid with chloroform or ether (Figure 1). The residue was dissolved in boiling water, and after filtration and concentration of the filtrate, a fine crystalline product was formed. Schardinger named this product dextrin-β. The second side-product was obtained from the filtrate by precipitation with alcohol and recrystallized from the water/alcohol mixture. This product was designated dextrin-α by Schardinger. It is necessary to point out that the name dextrin was used for any degradation product of starch at that time.[1] He characterized them by specific optical rotation and elemental analysis and distinguished them by preparing crystalline iodine complexes. Dextrin-α formed greenish needles and dextrin-β reddish brown crystals. Based on these results, Schardinger renamed these compounds to crystalline α-dextrin and crystalline β-dextrin. Only around 30% of starch was transformed into dextrins, but it was still around 10-times higher than Villiers's results. One hypothesis could be contamination of Villiers's *Bacillus amylobacter* by *Bacillus macerans*, which can be considered a great luck.[1]

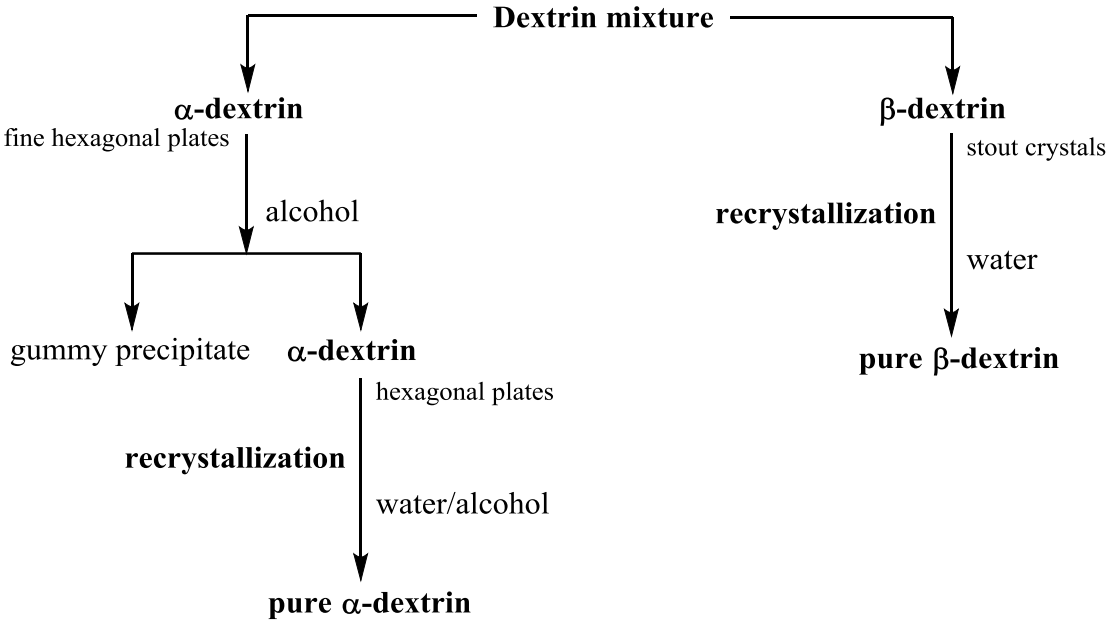


Figure 1. The fractionation and purification protocol proposed by Schardinger for producing dextrins (redraw from Crini[3]).

Later, Schardinger described their fundamental properties, hypothesized they are cyclic molecules, and suggested their complexation capabilities.[3] Freudenberg confirmed the hypothesis about their cyclic nature due to hydrolysis experiments some 30 years later.[4,5]

Schardinger's main contribution remains in discovering *Bacillus macerans*, an organism capable of synthesizing an enzyme today called CD glucanotransferase, which decomposes starch's amylose to CDs.[1]

In 1912, German chemist and biochemist Hans Pringsheim repeated experiments described by Schardinger and obtained pure α- and β-dextrin.[6] He showed their insolubility in alcohol, ether, and chloroform and their non-reductive abilities by Fehling's solution. His main contribution is the hypothesis of complex formation with various organic compounds, including halogens.[7,8]

Paul Karrer, a Swiss chemist and Nobel prize winner in 1937 for his research on vitamins, started to examine Schardinger's dextrins in the 1920s. He studied their interactions with ions like sodium[9] and potassium[10]. He proposed that dextrins are made of maltose units joined by α(1→ 4) glycosidic bonds.[11,12]

In the 1930s, German chemist Karl Johann Freudenberg started working with starch and its degradation products to elucidate its structure.[13,14] First, he considered Schardinger's dextrins to be linear and non-reductive.[13] Later, he utilized the cryoscopic method to determine their molecular weights and reported (inaccurately) the number of

glucose units: five for α-dextrin and six for β-dextrin.[5] In the second half of the 1930s, he confirmed dextrins' cyclic structures by hydrolysis, enzymatic hydrolysis, and acetolysis of permethylated dextrins. He stated that dextrins consist of maltose units joined by α(1→4) glycosidic bonds, as Karrer proposed in the 1920s.[15–17] Other groups confirmed this by X-ray crystallography.[18] In 1948, he discovered another type of dextrin (in the future, it will bear the name γ-CD) and elucidated its structure in 1950.[19]

The American chemist Dexter French also contributed to solving the mystery of Schardinger's dextrins. In 1942, due to X-ray diffraction and crystal density measurements, he elucidated the correct molecular weights and number of glucose units: six for α-dextrin and seven for β-dextrin.[20] In this article, French also introduced new cycloamylose-based terminology, which is still utilized today. He improved dextrins' purification by using specific precipitants like bromobenzene and *n*-propanol.[21,22]

Another great success in CDs' history was the discovery and isolation of the enzyme CD glucanotransferase (cycloamylose glucanotransferase) by Tilden and Hudson in the 1940s.[23,24]

At the end of the 1940s, Friedrich Cramer, a Polish chemist, and Freudenberg's Ph.D. student, was the first one who used the word *cyclodextrins* to define these compounds. It was the title of his doctoral thesis.[25] Later, he described new purification methods of native CDs by more efficient selective precipitations compared to French.[26]

Many famous chemists have come and continued in CD chemistry after these "pioneers" who have done enormous work. Without their effort, CDs would have never come into the light of modern chemistry and industry. Their successors have focused mostly on more precise structure elucidation, complexation phenomenon description and understanding, chemical modifications of native CDs, and utilization in the industry. Due to this, these great chemists will be mentioned in the following chapters.

3.1.2 Structure and properties

Today it is known that CDs are cyclic oligosaccharides composed of α-D-glucopyranose units (Figure 2). The three most common native CDs contain six, seven, and eight of these units and are called alpha-, beta-, and gamma-cyclodextrin, respectively (abbreviated as α-, β-, and γ-CD).[27] Synthetic CD possessing only five α-D-glucopyranose units was also prepared.[28] The smallest CDs made from three and four glucopyranose units were recently synthesized.[29] However, glucopyranoses are heavily twisted and

distorted due to the small ring size. The newest one of those three native CDs is γ-CD. Its structure was elucidated in 1950 by French.[30]

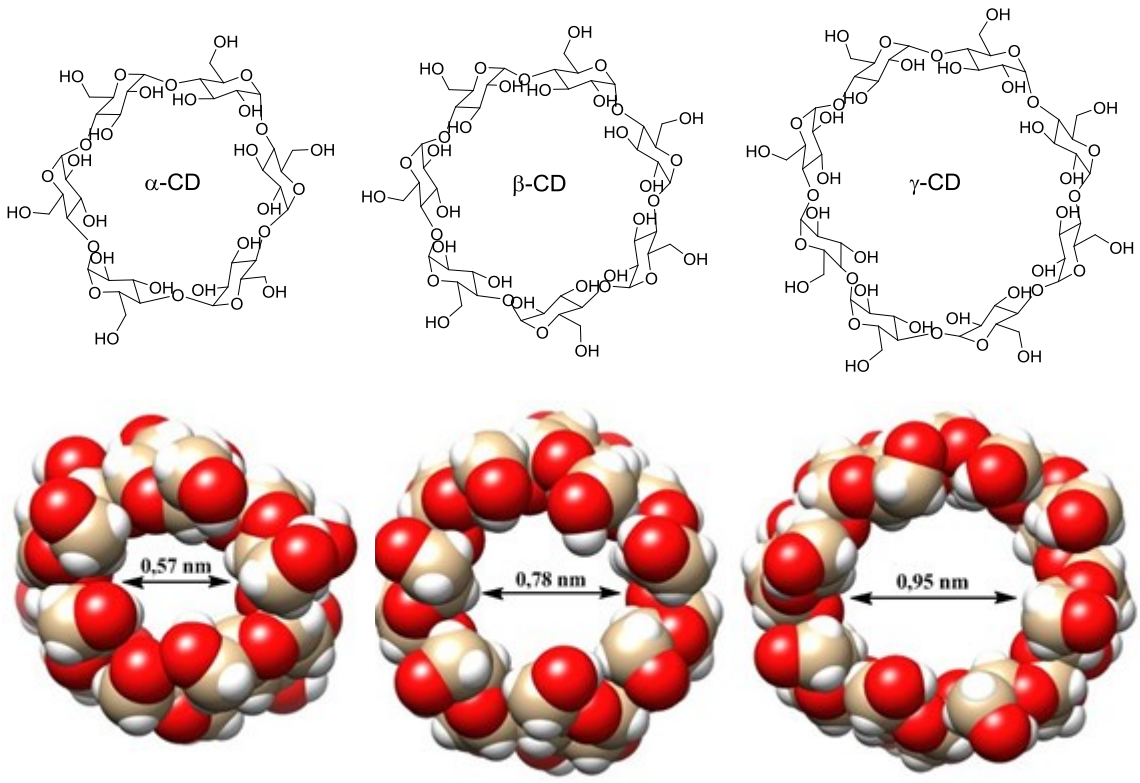


Figure 2. Structure and 3D molecular model of native CDs.

CDs containing more than eight units were also isolated. In 1961, French discovered delta-CD (δ-CD) and epsilon-CD (ε-CD) with nine and ten α-D-glucopyranose units.[31] In 1965, Thoma and Stewart described zeta-CD (ζ-CD) and eta-CD (η-CD), consisting of eleven and twelve α-D-glucopyranose units.[1]

Glucopyranose units have $^4C_1$ conformation, which was proved by Casu's IR and NMR measurements in the 1960s.[32–35]

CDs have the shape of a hollow truncated cone (Figure 3). Wolfram Saenger, the German biochemist and crystallographer, published their crystal structure analysis in the 1970s[36,37] and assigned NMR signals for individual protons in CDs.[38] The narrower upper edge is called primary because of primary hydroxyl groups on carbon C6. The wider lower edge is for the same reason called secondary rim because of secondary hydroxyl groups in positions C2 and C3. Glycosidic oxygens O4 connecting glucopyranose units are oriented to the inner space called the cavity. The same can be said about hydrogens H3 and H5.[39]

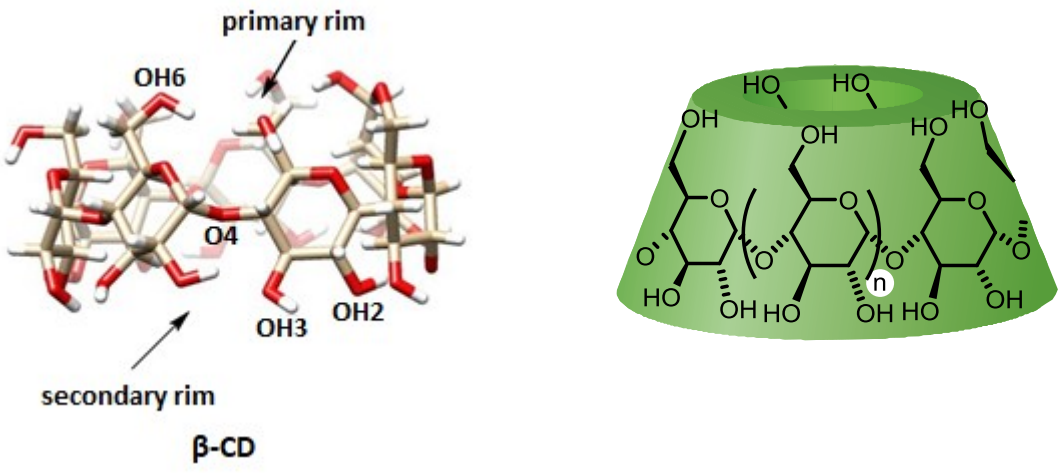


Figure 3. 3D picture of β-CD with marked hydroxyl groups, glycosidic oxygen O4 and both rims (left), the shape of α-CD (n=1), β-CD (n=2), and γ-CD (n=3) (right).

CDs have a hydrophilic surface due to the primary and secondary hydroxyl groups. On the other hand, glycosidic oxygens O4 and C-H bonds in positions 3 and 5 make the cavity hydrophobic.[27] Originally, Freudenberg was the one who came up with the CD's non-polar inner surface hypothesis. He and French (erroneously) expected the inner surface to have a hydrocarbon nature.[40] Thoma and Stewart refuted this in the 1960s.[1]

Hydrogen bonds between secondary hydroxyl groups of two neighboring glucopyranose units enhance the stability of the molecule. It is assumed that the difference in solubility is also caused by hydrogen bonding. The hydrogen bonding band is complete and the strongest in the case of β-CD with seven glucopyranose units. This hydrogen bonding band's incompleteness is why the water solubility of α- and γ-CD is higher than β-CD. The smallest native CD, α-CD, has six glucopyranose units, and because of this, it is not so compact, and hydrogen bonding is weaker than β-CD. Eight glucopyranose units in γ-CD make the molecule more flexible, and hydrogen bonds are weaker than β-CD due to this.[27] Comparison of water solubility and other properties are listed in Table 1.

Native CDs contain around 10 % water, as shown in Table 1. It was demonstrated by Claudy et al. that water molecules in CDs are not equally bonded. In other words, some water molecules are bound more firmly than others.[41]

Table 1. General parameters and properties of native CDs.[27]

CD	α	β	γ
number of glucopyranose units	6	7	8
relative molecular mass M_r	972	1135	1297
water solubility (g/100 mL at 25°C)	14.5	1.8	23.2
optical rotation $[\alpha]_D$ 25°C	150 ± 0.5	162.5 ± 0.5	177.4 ± 0.5
approximate cavity volume (Å^3)	174	262	427
crystal water (M_r %)	10.2	13.2 – 14.5	8.13 – 17.7

Native CDs with more than eight glucopyranose units become more and more flexible; thus, hydrogen bonds are much weaker and cannot make the molecules more rigid. For this reason, these molecules do not have the shape of a truncated cone, and in most cases, their cavity is smaller than γ-CD.[27]

3.1.3 Inclusion complexes

Due to the hydrophilic surface and hydrophobic cavity, CDs (hosts) can form supramolecular inclusion complexes with a broad spectrum of compounds (guests). These guest molecules belong to a diverse group of compounds, e.g., aromatic compounds[42] (benzene, naphthalene), steroid compounds[43] (cholesterol), long linear chain molecules[44] (polyethylene glycol), bulky organic compounds[45] (adamantane), and anions[46] (nitrate, sulfate).

One of the first significant contributions to this area of CDs' chemistry belongs to Cramer. In the 1950s, he studied the complexation abilities of CDs with iodine and dye compounds[47,48] and gases[49]. He also came up with the world "*inclusion complex/compound*".[26,50] Cramer and Freudenberg created the first patent dealing with CDs' inclusion compounds in 1953. The main topic was the protection of active compounds against air oxidation and the enhancement of their solubility.[51]

Another breakthrough came in the late 1960s when Hybl published the X-ray structure of the α-CD/KOAc inclusion complex as the first direct proof of complexation.[52]

Cramer continued his research from the 50s and focused on elucidating the formation of inclusion complexes.[53] According to his theory, the mechanism of formation consists of several steps:

1. The approach of the substrate to the molecule of CD

2. Breakdown of the water structure in the cavity and transport of some water molecules out of the cavity
3. Breakdown of the water structure around that part of the substrate, which is supposed to be included, and release of some water molecules into the solution
4. Interaction of the substituents of the substrate molecules with groups at the rim or inside the cavity
5. Possible formation of hydrogen bonds
6. Reconstitution of the water structure around the exposed parts of the substrate after the inclusion process

In the 1980s, Saenger also focused on this phenomenon and stated that van der Waals forces and hydrophobic interactions probably dominate in complex formation.[54]

In the same period, Bergeron and Rowan stated that London dispersion forces and expulsion of high-energy water from the cavity are the driving forces in complex formation between α-CD or β-CD and p-nitrophenolate.[55]

In 1982, József Szejtli, the Hungarian chemist, summarized and reformulated mostly Cramer's and Saenger's statements about this phenomenon.[1] According to him, water molecules are in a high-energy state due to polar-apolar interactions and tend to be replaced by less polar molecules. Organic molecules dissolved in water tend to find a more hydrophobic environment (cavity). The third energetical contribution comes from van der Waals forces, hydrogen bonding, and steric interactions. In summary, complexation results from more types of interactions between three components of the system (CD, guest, and solvent), leading to a more thermodynamically stable state.

In the 1990s and at the turn of the millennium, these statements were generally accepted, but there is still a debate over the size of the contribution of each interaction.[56,57] Rekharsky and Inoue stated that van der Waals interactions predominate over steric effects and hydrogen bonds in complex formation.[58] Liu and Guo in 2002 published a study in which they demonstrated that the water in the cavity did not intervene in the complex formation and questioned the main conclusion published by Szejtli in 1982.[59]

Examples of different stability of these complexes based on the steric match and for other non-binding interaction reasons can be included, e.g., 1-aminoadamantane inclusion complexes with α-, β-, and γ-CDs were characterized in aqueous solutions using NMR spectroscopy. The best steric match was found for β-CD, which was the reason for the largest measured complexation constant (5150 M^{-1}). Complexation constants for α- and γ-CD were much lower, 183 M^{-1} and 306 M^{-1}.[60] Calorimetric studies of benzoic acid

complexes with α-, β-, and γ-CDs showed the formation of fairly stable complexes with α- and β-CD (the benzoic acid-α-CD complex was the more stable one). In contrast, the complex with γ-CD was relatively unstable.[61] In the last example, hexanol forms a more stable complex than hexane, probably due to hydrogen bonding between CD's hydroxyl groups and hexanol.[62]

Stoichiometry of complexes can be 1:1 (guest: host), 1:2, 2:1, and many other ratios.[63] One of the extreme examples is compounds called pseudopolyrotaxanes[64] (Figure 4). These compounds contain many CD molecules complexed with only one guest molecule. This guest is a polymeric chain, e.g., polyethylene glycol, polypropylene glycol. Even in the case of pseudopolyrotaxanes, a trend of increasing complexation constant with the steric agreement can be observed. E.g., polyethylene glycol forms a stable complex with α-CD[65], but polypropylene glycol forms stable complexes only with β-CD and γ-CD[66]. Probably because side methyl groups make these molecules too bulky to fit in the α-CD cavity. With another set of side methyl groups, e.g., polydimethylsiloxane, the molecules are even bulkier and can form complexes only with γ-CD.[67]

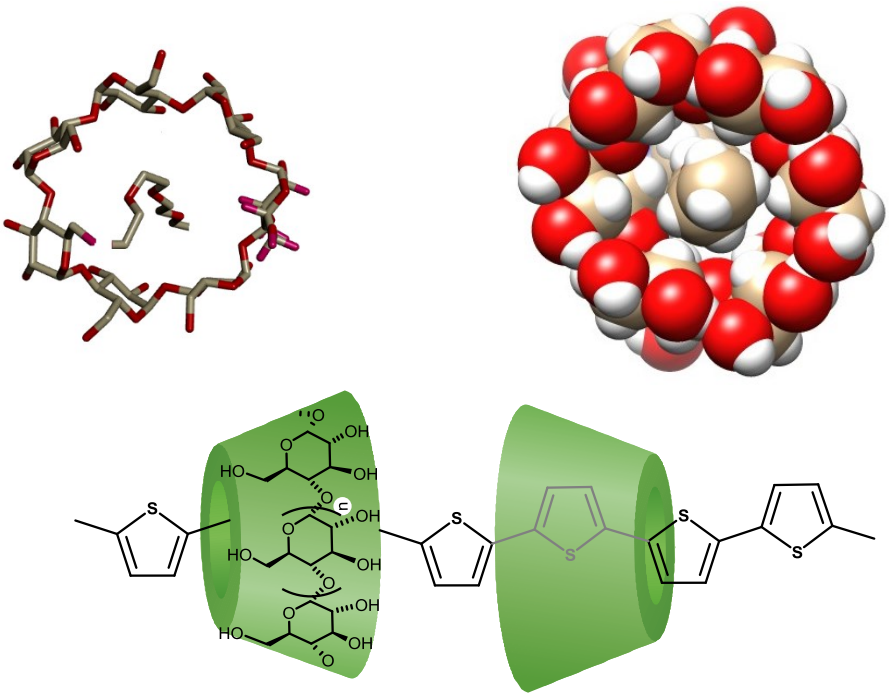


Figure 4. A general example of the CD host: guest complex with 1:1 stoichiometry (top), a representative example of pseudopolyrotaxane (bottom).

Before concluding this chapter, it is worth mentioning that CDs can also form non-inclusion complexes. Cramer suggested this already in 1956.[50] Due to hydroxyl groups

and their intermolecular hydrogen bonds, CD molecules can form molecular structures that "hide" lipophilic molecules from water. Another possibility is the formation of large CD aggregates with water-insoluble lipophilic molecules structurally like micelles. This area of CD research has only begun to be explored in the last decade.[68,69] Still, several papers and reviews can be found already.

3.1.3.1 Stability constant

A lot was written about the stability of different complexes, but their stability needs to be expressed quantitatively to compare complexes with each other. For this reason, scientists use the stability constant *K* defined in Eq. 1 and Eq. 2. Here it is shown for the simplest case when CD and guest create complex in 1:1 ratio. Often you can find in literature synonymous terms binding constant, formation constant, and equilibrium constant. The stability constant is mostly in a range of 10^2-10^5 M^{-1} in the case of CDs.[56]

$$[CD] + [guest] \leftrightarrows [CD \cdot guest] \quad \text{Eq. 1}$$

$$K = \frac{[CD \cdot guest]}{[CD] \cdot [guest]} \quad \text{Eq. 2}$$

Stability constants can be mainly determined by solubility methods[70], potentiometric[71], kinetic[72], and spectroscopic methods[73]. French in the 50s was one of the first to apply spectroscopic methods to study complexation between α-CD and iodine and iodide and to determine the stability constants.[74] In the 1970s, Thakkar and Demarco were the first to use NMR spectroscopy to study CDs' complexation with several organic compounds.[75] Their use will be described in more detail because spectroscopic methods, specifically UV-VIS and NMR spectroscopy, are the most common ones.

First, the stoichiometry of the complex needs to be determined because different stoichiometry means different basic equations (Eq. 3, Eq. 4, Eq. 5, Eq. 6, and Eq. 7) for the host-guest complexation.

$$a \cdot \mathrm{H} + b \cdot \mathrm{G} \leftrightarrows \mathrm{C} \qquad \text{Eq. 3}$$

$$K = \frac{[\mathrm{C}]}{[\mathrm{H}]^a \cdot [\mathrm{G}]^b} \qquad \text{Eq. 4}$$

$$[\mathrm{H}]_0 = [\mathrm{H}] + a \cdot [\mathrm{C}] \qquad \text{Eq. 5}$$

$$[\mathrm{G}]_0 = [\mathrm{G}] + b \cdot [\mathrm{C}] \qquad \text{Eq. 6}$$

$$K = \frac{[\mathrm{C}]}{([\mathrm{H}]_0 - a \cdot [\mathrm{C}])^a \cdot ([\mathrm{G}]_0 - b \cdot [\mathrm{C}])^b} \qquad \text{Eq. 7}$$

with H: host; G: guest; C: complex

a, b: stoichiometry coefficients

$[\mathrm{H}]_0$: initial concentration of host molecule

$[\mathrm{G}]_0$: initial concentration of guest molecule

[H], [G], [C]: equilibrium concentrations of host, guest, and complex

There are several methods for stoichiometry determination, e.g., the Slope Ratio Method[76], the Mole Ratio Method[77], and Continuous Variation Methods[78]. The last is the most popular, so further attention will focus on this.

By changing the initial host concentration and measuring the complex concentration (or a different parameter proportional to it), a Job's plot is obtained (

Figure ***5***). The stoichiometry from the x- coordinate at the curve's maximum can be determined.[79]

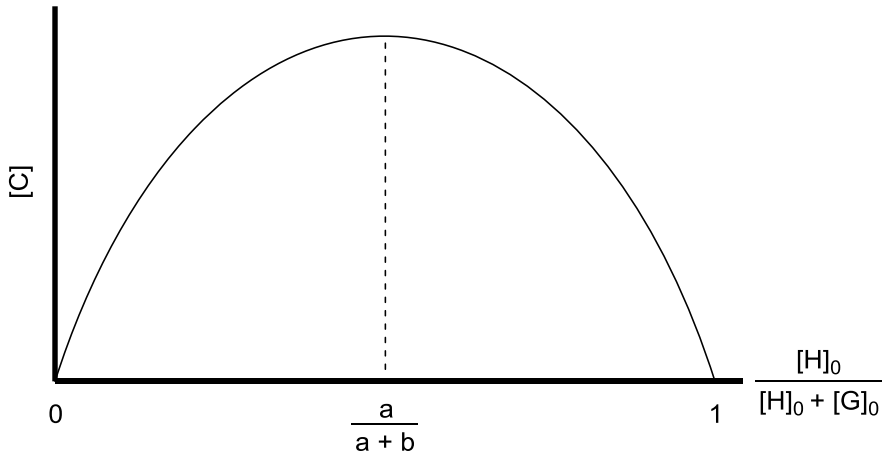


Figure 5. A general example of Job's plot.

The stoichiometry is known now, but the question is how to calculate K. Because even for 1:1 stoichiometry (a, b = 1) or 1:2 stoichiometry (a = 2 and b = 1, for example), quadratic (Eq. 8) and cubic (Eq. 9) equation are obtained from Eq. 7, respectively.

$$K \cdot [C]^2 - (K \cdot [G]_0 + K \cdot [H]_0 + 1) \cdot [C] + K \cdot [G]_0 \cdot [H]_0 = 0 \quad \text{Eq. 8}$$

$$4 \cdot K \cdot [\mathrm{C}]^3 - (4 \cdot K \cdot [\mathrm{G}]_0 + 4 \cdot K \cdot [\mathrm{H}]_0) \cdot [\mathrm{C}]^2 + (K \cdot [\mathrm{H}]_0{}^2 + 4 \cdot K \cdot [H]_0 \cdot [G]_0 + 1) \cdot [C] - K \cdot [H]_0{}^2 \cdot [G]_0 = 0 \quad \text{Eq. 9}$$

Several approximation methods have been developed in the past to overcome this inconvenience. The most common and used approximation methods are Benesi-Hildebrand[80] and Scatchard[81]. These approximations assume that $[\mathrm{G}]_0 = [\mathrm{G}]$ and $[\mathrm{G}]_0 \gg [\mathrm{H}]_0$ are also employed. The first condition is encountered for small *K*, and the second one is mostly fulfilled during experimental conditions. Applying these conditions on Eq. 7 together with 1:1 stoichiometry (a, b = 1), a simple linear Eq. 10 is obtained.

$$(K \cdot [G]_0 + 1) \cdot [C] - K \cdot [H]_0 \cdot [G]_0 = 0 \quad \text{Eq. 10}$$

When the assumption $[\mathrm{G}]_0 = [\mathrm{G}]$ cannot be applied, regression methods can be employed to make the situation less difficult. Some examples of these methods are the Rose-Drago[82], Nakano[83], and Creswell-Allred[84].

Although most of these approximation methods were put into practice in the first half of the last century, they are still used. In recent years, some chemists expressed doubts about these methods' accuracy and, in particular, the use of a Job's plot to determine stoichiometry.[85] They claim that Job's plot and linear approximations should never be used anymore because Job's plot is a low indicator of stoichiometry.[86,87] Due to a combination of modern computer processing power and sophisticated programs like *Python* or *Matlab*, these polynomials (Eq. 8 and Eq. 9) can be solved fast and precisely. The strategy should be as follows:

1. Estimate the stoichiometry of your host-guest system
2. Choose the binding model(s) for your system
3. Select the appropriate experimental method – mostly UV-VIS or NMR spectroscopy
4. Fit the obtained data to your binding model(s) to get the *K*-values of interest
5. Repeat the experiment at least 3, better 4, times to estimate the uncertainties

3.1.4 Cyclodextrin derivatives

Changing complexation properties and hence stability constant magnitude is one of the main driving forces for CDs' derivatization. Other reasons can include solubility changes, catalysis, etc..[88–90]

A short trip into history. Freudenberg started experimenting with CDs derivatization in 1922.[91] In the 80s, Breslow presented results about the utilization of modified CDs as artificial enzymes.[92] Many chemists began to publish works about modified CDs in the 80s. In 1981, Josef Pitha prepared hydroxypropyl-β-CD (randomly substituted), which was later commercialized as a solubilizer under the names Encapsin or Cavitron.[1] In the 1990s, the work about sulfobutylether-CDs was published.[93] These compounds are still produced by different companies (CYDEX Co., U.S., Cyclolab Kft., HU) as drug solubilizers. After the 2000s, per[6-(2-carboxyethylthio)-6-deoxy]-γ-CD sodium salt (Sugammadex) commercially known as Bridion® was synthesized and quickly became one of the most used CD derivatives in daily life due to its ability to reverse neuromuscular blockade in anesthesia.[94]

The number of CD's hydroxyl groups (18 for α-CD, 21 for β-CD, and 24 for γ-CD) can make someone think about limitless opportunities for modifications. The real situation is more complicated for several reasons. All OH groups have similar reactivity; the cavity can interact with reagent and thus change the reaction's course; the solubility of native (unmodified) CDs has its limits. These are only a few reasons why the preparation of new CD derivatives is difficult and challenging.[95,96]

There is a large group of CD derivatives with a non-uniform structure. These randomly substituted derivatives are mostly used in therapeutics as drug solubilizators and analytical chemistry as selectors.[97]

Over the years, chemists developed some methods and procedures leading to structurally unambiguous substituted CDs. As was written above, all OH groups have similar reactivity. But gifted chemists were able to find slight nuances between them and sort them into three groups.[98]

The primary hydroxyls at the C6 positions of glucopyranose units are the first group. They are the most basic ones, the most nucleophilic, and easily accessible. Because of these abilities, one needs only a weak base and bulky reagent for their modification.[98]

Secondary hydroxyls at positions C2 belong to the second group. These OH groups are considered the most acidic, having pK_a =12.2.[88] The strategies for their substitution

usually apply non-bulky reagents and one equivalent of a strong base. In most cases, modifications of primary hydroxyls at C6 positions are also observed. Thus, protection or pre-modification of these primary OH groups is necessary before C2 hydroxyls are modified.[98]

The last group covers the remaining secondary OH groups at positions C3. These hydroxyls suffer from inaccessibility, and in most cases, they cannot be modified without protecting at least primary hydroxyls at C6 positions.[98] Some exceptions can be found. Some reagents under special conditions predominantly react with the C3 position OH group. For example, (*E*)-cinnamyl bromide reacts with β-CD in NaOH water solution to give 3^{I}-*O*-cinnamyl-β-CD as the main product with more than 90% regioselectivity. An explanation for this phenomenon is the complexation of cinnamyl bromide's aromatic part into the β-CD cavity so that electrophilic carbon with bromide leaving group is oriented towards the C3 hydroxyl group.[99]

In general, it can be said that selective modification of OH groups located at the primary rim is more straightforward in terms of selectivity compared to a selective change of the secondary rim hydroxyls.

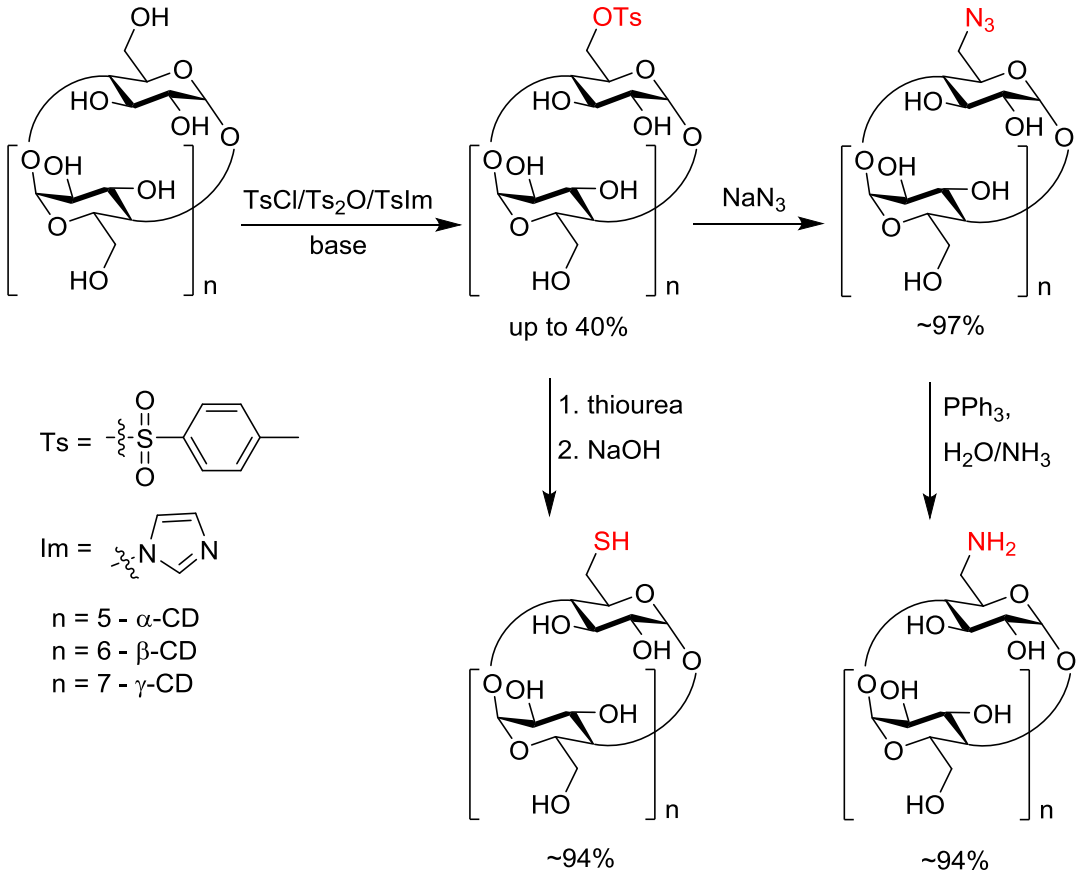
Because my work focuses on monosubstituted CDs on the primary side, persubstituted CDs on the primary side, and monosubstituted CDs on the secondary side with the persubstituted primary side, these three topics will be discussed in more detail in the following subchapters. For a more detailed review of various CD derivatives syntheses, reviews by Řezanka[100,101] can be recommended, a great inspiration for the following subchapters from an informative point of view.

3.1.4.1 Cyclodextrins monosubstituted on the primary side

There are two main approaches to preparing these types of CDs, direct and indirect methods.

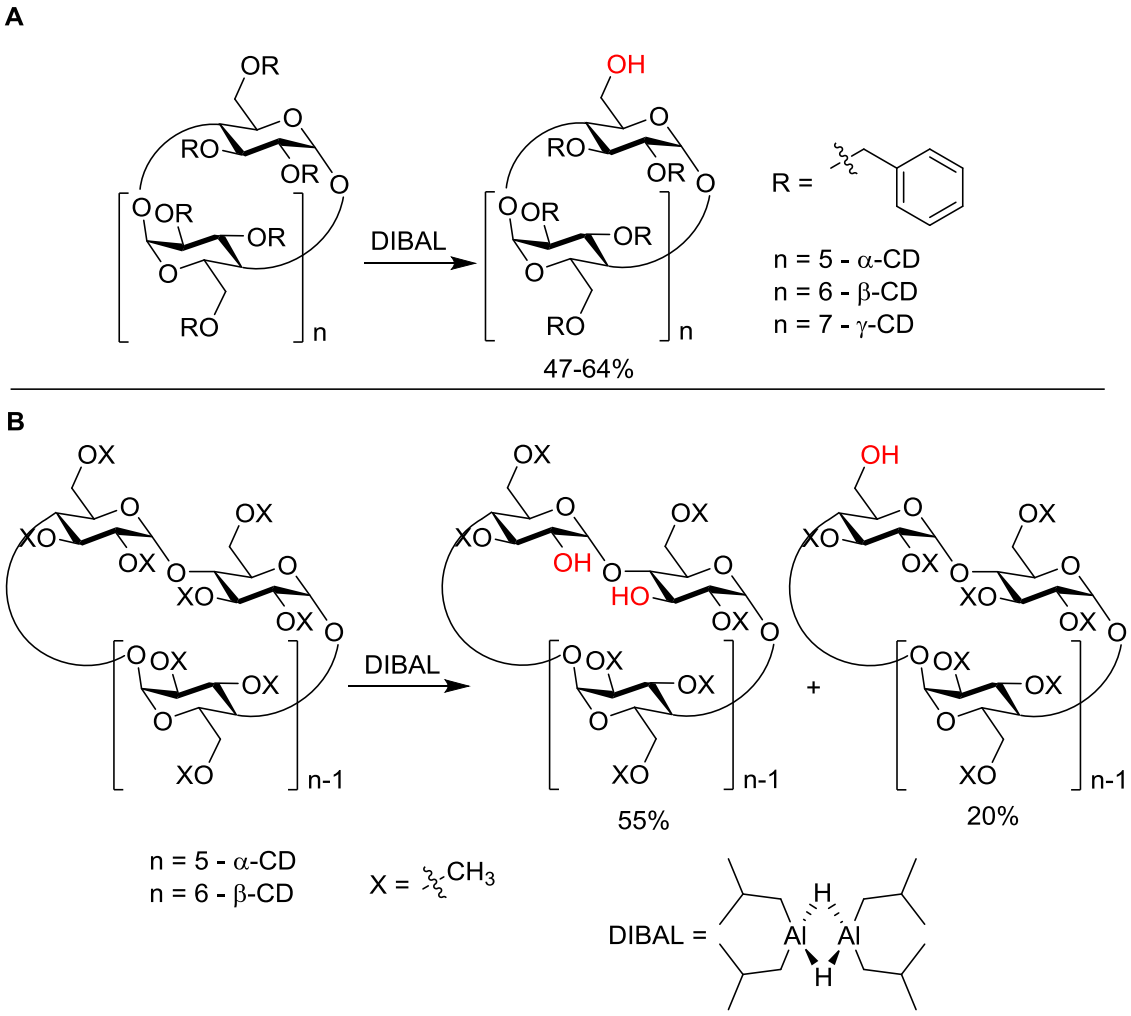
Direct methods utilize reactions between native CDs and appropriate reagents. In most cases, these reactions are not regiospecific, and chromatographic separations are necessary. As always, there are some exceptions. Tosylated CDs are an example of a regiospecific direct method (Scheme 1). According to the literature, regioselectivity highly depends on the type of CD and solvent.[102] These compounds are probably the most utilized precursors for further CD modifications, e.g., azido[103], amino[104,105], or thio[106] derivatives. The three most common tosylating agents are tosyl chloride[107,108], tosyl anhydride[109], and 1-tosylimidazole[110,111].

By the direct method, preparations of other useful monosubstituted CD derivatives, such as allyl, cinnamyl, and propargyl, are not regiospecific. Reactions are usually done in water with an excess of NaOH. These conditions lead to deprotonation of all hydroxyls, and because primary ones are the most basic (the least acidic), and the most sterically accessible, they react predominantly.[112–114]



Scheme 1. The preparation of tosylated CDs and their further modifications (partially redrawn from Řezanka[101]).

Indirect methods are based on high-yielding protection and deprotection steps. The most favorite one is selective DIBAL-promoted mono(de-*O*-benzylation) of perbenzylated CDs developed by Pearce and Sinaÿ (Scheme 2-A).[115] Another useful method is regioselective DIBAL-promoted bis-de-*O*-methylation of permethylated CDs. During this reaction with α-CD and β-CD derivatives, per-*O*-methyl-6^{I}-hydroxy-α/β-CD is formed as a side-product in 20% yield (Scheme 2-B).[116] A possible disadvantage of this latter indirect method is the lack of possibilities of deprotecting remaining methyl groups. That is not a problem in the case of the benzyl protecting groups.[117]



Scheme 2. The mono(de-O-benzylation) of perbenzylated CDs (A) and the mono and the bis-de-O-methylation of permethylated CDs (B) (redraw from Pearce and Sinay[115] (A) and du Roizel at al.[116] (B)).

In general, the synthesis of monosubstituted CDs has undergone little evolution. Low yielding and non-regiospecific direct methods (except tosylation) have been slowly but surely overcome by indirect methods due to their regiospecificity, high yields of protection, substitution, and deprotection steps.

The author of this thesis published a review concerning the synthesis of mono(6-substituted)-CDs.[118] The aim was to compile a general synthetic overview and point out common errors concerning isolated yields, purification methods, and the final purity of substances. The literature on the synthesis of these cyclodextrin derivatives is so full of misleading information that a synthetic chemist starting with these substances must at least be confused.

3.1.4.2 Cyclodextrins persubstituted on the primary side

Preparation of per(6-substituted) CDs utilizes bulky reagents reacting preferentially with the most accessible primary hydroxyl groups. Several common CD derivatives could be prepared in tens of grams (even hundreds) due to non-chromatographic purification procedures.

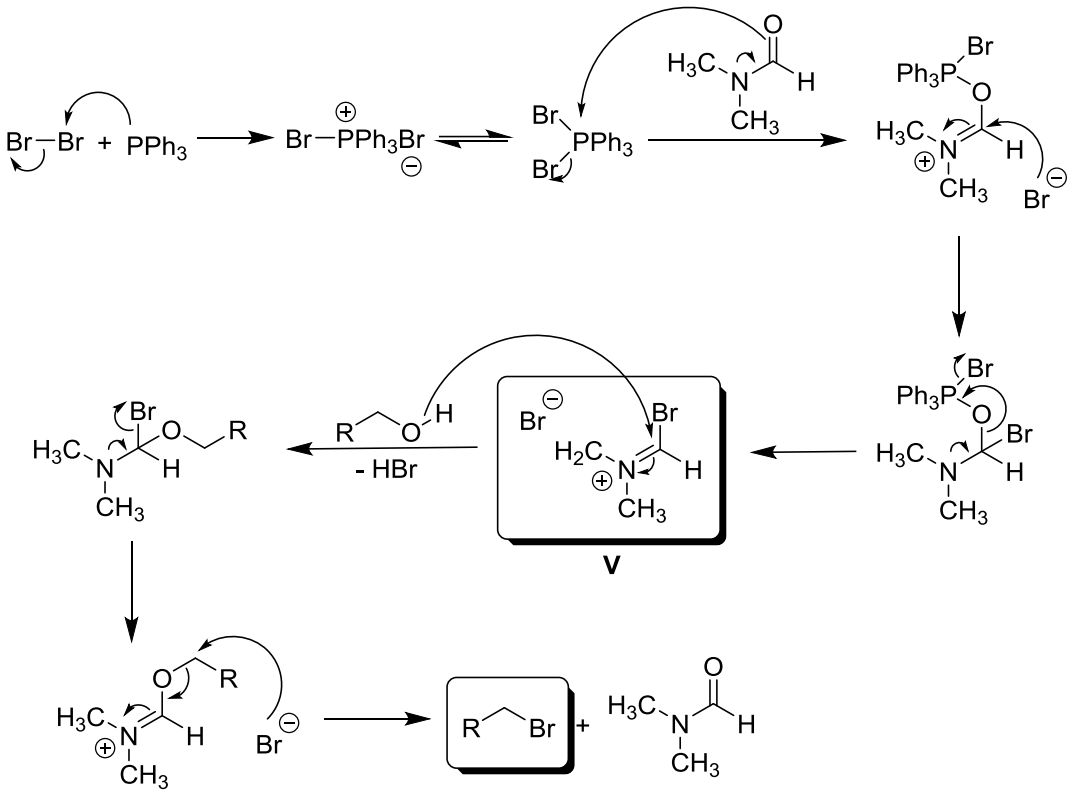
Some of the most utilized derivatives are per(6-*O*-(*tert*-butyldimethylsilyl))-α-, β- or γ-CDs. These compounds are usually used as an intermediate for mono-, partial, or perfunctionalization of the secondary rim. So, the TBDMS group works here as a protecting group that is removed in later steps. TBDMSCl is used as a reagent in all cases, but the base can differ. Pyridine[119] or imidazole[120] could be used as a base. Common drawback used to be product formation along with under- and oversilylated side-products, which led to complex and time-consuming separation. This difficulty was overcome in 2021 when Benkovics et al. described a protocol for large-scale preparation of these silylated CDs derivatives.[121] The key is hidden in the fact that per(6-*O*-silylated)- and oversilylated CDs significantly differ in their elution behavior during column chromatography separation. One elution mixture elutes only the desired product while the other washes out the side-product. The only thing needed is the addition of controlled excess of TBDMSCl to avoid the presence of undersilylated CDs. The authors could prepare more than 30 g of the desired product in 3 days.

After its installment and modification of the CD's secondary rim, the TBDMS group can be substituted with halogen *in situ* using PPh_3 and the source of electrophilic halogen (Br_2, I_2, etc.); the primary rim is then open to further modifications.[119]

This last information leads to the second most utilized group of per(6-substituted) CDs, per(6-halogeno-6-deoxy)-CDs. Methods for their preparation were developed between the 70s and 90s. They all utilize PPh_3 and source of halogen, Br_2[122], MsBr[123], I_2[119], etc. Later, reactions with safer reagents like N-halosuccinimides were developed.[124] For chloro analog, TsCl[125] or MsCl[126] are reagents of choice.

Concerning the mechanism, the use of DMF plays a vital role (Scheme 3). Vilsmeier type reagent like (bromomethylene)dimethyliminium bromide (compound **V** in Scheme 3) is formed during the reaction process and works as a brominating agent. Supports for this mechanism has come from various researchers over the years. Defaye et al. used pure crystalline Vilsmeier bromide **V**, prepared from PPh_3 and bromine in DMF, and obtained 6-bromo-6-deoxy-amylose.[127] Gadelle et al. repeated the same

procedure with iodine and used it to prepare per(6-deoxy-6-iodo)-α-, and β-CD. However, due to Vilsmeier iodide's greater reactivity, it was prepared only *in situ*.[128] Another proof is the necessity to quench the reaction with sodium methoxide to hydrolyze formate esters formed during the reaction.[128] Older papers even describe amylose and CDs' bromination or chlorination by using MsBr or MsCl, in DMF even without PPh_3.[123,129]



Scheme 3. The formation of Vilsmeier type reagent (bromomethylene)dimethyliminium bromide and its subsequent reaction with alcohol.

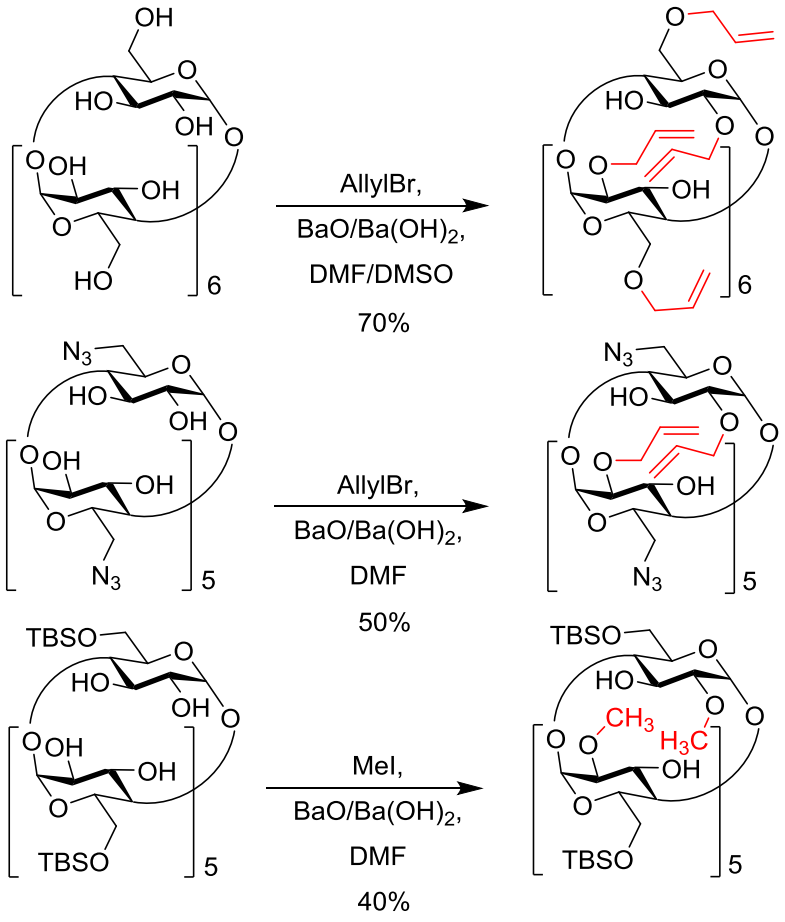
These per(6-halogeno-6-deoxy)-CDs are useful intermediates, and it is possible to let them react with azides[119] and then transform them to amines[130]. Azides could be further used in CuAAC reactions.[131] Analogically to monosubstituted CDs in the previous subchapter, thio derivatives could be prepared and further modified.[132] Recently, Jicsinzky et al. even published a paper about applying ball milling procedures under solvent-free conditions for these other per(6-halogeno-6-deoxy)-CDs' modifications.[125]

In general, these syntheses are sufficiently researched and robust, and no significant changes can be expected in the preparation of these substances.

3.1.4.3 Cyclodextrins selectively substituted on the secondary side with the persubstituted primary side

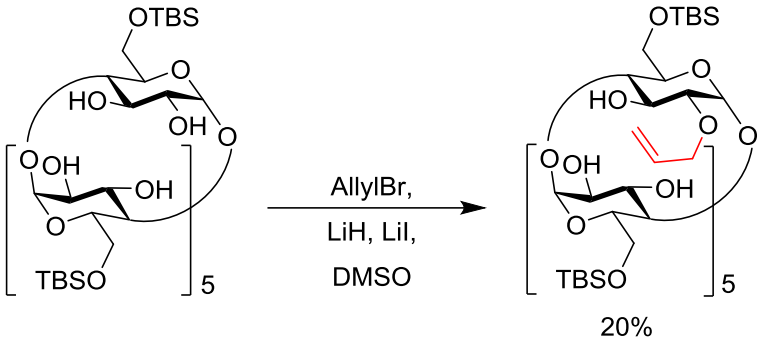
Compared to previous subchapters, examples of regioselective or even regiospecific modifications of the secondary rim are relatively rare. Reasons for this were already mentioned in the introduction of this chapter.

In the late 70s, Bergeron et al. published a paper about his work on CDs' selective modifications. According to his results, per(2,6-di-*O*-allyl)-β-CD could be prepared by reacting native β-CD with allyl bromide and barium oxide/hydroxide mixture in DMSO/DMF system (Scheme 4).[133] Later, Kraus et al. showed that it is possible to apply the same conditions to per(6-azido-6-deoxy)-α-CD, and only per(2-*O*-allylation) occurred.[134] The same results were observed by Takeo et al. when he tested conditions developed by Bergeron to methylate per(6-*O*-TBDMS)-α-CD selectively.[135] Tarver et al. applied these conditions to prepare a series of per(2-*O*-substituted-6-*O*-TBDMS)-β-and γ-CD.[136]



Scheme 4. Examples of CDs selectively substituted on the secondary rim and persubstituted primary rim (examples taken from Bergerone[133], Kraus[134], and Takeo[135]).

Hanessian et al. published in 1995 a paper where he described the regioselective allylation of α-CD. His conditions were based on LiH/LiI system and led to mono(2-*O*-allyl)- and mono(6-*O*-allyl)-α-CD in a 4:1 ratio.[137] This methodology also worked with per(6-*O*-TBDMS)-α-CD, native β-CD, and different alkyl halides (Scheme 5). Casas-Solvas and Vargas-Berenguel used the same conditions for the preparation of mono(2-*O*-propargyl)-β-CD.[138,139]



Scheme 5. Regioselective allylation of per(6-*O*-TBDMS)-α-CD described by Hanessian[137].

3.1.5 Positively charged cyclodextrins

Due to an enormously large number of compounds of this type in CD chemistry, a decision was made to write about derivatives that are the most structurally similar to CDs mentioned in this thesis. That means this chapter will be about structurally unambiguous CDs with permanent positive charge/s (no primary, secondary or tertiary amines, randomly substituted CDs, etc.)

The first group includes monosubstituted CDs, mostly primary rim substituted. This is probably the largest group due to the easiness of preparation, as mentioned in previous chapters. 6-Deoxy-6-trimethylammonium-CDs are one of the most common. They can be prepared directly from tosylated CDs by reaction with trimethylamine in a sealed ampule[140] (trimethylamine has a boiling point around 4°C, but reaction requires 80°C or even higher temperature) or by synthesizing 6-amino-6-deoxy-CDs from tosylated ones (Scheme 1) and its subsequent quaternization by using MeI.[141] Most CDs that fit into this group were synthesized from tosylated CDs. It includes compounds bearing other alkylammonium[142,143], pyridinium[144], imidazolium[145–147], and pyrrolidine[148,149] functional groups. Most of these CD derivatives were tested as chiral selectors in capillary electrophoresis[141,146,148] and catalysis[140].

Regioselectively disubstituted CDs with one or two charged groups belong to the second group. Yamamura et al. prepared AB, AC, and AD regioisomers of β-CD

possessing two trimethylammonium groups from diamino-β-CD precursors by using MeI for quaternization to study complexes with aromatic dicarboxylic acids.[150] Dai et al. published two papers about preparing AC regioisomer of β-CD bearing imidazolium charged group and amino group and tested its potential in capillary electrophoresis.[151,152] Zhou et al. also published synthesis and utilization in capillary electrophoresis of AC regioisomer of β-CD possessing imidazolium charged group.[153]

Some of these CD derivatives were also prepared in our laboratory. Popr et al. synthesized a series of products possessing one or two permanent positive charges[154] and studied their complexation abilities after deposition on Nafion® membrane[155]. Nafion® is a name for a sulfonated tetrafluoroethylene based fluoropolymer-copolymer.[156] It possesses a negative charge and can form electrostatic bonds with cationic compounds. Other types of solids show the same ability and will be discussed in the following chapter, but one more subchapter has to be included before that.

3.1.5.1 Amphiphilic cyclodextrins

CDs substituted on both rims with charged groups on one edge and lipophilic groups on the second form a special group of derivatives. These compounds are called amphiphilic CDs, and some time needs to be devoted to them because their synthesis is also part of this thesis.

Compounds of this type can form vesicles[157,158], nanoaggregates[159,160] in water, and Langmuir layers on a water/air interface[157]. They are mainly studied and tested as gene delivery systems due to their ability to encapsulate molecules of DNA[161–166], and RNA[167,168]. They can form nanoparticles with anionic porphyrins, and these systems could generate singlet oxygen useful for cancer therapy treatment.[169–171]

Concerning the structure and synthesis, charged groups can be on the primary side, and lipophilic groups on the secondary one[157,158], or vice versa[165,170,172–174], or both of them are situated on the primary rim[166,175]. Most of these compounds have completely substituted the primary rim and per(2-*O*-alkylated) secondary side.[176,177] This regioselective secondary rim modification is done either by applying strategies developed by Bergeron et al., which were discussed in the previous chapter[133,167,168,174,177], or by applying the synthetic procedure set by Mazzaglia et al.[178]. It is based on the reaction of per(6-substituted)-CDs with ethylene carbonate in the presence of potassium carbonate at elevated temperatures. These conditions lead to selective 2-*O*-modification with 8 to 22 oligo(ethylene glycol) units per CD. So, these compounds are not structurally

unambiguous. CD derivatives with both rims completely substituted are the second major group.[157,161–164,171,179] All compounds described in cited papers possess primary amines as a charged group, but some authors describe synthesis and utilization of compounds with quaternary ammonium salts.[180,181]

When dealing with amphiphilic CDs, which behave like *surfactants*, the most interesting feature is *micelles* formation and *critical micelle concentration* (CMC). Below CMC (usually in the range 10^{-5}-10^{-3} M), molecules of surfactant are in a unimolecular form. Above CMC, all newly added molecules associate into micelles to decrease the system's free energy. From a thermodynamical point of view, micelles' formation is driven by a relatively large positive entropy factor. To explain this, water molecules need to be taken into account. Water molecules that are in direct contact with the surfactant's lipophilic parts are heavily ordered. Micelle formation leads to exposure of these heavily organized water molecules to the bulk and thus a considerable increase of entropy.[182] The value of CMC can be determined in many ways. The surfactant's concentration is changed, and one of the physical-chemical properties of the system (density, osmotic pressure, turbidity, surface tension, molar conductivity, viscosity, absorbance, etc.) is measured. Then, the surfactant's concentration is determined, at which a slope change is observed.[183,184] The value of CMC depends on many factors but primarily on the structure of the molecules.

In the case of amphiphilic CDs, factors that increase the CMC are the growing number of alkyl chains in one molecule and weak ion-pairing between charged groups and their counter ions.[175] A higher number of alkyl chains mean more van der Waals interactions between them, and thus they are bound stronger together. Due to that, they do not allow water molecules to get between them. So, the system's enthalpy stabilization due to micelles formation is lower because it is already partially stabilized by van der Waals interactions.[175] Ion pairing plays a vital role also. Weak ion-pairing means more charges in one place during micelles formation when these charged groups are getting closer together. That leads to electrostatic repulsions, making micelle formation more difficult and energy demanding.[185]

3.2 Solid supports

It is time to discuss types of solid supports forming electrostatic bonds with ionic compounds. Anionic supports will be mentioned mainly because this thesis focuses on positively charged compounds.

In the beginning, these solids can be divided into two groups. The first group consists of solids that do not possess a negative charge. Applying a simple chemical or physical method can install a negative charge on the surface relatively easily. The gold surface is probably the most well-known representative of this group. Most authors create a negative charge by utilizing various mercapto-carboxylic acids due to forming a strong gold-sulfur bond.[186–192] After these modifications, various compounds with positive charges, e.g., modified CDs[186,187], polymers[188], proteins[189,191,192], and even DNA[190], can be attached and utilized for various purposes. Another possibility is gold nanoparticles possessing a negative charge on the surface. Amines[193] and even lysin with its amino group[194] could be attached to these nanoparticles.

The second group represents supports that already possess a negative charge, so it is unnecessary to modify them any further. Even so, examples of their modifications can be found in the literature. They are mostly done to enhance binding abilities or change the type of charges on the surface from negative to positive or vice versa. Silica gel is probably the most typical representative of the second group. It can adsorb many compounds, and electrostatic binding can play a vital role during these processes. The list of compounds capable of electrostatic interactions is extensive and includes compounds such as proteins[195–200], cationic polymers[201,202], cationic surfactants[203,204], DNA[205], and alkaloids[206]. It can also serve as a support matrix for anionic polymers, binding proteins.[207] It is possible to covalently modify silica gel by aminoalkyl chains (aminopropyl mostly) or alkyl chains with imidazolium moieties to install groups with positive charges onto the surface. This amino/imidazolium alkyl silica gel can form electrostatic bonds with oligonucleotides[208], anionic dyes[209], and proteins[210,211].

Polymers bearing acidic or basic functional groups are also members of the second group. An enormous number of these polymers can be found in literature, so some typical examples should be mentioned. One of the most utilized is sulfonated polystyrene.[212,213] Over the years, scientists have developed various procedures for manufacturing these polymers. Sulfonated polystyrene can be made in thin films[214], grafted on wool fibres[215], and covalently bound to polystyrene core to create an anionic shell[216]. Even polystyrene cuvettes can be sulfonated by simple exposure to sulfuric acid, and various cationic dyes can be immobilized on their surface with the potential for sensor application.[217] Sulfated natural polymers such as agarose and dextran are also commercially prepared and utilized in laboratories.[218,219] Various types of poly(methacrylate) and polyacrylonitrile polymers are also heavily manufactured and utilized.[220–222] They could be used as ionic

membranes[221,222]. Nafion® 117 membranes, utilized in our group[155,223,224], were already mentioned. The most extensive use of these polymers is probably as ion exchangers and in ion-exchange chromatography as stationary phases.[225]

Last but not least, alumina and, for example, its variants as nanoporous anodic alumina should be mentioned. The alumina surface is covered with various functional groups, including carboxylates, and can electrostatically bound different compounds, e.g., metal ions[226], humic substances[227], and their combinations[228]. The nanoporous anodic alumina has a structure of nanochannel arrays in a well-ordered hexagonal honeycomb-like arrangement and can form a bond with albumin.[229]

The next chapter will focus on two types of sorbents, Nafion® and silica gel, and their utilization in chiral separation systems. Nafion® has the potential to be used in chiral membrane separation systems. Silica gel is the most used sorbent for the majority of chiral HPLC columns and chiral TLCs; it bears the role of a matrix for chiral selectors, and was used for the same purpose by the author of this thesis. Due to this fact, the following chapters will be about utilizing silica gel as support for chiral selectors capable of forming electrostatic bonds or being physisorbed on it.

3.3 Chiral separations

Chiral separation of enantiomers is an alternative to enantioselective synthesis. Both of these approaches grow in importance with increasing demand for enantiopure pharmaceuticals. Many pharmaceuticals can exist as a mixture of two enantiomers (Figure 6). In some cases, both mirror images can be used as a racemic mixture for therapeutic treatment due to the inactivity or harmlessness of one enantiomer, e.g., ibuprofen[230]. However, many examples of harmful or even lethal mirror images of well-known and previously used drugs can be found, e.g., thalidomide[231], ethambutol[232], and naproxen[233].

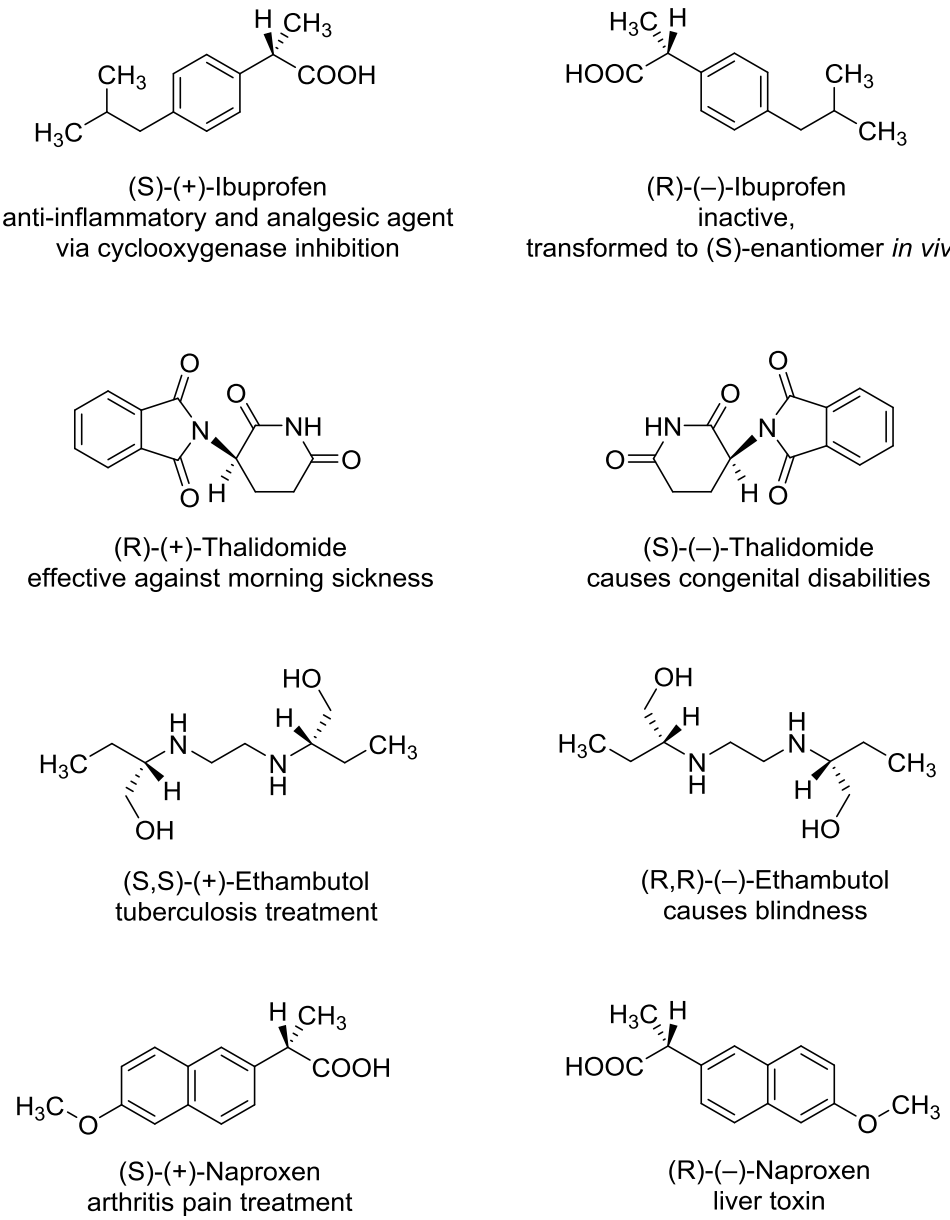


Figure 6. Examples of pharmaceutical enantiomers and their effects.

Due to these facts, it is necessary to test both mirror images of a new potential drug before it is launched on the market to maximize the product's effectiveness and minimize possible adverse effects.

Significant progress in this scientific field has been made over the years. Various methods for enantiomer separation have been developed, e.g., diastereomeric crystallization, chiral HPLC systems, supercritical fluid chromatography (SFC), and enzymatic resolution methods. However, most of the existing chiral resolution techniques suffer from several drawbacks. Diastereomeric crystallizations' main limitation is the necessity to use specific reagents, often effective only for a few or single systems.[234] Even

so, it is still the most widely used method on an industrial preparative scale. Enzymatic resolution methods deeply suffer from high costs and substrate specificity.[235] Both chromatographic methods, liquid chromatography[236] and SFC[237], are reasonable alternatives to crystallization. However, the main drawback is the high cost of chiral stationary phases with low capacity. Notwithstanding, there are heavily utilized on an analytical scale daily.

Among these solutions, membranes are regarded as excellent candidates for chiral separation processes, considering their low energy demand, easy scale-up, possible continuous processing, and limited environmental impact.

This chapter is divided into two parts. The first one gives a brief introduction to chiral membrane systems. The second part's primary goal is to describe chiral separation systems, emphasizing electrostatically bonded and physisorbed chiral selectors to silica gel as solid support concerning chiral HPLC chromatographic columns systems and the chiral thin-layer chromatography (TLC).

3.3.1 Chiral membrane systems

There are several types of membranes to be used according to the application. *Bulk liquid membranes* (BLM) are made of a relatively thick layer of immiscible fluid which is used to separate the feed and strip phases.[238] Chiral selective carriers can be incorporated within the liquid membrane phase and thus stereoselectively transport optical isomers.[238,239] Advantages of these membranes include easy operability and economic convenience. Disadvantages are long-term instability and low mass transport rates.

The latter problem can be overcome using *supported liquid* (SLM) or *emulsion membranes* (ELM). In the case of SLM, the immiscible fluid is enclosed on both sides by a non-selective material to keep the membrane in place.[240] The ELM consists of a strip phase emulsified inside the membrane phase, with the resulting emulsion stabilized by a surfactant. This emulsion is later dispersed in a continuous feed phase to form spherical membrane globules within the feed.[241] A great disadvantage could be significant leakage in the procedures.

Solid membranes usually show greater stability than liquid ones. *Inherently chiral polymers membranes* are the most common type due to their wide applicability.[242] However, this factor is often balanced by low enantioseparation. *Imprinted substrate membranes* are a much more selective alternative but with limited substrate scope.[243]

To combine good selectivity with a wide application range, the functionalization of an achiral membrane with a chiral selector could be a possible and promising strategy. Ongoing research in this field is trying to solve a problem related to the laborious preparation of these materials.[244]

The chiral selector, which ensures different interactions with each enantiomer, plays a crucial role in chiral separation using membrane technology. There is ongoing research to find more efficient chiral selectors to broaden the application field and with potential for scale-up.[245] In recent decades, novel chiral membranes have been developed and tested. Promising results and potential for preparative chiral separations make these materials highly attractive.

Ingole et al. achieved chiral separation of racemic α-amino acids utilizing enantioselective polymer membranes containing a chiral metal–Schiff base complex in a pressure-driven process.[246] D-enantiomers of α-amino acids lysine and arginine permeate preferentially through a composite membrane. High enantioselectivities, 94% and 83%, were observed for lysine and arginine, respectively, by using the Zn complex. This chiral separation came from a steric match of the enantiomers' conformation in the chiral space of the membrane and molecular interactions between racemate and membrane.

Chiral separation of racemic alcohols as (*R*,*S*)-2-amino-1-butanol could be done by applying a chitosan membrane crosslinked with glutaraldehyde.[247] The main advantage of this membrane is that the low operating pressures provide high enantiomeric excesses. At an operating pressure of 15 psi and a feed concentration of 500 ppm, the enantiomeric excess reached as high as 92 %. The information about feed concentration is crucial in this case because it affects enantiomeric enrichment. The use of a dilute feed solution is advantageous to achieving optimum enantioseparation. The influence of pressure cannot also be omitted. Lower trans-membrane pressure is advantageous for enantiomeric separation because higher trans-membrane pressure might reduce the intermolecular interaction between the membrane and enantiomers, thus affecting the enantioseparation adversely.

Ingole et al. also studied chiral polyamide-based thin film composite (TFC) membranes over polysulfone support for enantiomeric separation through chemical modification.[248] The concentration of chiral monomer in polymerization influences the enantiomeric excess. Optical resolution of lysine and asparagine amino acids was achieved. The enantiomeric excess of 92% and 68% were observed for lysine and asparagine, respectively. The ATR-FTIR, SEM, and AFM characterizations have

revealed that a thinner and smoother polyamide layer with a larger free volume is produced, leading to a higher volumetric flux, better mechanical stability, and greater enantioselectivity.

Another type of TFC membrane was again prepared by Ingole et al. by interfacial polymerization of *trans*-1,4-diaminocyclohexane and piperazine (in the aqueous phase) with trimesoyl chloride (in the non-aqueous phase) on the polysulfone membrane (support for thin chiral selective layer).[249] By applying a pressure-driven reverse osmosis process at 689.42 kPa pressure, the membrane showed enantioselectivity of over 78% for L-lysine monohydrochloride from aqueous solutions of a racemic mixture. A similar system developed in the same scientific group exhibited even better enantiomeric excess (90%) by utilizing L-lysine as the chiral selector (instead of the *trans*-1,4-diaminocyclohexane) and at trans-membrane pressure 172 kPa.[250]

However, despite the various types of chiral membranes already available, performing enantioseparation remains restricted to a small scale. Thus, further research is needed to tap into the undoubted potential of this field for industrial applications, for example, by utilizing negatively charged achiral membranes of optimal permeation properties to which suitable enantioselectors, such as positively charged CDs, can easily be bound.

3.3.2 Chiral HPLC systems

3.3.2.1 Introduction

The fundamental basis for separating enantiomers is their transformation to diastereomers or forming a diastereomeric relationship between ligated enantiomers and a chiral selector. Three common approaches can be named. The oldest one and barely used nowadays is called an *indirect approach*. It is a strategy when enantiomers are transformed to diastereomers by chemical reaction and later separated using an achiral column with achiral eluents.[251] The second one is called the *chiral mobile phase additive (CMPA) mode*. Enantiomers are separated on an achiral column applying the mobile phase with the pure chiral compound. Transient diastereomeric molecule associates are formed. This leads to inequivalent adsorption and retention for individual enantiomers.[252] The last and most utilized enantioseparation technique is named the *chiral stationary phase (CSP) mode*. A chiral selector (CS) is covalently bonded or physisorbed mostly to spherical porous silica support. Separation is based on the reversible formation of

transient diastereomeric molecular associates between CS and a selectand (SA) on the surface of the adsorbent.[253]

Basic principles of chiral recognition and separation were postulated in the 30s by Easson and Stedman in the *three-point attachment model*, and it is still the most prominent one.[254] This model states that a minimum of three configuration-dependent interactions between a chiral selector and a chiral substrate is required for chiral distinction (Figure 7).

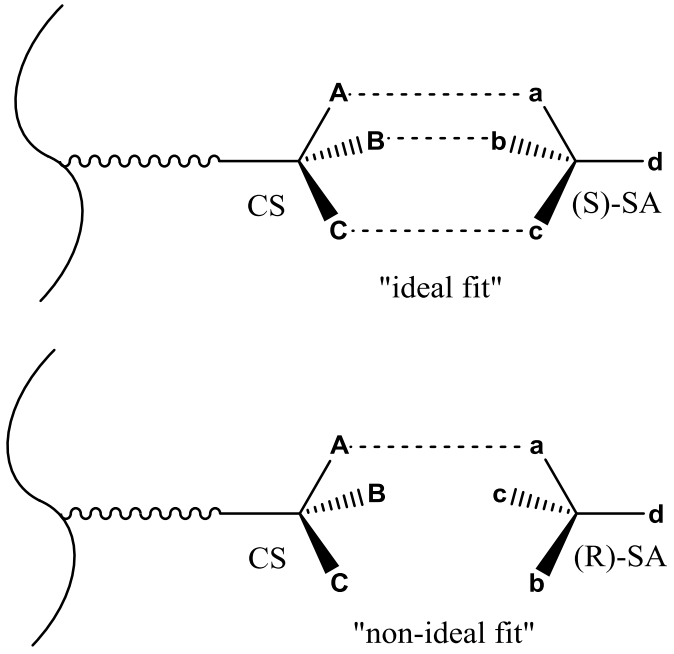


Figure 7. Three-point interaction model (redrawn from Lämmerhofer[255]).

3.3.2.2 Thermodynamic point of view

From a more sophisticated perspective, the retention and chiral recognition on CSPs are usually enthalpically controlled.[255] It means that strong binding between selector and substrate (represented by enthalpy change) overcomes an increase of order (entropic cost). In some cases entropically controlled separations can be found.[256,257] This phenomenon can be proven through temperature screening. As Eq. 13, called *van't Hoff equation*, and derived from Eq. 11 and Eq. 12, named *Gibbs-Helmholtz equation*, states: with negative enthalpy difference $\Delta_{R,S}H^{\circ}$ between the two enantiomers, while they interact with the stationary phase, an increase in temperature leads to decrease in selectivity a, but with positive $\Delta_{R,S}H^{\circ}$, an increase in temperature leads to increase in a.

$$\Delta_{R,S}G^{\circ} = -R \cdot T \cdot \ln a \quad \text{Eq. 11}$$

$$\Delta_{R,S}G^{\circ} = \Delta_{R,S}H^{\circ} - T \cdot \Delta_{R,S}S^{\circ} \quad \text{Eq. 12}$$

$$\ln a = -\frac{\Delta_{R,S}H^{\circ}}{R \cdot T} + \frac{\Delta_{R,S}S^{\circ}}{R} \quad \text{Eq. 13}$$

These thermodynamic parameters depend on many factors, e.g., solute, CSP, and the mobile phase. Due to this impossibility of generalization, they were determined for all important CSP with various sets of analytes.[258–260]

The situation is even more complicated because CSPs are heterogeneous supports with more than one type of adsorption site. The most straightforward division can be into enantioselective and non-enantioselective ones.[261,262] The latter have their origin in binding a substrate to the supporting matrix, linker groups, spacer unit residues from silanol end-capping, and last but not least, from the non-enantioselective binding site of the selector.

3.3.2.3 Modes and chiral stationary phases

As for the mobile phase, several modes differ in the solvents applied. The two most common ones are the *normal phase mode* (NP) and *reversed phase mode* (RP). The first utilizes the non-polar mobile phase (usually organic solvent with a small amount of alcohol) and polar stationary phase (such as silica gel) for polar compounds separation. The latter is based on the polar mobile phase (usually water solutions containing MeCN or MeOH) with a non-polar stationary phase (such as octadecyl modified silica gel) and is used to separate compounds possessing hydrophobic moieties.[263] The third one is called *hydrophilic interaction chromatography* (HILIC). This method, developed by Alpert, is the variation of NP and utilizes a hydrophilic stationary phase with an aqueous-polar organic solvent.[264]

Hundreds of CSPs have been developed and tested in laboratories. More than a hundred of them are offered by commercial suppliers, and around 30 of those are most frequently utilized in daily routine. CSP can be divided into several groups concerning the chiral selector type. Excellent reviews were published in the last years concerning this topic.[255,265]

The most commonly used CSPs in daily practice are *polysaccharide-based* CSPs.[266] Cellulose and amylose are the most used biopolymers. With their further chemical

modifications, these CSPs can separate numerous chiral compounds. *Macrocyclic antibiotics* CSPs introduced by Armstrong et al. in 1994 utilize glycopeptides vancomycin, teicoplanin, avoparcin, etc., as chiral selectors.[267] *Pirkle-type or donor-acceptor* CSPs consist of low molecular mass selectors developed by Pirkle et al. in the late 70s.[268] These CSPs will be discussed in more detail in the following subchapters, together with *cyclodextrin* CSPs. *Derivatized cyclofructan-based* CSPs are one of the newest developed CSPs introduced by Sun et al. in 2009.[269] *Crown ether-based* CSPs were prepared in the late 70s by Sogah and Cram and used for amino acid resolution.[270] Immobilized cinchona alkaloid and its derivatives developed mostly by Kacprzak et al. are among the most useful representatives of *ion-* and *ligand-exchange* CSPs.[271] These types of CSPs utilize electrostatic ion-ion interactions to guide analytes towards the chiral selector binding site. Finally, *protein-based* CSPs based mostly on human serum albumin, ovumocoid, cellobiohydrolase, etc., can be mentioned.[272]

This is just a list of some of the essential CSPs, and what will be discussed next, in more detail, are cyclodextrin and Pirkle-type CSPs.

3.3.2.4 Cyclodextrin chiral stationary phases

CDs are one of the most prevalent compounds used for chiral separation. Their advantage in HPLC systems is the ability to work as a chiral selector in all three previously mentioned modes.[265] The molecular recognition mechanism changes under these conditions. In RP mode, inclusion complexation driven by hydrophobic interactions is the main force governing the resolution.[273,274] On the other side, in NP mode, hydrogen bonding with CD OH groups and dipolar interactions contribute mostly to the recognition mechanism.[273]

Armstrong and DeMond introduced CD CSP in the 80s to separate dansylated amino acids and barbiturates.[275] Over the years, many CDs CSPs have been developed in laboratories. Some of them are even offered by commercial suppliers (*Cyclobond* columns, from ASTEC; *ChiraDex*, from Merck; *Ultron ES-CD* from Shinwa).

Wang and Ng have been very prolific in this field, synthesizing many CSPs containing native[276] and derivatized[277,278] CDs. Their primary strategy is the CuAAC reaction between CD's azido group and aminopropyl silica gel modified to 2-propynylamide. CDs' derivatization is mostly based on phenylcarbamoyl groups and their aromatic ring variations. These functional groups ensure π-π, dipole-dipole, and hydrophobic interactions in RP mode.[277] The majority of analytes tested on these CSPs

were dansylated amino acids, propionic acids with phenyl substituents, flavonoids, and other pharmaceutical compounds.

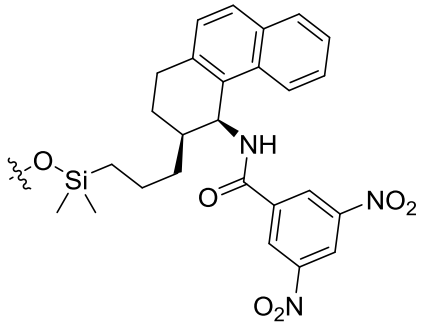
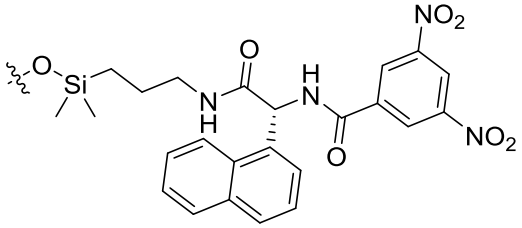
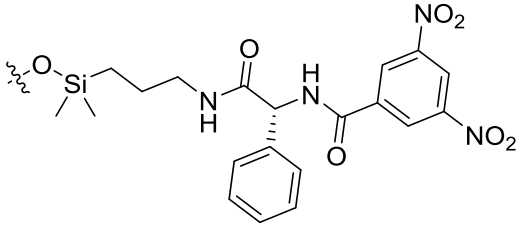
Another group of CD derivatives could easily belong in the group of *ion-* and *ligand-exchange* CSPs. These derivatives possess a positive charge close to CD moiety, and due to ion-ion interactions, molecules of the analyte are getting closer to a chiral CD molecule. Wang and Ng published several papers describing the preparation of these CSPs.[279–281] Several CSPs with cationic aromatic or aliphatic skeleton connected to the primary rim of CD and perphenylcarbamoylated on remaining hydroxyl groups were prepared and tested in HPLC systems under NP mode and *supercritical fluid chromatography* (SFC) *mode*. The resolution of flavonones, thiazides, and amino acid derivatives was observed. Other scientific groups also prepared similar CSPs based on similar principles and strategies. Zhou et al. prepared 1,2-dimethylimidazolium and 1-amino-1,2,3-triazolium substituted β-CD and tested their recognition capabilities after covalent attachment to silica gel.[282] Yao et al. prepared 1-allylimidazolium substituted β-CD and via thiol-ene reaction connected with thiol functionalized silica gel.[283,284] Wang group utilized Yao's strategy and resolute isoxazolines, dansylated amino acids, flavonoids, etc..[285,286]

3.3.2.5 Pirkle-type or donor-acceptor chiral stationary phases

As already written, these CSPs were developed in the late 70s by Pirkle et al., so they bear his name.[268] They were the first entirely synthetic CSPs. Structural elements that ensure rigidity, bulkiness, and necessary interactions are incorporated during synthesis.[287] They are compatible with all modes, but NP mode is the most utilized one. The NP mode activates donor-acceptor interactions, like hydrogen bonding, π-π, and dipole interactions, the most. A great advantage is a possibility of preparing a chiral selector in both enantiomeric forms and changing the order of analytes elution.[288]

The are several commercial columns on the market, and some of the important ones are listed in Table 2. The Whelk-O1 is a column with one of the broadest distributions in industrial and academic laboratories. Originally it was designed as a naproxen-specific CSPs.[289]

Table 2. Examples of commercially available donor-acceptor CSPs.[255]

Chiral selector	Column tradename
 3-[1-(3,5-dinitrobenzamido)-1,2,3,4-tetrahydrophenanthrene-2-yl]propyl-silica[290]	Whelk-O1
 (R)-3-[N-(3,5-dinitrobenzoyl)-1-naphthylglycine-amido]propyl-silica[291]	Chirex 3005
 (R)-3-[N-(3,5-dinitrobenzoyl)phenylglycine-amido]propyl-silica[292]	Phenylglycine DNBPG Chirex 3001 Phenomenex

Although most donor-acceptor CSPs were developed at the end of the last century, research into new types is still ongoing. In 2010, Pirkle and Lee prepared a new type derived from *a*-amino-β-lactam for β-blockers resolution.[293] Wei et al. prepared two new columns by applying benzoylated tartaric acid and 1,2-diphenylethylene diamine.[294]

3.3.2.6 Electrostatically bound and physisorbed chiral stationary phases

It is necessary to start again by mentioning Pirkle because of his pioneering work in this field. In 1981, he published the first paper in which he described a new CSP based on an electrostatically bonded *N*-3,5-dinitrobenzoyl-phenylglycine (DNBPG) to aminopropyl silica gel and showed its versatility by successful resolution of a broad spectrum of analytes.[295] In his following papers, he even demonstrated the use of this CSP on a preparative scale.[292,296,297] This CSP quickly became popular, which could be proved by Kasai et al., who utilized it in their paper, published in 1982, and stated they got it

from a commercial supplier.[298] By the end of the 80s, Horikawa et al. developed *N*-3,5-dinitrobenzoyl-naphthylglycine CSP as an analogy to Pirkle's commercial CSP (SUMIPAX OA-2000I). Both phases were tested, and Horikawa's was superior in a lot of cases.[291] Pirkle et al. prepared in 1992 a novel CSP based on (S)-naproxen electrostatically bonded to aminopropyl silica gel. This phase was tailored for naproxen resolution, and the authors utilized the reciprocity principle in this case.[299] Yang and Lin also broadened the chiral selectors' spectrum by preparing N-arylcarbamoyl derivatives of valine, alanine, and phenylglycine in their publications.[300,301] Some of these CSPs have become popular and commercially available (SUMICHIRAL OA-2000 series) over the years, even though mobile phase composition is limited. Only hexane (heptane)/*i*-PrOH mixtures should be used, and the amount of *i*-PrOH should not exceed 20 % w/w; otherwise, leaching of selectors from columns could be observed.[302]

Even though most of the work was done by the end of the last century, new papers dealing with new CSPs are published. In 2009, Lao and Gan developed a chiral phase with an ionically bonded diproline with aminopropyl or aminodipropyl silica gel.[303] Iuliano and Lecci, in their two publications from 2003 and 2005, synthesized new CSPs based on s-triazine covalently bound to silica gel.[304,305] The s-triazine was utilized as a scaffold used for connecting two different and differently modified amino acids. One was connected covalently, the second-one ionically (Figure 8). Separation of different racemic analytes, chosen among the racemates resolved by CSPs formed by the isolated amino acid derivatives, was tested. The authors observed no significant differences in the enantiodiscriminating capability between biselector and independent amino acid CSPs.

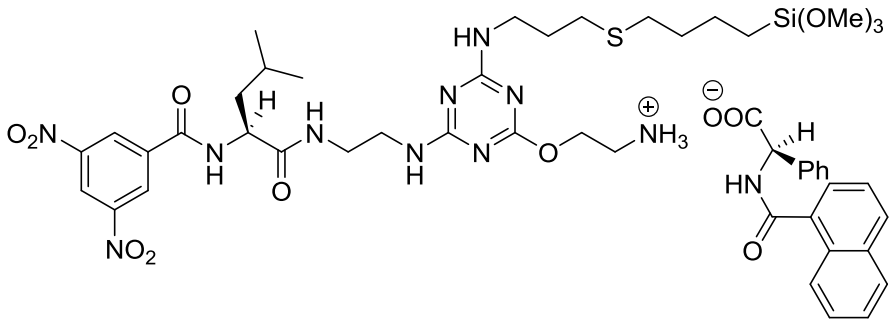


Figure 8. Example of CSP developed by Iuliano and Lecci (redrawn from Iuliano and Lecci[304]).

Concerning CDs, Ng et al. prepared series of cationic CDs in their pioneering work (Figure 9-A). They physisorbed their first cationic derivative onto silica gel and optimized the amount of sorbed selector. This CSP was tested in HPLC and SFC and compared with commercial column SINUPC containing chemically bonded mono(6-ureido-6-deoxy)-

perphenylcarbamoyl-β-CD. The ionically bonded CSPs were superior in terms of selectivity and resolution.[306,307] Later, authors prepared similar derivatives to test different aromatic substituents on CD moiety and alkyl substituents on cationic imidazolium part.[308] In all three papers, the analysis had to be done in the non-polar mobile phase to prevent selectors' leaching. The authors stated that in the hexane/*i*-PrOH mobile phase more than 3% w/w of *i*-PrOH led to noticeable selectors' losses.

Our group also contributed to this scientific field. Cationic CD PEMPDA-β-CD (Figure 9-B) synthesized by Popr et al.[154] was coated onto sulfonated silica gel, and this novel CSP was tested and characterized.[309]

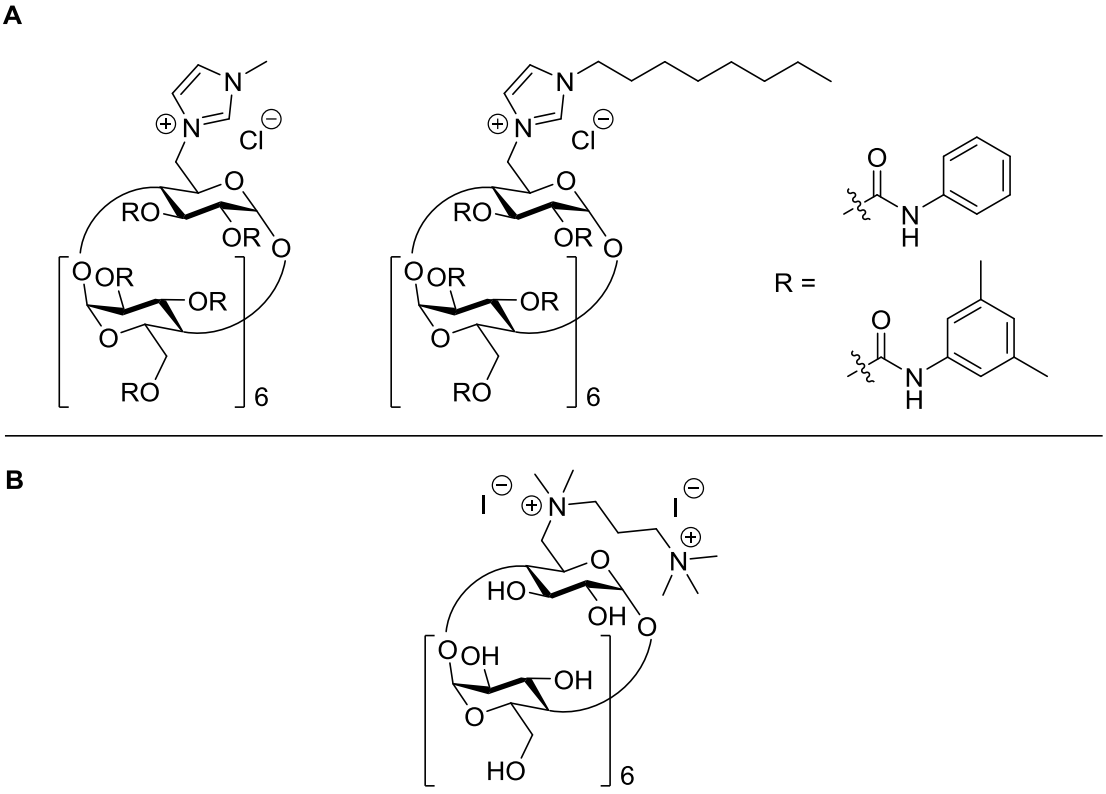


Figure 9. Compounds developed and utilized by Ng et al. (A) and Popr et al. (B) (redrawn from Ng et al.[306,308] and Popr et al.[309]).

3.3.3 Chiral thin-layer chromatography

This time, an introduction will be short. The reason is that what was written about HPLC systems can be sad about TLC.

There are three standard methods for TLC chiral separation.[310] The first one is the *chiral mobile phase additive (CMPA) mode*, which is utilized much more in this area than in HPLC systems. The main reason is the much lower consumption of the selector during a plate's evolution. The second one is the good old *chiral stationary phase (CSP) mode*,

based on chiral TLC plate and achiral mobile phase. In this case, stationary phases are constituted by the chiral selector and a binder as a minor component to ensure mechanical stability. There are only a few CSPs available on the market; they include native cellulose (CEL 300, Macherey-Nagel), microcrystalline cellulose (CEL 400, Macherey-Nagel), and acetylated cellulose (Analtech, Newark). On the other hand, many home-made CSPs have been prepared over the years, including CD-based CSPs.[311] The third method is named the *chiral-coated stationary phase (CCSP) mode*, and it is based on an achiral plate impregnated with the chiral selectors and achiral mobile phase. The CCSP can be subdivided into two methods. One is called the *slurry method* and is based on mixing the chiral selector with the achiral phase slurry (silica gel mostly) used for plate preparation.[312] The second one is named the *dipping method*, and as the name states, the chiral modification is done by dipping a TLC plate into the selector's solution.

The last-mentioned method is probably the most convenient for chemists due to no need for special chemicals or devices to modify plates. Chemists only need a synthesized or bought chiral selector and commercial achiral TLC plate. This is why testing the dipping method with compounds synthesized in this thesis is the most interesting option. Due to that, it will be discussed in more detail.

Most of CSPs prepared by the *dipping method* are *ion-* and *ligand-exchange* CSPs. Günther et al. prepared the first dipped CCSP plates in the 80s by dipping RP-18 TLC into copper(II) acetate solution and then proline derivative solution and utilized it for amino acid enantiomers.[313] Later, Günther et al. cooperated with Nacherey-Nagel, commercialized these plates, and sold them under the name CHIRALPLATE since then.[314] Other scientists contributed to this field before the millennia's beginning. Weinstein used alanine derivative instead of proline and resolved dansylated amino acids.[315] Marchelli et al. developed chiral TLC plates utilizing dimer of phenylalanine.[316] Remelli et al. used histidine derivative and successfully developed CCSP plates for amino acid resolutions.[317] Even recent publications concerning these *ligand-exchange* TLC CSPs can be found. Absalan et al. developed new plates by dipping silica gel TLC plate into gold nanoparticles solution, then to L-cysteine solution, and copper(II) acetate was added to the mobile phase.[318] This phenomenon of combining CCSP and mobile phases with other portions of chiral selectors or components needed for separation (copper(II) acetate, for example) is typical for papers published after the 2000s.

Plates different than RP-18 TLC were also utilized. Witherow et al. used TLC plates with aminopropyl silica gel. They dipped it into *N*-(3,5-dinitrobenzoyl)-L-leucine solution

and this TLC *Pirkle-type or donor-acceptor* CSP was used to resolve bi-β-naphthol compounds.[319] Aboul-Enein et al. developed novel chiral TLC by dipping silica gel TLC plate into L-serine or L-threonine solution to separate 2-arylpropionic acids.[320] Bhatt et al. tested L-tartaric acid as a chiral selector. The best results were obtained using impregnated silica gel TLC with L-tartaric acid and the same selector in the mobile phase.[321]

Two scientific groups contributed mainly to this field in new millennia. Sajewicz et al. have published several papers since 2004. They described the utilization of silica gel on a glass surface and L-arginine as a selector to resolve ibuprofen, 2-arylpropionic acids, naproxen, etc..[322–325] Bhushan et al. published many papers dealing with the chiral TLC plate prepared by the *slurry method*.[312] In several of them, authors compared these TLC plates and the ones prepared by the *dipping method*, stated that no general rule could be announced, and it depends on the analyte's nature.[326,327]

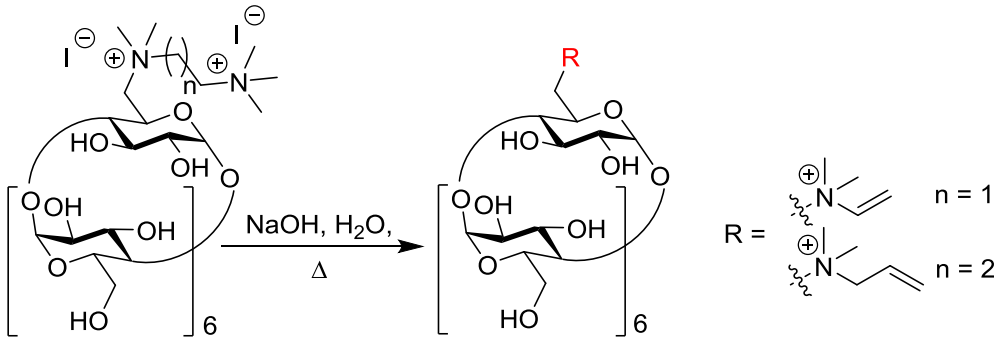
Concerning CDs, most publications deal with *chiral mobile phase additive (CMPA) mode*. Only one paper in which authors utilized at least a variation of the *dipping method* was found. Dąbrowska and Krzek evolved silica gel TLC plates in the β-CD solution to impregnate it. Later, they added β-CD also in the mobile phase and used this system to separate cefaclor epimers.[328]

4 RESULTS AND DISCUSSION

4.1 Charged neopentyl skeleton anchors

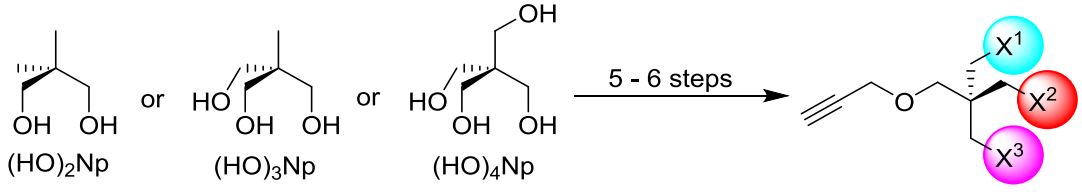
4.1.1 First generation synthesis

As was already written in the State of the Art chapter, the former colleague Martin Popr has prepared a series of permanently charged CD derivatives and tested their ability to adsorb onto Nafion® 117 membranes.[154,155] Possible disadvantage of these compounds was a lack of stability under basic conditions. Due to β-hydrogens next to alkylammonium groups, Hofmann elimination occurred (Scheme 6).



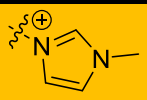
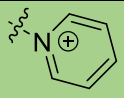
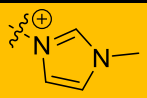
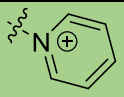
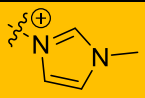
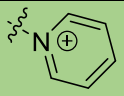
Scheme 6. Examples of CD derivatives prepared by Martin Popr and products of their thermal decomposition.[155]

Based on these findings, a decision to apply a different strategy was made. The effort was focused on preparing a series of multiply charged compounds (anchors) stable against Hoffmann elimination[329] and easily attachable to CDs and other compounds. For this purpose, neopentyl (Np) skeleton polyols $(HO)_2Np$, $(HO)_3Np$, and $(HO)_4Np$ (pentaerythritol) have been chosen to ensure the stability in a basic environment (Scheme 7), and several different types of anchors could be prepared (Table 3). Notwithstanding the difficulties described in the following chapters, their synthesis was developed and optimized, and subsequently, this new group of multiply charged substances was also patented.[330]



Scheme 7. Neopentyl skeleton polyols utilized for anchors synthesis.

Table 3. The list of prepared anchors.

Compound	X^1	X^2	X^3
Prg-O-MIM1 **1**	H		
Prg-O-PYR1 **2**	H		
Prg-O-TMA1 **3**	H		
Prg-O-MIM2 **4**	H		
Prg-O-PYR2 **5**	H		
Prg-O-TMA2 **6**	H		
Prg-O-MIM3 **7**			
Prg-O-PYR3 **8**			
Prg-O-TMA3 **9**			

The nomenclature should be mentioned here. For clarity, abbreviations for important functional groups and structural features are introduced (Figure 10). The meaning of the abbreviation can also be found in the list of abbreviations.

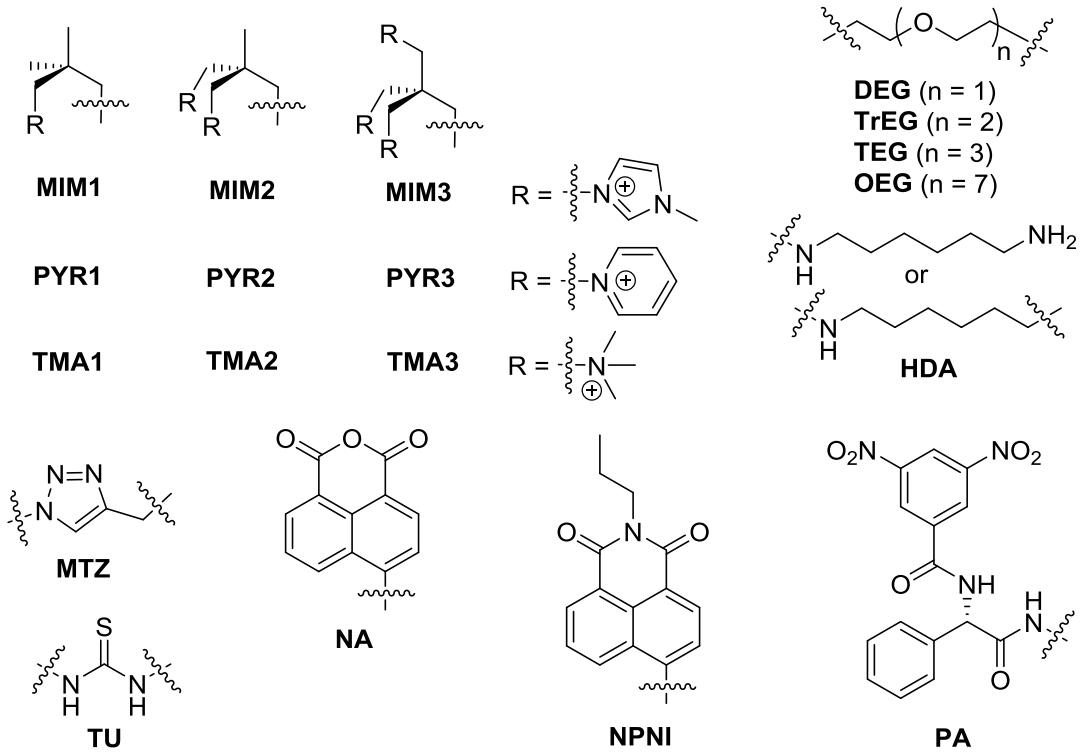
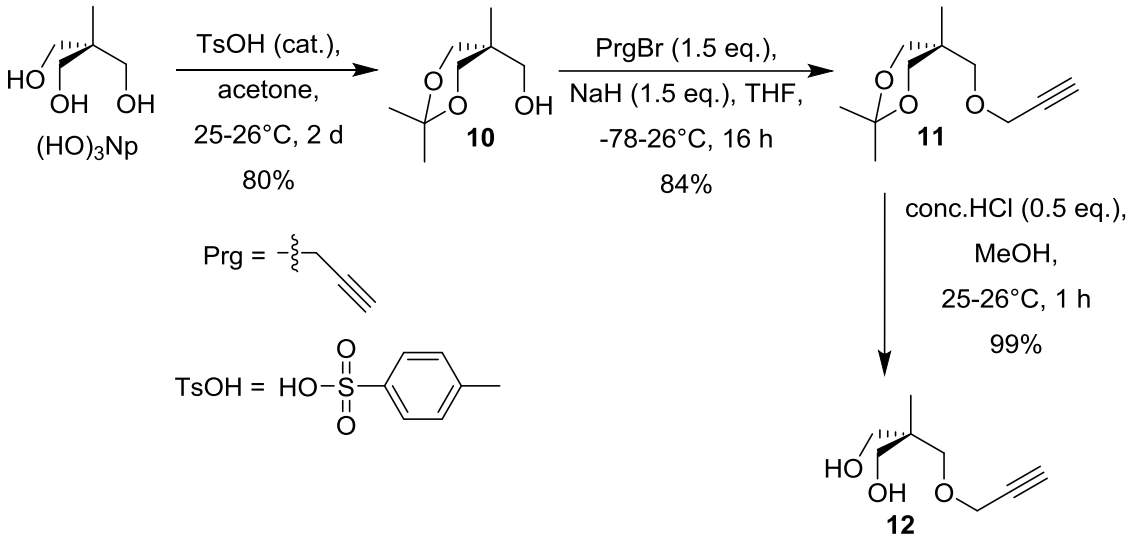


Figure 10. List of abbreviations for important functional groups and structural features used in this thesis

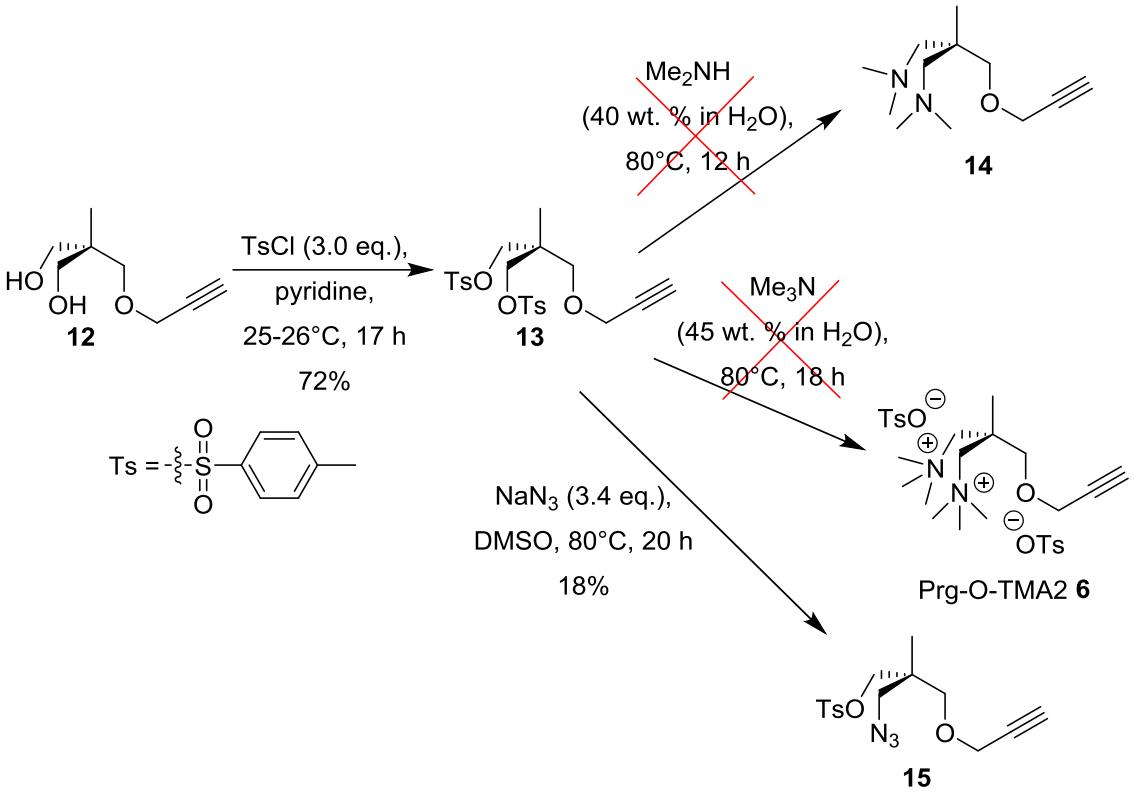
The synthesis of the first doubly charged anchor Prg-O-TMA2 **6**, possessing two trimethylammonium functional groups, started. The intermediate **12** was prepared according to the literature with slight modifications and improvements (Scheme 8). These improvements included purification of the compound **10** by vacuum distillation. This procedure was necessary due to the insufficient purity of the substance obtained by the described protocol.[331] The inspiration for this was the protocol for the second step leading to the compound **11**.[332] The propargyl group was chosen because of a decision to utilize the CuAAC click reaction concept, ensuring an easy connection with CD molecules. The deprotection step leading to **12** was first tried using a strong cation exchanger but better results were obtained by applying HCl.[333]



Scheme 8. Preparation of the intermediate **12**.

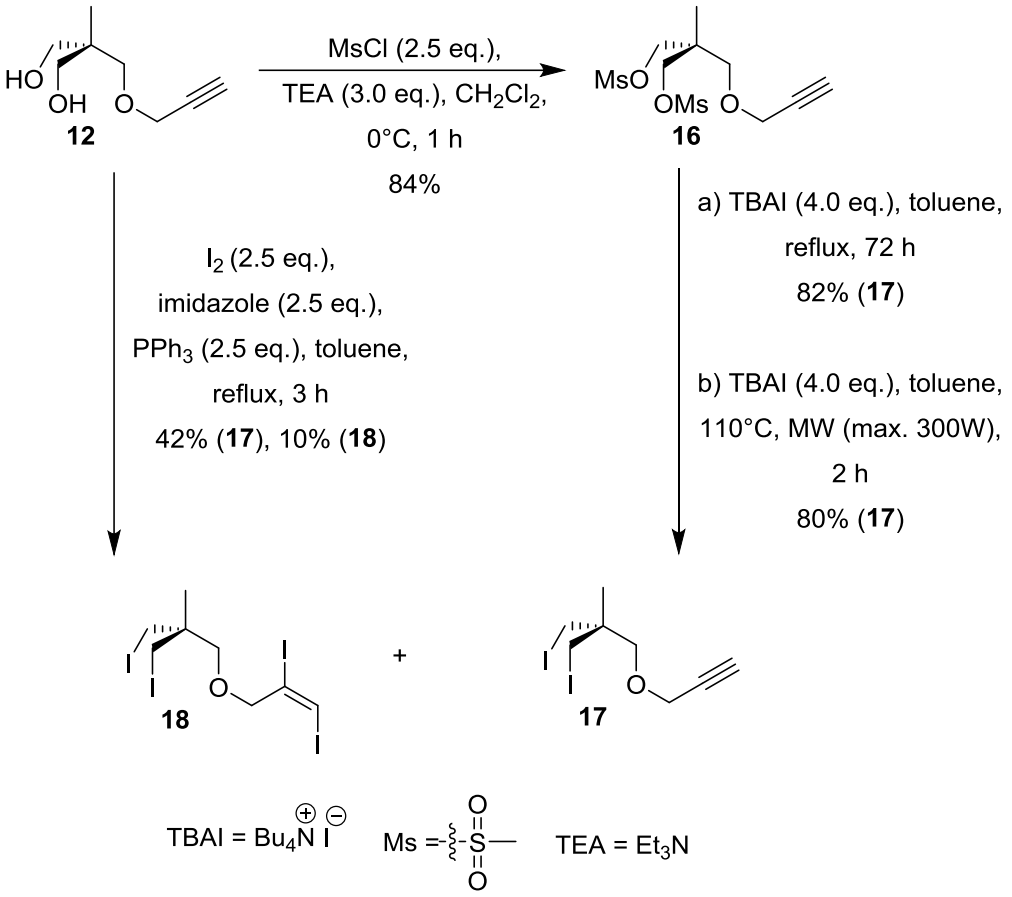
Then the proper leaving group had to be installed. Literature is a bit ambiguous concerning leaving group reactivity when being part of the neopentyl skeleton. In a nucleophilic substitution (S_N), the leaving group is replaced by a nucleophile. However, the neopentyl skeleton is too sterically hindered by the *tert*-butyl moiety for S_N2 to occur even though the leaving group is attached to the primary carbon atom, as shown by Whitmore and Rothrock.[334] Subsequently, Dostrovsky et al. observed that under S_N2 conditions, neopentyl bromide reacts approximately 10^5 times slower than other primary alkyl bromides.[335,336] However, under S_N1 conditions, the reaction rates are similar, but the resulted carbocation rearranges to a *tert*-amyl skeleton and then forms isoamylene via elimination. Later, Dostrovsky stated that this type of rearrangement could occur even in radical reactions.[336] In the following and recent years, more authors observed and studied this phenomenon. Sanderson and Mosher studied deuterated neopentyl alcohol rearrangement and concluded that this reaction is highly stereoselective and, therefore, cannot proceed via a free neopentyl cation.[337] Patrick et al. utilized ^{13}C labeling to verify the skeletal rearrangement mechanism in the reaction of neopentyl iodide with xenon difluoride.[338] Edwards et al. observed the rearranged product of neopentyl sulfate during their studies of the spontaneous hydrolysis of alkyl sulfates.[339]

The TsO^- leaving group was tested first[340], but unfortunately, the subsequent reactions with trimethylamine and dimethylamine were unsuccessful (Scheme 9). Only starting compound **12** was isolated. Just from curiosity, the reaction with NaN_3 (one of the best nucleophiles) was tested, and only a small amount of monoazido product **15** was formed and isolated (Scheme 9).



Scheme 9. Preparation of the compound **13** and unsuccessful subsequent reactions.

Next, a more reactive leaving group was tried. The compound **16** containing MsO^- leaving groups was prepared[341] and tested (Scheme 10). The result was basically the same compared to the previous compound **13** bearing TsO^- leaving group.

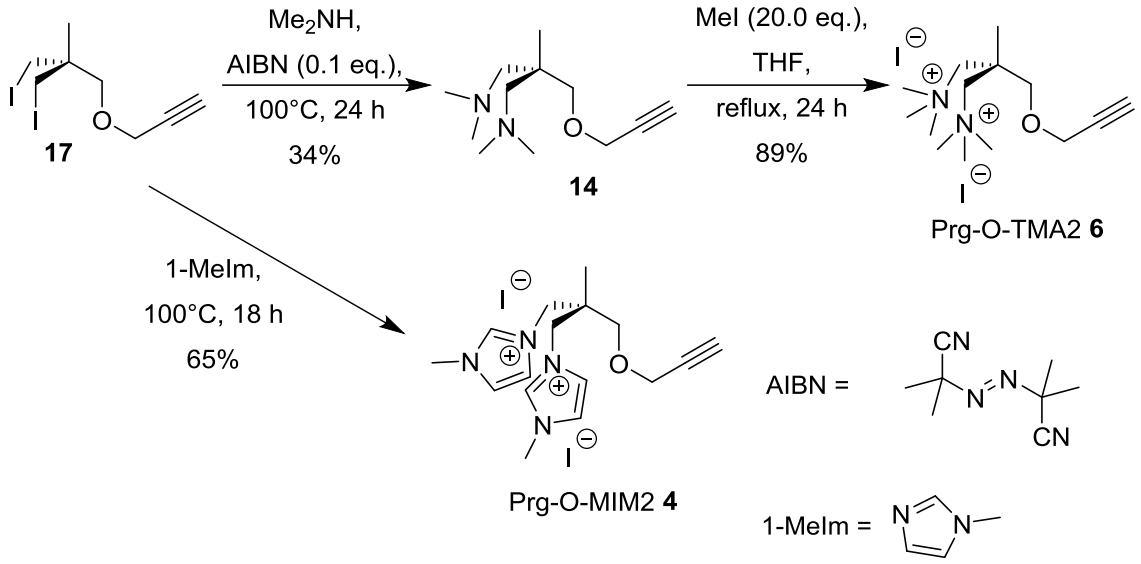


Scheme 10. Preparation of compounds **17** and **18**.

It was time to test iodide leaving group (Scheme 10). First, diiodo compound **17** was prepared directly from propargylated diol **12** by applying iodine, imidazole, and triphenyl phosphine protocol[342]. Unexpectedly, the formation of tetraiodo side-product **18** resulting from electrophilic addition to triple bond was observed. Due to difficult chromatographic separation of compounds **17** and **18**, this reaction pathway was abandoned. The mesylated compound **16**, prepared by standard procedure[341] was utilized instead and desired diiodo product **17** was prepared using TBAI. Standard oil bath heating[343] and microwave irradiation[344,345] were compared and both protocols could be considered equal concerning the yield of compound **17**. However, the reaction time is significantly shorter in the case of microwave irradiation conditions.

The compound **17** was utilized as an electrophilic partner in the reaction with trimethylamine and dimethylamine. The reaction proceeded only with freshly distilled and dried dimethylamine with yield around 30% of the diamine compound **14** (Scheme 11). If commercial dimethylamine aq. solution was used, hydrolysis of the starting compound **17** occurred. In the case of freshly distilled and dried trimethylamine, reaction

did not proceed at all possibly due to sterically hindrance. This phenomenon was already observed by other groups.[342]



Scheme 11. Preparation of first prototypes of anchors Prg-O-MIM2 **4** and Prg-O-TMA2 **6**.

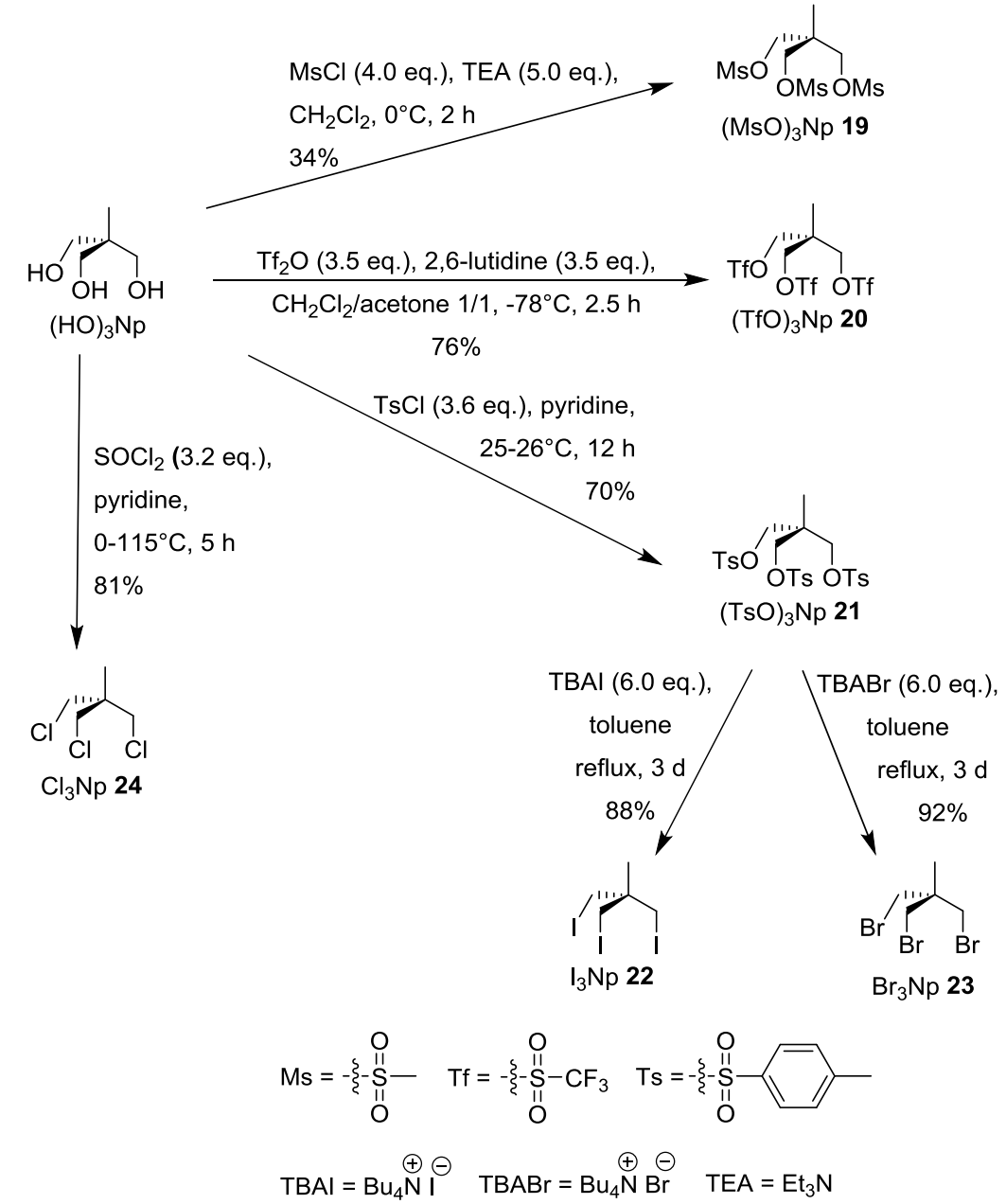
The last quaternization step had to be included due to no reaction with trimethylamine. According to the literature[346], for sterically hindered alkyl halides as neopentyl iodide, a radical nucleophilic substitution ($S_{RN}1$) could be an option to reach better conversion and higher yields in reactions with various nucleophiles. Due to this, the reaction with AIBN was tested. However, only a moderate yield of the desired compound was obtained, which was not too higher compared to the reaction without a radical initiator. The diamino compound **14** was synthesized and methylated applying MeI and the first permanently charged anchor Prg-O-TMA2 **6** was obtained (Scheme 11).

A decision to test the reaction of the diiodo compound **17** with 1-methylimidazole was made to obtain the second permanently charged anchor Prg-O-MIM2 **4** (Scheme 11). As expected, the reaction proceeded much more smoothly than with dimethylamine.

Although charged compounds Prg-O-MIM2 **4** and Prg-O-TMA2 **6** were obtained, there was only a little bit of potential for scale-up, and it was clear that column chromatography purification could not be avoided. Due to this, spending some time optimizing the protocol was necessary. Attention was focused on the type of the leaving group. Obtained results were published[347].

4.1.2 S_N2 reaction kinetic measurement of neopentyl skeleton compounds

The X_3Np derivatives were prepared using standard procedures (Scheme 12), starting from the triol $(HO)_3Np$. The leaving groups to introduce were selected from four leaving group types: alkylsulfonates – $(MsO)_3Np$ **19**, perfluoralkylsulfonates – $(TfO)_3Np$ **20**, arylsulfonates – $(TsO)_3Np$ **21**, and halides – I_3Np **22**, Br_3Np **23**, and Cl_3Np **24**.



Scheme 12. Preparation of compounds X_3Np for kinetic measurements.

Using published procedures, $(MsO)_3Np$ **19**[341], $(TsO)_3Np$ **21**[348], and Cl_3Np **24**[349] were prepared. In addition, I_3Np **22** was prepared from $(TsO)_3Np$ **21** with TBAI in toluene

under reflux, an approach inspired by literature[343]. Br_3Np **23** was prepared using the same procedure but with TBABr. Attempts to prepare $(TfO)_3Np$ **20** according to the literature[350] using TEA as a base were unsuccessful. An inseparable reaction mixture was obtained even at -20°C, most likely due to TEA quaternization. Thus, TEA was replaced with the more sterically hindered and less nucleophilic 2,6-lutidine, and the reaction ran smoothly and afforded the product in sufficient purity.

$(TsO)_3Np$ **21** was used to assess whether its reaction with NaN_3 in deuterated DMSO at 100°C can be followed by ^1H NMR spectroscopy. Results confirmed that the methyl signals of the starting compound $(TsO)_3Np$ **21**, monoazido $(TsO)_2N_3Np$, diazido $(TsO)(N_3)_2Np$, and triazido $(N_3)_3Np$ products are easily identified using this method (Figure 11). Therefore, ^1H NMR spectroscopy enables to follow the reaction and determine its rate by integrating the CH_3 signals over time.

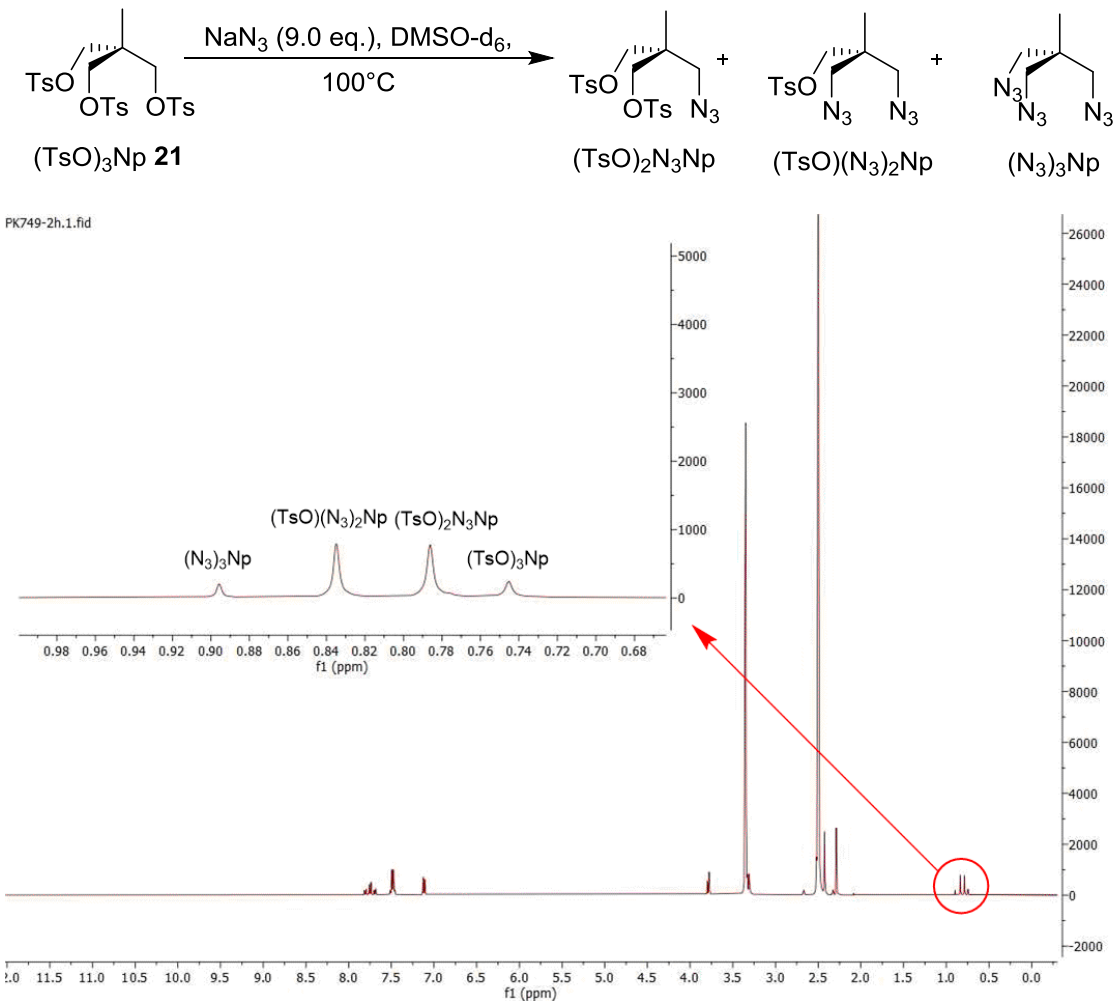


Figure 11. Reaction scheme and ^1H NMR of the reaction mixture showing sufficiently separated CH_3 group signals of starting compound $(TsO)_3Np$ **21**, monoazido $(TsO)_2N_3Np$, diazido $(TsO)(N_3)_2Np$, and triazido $(N_3)_3Np$ substituted products.

The reaction ran smoothly, and under these conditions, no unexpected side-products were observed, making it possible to monitor the reaction by the NMR technique.

In experiments, the reaction of X_3Np with azide was followed until its completion. The procedure was repeated three times for each starting compound to verify reproducibility. The resulting average values and standard deviations from the NMR kinetic experiments for $(TsO)_3Np$ are shown in the graph (Figure 12).

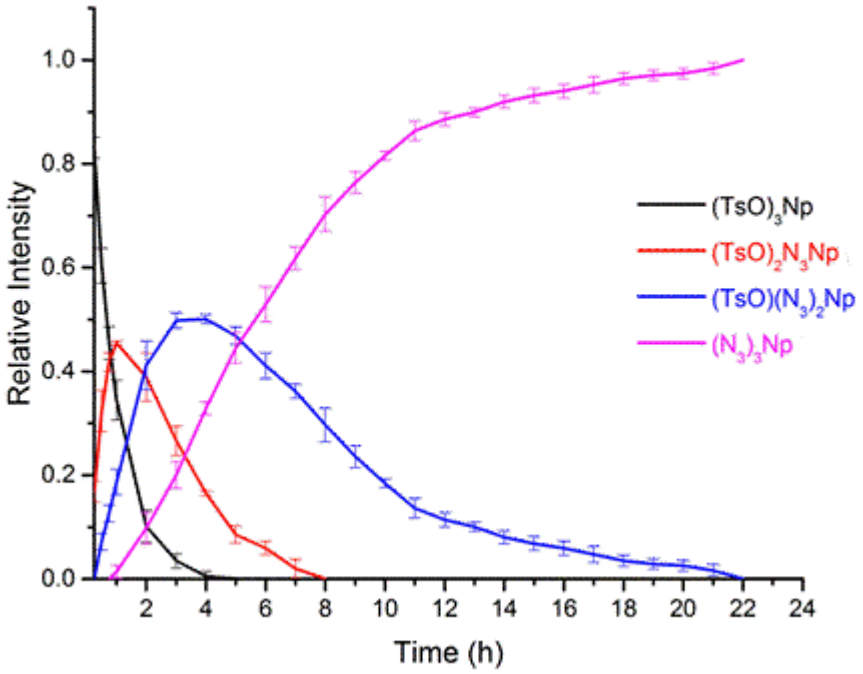


Figure 12. Composition of the reaction mixture in the reaction of $(TsO)_3Np$ **21** with an excess of NaN_3 in time.

Azide anion was used as a nucleophile and deuterated DMSO as the solvent to promote S_N2 reactions and to avoid skeletal rearrangements. No skeletal rearrangement was detected in any of the kinetic measurements monitoring the shifts and the splitting of the signals of the methyl and CH_2X groups, as these signals remained singlets throughout the analysis. If there were any skeletal rearrangements, these signals would change, e.g., become triplets due to the adjoining CH_2 group (Figure 13). Graphs of kinetic measurements of compounds $(MsO)_3Np$ **19**, $(TfO)_3Np$ **20**, I_3Np **22**, Br_3Np **23**, and Cl_3Np **24** are available in Supplemental Information (Figure S1 – Figure **S5**).

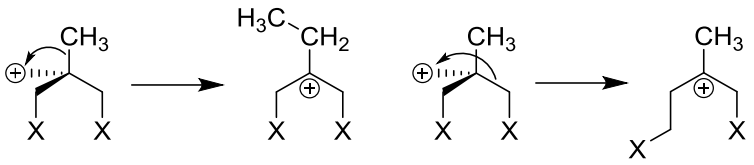


Figure 13. Possible skeletal rearrangements under S_N1 conditions.

The half-life of the X_3Np derivatives was defined as the time when 50% of the triazido product is formed. This information was retrieved from the kinetics graphs (Figure 12), and the collected data were plotted in two graphs for better visualization and comparison (Figure 14). Graph **A** shows data of compounds Br_3Np **23**, I_3Np **22**, and $(TfO)_3Np$ **20** and graph **B** of compounds Cl_3Np **24**, $(MsO)_3Np$ **19**, and $(TsO)_3Np$ **21**. The data were separated to better illustrate the large differences in rates between compound $(TfO)_3Np$ **20** and all others.

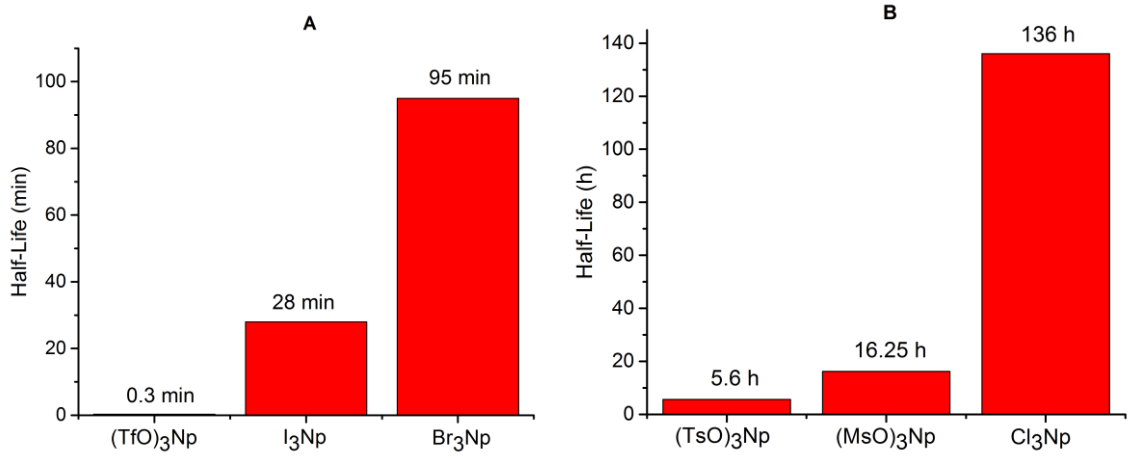


Figure 14. Graphs of half-lives: **A** – compounds Br_3Np **23**, I_3Np **22**, and $(TfO)_3Np$ **20**; **B** – compounds Cl_3Np **24**, $(MsO)_3Np$ **19**, and $(TsO)_3Np$ **21**.

To get a better overall view of the matter, the rate constant of the depletion of $(TsO)_3Np$ **21** starting compound was calculated. This rate constant in the studied reaction of $(TsO)_3Np$ **21** with NaN_3 in deuterated DMSO at 100°C was calculated from an integrated second-order kinetic equation (Eq. 14)[351] where $C_{A,0}$ and $C_{B,0}$ are initial concetrations of tritosylate and the azide; C_A and C_B are their concentrations at time t, and k is the rate constant. Using the percentage amounts of starting compound $(TsO)_3Np$ **21** and azido products $(TsO)_2(N_3)Np$, $(TsO)(N_3)_2Np$, and $(N_3)_3Np$ assessed at specific times of the „composition of the reaction mixture in time“ graph (Figure 12), $(TsO)_3Np$ **21** and NaN_3 concentrations were calculated and plotted as a function of time, using Eq. 14. This resulted in a linear curve whose slope is equal to the rate constant. The resulting graph

for $(TsO)_3Np$ **21** is shown in Figure 15, and the rate constant is equal to 0.00121 mol^{-1} dm^3 s^{-1}.

$$\frac{1}{C_{B,0}-C_{A,0}} ln \frac{C_{A,0}}{C_A} \frac{C_B}{C_{B,0}} = kt \qquad \text{Eq. 14}$$

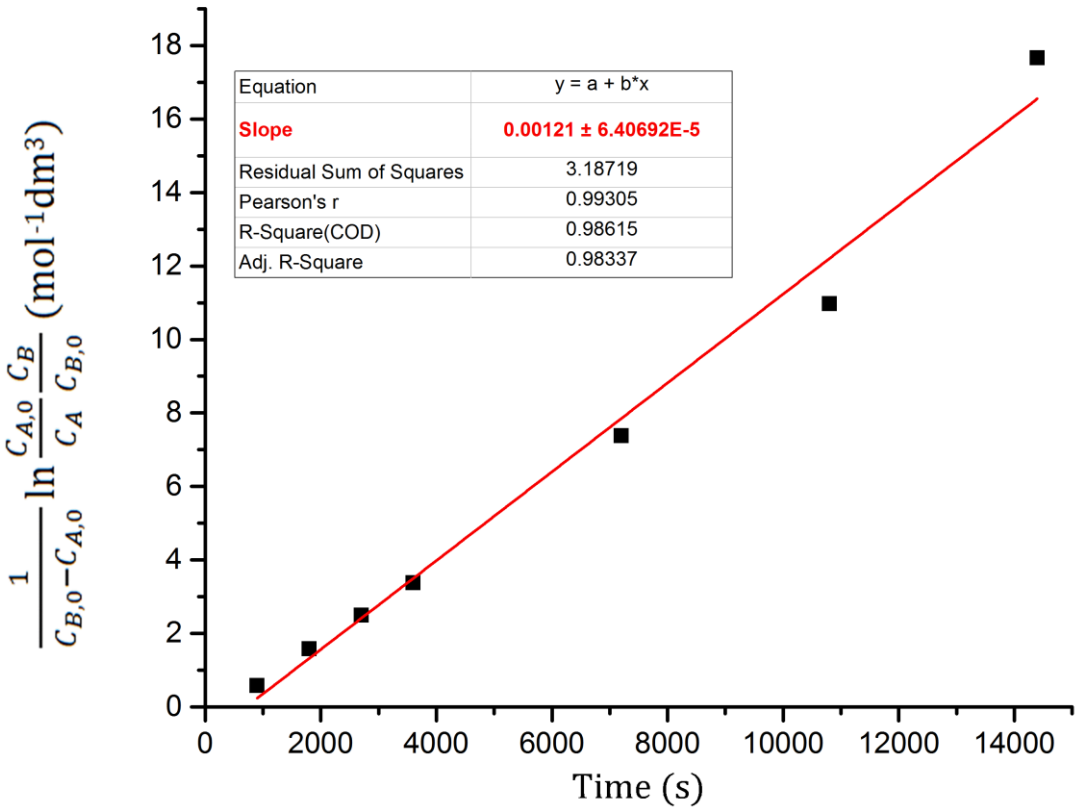


Figure 15. Variation of concentration of $(TsO)_3Np$ **21** and NaN_3 as a function of time.

The same strategy was used for the other four compounds $(MsO)_3Np$ **19**, I_3Np **22**, Br_3Np **23**, and Cl_3Np **24**. The resulting graphs are available in Supplemental Information (Figure S10 – Figure **S13**). In the case of the most reactive compound $(TfO)_3Np$ **20**, the reaction was completed in less than 1 minute. Due to that, there were not enough data to compile a graph similar to Figure 12, and the magnitude of the rate constant could be estimated only. Measurements were repeated for the same reaction at room temperature. Only the final $(N_3)_3Np$ product was observed after 5 minutes by ^1H NMR spectroscopy. All determined rate constants and their comparison can be found in Table 4.

Table 4. Comparison of rate constants of the formation of monoazido $(X)_2N_3Np$ products for the reaction at 100°C.

Derivative	Rate constant [mol^{-1} dm^{-3} $s^{-1}/10^{-5}$]
$(TfO)_3Np$	> 10000000
I_3Np	670
Br_3Np	240
$(TsO)_3Np$	120
$(MsO)_3Np$	30
Cl_3Np	3

Compounds I_3Np **22** and Br_3Np **23** possessing iodide and bromide leaving groups, have lower half-lives and higher rate constants than $(MsO)_3Np$ **19**, and $(TsO)_3Np$ **21** bearing MsO^- and TsO^- leaving groups. This trend is following the literature[352]. Due to an enormously high rate constant and short half-life in the case of $(TfO)_3Np$, a conclusion that the reactivity of the trifluoromethanesulfonate (triflate) leaving group is primarily affected by the electronic effect can be made. This effect suppresses any possible counter-steric effect, which slows down the reaction by making the electrophilic center less accessible. Similar reasoning can be used in the case of Cl_3Np. The chlorine atom is the smallest leaving group used in this study, so the steric effect is negligible. Even so, Cl_3Np reactivity is low due to the electronic effect.

Conversely, steric effects strongly affect the reactivity of bromo, iodo, MsO^-, and TsO^- leaving groups in the corresponding Np derivatives. Accordingly, the results of all four leaving groups analyzed are in good agreement with their A-values[353], which quantitatively express the bulkiness of these substituents and are derived from equilibrium measurements of monosubstituted cyclohexanes. More specifically, bromo, iodo, and Ms substituents have A-values of approximately 0.55, 0.5, and 2.50, respectively. Therefore, A-values can be used to predict the reactivity of bromo, iodo, and methanesulfonate leaving groups bound to a neopentyl.

In addition to electronic and steric effects, bond length and covalent radius may also affect leaving group reactivity. Atomic iodine has a radius of 140 pm; bromine, 115 pm; and oxygen, 73 pm.[354] Consequently, the electrophilic carbon is much more accessible to the nucleophile in Br_3Np **23** and I_3Np **22** bearing bromide and iodide leaving groups, than in oxygen derivatives $(MsO)_3Np$ **19** and $(TsO)_3Np$ **21**. The reactivity trend of these four leaving groups can also be explained from the perspective of the valence bond orbital theory[355]. The best orbital overlap occurs between carbon and oxygen due to their size match. Because iodine and bromine orbitals are larger and more diffused than carbon

orbitals, their overlap with carbon orbitals is poor, particularly in the case of iodine, which has the largest orbitals of all discussed atoms. Therefore, orbital overlap directly correlates with the length and strength of the bond between the leaving group and the electrophilic carbon.

Neighboring group participation is another possible explanation for this reactivity trend, as proposed by Dale et al..[356] In his study of the reactivity of $(TsO)_3Np$ **21**, Br_3Np **23**, and Cl_3Np **24**, he explains the higher reaction rates of the Br_3Np **23**, compared to the other two mentioned compounds, by the effect of neighboring groups (Figure 16). Bromine atoms have a 1,3 relationship and diffuse lone pairs, in contrast to the O and Cl atoms in $(TsO)_3Np$ **21** and Cl_3Np **24**. Due to the diffuse lone pairs, a four-membered ring of bromonium ion is formed. The formation of this four-membered ring relieves steric problems for both the nucleophile and the leaving group attached to the electrophilic carbon. Dale's findings and theories are in accordance with earlier observations and suggestions described by Doering and Levy[357], who described the reaction of I_3Np **22** and Br_3Np **23** derivatives with silver acetate and proposed a four-membered cyclic structure similar to Dale's for Br_3Np **23** as an explanation for non-observing a rearranged *tert*-amyl side-product.

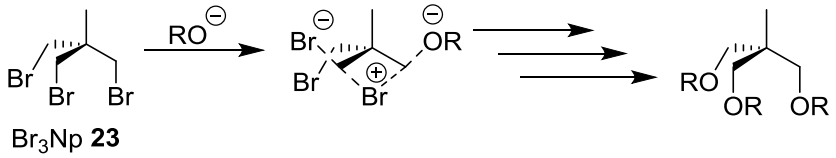


Figure 16. Neighboring group participation of compound Br_3Np **23** proposed by Dale et al..[356]

To test the countercation effects, CsN_3 and Me_4NN_3 were prepared. The analogous procedures described for $(TsO)_3Np$ **21** in previous paragraphs were performed for compounds I_3Np **22** and $(TsO)_3Np$ **21**, and the results were compared. The resulting graphs describing the composition of the reaction mixture in time and variation of total concentration as a function of time are available in Supplemental Information (Figure S6 – Figure **S9**, Figure **S14** –Figure **S17**). Table 5 outlines the calculated rate constants of the formation of monoazido $(X)_2N_3Np$ intermediates. These results indicate that the countercation has a small effect on the reaction rate because the reactions were performed in deuterated DMSO, a polar aprotic solvent that strongly solvates cations. Thus, its effect on the reaction rate should be negligible.

Table 5. Rate constants of compounds I_3Np **22** and $(TsO)_3$Np **21** for countercation comparison.

Derivative	Azide countercation	Rate constant [mol^{-1} dm^{-3} $s^{-1}/10^{-5}$]
I_3Np	Na^+	670
	Cs^+	640
	$(Me_4N)^+$	740
$(TsO)_3$Np	Na^+	120
	Cs^+	170
	$(Me_4N)^+$	140

The most important discovery is the long-term stability and, at the same time, the high reactivity of the derivative $(TfO)_3$Np **20**. TfO^- leaving group on primary carbons is known for its enormously high reactivity and very low stability. The tendency of the group to eliminate is enormous. A compound like $(TfO)_3$Np **20** and its bis-Tf and mono-Tf analogs possessing neopentane skeleton prepared during the work on this thesis do not show any signs of decomposition. These compounds can be stored in the freezer for months and on the shelf for several weeks. These properties make them ideal substances for robust and large-scale syntheses. They can be isolated by simple extractions in sufficient purity for further reactions, even purified by column chromatography, if necessary, stored, and can react smoothly and quickly with various nucleophiles.

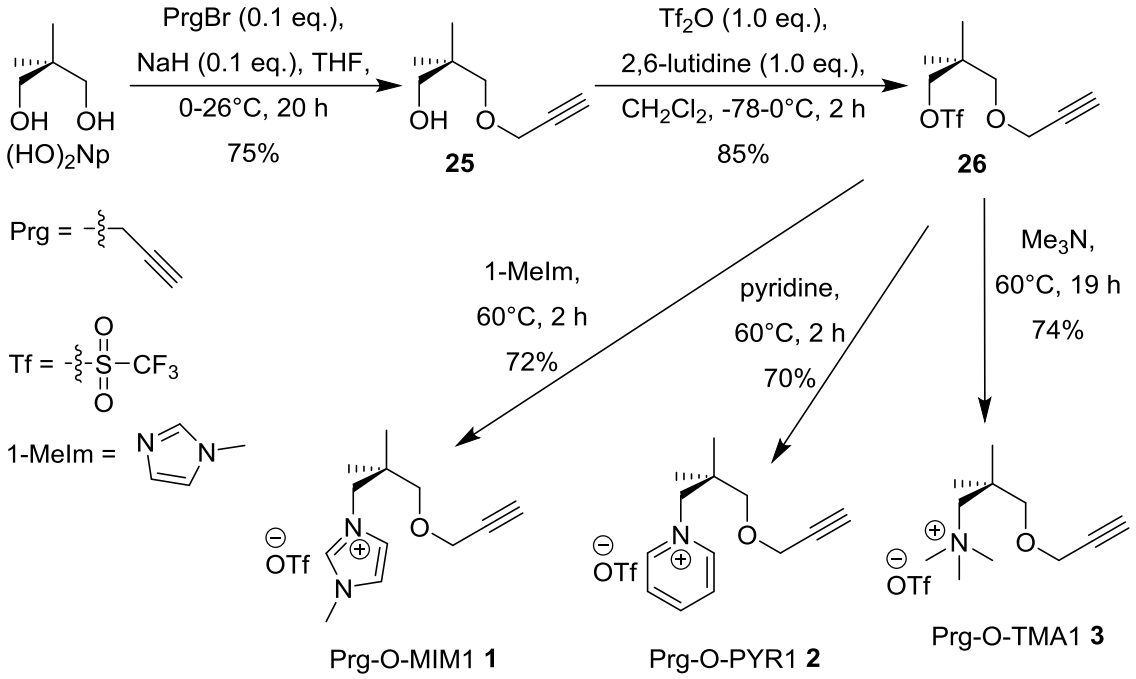
4.1.3 Second generation synthesis

4.1.3.1 Singly charged anchors

Due to results gathered from kinetic studies, a decision was made to base the whole synthesis on a TfO^- leaving group. Kinetic experiments showed that this leaving group is stable against hydrolysis, and no side-products derived from the S_N1 mechanism were observed.

First, anchors Prg-O-MIM1 **1**, Prg-O-PYR1 **2**, and Prg-O-TMA1 **3** possessing one charge were prepared. The diol $(HO)_2$Np was used as a starting compound and general intermediate **25** bearing propargyl group was synthetised according to compound **11** protocol[332]. TfO^- leaving group was installed using the modified procedure developed during kinetic experiments, thus utilizing 2,6-lutidine instead of TEA as a base, and final compounds Prg-O-MIM1 **1**, Prg-O-PYR1 **2**, and Prg-O-TMA1 **3** were obtained after the reaction of the compound **26** with 1-MeIm, pyridine, and trimethylamine (Scheme 13). The enhanced reactivity of the compound **26** in nucleophilic substitution reaction was proved in the reaction with distilled trimethylamine. Due to the bulkiness of

trimethylamine compared to 1-MeIm and pyridine, it was necessary to prolong the reaction time, but similar yields were achieved.



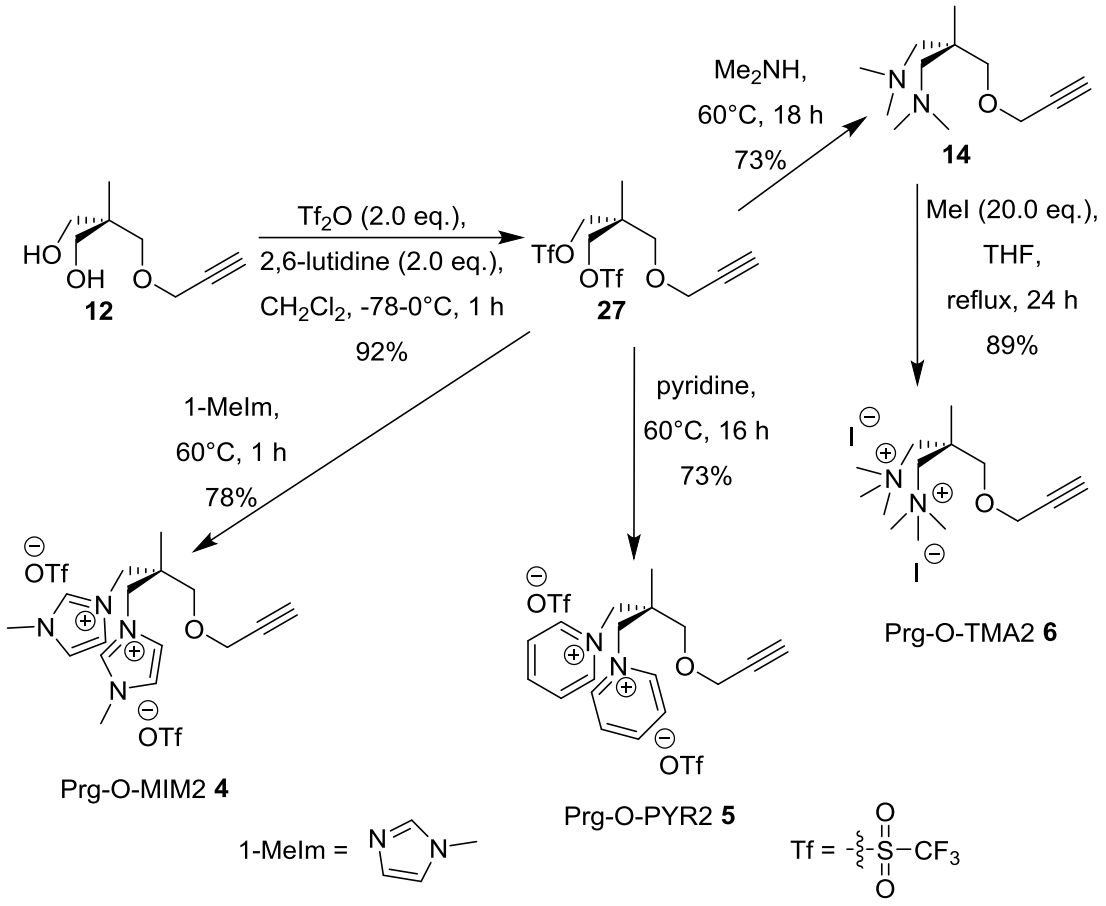
Scheme 13. Preparation of singly charged anchors Prg-O-MIM1 **1**, Prg-O-PYR1 **2**, and Prg-O-TMA1 **3** from mono-Tf intermediate **26**.

The whole synthetic processes could be done without any column chromatography separations and are therefore easy to scale-up.

4.1.3.2 Doubly charged anchors

The synthesis of doubly charged anchors Prg-O-MIM2 **4**, Prg-O-PYR2 **5**, and Prg-O-TMA2 **6** by applying the same Tf strategy logically followed.

The bis-Tf intermediate **27** was synthetized using an optimized procedure, and after the reaction with 1-MeIm and pyridine compounds, Prg-O-MIM2 **4** and Prg-O-PYR2 **5** were obtained (Scheme 14). By comparing both synthetic strategies leading to compound Prg-O-MIM2 **4** (from diiodo intermediate **17** (Scheme 10) and bis-Tf **27**), it can be stated that in the latter case, lower temperature and shorter reaction time are sufficient to obtain the desired compound in higher yield.



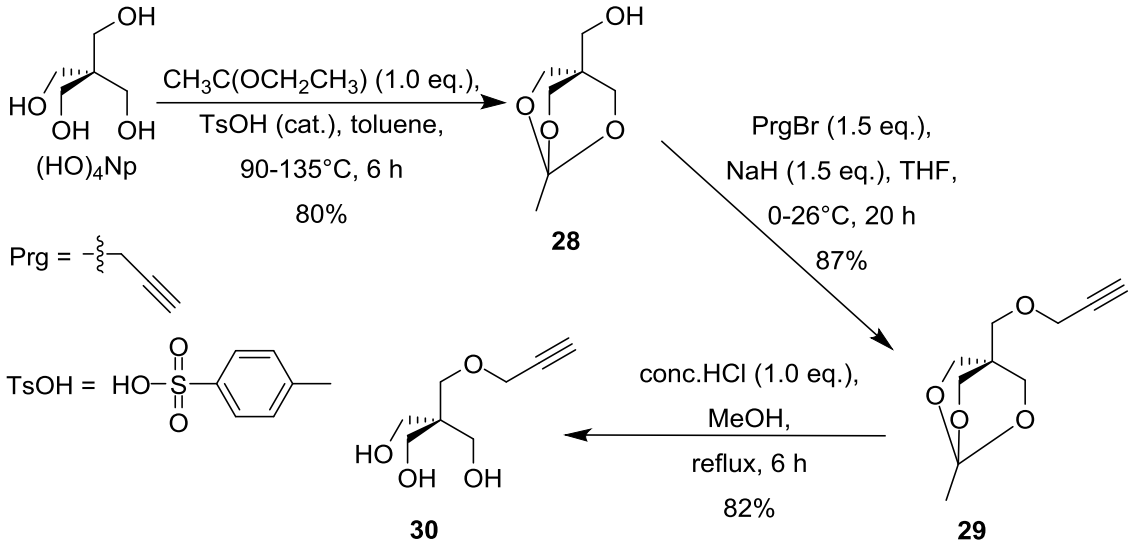
Scheme 14. Preparation of doubly charged anchors Prg-O-MIM2 **4**, Prg-O-PYR2 **5**, and Prg-O-TMA2 **6** from bis-Tf intermediate **27**.

The synthesis of the compound Prg-O-TMA2 **6** had to be prolonged by one more step due to trimethylamine unreactivity. Dimethylamine was used instead of trimethylamine, and the formed bis-tertial amine compound **14** was further quaternized by MeI (Scheme 14). It is the same strategy used for synthesizing the compound Prg-O-TMA2 **6** from diiodo intermediate **17** (Scheme 10). This Tf reaction sequence enabled to synthesize compound **14** in a shorter reaction time with lower temperature and with a considerable increase in the yield (from 34 to 73%).

Again, the whole synthetic procedure leading to doubly charged anchors Prg-O-MIM2 **4**, Prg-O-PYR2 **5**, and Prg-O-TMA2 **6** could be done without any column chromatographic separation. Thanks to this, it was not problematic for the author of this thesis to scale-up the whole process and for my colleague Attila Palágyi to reproduce protocols and prepare compounds Prg-O-MIM2 **4** and Prg-O-PYR2 **5** in quantities exceeding one hundred grams.

4.1.3.3 Triply charged anchors

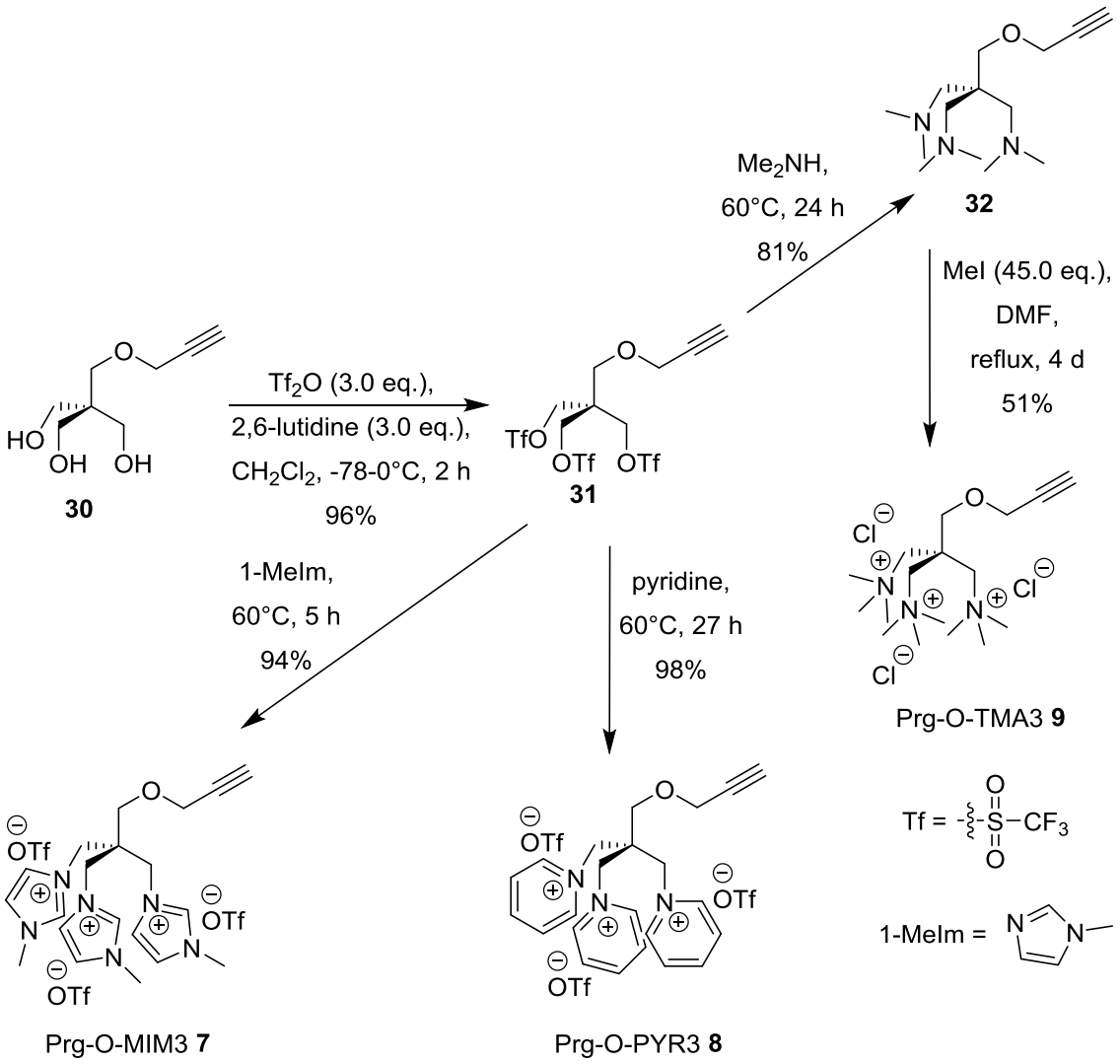
Last triply charged anchors Prg-O-MIM3 **7**, Prg-O-PYR3 **8**, and Prg-O-TMA3 **9** (Scheme 15) were prepared. The synthetic strategy was basically the same as in the case of doubly charged analogs.



Scheme 15. Preparation of propargylated pentaerythritol derivative **30**.

First, it was necessary to protect pentaerythritol $(HO)_4Np$ to have only one hydroxyl group to work with. Described orthoester protection strategy[358] was utilized to form intermediate **28**. Protocol for propargylation was adopted from procedures[332] described in previous paragraphs. Orthoester **29** was hydrolyzed according to literature[359], but with a modified procedure to avoid column chromatography purification.

With the compound **30** in hand, synthesis of triply charged derivatives Prg-O-MIM3 **7**, Prg-O-PYR3 **8**, and Prg-O-TMA3 **9** was performed by applying identical procedures as for doubly charged anchors. First, tris-Tf intermediate **31** was prepared using an optimized procedure. Subsequent reactions with 1-MeIm, and pyridine provided desired compounds Prg-O-MIM3 **7**, and Prg-O-PYR3 **8** (Scheme 16).



Scheme 16. Preparation of triply charged anchors Prg-O-MIM3 **7**, Prg-O-PYR3 **8**, and Prg-O-TMA3 **9** from tris-Tf intermediate **31**.

In the case of trimethylammonium derivative Prg-O-TMA3 **9**, the longer reaction sequence had to be used again (Scheme 16). The reaction of tris-Tf **31** with dimethylamine led to the formation of tris-tertial amine compound **32** in excellent yield considering the bulkiness of three dimethylamine groups. The subsequent MeI quaternization step provided the final compound Prg-O-TMA3 **9** in moderate yield with chloride anions. These anions resulted from purification by a weak cation exchanger Amberlite® CG50. The product was eluted by NH_4HCO_3 aqueous solution and subsequently neutralized by HCl. This yield can be considered successful due to enormous steric complexity of trimethylammonium groups comparable with *tert*-butyl moieties.

Again, there was no problem for the author of this thesis to scale-up the whole process and for the colleague Attila Palágyi to reproduce protocols and prepare tens of grams of compounds Prg-O-MIM3 **7**, Prg-O-PYR3 **8**, and Prg-O-TMA3 **9**. If necessary, compounds Prg-O-MIM3 **7**, Prg-O-PYR3 **8**, and Prg-O-TMA3 **9** can be purified via a weak cation exchanger by applying NH_4HCO_3 aq. solutions for elution.

All prepared anchors were subjected to crystallization efforts. However, the crystal formation was successful only in the case of triple charged compounds Prg-O-MIM3 **7**, Prg-O-PYR3 **8**, and Prg-O-TMA3 **9**. Compounds Prg-O-MIM3 **7** and Prg-O-TMA3 **9** had to be transformed into perchlorates to form crystals. X-ray structures are depicted in Figure 17. Compounds Prg-O-PYR3 **8** and Prg-O-TMA3 **9** are distorted compared to trismethylimidazolium anchor Prg-O-MIM3 **7**. However, the enormous bulkiness of trimethylammonium groups compared to planar pyridinium and methylimidazolium is clearly visible.

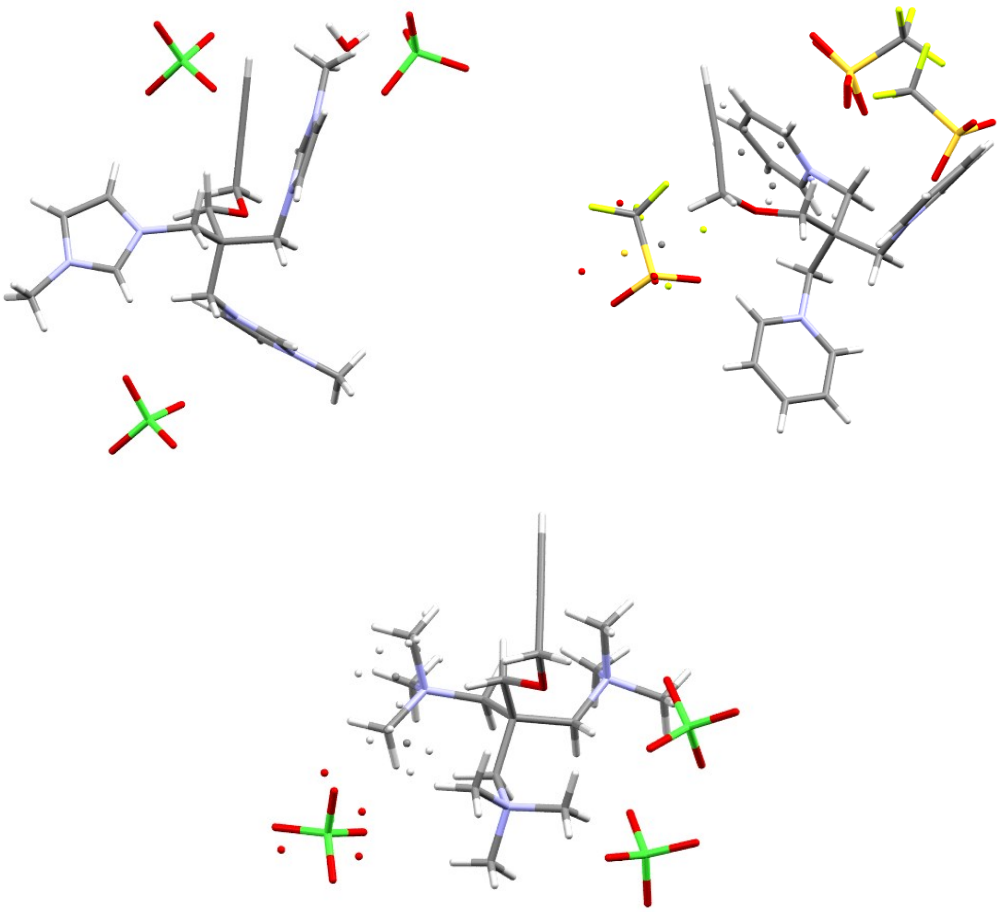
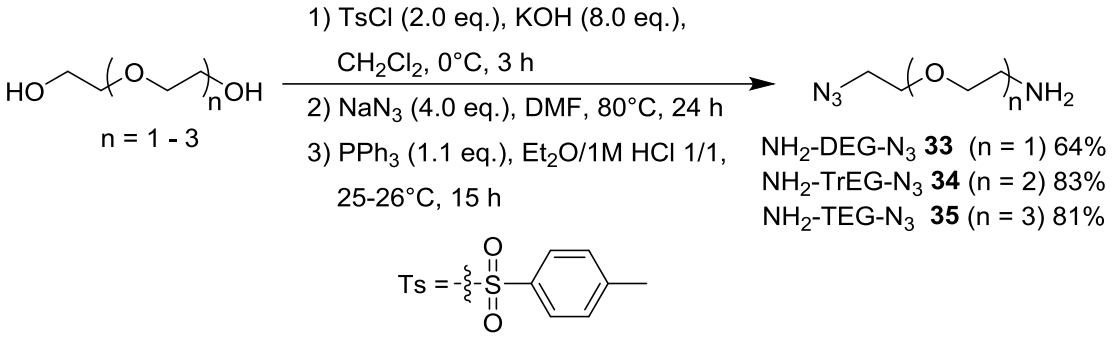


Figure 17. X-ray structures of compounds Prg-O-MIM3 **7**, Prg-O-PYR3 **8**, and Prg-O-TMA3 **9**.

4.2 Linkers

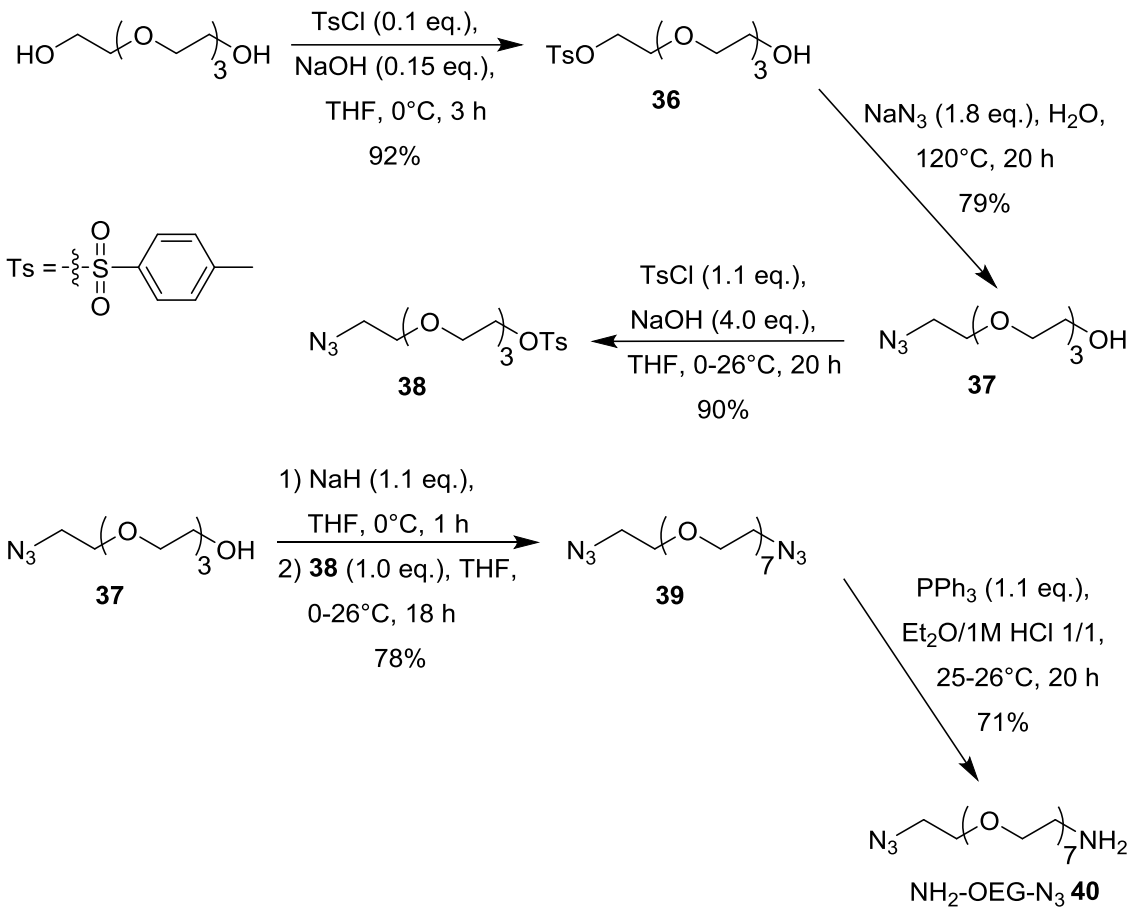
When the neopentyl charged anchors series were synthesized and ready to use, attention was focused on preparing suitable linkers. The main function of these linkers is to connect anchors with desirable compounds such as CD derivatives and fluorescent compounds.

A decision to utilize commercial oligo(ethylene glycols) was made due to the possibility of preparing linkers of different lengths, always by the same procedures. Azido amino oligo(ethylene glycols) NH_2-DEG-N_3 **33**, NH-TrEG-N **34**, and **35** derived from diethylene, triethylene, and tetraethylene glycol, were prepared (Scheme 17). Known procedures involving tosylation[360], azidation[360], and monoreduction of one azido group[361] were used. The process does not involve any chromatographic separation; therefore it was possible to prepare substances in quantities exceeding 50 grams.



Scheme 17. Preparation of azido amino oligo(ethylene glycols) NH_2-DEG-N_3 **33**, NH-TrEG-N **34**, and **35**.

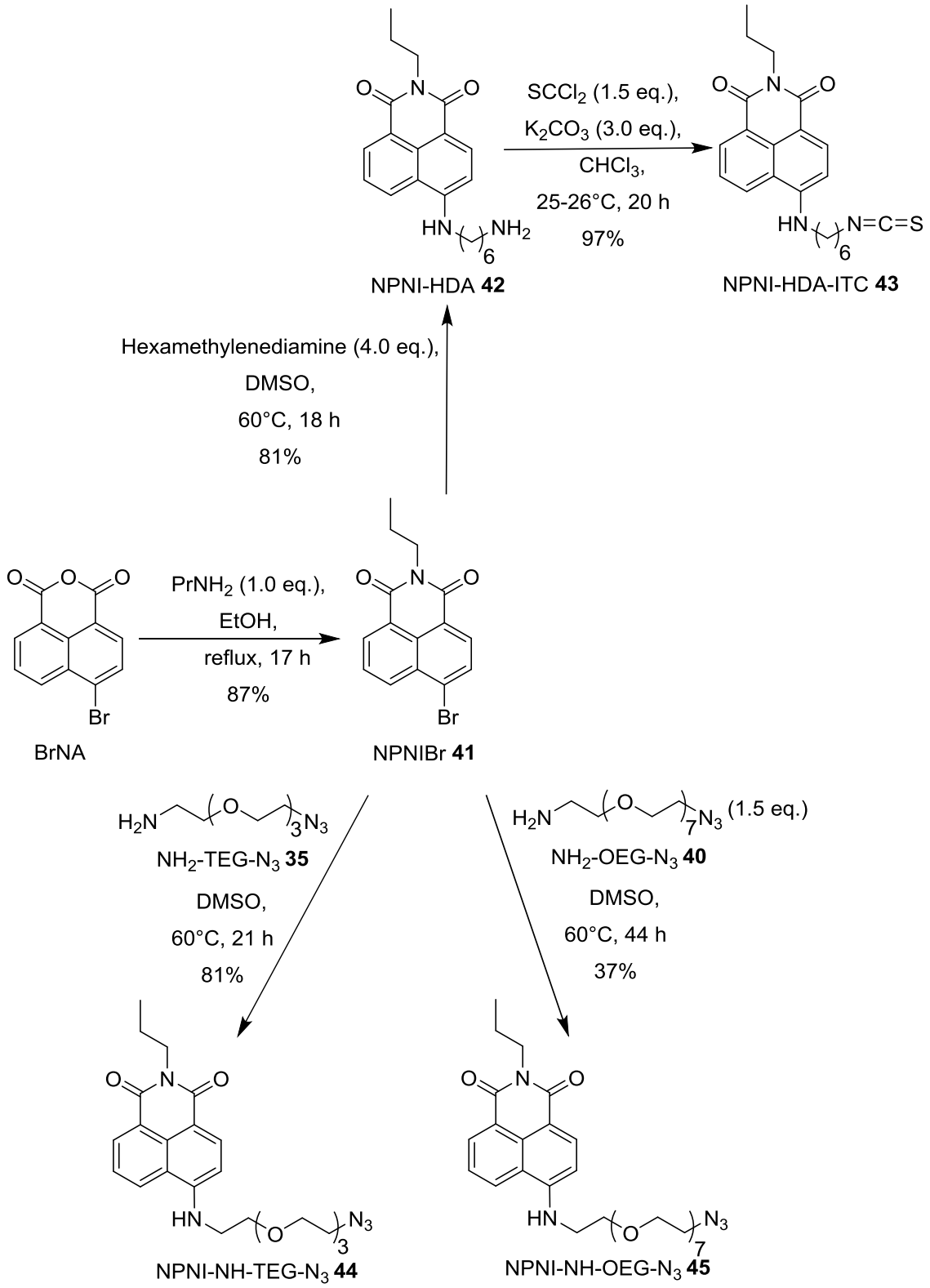
The last azido amino octaethylene glycol linker NH_2-OEG-N_3 **40** was not prepared by analogous procedure due to the price of octaethylene glycol. The strategy of connecting two tetraethylene glycol derivatives was utilized instead (Scheme 18). The synthesis was based on known procedures made up of monotosylation[362], monoazidation[363], second tosylation[364], connection of two monoazido glycols **37** and **38**[364], and already mentioned monoreduction of one azido group[361]. Again, the whole synthetic process was developed not to involve any chromatographic purification, so there is a possibility for scale-up.



Scheme 18. Preparation of azido amino octaethylene glycol NH_2-OEG-N_3 **40**.

4.3 Fluorophores

The next part was to prepare suitable fluorescent and UV active compounds which could be connected with charged anchors or with CD derivatives to test the bond strength of the anchors with various solid supports. Bromonaphthalic anhydride (BrNA) was chosen for this purpose, and by applying several reactions, the compound was transformed into desired final fluorescent products (Scheme 19).



Scheme 19. Preparation of naphthalimide derivatives NPNI-HDA-ITC **43**, NPNI-NH-TEG-N_3 **44**, and NPNI-NH-OEG-N_3 **45**.

First, the non-fluorescent compound BrNA was transformed into a more stable *N*-propylbromonaphthalimide (NPNIBr**) 41**.[365] This useful intermediate can become

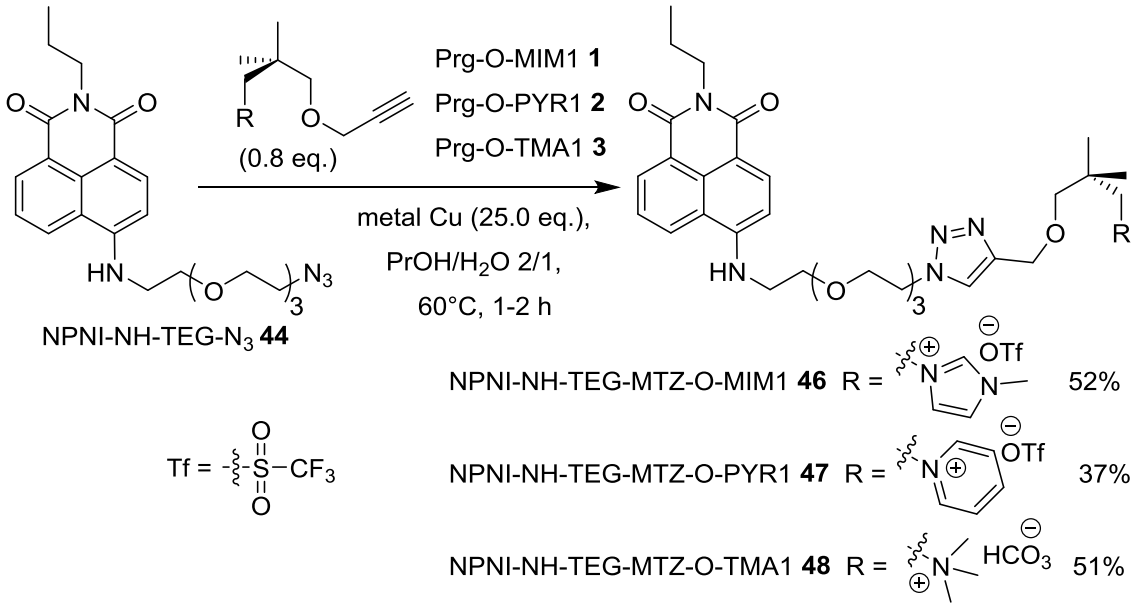
fluorescent when reacting with amines by an aromatic nucleophilic substitution reaction. So it was transformed into its hexamethylenediamine derivative NPNI-HDA **42**.[366] The remaining primary amine group was modified into isothiocyanate by general procedure taken from the literature[367] and the first suitable fluorescent compound, isothiocyanate NPNI-HDA-ITC **43** was synthesized.

The precursor NPNIBr **41** underwent the same reaction but with previously prepared azido amino oligo(ethylene glycols) linkers **35** and NH_2-OEG-N_3 **40**. The azido groups in the formed products NPNI-NH-TEG-N_3 **44** and NPNI-NH-OEG-N_3 **45** are suitable reaction partners for charged anchor's propargyl group.

4.4 Charged fluorophores

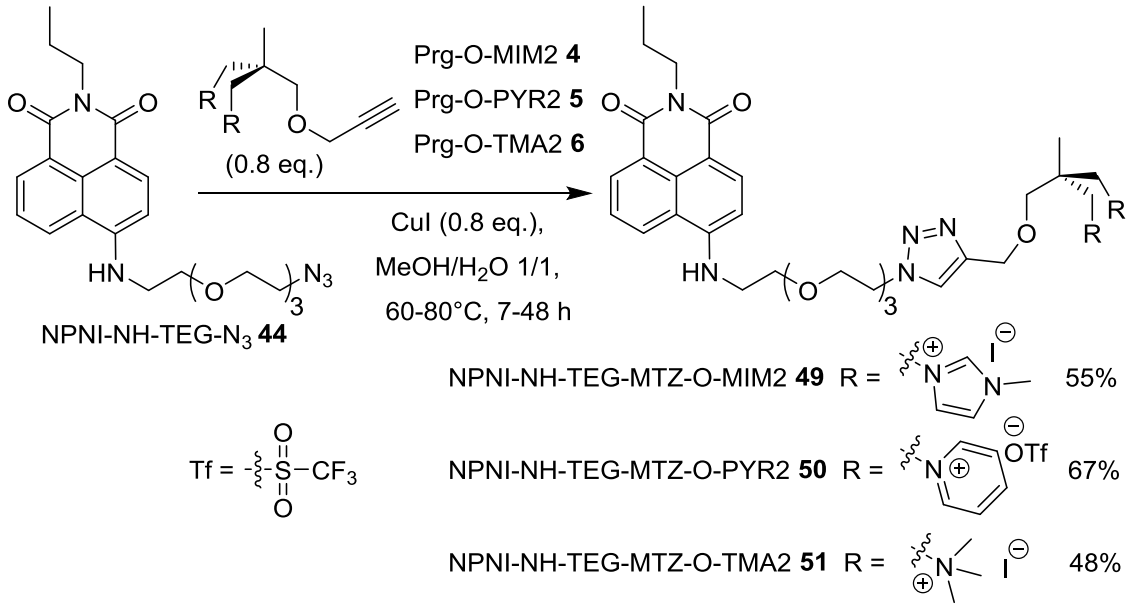
Next, the fluorescent derivative NPNI-NH-TEG-N_3 **44** was utilized as a starting compound for the click reaction with prepared anchors. Several standard methods for click reaction described in the review[368] were tested. No general method suitable for all anchors was found, but still, some trends were observed. The trend can be seen between the number of charges possessed by the anchor and the type of copper reagent.

Concerning the singly charged naphthalimide derivatives NPNI-NH-TEG-MTZ-O-MIM1 **46**, NPNI-NH-TEG-MTZ-O-PYR1 **47**, and NPNI-NH-TEG-MTZ-O-TMA1 **48**, conditions utilizing metal Cu[145] were the most suitable ones and afforded products in moderate yields after reverse-phase column chromatography purification (Scheme 20).



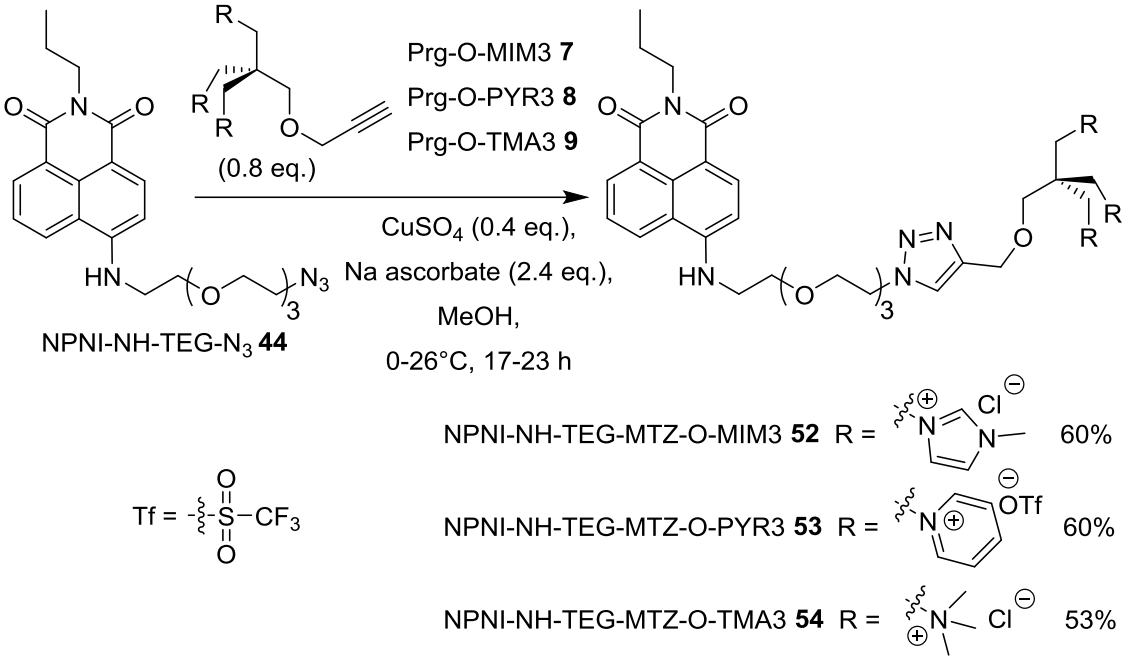
Scheme 20. Preparation of singly charged naphthalimide derivatives NPNI-NH-TEG-MTZ-O-MIM1 **46**, NPNI-NH-TEG-MTZ-O-PYR1 **47**, and NPNI-NH-TEG-MTZ-O-TMA1 **48**.

CuI[369] proved to be the best catalyst for the reaction with doubly charged anchors. Doubly charged naphthalimide derivatives NPNI-NH-TEG-MTZ-O-MIM2 **49**, NPNI-NH-TEG-MTZ-O-PYR2 **50**, and NPNI-NH-TEG-MTZ-O-TMA2 **51** were prepared by applying this reagent in moderate yields after necessary reverse-phase column chromatography purification (Scheme 21).



Scheme 21. Preparation of doubly charged naphthalimide derivatives NPNI-NH-TEG-MTZ-O-MIM2 **49**, NPNI-NH-TEG-MTZ-O-PYR2 **50**, and NPNI-NH-TEG-MTZ-O-TMA2 **51**.

For the preparation of triply charged naphthalimide derivatives NPNI-NH-TEG-MTZ-O-MIM3 **52**, NPNI-NH-TEG-MTZ-O-PYR3 **53**, and NPNI-NH-TEG-MTZ-O-TMA3 **54**, probably the most common method[370] with $CuSO_4 \cdot 5H_2O$ and sodium ascorbate gave the best results (Scheme 22). Products had to be purified by ion-exchange chromatography utilizing a weak cation exchanger.



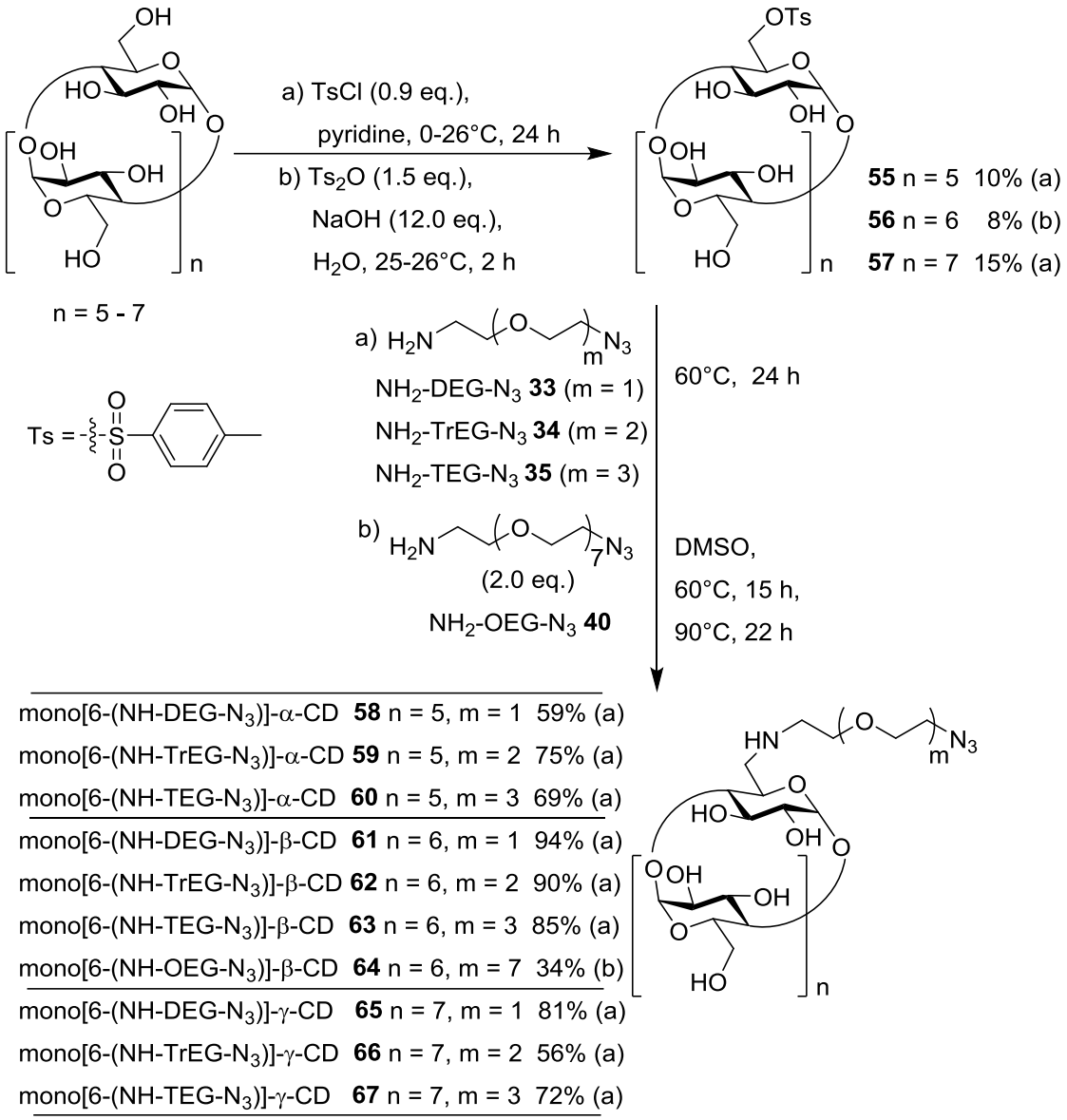
Scheme 22. Preparation of triply charged naphthalimide derivatives NPNI-NH-TEG-MTZ-O-MIM3 **52**, NPNI-NH-TEG-MTZ-O-PYR3 **53**, and NPNI-NH-TEG-MTZ-O-TMA3 **54**.

Reasons for moderate yields for singly, doubly, and triply charged naphthalimide derivatives were necessary chromatographic purifications due to the formation of unidentified side-products. Reactions' conditions were not further optimized because products were obtained in sufficient amounts for further testing.

Before the start of the bond strength testing of fluorescently marked anchors with various solid supports, a synthesis of some fluorescently labeled CD derivatives was done. This step should enable to determine if the group's bulkiness attached to the anchor can somehow influence the mentioned bond strength.

4.5 Azido amino oligo(ethylene glycols) cyclodextrins

It was necessary to install an amino group into the CD molecule to attach naphthalimide derivative NPNI-HDA-ITC **43**. Azido amino oligo(ethylene glycols) linkers NH_2-DEG-N_3 **33**, NH-TrEG-N **34**, **35**, and NH_2-OEG-N_3 **40** were utilized together with series of mono(6-*O*-Ts)-CDs **55**, **56**, and **57** (Scheme 23).



Scheme 23. Preparation of azido amino oligo(ethylene glycols) CDs (mono[6-(NH-EG-N_3]-CDs) **58** – **67** series.

First, some of the most common CD derivatives, mono(6-*O*-Ts)-CDs **55**, **56**, and **57** were prepared according to literature procedures[105,154].

A decision was made to use solvent-free conditions for diethylene, triethylene, and tetraethylene glycol derivatives NH_2-DEG-N_3 **33**, NH-TrEG-N **34**, and **35**. Tosylated CDs were suspended in these glycols and heated to 60°C. CDs dissolved sequentially, and homogeneous mixtures were stirred for a couple of hours at this temperature. This strategy enforced desired products' formation and suppressed the formation of side-products as native CD (hydrolysis) and 3^A,6^A-anhydro-CD. This side-product is typical for nucleophilic substitution reactions. It results from 3-OH group deprotonation by base

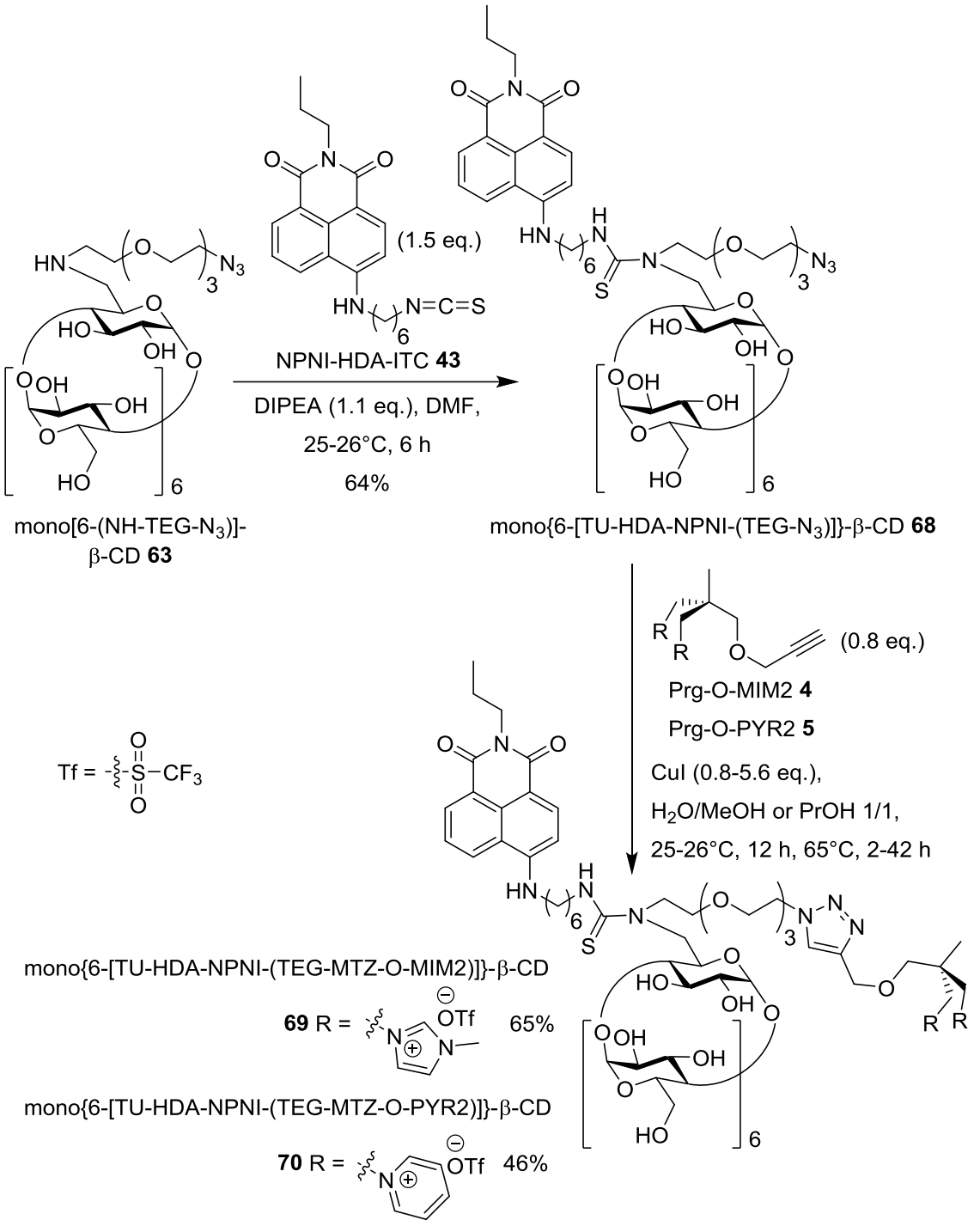
(amine) and subsequent intramolecular nucleophilic substitution between deprotonated hydroxyl and the carbon with TsO^- leaving group.[371] Products are easily purified by utilizing strong cation exchanges.

A different strategy was applied to prepare the derivative **64** by the reaction of mono(6-*O*-Ts)-β-CD **56** and NH_2-OEG-N_3 **40**. The reason was more solid form of this glycol derivative compared to previous three used azido amino oligo(ethylene glycols). The glycol was diluted with DMSO, and as can be seen from the yield (Scheme 23), the formation of previously mentioned side-products was more significant.

Concerning the preparation of azido amino oligo(ethylene glycols) CDs (mono[6-(NH-EG-N_3)]-CDs) **58** – **67**, only synthesis of mono[6-(NH-TrEG-N_3)]-β-CD could be found in the literature. Wang et al. reacted mono(6-*O*-Ts)-β-CD **56** with diamino triethylene glycol derivative and further transformed the remaining primary amino group into azido by the reaction with imidazole-1-sulfonyl azide hydrochloride under $CuSO_4 \cdot 5H_2O$ catalysis.[372]

4.6 Charged fluorescent cyclodextrin derivatives

With CD derivatives mono[6-(NH-EG-N_3)]-CDs **58** – **67** synthesized, it was time to attach naphthalimide derivative NPNI-HDA-ITC **43** to the amino group, which was installed into them (Scheme 24).



Scheme 24. Preparation of charged fluorescent CD derivatives mono{6-[TU-HDA-NPNI-(TEG-MTZ-O-MIM2)]}-β-CD **69** and mono{6-[TU-HDA-NPNI-(TEG-MTZ-O-PYR2)]}-β-CD **70**.

CD derivative mono[6-(NH-TEG-N_3)]-β-CD **63**, which possesses azido amino tetraethylene glycol linker, was utilized and connected with fluorescent naphthalimide NPNI-HDA-ITC **43** via thiourea moiety[373,374]. This high-yielding and thus quite favorite reaction proceeded well. Product mono{6-[TU-HDA-NPNI-(TEG-N_3)]}-β-CD **68** was purified by reverse-phase column chromatography and obtained in moderate yield.

Next, two doubly charged anchors, Prg-O-MIM2 **4** and Prg-O-PYR2 **5**, bearing imidazolium and pyridinium groups, were attached. CuI[369] proved to be the best option for this combination of compounds. Both compounds mono{6-[TU-HDA-NPNI-(TEG-MTZ-O-MIM2)]}-β-CD **69** and mono{6-[TU-HDA-NPNI-(TEG-MTZ-O-PYR2)]}-β-CD **70** were obtained in moderate yields after reverse-phase column chromatography purification.

4.7 Tests of stability

4.7.1 Thermal and pH stability

As written at the beginning of the Results and Discussion chapter, the charged compounds prepared by the former colleague Martin Popr were not stable enough. They were degraded by Hofmann elimination under basic and thermal conditions. The compounds synthesized in this work should lack this instability. Due to that, the prepared charged compounds were subjected to thermal and pH stability testing to confirm this claim.

The thermal stability under various pH of some of the prepared compounds was tested. Three pH values were tested, namely 1, 7, and 9. Measurements were done in D_2O, either acidified to pH 1 by HCl or left neutral or basified to pH 9 by NaOH. Some of the doubly and triply charged anchors were measured. During each measurement, the temperature was changed by 20°C, and the sample was kept at this temperature for 1 hour. Spectra were measured every 10 minutes. The highest temperature was 80°C. Spectra at higher temperatures were not evaluated due to technical parameters of the 600MHz NMR probe.

From the results for doubly charged methylimidazolium anchor Prg-O-MIM2 **4** (Figure 18), a conclusion can be made that the compound is stable up to 80°C in the whole pH range.

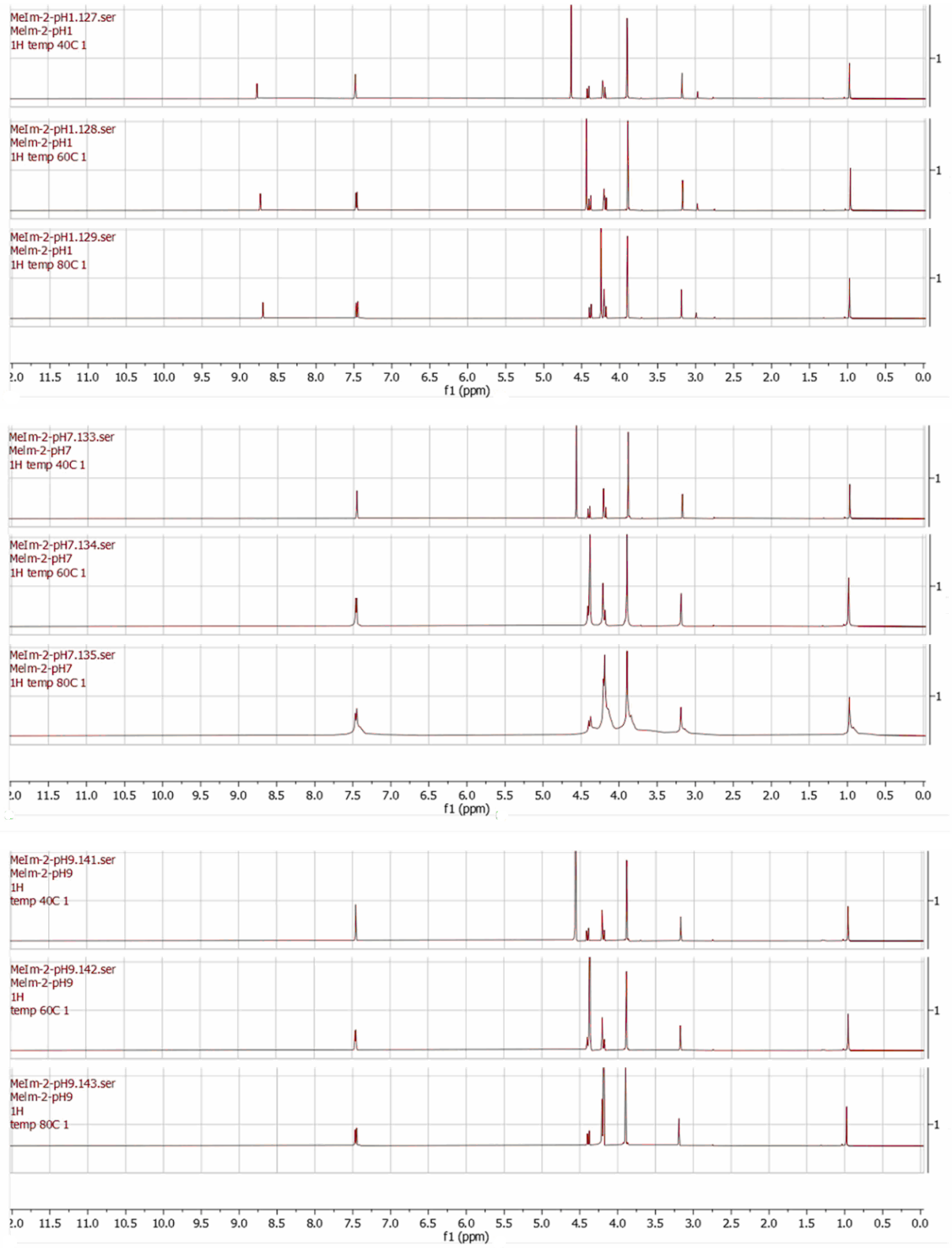


Figure 18. Thermal stability of doubly charged methylimidazolium anchor Prg-O-MIM2 **4** under pH 1 and temperature of 40, 60, and 80°C (upper three spectra), pH 7 and temperature of 40, 60, and 80°C (middle three spectra), and pH 9 and temperature of 40, 60, and 80°C (lower three spectra).

The same can be said about its triply charged analog Prg-O-MIM3 **7** (Figure 19). Some other anchors were also measured, and neither showed any degree of instability

under thermal and pH conditions. Their NMR are available in Supplemental Information (Figure S18 – Figure S19).

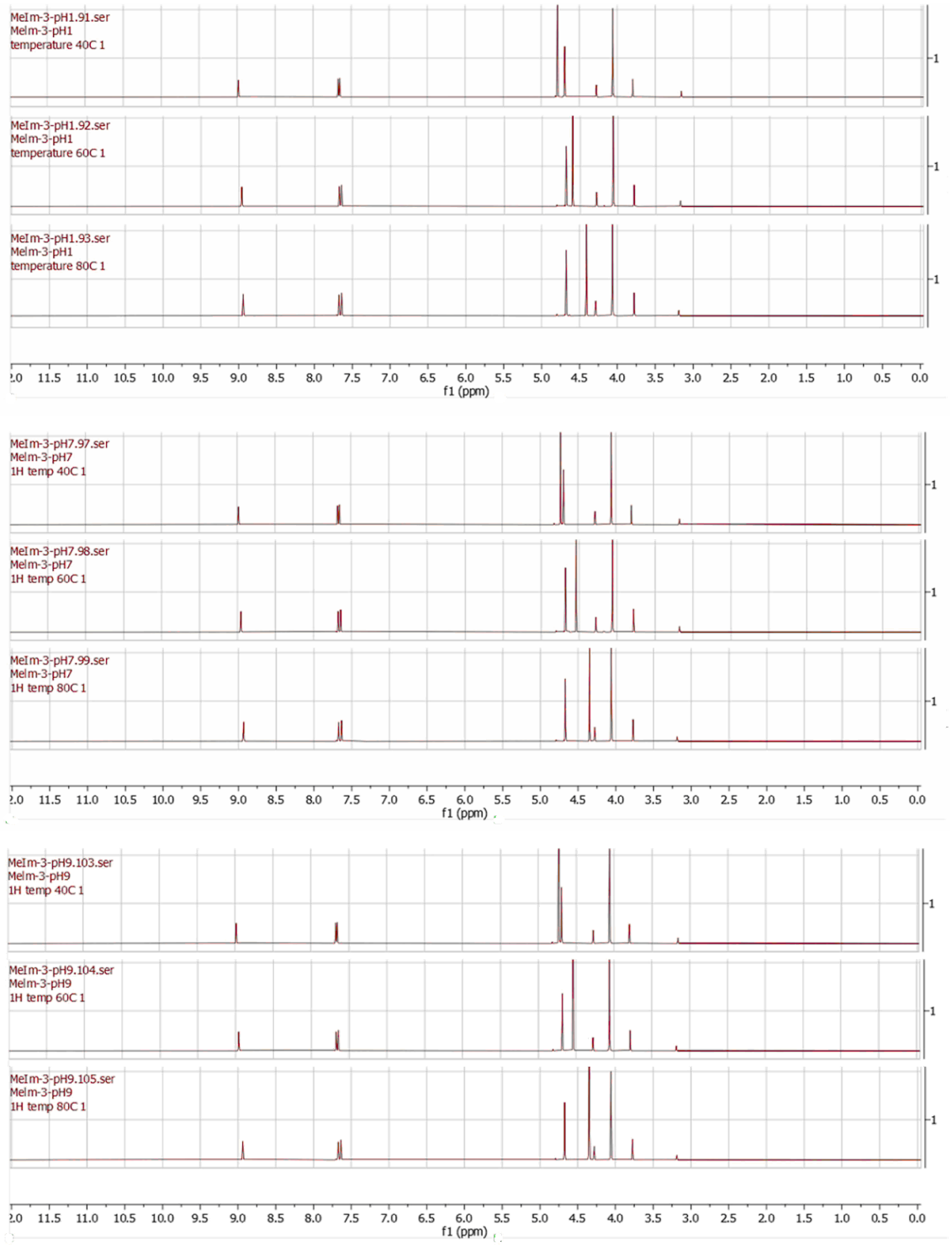


Figure 19. Thermal stability of triply charged methylimidazolium anchor Prg-O-MIM3 **7** under pH 1 and temperature of 40, 60, and 80°C (upper three spectra), pH 7 and temperature of 40, 60, and 80°C (middle three spectra), and pH 9 and temperature of 40, 60, and 80°C (lower three spectra).

Anchor Prg-O-PYR3 **8**, possessing three pyridinium groups, was picked up, and measurements at a temperature higher than 80°C were conducted. Three NMR cuvettes with anchor dissolved in D_2O (again, pH 1, 7, and 10) were oil bath heated for one hour at a given temperature. After cooling, NMR spectra were measured. Anchor showed extreme stability up to 120°C. Higher temperature experiments were not performed due to D_2O boiling point.

Based on the results, it can be stated that synthesized anchors are stable under tested conditions. In some cases, only the exchange of acidic hydrogens (terminal triple bond hydrogen and imidazolium hydrogen situated between nitrogen atoms) for deuterium was observed. This resulted in the disappearance of the signal. The only signal which was shifted was the signal of HDO due to H-bond temperature dependence.

4.7.2 Anchor/solid support bond strength

Charged fluorescent naphthalimide compounds NPNI-NH-TEG-MTZ-O-MIM1 **46**, NPNI-NH-TEG-MTZ-O-MIM2 **49**, and NPNI-NH-TEG-MTZ-O-MIM3 **52** were subjected to bond strength tests with various solid supports. It would be worth recalling here the information from the State of the Art chapter that the original Pirkl columns based on salt formation between chiral amino acid and aminopropyl silica gel were stable only in heptane/*i*-PrOH mixtures with an alcohol content not exceeding 20% w/w. Silica gel was tested as the first solid support. Compounds possessing methylimidazolium groups and differing in the number of charges from one to three were coated onto silica gel. The coating was performed by mixing silica gel (50 mg) and one of the three fluorescent napthalimide charged compounds (3 μmol) in a 0.01 M NH_4HCO_3 aq. Solution (0.5 mL). Coated silica gel was then transfered into a glass Pasteur pipette, and various solutions were pumped through in the order given in Table 6. The total volume of the mixtures was 5 mL, and 1 mL fractions were collected. UV-VIS measurements of every fraction were performed, and the concentration of charged fluorescent naphthalimide compounds in eluents was calculated after calibration measurements for compounds NPNI-NH-TEG-MTZ-O-MIM1 **46**, NPNI-NH-TEG-MTZ-O-MIM2 **49**, and NPNI-NH-TEG-MTZ-O-MIM3 **52** were done. Results are summarized in Table 6. It should also be mentioned why only compounds with methylimidazolium anchors were tested. These anchors were prepared in the most abundant quantities. For the sake of consistency and not to bring more variables into subsequent experiments, all further

syntheses of the main compounds, assays, and measurements were based on methylimidazolium anchors.

Table 6. Comparison of the bond stability of charged fluorescent methylimidazolium compounds NPNI-NH-TEG-MTZ-O-MIM1 **46**, NPNI-NH-TEG-MTZ-O-MIM2 **49**, and NPNI-NH-TEG-MTZ-O-MIM3 **52** with different numbers of charges on silica gel in an acidic and a basic environment.

Compound	NPNI-NH-TEG-MTZ-O-MIM1 **46**	NPNI-NH-TEG-MTZ-O-MIM2 **49**	NPNI-NH-TEG-MTZ-O-MIM3 **52**
Elution mixture			
1. H_2O	7	4	119
	nd	22	575
	nd	40	220
	nd	33	210
	nd	14	207
2. 0.01 M NH_4HCO_3 aq. sol.	3	11	18
	10	nd	nd
	4	nd	nd
	nd	nd	nd
	nd	nd	nd
3. 0.10 M NH_4HCO_3 aq. sol.	4	nd	11
	5	nd	nd
	4	nd	nd
	3	nd	nd
	3	nd	nd
4. 1.00 M NH_4HCO_3 aq. sol.	13	nd	nd
	20	nd	nd
	24	nd	nd
	33	nd	nd
	53	nd	nd
5. HCl aq. sol.	15	2	3
	49	4	103
	285	192	506

pH 2	241	170	171
	188	94	81
6. 0.01 M NH_4Cl aq. sol. pH 2 (HCl)	111	29	28
	81	20	17
	57	18	16
	59	16	16
	62	16	10
7. 0.10 M NH_4Cl aq. sol. pH 2 (HCl)	22	4	nd
	17	2	nd
	15	2	nd
	16	2	nd
	15	1	nd
8. 1.00 M NH_4Cl aq. sol. pH 2 (HCl)	6	nd	nd
	4	nd	nd
	5	nd	nd
	5	nd	nd
	4	nd	nd
9. 0.10 M NH_4Cl MeOH/H_2O 1/1 pH 2 (HCl)	1414	523	537
	137	110	147
	17	17	28
	7	12	19
	4	10	5
10. 1.00 M NH_4Cl MeOH/H_2O 1/1 pH 2 (HCl)	3	1	7
	2	nd	3
	9	1	nd
	nd	nd	nd
	nd	nd	nd

nd = not detected (no elution)

Concentration 1-10 µM

Concentration 11-100 µM

Concentration 101-1000 µM

Concenration > 1000 μM

Results indicate low bond stability of monocharged compound NPNI-NH-TEG-MTZ-O-MIM1 **46** on silica gel in all basic NH_4HCO_3 aq. solutions, compared to two compounds bearing two and three permanent positive charges. No difference in bond stability with this solid support was observed compared to doubly chargedNPNI-NH-TEG-MTZ-O-MIM2 **49** and triply charged NPNI-NH-TEG-MTZ-O-MIM3 **52** compounds in these solutions.

When acidic elution mixtures were tested, a dramatic increase in all three modifiers' elution was observed. An even higher elution rate was detected when acidic NH_4Cl aq. solution elution mixtures were replaced by their 50% MeOH aq. solution analogs.

After observing this dramatic increase of elution in MeOH solution, another experiment was performed with newly coated columns prepared similarly. MeOH and MeCN aq. solution elution mixtures were tested, and the results are summarized in Table 7.

Table 7. Comparison of the bond stability of charged fluorescent methylimidazolium compounds NPNI-NH-TEG-MTZ-O-MIM1 **46**, NPNI-NH-TEG-MTZ-O-MIM2 **49**, and NPNI-NH-TEG-MTZ-O-MIM3 **52** with different numbers of charges on silica gel in various MeOH and MeCN environments.

Compound	NPNI-NH-TEG-MTZ-O-MIM1 **46**	NPNI-NH-TEG-MTZ-O-MIM2 **49**	NPNI-NH-TEG-MTZ-O-MIM3 **52**
Elution mixture			
1. 0.01 M NH_4HCO_3 aq. sol.	3	nd	36
	nd	nd	4
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
2. 0.01 M NH_4HCO_3 MeOH/H_2O 1/1	255	11	32
	291	nd	3
	272	nd	nd
	245	nd	nd
	226	nd	nd

3. 0.01 M NH_4HCO_3 MeOH/H_2O 3/1	462	nd	nd
	372	nd	nd
	264	nd	nd
	147	nd	nd
	60	nd	nd
4. 0.01 M NH_4HCO_3 MeOH	10	nd	nd
	7	nd	nd
	4	nd	nd
	nd	nd	nd
	nd	nd	nd
5. 0.01 M NH_4HCO_3 MeCN/H_2O 1/1	nd	3	nd
	nd	4	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
6. 0.01 M NH_4HCO_3 MeCN/H_2O 6/1	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
7. 0.01 M NH_4OAc aq. sol.	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
8. MeCN	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
9. *i*-PrOH	nd	nd	nd
	nd	nd	nd

	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
10. PrOH	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
11. EtOH	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
12. MeOH	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
nd = not detected (no elution)			
Concentration 1-10 μM			
Concentration 11-100 μM			
Concentration 101-1000 μM			

The bond instability of singly charged compound NPNI-NH-TEG-MTZ-O-MIM1 **46** in all basic NH_4HCO_3 MeOH solutions can be observed from the data. The bond between silica gel and doubly charged compound NPNI-NH-TEG-MTZ-O-MIM2 **49** and triply charged analog NPNI-NH-TEG-MTZ-O-MIM3 **52** are firmly stable under the same conditions. MeCN solutions do not cause elution, and the same can be said about various pure polar alcohols.

Next, doubly charged naphthalimide CD derivative mono{6-[TU-HDA-NPNI-(TEG-MTZ-O-MIM2)]}-β-CD **69** was coated onto silica gel by applying the already mentioned protocol. The stability of this column was tested and compared with the

already studied column modified with doubly charged naphthalimide derivative NPNI-NH-TEG-MTZ-O-MIM2 **49**. Results are depicted in Table 8. From the data, a conclusion can be made that type and bulkiness of the molecule connected to the anchor have no dramatic effect on stability with silica gel. This fact would indicate the primary source of bond stability between anchors and solid supports are charges built into the molecules.

Table 8. Comparison of the bond stability of charged fluorescent methylimidazolium compound NPNI-NH-TEG-MTZ-O-MIM2 **49**, and charged fluorescent methylimidazolium CD compound mono{6-[TU-HDA-NPNI-(TEG-MTZ-O-MIM2)]}-β-CD **69** on silica gel in various MeOH and MeCN environments.

Compound	NPNI-NH-TEG-MTZ-O-MIM2 **49**	mono{6-[TU-HDA-NPNI-(TEG-MTZ-O-MIM2)]}-β-CD **69**
Elution mixture		
1. 0.01 M NH_4HCO_3 aq. sol.	nd	26
	nd	6
	nd	nd
	nd	nd
	nd	nd
2. 0.01 M NH_4HCO_3 MeOH/H_2O 1/1	11	68
	nd	36
	nd	21
	nd	15
	nd	12
3. 0.01 M NH_4HCO_3 MeOH/H_2O 3/1	nd	13
	nd	11
	nd	7
	nd	5
	nd	nd
4. 0.01 M NH_4HCO_3 MeOH	nd	nd
	nd	nd
	nd	nd
	nd	nd
	nd	nd

5. 0.01 M NH_4HCO_3 MeCN/H_2O 1/1	3	26
	4	19
	nd	12
	nd	9
	nd	7
6. 0.01 M NH_4HCO_3 MeCN/H_2O 6/1	nd	nd
	nd	nd
	nd	nd
	nd	nd
	nd	nd
7. 0.01 M NH_4OAc aq. sol.	nd	12
	nd	nd
	nd	nd
	nd	nd
	nd	nd
8. MeCN	nd	nd
	nd	nd
	nd	nd
	nd	nd
	nd	nd
9. *i*-PrOH	nd	nd
	nd	nd
	nd	nd
	nd	nd
	nd	nd
10. PrOH	nd	nd
	nd	nd
	nd	nd
	nd	nd
	nd	nd
EtOH	nd	nd
	nd	nd

	nd nd nd	nd nd nd
11. MeOH	nd nd nd nd nd	nd nd nd nd nd
nd = not detected (no elution)		
Concentration 1-10 μM		
Concentration 11-100 μM		

Next, triply charged analog NPNI-NH-TEG-MTZ-O-MIM3 **52** was subjected to another stability testing. Three solid support types, silica gel, sulfonated silica gel, and strong cation exchanger DOWEX® 50W-X8, were coated with the compound **52** by applying the already mentioned protocol, and stability in an acidic environment was studied. Results are summarized in Table 9.

Table 9. Comparison of the bond stability of triply charged fluorescent methylimidazolium compound NPNI-NH-TEG-MTZ-O-MIM3 **52** on silica gel, sulfonated silica gel, and DOWEX® 50W-X8 in an acidic environment.

Compound	NPNI-NH-TEG-MTZ-O-MIM3 **52**		
Solid support	Silica gel	Sulfonated silica gel	DOWEX® 50W-X8
Elution mixture			
1. H_2O	747	52	230
	320	24	10
	254	15	3
	287	17	1
	137	9	nd
2.	318	13	3

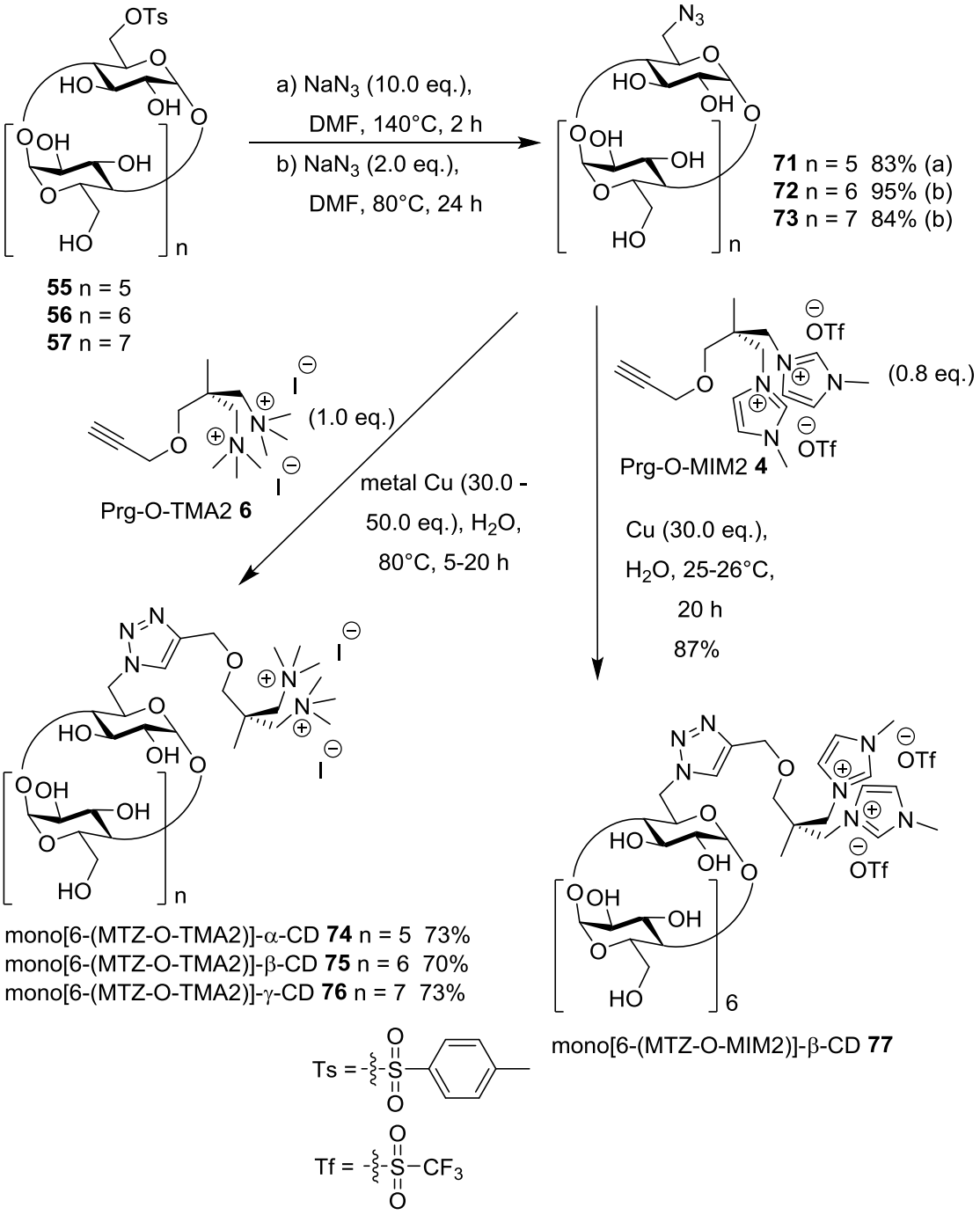
HCl aq. sol. pH 2	184	3	nd
	148	3	nd
	107	2	nd
	42	nd	nd
3. 0.01 M NH_4Cl aq. sol. pH 2 (HCl)	32	nd	nd
	33	nd	nd
	30	nd	nd
	22	nd	nd
	18	nd	nd
4. 0.10 M NH_4Cl aq. sol. pH 2 (HCl)		nd	nd
		nd	nd
		nd	nd
		nd	nd
		nd	nd
5. 1.00 M NH_4Cl aq. sol. pH 2 (HCl)		46	nd
		48	nd
		47	nd
		46	nd
		41	nd
6. 0.01 M $CaCl_2$ aq. sol. pH 2 (HCl)		7	nd
		nd	nd
		nd	nd
		nd	nd
		nd	nd
7. 0.10 M $CaCl_2$ aq. sol. pH 2 (HCl)		nd	nd
		nd	nd
		nd	nd
		nd	nd
		nd	nd
8. 1.00 M $CaCl_2$ aq. sol.		39	nd
		54	nd
		53	nd

pH 2 (HCl)		55	nd
		54	nd
nd = not detected (no elution)			
Concentration 1-10 μM			
Concentration 11-100 μM			
Concentration 101-1000 μM			

An interesting conclusion can be made from these data. First, the bond stability of compound NPNI-NH-TEG-MTZ-O-MIM3 **52** with silica gel is weak and insufficient. An assumption that the same or worse results would be obtained for singly charged NPNI-NH-TEG-MTZ-O-MIM1 **46** and doubly charged NPNI-NH-TEG-MTZ-O-MIM2 **49** analogs can be made. The majority of the compound **52** was already eluted from the silica gel by HCl aqueous solution. Due to that, after quantification of 0.01 M NH_4Cl acidic solutions, later solutions were not quantified. Binding stability with sulfonated silica gel is much stronger. Elution was observed only in 1 M NH_4Cl and $CaCl_2$ acidic solutions. It is also apparent that cation´s charge has no significant influence. The most stable bond is formed with a strong cation exchanger DOWEX® 50W-X8. No elution was observed in tested elution solutions. The explanation for this can be the higher density of sulfonated groups compared to sulfonated silica gel. All three charges in the compound form an electrostatic bond with the support. In the case of the lower density of sulfonated groups, some charged parts from the molecule cannot form an electrostatic bond. Thus, the system behaves like only singly, or doubly charged compound was attached to the support. The difference in stability between silica gel and its sulfonated analog is that the electrostatic bond with the sulfonate group is much stronger than the bond with hydroxyl groups of silica gel due to higher dissociation/ionization of sulfonic acid groups in a large pH range.

4.8 Doubly charged cyclodextrins

After the stability test and results, the first non-fluorescent doubly charged CDs **74** – **77** were synthesized (Scheme 25) to test their capability to work as a chiral selectors in membrane systems.



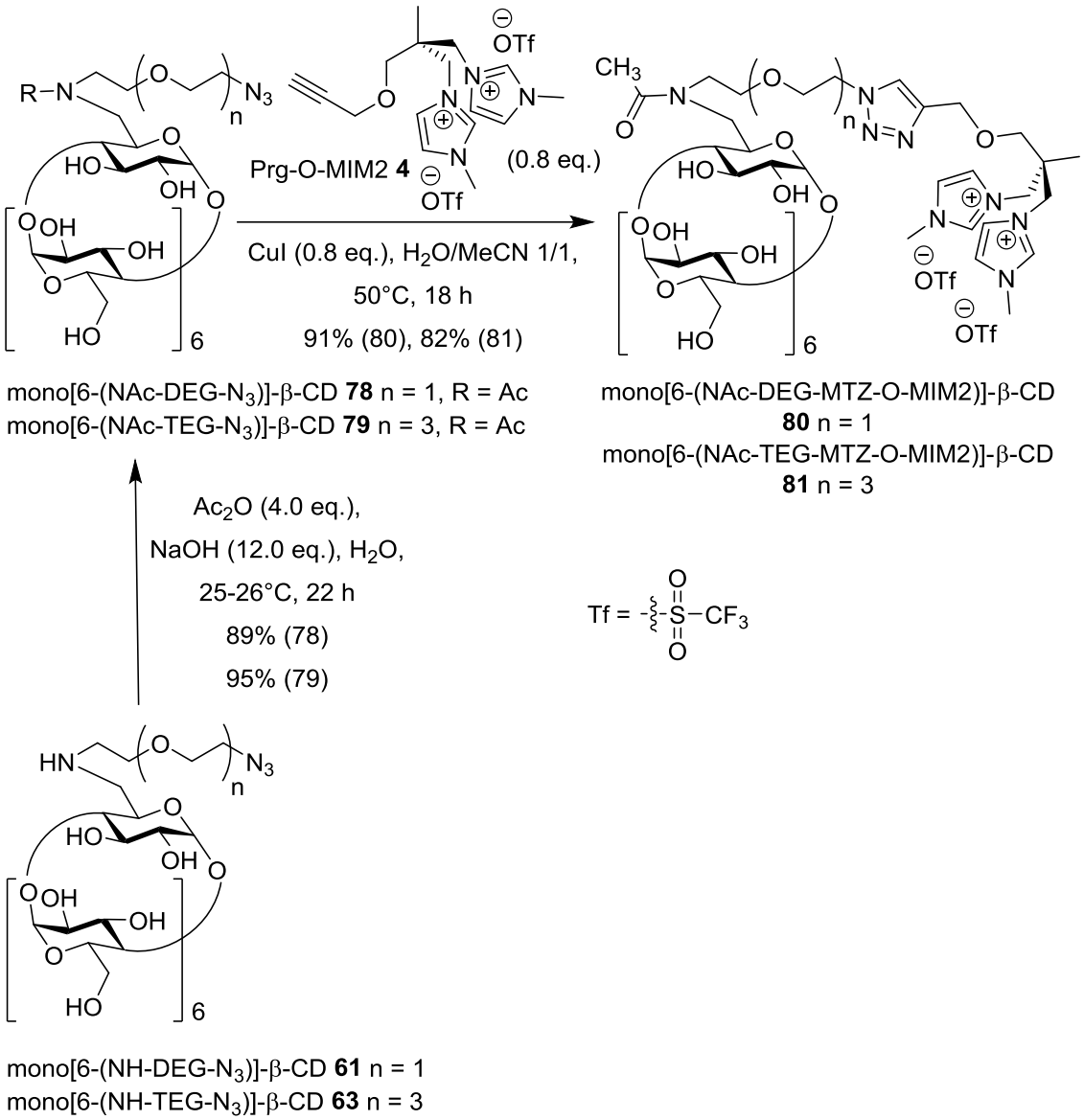
Scheme 25. Preparation of compounds **74** – **77** via azidation and subsequent click reaction.

First, the azido group was installed, and azidated CD derivatives **71** – **73** were obtained. For the preparation of mono(6-N_3)-*a*-CD **71**, huge excess (10 eq.) of NaN_3 was utilized according to literature[375]. Due to that and traces of starting *a*-CD, column chromatography purification was necessary. NaN_3 amount and temperature were lowered and reaction time prolonged to avoid column chromatography. In the case of mono(6-N_3)-β-CD **72**, no chromatographic purification was needed, and the product was obtained in pure form after repeated precipitation from acetone. The γ-CD product had to be

purified by column chromatography because native γ-CD was formed by partial hydrolysis and separated from the product mono(6-N_3)-γ-CD **73**.

Then, the complete series of mono[6-(MTZ-O-TMA2)]-CDs **74** – **76** with doubly charged trimethylammonium anchor Prg-O-TMA2 **6** was synthesized using metal Cu[145]. Products were obtained in moderate yields after reverse-phase column chromatography purification. Mono[6-(MTZ-O-MIM2)]-β-CD **77** possessing doubly charged methylimidazolium anchor Prg-O-MIM2 **4** was prepared similarly. Only the temperature was lowered because it proved unnecessary to carry out the reaction at an elevated temperature. Again, the product had to be purified by reverse-phase column chromatography and was obtained at a moderate yield.

Next, a decision was made to modify the distance between the anchor and the CD moiety to test the influence of a CD-solid support distance on the separation ability. Doubly charged CD compounds mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80** and mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81** with methylimidazolium anchor were prepared (Scheme 26). So, mono[6-(NH-DEG-N_3)]-β-CD **61** and mono[6-(NH-TEG-N_3)]-β-CD **63**, bearing diethylene and tetraethylene glycol were utilized. First, the secondary amine in these compounds was acetylated to avoid any problems with its basicity. The modified Lumière-Barbier method[376] with NaOH instead of sodium acetate was utilized. Excess of acetic anhydride was used to acetylate the amino group completely. Then the excess of NaOH was added to hydrolyze acetic acid esters, which were also formed. Acetylated compounds mono[6-(NAc-DEG-N_3)]-β-CD **78** and mono[6-(NAc-TEG-N_3)]-β-CD **79** were separated from traces of unacetylated amines mono[6-(NH-DEG-N_3)]-β-CD **61** and mono[6-(NH-TEG-N_3)]-β-CD **63** using a strong cation exchanger. Amines were attached to the resin by salt formation, while acetylated products went through because they lack basic nitrogen.



Scheme 26. Preparation of doubly charged compounds mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80** and mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81** via acetylation and subsequent click reaction.

4.9 Chiral Nafion® 117 membranes preparation and tryptophan racemic mixtures' separation

This part of the thesis was done in cooperation with Pavel Izák at Institute of Chemical Process Fundamentals of the Czech Academy of Sciences. Modification of Nafion® 117 membranes was performed by the author of this thesis while chiral measurements were done in the laboratory of Pavel Izák. Results were published in two articles[223,224].

4.9.1 Preferential sorption

Charged CD compounds mono[6-(MTZ-O-MIM2)]-β-CD **77**, mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80**, and mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81** bearing doubly charged methylimidazolium anchor and differing by the spacer length between CD moiety and the anchor were ionically bound to a Nafion® 117 membrane. First, their preferential enantiomer sorption of the racemic tryptophan mixture was tested.[223]

The modification was ensured by stirring a 1% aq. solution of CD modifier mono[6-(MTZ-O-MIM2)]-β-CD **77**, mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80**, or mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81** with a membrane for one day. This membrane was freshly activated by refluxing in concentrated nitric acid and then transformed into an NH_4^+ cycle before its use.

The amount of ionically attached CD modifiers per cm^2 of the membrane was determined by gravimetry after evaporating the solution of the unattached modifier and drying to constant weight at 60°C. Results are summarized in Table 10.

Table 10. The amount of ionically attached CD modifiers mono[6-(MTZ-O-MIM2)]-β-CD **77**, mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80**, and mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81** per cm^2 of the membrane.

CD modifier	77	80	81
Attached amount (μmol/cm^2)	0.57	1.54	1.85

The results show that the longer the linker connecting the charged anchor and the CD unit, the larger the amount of the attached modifier. The most probable explanation is the steric repulsion between the bulky CD unit and the membrane.

Membranes were subjected to preferential sorption experiments. The sorption experimental setup is illustrated in Figure 20. A piece of the membrane was placed in a 100 mL dark glass bottle containing a racemic solution of tryptophan at a concentration of 2 g/L in H_2O. The bottle was left in a rolling mill.

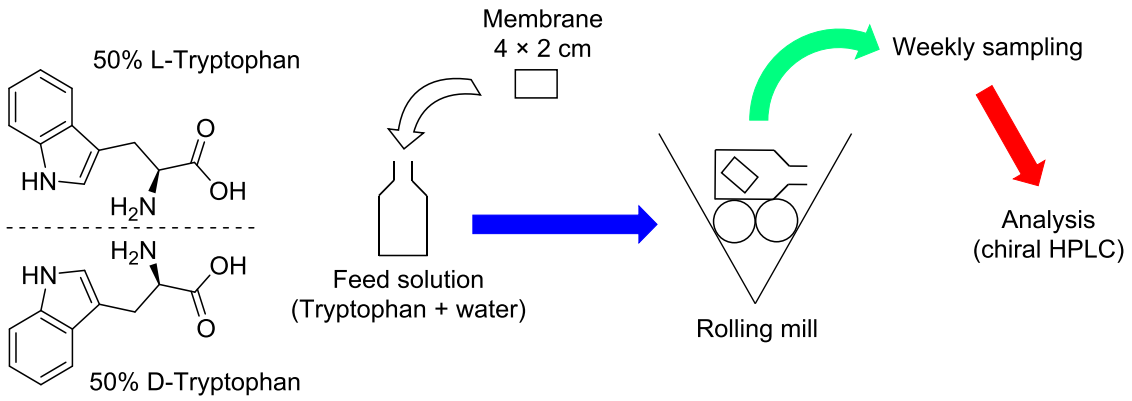


Figure 20. Preferential sorption setup.

The desired chiral resolution performance of the membrane depends on the specific interaction between the membrane recognition sites filled with chiral selector molecules and the enantiomers.[377] Over time, samples (1 mL) were taken and analyzed via chiral HPLC and compared to the racemic mixture. Results indicated preferential sorption of L-enantiomer.

The Nafion® 117 membrane without CD modification has also been studied as a reference point. No enantiomer separation was observed; the tryptophan solution contained equal amounts of D and L isomers throughout the sorption experiment.

The kinetics of preferential sorption of racemic tryptophan on each CD modified membrane is shown in Figure 21, representing the variation in the percentages of enantiomer concentration area (Y1) and of enantiomeric excess (*ee*) of both enantiomers (Y2) as a function of soaking time (X). The data reveal that the kinetics of the separation is very slow. However, it is caused by the considerable thickness of the membrane (0.19 mm). This problem can be solved by preparing the composite membrane with an ultra-thin selective layer from Nafion® 117 and CD. After 280 days of preferential sorption, the peak ratio continually and significantly increased from 49.5:50.5 to 57:43, 72:28, and 54:46 for mono[6-(MTZ-O-MIM2)]-β-CD **77**, mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80**, and mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81** membranes at the end of the sorption tests, respectively. From these proportions, variable enantiomeric excesses of 14, 44, and 8 % were calculated for mono[6-(MTZ-O-MIM2)]-β-CD **77**, mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80**, and mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81** membranes, respectively.

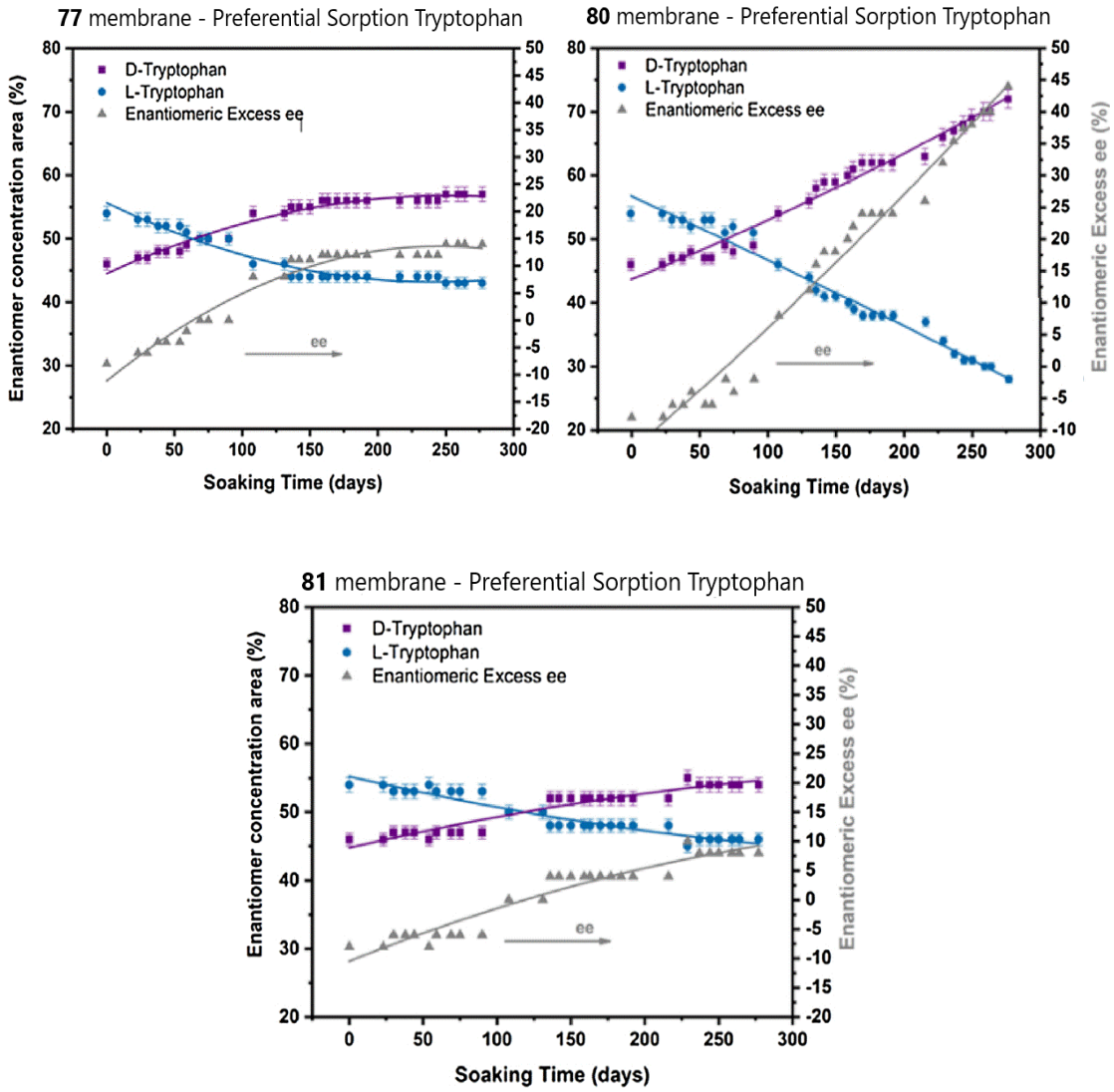


Figure 21. Kinetics of preferential sorption of a racemic mixture of tryptophan on Nafion® 117 membranes modified with CD derivatives mono[6-(MTZ-O-MIM2)] **77**, mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80**, mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81**.

The mono[6-(MTZ-O-MIM2)]-β-CD **77** membrane was prepared with modified CD without a linker. This configuration kept the CD molecule very close to the membrane (and to each other), thereby preventing the tryptophan enantiomers from accessing the densified CD rings and inhibiting enantioseparation. The lowest density of CD rings on the mono[6-(MTZ-O-MIM2)]-β-CD **77** membrane matches the lowest amount of ionically bound CD modifier per cm^2 (0.57 μmol/cm^2), in contrast to the most selective membrane, mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80** (1.54 μmol/cm^2).

The mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80** membrane was prepared with modified CD with a medium linker length. This membrane had the highest selectivity of the three membranes tested, as shown by the preferential sorption results – 44 % enantiomeric excess.

The mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81** membrane had the longest modified CD linker, thereby keeping the CD molecule far from the membrane. The interaction with the Nafion® 117 support presumably has a synergic effect necessary for the preferential sorption of enantiomers, which is weakened in long linkers, thus hampering the preferential adsorption of the tryptophan enantiomers. Moreover, this membrane contained the highest amount of ionically bound CD modifier per cm^2 (1.83 $\mu mol/cm^2$), which could also cause some sterical obstructions.

The data reveal relatively slow kinetics. Throughout the experiment of D, L-tryptophan preferential sorption, lasting 280 days, the peak ratio of D-Trp:L-Trp continually and significantly increased.

4.9.2 Pertraction

Next, pertraction experiments were tested.[224] The setup for pertraction experiments is depicted in Figure 22. The solid membrane separated the two compartments of the pertraction device. Membranes (2.5 cm diameter) were cut and loaded in the middle of pertraction test cells. The two chambers of 60 mL each were filled with the racemic solution in H_2O (2 g/L) on the feed side and fresh solvent (ultrapure H_2O) on the stripping side. A thermostat kept each chamber at a stable temperature, and liquids inside the cell were stirred by magnetic bars. The concentration of each enantiomer in the permeate was determined by chiral HPLC again.

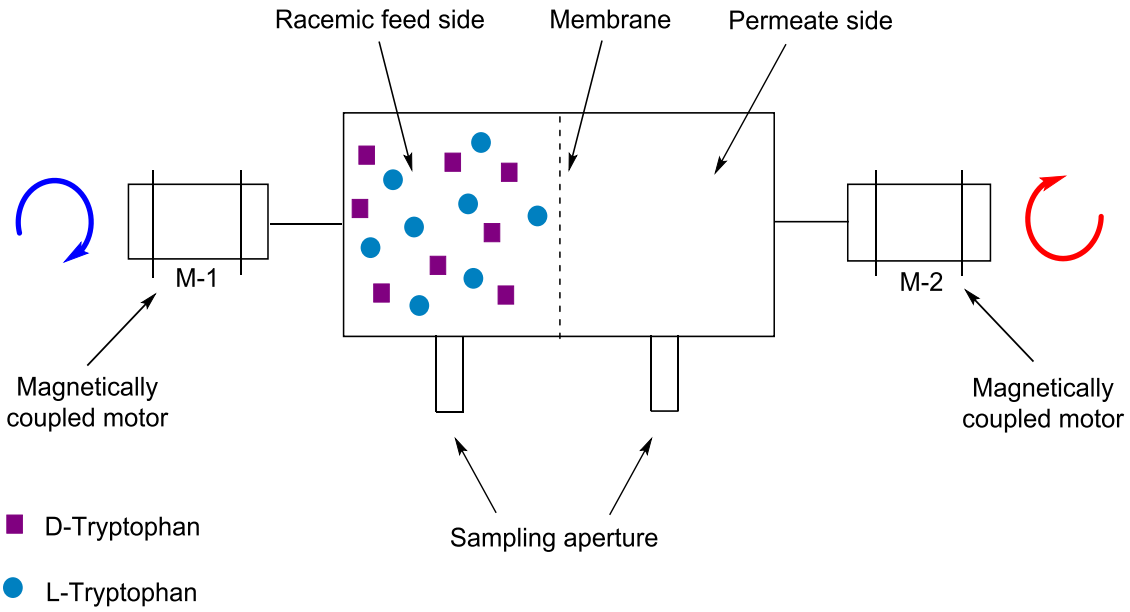


Figure 22. Pertraction setup.

The evolution of peak area of Trp enantiomers during pertraction is depicted in Figure 23 for all membranes. Graphs for the feeds are on the left, and permeates are on the right. The first line shows the results of mono[6-(MTZ-O-MIM2)]-β-CD **77**, the membrane with no spacer in the CD modifier, the second one is attributed to mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80** with the short spacer, and the third belongs to mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81** with the long spacer.

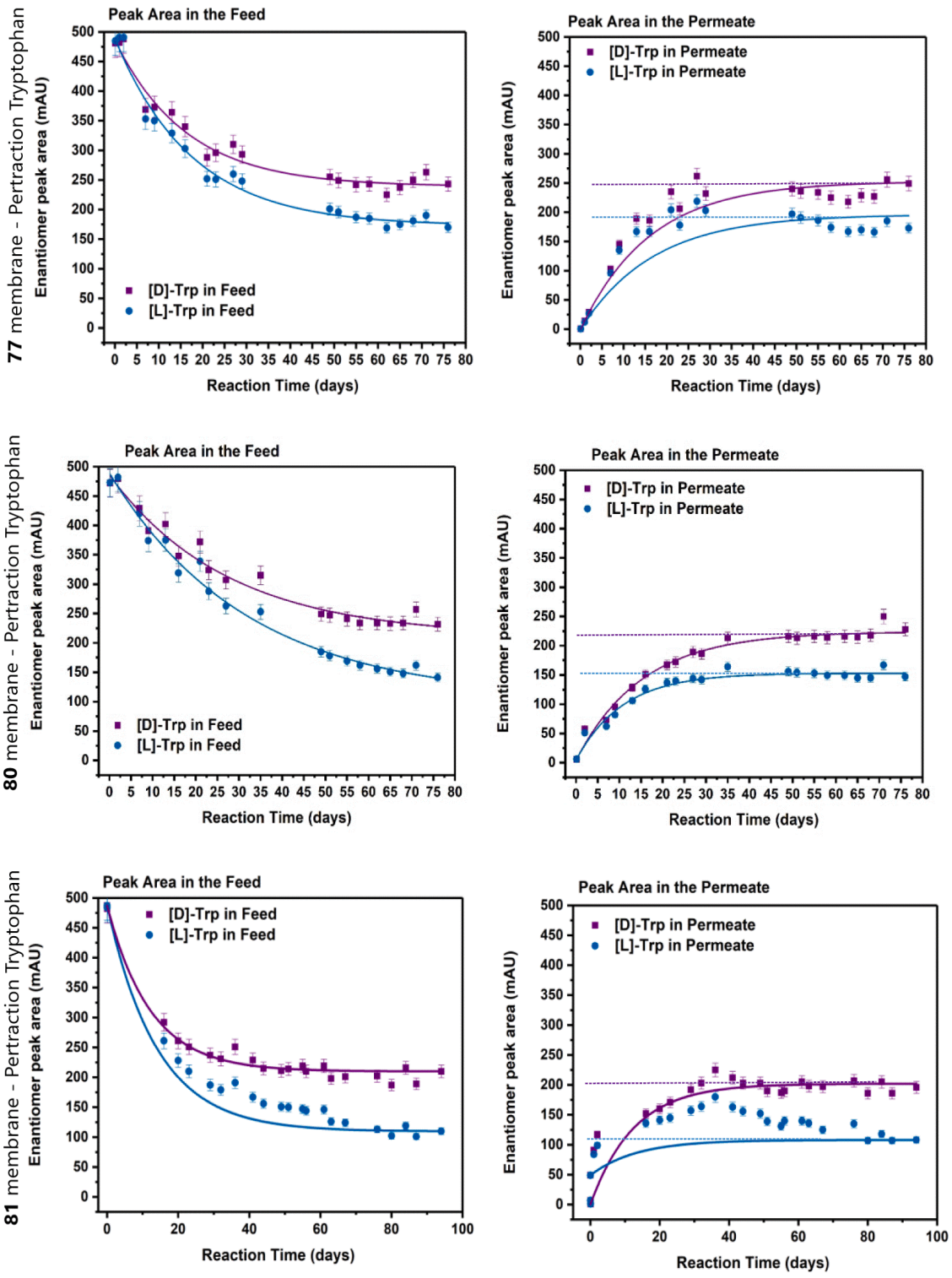


Figure 23. Evolution of peak area of Trp enantiomers during pertraction experiments. Left graphs – situation in feed, at right – permeate site. The first line for mono[6-(MTZ-O-MIM2)]-β-CD **77**, the second line for mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80** and the third line for mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81** membrane.

For the three types of membranes, the equilibrium of Trp in two chambers has been reached within 80 days. In accordance with the preferential sorption tests, all membranes preferentially sorb L-Trp.

Figure 24 shows the kinetics of pertraction of racemic Trp through the mono[6-(MTZ-O-MIM2)]-β-CD **77**, mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80**, and mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81** membranes. A detailed evolution of the enantiomer peak area in the feed and in permeate is given along with the enantiomer concentration and the enantiomeric excess for mono[6-(MTZ-O-MIM2)]-β-CD **77** (the 1st line), mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80** (the 2nd line), and mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81** membrane (the 3rd line). As the enantiomers pass through the membrane, their concentration (peak area) decreases in the feed and increases in permeate. The concentration of both enantiomers decreased, significantly more for the L-Trp, indicating preferential sorption of the L-enantiomer in the membrane described above. The completely new behavior of the membranes was observed – the unchanged ratio of enantiomers was transported from the feed to permeate using these membranes. The ratio in the permeate changed from 51:49 to 59:41 (D-Trp:L-Trp) using mono[6-(MTZ-O-MIM2)]-β-CD **77**, from 51:49 to 61:39 applying mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80**, and from initial 51:49 up to 63:37 with mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81** membrane. Enantiomeric excess was calculated as 18, 22, and 27% in favor of the D-enantiomer for mono[6-(MTZ-O-MIM2)]-β-CD **77**, mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80**, and mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81**, respectively. However, the enantio-separation process occurs exclusively during the sorption part of the pertraction process, followed by simple diffusion transport of the Trp mixture.

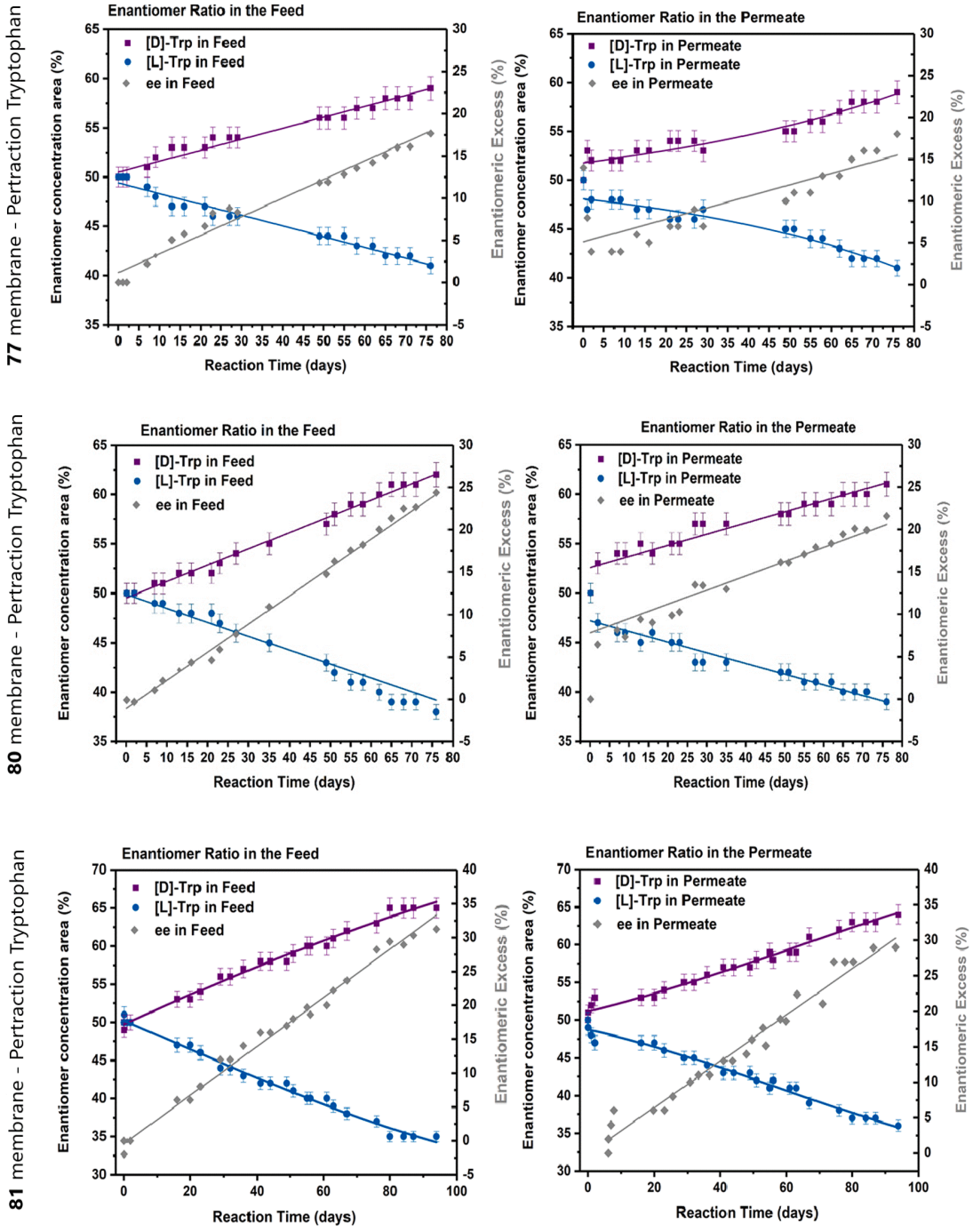


Figure 24. Kinetics of pertraction of a racemic mixture of tryptophan on modified Nafion® 117 membranes The first line for mono[6-(MTZ-O-MIM2)]-β-CD **77**, the second line for mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80** and the third line for mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81** membrane.

Last but not least, an important fact about pertraction can be revealed compared to simple sorption experiments. The enantiomeric excess of 44% was reached in the sorption process using CD modifier mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80** with the short spacer. However, the experiment took 280 days. Pertraction tests lasted around 80 days

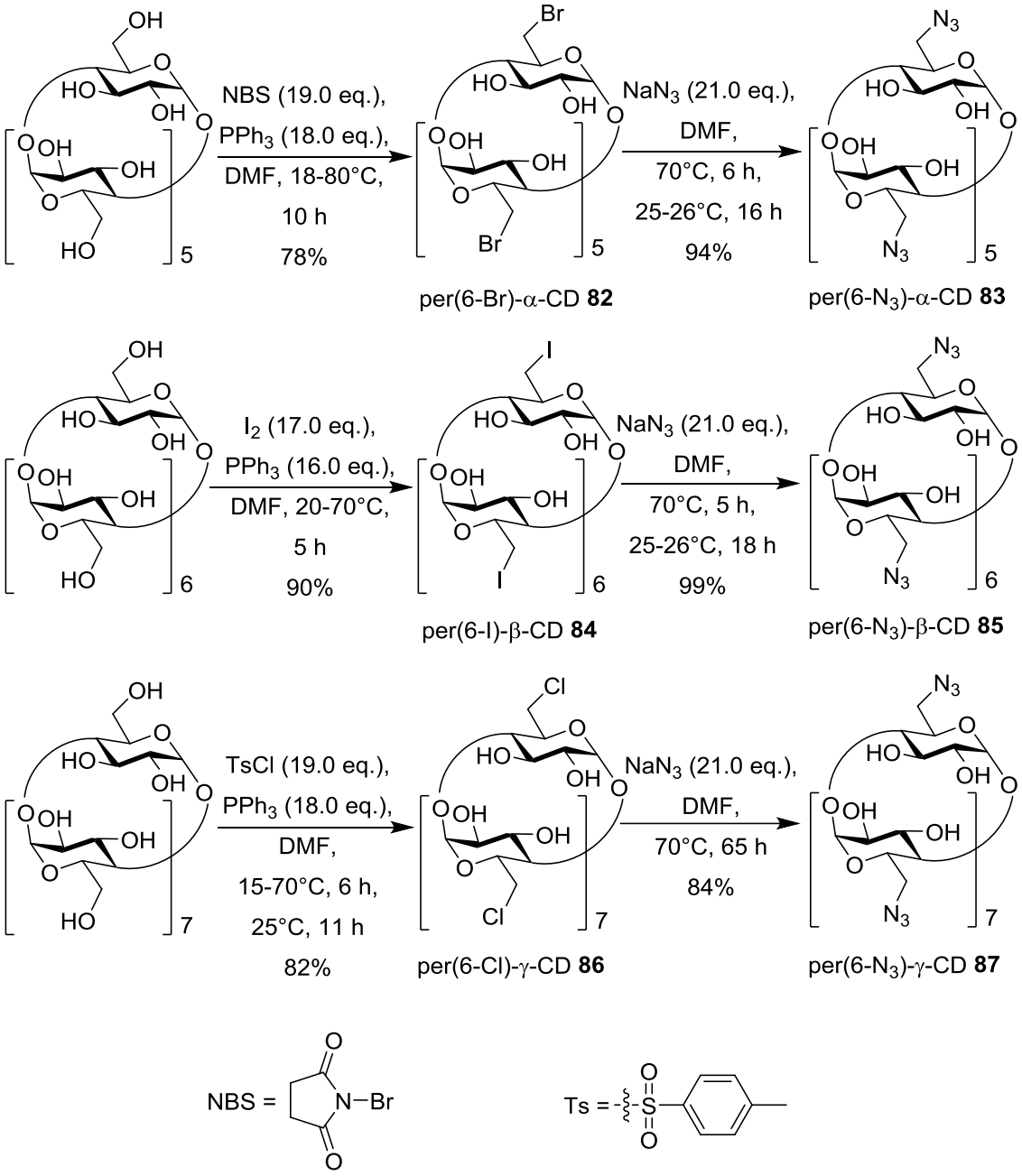
to reach a steady state, which matches to *ee* close to 13% only for the sorption experiment. Corresponding performance of mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80** membrane was higher, *ee* equal 22% and confirm, that pertraction process is more suitable for selective elimination of L-enantiomer of Trp from H_2O.

4.10 Multiply charged cyclodextrins

4.10.1 Fluorescent multiply charged cyclodextrins

Following the results from the subchapter "Anchor/solid support bond strength", it was apparent that more charges must be included into CD molecules if sufficient stability with a silica gel solid support should be achieved. The per(6-substitution) strategy was chosen due to its easiness, non-chromatographic purification, and, thus, the potential for large-scale synthesis.

Starting per(6-N_3)-CDs **83**, **85**, and **87** were prepared according to literature[119,124,125] through per(6-halogenation) and nucleophilic substitution sequence (Scheme 27). The advantage of this synthesis is no need for chromatographic purification. The solubility of per(6-substituted) products is entirely different compared to over-reacted or unreacted side-products. Due to that, compounds were prepared on more than 50 g scale.

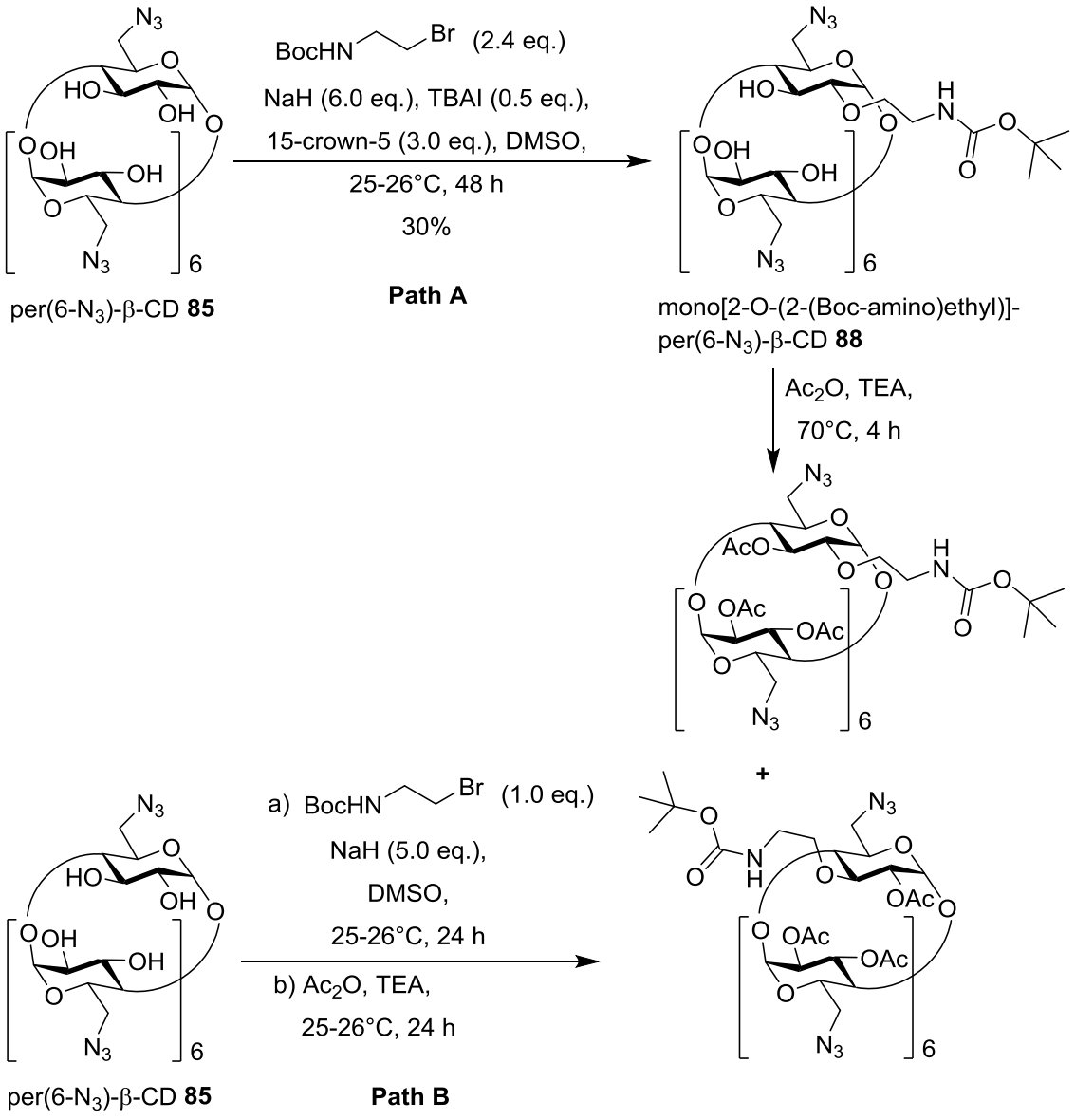


Scheme 27. Preparation of per(6-N_3)-CDs **83**, **85**, and **87**.

To attach the fluorescent naphthalimide moiety, the already prepared isothiocyanate compound NPNI-HDA-ITC **43** was utilized **(**Scheme 19). Isothiocyanate should be connected via thiourea moiety, so it was necessary to introduce the amino group into per(6-N_3)-CDs **83**, **85**, and **87**.

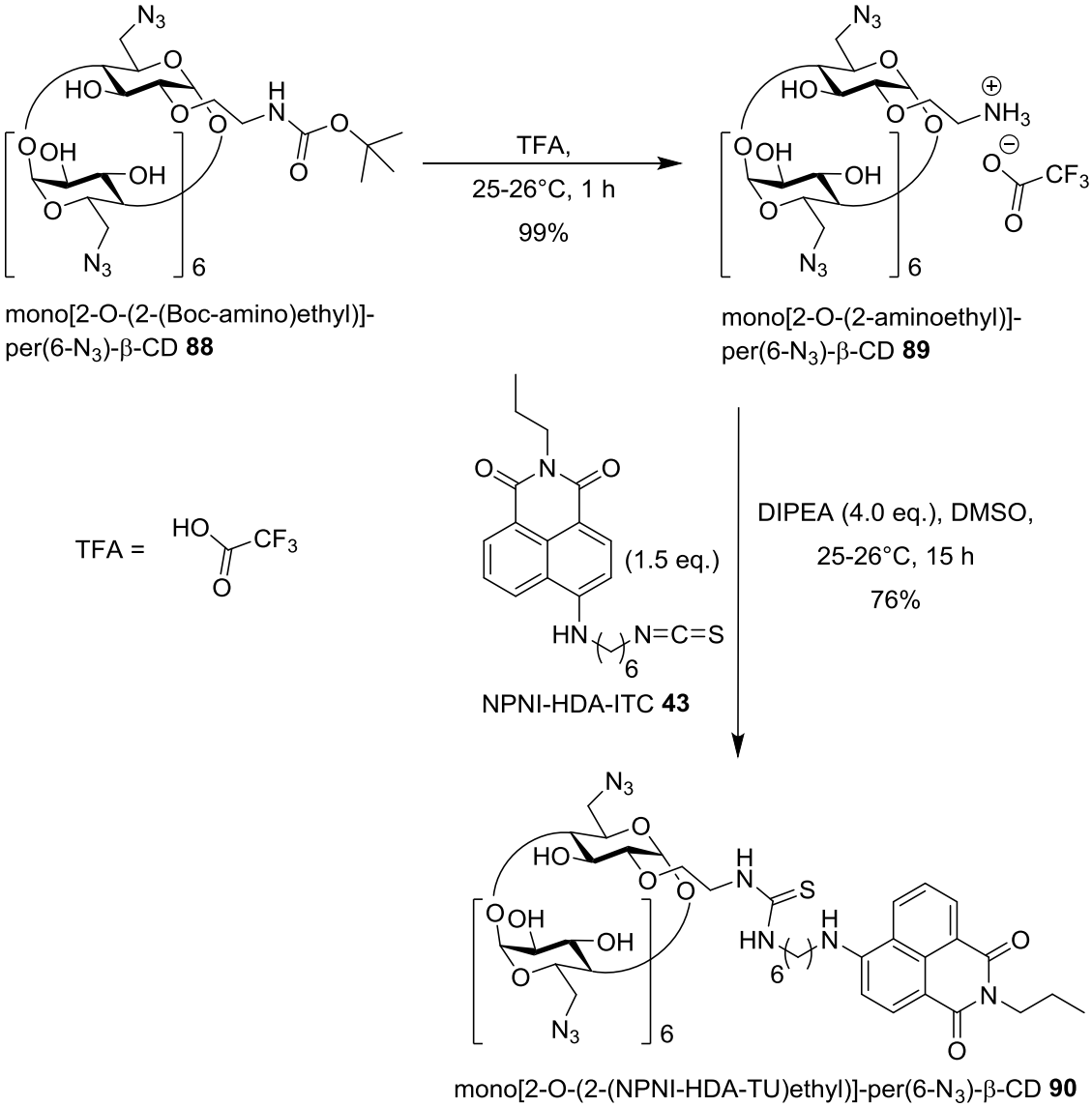
Per(6-N_3)-β-CD **85** was alkylated on the secondary rim with Boc-protected aminoethylbromide. As mentioned in the State of the Art chapter concerning alkylation reactions, a mixture of all possible regioisomers is usually formed with one of them as a major product.

Reaction conditions were developed under which only 2-*O*- regioisomer was formed (Scheme 28, Path A). The key was to utilize additives, which enhanced the reactivity of the alcoholate (15-crown-5) or the electrophile (TBAI). When the product **88** was subjected to acetylation under these conditions, only one compound was detected by TLC, indicating the formation of only one regioisomer. The same reaction was performed without additives (Scheme 28, Path B). After isolation of the monosubstituted product and its acetylation, two compounds were detected by TLC. One of them was matching with the compound prepared by Path A. Later, it was proven by 2D NMR of mono[2-*O*-(2-aminoethyl)]-per(6-N_3)-β-CD **89** that the isomer prepared by Path A was 2-*O*- regioisomer. NMR spectra are available in Supplemental Information (Figure S20 – Figure S24).



Scheme 28. Regioselective preparation of mono[2-*O*-(2-(Boc-amino)ethyl)]-per(6-N_3)-β-CD **88**.

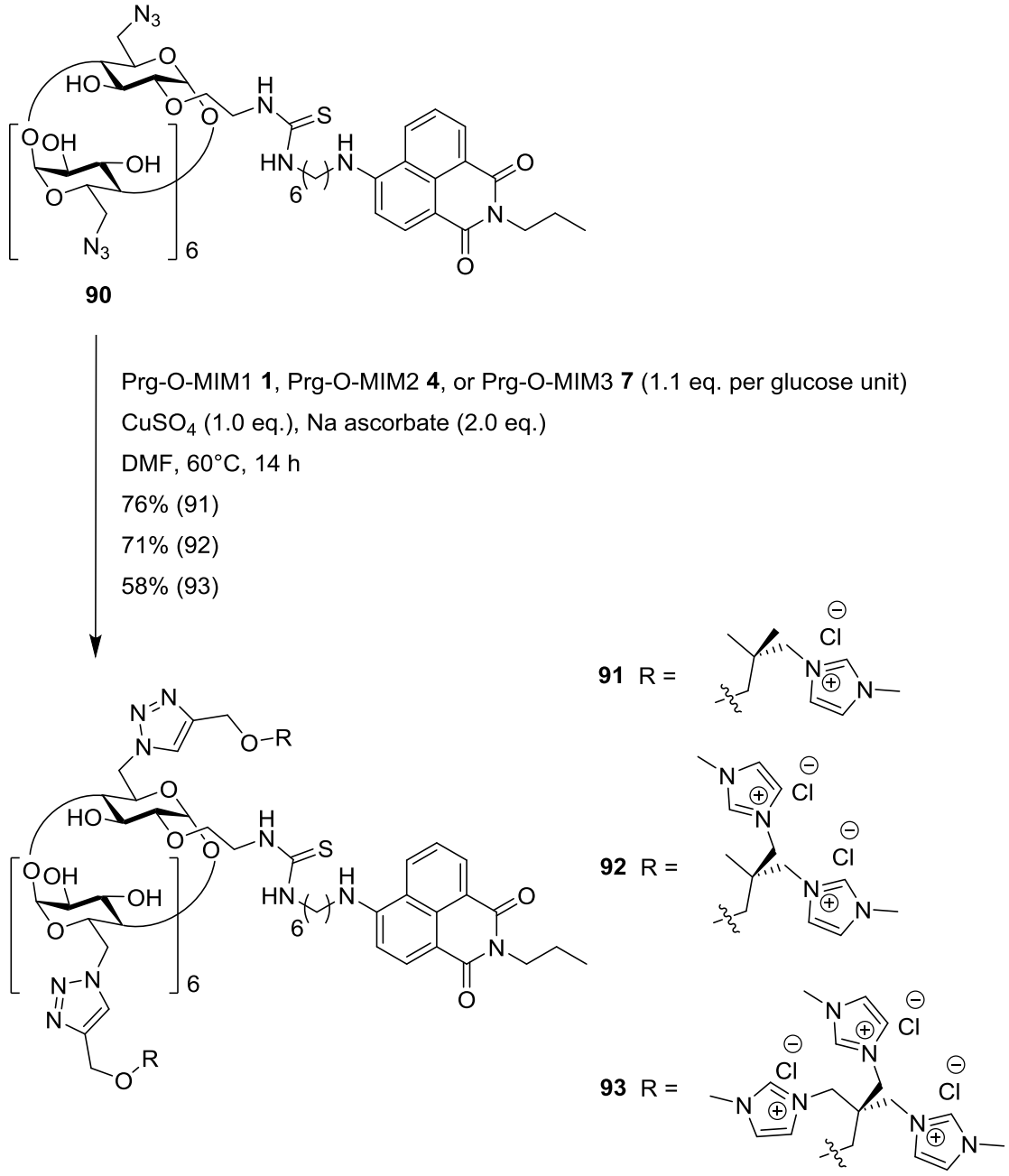
Next, Boc protecting group was removed applying literature protocol[378] by dissolving mono[2-*O*-(2-(Boc-amino)ethyl)]-per(6-N_3)-β-CD **88** in trifluoroacetic acid and stirring at room temperature for 1 hour. This procedure afforded the primary amino CD derivative mono[2-*O*-(2-aminoethyl)]-per(6-N_3)-β-CD **89** in quantitative yield. This derivative was then subjected to the thiourea formation reaction[373,374] with isothiocyanate NPNI-HDA-ITC **43**, and the final product mono[2-*O*-(2-(NPNI-HDA-TU)ethyl)]-per(6-N_3)-β-CD **90** was formed in moderate yield (Scheme 29).



Scheme 29. Boc group removal and preparation of mono[2-*O*-(2-(NPNI-HDA-TU)ethyl)]-per(6-N_3)-β-CD **90**.

The last step of this sequence was the attachment of the anchors. To compare the effect of the number of charges with the results from the chapter "Anchor/solid support

bond strength", singly, doubly, and triply charged methylimidazolium anchors Prg-O-MIM1 **1**, Prg-O-MIM2 **4**, and Prg-O-MIM3 **7** were used (Scheme 30).



Scheme 30. Preparation of naphthalimide monosubstituted multiply charged CDs **91**, **92**, and **93**.

The best conditions showed to be $CuSO_4 \cdot 5H_2O$ and sodium ascorbate[370], probably the most common method. The final products mono[2-*O*-(2-(NPNI-HDA-TU)ethyl)]-per[6-(MTZ-O-MIM1)]-β-CD **91**, mono[2-*O*-(2-(NPNI-HDA-TU)ethyl)]-per[6-(MTZ-O-MIM2)]-β-CD **92**, and mono[2-*O*-(2-(NPNI-HDA-TU)ethyl)]-per[6-(MTZ-O-MIM3)]-β-CD **93**, bearing 7, 14, and 21 permanent positive charges, respectively, were isolated and purified by basic alumina column chromatography.

Problems occurred with the NMR characterization of these compounds. Spectra were broad, and it was hard to distinguish various peaks. The possible reason is the micelle formation because the CD secondary rim now possesses a bulky lipophilic substituent. In contrast, the primary rim is fully charged and so highly polar as a consequence.

4.10.2 Silica gel bond strength

With compounds **91**, **92**, and **93** synthesized, the same sorption and bond strength stability test described in the previous chapter for compounds with one to three charges was performed.

Silica gel was tested as solid support. As described in the previous subchapter, the coating was performed by mixing silica gel (50 mg) and one of the three fluorescent naphthalimide CD charged compounds (3 μmol) in a 0.01 M NH_4HCO_3 aq. Solution (0.5 mL). Coated silica gel was then transformed into a Pasteur pipette, and various basic and acidic polar solutions were pumped through. The total volume of the mixtures was 5 mL, and 1 mL fractions were collected. UV-VIS spectra of every fraction were measured, and the concentration of charged fluorescent naphthalimide CD compounds in eluents was calculated after calibration measurements for compounds **91**, **92**, and **93** were done. Results are summarized in Table 11.

Table 11. Comparison of the bond stability of multiply charged fluorescent methylimidazolium CD compounds **91**, **92**, and **93** with 7 to 21 charges on silica gel in an acidic and a basic polar environment.

Compound	**91** 7 charges	**92** 14 charges	**93** 21 charges
Elution mixture			
1. 0.01 M NH_4HCO_3 aq. sol.	1026	1354	1782
	72	51	92
	5	3	4
	3	nd	3
	3	nd	3
2. 0.1 M NH_4HCO_3 aq. sol.	11	3	4
	4	2	4
	4	nd	4

	3	nd	3
	nd	nd	3
3. 1 M NH_4HCO_3 aq. sol.	11	6	8
	7	nd	4
	3	nd	3
	nd	nd	3
	nd	nd	nd
4. MeOH/H_2O/NH_4OAc/TEA 95/5/6.0 g/L/1	101	38	47
	2	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
5. MeCN/H_2O/HCOOH 95/5/0.1	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
6. MeCN/H_2O/AcOH/ TFA 93/7/1/0.025	76	nd	nd
	91	nd	nd
	49	nd	nd
	34	nd	nd
	27	nd	nd
7. MeOH	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
8. MeCN	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd

9. H_2O	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
	nd	nd	nd
nd = not detected (no elution)			
Concentration 1-10 μM			
Concentration 11-100 μM			
Concentration 101-1000 μM			
Concenration > 1000 μM			

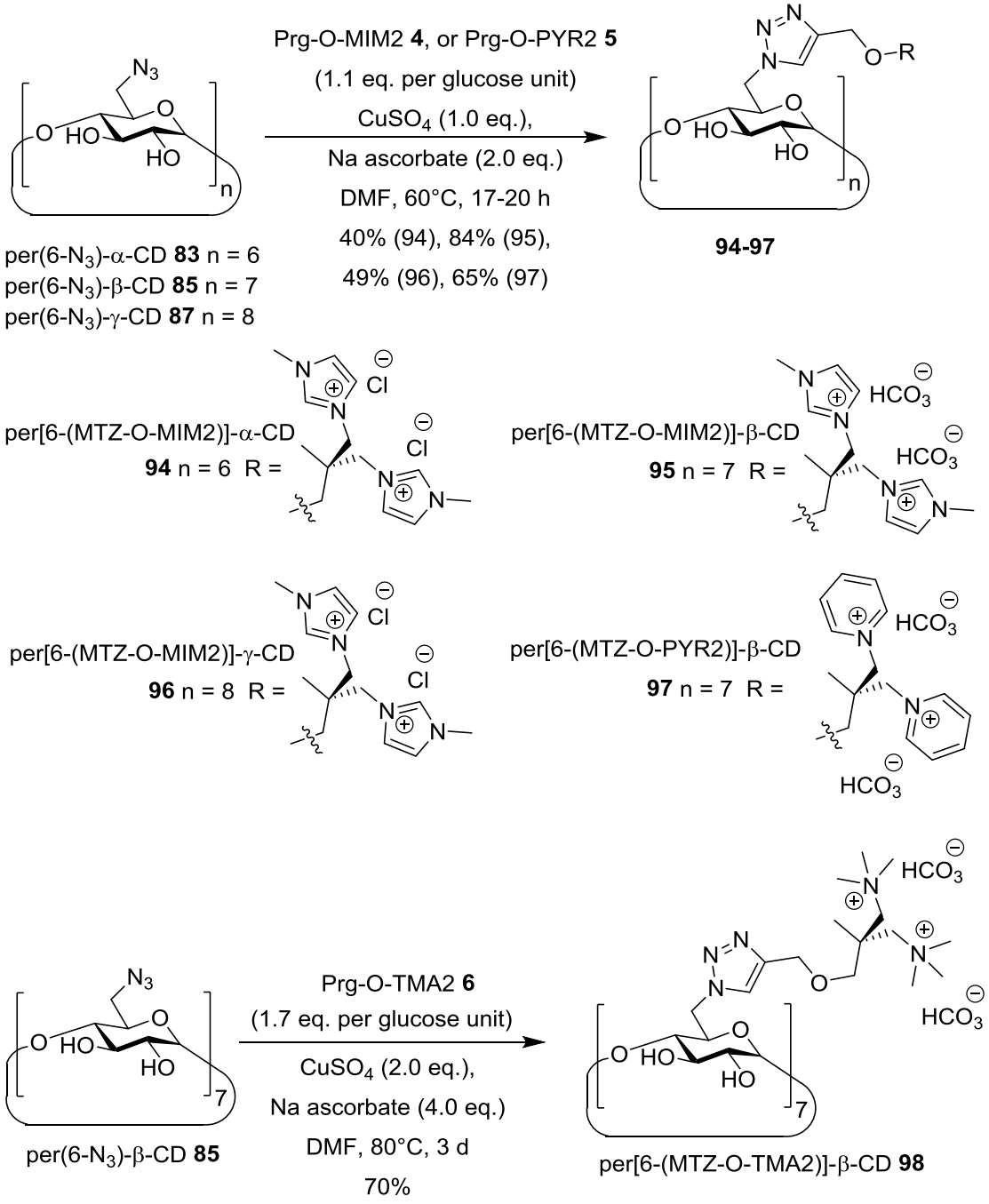
The results indicate excellent stability of multiply charged CDs onto silica gel support. The initial high concentrations in 0.01 M NH_4HCO_3 aq. solution result from significant excess of starting CD compounds **91**, **92**, and **93**. Later, when an excess of these compounds is washed away, a stable bond in a basic polar mobile phase commonly used in HILIC chromatography[379] (MeOH/H_2O/NH_4OAc/TEA 95/5/6.0 g/L/1) can be observed. The mild acidic HILIC mobile phase containing MeCN/H_2O/HCOOH 95/5/0.1 also did not present problems in terms of stability and modifier washout. The only limitation is the utilization of highly acidic MeCN/H_2O/AcOH/TFA 93/7/1/0.025 mixture in the case of the compound **91**, containing 7 permanent charges. On the other hand, compounds **92** and **93** with 14 and 21 charges do not elute even under these conditions.

To further test the stability of compounds **92** and **93** with the silica gel, the columns in Pasteur pipettes were washed with one liter of MeCN/H_2O/AcOH/TFA 93/7/1/0.025 mixture, and eluents were concentrated, and their fluorescence was checked. Neither of both eluents was fluorescent. An indication that multiply charged compounds **92** and **93** were not eluting.

It is evident from these results that increasing the number of charges led to higher stability with silica gel support. Also, it was clear that 14 charges were enough for most of the intended experiments concerning silica gel.

4.10.3 Multiply charged cyclodextrins

After these findings, the whole series of multiply charged CDs was synthesized to check the scope of the reaction (Scheme 31).



Scheme 31. Preparation of multiply charged CDs **94** – **98**.

As can be seen from the Scheme 31, the reaction runs smoothly with per(6-N_3)-CDs **83**, **85**, and **87** and doubly charged methylimidazolium anchor Prg-O-MIM2 **4**. Yields ranged from 40 to 65%, but after isolation and purification optimization, the yield was increased to over 84% (per[6-(MTZ-O-MIM2)]-β-CD **95**).

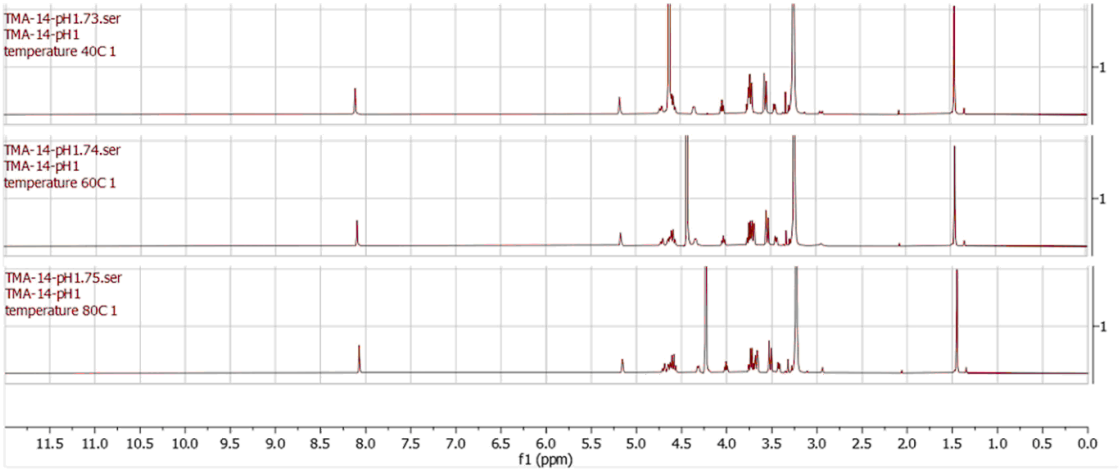
The reaction with doubly charged pyridinium anchor Prg-O-PYR2 **5** ran smoothly, and multiply charged per[6-(MTZ-O-PYR2)]-β-CD **97** was obtained in moderate yield.

However, when bulkier doubly charged trimethylammonium anchor Prg-O-TMA2 **6** was utilized, more equivalents of the anchor, $CuSO_4 \cdot 5H_2O$, and sodium ascorbate had to be used. Even the reaction time needed to be prolonged, and the temperature had to be raised to 80°C. It was evident that more sterically demanding anchors required harsher reaction conditions.

Compared to naphthalimide compounds **91**, **92**, and **93**, NMR spectra of these new compounds **94** – **98** were nice and sharp, resembling one glucose unit due to symmetry. Also, the micelle hypothesis would explain these observations because compared to compounds **91-93**, these new CD derivatives have an unmodified secondary rim. They have a polar environment on both rims and cannot behave like amphiphilic molecules.

Even in this case, the thermal and pH stability of some of the prepared compounds was tested. The protocol was the same as described in the previous subchapter, "Thermal and pH stability". So, three different pH values were tested, particularly pH 1, 7, and 9. The temperature varied from 25 to 80°C through 40 and 60°C. Samples stayed at the defined temperature for 1 hour, and spectra were recorded every 10 minutes.

Figure 25 shows results for compound per[6-(MTZ-O-TMA2)]-β-CD **98**, multiply charged CD possessing seven doubly charged trimethylammonium anchors. It was evident that even these multiply charged compounds show the same level of stability as anchors themselves.



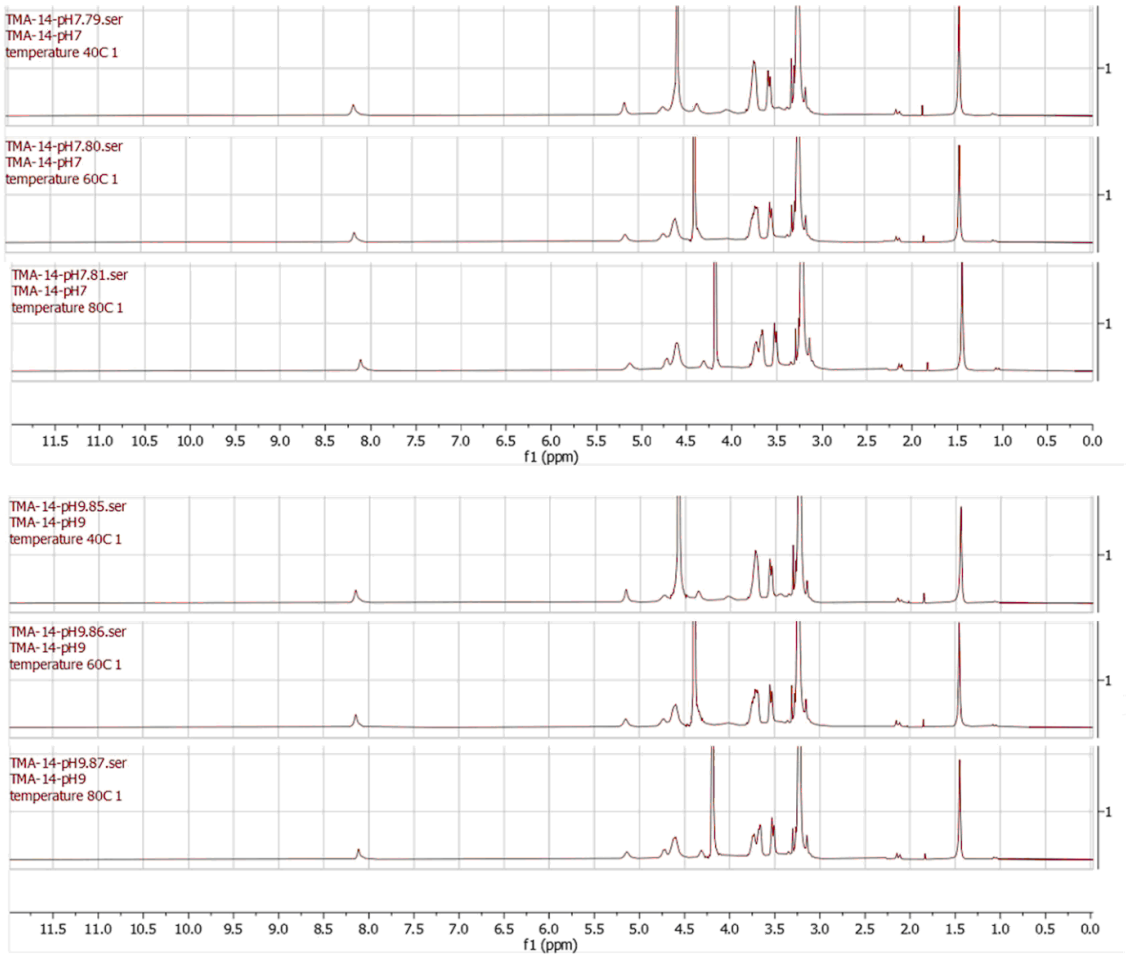
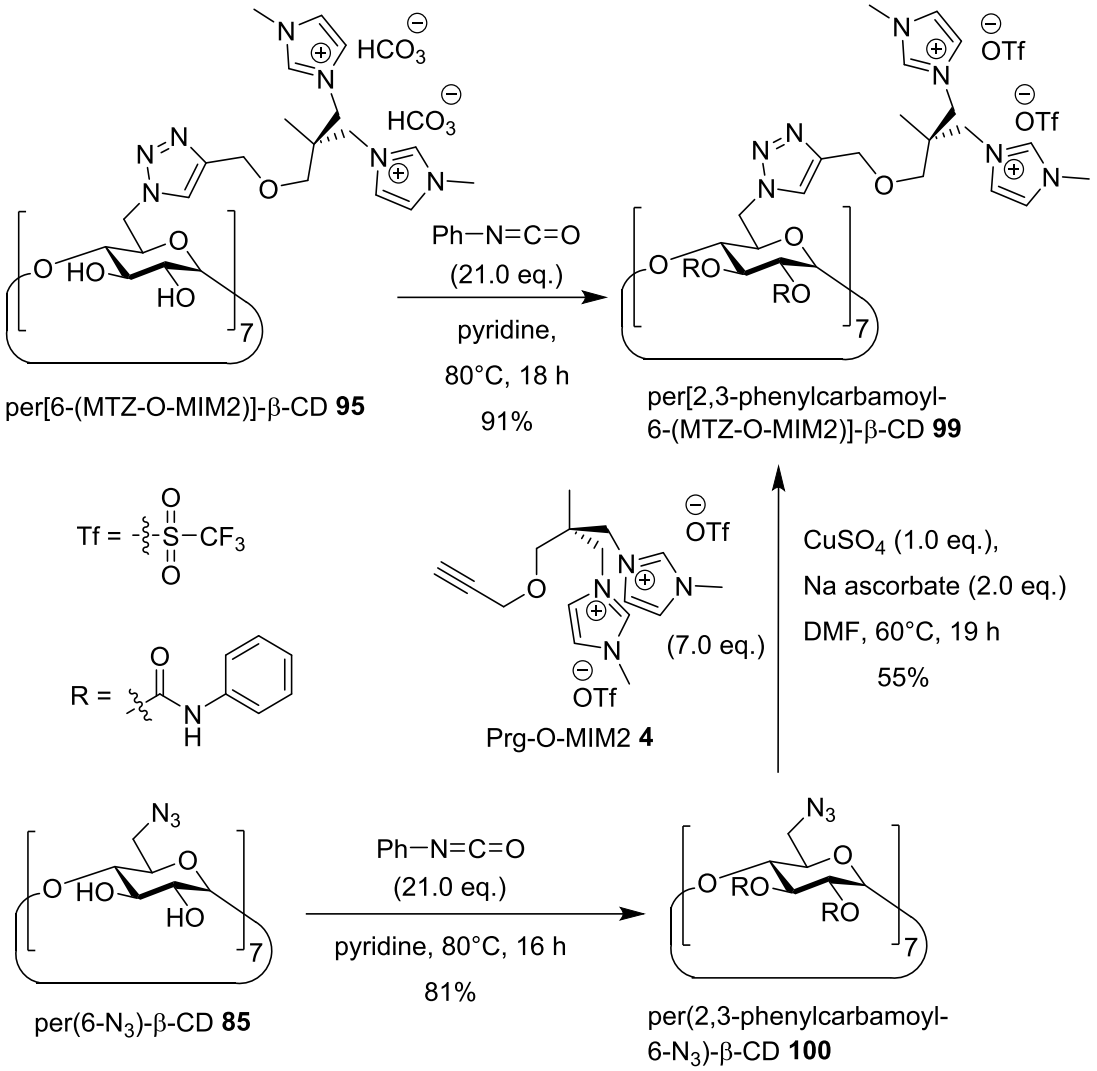


Figure 25. Thermal stability of multiply charged per[6-(MTZ-O-TMA2)]-β-CD **98** under pH 1 and temperature of 40, 60, and 80°C (upper three spectra), pH 7 and temperature of 40, 60, and 80°C (middle three spectra), and pH 9 and temperature of 40, 60, and 80°C (lower three spectra).

4.10.4 Secondary rim modification

After successfully synthesizing multiply charged CDs **94** – **98**, another logical step was the secondary rim modification. The modification is based on carbamate moiety. The reaction with isocyanates can easily form these functional groups, and no side-products are formed during the reaction.

Inspired by literature[278], the multiply charged per[6-(MTZ-O-MIM2)]-β-CD **95** was mixed with phenyl isocyanate in pyridine and heated for a couple of hours (Scheme 32). Per[2,3-phenylcarbamoyl-6-(MTZ-O-MIM2)]-β-CD **99** suffered from the same NMR characterization problem as fluorescent multiply charged CD derivatives **91**, **92**, and **93** (Scheme 30). The secondary rim modification obviously increased the tendency to micellization and worsened the quality of NMR spectra.



Scheme 32. Secondary rim modification and preparation of per[2,3-phenylcarbamoyl-6-(MTZ-O-MIM2)]-β-CD **99**.

To prove the hypothesis, per[2,3-phenylcarbamoyl-6-(MTZ-O-MIM2)]-β-CD **99** was synthesized by the reverse reaction sequence (Scheme 32). First, the per(6-N_3)-β-CD **85** reacted with phenyl isocyanate in pyridine at an elevated temperature, according to the literature[278]. After purification of per(2,3-phenylcarbamoyl-6-N_3)-β-CD **100** and NMR measurements, it was apparent that spectra were sharp and clean. Next, the click reaction of the doubly charged methylimidazolium anchor Prg-O-MIM2 **4** was conducted, the product was purified, and NMR spectra were measured. Again, the quality of spectra was much worse. However, the full match of NMR and IR spectra of per[2,3-phenylcarbamoyl-6-(MTZ-O-MIM2)]-β-CD **99** prepared by both possible synthetic pathways was observed.

Temperature NMR test was conducted with the compound **99** in deuterated DMSO. The reason behind this was to find possible temperature-structural change dependence. The sample was heated to 110°C and then cooled back to 25°C. The monitoring revealed a partial decomposition at 70°C and higher temperatures before any possible structural change occurred.

Two conclusions can be done from these findings. First, the complete secondary rim modification was achieved. This was verified by a full match in NMR and IR spectra. Second, an apparent tendency for micellization of these amphiphilic CD derivatives is induced by installing charged groups into the primary rim and modifying the secondary rim with lipophilic groups.

4.10.5 Reverse-phase modifier

When secondary rim modification via carbamate moiety was developed, preparing a functional derivative that could serve as a suitable and practical silica gel modifier was the next step.

The main idea behind this approach is illustrated in Figure 26. If the CD primary rim is entirely substituted by charged anchors and adsorbed to a silica gel, then the secondary rim points in the open space. So, suitable secondary rim modification allows silica gel properties to be changed according to the desired purpose.

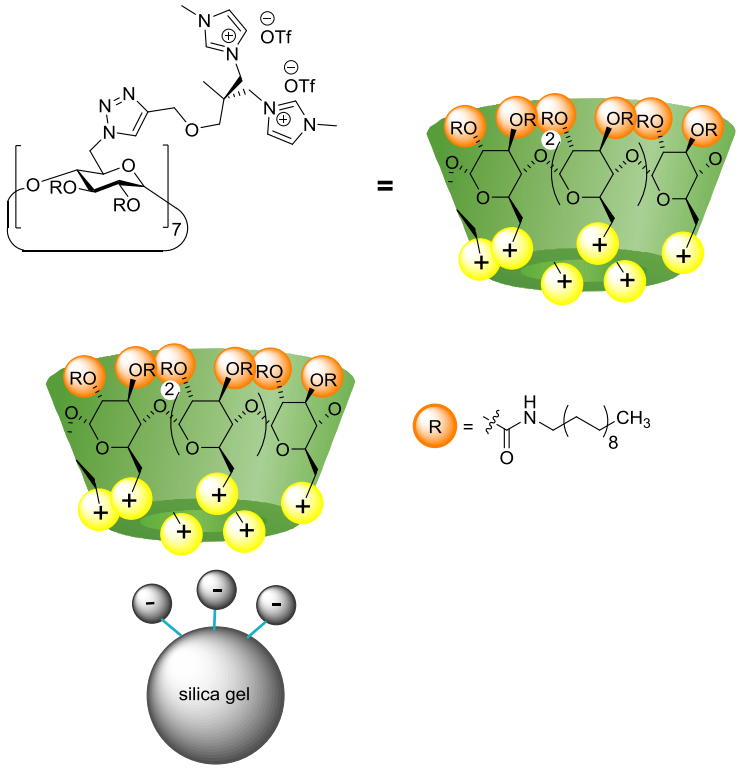
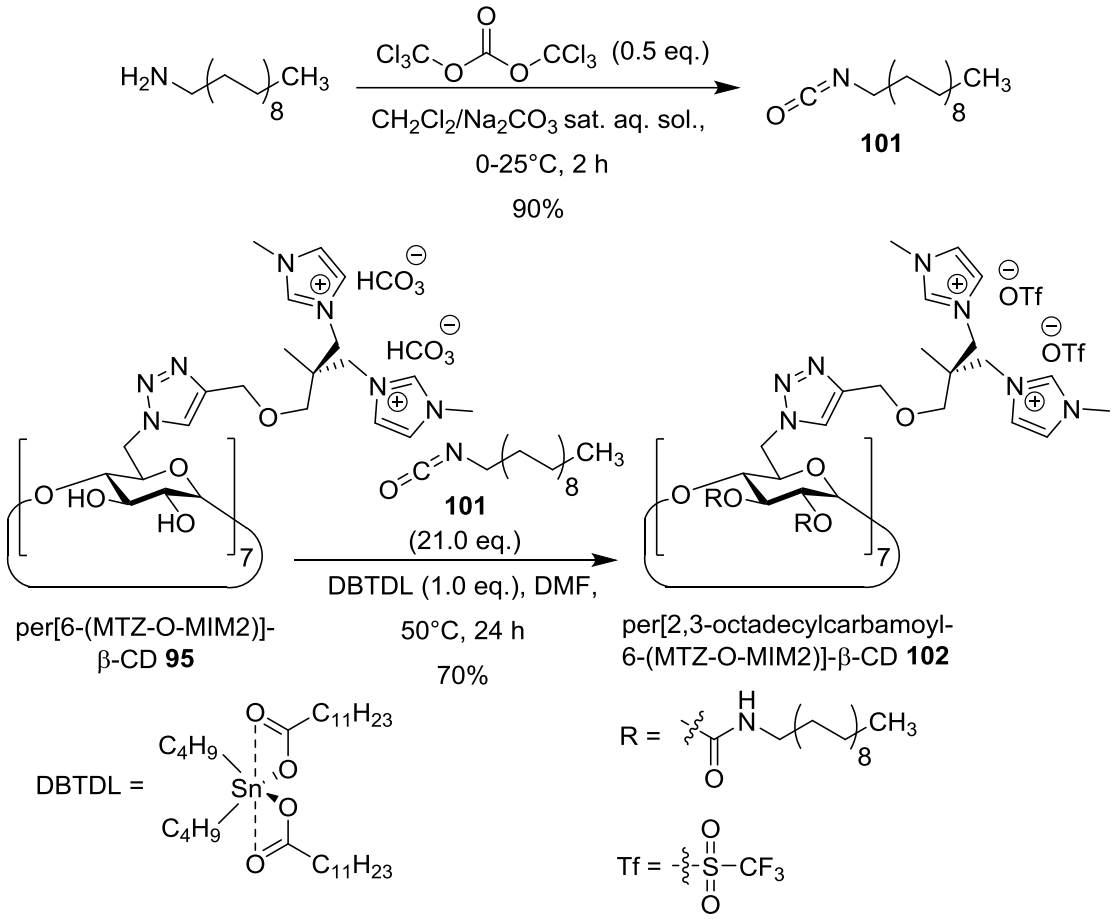


Figure 26. One of the possible silica gel modifiers and its adsorption to silica gel.

A decision was made to test the hypothesis by substituting the secondary rim with octadecylcarbamoyl moieties (Scheme 33). First, octadecylamine was transformed to its isocyanate **101** by reaction with triphosgene, according to literature[380]. Then, a reaction with multiply charged per[6-(MTZ-O-MIM2)]-β-CD **95** was tested. Applying the same reaction conditions as in the case of phenyl isocyanate (Scheme 32) did not lead to the desired product. Only starting CD compound was isolated.



Scheme 33. Preparation of reverse-phase modifier per[2,3-octadecylcarbamoyl-6-(MTZ-O-MIM2)]-β-CD **102**.

After a literature search, the DBTDL catalyst was tested. This Lewis acid is a common activator of isocyanates in macromolecular chemistry. Even CD polymers are manufactured by reaction with hexamethylene diisocyanate in the presence of DBTDL.[381] Under these reaction conditions, the desired amphiphilic per[2,3-octadecylcarbamoyl-6-(MTZ-O-MIM2)]-β-CD **102** was formed and isolated in moderate yield. Again, the ^{1}H NMR spectrum was too broad, so it was impossible to determine if the secondary rim substitution was complete. However, mass spectrometry revealed the degree of substitution from 8 to 13, with the prominent peaks corresponding to 10 and 11 octadecylcarbamoyl substituents.

The next phase was silica gel modification. A normal phase HPLC column (250 × 4.6 mm) filled with Kromasil 100-5-SIL silica gel was chosen as a testing column. The coating was done as depicted in Figure 27. The stock solution containing the reverse-phase modifier **102** was pumped through the column. When no concentration change was observed in the stock solution, pumping was stopped, and the coated column was washed with an excess of solvent to remove unabsorbed reverse-phase modifier **102**. Stock and washing solutions were evaporated, and the solid was dried. This gravimetry analysis determined that 150 mg (16 µmol) of the modifier was adsorbed. This amount corresponds to a 2% carbon load if only the octadecyl chains are counted and not the CD molecules. The silica gel density in the HPLC column is approximately 1 g/mL, so the column contains around 3 g of sorbent. For comparison, commercial RP columns have a carbon load of around 20-25%.

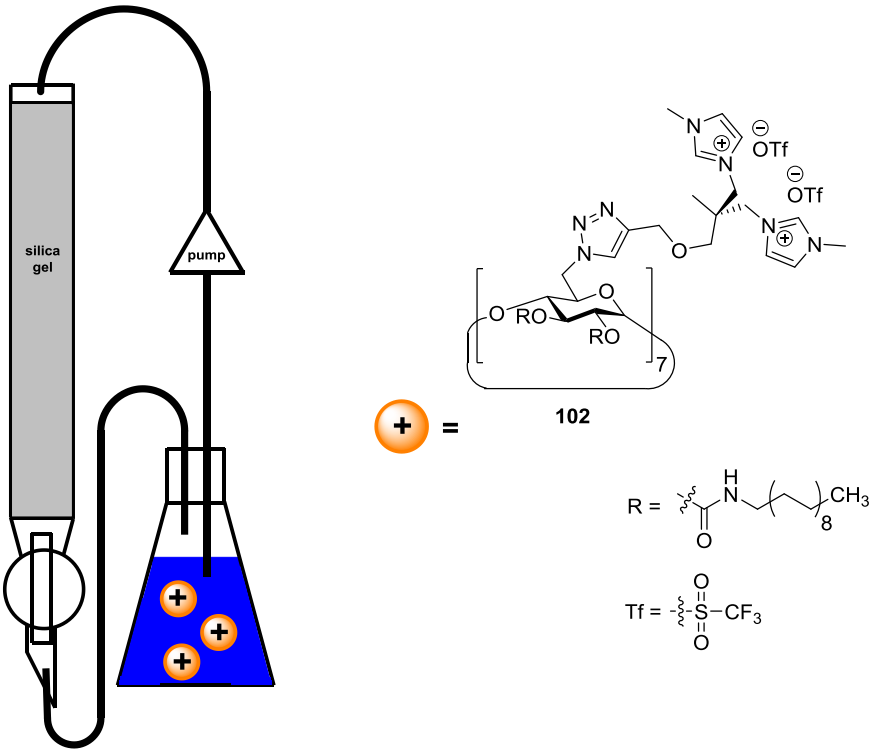


Figure 27. Schematic representation of HPLC column coating by reverse-phase modifier **102**.

The coated column was subjected to separation capability testing. A standard mixture of acetone, benzene, toluene was used for testing. Results are depicted in Figure 28. As a reference Supelco Analytical Ascentis Express C18 (150 × 4.6 mm), with 5 µm silica gel, was used. Chromatogram A depicts the baseline separation of acetone, benzene, and toluene mixture applying this commercial RP column. The mobile phase was MeCN/H_2O 70/30. The coated column did not separate the mixture using the same mobile

phase as can be seen in chromatogram B. Everything eluted with the dead volume. These results and the carbon loading calculations showed that the column is less lipophilic than the commercial one, so a slower or more polar mobile phase is needed. Chromatogram C depicted baseline separation of acetone, benzene, and toluene using coated column and MeCN/H_2O 20/80 mobile phase. As the last check, the uncoated normal phase HPLC column (250 × 4.6 mm) filled with Kromasil 100-5-SIL silica gel, the same one used for coating, was tested in MeCN/H_2O 20/80 mobile phase. Results are depicted in chromatogram D, and only partial separation was observed.

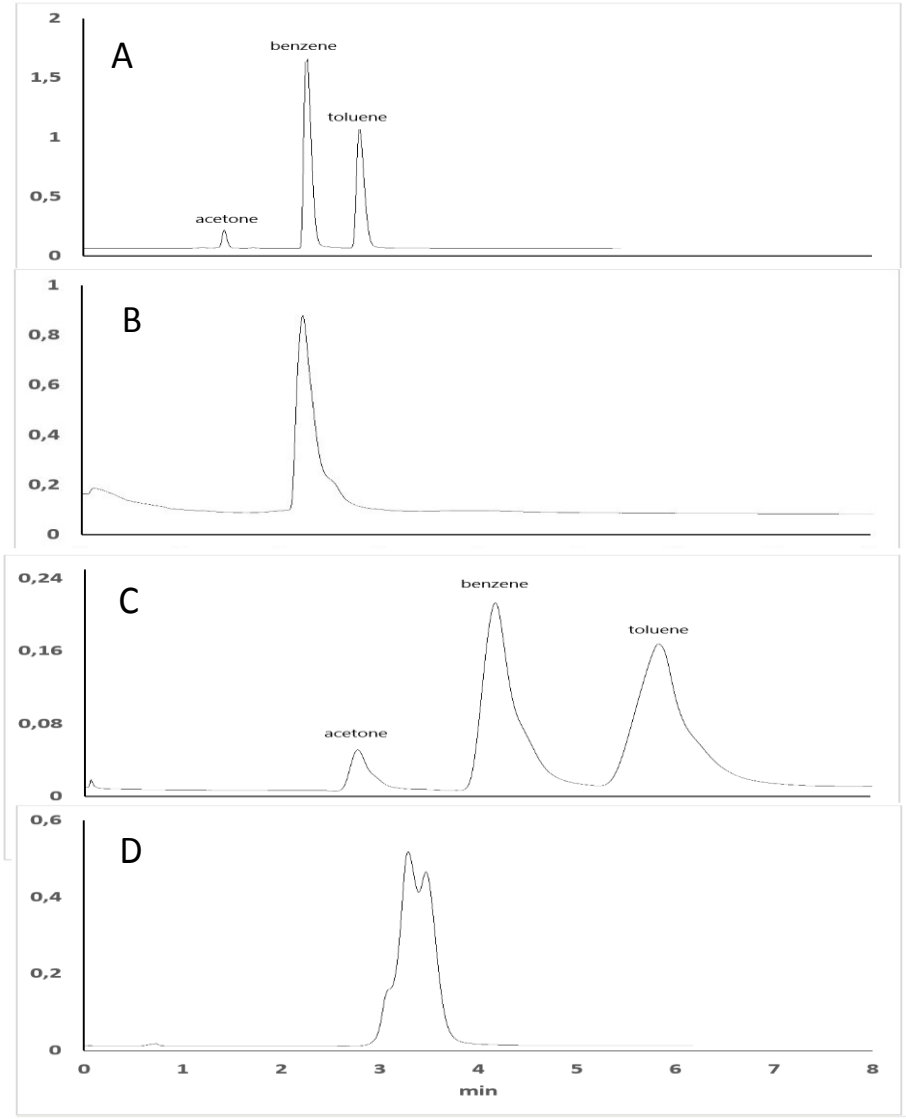
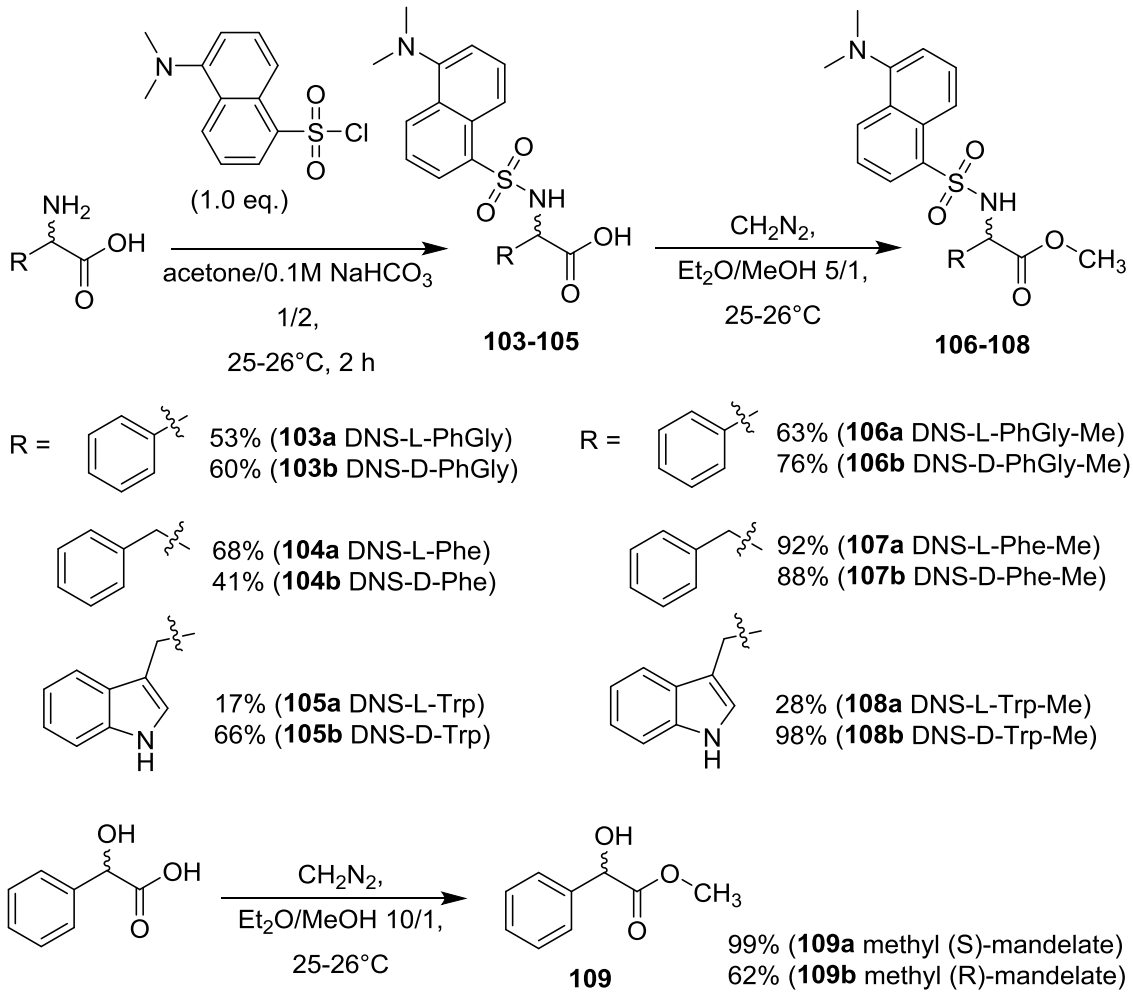


Figure 28. Chromatograms of reverse-phase modifier **102** coated column testing. **A** – commercial RP column in MeCN/H_2O 70/30 mobile phase; **B** – coated column in MeCN/H_2O 70/30 mobile phase; **C** – coated column in MeCN/H_2O 20/80 mobile phase; **D** – uncoated commercial NP column in MeCN/H_2O 20/80 mobile phase; flow 1mL/min; temperature 25°C, concentration 10μL/mL; injection 10 μL; UV (220 nm) detection.

Baseline separation was achieved, and so the concept was proven. The coated column can serve as an intermediate column for retained or very slowly eluted compounds on commercial RP columns. However, a significant broadening of the peaks is observed compared to the commercial RP column. The slow exchange of analyte molecules between stationary and mobile phases could explain this phenomenon. Another possible reason could be more than one interaction mechanism between the selector and analytes.

4.10.6 Chiral-phase modifier

The logical continuation was the development of a chiral column using the described coating method. First, per[2,3-phenylcarbamoyl-6-(MTZ-O-MIM2)]-β-CD **99** bearing phenylcarbamoyl groups was tested. For this purpose, a series of chiral analytes derived from phenylglycine, phenylalanine, and tryptophan was prepared (Scheme 34). According to the literature[278,285], enantiomers of dansylated amino acids were fully separated using columns with these kinds of CD-based chiral selectors.



Scheme 34. Preparation of chiral analytes **106** – **109** derived from phenylglycine, phenylalanine, tryptophan, and mandelic acid.

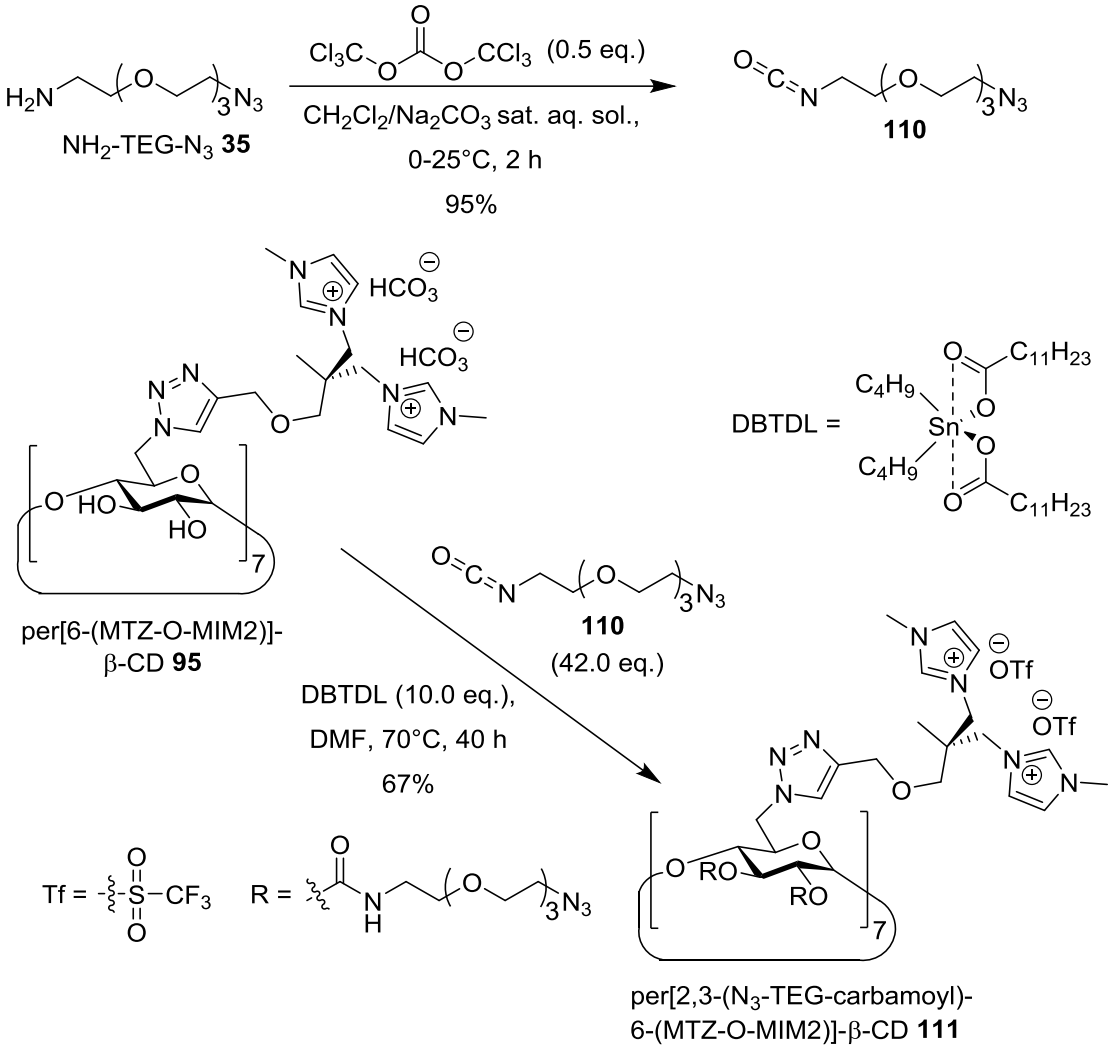
Amino acids were dansylated following literature protocols[382,383], and compounds **103** – **105** were obtained. To prevent partial racemization of enantiopure dansylated amino acids, esterification was done under neutral conditions utilizing freshly prepared diazomethane[384]. Inspired by literature[385], compounds **106** – **108** were synthesized. Both mandelic acid enantiomers were methylated by the same procedure to obtain the compound **109**.

The chiral-phase modifier per[2,3-phenylcarbamoyl-6-(MTZ-O-MIM2)]-β-CD **99** was adsorbed to normal phase column type as reverse-phase modifier **102** (HPLC column (250 × 4.6 mm) filled with Kromasil 100-5-SIL silica gel) using the same protocol described in the previous chapter concerning the reverse-phase modifier (Figure 27). According to gravimetric analysis, 74 mg (10.5 μmol) of the chiral-phase modifier **99** was adsorbed on the column.

Coated column chiral separation capability was tested in cooperation with Zuzana Bosáková. No chiral separation of synthesized analytes was observed, and coated column did not show any promising results.

After these negative results, the strategy was changed and multiply charged CDs were utilized as a scaffold to focus chiral selector moieties in one place. In other words, the strategy is the same as depicted in Figure 26. CDs' secondary side should be oriented into the space, away from the silica gel surface, and chiral moieties connected to it should ensure chiral separation.

Our vision was to prepare a versatile intermediate with suitable functional groups to connect final chiral selectors in the last step. Ideally, potential users of these chiral-phase modifiers could vary chiral selectors according to their needs just in the last step and then proceed directly to the coating procedure. For this purpose, general multiply charged per[2,3-(N_3-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **111** was synthesized as described in Scheme 35.

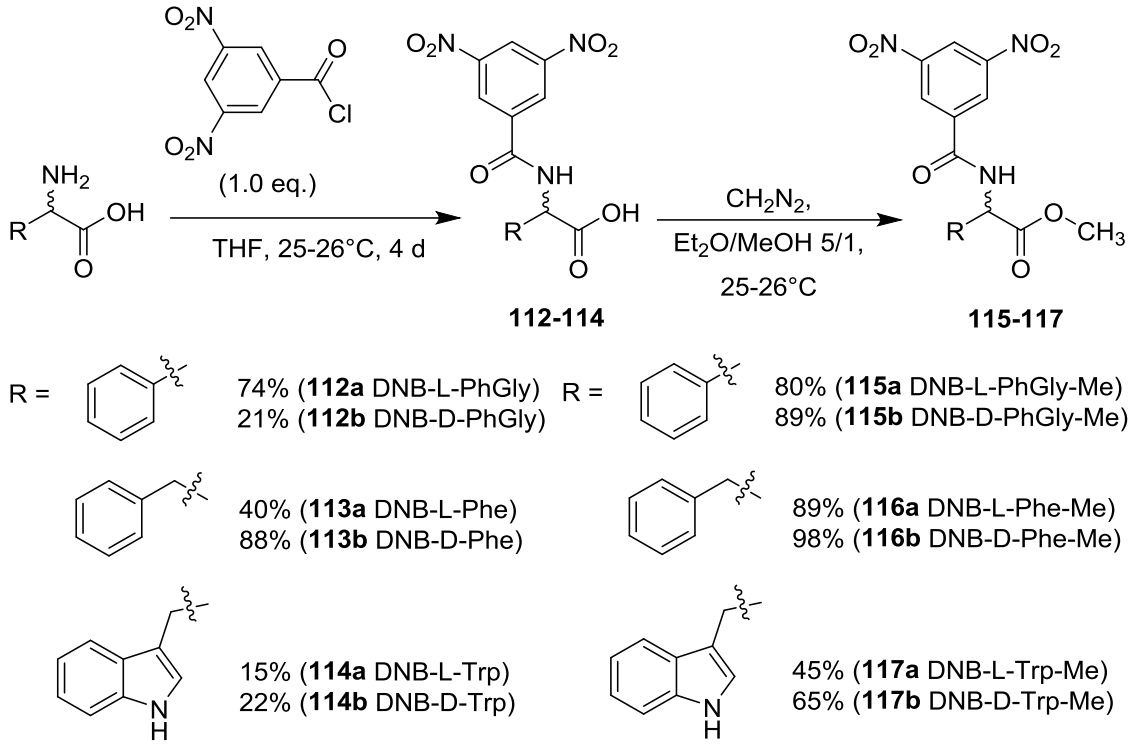


Scheme 35. Preparation of general multiply charged per[2,3-(N_3-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **111**.

The synthetic strategy was the same as for the reverse-phase modifier **102**. First, **35** was transformed into isocyanate **110** using the same literature protocol for compound **101**[380]. Then, the reaction with multiply charged per[6-(MTZ-O-MIM2)]-β-CD **95** was performed under similar conditions as described before, utilizing DBTDL for isocyanate activation. The desired product per[2,3-(N_3-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **111** was synthesized and isolated in a moderate yield. Again, the same problem with NMR characterization occurred, indicating the micelle formation phenomenon mentioned earlier.

The newly installed azido groups can be utilized to attach any desired molecules. One of Pirkle amides derivatives was tested as a chiral selector. According to the literature[299,300,304,386], typical analytes for Pirkle-based chiral columns are 3,5-dinitrobenzoylated amino acids. So a decision was made not just to test already prepared

analytes **106** – **109** possessing the dansyl group but also to prepare a new set of analytes based on 3,5-dinitrobenzoylated phenylglycine, phenylalanine, and tryptophan (Scheme 36).

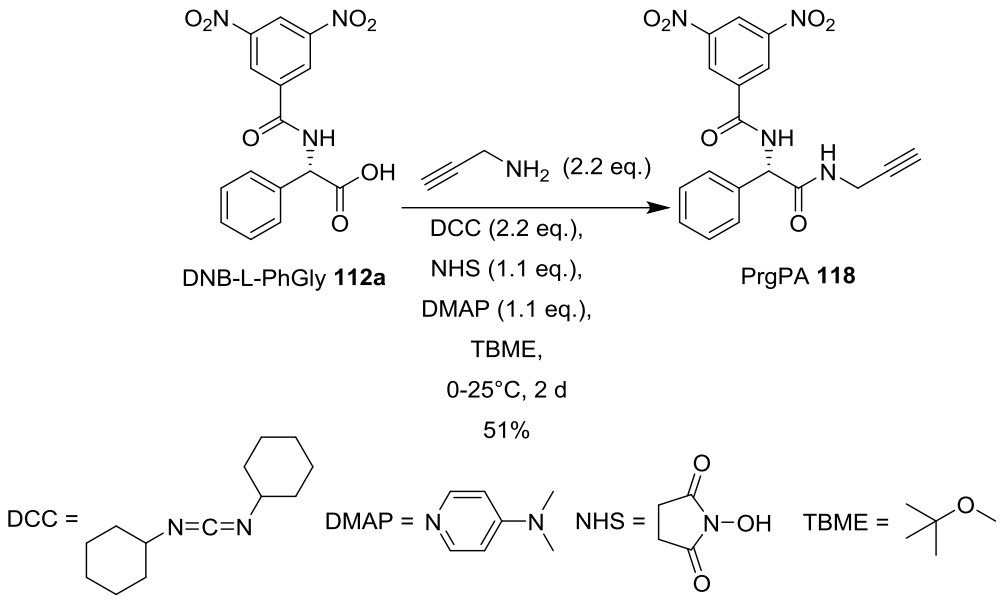


Scheme 36. Preparation of 3,5-dinitrobenzoylated chiral analytes **115** – **117** derived from phenylglycine, phenylalanine, tryptophan.

First, amino acids were acylated by 3,5-dinitrobenzoyl chloride following the original Pirkle protocol[296] to get compounds **112** – **114**. Next, acids were esterified by freshly prepared CH_2N_2[384] following the literature protocol[385].

The chiral selector is based on a 3,5-dinitrobenzoylated L-phenylglycine derivative. Its synthesis is depicted in Scheme 37. The advantage is that the already prepared chiral analyte DNB-L-PhGly **112a** could be utilized. The second step, the amidation by propargylamine, proved problematic. Several strategies, including transformation to acyl chloride and subsequent reaction with propargylamine or coupling reagents like COMU[387], EEDQ[304], DCC/HOBt combination[388], EDC/HOBt combination[389], were tested. However, reactions did not proceed at all, or in other cases, conversion was insufficient. It was also necessary to separate the product from coupling reagent side-products using column chromatography. Even the side-product resulting from the transamidation reaction was observed in some cases. In the end, the optimal conditions consisting of using DCC/NHS combination with DMAP in TBME as a solvent were

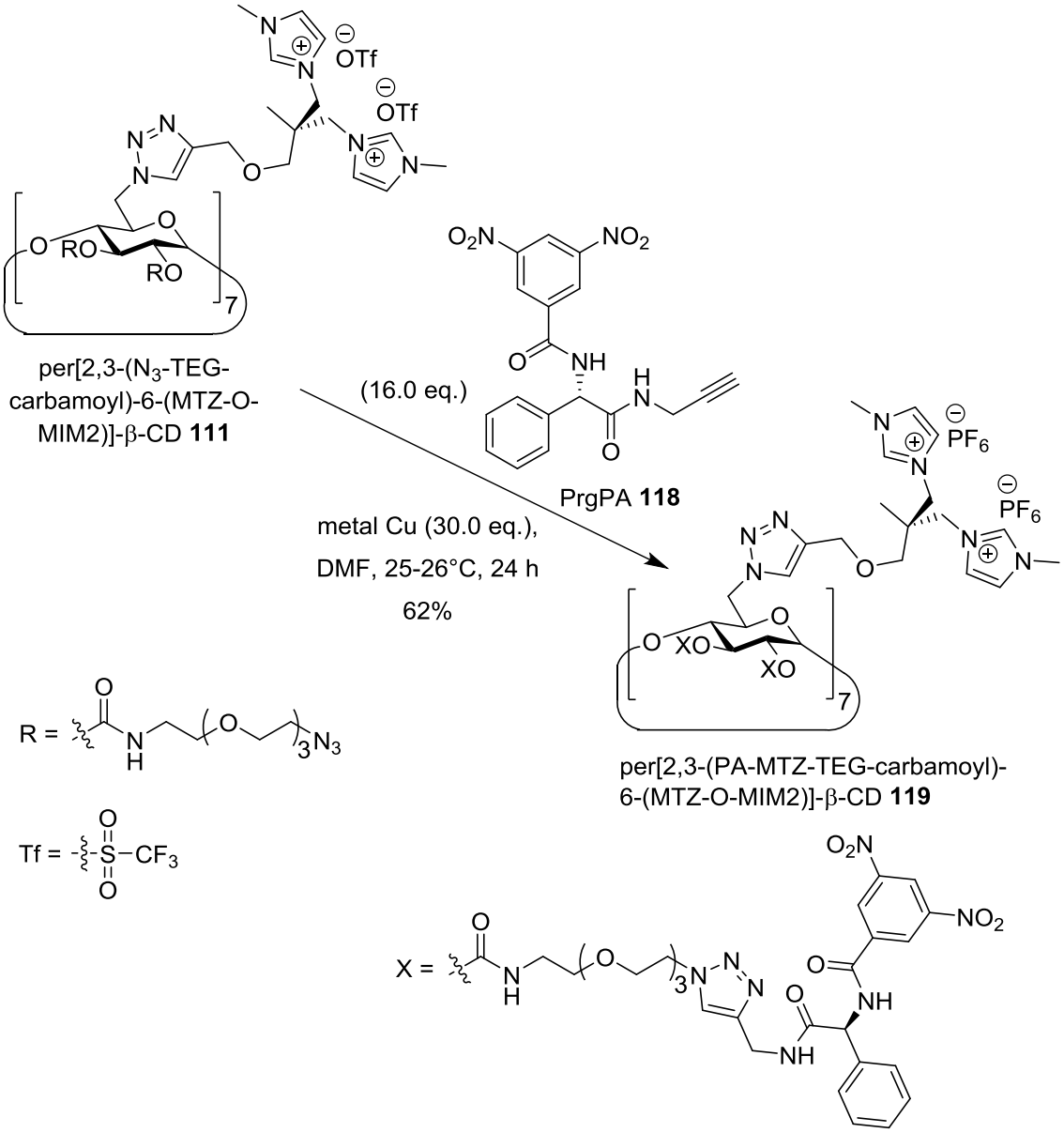
found. The final product Pirkle propargylamide (PrgPA) **118** could be purified by simple precipitation from EtOAc and was obtained in a moderate yield of around 50%.



Scheme 37. Preparation of PrgPA **118**, its reaction with general multiply charged per[2,3-(N_3-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **111**, and formation of chiral-phase modifier per[2,3-(PA-MTZ-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **119**.

Product PrgPA **118** was prepared in racemic form also to check the enantiomeric purity of the product prepared from L-phenylglycine. Both products were measured using a commercial Daicel Chiralpak IA HPLC column. Chromatograms are available in Supplemental Information (Figure S25). During the two-step reaction sequence, no racemization occurred from L-phenylglycine to PrgPA **118**.

In the next phase, PrgPA **118** was connected to multiply charged per[2,3-(N_3-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **111** via click reaction (Scheme 38). Metal Cu[145] proved to be the best option in terms of isolation and yield of final chiral-phase modifier per[2,3-(PA-MTZ-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **119**. Again, to prove that no partial racemization occurred during the reaction. Excess of used PrgPA **118** was isolated back, and its enantiomeric purity was remeasured. The Result matched the chromatogram depicted in Figure S25. Based on these data, the possibility of partial racemization during the click reaction can be ruled out.



Scheme 38. The reaction of PrgPA **118** with general multiply charged per[2,3-(N_3-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **111**, and formation of chiral-phase modifier per[2,3-(PA-MTZ-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **119**.

The chiral-phase modifier per[2,3-(PA-MTZ-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **119** was adsorbed to a normal phase HPLC column (150 × 4.6 mm) filled with Kromasil 100-5-SIL silica gel using the same protocol described in the previous chapter concerning the reverse-phase modifier **102** (Figure 27). According to gravimetric analysis, 170 mg (12 μmol) of the chiral-phase modifier was adsorbed on the column.

Prepared analytes were subjected to chiral separation testing utilizing the newest coated column. Various *i*-PrOH and heptane ratios were tested as the mobile phase. Temperature and flow rate were also varied to find optimal conditions. After many

attempts, only partial separation of dansylated phenylalanine derivatives **107a** and **107b** mixture was observed in the heptane/*i*-PrOH 90/10 mixture, as depicted in Figure 29. Dansylated phenylglycine derivatives **106a** and **106b** and DNB phenylalanine derivatives **116a** and **116b** separation tendency was negligible. Tryptophan **108a** and **108b**, DNB phenylglycine **115a** and **115b**, and mandelic acid **109a** and **109b** mixtures did not show any resolution. By analyzing these results, a conclusion can be made that separation capability is worse than original Pirkle columns based on electrostatic interaction. The only advantage is the possibility of utilizing more polar mixtures than original columns where the selector leaking was observed with higher *i*-PrOH content than 20%.

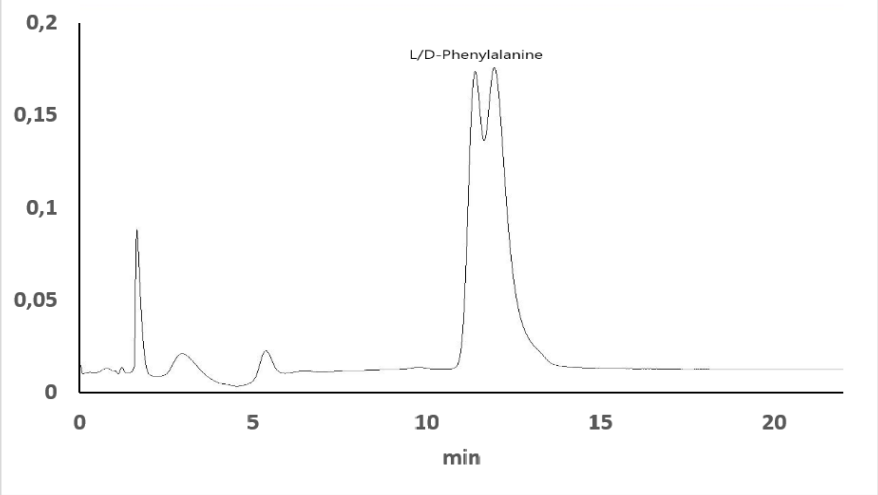


Figure 29. Chromatogram of L/D-phenylalanine derivative **107** partial resolution using coated HPLC column with chiral-phase modifier per[2,3-(PA-MTZ-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **119**; heptane/*i*-PrOH 90/10 mobile phase; flow 1mL/min; temperature 30°C, concentration 300μg/mL; injection 10 μL; UV (220 nm) detection.

It is evident from these results gathered during HPLC chiral and non-chiral testing that a higher amount of selectors needs to be coated on a silica gel column. Otherwise, the potential for baseline separation is negligible. One possible option could be a batch coating, where a silica gel is stirred in a selector's solution. Then this modified silica gel would have to be put into an HPLC column under high pressure. Not every scientist has this option, so this protocol lacks the advantages of the method described in this thesis.

One of the theories why such low selectors' amounts were adsorbed is their micelle or close ion-pairs formation. These structures could still be present during the coating procedure. Hence, selectors' permanent charges are not free to bound with silica gel. Solutions with various ionic power should be tested in combination with dynamic light scattering to support this hypothesis.

4.10.7 Chiral thin-layer chromatography

Another chiral separation abilities tests of multiply charged cyclodextrins were performed. This time, chiral TLC systems were developed and tested.

The dipping method, described in the State of the Art chapter, was utilized. The experimental part describes the detailed protocol for the preparation and evolution of these TLC systems.

First, per[6-(MTZ-O-MIM2)]-β-CD **95** was chosen as a chiral selector. Two solutions with different selector concentrations (1 mM and 10 mM) were tested. Dansylated amino acids **103** – **105** were utilized as analytes. Results can be seen in Table 12.

Table 12. TLC chiral separation tests with chiral selector per[6-(MTZ-O-MIM2)]-β-CD **95** (NS = no separation)

Selector (Concentration)	per[6-(MTZ-O-MIM2)]-β-CD **95** **1 mM in H_2O**		
Analyte (Concentration)	DNS-L-PhGly **103a** DNS-D-PhGly **103b** **0.1 mM in MeOH**	DNS-L-Phe **104a** DNS-D-Phe **104b** **0.1 mM in MeOH**	DNS-L-Trp **105a** DNS-D-Trp **105b** **0.1 mM in MeOH**
Mobile phase			
$CHCl_3$/Dioxane 1/2	NS - tailing	NS - tailing	NS - tailing
$CHCl_3$/Dioxane 1/1	NS - tailing	NS - tailing	NS - tailing
$CHCl_3$/MeOH 5/1		start	
$CHCl_3$/MeOH 2/1		NS - tailing	
Analyte (Concentration)	DNS-L-PhGly **103a** DNS-D-PhGly **103b** **0.01 mM in MeOH**	DNS-L-Phe **104a** DNS-D-Phe **104b** **0.01 mM in MeOH**	DNS-L-Trp **105a** DNS-D-Trp **105b** **0.01 mM in MeOH**
Mobile phase			
$CHCl_3$/MeOH 5/1		start	
$CHCl_3$/MeOH 4/1		front	
$CHCl_3$/MeOH 3/1	start	front	start
$CHCl_3$/MeOH 2/1	front		front

$CHCl_3$/EtOH 2/1		start	
$CHCl_3$/EtOH 1/1	NS	NS	NS
MeOH/H_2O/NH_4OAc /TEA 95/5/6.0 g/L/1		front	
MeCN/H_2O/HCOOH 95/5/0.1		front	
MeCN/H_2O/AcOH/ TFA 93/7/1/0.025		front	
Selector (Concentration)	per[6-(MTZ-O-MIM2)]-β-CD **95** **10mM in H_2O**		
Analyte (Concentration)	DNS-L-PhGly **103a** DNS-D-PhGly **103b** **0.01 mM in MeOH**	DNS-L-Phe **104a** DNS-D-Phe **104b** **0.01 mM in MeOH**	DNS-L-Trp **105a** DNS-D-Trp **105b** **0.01 mM in MeOH**
MeOH/H_2O/NH_4OAc /TEA 95/5/6.0 g/L/1		NS	
MeCN/H_2O/HCOOH 95/5/0.1		start	
MeCN/H_2O/AcOH/ TFA 93/7/1/0.025		NS	
MeOH/AcOH/1% NH_4OAc 10/1/9	NS	NS	NS
$CHCl_3$/EtOH 1/1			start

Results show no chiral separation with 1 mM chiral selector per[6-(MTZ-O-MIM2)]-β-CD **95**. When analytes concentration was 0.1 mM (injected volume was in all cases 1 µl), tailing was observed. This phenomenon made following at least partial separation difficult, so 0.01 mM concentration was tested.$CHCl_3$/MeOH mobile phases were tested. However, MeOH showed to be too polar for these TLC systems. Even small addition pushed analytes from the start to the front. EtOH was utilized instead, and results were much better regarding mobile phase polarity adjustments. Despite this, no chiral separation was observed.

Polar mobile phases used in HILIC chromatography were also tested. Still, they showed too polar and pushed analytes in the front. When 10 mM chiral selector per[6-

(MTZ-O-MIM2)]-β-CD **95** was tested with HILIC polar mobile phases, analytes were not moving in the front. However, no chiral separation was observed.

Several conclusions can be drawn from these results. Polar mixtures do not cause tailing compared to $CHCl_3$/MeOH mobile phases. Higher concentration of the selector per[6-(MTZ-O-MIM2)]-β-CD **95** increases the polarity of the TLC stationary phase, most probably due to the higher density of OH groups situated on the CD secondary rim.

In the next phase, chiral selector per[2,3-(PA-MTZ-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **119** which contains Pirkle amide was tested. Based on the results obtained during HPLC chiral separation tests, DNS-Phe **104a** and **104b** and DNS-Phe-Me **107a** and **107b**, together with DNS-Trp-Me **108a** and **108b** were tested as chiral analytes. HPTLC plates were tested instead of standard TLCs to increase the chances for chiral separation. Results are depicted in Table 13.

Table 13. TLC chiral separation tests with chiral selector per[2,3-(PA-MTZ-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **119** (NS = no separation)

Selector (Concentration)	per[2,3-(PA-MTZ-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **119** **1 mM in H_2O**	
Analyte (Concentration)	DNS-L-Phe **104a** DNS-D-Phe **104b** **10 mM in MeOH**	
Mobile phase		
$CHCl_3$/EtOH 5/1	NS	
$CHCl_3$/EtOH 1/1	NS	
H_2O/EtOH 1/1	front	
Analyte (Concentration)	DNS-L-Phe-Me **107a** DNS-D-Phe-Me **107b** **1 mM in $CHCl_3$**	DNS-L-Trp-Me **108a** DNS-D-Trp-Me **108b** **0.1 mM in $CHCl_3$**
Mobile phase		
Hexane/*i*-PrOH 3/1	front	
Hexane/*i*-PrOH 10/1		NS
Hexane/*i*-PrOH 15/1	NS	

Hexane/*i*-PrOH 20/1		start
Hexane/*i*-PrOH 30/1	NS	
Hexane/*i*-PrOH 50/1	NS	

In the case of polar analyte DNS-Phe **104a** and **104b**, $CHCl_3$/EtOH mobile phases were tested. For Me ester derivatives DNS-Phe-Me **107a** and **107b** and DNS-L-Trp-Me **108a** and **108b**, hexane/*i*-PrOH mobile phases were utilized to mimic chiral HPLC tests described in the previous chapter. Nevertheless, no chiral separation was observed.

5 CONCLUSION

The work and research described and discussed in this thesis were divided into the following stages:

1. Preparation of neopentyl skeleton substances containing one to three permanent positive charges (anchors) and their connection with fluorescent CDs and compounds without CD.
2. Testing of bond strength synthesized charged compounds with solid supports.
3. Preparing charged CD derivatives, their sorption on solid supports, and testing their chiral and non-chiral separation abilities.

In the first part of this research, a method for large-scale preparation of anchors was developed. This part also included the NMR kinetic study of S_N2 reaction between azides and neopentyl skeleton compounds possessing different types of leaving groups. Based on the results of this study, the TfO^- group was selected as the most suitable leaving group due to its reactivity and, at the same time, surprising stability. In some cases, the final desired compounds were synthesized in the amount exceeding 100 g. Next, fluorescent compounds derived from naphthalimide were synthesized and connected with anchors via CuAAC reaction. The same strategy was used to prepare fluorescent charged CD derivatives.

In the second part, bond strength tests were performed. Fluorescent compounds synthesized in the first part were sorbed onto solid supports like silica gel, sulfonated silica gel, and a strong cation exchanger. It was investigated how firmly these substances hold on these supports in an environment with different pH and ionic strength. Results showed that the bond between sulfonated solid supports and our compounds is strong enough to prevent any leaking of the compound from the support. However, interactions with unmodified silica gel were weaker, and it was apparent that more permanent charges were necessary. So, fluorescent CD derivatives bearing 7, 14, and 21 permanent positive charges on their primary rim were synthesized. These compounds were subjected to the same solid support bond strength tests as previous compounds. The number of charges dramatically increased the bond strength between silica gel and these multiply charged CD derivatives.

In the third and the last part, CD derivatives with 2 permanent positive charges were synthesized and utilized for Nafion® 117 membrane coating. These modified membranes

were tested in chiral separation membrane systems. Preferential sorption and pertraction experiments were performed, and the racemic tryptophan mixture was partially separated. However, the disadvantage of this experiment is the very long separation time (in the order of hundreds of days). Next, multiply charged CD derivatives with 14 charges and modified secondary rim with suitable chiral and non-chiral moieties were synthesized and coated onto silica gel columns. One column was tested as a reverse-phase column, and baseline separation of acetone, benzene, and toluene mixture was achieved. The other two columns possessing CD derivatives with phenyl carbamates substituents or enantiopure Pirkle amides on their secondary rim were tested as chiral columns. Partial separation of racemic dansylated phenylalanine methyl ester was achieved. At last, tests of chiral TLCs prepared by the dipping method were also performed. No chiral separation was observed.

Although chiral separation has not yet been achieved, the applicability of multiply charged CDs for stable electrostatic modification of negatively charged solid supports, including silica gel, has been demonstrated.

6 EXPERIMENTAL SECTION

6.1 General information, instruments, and materials

Organic solvents were distilled before use. Solvents were dried according to procedures described in Advanced Practical Organic Chemistry textbook. Deionized H_2O was utilized for reactions, chromatography purifications, and sorption/desorption tests. Native CDs (α-,β-, and γ-CDs) were purchased from Wacker Chemie (Germany). Other reagents were purchased from common commercial sources (Sigma, Penta Chemicals, Lach-Ner, Fluorochem) and used without further purification. The laboratory glassware was dried at 180°C in an oven before use if the reaction required dry conditions.

Argon or nitrogen were used as an inert gas. Silica gel 60 (0.040–0.063 mm) and basic aluminum oxide Brockmann I were used for column chromatography, both purchased from Merck, Germany or Silicycle, Canada. TLC was performed on aluminium sheets with a layer of silica gel 60 F254, purchased from Merck, Germany. Sulfonated silica gel was prepared by former colleague Veronika Garbárová. Chiral TLC experiments were performed on HPTLC Silica gel 60 F254 from Merck. The solvent ratio in elution mixtures is given as volume/volume. Plates were developed in a saturated chamber; the mobile phases are given at each procedure in volume/volume ratio.

Spots on TLC plates were detected by using several different methods:

- M1 = a UV lamp (λ = 254 nm and λ = 366 nm for naphthalimide derivatives).
- M2 = dipping the TLC plate in a basic $KMnO_4$ aq. solution ($KMnO_4$ (1.5 g), K_2CO_3 (10 g), 10% w/w NaOH aq. solution (1.25 mL), and H_2O (200 mL)), followed by heating to 250°C by a heat gun.
- M3 = dipping the TLC plate in a 1% w/w EtOH solution of NBP, followed by heating of the plate to 250°C by a heat gun and dipping in a conc. NH_3 aq. solution.
- M4 = dipping the TLC plate in a ninhydrin solution (ninhydrin (0.2 g), 10% w/w AcOH aq. solution (5 mL), and BuOH (95 mL)), followed by heating to 250°C by a heat gun.
- M5 = dipping the TLC plate in a 50% w/w H_2SO_4 aq. solution, followed by heating to 250°C by a heat gun.
- M6 = dipping the TLC plate in a CAM mixture ($Ce(SO_4)_2 \cdot 4H_2O$ (0.5 g), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (2.5 g), H_2SO_4 (5 mL), and H_2O (45 mL)), followed by heating to 250°C by a heat gun.

- M7 = putting the TLC plate into a sealed bottle, filled with few I_2 crystals and silica gel, and shaking until brown spots appear

HPLC chiral measurements and reverse phase separations utilizing self made columns were carried out on an Shimadzu LC-20AD HPLC pump and UV-VIS detector Ingos LCD 5000. The temperature was maintained by thermostat Spart Mistral 880. HPLC grade solvents (HiPerSolv CHROMANORM) were used. The chiral analyses of compounds DNB-L-PhGly **112a** and PrgPA **118** were performed with a Shimadzu liquid chromatograph with a spectrophotometric detector (SPD-M20A). Chiral column Daicel Chiralpak IA was used for chiral separation of compounds **112a** and **118**, together with their racemic mixtures. Specific optical rotations were measured with AUTOMATIC polarimetry, Autopol III (Rudolph research, Flandres, New Jersey) at 589 nm (sodium D line) and the values of $[\alpha]^{25}_{D}$ are reported together with the concentration (*c*, g/100 mL) and solvent. Infrared spectroscopy spectra were measured with a Nicolet Avatar 370 FTIR. The method used for measuring was a diffuse reflectance (DRIFT) in KBr or Attenuated Total Reflectance (ATR) with Ge crystal. IR absorptions are given in wavenumbers as cm^{-1}. UV-VIS spectroscopy spectra were measured with Thermo Scientific Helios γ with wolfram and deuterium lamp or Thermo Scientific Evolution 201 with Hg lamp. Wavelength range was 190-800 nm. Low resolution mass spectra were measured with a Shimadzu LCMS-2020. Samples were ionized by electrospray technique (ESI) and analysed by quadrupole. Drying and nebulizer gas was nitrogen. High resolution mass spectra were measured with a Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS Samples were ionized by electrospray technique (ESI) and analysed by quadrupole or TOF. Drying and nebulizer gas was nitrogen. The pH-meter TESTO 206 PH1 was used when solutions with defined pH were prepared.

^{1}H NMR spectra were acquired on Bruker AVANCE III spectrometer at 600 MHz or 400 MHz, ^{13}C NMR spectra at 151 MHz or 101 MHz, with DEPT and 2D NMR measurements (^{1}H, ^{1}H-COSY, HSQC, and HMBC), and ^{19}F NMR spectra at 376 MHz. ^{1}H and ^{13}C NMR spectra of some of the simpler compounds were acquired on Varian VNMRS 300 at 300 MHz and 75 MHz, respectively. Samples were dissolved in $CDCl_3$, DMSO-d_6, CD_3OD or D_2O with a drop of *tert*-butanol. Chemical shifts are given in ppm; coupling constants *J* are given in Hz.

6.2 Kinetic measurements

All reactions were performed in deuterated DMSO, and the concentration of the starting compound was 35.6 mM (0.356 mmol in 10 mL or 0.178 mmol in 5 mL). The molar amount of azide was 3.2 mmol (10 mL of DMSO-d_6) or 1.6 mmol (5 mL of DMSO-d_6). The starting compound was dissolved in DMSO, subsequently adding azide. Then, the mixture was immersed in an oil bath tempered at 100°C, and the time was recorded from that moment. The volume of samples taken from the reaction mixture was 50 µL. Those samples were immediately diluted in 400 µL of deuterated DMSO and measured. The samples of $(TfO)_3Np$ **20** had to be frozen in an ice bath first, then diluted, and immediately measured.

6.3 Thermal and pH stability

Spectra were acquired on Bruker AVANCE III spectrometer at 600 MHz. Samples were subsequently heated to 40°C, 60°C, and 80°C. Samples were left at the given temperatures for 1 hour, and spectra were measured every 10 minutes. The weight of the substances was from 10 to 13 mg, and they were dissolved in 0.5 mL of acidic, neutral, or basic D_2O. Acidic D_2O solution was prepared by adding conc. HCl into D_2O until pH was 1, according to the pH-meter TESTO 206 PH1. Basic D_2O solution was prepared by adding solid NaOH into D_2O until pH was 9-10, according to the pH-meter TESTO 206 PH1.

6.4 Anchor/solid support bond strength

First, calibration measurements of compounds (modifiers) **46**, **49**, **52**, **69**, **91**, **92**, and **93** were done. Initial aqueous 6 mM solutions were prepared. Each solution was diluted to half until 0.7 nM concentration was reached. UV-VIS spectra of all solutions were measured at 450 nm wavelength, and an area of linearity was found. Graphs are available in Supplemental Information (Figure S26 – Figure **S32**).

A modifier (3 µmol) was dissolved in 0.01 M NH_4HCO_3 aq. solution or H_2O (0.5 mL), and solid support (50 mg) was added. The suspension was stirred for 10 minutes. Then it was placed in a glass Pasteur pipette with a piece of cotton at the bottom, washed with 0.01 M NH_4HCO_3 aq. solution or H_2O (5 mL), and subsequently with other solutions and mixtures described in the tables, which can be found in the given chapters. The final volume of mixtures was always 5 mL, but five 1 mL fractions were collected. UV-VIS spectra of all fractions were measured and diluted to reach the linearity area if necessary.

Concentrations were calculated from measured absorbance values and put in tables in given chapters.

6.5 Nafion® membrane coating

Membrane activation

Membranes were activated by one-hour reflux in a 25 % HNO_3 aq. solution. Membranes were then washed 3 times in H_2O and once in a 5 % NH_3 aq. solution (all volumes were just sufficient to submerge the whole membrane).

Membrane coating

mono[6-(MTZ-O-MIM2)]-β-CD **77** membrane.

The compound (0.146 g) was dissolved in H_2O (84 mL); the membrane (4.5 × 8.5 cm) was added to the solution, and the mixture was shaken for 3 days. The membrane was washed 3 times with H_2O. The solution of the compound and washing H_2O solutions were evaporated on a rotary vacuum evaporator at 50°C. The solid was dried at 60°C for 5 h using a vacuum oil pump. The gravimetric analysis showed that 37.1 mg (21.8 μmol) of the compound attached to the membrane, i.e. 0.57 μmol/cm^2.

mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80** membrane.

The procedure was identical to that previously described. The compound (1.070 g) was dissolved in H_2O (146 mL), and the membrane (8.5 × 7.0 cm) was used. The solid was dried at 70°C for 5 h. The amount of the compound attached to the membrane was 0.168 g (91.8 μmol), i.e. 1.54 μmol/cm^2.

mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81** membrane.

The procedure was identical to that previously described. The compound (0.505 g) was dissolved in H_2O (66 mL), and the membrane (4.5 × 8.0 cm) was used. The solid was dried at 70°C for 9 h. The amount of the compound attached to the membrane was 0.128 g (66.7 μmol), i.e. 1.85 μmol/cm^2.

Preferential sorption and pertraction

Experiments were performed in the laboratory of Pavel Izák at Institute of Chemical Process Fundamentals of the Czech Academy of Sciences. More details could be found in published articles[223,224].

6.6 HPLC column coating and separation ability testing

General HPLC column coating procedure

The charged CD derivative was dissolved in the solvent. 1 ml of solution was taken on a TLC standard. The column was pre-washed with the same solvent. The suction hose with the filter was placed in the CD solution. The tube leading from the column was also introduced into the same CD solution. A pump with a flow rate of 1 ml/min was switched on. The course of the coating was monitored by TLC. TLC monitoring involved loading the standard and coating solution (both 1 μl) onto a TLC plate. The plate was not developed, but the substances were directly visualized by one of the methods described in the general part. After a loss of CD derivative in the coating solution was no longer observed, the suction hose with the filter was transferred to pure solvent, and the entire HPLC system and column were washed. During the wash, the tube leading from the column was still placed in the original coating solution. The elution of excess CD derivative was checked by an identical TLC method. The CD derivative solution was evaporated on a rotary evaporator at 40°C. The residue was then lyophilized or dried using an oil rotary pump, and the amount of adsorbed CD derivative was determined gravimetrically.

HPLC column coating by reverse-phase modifier per[2,3-octadecylcarbamoyl-6-(MTZ-O-MIM2)]-β-CD (102)

Per[2,3-octadecylcarbamoyl-6-(MTZ-O-MIM2)]-β-CD **102** (0.258 g, 27.0 μmol) was dissolved in $CHCl_3$ (35 mL) in a tempered bath at a temperature of 40-45°C, in which the solution was immersed throughout the coating. The column Kromasil 100-5-SIL (250 × 4.6 mm) was pre-washed with $CHCl_3$. During coating, the initial pressure was 6 MPa and gradually stabilized at 20 MPa. Spots on TLC plates were detected by the method M5. The coating was stopped after approximately 5.5 hours. The column was washed with $CHCl_3$ and then with MeCN. The residue obtained after evaporation was dissolved in benzene (20 mL) and freeze-dried. The weight of the obtained CD derivative was 0.104 g. Thus, 0.154 g (16.1 μmol) was adsorbed on the column.

HPLC column coating by chiral-phase modifier per[2,3-phenylcarbamoyl-6-(MTZ-O-MIM2)]-β-CD (99)

Per[2,3-phenylcarbamoyl-6-(MTZ-O-MIM2)]-β-CD **99** (0.251 g, 35.6 μmol) was dissolved in acetone (40 mL) at room temperature. The column Kromasil 100-5-SIL (250 × 4.6 mm) was pre-washed with acetone. During coating, the initial pressure was 5 MPa and gradually stabilized at 7 MPa. Spots on TLC plates were detected by the method M1. The coating was stopped after approximately 3 hours. The column was washed with

acetone and then with hexane. The residue obtained after evaporation was dried at 60°C using an oil rotary pump. The weight of the obtained CD derivative was 0.177 g. Thus, 0.074 g (10.5 μmol) was adsorbed on the column.

HPLC column coating by chiral-phase modifier per[2,3-(PA-MTZ-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD (119)

Per[2,3-(PA-MTZ-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **119** (0.204 g, 14.5 μmol) was dissolved in MeCN/H_2O 1/1 (30 mL) at room temperature. The column Kromasil 100-5-SIL (150 × 4 mm) was pre-washed with acetone. During coating, the initial pressure was 5 MPa and gradually stabilized at 10 MPa. Spots on TLC plates were detected by the method M1. The coating was stopped after approximately 24 hours. The column was washed with MeCN and then with hexane. The residue obtained after evaporation was dried at 60°C using an oil rotary pump. The weight of the obtained CD derivative was 0.030 g. Thus, 0.174 g (12.2 μmol) was adsorbed on the column.

Separation ability testing of reverse-phase modifier per[2,3-octadecylcarbamoyl-6-(MTZ-O-MIM2)]-β-CD (102) coated column

The mixture of acetone, benzene, and toluene in MeCN (10 μL/mL) was prepared. The injection was 10 μL, the detection was provided by a UV detector (220 nm), the flow was 1 mL/min, and the temperature was maintained at 25°C. Mobile phases are mentioned and discussed in appropriate chapters in the results and discussion part. All measurements were done in triplicate.

Separation ability testing of chiral-phase modifier per[2,3-phenylcarbamoyl-6-(MTZ-O-MIM2)]-β-CD (99) and per[2,3-(PA-MTZ-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD (119) coated columns

Stock solutions of chiral analytes (3 mg/mL) were prepared. For compounds **106** – **109**, *i*-PrOH was used, for compounds **115** and **116**, heptane/*i*-PrOH 1/1 mixture was used, the compound **117** was insoluble in heptane or *i*-PrOH, and the compound **118** was dissolved in EtOAc. Diluted solutions (300 μg/mL) were prepared in *i*-PrOH. The injection was 10 μL, the detection was provided by a UV detector (210 or 220 nm), the flow was 1 mL/min, and measurements were performed at different temperatures ranging from 10 to 40°C. Mobile phases are mentioned and discussed in appropriate chapters in the results and discussion part. All measurements where partial separation was observed

were done in triplicate. New solutions of these compounds were also prepared and tested again to eliminate misleading results.

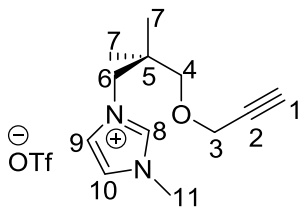
6.7 Chiral thin-layer chromatography

TLC plate (1 × 10 cm) was immersed in the selector's solution for 5 seconds. The plate was then washed with MeOH 3-times and dried by heating to 250°C by a heat gun. TLC plates used for chiral selector per[2,3-(PA-MTZ-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **119** were immersed in the solution of this substance only to a depth of 6 cm in order to be able to detect analytes still. Compound **119** is UV active, and so analytes would not be detectable. By this protocol, analytes can be detected when they reach the uncoated area and would be separated if there was any separation in the coated area. Analytes' solutions (1 μL) were placed on the plate's start, and the whole plate was immersed in a chamber filled with appropriate mobile phase. When the evolution was finished, the plate was dried by heating to 250°C by a heat gun and detected by a UV lamp (254 or 366 nm).

6.8 Synthesis

6.8.1 Anchors

3-(2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propyl)-1-methyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (Prg-O-MIM1 **1).** 2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propyl trifluoromethanesulfonate **26** (2.04 g, 7.44 mmol) was dissolved in 1-methylimidazole (38 mL). The reaction mixture was heated to 60°C and stirred at this temperature for 2 hours. The reaction mixture was monitored by TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by the method M2. 1-Methylimidazole was distilled from the reaction mixture under reduced pressure (1-10 mbar) at 80°C. The crude product was dissolved in H_2O (60 mL) and the solution was washed with toluene (160 mL). The aqueous phase was evaporated on a rotary evaporator at 55°C. The product was dried at 85°C using an oil rotary pump and obtained as a light brown oil in a 72% yield (1.93 g). **IR(DRIFT)**: 3150 ν(C-H alkyne), 3117, 2968, 2875, 2113 ν(C-C alkyne), 1628, 1580, 1482, 1431, 1356, 1254, 1225, 1159, 1099 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 8.98 (s, 1H, H-8), 7.71 (s, 1H, H-10), 7.61 (s, 1H, H-9), 4.18 (d, J = 2.4 Hz, 2H, H-3), 4.06 (s, 2H, H-6), 3.87 (s, 3H, H-11), 3.49 (t, J = 2.4 Hz, 1H, H-1), 3.16 (s, 2H, H-4), 0.90 (s, 6H, H-7) ppm.



^{13}C NMR (101 MHz, DMSO-d_6): δ = 137.32 (C-8), 123.71 (C-9), 123.17 (C-10), 80.04 (C-2), 77.54 (C-1), 74.90 (C-4), 57.94 (C-3), 55.62 (C-6), 35.83 (C-11), 35.68 (C-5), 22.17 (C-7) ppm. **^{19}F NMR** (376 MHz, DMSO-d_6, C_6F_6): δ = -80.05, -164.90 (C_6F_6) ppm. **UV-VIS** (H_2O), λ_{max1}, nm: 209.0, λ_{max2}, nm: 260.0, 1*10^{-5} M. **ESI MS**: for $C_{12}H_{19}N_2O^+$ calcd: *m/z* 207.1, found 207.2 [M^+]. **HRMS**: for $C_{12}H_{19}N_2O^+$ calcd: *m/z* 207.1492, found 207.1502 [M^+], Δ 4.8 ppm.

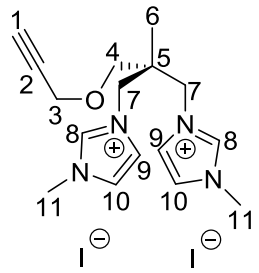
1-(2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propyl)pyridin-1-ium trifluoromethanesulfonate (Prg-O-PYR1 **2).** 2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propyl trifluoromethanesulfonate **26** (0.31 g, 1.13 mmol) was dissolved in dry pyridine (6 mL). The reaction mixture was heated to 60°C and stirred at this temperature for 2 hours. The reaction mixture was monitored by TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by the method M2. Pyridine was distilled from the reaction mixture under reduced pressure (1-10 mbar) at 60°C. The crude product was dissolved in H_2O (20 mL) and the solution was washed with toluene (20 mL). The aqueous phase was evaporated on a rotary evaporator at 50°C. The product was dried at 50°C using an oil rotary pump and obtained as a light brown oil in a 70% yield (0.28 g). **IR(DRIFT)**: 3258, 3141 ν(C-H alkyne), 3091, 2971, 2878, 2116 ν(C-C alkyne), 1634, 1494, 1257, 1228, 1162, 1096, 1033 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 8.90 (m, 2H, H-8), 8.66 (tt, *J* = 7.8, 1.4 Hz, 1H, H-10), 8.16 (dd, *J* = 7.9, 6.5 Hz, 2H, H-9), 4.54 (s, 2H, H-6), 4.16 (d, *J* = 2.4 Hz, 2H, H-3), 3.51 (t, *J* = 2.4 Hz, 1H, H-1), 3.21 (s, 2H, H-4), 0.94 (s, 6H, H-7) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 145.92 (C-10), 145.73 (C-8), 127.67 (C-9), 120.67 (q, *J* = 322.2 Hz, CF_3), 79.85 (C-2), 77.77 (C-1), 74.59 (C-4), 66.93 (C-6), 57.87 (C-3), 36.52 (C-5), 21.98 (C-7) ppm. **^{19}F NMR** (376 MHz, DMSO-d_6, C_6F_6): δ = -80.05, -164.90 (C_6F_6) ppm. **UV-VIS** (H_2O), λ_{max}, nm: 260.0, 1*10^{-5} M. **ESI MS**: for $C_{13}H_{18}NO^+$ calcd: *m/z* 204.1, found 204.2 [M^+]. **HRMS**: for $C_{13}H_{18}NO^+$ calcd: *m/z* 204.1383, found 204.1378 [M^+], Δ 2.4 ppm.

N,N,N,2,2-Pentamethyl-3-(prop-2-yn-1-yloxy)-propan-1-aminium trifluoromethanesulfonate (Prg-O-TMA1 **3).** 2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propyl trifluoromethanesulfonate **26** (0.84 g, 3.06 mmol) was mixed with freshly distilled and dried trimethylamine (20 mL) at -78°C. The reaction vessel was tightly sealed, heated to

60°C, and stirred for 19 hours. The reaction mixture was monitored by TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by the method M2. After cooling to -78°C, the reaction vessel was opened. The reaction mixture was poured into H_2O (60 mL) and the solution was washed with toluene (70 mL). The aqueous phase was separated and evaporated on a rotary evaporator at 60°C. The residue (1.13 g) was dissolved in H_2O (25 ml) and freeze-dried. The product was obtained as a light brown solid in a 74% yield (0.76 g). **IR(DRIFT)**: 3257 ν(C-H alkyne), 3043, 2978, 2929, 2895, 2112 ν(C-C alkyne), 1506, 1485, 1415, 1367, 1261, 1225, 1159, 1090, 1032 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 4.19 (d, J = 2.4 Hz, 2H, H-3), 3.50 (t, J = 2.4 Hz, 1H, H-1), 3.34 (s, 2H, H-4), 3.31 (s, 2H, H-6, solvent overlay), 3.15 (s, 9H, H-8), 1.11 (s, 6H, H-7) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 120.67 (q, J = 322.2 Hz, CF_3), 79.80 (C-2), 77.70 (C-1), 76.17 (C-4), 72.31 (C-6), 57.91 (C-3), 55.14 – 54.11 (C-8), 36.68 (C-5), 24.85 (C-7) ppm. **^{19}F NMR** (376 MHz, DMSO-d_6, C_6F_6): δ = -80.04, -164.90 (C_6F_6) ppm. **ESI MS**: for $C_{11}H_{22}NO^+$ calcd: *m/z* 184.2, found 184.2 [M^+]. **HRMS**: for $C_{11}H_{22}NO^+$ calcd: *m/z* 184.1696, found 184.1693 [M^+], Δ 1.6 ppm.

3,3'-(2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diyl)bis(1-methyl-1*H*-imidazol-3-ium) diiodide/bis(trifluoromethanesulfonate) (Prg-O-MIM2 **4).**

From 3-(3-iodo-2-(iodomethyl)-2-methylpropoxy)prop-1-yne **17**:



3-(3-Iodo-2-(iodomethyl)-2-methylpropoxy)prop-1-yne **17** (1.80 g, 4.76 mmol) was dissolved in 1-methylimidazole (7.6 mL), and the mixture was heated to 100°C and stirred for 18 hours. The reaction mixture was monitored by TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture for the product and toluene/hexane 1/20 mixture for the starting compound. Spots were detected by the method M2. 1-Methylimidazole was distilled from the reaction mixture under reduced pressure at 110°C. The crude product was dissolved in H_2O (70 mL) and the solution was washed with $CHCl_3$ (11 × 70 mL). The aqueous phase was evaporated on a rotary evaporator at 50°C. The product was dried at 60°C using an oil rotary pump. The product was obtained as a light brown oil in a 65% yield (1.70 g). **IR(DRIFT)**: 3458, 3156 ν(C-H alkyne), 3144, 2881, 2110 ν(C-C alkyne), 1616, 1574, 1559, 1455, 1422, 1278, 1171, 1090 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 9.07 (s, 2H, H-8), 7.78 (t, J = 1.8 Hz, 2H, H-10), 7.66 (t, J = 1.8 Hz, 2H, H-9), 4.32 (d, J = 14.1 Hz, 2H, H-7), 4.26 – 4.19 (m, 4H, H-3, H-7), 3.89 (s, 6H, H-11), 3.61 (t, J = 2.3 Hz, 1H, H-1), 3.14 (s, 2H, H-

4), 0.89 (s, 3H, H-6) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 137.70 (C-8), 123.78 (C-9), 123.50 (C-10), 79.53 (C-2), 78.25 (C-1), 70.02 (C-4), 57.96 (C-3), 52.47 (C-7), 39.67 (C-5, solvent overlay),36.04 (C-11), 17.33 (C-6) ppm. **UV-VIS** (H_2O), λ_{max1}, nm: 224.0, $1*10^{-5}$ M. **ESI MS**: for $C_{16}H_{24}N_4O^{2+}$ calcd: *m/z* 144.1, found 144.2 [M^{2+}]. **HRMS**: for $C_{16}H_{24}N_4O^{2+}$ calcd: *m/z* 144.0970, found 144.0978 [M^{2+}], Δ 5.5 ppm.

From 2-methyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diyl bis(trifluoromethanesulfonate) **27**:

2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diyl bis(trifluoromethanesulfonate) **27** (0.21 g, 0.49 mmol) was dissolved in 1-methylimidazole (2 mL), the mixture was heated to 60°C and stirred at this temperature for 1 hour. The reaction mixture was monitored by TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by the method M2. 1-Methylimidazole was distilled from the reaction mixture under reduced pressure (1-10 mbar) at 100°C. Residues of 1-methylimidazole were extracted with EtOAc (2 × 5 mL). The crude product was dissolved in H_2O (5 mL) and the solution was washed with $CHCl_3$ (9 × 5 mL). The aqueous phase was evaporated on a rotary evaporator at 50°C. The product was dried at 70°C using an oil rotary pump and obtained as a light brown oil in a 78% yield (0.23 g). **IR(DRIFT)**: 3557, 3153 ν(C-H alkyne), 3111, 2968, 2869, 2116 ν(C-C alkyne), 1628, 1577, 1562, 1455, 1431, 1260, 1168, 1096 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 9.03 (s, 2H, H-8), 7.76 (t, *J* = 1.8 Hz, 2H, H-10), 7.63 (t, *J* = 1.8 Hz, 2H, H-9), 4.31 (d, *J* = 14.1 Hz, 2H, H-7), 4.22 (d, *J* = 2.4 Hz, 2H, H-3), 4.18 (d, *J* = 14.1 Hz, 2H, H-7), 3.88 (s, 6H, H-11), 3.59 (t, *J* = 2.4 Hz, 1H, H-1), 3.13 (s, 2H, H-4), 0.87 (s, 3H, H-6) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 137.74 (C-8), 123.80 (C-9), 123.52 (C-10), 120.66 (q, *J* = 322.3 Hz, CF_3), 79.53 (C-2), 78.23 (C-1), 69.95 (C-4), 57.93 (C-3), 52.50 (C-7), 39.64 (C-5, solvent overlay), 35.95 (C-11), 17.29 (C-6) ppm. **^{19}F NMR** (376 MHz, DMSO-d_6, C_6F_6): δ = -80.04, -164.90 (C_6F_6) ppm. **UV-VIS** (H_2O), λ_{max1}, nm: 210.0, $1*10^{-5}$ M. **ESI MS**: for $C_{16}H_{24}N_4O^{2+}$ calcd: *m/z* 144.1, found 144.3 [M^{2+}]. **HRMS**: for $C_{16}H_{24}N_4O^{2+}$ calcd: *m/z* 144.0970, found 144.0975 [M^{2+}], Δ 3.4 ppm.

1,1'-(2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl)bis(pyridin-1-ium) bis(trifluoromethanesulfonate) (Prg-O-PYR2 **5).** 2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diyl bis(trifluoromethanesulfonate) **27** (1.9 g, 4.57 mmol) was dissolved in dry pyridine (35 mL), the mixture was warmed to 60°C and stirred for 16 hours. The reaction mixture was monitored by TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by the method M2. Pyridine was distilled from the reaction mixture under reduced pressure (1-10 mbar) at 60°C. The crude product was dissolved in H_2O (50 mL) and the solution was washed with $CHCl_3$ (2 × 50 mL). The aqueous phase was evaporated on a rotary evaporator at 40°C. The product was dried at 60°C using an oil rotary pump and obtained as a brownish oil in a 73% yield (1.9 g). **IR(DRIFT)**: 3261, 3141 ν(C-H alkyne), 3094, 2974, 2105 ν(C-C alkyne), 1637, 1613, 1491, 1267, 1228, 1171, 1096, 1030 cm^{-1}. **^{1}H NMR** (400 MHz, D_2O): δ = 8.83 (m, 4H, H-8), 8.62 (tt, J = 7.9, 1.4 Hz, 2H, H-10), 8.12 (dd, J = 8.0, 6.6 Hz, 4H, H-9), 4.95 (d, J = 13.6 Hz, 2H, H-7), 4.68 (d, J = 13.6 Hz, 2H, H-7), 4.23 (d, J = 2.4 Hz, 2H, H-3), 3.18 (s, 2H, H-4), 2.94 (t, J = 2.4 Hz, 1H, H-1), 1.01 (s, 3H, H-6) ppm. **^{13}C NMR** (101 MHz, D_2O, *t*BuOH): δ = 147.55 (C-10), 146.53 (C-8), 129.11 (C-9), 120.32 (q, J = 317.1 Hz, CF_3), 79.27 (C-2), 77.76 (C-1), 70.48 (*t*BuOH), 67.99 (C-4), 64.65 (C-7), 58.52 (C-3), 42.20 (C-5), 30.29 (*t*BuOH), 17.01 (C-6) ppm. **^{19}F NMR** (376 MHz, DMSO-d_6, C_6F_6): δ = -80.03, -164.90 (C_6F_6) ppm. **UV-VIS** (H_2O), λ_{max1}, nm: 214.0, λ_{max2}, nm: 260.0, $1*10^{-5}$ M. **ESI MS**: for $C_{18}H_{22}N_2O^{2+}$ calcd: *m/z* 141.1, found 141.2 [M^{2+}]. **HRMS**: for $C_{18}H_{22}N_2O^{2+}$ calcd: *m/z* 141.0861 (for [M^{2+}-H^+]$^+$ calcd: *m/z* 281.1648), found 281.1642 [M^{2+}-H^+]$^+$, Δ 2.1 ppm.

N^1,N^1,N^1,N^3,N^3,N^3,2-Heptamethyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diamine diiodide (Prg-O-TMA2 **6**). N^1, N^1, N^3, N^3,2-Pentamethyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diamine **14** (0.38 g, 1.79 mmol) was dissolved in dry THF (10 mL) and MeI (5.08g, 35.8 mmol) was added slowly. The reaction mixture was heated to reflux and stirred for 24 hours. The reaction mixture was monitored by TLC using $CHCl_3$/MeOH/conc. NH_3 aq. solution 90/10/0.5 mixture for compound **14** and MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture for the product. Spots were detected by the method M2. A precipitate formed during stirring.

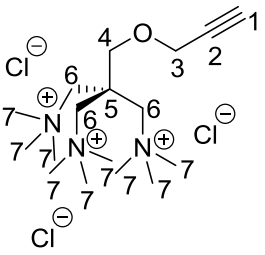
THF was filtered off, and the precipitate was washed with THF (3 × 8 mL). The precipitate was dissolved in H_2O and evaporated on a rotary evaporator at 50°C. The product was dried at 50°C using an oil rotary pump and obtained as a yellowish oil in an 89% yield (0.79 g). **IR(DRIFT)**: 3219 ν(C-H alkyne), 3010, 2971, 2878, 2116 ν(C-C alkyne), 1482, 1416, 1368, 1269, 1099 cm^{-1}. **^{1}H NMR** (400 MHz, D_2O): δ = 4.36 (d, J = 2.4 Hz, 2H, H-3), 3.86 (s, 2H, H-4), 3.84 (d, J = 14.2 Hz, 2H, H-7), 3.63 (d, J = 14.2 Hz, 2H, H-7), 3.34 (s, 18H, H-8), 2.98 (t, J = 2.4 Hz, 1H, H-1), 1.54 (s, 3H, H-6) ppm. **^{13}C NMR** (101 MHz, CD_3OD): δ = 78.97 (C-2), 78.13 (C-1), 73.37 (C-7), 72.78 (C-4), 59.19 (C-3), 56.89 (C-8), 44.98 (C-5), 22.14 (C-6) ppm. **ESI MS**: for $C_{14}H_{30}N_2O^{2+}$ calcd: *m/z* 121.1 (for $[M^{2+}+I^-]^+$ calcd: *m/z* 369.1), found 121.0 $[M^{2+}]$ and 370.0 $[M^{2+}+I^-]^+$. **HRMS**: for $C_{14}H_{30}N_2O^{2+}$ calcd: *m/z* 121.1174 (for $[M^{2+}+I^-]^+$ calcd: *m/z* 369.1397), found 369.1404 $[M^{2+}+I^-]^+$, Δ 1.9 ppm.

3,3'-(2-((1-Methyl-1*H*-imidazol-3-ium-3-yl)methyl)-2-((prop-2-yn-1-yloxy)methyl)propane-1,3(diyl)bis(1-methyl-1*H*-imidazol-3-ium) tris(trifluoromethanesulfonate) (Prg-O-MIM3 **7).** 2-((Prop-2-yn-1-yloxy)methyl)-2-((((trifluoromethyl)sulfonyl)oxy)methyl)propane-1,3-diyl bis(trifluoromethanesulfonate) **31** (2.0 g, 3.51 mmol) was dissolved in 1-methylimidazole (25 mL). The reaction mixture was warmed to 60°C and allowed to stir for 5 hours. The reaction mixture was monitored by TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by the method M2. 1-Methylimidazole was distilled from the reaction mixture under reduced pressure (1-10 mbar) at 80°C. The crude product was dissolved in H_2O (70 mL) and the solution was washed with $CHCl_3$ (4 × 100 mL). The aq. solution was evaporated on a rotary evaporator at 50°C. The residue (4.17 g) was dissolved in H_2O (70 mL) and freeze-dried. The product was further dried at 80°C using an oil rotary pump and obtained as a brownish glassy substance in a 94% yield (2.7 g). **IR(DRIFT)**: 3292 ν(C-H alkyne), 3155, 3122, 3084, 2962, 2914, 2852, 2117 ν(C-C alkyne), 1728, 1579, 1562, 1504, 1452, 1427, 1344, 1282, 1250, 1225, 1095, 1030 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 9.06 (s, 3H, H-7), 7.82 (t, J = 1.7 Hz, 3H, H-9), 7.65 (t, J = 1.8 Hz, 3H, H-8), 4.40 (s, 6H, H-6), 4.07 (d, J = 2.4 Hz, 2H, H-3), 3.90 (s, 9H, H-10), 3.64 (t, J = 2.3 Hz, 1H, H-1), 3.45 (s, 2H, H-4) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 138.15 (C-7), 123.96 (C-8), 123.79 (C-9), 120.66 (q, J = 322.2 Hz, CF_3), 79.02

(C-2), 78.50 (C-1), 68.01 (C-4), 57.97 (C-3), 50.10 (C-6), 42.28 (C-5), 36.09 (C-10) ppm. **^{19}F NMR** (376 MHz, DMSO-d_6, C_6F_6): δ = -80.05, -164.90 (C_6F_6) ppm. **UV-VIS** (H_2O), λ_{max}, nm: 212.5, 1*10^{-5} M. **ESI MS**: for $C_{20}H_{29}N_6O^{3+}$ calcd: *m/z* 123.1 (for $[M^{3+}+OTf^-]^{2+}$ calcd: *m/z* 259.1, for $[M^{3+}+2\times OTf^-]^+$ calcd: *m/z* 667.1), found 259 $[M^{3+}+OTf^-]^{2+}$ and 667 $[M^{3+}+2\times OTf^-]^+$. **HRMS**: for $C_{20}H_{29}N_6O^{3+}$ calcd: *m/z* 123.0795 (for $[M^{3+}-2\times H^+]^+$ calcd: *m/z* 367.2241), found 367.2231 $[M^{3+}-2\times H^+]^+$, Δ 2.7 ppm.

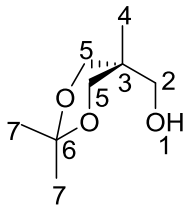
1,1'-(2-((Prop-2-yn-1-yloxy)methyl)-2-(pyridin-1-ium-1-ylmethyl)propane-1,3-diyl)bis(pyridin-1-ium) tris(trifluoromethanesulfonate) (Prg-O-PYR3 **8).** 2-((Prop-2-yn-1-yloxy)methyl)-2-((((trifluoromethyl)sulfonyl)oxy)methyl)propane-1,3-diyl bis(trifluoromethanesulfonate) **31** (2.0 g, 3.51 mmol) was dissolved in dry pyridine (25 mL) and the reaction mixture was heated to 60°C and stirred for 27 hours. The reaction mixture was monitored by TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by the method M2. Pyridine was distilled from the reaction mixture under reduced pressure (1-10 mbar) at 60°C. The crude product was dissolved in H_2O (70 mL) and the solution was washed with $CHCl_3$ (2 × 100 mL). The aq. solution was evaporated on a rotary evaporator at 50°C. The residue (3.3 g) was dissolved in H_2O (50 mL) and freeze-dried. The product was further dried at 50°C using an oil rotary pump and obtained as a brownish glassy substance in a 98% yield (2.8 g). **IR(DRIFT)**: 3259 ν(C-H alkyne), 3140, 3093, 3072, 2987, 2875, 2117 ν(C-C alkyne), 1635, 1583, 1495, 1462, 1259, 1227, 1165, 1093, 1030 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 8.88 (d, *J* = 5.3 Hz, 6H, H-7), 8.79 (t, *J* = 7.8 Hz, 3H, H-9), 8.27 (dd, *J* = 7.8, 6.5 Hz, 6H, H-8), 5.00 (s, 6H, H-6), 4.01 (d, *J* = 2.4 Hz, 2H, H-3), 3.81 (s, 2H, H-4), 3.66 (t, *J* = 2.3 Hz, 1H, H-1) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 147.17 (C-9), 146.44 (C-7), 128.47 (C-8), 120.66 (q, *J* = 322.1 Hz, CF_3), 79.24 (C-1), 78.54 (C-2), 66.83 (C-4), 61.49 (C-6), 57.80 (C-3), 43.50 (C-5) ppm. **^{19}F NMR** (376 MHz, DMSO-d_6, C_6F_6): δ = -80.03, -164.90 (C_6F_6) ppm. **UV-VIS** (H_2O), λ_{max1}, nm: 217.0, λ_{max2}, nm: 260.0, 3*10^{-5} M. **ESI MS**: for $C_{23}H_{26}N_3O^{3+}$ calcd: *m/z* 120.1 (for $[M^{3+}+OTf^-]^{2+}$ calcd: *m/z* 254.6, for $[M^{3+}+2\times OTf^-]^+$ calcd: *m/z* 658.1), found 255 $[M^{3+}+OTf^-]^{2+}$ and 658 $[M^{3+}+2\times OTf^-]^+$. **HRMS**: for $C_{23}H_{26}N_3O^{3+}$ calcd: *m/z* 120.0686 (for $[M^{3+}-2\times H^+]^+$ calcd: *m/z* 358.1914), found 358.1903 $[M^{3+}-2\times H^+]^+$, Δ 4.7 ppm.

N^1,N^1,N^1,N^3,N^3,N^3-Hexamethyl-2-((prop-2-yn-1-yloxy)methyl)-2-((trimethyl-14-azaneyl)methyl)propane-1,3-diamine trichloride (Prg-O-TMA3 **9).** 2-



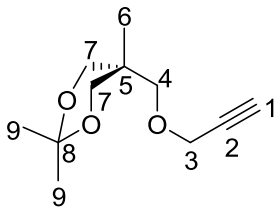
((Dimethylamino)methyl)-N^1, N^1, N^3, N^3-tetramethyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diamine **32** (0.60 g, 2.35 mmol) was dissolved in dry DMF (15 mL) and MeI (4.4 mL, 70.5 mmol) was added dropwise. The mixture was heated to 70°C and allowed to stir for 23 hours. The reaction mixture was monitored by TLC using $CHCl_3$/MeOH/conc. NH_3 aq. solution 90/50/0.5 mixture for the starting compound and MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture for the product. Spots were detected by the method M2. The reaction was not finished, it was cooled down to room temperature, and another portion of MeI (2.2 mL, 35.3 mmol) was added dropwise. The mixture was heated to 70°C and allowed to stir for another 72 hours. DMF was distilled from the reaction mixture under reduced pressure (1-10 mbar) at 70°C. The crude product was then dissolved in H_2O (90 mL) and purified using a weak cation exchanger Amberlite® CG50 (30 g, NH_4^+ form). The elution solutions were successively H_2O, 0,01 M NH_4HCO_3, 0,1 M NH_4HCO_3, and 1 M NH_4HCO_3 aq. solutions. Fractions containing pure product were evaporated on a rotary evaporator at 50°C. The residue was suspended in MeOH (100 mL), filtered, neutralized with 1 M HCl and evaporated on a rotary evaporator at 50°C. The residue (0.75 g) was dissolved in H_2O (20 mL) and freeze-dried. The product was obtained as a slightly yellow glassy substance in a 51% yield (0.49 g). **IR(DRIFT)**: 3363, 3199, 3109, 3014, 2112 ν(C-C alkyne), 1635, 1483, 1410, 1263, 1238, 1103, 1020 cm^{-1}. **^{1}H NMR** (400 MHz, D_2O, *t*BuOH): δ = 4.49 (d, J = 2.4 Hz, 2H, H-3), 4.42 (s, 2H, H-4), 4.12 (s, 6H, H-6), 3.47 (s, 27H, H-7), 3.05 (t, J = 2.4 Hz, 1H, H-1), 1.24 (s, *t*BuOH) ppm. **^{13}C NMR** (101 MHz, D_2O, *t*BuOH): δ = 78.86 (C-1), 77.52 (C-2), 70.40 (*t*BuOH), 70.24 (C-6), 68.65 (C-4), 58.81 (C-3), 57.54 (C-7), 52.67 (C-5), 30.29 (*t*BuOH) ppm. **ESI MS**: for $C_{17}H_{38}N_3O^{3+}$ calcd: *m/z* 100.1 (for $[M^{3+}+Cl^-]^{2+}$ calcd: *m/z* 167.6, for $[M^{3+}+2\times Cl^-]^+$ calcd: *m/z* 370.2), found 168 $[M^{3+}+Cl^-]^{2+}$ and 370 $[M^{3+}+2\times Cl^-]^+$. **HRMS**: for $C_{17}H_{38}N_3O^{3+}$ calcd: *m/z* 100.0999 (for $[M^{3+}+2\times Cl^-]^+$ calcd: *m/z* 370.2386), found 370.2385 $[M^{3+}+2\times Cl^-]^+$, Δ 0.3 ppm.

5-(Hydroxymethyl)-2,2,5-trimethyl-1,3-dioxane (10). Compound **10** was prepared



according to the previously published procedure[331], with some modifications of the purification process. 1,1,1-Tris(hydroxymethyl)ethane (60 g, 0.5 mol) and TsOH (60 mg, 315 μmol) were dissolved in dry acetone (600 mL). The reaction mixture was stirred at room temperature for 2 days. The reaction mixture was monitored by TLC using $CHCl_3$/MeOH 20/1 mixture. Spots were detected by the method M6. The reaction mixture was neutralized with K_2CO_3 (1.5 g, 11 mmol), filtered, and evaporated on a rotary evaporator at 40°C. The product was purified by distillation under reduced pressure (130°C, 1.5 mbar). The product was obtained as colorless oil in an 80% yield (64.5 g). **IR(DRIFT)**: 3518, 3452, 3381, 2995, 2947, 2872, 1658, 1455, 1374, 1263, 1210, 1156 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 3.61 (m, 6H, H-2, H-5), 2.50 (t, J = 5.6 Hz, 1H, H-1), 1.41 (s, 3H, H-7), 1.36 (s, 3H, H-7), 0.80 (s, 3H, H-4) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 98.07 (C-6), 66.38 (C-5), 65.77 (C-2), 34.84 (C-3), 27.38 (C-7), 20.23 (C-7), 17.67 (C-4) ppm. **ESI MS**: for $C_8H_{16}O_3$ calcd: *m/z* 160.1 (for $[M+K]^+$ calcd: *m/z* 199.1), found 198.2 $[M+K]^+$. **HRMS**: for $C_8H_{16}O_3$ calcd: *m/z* 160.1099 (for $[M+H]^+$ calcd: *m/z* 161.1172), found 161.1179 $[M+H]^+$, Δ 4.3 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[332].

2,2,5-Trimethyl-5-((prop-2-yn-1-yloxy)methyl)-1,3-dioxane (11). Compound **11** was

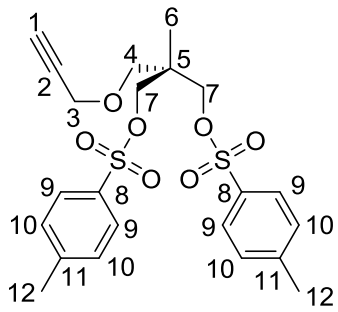


prepared according to the previously published procedure[332]. 5-(Hydroxymethyl)-2,2,5-trimethyl-1,3-dioxane **10** (30.6 g, 191 mmol) was dissolved in dry THF (270 mL) and cooled to 0°C. NaH (11.5 g, 287 mmol, 60% dispersion in oil) was added to the solution over 30 minutes. The suspension was stirred at 0°C for 2 hours. It was then cooled to -78°C, and PrgBr (32 mL, 287 mmol, 80% solution in toluene) was added dropwise over 30 minutes. The reaction mixture was warmed to room temperature and allowed to stir for 14 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 5/1 mixture. Spots were detected by the method M6. The reaction mixture was filtered through celite and concentrated on a rotary evaporator at 40°C. The product was purified by distillation under reduced pressure (115 ° C, 1.5 mbar). The product was obtained as colorless oil in an 84% yield (32.0 g). **IR(DRIFT)**: 3285 ν(C-H alkyne), 2992, 2953, 2860, 2113 (C-C alkyne), 1658, 1449, 1374, 1263, 1210, 1090 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.16 (d, J = 2.4 Hz, 2H, H-3), 3.70 (d, J = 12.0 Hz,

2H, H-7), 3.55 (d, *J* = 12.0 Hz, 2H, H-7), 3.52 (s, 2H, H-4), 2.41 (t, *J* = 2.4 Hz, 1H, H-1), 1.43 (s, 3H, H-9), 1.40 (s, 3H, H-9), 0.88 (s, 3H, H-6) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 98.01 (C-8), 80.06 (C-2), 74.30 (C-1), 73.07 (C-4), 66.63 (C-7), 58.85 (C-3), 34.34 (C-5), 26.58 (C-9), 21.20 (C-9), 18.32 (C-6) ppm. **ESI MS**: for $C_{11}H_{18}O_3$ calcd: *m/z* 198.1 (for $[M+Na]^+$ calcd: *m/z* 221.1), found 221.1 $[M+Na]^+$. **HRMS**: for $C_{11}H_{18}O_3$ calcd: *m/z* 198.1256 (for $[M-C_3H_6+H]^+$ calcd: *m/z* 159.1016), found 159.1023 $[M-C_3H_6+H]^+$, Δ 4.4 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[332].

2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diol (12). Compound **12** was prepared according to the previously published procedure[333], with some modifications of the purification process to avoid column chromatography. 2,2,5-Trimethyl-5-((prop-2-yn-1-yloxy)methyl)-1,3-dioxane **11** (1.0 g, 5.0 mmol) was dissolved in MeOH (6 mL) and a conc. HCl (0.2 mL, 2.5 mmol) was added and the reaction mixture was stirred for 1 hour. The reaction mixture was monitored by TLC using hexane/EtOAc 1/2 mixture. Spots were detected by the method M2. The reaction mixture was neutralized with 50% w/w NaOH aq. solution. The resulting NaCl precipitate was filtered off. The filtrate was evaporated on a rotary evaporator at 40°C. The residue was suspended in $CHCl_3$ (3 mL), and the remaining traces of NaCl were separated from the solution by filtration. The solution was evaporated on a rotary evaporator at 40°C. The product was obtained as a light brown oil in a 99% yield (0.8 g). **IR(DRIFT)**: 3485, 3351, 3094, 2986, 2965, 2938, 2875, 2860, 2122 ν(C-C alkyne), 1718, 1706, 1658, 1622, 1353, 1344, 1248, 1204, 1099, 1048 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.13 (d, *J* = 2.4 Hz, 2H, H-3), 3.64 (d, *J* = 11.0 Hz, 2H, H-7), 3.55 (d, *J* = 11.0 Hz, 2H, H-7), 3.50 (s, 2H, H-4), 2.98 (bs, 2H, H-8), 2.45 (t, *J* = 2.4 Hz, 1H, H-1), 0.82 (s, 3H, H-6) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 79.65 (C-2), 74.87 (C-1), 74.72 (C-4), 67.66 (C-7), 58.86 (C-3), 40.86 (C-5), 17.17 (C-6) ppm. **ESI MS**: for $C_8H_{14}O_3$ calcd: *m/z* 158.1 (for $[M+Na]^+$ calcd: *m/z* 181.1), found 181.0 $[M+Na]^+$. **HRMS**: for $C_8H_{14}O_3$ calcd: *m/z* 158.0943 (for $[M+H]^+$ calcd: *m/z* 159.1016), found 159.1022 $[M+H]^+$, Δ 4.0 ppm. ^{1}H NMR spectrum is in accordance with the literature[333].

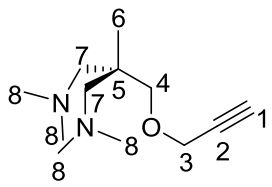
2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diyl bis(4-methylbenzenesulfonate) (13). 2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diol **12** (0.46 g, 2.91 mmol) was dissolved in dry pyridine (15 mL). TsCl (1.67 g, 8.74 mmol) was added, and the reaction mixture was stirred for 17 hours at room temperature. The reaction mixture was monitored by TLC using hexane/EtOAc 1/2 mixture. Spots were detected by the method M6. Pyridine was distilled from the reaction mixture under reduced pressure at 50°C. The residue (3.36 g) was dissolved in the smallest possible amount of $CHCl_3$, silica gel (15 g) was added, and the mixture was evaporated on a rotary evaporator at 40°C. The adsorbed crude product was purified by column chromatography (130 g silica gel) eluting with hexane/EtOAc 3/1. After purification, fractions with product were evaporated on a rotary evaporator at 40°C. The product was dried at room temperature using an oil rotary pump. The final product was obtained as a white solid in a 72% yield (0.99 g). **IR(DRIFT)**: 3279 ν(C-H alkyne), 2989, 2950, 2896, 2869, 1598, 1473, 1359 , 1183, 1102 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.74 (m, 4H, H-9), 7.35 (m, 4H, H-10), 3.95 (d, J = 2.4 Hz, 2H, H-3), 3.83 (s, 4H, H-7), 3.28 (s, 2H, H-4), 2.46 (s, 6H, H-12), 2.40 (t, J = 2.4 Hz, 1H, H-1), 0.92 (s, 3H, H-6) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 145.97 (C-8), 132.38 (C-11), 129.94 (C-10), 127.96 (C-9), 79.05 (C-2), 74.89 (C-1), 71.03 (C-7), 70.44 (C-4), 58.48 (C-3), 39.72 (C-5), 21.69 (C-12), 16.48 (C-6) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 201.0, λ_{max2}, nm: 225.0, λ_{max3}, nm: 267.0, λ_{max4}, nm: 273.0, $1*10^{-5}$ M. **ESI MS**: for $C_{22}H_{26}O_7S_2$ calcd: *m/z* 466.1 (for $[M+Na]^+$ calcd: *m/z* 489.1), found 489.1 $[M+Na]^+$. **HRMS**: for $C_{22}H_{26}O_7S_2$ calcd: *m/z* 466.1120 (for $[M+NH_4]^+$ calcd: *m/z* 484.1458), found 484.1456 $[M+NH_4]^+$, Δ 0.5 ppm.



N^1,N^1,N^3,N^3,2-Pentamethyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diamine (14).

From 3-(3-iodo-2-(iodomethyl)-2-methylpropoxy)prop-1-yne **17**:

3-(3-Iodo-2-(iodomethyl)-2-methylpropoxy)prop-1-yne **17** (1.59 g, 4.21 mmol) and AIBN (69 mg, 0.42 mmol) were mixed with freshly distilled and dried dimethylamine (22 mL) at -78°C. The reaction vessel was tightly sealed, and the mixture was heated to 100°C and allowed to stir for 24 hours. The reaction vessel was cooled to -78°C and opened. The reaction mixture was monitored by TLC using $CHCl_3$/MeOH/conc. NH_3 aq.



solution 90/10/0.5. Spots were detected by the method M2. The reaction mixture was warm up to room temperature and left open for a couple of hours to volatilize the excess of dimethylamine. The reaction mixture was suspended in H_2O (150 mL) and codistilled at 110°C. The distillate (approximately 100 mL) was neutralized with 1 M HCl and washed with $CHCl_3$ (2 × 70 mL). The aqueous phase was evaporated on a rotary evaporator at 50°C. The residue was dissolved in a saturated $NaHCO_3$ aq. solution (50 mL) and the solution was washed with CH_2Cl_2 (3 × 60mL). The organic phase was evaporated on a rotary evaporator at 40°C and dried at room temperature using an oil rotary pump. The product was obtained as colorless oil in a 34% yield (0.305 g).

From 2-methyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diyl bis(trifluoromethanesulfonate) **27**:

2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diyl bis(trifluoromethanesulfonate) **27** (0.30 g, 0.71 mmol) was mixed with freshly distilled and dried dimethylamine (3 mL) at -78°C. The reaction vessel was tightly sealed, and the mixture was heated to 60°C and allowed to stir for 18 hours. The reaction vessel was cooled to -78°C and opened. The reaction mixture was monitored by TLC using $CHCl_3$/MeOH/conc. NH_3 aq. solution 90/10/0.5. Spots were detected by the method M2. The reaction mixture was dissolved in CH_2Cl_2 (10 mL) and the solution was washed with 5% w/w NaOH aq. solution (10 mL). The organic phase was evaporated on a rotary evaporator at room temperature. The residue was suspended in H_2O (20 mL) and codistilled at 110°C. The distillate was washed with CH_2Cl_2 (20 mL). The organic phase was dried with $MgSO_4$ (0.1 g), filtered, and evaporated on a rotary evaporator at room temperature. The product was obtained as colorless oil in a 73% yield (0.113 g). **IR(DRIFT)**: 3303 ν(C-H alkyne), 2968, 2938, 2851, 2815, 2764, 2119 ν(C-C alkyne), 1467, 1452, 1359, 1266, 1093, 1045 cm^{-1}. **^{1}H NMR** (400 MHz, D_2O): $\delta = 4.34$ (d, $J = 2.4$ Hz, 2H, H-3), 3.77 (s, 2H, H-4), 3.51 (d, $J = 14.1$ Hz, 2H, H-7), 3.36 (d, $J = 14.1$ Hz, 2H, H-7), 3.01 (d, $J = 4.1$ Hz, 12H, H-8), 2.99 (t, $J = 2.4$ Hz, 1H, H-1), 1.28 (s, 3H, H-6) ppm. **^{13}C NMR** (101 MHz, D_2O, *t*BuOH): δ = 79.21 (C-2), 77.73 (C-1), 72.26 (C-4), 70.51 (*t*BuOH), 64.88 (C-7), 59.03 (C-3), 47.92 (C-8), 47.52 (C-8), 38.75 (C-5), 30.29 (*t*BuOH), 17.97 (C-6) ppm. The NMR sample was converted to the hydrochloric acid salt to obtain better spectra. **ESI MS**: for $C_{12}H_{24}N_2O$ calcd: *m/z* 213.0 (for $[M+H]^+$ calcd: *m/z* 213.2), found 213 $[M+H]^+$. **HRMS**: for $C_{12}H_{24}N_2O$ calcd: *m/z* 212.1889 (for $[M+H]^+$ calcd: *m/z* 213.1961), found 213.1968 $[M+H]^+$, Δ 3.3 ppm.

3-Azido-2-methyl-2-((prop-2-yn-1-yloxy)methyl)propyl 4-methylbenzenesulfonate (15). 2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diyl bis(4-methylbenzenesulfonate) **13** (0.159 g, 0.341 mmol) was dissolved in DMSO (3 mL) and NaN_3 (75 mg, 1.16 mmol) was added. The reaction mixture was stirred at 80°C for 20 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 4/1 mixture. Spots were detected by methods M1 and M2. The reaction mixture was cooled down to room temperature, poured into toluene (5 mL), and the solution was washed with H_2O (5 mL). The organic phase was washed with 1 M HCl (5 mL) and brine (5 mL), respectively. The organic phase was dried with $MgSO_4$ (0.16 g), filtered, and organic solvents were removed on a rotary evaporator at 50°C. The residue (0.12 g) was dissolved in the smallest possible amount of $CHCl_3$, silica gel (0.6 g) was added, and the mixture was evaporated on a rotary evaporator at 40°C. The adsorbed crude product was purified by column chromatography (3.3 g silica gel) eluting with hexane/EtOAc 4/1. After purification, fractions with product were evaporated on a rotary evaporator at 40°C. The product was dried at room temperature using an oil rotary pump. The final product was obtained as colorless oil in an 18% yield (21.6 mg). **IR(DRIFT)**: 3282 ν(C-H alkyne), 2959, 2920, 2866, 2104 ν(azide), 1595, 1455, 1359, 1186, 1102 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.78 (m, 2H, H-9), 7.36 (m, 2H, H-10), 4.04 (d, J = 2.4 Hz, 2H, H-3), 3.85 (s, 2H, H-7), 3.30 (d, J = 3.4 Hz, 2H, H-13), 3.27 (s, 2H, H-4), 2.45 (s, 3H, H-12), 2.42 (t, J = 2.4 Hz, 1H, H-1), 0.94 (s, 3H, H-6) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 145.08 (C-8), 132.71 (C-11), 130.02 (C-10), 128.11 (C-9), 79.31 (C-2), 74.92 (C-1), 72.11 (C-7), 71.46 (C-13), 58.64 (C-3), 54.70 (C-4), 40.30 (C-5), 21.81 (C-12), 17.56 (C-6) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 224.0, λ_{max2}, nm: 260.0, $1*10^{-5}$ M. **ESI MS**: for $C_{15}H_{19}N_3O_4S$ calcd: *m/z* 337.1 (for $[M+Na]^+$ calcd: *m/z* 360.1), found 359.9 $[M+Na]^+$. **HRMS**: for $C_{15}H_{19}N_3O_4S$ calcd: *m/z* 337.1096 (for $[M+H]^+$ calcd: *m/z* 338.1169), found 338.1162 $[M+H]^+$, Δ 2.0 ppm.

2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propan-1,3-diyl dimethanesulfonate (16). 2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diol **12** (0.338 g, 2.14 mmol) was dissolved in dry CH_2Cl_2 (10 mL). TEA (0.9 mL, 6.42 mmol) was added, and the mixture was cooled to 0°C. MsCl (0.42 mL, 5.35 mmol) was added dropwise, and the mixture was stirred at 0°C for 1 hour. The reaction was monitored by TLC with a hexane/EtOAc

1/1 mixture. Spots were detected by the method M6. The reacting mixture was washed with cold H_2O, cold 1 M HCl, a saturated Na_2CO_3 aq. solution, brine (all 10 mL), and dried with $MgSO_4$ (1.5 g). Organic extracts were evaporated on a rotary evaporator at 40°C. The crude product (0.680 g) was dissolved in the smallest amount of $CHCl_3$ and adsorbed onto silica gel (3.4 g). The adsorbed crude product was purified by column chromatography (21 g silica gel) eluting with hexane/EtOAc 1/1. Fractions with the product were evaporated on a rotary evaporator at 40°C, and the pure product was dried at room temperature using an oil rotary pump. The final product was obtained as a slightly yellow oil in an 84% yield (0.567 g). **IR(DRIFT)**: 3276 ν(C-H alkyne), 3099, 2977, 2944, 2896, 2113 ν(C-C alkyne), 1476, 1347, 1174, 1099 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 4.16 (d, J = 2.4 Hz, 2H, H-3), 4.14 (s, 4H, H-7), 3.45 (s, 2H, H-4), 3.05 (s, 6H, H-8), 2.46 (t, J = 2.4 Hz, 1H, H-1), 1.11 (s, 3H, H-6) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 78.99 (C-2), 75.17 (C-1), 70.63 (C-7, C-4), 58.67 (C-3), 39.80 (C-5), 37.16 (C-8), 16.65 (C-6) ppm. **ESI MS**: for $C_{10}H_{18}O_7S_2$ calcd: *m/z* 314.0 (for $[M+Na]^+$ calcd: *m/z* 337.0), found 337.1 $[M+Na]^+$. **HRMS**: for $C_{10}H_{18}O_7S_2$ calcd: *m/z* 314.0494 (for $[M+NH_4]^+$ calcd: *m/z* 332.0832), found 332.0839 $[M+NH_4]^+$, Δ 2.0 ppm.

3-(3-Iodo-2-(iodomethyl)-2-methylpropoxy)prop-1-yne (17).

A) microwave oven

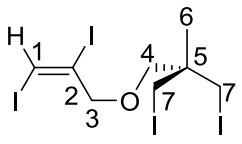
2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diyl dimethanesulfonate **16** (0.139 g, 0.443 mmol) was dissolved in dry toluene (2.6 mL), TBAI (0.65 g, 1.77 mmol) was added, and the mixture was heated to 110°C in a microwave oven for 2 hours (maximum power was 300 W). The reaction mixture was monitored by TLC using toluene/hexane 1/20 mixture for the product, EtOAc/hexane 5/1 mixture for the monoiodo intermediate compound, and EtOAc/hexane 1/1 mixture for the starting compound. Spots were detected by the method M2. After 2 hours, the reaction mixture was cooled to room temperature, diluted with toluene (3 mL), and washed with H_2O (6 mL). The aqueous phase was washed with toluene (5 × 4 mL). Organic extracts were combined and washed with a saturated $Na_2S_2O_3$ aq. solution (15 mL), brine (15 mL), and then dried with $MgSO_4$ (2 g). After filtration, toluene was removed on a rotary evaporator at 40°C. The crude product was dissolved in the smallest possible amount of $CHCl_3$ and adsorbed onto silica gel (1.3 g). The adsorbed crude product was purified by column chromatography (5.3 g silica gel) eluting with hexane/toluene 20/1. Fractions with the product were evaporated on a rotary

evaporator at 40°C, and the pure product was dried at room temperature using an oil rotary pump. The final product was obtained as a slightly yellow oil in an 80% yield (0.134 g).

B) oil bath

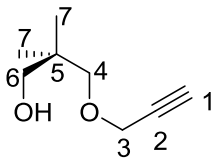
2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diyl dimethanesulfonate **16** (4.3 g, 13.6 mmol) was dissolved in dry toluene (90 mL), TBAI (20.2 g, 54,7 mmol) was added, and the mixture was heated to reflux in an oil bath for 3 days. The reaction mixture was monitored by TLC using toluene/hexane 1/20 mixture for the product, EtOAc/hexane 5/1 mixture for the monoiodo intermediate compound, and EtOAc/hexane 1/1 mixture for the starting compound. Spots were detected by the method M2. The mixture was cooled to room temperature, diluted with toluene (100 mL), and washed with H_2O (200 mL). The aqueous phase was washed with toluene (4 × 100 mL). Organic phases were combined and washed with a saturated $Na_2S_2O_3$ aq. solution (500 mL), brine (500 mL), and then dried with $MgSO_4$ (30 g). After filtration, toluene was removed on a rotary evaporator at 40°C. The crude product (6.7 g) was dissolved in $CHCl_3$ (200 mL) and adsorbed onto silica gel (34 g). The adsorbed crude product was purified by column chromatography (134 g silica gel) eluting with hexane/toluene 20/1. Fractions with the product were evaporated on a rotary evaporator at 40°C, and the pure product was dried at room temperature using an oil rotary pump. The product was obtained as a slightly yellow oil in an 82% yield (4.2 g). **IR(DRIFT)**: 3291 ν(C-H alkyne), 2962, 2926, 2896, 2851, 2116 ν(C-C alkyne), 1473, 1356, 1210, 1177, 1102 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 4.17 (d, J = 2.4 Hz, 2H, H-3), 3.47 (s, 2H, H-4), 3.31 (s, 4H, H-7), 2.45 (t, J = 2.4 Hz, 1H, H-1), 1.18 (s, 3H, H-6) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 79.42 (C-2), 74.80 (C-1), 73.98 (C-4), 58.66 (C-3), 37.39 (C-5), 22.59 (C-7), 15.80 (C-6) ppm. EI MS: for $C_8H_{12}I_2O$ calcd: m/z 377.9, found 378 [MO], 251 [MO-I].

(E)-1,2-Diiodo-3-(3-iodo-2-(iodomethyl)-2-methylpropoxy)prop-1-ene (18). 2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diol **12** (0.50 g, 3.16 mmol) was dissolved in dry toluene (40 mL). I_2 (1.99 g, 7.91 mmol), imidazole (0.54 g, 7.91 mmol), and PPh_3 (2.07 g, 7.91 mmol) were slowly added to the solution. The reaction mixture was heated to reflux and stirred at this temperature for 3 hours. The reaction mixture was monitored by TLC using a toluene/hexane 1/20 mixture. Spots were detected by the method M2. The reaction mixture was cooled to room temperature and washed with a saturated Na_2CO_3 aq. solution, a saturated $Na_2S_2O_3$ aq. solution, and brine (all 40 mL). The organic phase was



dried with $MgSO_4$ (3 g), filtered, and toluene was removed on a rotary evaporator at 40°C. The crude solid (3.91 g) was dissolved in $CHCl_3$ (60 mL) and adsorbed onto silica gel (20 g). The adsorbed crude solid was purified by column chromatography (280 g silica gel) eluting with hexane/toluene 20/1. After purification, fractions with compounds **17** and **18** were evaporated separately on a rotary evaporator at 40°C. Compounds were dried at room temperature using an oil rotary pump. The compound **18** was obtained as a slightly yellow oil in a 10% yield (0.212 g). Compound **17** was obtained in an approximate 42% yield due to impurities; PPh_3 signals were visible in the NMR spectrum. **IR(DRIFT)**: 3058 ν(C-H alkene), 2965, 2944, 2929, 2881, 2854, 1461, 1347, 1222, 1180, 1105 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.14 (t, J = 0.9 Hz, 1H, H-1), 4.20 (d, J = 0.9 Hz, 2H, H-3), 3.40 (s, 2H, H-4), 3.35 (d, J = 3.6 Hz, 4H, H-7), 1.21 (s, 3H, H-6) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 100.45 (C-2), 81.91 (C-1), 77.76 (C-3), 74.07 (C-4), 37.84 (C-5), 22.96 (C-6), 16.13 (C-7) ppm. EI MS: for $C_8H_{12}I_4O$ calcd: *m/z* 631.7, found 632 [MO], 505 [MO-I], 254 [MO-3I], 127 [MO-4I].

2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propan-1-ol (25). 2,2-Dimethylpropane-1,3-diol



(70.0 g, 0.67 mol) was suspended in dry THF (100 mL). The mixture was cooled to 0°C, and NaH (2.7 g, 67.2 mmol, 60% dispersion in mineral oil) was carefully added. The reaction mixture was stirred at 0°C for 1 hour and became homogeneous. PrgBr (7.50 mL, 67.2 mmol, 80% solution in toluene) was added dropwise, and the reaction mixture was stirred for 20 hours at room temperature. The reaction mixture was monitored by TLC using $CHCl_3$/MeOH 30/1 mixture. Spots were detected by the method M2. The reaction mixture was diluted with toluene (250 mL), the solution was washed with H_2O (4 × 250 mL), dried with $MgSO_4$ (2.5 g), filtered, and evaporated on a rotary evaporator at 50°C. The product was dried at room temperature using an oil rotary pump and obtained as a yellowish oil in a 75% yield (7.2 g, calculated with respect to PrgBr). **IR(DRIFT)**: 3433, 3287 ν(C-H alkyne), 2963, 2921, 2866, 2120 ν(C-C alkyne), 1476, 1455, 1434, 1357, 1270, 1099, 1044 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.14 (d, J = 2.4 Hz, 2H, H-3), 3.44 (d, J = 6.1 Hz, 2H, H-6), 3.37 (s, 2H, H-4), 2.43 (t, J = 2.4 Hz, 1H, H-1), 2.20 (t, J = 6.1 Hz, 1H, OH), 0.93 (s, 6H, H-7) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 78.56 (C-2), 78.34 (C-4), 74.46 (C-1), 71.02 (C-6), 58.66 (C-3), 38.16 (C-5), 21.78 (C-7) ppm. **ESI MS**: for $C_8H_{14}O_2$ calcd: *m/z* 142.1 (for $[M+H]^+$ calcd: *m/z* 143.1), found 143.0 $[M+H]^+$.

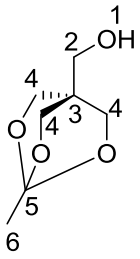
HRMS: for $C_8H_{14}O_2$ calcd: *m/z* 142.0994 (for $[M+Na]^+$ calcd: *m/z* 165.0886), found 165.0884 $[M+Na]^+$, Δ 1.2 ppm.

2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propyl trifluoromethanesulfonate (26). 2,2-Dimethyl-3-(prop-2-yn-1-yloxy)propan-1-ol **25** (0.68 g, 4.83 mmol) was dissolved in dry CH_2Cl_2 (22 mL) and 2,6-lutidine (0.55 mL, 4.83 mmol) was added. The reaction mixture was cooled to -78°C, Tf_2O (0.81 mL, 4.83 mmol) was added dropwise, and the reaction mixture was stirred at this temperature for 2 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 10/1 mixture. Spots were detected by methods M2 and M3. The reaction mixture was diluted with Et_2O (60 mL), and the solution was washed with 1 M HCl (60 mL). The organic phase was then washed with a saturated $NaHCO_3$ aq. solution (60 mL) and brine (60 mL). The organic phase was dried with $MgSO_4$ (0.4 g), filtered, and evaporated on a rotary evaporator at 30°C. The product was dried at room temperature using an oil rotary pump and obtained as a light brown oil in an 85% yield (1.12 g). **IR(DRIFT)**: 3300 ν(C-H alkyne), 2968, 2926, 2854, 2122 ν(C-C alkyne), 1482, 1422, 1248, 1204, 1150, 1102 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.33 (s, 2H, H-6), 4.14 (d, J = 2.4 Hz, 2H, H-3), 3.31 (s, 2H, H-4), 2.43 (t, J = 2.4 Hz, 1H, H-1), 1.02 (s, 6H, H-7) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 118.83 (q, J = 319.7 Hz, C-8), 81.84 (C-6), 79.46 (C-2), 74.77 (C-1), 74.18 (C-4), 58.63 (C-3), 36.15 (C-5), 21.44 (C-7) ppm. EI MS: for $C_9H_{13}F_3O_4S$ calcd: *m/z* 274.0, found 149 [MO-$C_8H_{13}O$], 126 [MO- CF_3O_3S].

2-Methyl-2-((prop-2-yn-1-yloxy) methyl)propane-1,3-diyl bis(trifluoromethanesulfonate) (27). 2-Methyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diol **12** (1.04 g, 6.58 mmol) was dissolved in dry CH_2Cl_2 (30 mL) and 2,6-lutidine (1.53 mL, 13 mmol) was added. The reaction mixture was cooled to -78°C, and Tf_2O (2.2 mL, 13 mmol) was added dropwise to the reaction mixture. The reaction mixture was stirred at this temperature for 1 hour. The reaction mixture was monitored by TLC using hexane/EtOAc 10/1 mixture. Spots were detected by the method M3. The reaction mixture was diluted with Et_2O (60 mL), and the solution was washed with 1 M HCl (40 mL). The organic phase was then washed with a saturated $NaHCO_3$ aq. solution (40 mL) and brine (40 mL). The organic phase was dried with $MgSO_4$ (1 g), filtered, and evaporated on a rotary evaporator at room

temperature. The product was dried at room temperature using an oil rotary pump and obtained as a light brown oil in a 92% yield (2.55 g). **IR(DRIFT)**: 3303 ν(C-H alkyne), 2983, 2911, 2869, 2122 ν(C-C alkyne), 1422, 1248, 1207, 1144, 1108 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.45 (s, 4H, H-7), 4.17 (d, J = 2.4 Hz, 2H, H-3), 3.48 (s, 2H, H-4), 2.48 (t, J = 2.4 Hz, 1H, H-1), 1.17 (s, 3H, H-6) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 118.72 (q, J = 319.7 Hz, C-8), 78.44 (C-2), 76.68 (C-7), 75.76 (C-1), 69.74 (C-4), 58.80 (C-3), 40.63 (C-5), 16.34 (C-6) ppm. **^{19}F NMR** (376 MHz, $CDCl_3$, C_6F_6): δ = -77.33, -164.90 (C_6F_6) ppm. **ESI MS**: for $C_{10}H_{12}F_6O_7S_2$ calcd: *m/z* 422.0 (for $[M+Na]^+$ calcd: *m/z* 445.0), found 445.0 $[M+Na]^+$. **HRMS**: for $C_{10}H_{12}F_6O_7S_2$ calcd: *m/z* 421.9929 (for $[M+Na]^+$ calcd: *m/z* 444.9821), found 444.9816 $[M+Na]^+$, Δ 1.1 ppm.

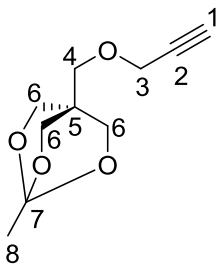
(1-Methyl-2,6,7-trioxabicyclo[2.2.2]octan-4-yl)methanol (28). Compound **28** was



prepared according to the previously published procedure[358]. Pentaerythritol (15 g, 0.11 mol) was suspended in toluene (11 mL). Triethyl orthoacetate (20.2 mL, 0.11 mol) and TsOH (55 mg, 0.28 mmol) were added to the mixture. The reaction mixture was heated to 90°C, and the resulting EtOH was gradually distilled from the reaction mixture by increasing the temperature from 90 to 100°C. After distilling the EtOH, the temperature was raised to 135°C, and toluene was also distilled from the reaction mixture. The gelled residue was transferred into an elongated flask, and the product was sublimed in Kugelrohr (180-190°C, 5 mbar). The product was obtained as a white solid in an 80% yield (14,9 g). **IR(DRIFT)**: 3452, 3351, 2956, 2935, 2887, 1718, 1655, 1365, 1245, 1153, 1036 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 4.02 (s, 6H, H-4), 3.47 (d, J = 4.7 Hz, 2H, H-2), 1.51 (t, J = 4.7 Hz, 1H, H-1), 1.46 (s, 3H, H-6) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 108.65 (C-5), 69.41 (C-4), 61.40 (C-2), 35.71 (C-3), 23.51 (C-6) ppm. **ESI MS**: for $C_7H_{12}O_4$ calcd: *m/z* 160.1 (for $[M+H]^+$ calcd: *m/z* 161.1), found 161 $[M+H]^+$. **HRMS**: for $C_7H_{12}O_4$ calcd: *m/z* 160.0736 (for $[M+H]^+$ calcd: *m/z* 161.0808), found 161.0802 $[M+H]^+$, Δ 3.7 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[358].

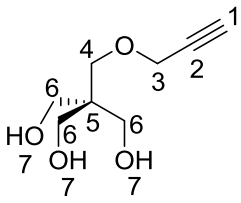
1-Methyl-4-((prop-2-yn-1-yloxy)methyl)-2,6,7-trioxabicyclo[2.2.2]octane (29). Compound **29** was prepared according to the previously published procedure[332] originally described for compound **11**. (1-Methyl-2,6,7-trioxabicyclo[2.2.2]octan-4-yl)methanol **28**

(16.0 g, 0.10 mol) was dissolved in dry THF (150 mL), and the solution was cooled to 0°C. NaH (6.0 g, 0.15 mol, 60% dispersion in mineral oil) was carefully added, and the



mixture was stirred at 0°C for 2 hours. PrgBr (16.7 mL, 0.15 mol, 80% solution in toluene) was slowly added dropwise, and the mixture was stirred at room temperature for 20 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 5/1 mixture. Spots were detected by the method M2. The mixture was filtered through celite, and the filtrate was evaporated on a rotary evaporator at 50°C. The residue was transferred into an elongated flask, and the product was sublimed in Kugelrohr (170-180°C, 5 mbar). The product was obtained as a white solid in an 87% yield (17.3 g). **IR(DRIFT)**: 3261 ν(C-H alkyne), 3007, 2932, 2881 , 2851, 2122 ν(C-C alkyne), 1476, 1410, 1356, 1299, 1269, 1132, 1102, 1048 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 4.09 (s, 2H, H-3), 4.00 (s, 2H, H-6), 3.29 (s, 2H, H-4), 2.44 (s, 1H, H-1), 1.45 (s, 3H, H-8) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 108.70 (C-7), 78.96 (C-2), 75.34 (C-1), 69.54 (C-6), 68.11 (C-4), 58.87 (C-3), 34.84 (C-5), 23.56 (C-8) ppm. **ESI MS**: for $C_{10}H_{14}O_4$ calcd: *m/z* 198.1 (for $[M+H]^+$ calcd: *m/z* 199.1), found 199.0 $[M+H]^+$. **HRMS**: for $C_{10}H_{14}O_4$ calcd: *m/z* 198.0892 (for $[M+H]^+$ calcd: *m/z* 199.0965), found 199.0959 $[M+H]^+$, Δ 3.0 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[390].

2-(Hydroxymethyl)-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diol (30). Compound

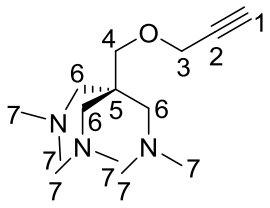


30 was prepared according to the previously published procedure[359], with some modifications of the purification process. 1-Methyl-4-((prop-2-yn-1-yloxy)methyl)-2,6,7-trioxabicyclo[2.2.2]octane **29** (17.3 g, 87.3 mmol) was dissolved in MeOH (350 mL) and conc. HCl (7.2 mL, 87.3 mmol) was added. The mixture was heated to reflux and stirred for 6 hours. The reaction mixture was monitored by TLC using $CHCl_3$/MeOH 15/1 mixture. Spots were detected by the method M2. The reaction mixture was cooled to room temperature, neutralized with 5% w/w NaOH aq. solution, and evaporated on a rotary evaporator at 50°C. The residue (31.9 g) was dissolved in H_2O (500 mL), and the solution was washed with $CHCl_3$ (3 × 500 mL). The aqueous phase was evaporated on a rotary evaporator at 50°C, and the residue (18.0 g) was suspended in acetone (200 mL). The mixture was filtered, and the filtrate evaporated on a rotary evaporator at 40°C. The product was dried at 80°C using an oil rotary pump and obtained as a light brown viscous

oil in an 82% yield (12.5 g). **IR(DRIFT)**: 3357, 2935, 2881, 2116 ν(C-C alkyne), 1721, 1649, 1365, 1245, 1093, 1042 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.15 (d, J = 2.4 Hz, 2H, H-3), 3.72 (s, 6H, H-6), 3.57 (s, 2H, H-4), 2.47 (t, J = 2.4 Hz, 1H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 79.41 (C-2), 75.16 (C-1), 71.59 (C-4), 64.57 (C-6), 59.04 (C-3), 45.15 (C-5) ppm. **ESI MS**: for $C_8H_{14}O_4$ calcd: *m/z* 174.1 (for $[M+H]^+$ calcd: *m/z* 175.1), found 175.0 $[M+H]^+$. **HRMS**: for $C_8H_{14}O_4$ calcd: *m/z* 174.0892 (for $[M+Na]^+$ calcd: *m/z* 197.0784), found 197.0778 $[M+Na]^+$, Δ 3.0 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[390].

2-((Prop-2-yn-1-yloxy)methyl)-2-((((trifluoromethyl)sulfonyl)oxy)methyl)propane-1,3-diyl bis(trifluoromethanesulfonate) (31). 2-(Hydroxymethyl)-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diol **30** (4.0 g, 23.0 mmol) was dissolved in dry CH_2Cl_2 (120 mL) and 2,6-lutidine (8.0 mL, 68.9 mmol) was added. The reaction mixture was cooled to -78°C, and Tf_2O (11.6 mL, 68.9 mmol) was added dropwise to the reaction mixture. The reaction mixture was stirred at this temperature for 2 hours. The reaction mixture was monitored by TLC using $CHCl_3$/MeOH 15/1 mixture for the starting triol and hexane/EtOAc 10/1 for the product. The starting compound was detected by the method M2. The product was detected by the method M3. The reaction mixture was diluted with Et_2O (240 mL), and the solution was washed with 1 M HCl (240 mL). The organic phase was washed with a saturated $NaHCO_3$ aq. solution (240 mL), brine (240 mL), dried with $MgSO_4$ (4 g), filtered, and evaporated on a rotary evaporator at 30°C. The product was dried at room temperature using an oil rotary pump and obtained as a light brown solid in a 96% yield (12.7 g). **IR(DRIFT)**: 3303 ν(C-H alkyne), 2980, 2905, 2122 ν(C-C alkyne), 1428, 1407, 1245, 1210, 1147, 1108 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.57 (s, 6H, H-6), 4.22 (d, J = 2.4 Hz, 2H, H-3), 3.64 (s, 2H, H-4), 2.54 (t, J = 2.4 Hz, 1H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 118.65 (q, J = 319.9 Hz, C-7), 77.16 (C-2), 76.75 (C-1), 71.43 (C-6), 64.61 (C-4), 59.05 (C-3), 44.84 (C-5) ppm. **^{19}F NMR** (376 MHz, $CDCl_3$, C_6F_6): δ = -76.95, -164.90 (C_6F_6) ppm. **ESI MS**: for $C_{11}H_{11}F_9O_{10}S_3$ calcd: *m/z* 569.9 (for $[M+NH_4]^+$ calcd: *m/z* 588.0), found 587.9 $[M+NH_4]^+$. **HRMS**: for $C_{11}H_{11}F_9O_{10}S_3$ calcd: *m/z* 569.9371 (for $[M+Na]^+$ calcd: *m/z* 592.9), found 592.9265 $[M+Na]^+$, Δ 0.3 ppm.

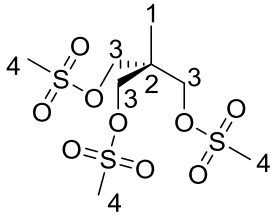
2-((Dimethylamino)methyl) -N^1,N^1,N^3,N^3-tetramethyl-2-((prop-2-yn-1-yloxy)methyl)propane-1,3-diamine (32). 2-((Prop-2-yn-1-yloxy)methyl)-2-((((trifluoromethyl)sulfonyl)oxy)methyl)propane-1,3-diyl bis(trifluoromethanesulfonate)



31 (0.36 g, 0.62 mmol) was mixed with freshly distilled and dried dimethylamine (3.6 mL) at -78°C. The reaction vessel was tightly sealed, and the mixture was heated to 60°C and stirred for 24 hours. The reaction vessel was cooled to -78°C and opened. The reaction mixture was monitored by TLC using $CHCl_3$/MeOH/conc. NH_3 aq. solution 90/10/0.5 mixture. Spots were detected by the method M2. The reaction mixture was poured into CH_2Cl_2 (12 mL), and the solution was washed with a 5% w/w NaOH aq. solution (12 mL). The organic phase was evaporated on a rotary evaporator at room temperature. The residue (0.18 g) was suspended in H_2O (30 mL) and codistilled at 120°C. The distillate was washed with $CHCl_3$ (30 mL). The organic phase was dried with $MgSO_4$ (0.7 g), filtered, and evaporated on a rotary evaporator at room temperature. The product was dried at room temperature using an oil rotary pump and obtained as colorless oil in an 81% yield (0.13 g). **IR(DRIFT)**: 3309 ν(C-H alkyne), 2971, 2941, 2860, 2815, 2768, 2107 ν(C-C alkyne), 1458, 1263, 1096, 1036 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.10 (d, J = 2.4 Hz, 2H, H-3), 3.46 (s, 2H, H-4), 2.38 (d, J = 2.4 Hz, 1H, H-1), 2.37 (s, 6H, H-6), 2.27 (s, 18H, H-7) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 80.12 (C-2), 74.32 (C-1), 72.55 (C-4), 61.86 (C-6), 58.42 (C-3), 49.11 (C-7), 46.98 (C-5) ppm. **ESI MS**: for $C_{14}H_{29}N_3O$ calcd: *m/z* 255.2 (for $[M+H]^+$ calcd: *m/z* 256.2), found 256.3 $[M+H]^+$. **HRMS**: for $C_{14}H_{29}N_3O$ calcd: *m/z* 255.2311 (for $[M+H]^+$ calcd: *m/z* 256.2383), found 256.2389 $[M+H]^+$, Δ 2.3 ppm.

6.8.2 Kinetic measurements

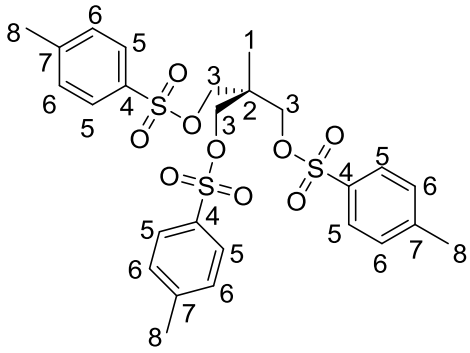
2-Methyl-2-(((methylsulfonyl)oxy)methyl)propane-1,3-diyl dimethanesulfonate (($(MsO)_3Np$ **19).** Compound $(MsO)_3Np$ **19** was prepared according to the previously published procedure[341]. 1,1,1-Tris(hydroxymethyl)ethane (1.0 g, 8.3 mmol) was suspended in CH_2Cl_2 (30 mL), TEA (5.8 mL, 42 mmol) was added, and the mixture was cooled to 0°C. For 5 minutes, MsCl (2.6 mL, 33 mmol) was added dropwise, and the mixture was stirred at 0°C. After 1.5 hours, reaction completion was confirmed by NMR. The mixture was extracted between CH_2Cl_2 and 1 M HCl (both 50 mL). The organic phase was dried with $MgSO_4$ (0.5 g). The crude product (1.97 g)



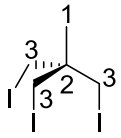
was purified by column chromatography (25 g silica gel) eluting with hexane/EtOAc 1/3. Fractions with the product were collected and evaporated on a rotary evaporator at room temperature and then dried at room temperature using an oil rotary pump. The product was obtained as colorless oil in a 34% yield (1.0 g). **IR(DRIFT)**: 3022, 2944, 1470, 1413, 1356, 1341, 1329, 1171, 1006 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.15 (s, 6H, H-3), 3.07 (s, 9H, H-4), 1.16 (s, 3H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 69.47 (C-3), 39.70 (C-2), 37.50 (C-4), 16.39 (C-1) ppm. **ESI MS**: for $C_8H_{18}O_9S_3$ calcd: *m/z* 354.0 (for $[M+Na]^+$ calcd: *m/z* 377.0), found 377.0 $[M+Na]^+$. **HRMS**: for $C_8H_{18}O_9S_3$ calcd: *m/z* 354.0113 (for $[M+NH_4]^+$ calcd: *m/z* 372.0451), found 372.0466 $[M+NH_4]^+$, Δ 4.0 ppm. ^{1}H spectrum is in accordance with the literature[391].

2-Methyl-2-((((trifluoromethyl)sulfonyl)oxy)methyl)propane-1,3-diyl bis(trifluoromethanesulfonate) ($(TfO)_3Np$ **20).** 1,1,1-Tris(hydroxymethyl)ethane (0.3 g, 2.5 mmol) was suspended in CH_2Cl_2/acetone 1/1 mixture (60 mL), 2,6-lutidine (1.0 mL, 8.7 mmol) was added, and the mixture was cooled to -78°C. Tf_2O (1.5 mL, 8.7 mmol) was added dropwise. After 30 minutes of stirring at this temperature, the mixture became homogeneous. The mixture was stirred for another 2 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 10/1 mixture. Spots were detected by the method M3. The mixture was washed with 1 M HCl (2 × 80 mL), a saturated $NaHCO_3$ aq. solution (80 mL), and brine (80 mL). The organic phase was dried with $MgSO_4$ (4.0 g). After filtration, the filtrate was evaporated at room temperature on a rotary evaporator and dried at room temperature using an oil rotary pump. The product (1.21 g, 93% yield) was obtained in the orange oil form in sufficient purity according to ^{1}H NMR. However, for kinetic experiments, the product was further purified by column chromatography (24 g silica gel) eluting with hexane/EtOAc 10/1. Fractions with the product were collected and evaporated on a rotary evaporator at room temperature. The product was then dried at room temperature using an oil rotary pump and obtained as an orange oil in a 76% yield (0.99 g). **IR(DRIFT)**: 2983, 1419, 1251, 1216, 1144, 952 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 4.45 (s, 6H, H-3), 1.28 (s, 3H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 118.67 (q, J = 319.7 Hz, C-4), 74.38 (C-3), 40.71 (C-2), 15.81 (C-1) ppm. **^{19}F NMR** (376 MHz, $CDCl_3$, C_6F_6): δ = -76.99, -164.90 (C_6F_6) ppm. **ESI MS**: for $C_8H_9F_9O_9S_3$ calcd: *m/z* 515.9 (for $[M+Na]^+$ calcd: *m/z* 538.9), found 539.0 $[M+Na]^+$.

2-Methyl-2-((tosyloxy)methyl)propane-1,3-diyl bis(4-methylbenzenesulfonate) (($(TsO)_3Np$ **21).** Compound $(TsO)_3Np$ **21** was prepared according to the previously published procedure[348]. 1,1,1-Tris(hydroxymethyl)ethane (10 g, 83.2 mmol) was dissolved in pyridine (40 mL). For 1 hour, TsCl (58 g, 300 mmol) in pyridine (180 mL) was added dropwise, and the mixture was stirred at room temperature for 12 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 1/1 mixture. Spots were detected by the method M2. The reaction mixture was poured into a mixture of H_2O (80 mL), MeOH (160 mL), and conc. HCl (64 mL). The resulting precipitate was collected by filtration and washed with H_2O and MeOH. The crude product was dried at 55°C using an oil rotary pump and then recrystallized from acetone (45 mL). The product was collected by filtration and dried at room temperature using an oil rotary pump. The product was obtained as a white crystalline powder in a 70% yield (33.8 g). **IR(DRIFT)**: 3064, 3052, 3004, 2962, 2926, 1924, 1598, 1476, 1356, 1293, 1180, 1099 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 7.69 (m, 6H, H-5), 7.35 (m, 6H, H-6), 3.75 (s, 6H, H-3), 2.46 (s, 9H, H-8), 0.88 (s, 3H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 145.50 (C-4), 131.99 (C-7), 130.20 (C-6), 128.02 (C-5), 69.86 (C-3), 39.53 (C-2), 21.81 (C-8), 16.21 (C-1) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 201.5, λ_{max2}, nm: 225.5, λ_{max3}, nm: 262.0, λ_{max4}, nm: 273.0, $1*10^{-5}$ M. **ESI MS**: for $C_{26}H_{30}O_9S_3$ calcd: *m/z* 582.1 (for $[M+Na]^+$ calcd: *m/z* 605.1), found 605.1 $[M+Na]^+$. **HRMS**: for $C_{26}H_{30}O_9S_3$ calcd: *m/z* 582.1052 (for $[M+Na]^+$ calcd: *m/z* 605.0944), found 605.0970 $[M+Na]^+$, Δ 4.3 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[392].

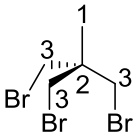


1,3-Diiodo-2-(iodomethyl)-2-methylpropane (I_3Np **22).** $(TsO)_3Np$ **21** (1.5 g, 2.6 mmol) and TBAI (5.7 g, 15 mmol) were dissolved in toluene (45 mL), heated to reflux, and the mixture was stirred for 3 days. The reaction mixture was monitored by TLC using hexane/EtOAc 1/1 mixture for the starting compound and hexane for the product. Spots were detected by the method M2. The mixture was cooled to room temperature and filtered. The mixture was extracted between toluene and H_2O (both 60 mL). The organic phase was washed with a saturated $Na_2S_2O_3$ aq. solution (60 mL), dried with $MgSO_4$ (1 g), filtered, and evaporated at 40°C on a rotary evaporator. The crude product (1.53 g) was purified by column chromatography (15 g silica gel)

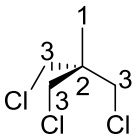


eluting with hexane. Fractions with the product were collected and evaporated on a rotary evaporator at room temperature and then dried at room temperature using an oil rotary pump. The product was obtained as colorless oil in an 88% yield (1.02 g). **IR(DRIFT)**: 2965, 2941, 2926, 2878, 1455, 1413, 1377, 1237, 1204, 1174, 1156 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 3.37 (s, 6H, H-3), 1.37 (s, 3H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 35.71 (C-2), 24.37 (C-1), 16.11 (C-3) ppm. ^{1}H spectrum is in accordance with the literature[393].

1,3-Dibromo-2-(bromomethyl)-2-methylpropane (Br_3Np **23).** $(TsO)_3Np$ **21** (2.0 g, 3.4 mmol) and TBABr (6.6 g, 21 mmol) were dissolved in toluene (60 mL), heated to reflux, and the mixture was stirred for 3 days. NMR confirmed reaction completion. The mixture was cooled to room temperature and extracted between toluene (30 mL) and H_2O (60 mL). The organic phase was washed with H_2O (2 × 60 mL), 1 M HCl (60 mL), and brine (60 mL) and dried with $MgSO_4$ (0.6 g). The organic phase was filtered and evaporated at 40°C on a rotary evaporator and then dried at room temperature using an oil rotary pump. The product was obtained as colorless oil in a 92% yield (0.97 g). **IR(DRIFT)**: 2971, 2956, 2935, 2866, 1458, 1428, 1374, 1269, 1242, 1210, 1189 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 3.50 (s, 6H, H-3), 1.29 (s, 3H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 39.69 (C-2), 39.09 (C-3), 21.70 (C-1) ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[394].



1,3-Dichloro-2-(chloromethyl)-2-methylpropane (Cl_3Np **24).** Compound Cl_3Np **24** was prepared according to the previously published procedure[349], with some modifications of the purification process to avoid column chromatography. 1,1,1-Tris(hydroxymethyl)ethane (5 g, 42 mmol) was dissolved in pyridine (10 mL). This solution and $SOCl_2$ (9.7 mL, 133 mmol) were simultaneously added dropwise to pyridine (17 mL) cooled to 0°C. After addition, the mixture was warmed to room temperature and then to 50°C. After 1 hour, the mixture was warmed to 115°C and kept under stirring for 4 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 1/3 mixture for the starting compound. Spots were detected by the method M2. The reaction mixture was extracted between Et_2O and 1 M HCl (both 70 mL). The organic phase was diluted with another 50 mL of Et_2O and subsequently extracted with brine (70 mL). The organic phase was dried with $MgSO_4$ (0.5 g), filtered, and evaporated at room temperature on a rotary evaporator. The product was dried at room temperature using an oil rotary pump. The product was obtained as colorless oil in an 81% yield (5.85



g). **IR(DRIFT)**: 2968, 2923, 2845, 1559, 1410, 1365, 1174 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 3.58 (s, 6H, H-3), 1.20 (s, 3H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 48.46 (C-3), 42.13 (C-2), 19.48 (C-1) ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[395].

Preparation of CsN_3

NaN_3 (2.60 g, 0.040 mol) was dissolved in H_2O (50 mL) and poured into a strong cation exchanger column DOWEX® 50W-X8 (60 mL, H^+ form). The column was washed with H_2O until the solution was weakly acidic. Cs_2CO_3 (5.2 g, 0.016 mol) was dissolved in H_2O (100 mL), and the aqueous solution of HN_3 was slowly poured into this solution. The solution was evaporated on a rotary evaporator at 50°C. The product was dried at 70°C using an oil rotary pump. The product was obtained as a white solid in a 97% yield (5.48 g).

Preparation of Me_4NN_3

NaN_3 (3.12 g, 0.048 mol) was dissolved in H_2O (50 mL) and poured into a strong cation exchanger column DOWEX® 50W-X8 (60 mL, H^+ form). The column was washed with H_2O until the solution was weakly acidic. Me_4NBr (5.0 g, 0.032 mol) was dissolved in H_2O (50 mL) and poured into a strong anion exchanger column DOWEX® 1-8 (60 mL, OH^- form). The column was washed with H_2O until the solution was basic. Then, the aqueous solution of HN_3 was slowly poured into this solution. The solution was evaporated on a rotary evaporator at 50°C. The product was dried at 70°C using an oil rotary pump. The product was obtained as a white solid in a 98% yield (3.67 g).

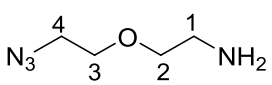
6.8.3 Linkers

General procedure for preparation of azido amino oligo(ethylene glycols) (GP1).

Compounds NH_2-DEG-N_3 **33**, NH-TrEG-N **34**, and **35** were prepared according to the previously published procedure, involving tosylation[360], azidation[360], and monoreduction[361], with some modifications of the process to avoid chromatographic column. Glycol (50.0 g, 0.47 mol for **DEG**, 0.33 mol for **TrEG**, 0.26 mol for **TEG**) was dissolved in CH_2Cl_2 (430 mL) and TsCl (2.0 eq.) was added. The solution was cooled to 0°C and crushed KOH (8.0 eq.) was slowly added. The suspension was further stirred at 0°C for 3 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 1/1 mixture. Spots were detected by methods M1 and M2. The mixture was warmed to room temperature and was diluted with $CHCl_3$ (400 mL). The mixture was washed with H_2O

(3 × 400 mL) and the organic phase was dried with $MgSO_4$ (25 g). The desiccant was filtered off and the filtrate was evaporated on a rotary evaporator at 40°C. The product was dried at room temperature using an oil rotary pump. Glycol ditosylate was dissolved in DMF (680 mL) and NaN_3 (4.0 eq., calculated to starting glycols) was added. The suspension was stirred at 80°C for 24 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 1/1 mixture. Spots were detected by the method M2. The suspension was cooled to room temperature and was diluted with H_2O (670 mL). The solution was washed with toluene (2 × 1400 mL). The organic phase was then washed with H_2O (4 × 1400 mL). It was verified by ^1H NMR that the organic phase was free of DMF residues. The organic phase was dried with $MgSO_4$ (50 g), the desiccant was filtered off and the filtrate was evaporated on a rotary evaporator at 40°C. The residue was dried at room temperature using an oil rotary pump. **Hazardous diethylene glycol derivative** reaction mixture was quenched differently. The suspension was cooled to room temperature and was diluted with H_2O (750 mL). The solution was washed with Et_2O (1600 mL). The organic phase was then washed with H_2O (3 × 1600 mL). It was verified by ^1H NMR that the organic phase was free of DMF residues. The organic phase was then concentrated to a volume of approximately 800 mL on a rotary evaporator at room temperature. Glycol diazide derivative was dissolved in Et_2O (800 mL). To the solution was added 1 M HCl (800 mL), and the biphasic mixture was stirred vigorously. PPh_3 (1.1 eq., calculated to starting glycols) was then added in small portions and the mixture was stirred for 15 hours at room temperature. The reaction mixture was monitored by TLC using hexane/EtOAc 1/1 mixture for the starting diazido compound, CH_2Cl_2/MeOH/conc. NH_3 aq. solution 3/3/1 mixture for the product. Spots were detected by methods M2 and M4. The precipitated PPh_3 oxide was filtered off and washed with H_2O. The organic phase was separated, and the aqueous solution was subsequently washed with Et_2O (3 × 500 mL). The aqueous solution was cooled to 0°C and KOH (300 g) was added slowly. The basic aqueous solution was then washed with CH_2Cl_2 (6 × 600 mL). The organic phase was dried with $MgSO_4$ (18 g), the desiccant was filtered off and the filtrate was evaporated at 30°C on a rotary evaporator. The product was dried at room temperature using an oil rotary pump.

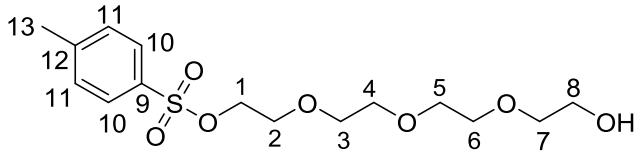
2-(2-Azidoethoxy)ethan-1-amine (NH_2-DEG-N_3 **33).** Compound **33** was prepared according to the general procedure (**GP1**). The product was obtained as a yellowish oil in a 64% yield (39 g). **IR(DRIFT)**:



3357, 2860, 2101 ν(azide), 1595, 1440, 1344, 1269, 1120 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 3.65 (t, *J* = 5.2 Hz, 2H, H-3), 3.52 (t, *J* = 5.1 Hz, 2H, H-2), 3.39 (t, *J* = 5.1 Hz, 2H, H-4), 2.88 (t, *J* = 5.1 Hz, 2H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 73.15 (C-2), 70.00 (C-3), 50.80 (C-4), 41.73 (C-1) ppm. **ESI MS**: for $C_4H_{10}N_4O$ calcd: *m/z* 130.1 (for $[M+H]^+$ calcd: *m/z* 131.1), found 131.2 $[M+H]^+$. **HRMS**: for $C_4H_{10}N_4O$ calcd: *m/z* 130.0855 (for $[M+H]^+$ calcd: *m/z* 131.0927), found 131.0933 $[M+H]^+$, Δ 4.6 ppm. ^{1}H NMR spectrum is in accordance with the literature[396].

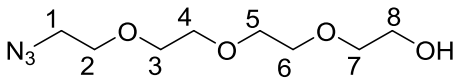
2-(2-(2-Azidoethoxy)ethoxy)ethan-1-amine (NH-TrEG-N **34).** Compound **34** was prepared according to the general procedure (**GP1**). The product was obtained as a yellowish oil in an 83% yield (48 g). **IR(DRIFT)**: 3369, 2908, 2881, 2104 ν(azide), 1598, 1440, 1344, 1266, 1120 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 3.70-3.62 (m, 6H, H-3, H-4, H-5), 3.52 (t, *J* = 5.2 Hz, 2H, H-2), 3.39 (t, *J* = 5.1 Hz, 2H, H-6), 2.87 (t, *J* = 5.2 Hz, 2H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 72.98 (C-2), 70.68 – 70.08 (C-3, C-4, C-5), 50.71 (C-6), 41.60 (C-1) ppm. **ESI MS**: for $C_6H_{14}N_4O_2$ calcd: *m/z* 174.1 (for $[M+H]^+$ calcd: *m/z* 175.1), found 175.2 $[M+H]^+$. **HRMS**: for $C_6H_{14}N_4O_2$ calcd: *m/z* 174.1117 (for $[M+H]^+$ calcd: *m/z* 175.1190), found 175.1189 $[M+H]^+$, Δ 0.6 ppm. ^{1}H NMR spectrum is in accordance with the literature[396].

2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethan-1-amine (35). Compound **35** was prepared according to the general procedure (**GP1**). The product was obtained as a yellowish oil in an 81% yield (45 g). **IR(DRIFT)**: 3393, 2878, 2095 ν(azide), 1601, 1347, 1272, 1254, 1117 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 3.69 – 3.61 (m, 10H, H-3, H-4, H-5, H-6, H-7), 3.51 (t, *J* = 5.2 Hz, 2H, H-2), 3.39 (t, *J* = 5.1 Hz, 2H, H-8), 2.86 (t, *J* = 5.2 Hz, 2H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 73.25 (C-2), 70.72 – 70.05 (C-3, C-4, C-5, C-6, C-7), 50.70 (C-8), 41.71 (C-1) ppm. **ESI MS**: for $C_8H_{18}N_4O_3$ calcd: *m/z* 218.1 (for $[M+H]^+$ calcd: *m/z* 219.1), found 219.2 $[M+H]^+$. **HRMS**: for $C_8H_{18}N_4O_3$ calcd: *m/z* 218.1379 (for $[M+H]^+$ calcd: *m/z* 219.1452), found 219.1454 $[M+H]^+$, Δ 0.9 ppm. ^{1}H NMR spectrum is in accordance with the literature[396].

2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (36). 

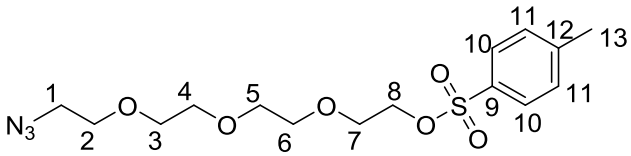
Compound **36** was prepared according to the previously published procedure[362]. **TEG** (89.3 g, 0.46 mol) was dissolved in dry THF (40 mL) and NaOH solution (2.8 g in 16 mL of H_2O) was added. The reaction mixture was cooled to 0°C and TsCl (7.8 g, 41.8 mmol) in dry THF (80 mL) was added dropwise. The reaction mixture was further stirred at 0°C for 3 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 1/10 mixture. Spots were detected by the method M2. The reaction mixture was poured into ice cold H_2O (280 mL) and the solution was washed with CH_2Cl_2 (3 × 200 mL). The organic phase was washed with H_2O (2 × 200 mL) and dried with $MgSO_4$ (10 g). After filtration, the solution was evaporated on a rotary evaporator at 40°C. The product was dried at room temperature using an oil rotary pump. The product was obtained as colorless oil in a 92% yield (13.3 g). **IR(DRIFT)**: 3348, 2945, 2871, 1930, 1597, 1454, 1354, 1175, 1018 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.80 (m, 2H, H-10), 7.34 (m, 2H, H-11), 4.19 – 4.15 (m, 2H, H-1), 3.76 – 3.58 (m, 14H, H-2, H-3, H-4, H-5, H-6, H-7, H-8), 2.45 (s, 3H, H-13) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 144.96 (C-9), 133.15 (C-12), 129.97 (C-11), 128.14 (C-10), 72.61 – 68.87 (C-2, C-3, C-4, C-5, C-6, C-7), 69.38 (C-1), 61.90 (C-8), 21.79 (C-13) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 225.0, $1*10^{-5}$ M. **ESI MS**: for $C_{15}H_{24}O_7S_2$ calcd: *m/z* 348.1 (for $[M+Na]^+$ calcd: *m/z* 371.1), found 371 $[M+Na]^+$. **HRMS**: for $C_{15}H_{24}O_7S$ calcd: *m/z* 348.1243 (for $[M+H]^+$ calcd: *m/z* 349.1316), found 349.1317 $[M+H]^+$, Δ 0.3 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[362].

2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethan-1-ol (37). Compound **37** was prepared according to the previously published procedure[363]. 2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate **36** (6.4 g; 18.4 mmol) was suspended in distilled H_2O (130 mL) and NaN_3 (2.15 g; 33.1 mmol) was added. The reaction mixture was heated to reflux (120°C) and stirred for 20 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 1/10 mixture. The starting compound and the product had the same R_f. The starting compound was detected by methods M1 and M2. The product was detected by the method M2. The reaction mixture was washed with $CHCl_3$ (3 × 250 mL). The organic phase was dried with $MgSO_4$ (15 g),



filtered, and evaporated on a rotary evaporator at 40°C, the product was dried at room temperature using an oil rotary pump. The product was obtained as a yellow oil in a 79% yield (3.2 g). **IR(DRIFT)**: 3506, 2959, 2869, 2095 ν(azide), 1649, 1350, 1251, 1129 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 3.74 – 3.69 (m, 2H, H-8), 3.67 – 3.65 (m, 10H, H-3, H-4, H-5, H-6, H-7), 3.61 – 3.57 (m, 2H, H-2), 3.38 (t, *J* = 4.8 Hz, 2H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 72.62 (C-2), 70.79 – 70.13 (C-3, C-4, C-5, C-6, C-7), 61.83 (C-8), 50.78 (C-1) ppm. **ESI MS**: for $C_8H_{17}N_3O_4$ calcd: *m/z* 219.1 (for $[M+Na]^+$ calcd: *m/z* 242.1), found 242.1 $[M+Na]^+$. **HRMS**: for $C_8H_{17}N_3O_4$ calcd: *m/z* 219.1219 (for $[M+Na]^+$ calcd: *m/z* 242.1111), found 242.1099 $[M+Na]^+$, Δ 4.9 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[397].

2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (38). Compound **38** was prepared according to the previously published procedure[364]. 2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethan-1-ol **37** (4.5 g, 20.5 mmol) was dissolved in dry THF (92 mL) and TsCl (4.3 g, 22.6 mmol) was added. The reaction mixture was cooled to 0°C and NaOH solution (3.3 g in 17 mL of H_2O) was added dropwise. The cooling bath was taken away and the reaction mixture was further stirred at room temperature for 20 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 1/1 mixture. Spots were detected by methods M1 and M2. The reaction mixture was neutralized with 1 M HCl. THF was evaporated from the reaction mixture on a rotary evaporator at 30°C and the aq. residue was washed with CH_2Cl_2 (100 mL). The organic phase was then washed with H_2O (40 mL) and dried with $MgSO_4$ (2.5 g), the desiccant was filtered off and the filtrate was evaporated on a rotary evaporator at 40°C. The product was dried at room temperature using an oil rotary pump. The product was obtained as a yellow oil in a 90% yield (6.9 g). **IR(DRIFT)**: 2872, 2104 ν(azide), 1353, 1296, 1174 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.79 (m, 2H, H-10), 7.33 (m, 2H, H-11), 4.17 – 4.13 (m, 2H, H-8), 3.69 – 3.59 (m, 12H, H-2, H-3, H-4, H-5, H-6, H-7), 3.38 (t, *J* = 5.2 Hz, 2H, H-1), 2.44 (s, 3H, H-13) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 144.92 (C-9), 133.12 (C-12), 129.93 (C-11), 128.09 (C-10), 70.87 – 68.79 (C-2, C-3, C-4, C-5, C-6, C-7), 69.36 (C-8), 50.80 (C-1), 21.76 (C-13) ppm. **UV-VIS** (MeCN), λ_{max1}, nm: 202.5, λ_{max2}, nm: 225.0, λ_{max3}, nm: 229.5, 8*10^{-5} M. **ESI MS**: for $C_{15}H_{23}N_3O_6S$ calcd: *m/z* 373.1 (for $[M+Na]^+$ calcd: *m/z*



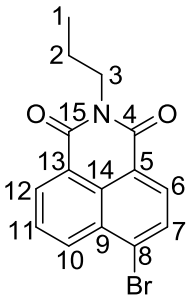
396.1), found 396.2 $[M+Na]^+$. **HRMS**: for $C_{15}H_{23}N_3O_6S$ calcd: *m/z* 373.1308 (for $[M+Na]^+$ calcd: *m/z* 396.1200), found 396.1205 $[M+Na]^+$, Δ 1.3 ppm. ^1H and ^{13}C NMR spectra are in accordance with the literature[364].

1,23-Diazido-3,6,9,12,15,18,21-heptaoxatricosane (39). Compound **39** was prepared according to the previously published procedure[364]. 2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethan-1-ol **37** (4.1 g, 18.5 mmol) was dissolved in dry THF (130 mL) and the solution was cooled to 0°C. NaH (0.81 g, 20.3 mmol, 60% dispersion in oil) was added and the mixture was stirred for 1 hour at 0°C. Then, the solution of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate **38** (6.9 g, 18.5 mmol) in THF (130 mL) was added dropwise. The cooling bath was taken away and the reaction mixture was further stirred at room temperature for 18 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 1/10 mixture. Spots were detected by methods M1 and M2. The reaction mixture was neutralized with 1 M HCl. THF was evaporated from the reaction mixture on a rotary evaporator at 30°C and the aq. residue was washed with $CHCl_3$ (150 mL). The organic phase was washed with 1 M HCl, a saturated $NaHCO_3$ aq. solution, brine (all 100 mL), and dried with $MgSO_4$ (3.0 g). The desiccant was filtered off and the filtrate was evaporated at 40°C on a rotary evaporator. The residue (9.3 g) was dissolved in H_2O (150 mL), the solution was washed with hexane (2 ×150 mL), and evaporated on a rotary evaporator at 50°C. The residue was re-evaporated once more from MeOH and $CHCl_3$, subsequently. The product was dried at room temperature using an oil rotary pump. The product was obtained as a brown oil in a 78% yield (6.1 g). **IR(DRIFT)**: 2950, 2911, 2887, 2869, 2107 ν(azide), 1640, 1449, 1350, 1281, 1254, 1108 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 3.68 – 3.64 (m, 28H, H-2, H-3, H-4, H-5, H-6, H-7, H-8), 3.38 (t, *J* = 5.1 Hz, 4H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 70.83 – 70.16 (C-2, C-3, C-4, C-5, C-6, C-7, C-8), 50.82 (C-1) ppm. **ESI MS**: for $C_{16}H_{32}N_6O_7$ calcd: *m/z* 420.2 (for $[M+K]^+$ calcd: *m/z* 459.2), found 459.2 $[M+K]^+$. **HRMS**: for $C_{16}H_{32}N_6O_7$ calcd: *m/z* 420.2332 (for $[M+NH_4]^+$ calcd: *m/z* 438.2671), found 438.2641 $[M+NH_4]^+$, Δ 6.8 ppm. ^1H and ^{13}C NMR spectra are in accordance with the literature[398].

23-Azido-3,6,9,12,15,18,21-heptaoxatricosan-1-amine (NH_2-OEG-N_3 **40).** Compound NH_2-OEG-N_3 **40** was prepared according to the previously published procedure[361], described for compounds NH_2-DEG-N_3 **33**, NH-TrEG-N **34**, and **35**. 1,23-Diazido-3,6,9,12,15,18,21-heptaoxatricosane **39** (6.1 g, 14.5 mmol) was dissolved in Et_2O (140 mL). To the solution was added 1 M HCl (140 mL), and the biphasic mixture was stirred vigorously. PPh_3 (4.2 g, 16.0 mmol) was then added in small portions and the mixture was stirred for 20 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 1/10 mixture for the starting diazido compound, CH_2Cl_2/MeOH/conc. NH_3 aq. solution 3/3/1 mixture for the product. Spots were detected by methods M2 and M4. The precipitated PPh_3 oxide was filtered off and washed with H_2O. The organic phase was separated, and the aqueous solution was subsequently washed with Et_2O (2 × 200 mL). The aqueous solution was cooled to 0°C and KOH (40 g) was added slowly. The basic aqueous solution was then washed with CH_2Cl_2 (200 mL). The organic phase was dried with $MgSO_4$ (9 g), the desiccant was filtered off, and the filtrate was evaporated at 40°C on a rotary evaporator. The product was dried at room temperature using an oil rotary pump. The product was obtained as a light brown oil in a 71% yield (4.1 g). **IR(DRIFT)**: 3479, 2866, 2101 ν(azide), 1640, 1350, 1245, 1114 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 3.65 – 3.61 (m, 26H, H-3, H-4, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-15), 3.53 (t, J = 5.1 Hz, 2H, H-2), 3.36 (t, J = 5.1 Hz, 2H, H-16), 2.87 (t, J = 5.2 Hz, 2H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 72.55 (C-2), 70.72 – 70.06 (C-3, C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-11, C-12, C-13, C-14, C-15), 50.74 (C-16), 41.63 (C-1) ppm. **ESI MS**: for $C_{16}H_{34}N_4O_7$ calcd: *m/z* 394.2 (for $[M+H]^+$ calcd: *m/z* 395.3), found 395.3 $[M+H]^+$. **HRMS**: for $C_{16}H_{34}N_4O_7$ calcd: *m/z* 394.2427 (for $[M+H]^+$ calcd: *m/z* 395.2500), found 395.2509 $[M+H]^+$, Δ 2.2 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[398].

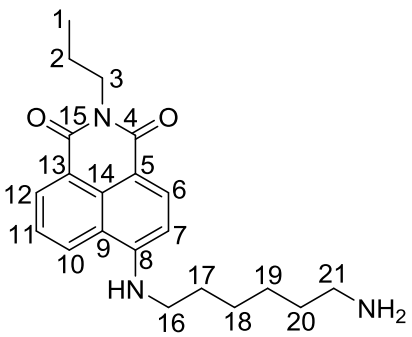
6.8.4 Fluorophores

6-Bromo-2-propyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (NPNIBr **41).**



Compound NPNIBr **41** was prepared according to the previously published procedure[365]. 4-Bromo-1,8-naphthalic anhydride (3.0 g, 10.8 mmol) was suspended in EtOH (125 mL) and $PrNH_2$ (0.64 g, 10.8 mmol) was added. The mixture was heated to reflux and stirred for 17 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 2/1 mixture. Spots were detected by the method M1. The reaction mixture was evaporated on a rotary evaporator at 40°C. The residue (3 g) was dissolved in the smallest possible amount of $CHCl_3$, silica gel (15 g) was added, and the mixture was evaporated on a rotary evaporator at 40°C. The adsorbed crude was purified by column chromatography (65 g silica gel) eluting with hexane/EtOAc 20/1 and 10/1. Fractions containing the product were evaporated on a rotary evaporator at 40°C. The product was dried at room temperature using an oil rotary pump and obtained as a yellow solid in an 87% yield (2.99 g). **IR(DRIFT)**: 3082, 3058, 2968, 2950, 2872, 1703, 1658, 1619, 1586, 1571, 1359, 1290, 1242 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 8.65 (dd, J = 7.3, 1.1 Hz, 1H, H-12), 8.56 (dd, J = 8.5, 1.2 Hz, 1H, H-10), 8.41 (d, J = 7.9 Hz, 1H, H-6), 8.03 (d, J = 7.9 Hz, 1H, H-7), 7.84 (dd, J = 8.5, 7.3 Hz, 1H, H-11), 4.16 – 4.11 (m, 2H, H-3), 1.76 (h, J = 7.6 Hz, 2H, H-2), 1.01 (t, J = 7.4 Hz, 3H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 163.65 (C-15), 163.63 (C-4), 133.21 (C-10), 132.05 (C-12), 131.23 (C-6), 131.13 (C-7), 130.62 (C-8), 130.22 (C-9), 129.00 (C-14), 128.12 (C-11), 123.19 (C-13), 122.33 (C-5), 42.17 (C-3), 21.47 (C-2), 11.64 (C-1) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 201.1, λ_{max2}, nm: 236.5, λ_{max3}, nm: 342.9, $3*10^{-7}$ M. **ESI MS**: for $C_{15}H_{12}BrNO_2$ calcd: *m/z* 317.0 (for $[M+Na]^+$ calcd: *m/z* 340.0), found 340.0 $[M+Na]^+$. **HRMS**: for $C_{15}H_{12}BrNO_2$ calcd: *m/z* 317.0051 (for $[M+H]^+$ calcd: *m/z* 318.0124), found 318.0137 $[M+H]^+$, Δ 4.1 ppm. ^{1}H NMR spectrum is in accordance with the literature[399].

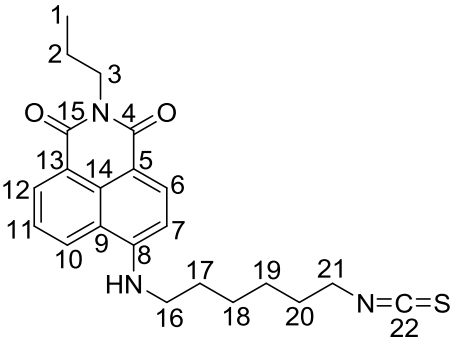
6-((6-Aminohexyl)amino)-2-propyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione



(NPNI-HDA **42).** Compound NPNI-HDA **42** was prepared according to the previously published procedure[366], with some modifications. NPNIBr **41** (1.14 g, 3.61 mmol) was dissolved in DMSO (24 mL) and hexamethylenediamine (1.68 g, 14.4 mmol) was added. The solution was heated to 60°C and stirred for

18 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 20/1 mixture for the starting compound and $CHCl_3$/MeOH 1/1 mixture for the product. Spots were detected by the method M1. DMSO was distilled from the reaction mixture at 90°C using an oil rotary pump. The crude product was dissolved in $CHCl_3$ (40 mL) and washed with 5% w/w NaOH aq. solution (40 mL). The aqueous phase was extracted with $CHCl_3$ (2 × 40 mL). The organic extracts were combined and dried with $MgSO_4$ (0.5 g). The desiccant was removed by filtration and the filtrate was evaporated at 40°C on a rotary evaporator. The crude product (2.2 g) was dissolved in the smallest possible amount of $CHCl_3$ and silica gel (11 g) was added. The mixture was evaporated at 40°C on a rotary evaporator. The adsorbed product was purified by column chromatography (47 g silica gel) eluting with $CHCl_3$/MeOH 1/1. Fractions containing the product were evaporated at 40°C on a rotary evaporator. The product was dried at room temperature using an oil rotary pump and obtained as a yellow oil in an 81% yield (1.04 g). **IR(DRIFT)**: 3381, 2929, 2872, 2848, 1682, 1643, 1580, 1548, 1353, 1248 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 8.57 (dd, *J* = 7.3, 1.1 Hz, 1H, H-12), 8.45 (d, *J* = 8.4 Hz, 1H, H-6), 8.08 (dd, *J* = 7.3, 1.0 Hz, 1H, H-10), 7.61 (dd, *J* = 8.4, 7.3 Hz, 1H, H-11), 6.71 (d, *J* = 8.5 Hz, 1H, H-7), 5.27 (t, *J* = 5.1 Hz, 1H, NH), 4.17 – 4.09 (m, 2H, H-3), 3.40 (td, *J* = 7.2, 5.1 Hz, 2H, H-16), 2.71 (t, *J* = 6.6 Hz, 2H, H-21), 1.87 – 1.69 (m, 4H, H-2, H-17), 1.56 – 1.38 (m, 6H, H-18, H-19, H-20), 1.00 (t, *J* = 7.4 Hz, 3H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 164.79 (C-15), 164.25 (C-4), 149.52 (C-8), 134.52 (C-6), 131.13 (C-12), 129.89 (C-13), 125.90 (C-10), 124.70 (C-11), 123.27 (C-14), 120.27 (C-9), 110.33 (C-5), 104.37 (C-7), 43.76 (C-16), 42.19 (C-21), 41.77 (C-3), 33.72 (C-20), 29.06 (C-17), 27.17 – 26.76 (C-18, C-19), 21.57 (C-2), 11.68 (C-1) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 203.6, λ_{max2}, nm: 229.7, λ_{max3}, nm: 259.9, λ_{max4}, nm: 283.2, λ_{max5}, nm: 442.2, $3*10^{-5}$ M. **ESI MS**: for $C_{21}H_{27}N_3O_2$ calcd: *m/z* 353.2 (for $[M+H]^+$ calcd: *m/z* 354.2), found 354.2 $[M+H]^+$. **HRMS**: for $C_{21}H_{27}N_3O_2$ calcd: *m/z* 353.2103 (for $[M+H]^+$ calcd: *m/z* 354.2176), found 354.2186 $[M+H]^+$, Δ 2.8 ppm.

6-((6-Isothiocyanatohexyl)amino)-2-propyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (NPNI-HDA-ITC **43).** NPNI-HDA **42** (0.76 g, 2.16 mmol) was dissolved in $CHCl_3$ (130 mL) and K_2CO_3 (0.90 g, 6.48 mmol) was added. Thiophosgene (0.25 mL, 3.23 mmol) was added dropwise and the reaction mixture was stirred at room temperature for 20 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 2/1 mixture for the product and $CHCl_3$/MeOH 1/1 mixture for the starting compound. Spots were detected by the method M1. The reaction mixture was diluted with Et_2O (200 mL) and the solution was washed with H_2O (80 mL). The organic phase was washed with H_2O (200 mL) again and dried with $MgSO_4$ (4.7 g). The desiccant was removed by filtration and the filtrate was evaporated at 40°C on a rotary evaporator. The product was dried at room temperature using an oil rotary pump and obtained as an orange solid in a 97% yield (0.83 g). **IR(DRIFT)**: 3387, 2962, 2938, 2872, 2857, 2187 ν(isothiocyanate), 2125 ν(isothiocyanate), 1676, 1634, 1619, 1589, 1574, 1551, 1395, 1374, 1365, 1350, 1245 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 8.58 (dd, J = 7.3, 1.1 Hz, 1H, H-12), 8.46 (d, J = 8.3 Hz, 1H, H-6), 8.09 (dd, J = 8.5, 1.1 Hz, 1H, H-10), 7.62 (dd, J = 8.4, 7.3 Hz, 1H, H-11), 6.72 (d, J = 8.4 Hz, 1H, H-7), 5.25 (t, J = 5.2 Hz, 1H, NH), 4.17 – 4.07 (m, 2H, H-3), 3.55 (t, J = 6.4 Hz, 2H, H-21), 3.43 (td, J = 7.1, 5.1 Hz, 2H, H-16), 1.89 – 1.81 (m, 2H, H-17), 1.80 – 1.70 (m, 4H, H-2, H-20), 1.56 – 1.53 (m, 4H, H-18, H-19), 1.00 (t, J = 7.4 Hz, 3H, H-1) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 164.82 (C-15), 164.29 (C-4), 149.34 (C-8), 134.53 (C-6), 131.24 (C-12), 129.92 (C-13, C22), 125.82 (C-10), 124.89 (C-11), 123.39 (C-14), 120.30 (C-9), 110.65 (C-5), 104.48 (C-7), 45.09 (C-21), 43.64 (C-16), 41.82 (C-3), 29.95 (C-20), 28.97 (C-17), 26.55 (C-18, C-19), 21.60 (C-2), 11.72 (C-1) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 202.9, λ_{max2}, nm: 229.9, λ_{max3}, nm: 259.7, λ_{max4}, nm: 282.8, λ_{max5}, nm: 442.0, $2*10^{-5}$ M. **ESI MS**: for $C_{22}H_{25}N_3O_2S$ calcd: *m/z* 395.2 (for $[M+H]^+$ calcd: *m/z* 396.2), found 396.2 $[M+H]^+$. **HRMS**: for $C_{22}H_{25}N_3O_2S$ calcd: *m/z* 395.1667 (for $[M+H]^+$ calcd: *m/z* 396.1740), found 396.1737 $[M+H]^+$, Δ 0.8 ppm.



6-((2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl)amino)-2-propyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (NPNI-NH-TEG-N_3 **44).** NPNIBr **41** (1.0 g, 3.15 mmol) and **35** (5.0 mL) were dissolved in DMSO (10 mL) and the mixture was heated

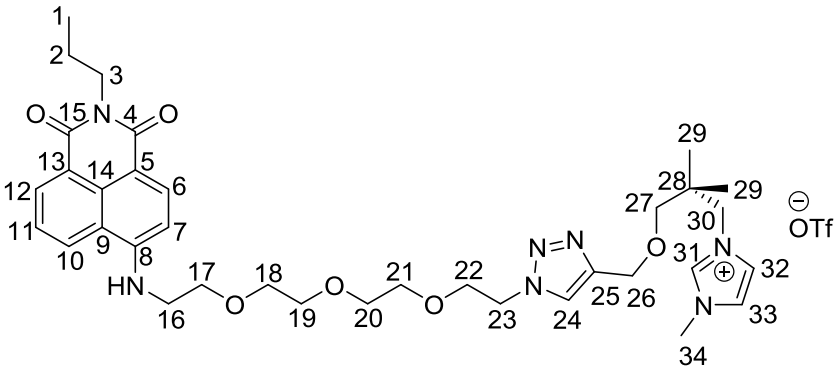
to 60°C for 21 hours. The reaction was monitored by TLC using hexane/EtOAc 2/1 mixture for the starting compound and $CHCl_3$/MeOH 40/1 mixture for the product. Spots were detected by the method M1. The solvent was distilled from the reaction mixture under reduced pressure at 80°C using an oil rotary pump. The crude product was dissolved in $CHCl_3$ (100 mL) and the solution was washed with H_2O (100 mL). The organic phase was washed with H_2O (200 mL) again and dried with $MgSO_4$ (1 g). The desiccant was removed by filtration and the filtrate was evaporated at 40°C on a rotary evaporator. The crude product (4.18 g) was dissolved in the smallest possible amount of $CHCl_3$, silica gel (20 g) was added, and the suspension was evaporated at 40°C on a rotary evaporator. The adsorbed product was purified by column chromatography (160 g silica gel) eluting with $CHCl_3$/MeOH 40/1. Fractions with the pure product were evaporated on a rotary evaporator at 40°C. The product (1.25 g) was dissolved in benzene (25 mL) and freeze-dried. The product was obtained as a yellow solid in an 81% yield (1.17 g). **IR(DRIFT)**: 3402, 2959, 2884, 2119 ν(azide), 1673, 1637, 1616, 1577, 1559, 1389, 1359, 1350, 1281, 1245 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 8.58 (d, J = 8.4 Hz, 1H, H-12), 8.46 (d, J = 8.4 Hz, 1H, H-6), 8.18 (d, J = 9.5 Hz, 1H, H-10), 7.64 – 7.59 (m, 1H, H-11), 6.71 (d, J = 8.4 Hz, 1H, H-7), 5.90 (bs, 1H, NH), 4.15 – 4.11 (m, 2H, H-3), 3.90 (t, J = 5.2 Hz, 2H, H-17), 3.74 – 3.62 (m, 10H, H-18, H-19, H-20, H-21, H-22), 3.58 (q, J = 5.0 Hz, 2H, H-16), 3.33 (t, J = 4.9 Hz, 2H, H-23), 1.76 (h, J = 7.6 Hz, 2H, H-2), 1.00 (t, J = 7.4 Hz, 3H, H-1) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 164.85 (C-15), 164.31 (C-4), 149.65 (C-8), 134.50 (C-6), 131.23 (C-12), 129.95 (C-13), 126.48 (C-10), 124.77 (C-11), 123.28 (C-14), 120.66 (C-9), 110.74 (C-5), 104.53 (C-7), 70.85 – 70.19 (C-18, C-19, C-20, C-21, C-22), 68.78 (C-17), 50.79 (C-23), 43.26 (C-16), 41.81 (C-3), 21.59 (C-2), 11.71 (C-1) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 203.6, λ_{max2}, nm: 229.4, λ_{max3}, nm: 259.4, λ_{max4}, nm: 282.1, λ_{max5}, nm: 438.4, $4*10^{-5}$ M. **ESI MS**: for $C_{23}H_{29}N_5O_5$ calcd: *m/z* 455.2 (for $[M+H]^+$ calcd: *m/z* 456.2), found 456.2 $[M+H]^+$. **HRMS**: for $C_{23}H_{29}N_5O_5$ calcd: *m/z* 455.2169 (for $[M+H]^+$ calcd: *m/z* 456.2241), found 456.2239 $[M+H]^+$, Δ 0.4 ppm.

6-((23-Azido-3,6,9,12,15,18,21-heptaoxatricosyl)amino)-2-propyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (NPNI-NH-OEG-N_3 **45).** NPNIBr **41** (0.44 g, 1.40 mmol) and NH_2-OEG-N_3 **40** (0.82 g, 2.08 mmol) were dissolved in DMSO (5 mL) and the mixture was heated to 60°C for 20 hours. The reaction was monitored by TLC using hexane/EtOAc 2/1 mixture for the starting compound and $CHCl_3$/MeOH 40/1 mixture for the product. Spots were detected by the method M1. The reaction was not completed and so the mixture was stirred for another 24 hours at 60°C. Then, the reaction mixture was diluted with $CHCl_3$ (50 mL) and washed with H_2O (50 mL), brine (50 mL), and dried with $MgSO_4$ (0.5 g). The desiccant was filtered off and the filtrate was evaporated on a rotary evaporator at 50°C. The residue (1.19 g) was dissolved in the smallest possible amount of $CHCl_3$, silica gel (6.0 g) was added, and the mixture was evaporated on a rotary evaporator at 40°C. The adsorbed crude product was purified by column chromatography (35 g silica gel) eluting with $CHCl_3$/MeOH 40/1. Fractions with the pure product were evaporated on a rotary evaporator at 50°C. The product was dried at 90°C using an oil rotary pump. The final product was obtained as a yellow solid in a 37% yield (0.33 g). **IR(DRIFT)**: 3363, 2869, 2104 ν(azide), 1685, 1646, 1583, 1344, 1281, 1248 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 8.57 (dd, J = 7.3, 1.1 Hz, 1H, H-12), 8.45 (d, J = 8.4 Hz, 1H, H-6), 8.28 (dd, J = 8.5, 1.2 Hz, 1H, H-10), 7.63 – 7.59 (m, 1H, H-11), 6.69 (d, J = 8.4 Hz, 1H, H-7), 6.19 (t, J = 5.1 Hz, 1H, NH), 4.18 – 4.08 (m, 2H, H-3), 3.89 (t, J = 4.9 Hz, 2H, H-17), 3.74 – 3.56 (m, 28H, H-16, H-18, H-19, H-20, H-21, H-22, H-23, H-24, H-25, H-26, H-27, H-28, H-29, H-30), 3.36 (t, J = 5.1 Hz, 2H, H-31), 1.75 (h, J = 7.4 Hz, 2H, H-2), 1.00 (t, J = 7.4 Hz, 3H, H-1) ppm. **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 164.99 (C-15), 164.39 (C-4), 149.87 (C-8), 134.53 (C-6), 131.22 (C-12), 129.99 (C-13), 126.93 (C-10), 124.75 (C-11), 123.15 (C-14), 120.73 (C-9), 110.49 (C-5), 104.37 (C-7), 70.83 – 70.14 (C-18, C-19, C-20, C-21, C-22, C-23, C-24, C-25, C-26, C-27, C-28, C-29, C-30), 68.78 (C-17), 50.80 (C-31), 43.36 (C-16), 41.79 (C-3), 21.60 (C-2), 11.71 (C-1) ppm. **UV-VIS** ($CHCl_3$), λ_{max1},

nm: 428.5, 4*10^{-4} M. **ESI MS**: for $C_{31}H_{45}N_5O_9$ calcd: *m/z* 631.3 (for $[M+Na]^+$ calcd: *m/z* 654.3), found 654.3 $[M+Na]^+$. **HRMS**: for $C_{31}H_{45}N_5O_9$ calcd: *m/z* 631.3217 (for $[M+H]^+$ calcd: *m/z* 632.3290), found 632.3295 $[M+H]^+$, Δ 0.8 ppm.

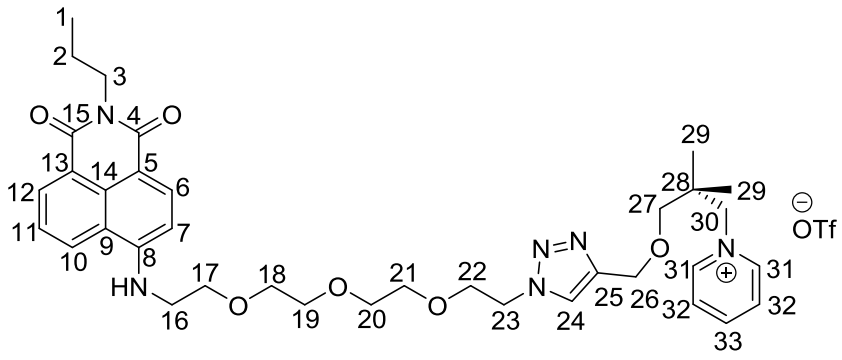
6.8.5 Charged fluorophores

3-(3-((1-(2-(2-(2-(2-((1,3-Dioxo-2-propyl-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-2,2-dimethylpropyl)-1-methyl-1*H*-imidazol-3-ium trifluoromethanesulfonate (NPNI-NH-TEG-MTZ-O-MIM1 **46).** Prg-O-MIM1 trifluoromethanesulfonate **1** (0.1 g, 0.28 mmol) and NPNI-NH-TEG-N_3 **44** (0.153 g, 0.34 mmol) were dissolved in PrOH/H_2O 2/1 mixture (4.5 mL). A metal Cu (0.53 g, 8.4 mmol) was added, and the reaction mixture was stirred for 1 hour at 60°C. The reaction was monitored by TLC and RP-18 TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by methods M1 and M2. The reaction mixture was filtered through celite and evaporated on a rotary evaporator at 50°C. The crude product was purified by a C18 RP column chromatography (2.0 g silica gel) using 20-40% w/w MeOH aq. solution for elution. Fractions with the pure product were evaporated on a rotary evaporator at 50°C. The product (0.12 g) was dissolved in H_2O (2.5 mL) and freeze-dried. The product was obtained as a yellow-orange oil-like compound in a 52% yield (0.11 g). **IR(DRIFT)**: 3390, 2959, 2875, 1679, 1646, 1586, 1431, 1395, 1359, 1248, 1159, 1033 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 8.95 (s, 1H, H-31), 8.67 (d, *J* = 8.4 Hz, 1H, H-12), 8.43 (d, *J* = 7.2 Hz, 1H, H-6), 8.25 (d, *J* = 8.5 Hz, 1H, H-10), 8.05 (s, 1H, H-24), 7.76 (t, *J* = 5.6 Hz, 1H, NH), 7.71 – 7.65 (m, 2H, H-11, H-33), 7.54 (s, 1H, H-32), 6.83 (d, *J* = 8.6 Hz, 1H, H-7), 4.50 (s, 2H, H-26), 4.48 (t, *J* = 5.5 Hz, 2H, H-23), 4.01 (s, 2H, H-30), 3.97 (t, *J* = 7.4 Hz, 2H, H-3), 3.84 (s, 3H, H-34), 3.78 (t, *J* = 5.2 Hz, 2H, H-22), 3.71 (t, *J* = 5.7 Hz, 2H, H-17), 3.61 – 3.43 (m, 10H, H-16, H-18, H-19, H-20, H-21), 3.08 (s, 2H, H-27), 1.62 (h, *J* = 7.5 Hz, 2H, H-2), 0.90 (t, *J* = 7.5 Hz, 3H, H-1), 0.84 (s, 6H, H-29) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 163.73 – 162.92 (C-4, C-15), 150.58 (C-8), 143.37 (C-



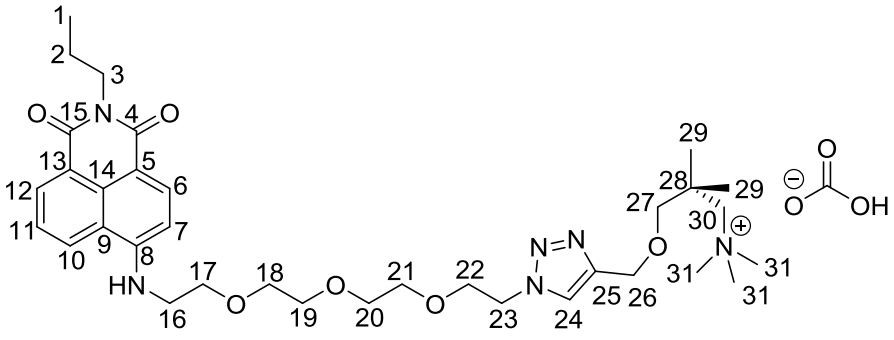
25), 137.28 (C-31), 134.14 (C-10), 130.68 (C-6), 129.38 (C-13), 128.48 (C-12), 124.32 (C-24), 123.58 – 123.09 (C-32, C-33, C-11), 121.88 (C-14), 120.10 (C-9), 107.77 (C-5), 103,96 (C-7), 75.16 (C-27), 69.80 – 69.48 (C-18, C-19, C-20, C-21), 68.64 (C-22), 68.17 (C-17), 63.66 (C-26), 55.58 (C-30), 49.31 (C-23), 42.71 (C-16), 40.74 (C-3), 39.52 (C-28, solvent overlay), 35.72 (C-34), 22.18 (C-29), 20.97 (C-2), 11.39 (C-1) ppm. **^{19}F NMR** (376 MHz, DMSO-d_6, C_6F_6): δ = -80.07, -164.90 (C_6F_6) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 205.3, λ_{max2}, nm: 259.6, λ_{max3}, nm: 282.0, λ_{max4}, nm: 438.0, $1*10^{-5}$ M. **ESI MS**: for $C_{35}H_{48}N_7O_6^+$ calcd: *m/z* 662.4, found 662.3 [M^+]. **HRMS**: for $C_{35}H_{48}N_7O_6^+$ calcd: *m/z* 662.3661, found 662.3668 [M^+], Δ 1.0 ppm.

1-(3-((1-(2-(2-(2-(2-((1,3-Dioxo-2-propyl-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-2,2-dimethylpropyl)pyridin-1-ium trifluoromethanesulfonate (NPNI-NH-TEG-MTZ-O-PYR1 **47).** Prg-O-PYR1 trifluoromethanesulfonate **2** (0.062 g, 0.18 mmol) and NPNI-NH-TEG-N_3 **44** (0.10 g, 0.22 mmol) were dissolved in PrOH/H_2O 2/1 mixture (3.0 mL). The solution was bubbled with nitrogen for 30 minutes, a metal Cu (0.33 g, 5.3 mmol) was added, and the reaction mixture was stirred for 2 hours at 60°C. The reaction was monitored by TLC and RP-18 TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by methods M1 and M2. The reaction mixture was filtered through celite and evaporated on a rotary evaporator at 50°C. The crude product (0.14 g) was purified by a C18 RP column chromatography (1.5 g silica gel) using 20-40% w/w MeOH aq. solution for elution. Fractions with the pure product were evaporated on a rotary evaporator at 50°C. The product (0.08 g) was dissolved in H_2O (2 mL) and freeze-dried. The product was obtained as a yellow-orange oil-like compound in a 37% yield (0.054 g). **IR(DRIFT)**: 3531, 3367, 3136, 3091, 3064, 2960, 2875, 1682, 1645, 1581, 1552, 1489, 14645, 1429, 1394, 1358, 1259, 1161, 1124, 1093, 1032 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 8.82 (d, *J* = 6.0 Hz, 2H, H-31), 8.67 (d, *J* = 8.4 Hz, 1H, H-12), 8.59 (t, *J* = 7.8 Hz, 1H, H-33), 8.43 (d, *J* = 7.3 Hz, 1H, H-6), 8.25 (d, *J* = 8.5 Hz, 1H, H-10), 8.06 (t, *J* = 7.1 Hz, 2H, H-32), 8.04 (s, 1H, H-24), 7.78



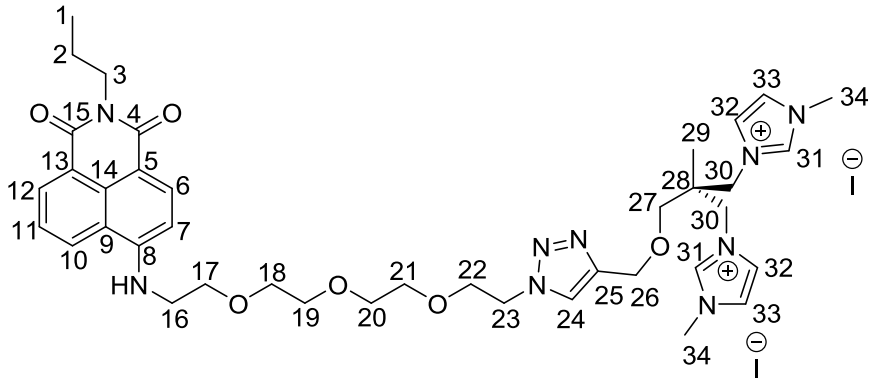
(bs, 1H, NH), 7.67 (t, *J* = 7.9 Hz, 1H, H-11), 6.82 (d, *J* = 8.6 Hz, 1H, H-7), 4.50 – 4.48 (m, 6H, H-23, H-30, H-26), 3.97 (t, *J* = 7.6 Hz, 2H, H-3), 3.78 (t, *J* = 5.2 Hz, 2H, H-22), 3.70 (t, *J* = 5.7 Hz, 2H, H-17), 3.58 – 3.46 (m, 10H, H-16, H-18, H-19, H-20, H-21), 3.12 (s, 2H, H-27), 1.61 (h, *J* = 7.4 Hz, 2H, H-2), 0.90 (t, *J* = 7.6 Hz, 3H, H-1), 0.88 (s, 6H, H-29) ppm. **^{13}C NMR** (100 MHz, DMSO-d_6): δ = 163.77 (C-15), 162.95 (C-4), 150.61 (C-8), 145.85 (C-33), 145.64 (C-31), 143.12 (C-25), 134.19 (C-10), 130.74 (C-6), 129.41 (C-13), 128.54 (C-12), 127.56 (C-32), 124.49 (C-24), 124.38 (C-11), 121.90 (C-14), 120.12 (C-9), 107.77 (C-5), 104,00 (C-7), 74.79 (C-27), 69.83 – 69.50 (C-18, C-19, C-20, C-21), 68.68 (C-22), 68.18 (C-17), 66.91 (C-26), 63.58 (C-30), 49.34 (C-23), 42.73 (C-16), 40.78 (C-3), 36.57 (C-28), 22.05 (C-29), 21.01 (C-2), 11.44 (C-1) ppm. **^{19}F NMR** (376 MHz, DMSO-d_6, C_6F_6): δ = -80.07, -164.90 (C_6F_6) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 203.7, λ_{max2}, nm: 259.7, λ_{max3}, nm: 281.8, λ_{max4}, nm: 438.2, 3*10^{-5} M. **ESI MS**: for $C_{36}H_{47}N_6O_6^+$ calcd: *m/z* 659.4, found 659.3 [M^+]. **HRMS**: for $C_{36}H_{47}N_6O_6^+$ calcd: *m/z* 659.3552, found 659.3532 [M^+], Δ 3.0 ppm.

3-((1-(2-(2-(2-(2-((1,3-Dioxo-2-propyl-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-N,N,N,2,2-pentamethylpropan-1-aminium bicarbonate (NPNI-NH-TEG-MTZ-O-TMA1 **48).** Prg-O-TMA1 hydrogen carbonate **3** (0.10 g, 0.41 mmol) and NPNI-NH-TEG-N_3 **44** (0.22 g, 0.49 mmol) were dissolved in PrOH/H_2O 2/1 mixture (4.5 mL). A metal Cu (0.77 g, 12.0 mmol) was added, and the reaction mixture was stirred for 2 hours at 60°C. The reaction was monitored by TLC and RP-18 TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by methods M1 and M2. The reaction mixture was filtered through celite and evaporated on a rotary evaporator at 50°C. The crude product (0.35 g) was purified by a C18 RP column chromatography (7.0 g silica gel) using 20-40% w/w MeOH aq. solution for elution. Fractions with the product were evaporated on a rotary evaporator at 50°C. The product (0.16 g) was dissolved in H_2O (3.5 mL) and freeze-dried. The product was obtained as a yellow-orange oil-like compound in a 51%



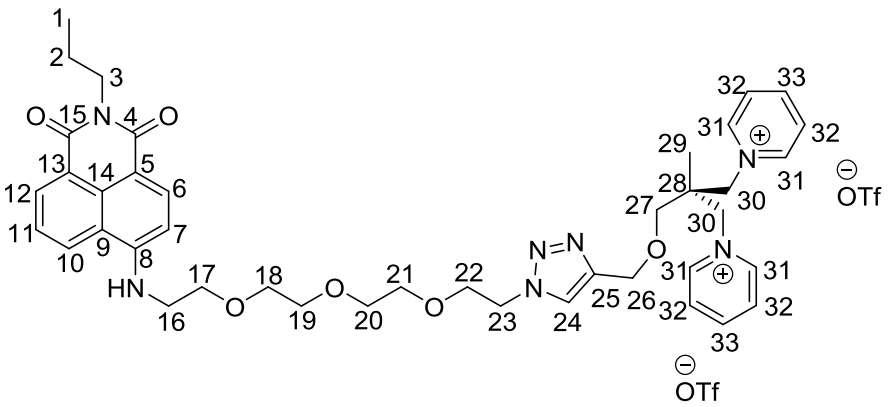
yield (0.15 g). **IR(DRIFT)**: 3419, 2959, 2914, 2869, 2110 ν(C=O), 1682, 1586 ν(C=O), 1434, 1395, 1359, 1248, 1099 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 8.80 (d, J = 8.4 Hz, 1H, H-12), 8.42 (d, J = 7.3 Hz, 1H, H-6), 8.24 (d, J = 8.5 Hz, 1H, H-10), 8.11 (s, 1H, H-24), 7.66 (dd, J = 8.5, 7.3 Hz, 1H, H-11), 6.82 (d, J = 8.7 Hz, 1H, H-7), 4.52 (s, 2H, H-26), 4.48 (t, J = 5.2 Hz, 2H, H-23), 3.98 (t, J = 6.1 Hz, 2H, H-3), 3.77 (t, J = 5.2 Hz, 2H, H-22), 3.71 (t, J = 5.8 Hz, 2H, H-17), 3.61 – 3.44 (m, 10H, H-16, H-18, H-19, H-20, H-21), 3.31 (s, 2H, H-27), 3.30 (s, 2H, H-30), 3.13 (s, 9H, H-31), 1.61 (h, J = 7.5 Hz, 2H, H-2), 1.04 (s, 6H, H-29), 0.89 (t, J = 7.4 Hz, 3H, H-1) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 163.75 (C-15), 162.92 (C-4), 150.72 (C-8), 143.19 (C-25), 134.17 (C-10), 130.68 (C-6), 129.41 (C-13), 128.84 (C-12), 124.40 (C-24), 124.25 (C-11), 121.80 (C-14), 120.15 (C-9), 107.63 (C-5), 103,91 (C-7), 76.29 (C-27), 72.31 (C-30), 69.79 – 69.48 (C-18, C-19, C-20, C-21), 68.65 (C-22), 68.18 (C-17), 63.73 (C-26), 54.45 (C-31), 49.30 (C-23), 42.61 (C-16), 40.73 (C-3), 36.76 (C-28), 24.94 (C-29), 20.97 (C-2), 11.40 (C-1) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 203.9, λ_{max2}, nm: 259.6, λ_{max3}, nm: 282.0, λ_{max4}, nm: 438.2, $3*10^{-5}$ M. **ESI MS**: for $C_{34}H_{51}N_6O_6^+$ calcd: *m/z* 639.4, found 639.3 [M^+]. **HRMS**: for $C_{34}H_{51}N_6O_6^+$ calcd: *m/z* 639.3865, found 639.3863 [M^+], Δ 0.3 ppm.

3,3'-(2-(((1-(2-(2-(2-(2-((1,3-Dioxo-2-propyl-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2-methylpropane-1,3-diyl)bis(1-methyl-1*H*-imidazol-3-ium) diiodide (NPNI-NH-TEG-MTZ-O-MIM2 **49).** Prg-O-MIM2 diiodide **4** (0.147 g, 0.27 mmol) and NPNI-NH-TEG-N_3 **44** (0.147 g, 0.33 mmol) were dissolved in MeOH/H_2O 1/1 mixture (8.0 mL). The reaction mixture was bubbled with argon for 5 minutes. CuI (0.052 g, 0.27 mmol) was added, and the mixture was stirred for 2 days at 60°C. The reaction was monitored by TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by methods M1 and M2. The reaction mixture was evaporated on a rotary evaporator at 50°C. The crude product was purified by a C18 RP column chromatography (2.7 g silica gel) using H_2O and 10%



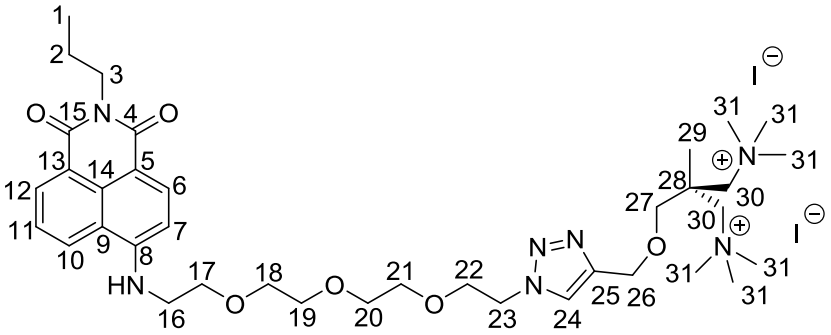
w/w MeOH aq. solution for elution. Fractions with the product were evaporated on a rotary evaporator at 50°C. The product was dried at 50°C using an oil rotary pump. The product was obtained as a yellow glassy compound in a 55% yield (0.15 g). **IR(DRIFT)**: 3437, 3082, 2956, 2866, 1679, 1643, 1610, 1580, 1425, 1392, 1356, 1245, 1174, 1093 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 9.03 (s, 2H, H-31), 8.69 (dd, *J* = 8.6, 1.2 Hz, 1H, H-12), 8.44 (dd, *J* = 7.4, 1.0 Hz, 1H, H-6), 8.26 (d, *J* = 8.5 Hz, 1H, H-10), 8.14 (s, 1H, H-24), 7.77 (t, *J* = 5.7 Hz, 1H, NH), 7.74 (s, 2H, H-33), 7.68 (dd, *J* = 8.4, 7.3 Hz, 1H, H-11), 7.59 (s, 2H, H-32), 6.84 (d, *J* = 8.7 Hz, 1H, H-7), 4.54 (s, 2H, H-26), 4.51 (t, *J* = 5.2 Hz, 2H, H-23), 4.29 (d, *J* = 14.0 Hz, 2H, H-30), 4.17 (d, *J* = 14.0 Hz, 2H, H-30), 3.98 (t, *J* = 6.3 Hz, 2H, H-3), 3.86 (s, 6H, H-34), 3.80 (t, *J* = 5.2 Hz, 2H, H-22), 3.71 (t, *J* = 5.7 Hz, 2H, H-17), 3.59 – 3.45 (m, 10H, H-16, H-18, H-19, H-20, H-21), 3.08 (s, 2H, H-27), 1.62 (h, *J* = 7.4 Hz, 2H, H-2), 0.90 (t, *J* = 7.4 Hz, 3H, H-1), 0.83 (s, 3H, H-29) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 163.80 (C-15), 162.99 (C-4), 150.65 (C-8), 142.91 (C-25), 137.73 (C-31), 134.21 (C-10), 130.78 (C-6), 129.45 (C-13), 128.57 (C-12), 124.66 (C-24), 124.42 (C-11), 123.74 (C-32), 123.52 (C-33), 121.95 (C-14), 120.16 (C-9), 107.83 (C-5), 104,05 (C-7), 70.43 (C-27), 69.87 – 69.53 (C-18, C-19, C-20, C-21), 68.70 (C-22), 68.24 (C-17), 63.45 (C-26), 52.58 (C-30), 49.44 (C-23), 42.77 (C-16), 40.82 (C-3), 39.59 (C-28, solvent overlay), 36.06 (C-34), 21.04 (C-2), 17.39 (C-29), 11.47 (C-1) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 203.3, λ_{max2}, nm: 216.6, λ_{max3}, nm: 259.4, λ_{max4}, nm: 280.8, λ_{max5}, nm: 438.4, 3*10^{-5} M. **ESI MS**: for $C_{39}H_{53}N_9O_6^{2+}$ calcd: *m/z* 371.7, found 371.9 [M^{2+}]. **HRMS**: for $C_{39}H_{53}N_9O_6^{2+}$ calcd: *m/z* 371.7054, found 371.7070 [M^{2+}], Δ 4.3 ppm.

1,1'-(2-(((1-(2-(2-(2-(2-((1,3-Dioxo-2-propyl-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2-methylpropane-1,3-diyl)bis(pyridin-1-ium) bistrifluoromethanesulfonate (NPNI-NH-TEG-MTZ-O-PYR2 **50).** Prg-O-PYR2 bistrifluoromethanesulfonate **5** (0.051 g, 87.5 µmol) and NPNI-NH-TEG-N_3 **44** (0.048 g, 0.11 mmol) were dissolved in MeOH/H_2O 1/1 mixture (3.0



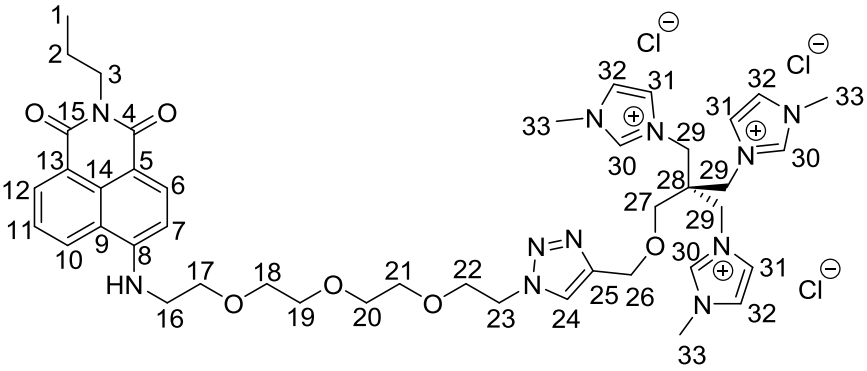
mL). The mixture was bubbled with argon for 5 minutes. CuI (0.017 g, 87.5 μmol) was added, and the reaction mixture was stirred for 1 day at 80°C. The reaction was monitored by TLC and RP-18 TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by methods M1 and M2. The reaction mixture was filtered and evaporated on a rotary evaporator at 50°C. The crude product (0.10 g) was purified by a C18 RP column chromatography (2.0 g silica gel) using 20% w/w MeOH aq. solution for elution. Fractions with the product were evaporated on a rotary evaporator at 50°C. The product (0.064 g) was dissolved in H_2O (1.0 mL) and freeze-dried. The product was obtained as a yellow-orange powder in a 67% yield (0.062 g). **IR(DRIFT)**: 3437, 3387, 2959, 2875, 1679, 1643, 1610, 1583, 1491, 1398, 1356, 1257, 1225, 1165, 1030 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 8.86 (d, *J* = 5.3 Hz, 4H, H-31), 8.69 – 8.65 (m, 3H, H-12, H-33), 8.43 (dd, *J* = 7.3, 1.0 Hz, 1H, H-6), 8.25 (d, *J* = 8.5 Hz, 1H, H-10), 8.14 (dd, *J* = 7.3, 1.0 Hz, 4H, H-32), 8.08 (s, 1H, H-24), 7.76 (t, *J* = 5.6 Hz, 1H, NH), 7.68 (dd, *J* = 8.5, 7.3 Hz, 1H, H-11), 6.83 (d, *J* = 8.6 Hz, 1H, H-7), 4.82 (d, *J* = 13.3 Hz, 2H, H-30), 4.70 (d, *J* = 13.2 Hz, 2H, H-30), 4.52 – 4.49 (m, 4H, H-23, H-26), 3.98 (t, *J* = 6.1 Hz, 2H, H-3), 3.80 (t, *J* = 5.2 Hz, 2H, H-22), 3.71 (t, *J* = 5.7 Hz, 2H, H-17), 3.59 – 3.45 (m, 10H, H-16, H-18, H-19, H-20, H-21), 3.14 (s, 2H, H-27), 1.61 (h, *J* = 7.4 Hz, 2H, H-2), 0.90 (t, *J* = 7.4 Hz, 3H, H-1), 0.87 (s, 3H, H-29) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 163.81 (C-15), 163.00 (C-4), 150.66 (C-8), 146.52 (C-33), 146.05 (C-31), 142.44 (C-25), 134.22 (C-10), 130.79 (C-6), 129.45 (C-13), 128.55 (C-12), 128.12 (C-32), 124.88 (C-24), 124.43 (C-11), 121.96 (C-14), 120.16 (C-9), 107.84 (C-5), 104,05 (C-7), 69.87 – 69.53 (C-18, C-19, C-20, C-21, C-27), 68.71 (C-22), 68.25 (C-17), 63.73 (C-26), 63.31 (C-30), 49.43 (C-23), 42.79 (C-16), 41.09 (C-28), 40.83 (C-3), 21.05 (C-2), 16.62 (C-29), 11.47 (C-1) ppm. **^{19}F NMR** (376 MHz, DMSO-d_6, C_6F_6): δ = -80.07, -164.90 (C_6F_6) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 203.1, λ_{max2}, nm: 259.8, λ_{max3}, nm: 281.1, λ_{max4}, nm: 438.4, $3*10^{-5}$ M. **ESI MS**: for $C_{41}H_{51}N_7O_6^{2+}$ calcd: *m/z* 368.7, found 368.9 [M^{2+}]. **HRMS**: for $C_{41}H_{51}N_7O_6^{2+}$ calcd: *m/z* 368.6945, found 368.6945 [M^{2+}], Δ 0.0 ppm.

2-(((1-(2-(2-(2-(2-((1,3-Dioxo-2-propyl-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-N^1,N^1,N^1,N^3,N^3,N^3,2-heptamethylpropane-1,3-diaminium diiodide (NPNI-NH-TEG-MTZ-O-TMA2 **51).** Prg-O-TMA2 diiodide **6** (0.174 g, 0.35 mmol) and NPNI-NH-TEG-N_3 **44** (0.20 g, 0.44 mmol) were dissolved in MeOH/H_2O 1/1 mixture (11.0 mL). The mixture was bubbled with argon for 30 minutes. CuI (0.067 g, 0.35 mmol) was added, and the reaction mixture was stirred for 7 hours at 60°C. The reaction was monitored by TLC and RP-18 TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by methods M1 and M2. The reaction mixture was filtered and evaporated on a rotary evaporator at 50°C. The crude product (0.39 g) was purified by a C18 RP column chromatography (7.0 g silica gel) using 10% w/w MeOH aq. solution for elution. Fractions with the product were evaporated on a rotary evaporator at 50°C. The product (0.20 g) was dissolved in H_2O (40 mL), and the solution was washed with $CHCl_3$ (40 mL). The aqueous phase was evaporated on a rotary evaporator at 50°C, and the product was dried at 70°C using an oil rotary pump. The product was obtained as a yellow-orange glassy solid in a 48% yield (0.16 g). **IR(DRIFT)**: 3449, 2923, 2872, 1637, 1583, 1347, 1245, 1105 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 8.70 (dd, J = 8.5, 1.3 Hz, 1H, H-12), 8.44 (dd, J = 7.4, 1.0 Hz, 1H, H-6), 8.26 (d, J = 8.6 Hz, 1H, H-10), 8.14 (s, 1H, H-24), 7.79 (t, J = 5.6 Hz, 1H, NH), 7.69 (dd, J = 8.5, 7.3 Hz, 1H, H-11), 6.84 (d, J = 8.6 Hz, 1H, H-7), 4.58 (s, 2H, H-26), 4.48 (t, J = 5.2 Hz, 2H, H-23), 3.97 (t, J = 7.3 Hz, 2H, H-3), 3.78 (t, J = 5.3 Hz, 2H, H-22), 3.71 (t, J = 5.7 Hz, 2H, H-17), 3.65 – 3.44 (m, 16H, H-16, H-18, H-19, H-20, H-21, H-27, H-30), 3.19 (s, 18H, H-31), 1.62 (h, J = 7.4 Hz, 2H, H-2), 1.39 (s, 3H, H-29), 0.90 (t, J = 7.4 Hz, 3H, H-1) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 163.75 (C-15), 162.94 (C-4), 150.60 (C-8), 142.48 (C-25), 134.16 (C-10), 130.72 (C-6), 129.39 (C-13), 128.53 (C-12), 124.69 (C-24), 124.37 (C-11), 121.89 (C-14), 120.11 (C-9), 107.77 (C-5), 104,01 (C-7), 72.32 – 69.24 (C-18, C-19, C-20, C-21, C-27, C-30), 68.65 (C-22), 68.18 (C-17), 63.38 (C-26), 52.28 (C-31), 49.34 (C-23), 42.88 (C-16), 42.70 (C-28), 40.76 (C-3), 21.15 (C-29), 20.98 (C-2), 11.41 (C-1) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 203.2, λ_{max2}, nm:



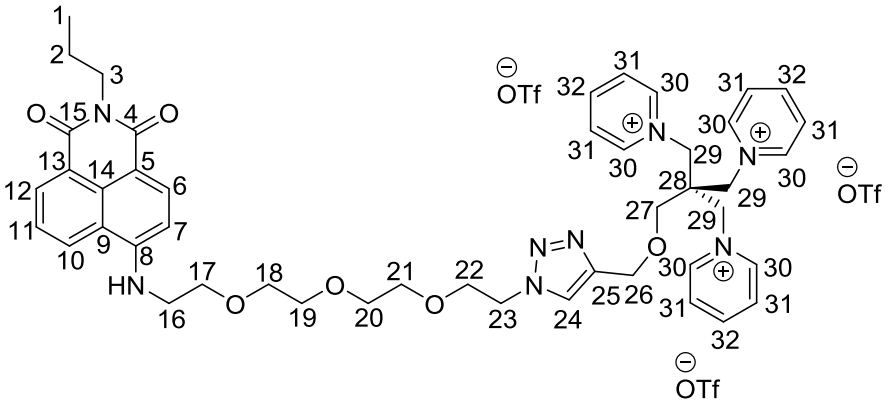
217.2, λ_{max3}, nm: 259.4, λ_{max4}, nm: 281.8, λ_{max5}, nm: 438.2, $3*10^{-5}$ M. **ESI MS**: for $C_{37}H_{59}N_7O_6^{2+}$ calcd: *m/z* 348.7, found 348.7 [M^{2+}]. **HRMS**: for $C_{37}H_{59}N_7O_6^{2+}$ calcd: *m/z* 348.7258, found 348.7262 [M^{2+}], Δ 1.1 ppm.

3,3'-(2-(((1-(2-(2-(2-(2-((1,3-Dioxo-2-propyl-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2-((1-methyl-1*H*-imidazol-3-ium-3-yl)methyl)propane-1,3-diyl)bis(1-methyl-1*H*-imidazol-3-ium) trichloride (NPNI-NH-TEG-MTZ-O-MIM3 **52).** Prg-O-MIM3 trichloride **7** (0.057 g, 0.12 mmol) and NPNI-NH-TEG-N_3 **44** (0.066 g, 0.15 mmol) were dissolved in MeOH (4.0 mL). The mixture was cooled down to 0°C and bubbled with argon for 20 minutes. $CuSO_4 \cdot 5H_2O$ (15.0 mg, 61.0 μmol) and sodium ascorbate (72.0 mg, 0.36 mmol) were added, and the reaction mixture was stirred at 0°C for 17 hours. The reaction was monitored by TLC and RP-18 TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/10/9 mixture. Spots were detected by methods M1 and M2. The reaction mixture was filtered and evaporated on a rotary evaporator at 40°C. The crude product (0.15 g) was purified by column chromatography (5.5 g silica gel) eluting with MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/10/9 mixture. Fractions with the product were evaporated on a rotary evaporator at 60°C. The product (0.8501 g) was dissolved in H_2O (100 mL) and purified using a weak cation exchanger Amberlite® CG50 (11 mL, NH_4^+ form). The elution solutions were successively H_2O and 1.4 M NH_4HCO_3 aq. solution (both 200 mL). Fractions containing the product were evaporated on a rotary evaporator at 50°C. The residue (0.18 g) was suspended in MeOH (5 mL), filtered, neutralized with 1 M HCl, and evaporated on a rotary evaporator at 50°C. The product (0.086 g) was dissolved in H_2O (15 mL) and freeze-dried. The product was obtained as an orange solid in a 60% yield (0.068 g). **IR(DRIFT)**: 3408, 3097, 2956, 2923, 2866, 1676, 1640, 1586, 1431, 1398, 1353, 1248, 1174, 1117 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 9.52 (s, 3H, H-30), 8.85 (dd, *J* = 8.5, 1.1 Hz, 1H, H-12), 8.42 (dd, *J* = 7.3, 1.0 Hz, 1H, H-6), 8.24 (d, *J* = 8.6 Hz, 1H, H-10), 8.20 (s, 1H, H-24), 7.80 (s, 3H, H-32), 7.75 (s, 3H, H-31), 7.66 (dd, *J* =



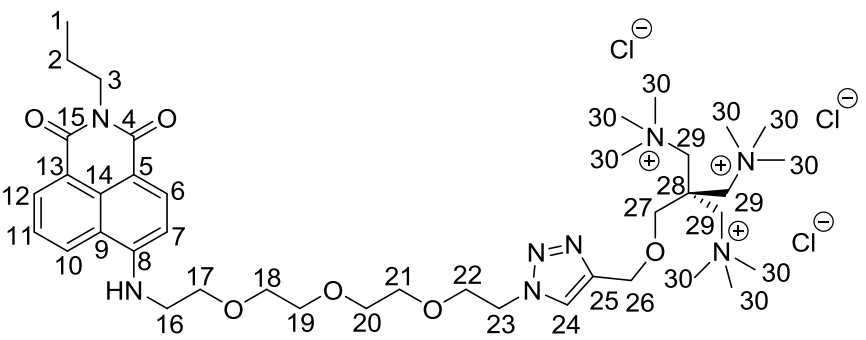
8.4, 7.3 Hz, 1H, H-11), 6.82 (d, *J* = 8.7 Hz, 1H, H-7), 4.59 (s, 6H, H-29), 4.51 (t, *J* = 5.2 Hz, 2H, H-23), 4.42 (s, 2H, H-26), 3.98 (t, *J* = 6.1 Hz, 2H, H-3), 3.87 (s, 9H, H-33), 3.81 (t, *J* = 5.2 Hz, 2H, H-22), 3.71 (t, *J* = 5.9 Hz, 2H, H-17), 3.64 (s, 2H, H-27), 3.58 – 3.47 (m, 10H, H-16, H-18, H-19, H-20, H-21), 1.61 (h, *J* = 7.5 Hz, 2H, H-2), 0.89 (t, *J* = 7.4 Hz, 3H, H-1) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 164.28 (C-15), 163.44 (C-4), 151.28 (C-8), 143.03 (C-25), 138.85 (C-30), 134.71 (C-10), 131.22 (C-6), 129.93 (C-13), 129.50 (C-12), 125.08 (C-24), 124.76 (C-11), 124.33 (C-31), 123.92 (C-32), 122.29 (C-14), 120.67 (C-9), 108.07 (C-5), 104.42 (C-7), 70.28 – 69.97 (C-18, C-19, C-20, C-21, C-27), 69.10 (C-22), 68.65 (C-17), 63.94 (C-26), 50.39 (C-29), 49.88 (C-23), 43.06 (C-16), 41.24 (C-3), 39.59 (C-28, solvent overlay), 36.47 (C-33), 21.48 (C-2), 11.91 (C-1) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 203.9, λ_{max2}, nm: 259.5, λ_{max3}, nm: 281.9, λ_{max4}, nm: 438.3, 3*10^{-5} M. **ESI MS**: for $C_{43}H_{58}N_{11}O_6^{3+}$ calcd: *m/z* 274.8, found 275.0 [M^{3+}]. **HRMS**: for $C_{43}H_{58}N_{11}O_6^{3+}$ calcd: *m/z* 274.8185 (for [M^{3+}-H^+]$^{2+}$ calcd: *m/z* 411.7241), found 411.7256 [M^{3+}-H^+]$^{2+}$, Δ 3.6 ppm.

1,1'-(2-(((1-(2-(2-(2-(2-((1,3-Dioxo-2-propyl-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-2-(pyridin-1-ium-1-ylmethyl)propane-1,3-diyl)bis(pyridin-1-ium) tris(trifluoromethanesulfonate) (NPNI-NH-TEG-MTZ-O-PYR3 **53).** Prg-O-PYR3 tris(trifluoromethanesulfonate) **8** (0.14 g, 0.18 mmol) and NPNI-NH-TEG-N_3 **44** (0.1 g, 0.22 mmol) were dissolved in MeOH (6.0 mL). The mixture was cooled down to 0°C and bubbled with argon for 20 minutes. $CuSO_4 \cdot 5H_2O$ (22.0 mg, 87.8 μmol) and sodium ascorbate (0.10 g, 0.53 mmol) were added, and the reaction mixture was stirred at 0°C for 23 hours. The reaction was monitored by TLC and RP-18 TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/10/9 mixture. Spots were detected by methods M1 and M2. The reaction mixture was filtered and evaporated on a rotary evaporator at 40°C. The crude product (0.26 g) was dissolved in H_2O (30 mL) and washed with $CHCl_3$ (3 × 40 mL). The product



in the aqueous phase was purified by a C18 RP column chromatography (6.0 g silica gel) using H_2O and 10-20% w/w MeOH aq. solution for elution. Fractions with the product were evaporated on a rotary evaporator at 50°C. The product was dissolved in H_2O (5.0 mL) and freeze-dried. The product was obtained as an orange glassy solid in a 60% yield (0.13 g). **IR(DRIFT)**: 3564, 3373, 3134, 3064, 2966, 2873, 1682, 1633, 1581, 1549, 1495, 1462, 1431, 1394, 1360, 1257, 1165, 1030 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 9.03 (bs, 6H, H-30), 8.74 – 8.65 (m, 4H, H-12, H-32), 8.48 – 8.41 (m, 1H, H-6), 8.25 (d, *J* = 8.5 Hz, 1H, H-10), 8.18 (bs, 6H, H-31), 8.03 (s, 1H, H-24), 7.78 (t, *J* = 5.5 Hz, 1H, NH), 7.68 (t, *J* = 7.8 Hz, 1H, H-11), 6.82 (d, *J* = 8.7 Hz, 1H, H-7), 5.20 (bs, 6H, H-29), 4.50 (t, *J* = 5.2 Hz, 2H, H-23), 4.43 (s, 2H, H-26), 4.01 – 3.93 (m, 2H, H-3), 3.86 (bs, 2H), 3.80 (t, *J* = 5.2 Hz, 2H, H-22), 3.70 (t, *J* = 5.6 Hz, 2H, H-17), 3.58 – 3.45 (m, 12H, H-16, H-18, H-19, H-20, H-21, H-27), 1.62 (h, *J* = 7.6 Hz, 2H, H-2), 0.90 (t, *J* = 7.4 Hz, 3H, H-1) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 163.76 (C-15), 162.96 (C-4), 150.63 (C-8), 146.88 (C-32), 146.54 (C-30), 141.89 (C-25), 134.16 (C-10), 130.74 (C-6), 129.39 (C-13), 128.53 (C-12), 128.24 (C-31), 124.80 (C-24), 124.37 (C-11), 121.90 (C-14), 120.11 (C-9), 107.77 (C-5), 103.98 (C-7), 69.79 – 69.46 (C-18, C-19, C-20, C-21, C-27), 68.67 (C-22), 68.19 (C-17), 63.16 (C-26), 61.17 (C-29), 49.37 (C-23), 43.29 (C-28), 42.70 (C-16), 40.76 (C-3), 20.98 (C-2), 11.40 (C-1) ppm. **^{19}F NMR** (376 MHz, DMSO-d_6, C_6F_6): δ = -80.05, -164.90 (C_6F_6) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 202.8, λ_{max2}, nm: 260.3, λ_{max3}, nm: 438.3, 2*10^{-5} M. **ESI MS**: for $C_{46}H_{55}N_8O_6^{3+}$ calcd: *m/z* 271.8 (for $[M^{3+}+HOTf]^{3+}$ calcd: *m/z* 321.8), found 321 $[M^{3+}+HOTf]^{3+}$. **HRMS**: for $C_{46}H_{55}N_8O_6^{3+}$ calcd: *m/z* 271.8076 (for $[M^{3+}-H^+]^{2+}$ calcd: *m/z* 407.2078), found 407.2066 $[M^{3+}-H^+]^{2+}$, Δ 2.9 ppm.

2-(((1-(2-(2-(2-(2-((1,3-Dioxo-2-propyl-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-N^1,N^1,N^1,N^3,N^3,N^3-hexamethyl-2-((trimethylammonio)methyl)propane-1,3-diaminium trichloride (NPNI-NH-TEG-MTZ-O-TMA3 **54).** Prg-O-TMA3 trichloride



9 (0.14 g, 0.35 mmol) and NPNI-NH-TEG-N_3 **44** (0.19 g, 0.42 mmol) were dissolved in

MeOH (10.0 mL). The mixture was bubbled with argon for 1 hour. $CuSO_4 \cdot 5H_2O$ (43.0 mg, 0.17 mmol) and sodium ascorbate (0.205 g, 1.04 mmol) were added, and the reaction mixture was stirred at room temperature for 17 hours. The reaction was monitored by TLC and RP-18 TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/10/9 mixture. Spots were detected by methods M1 and M2. The reaction mixture was filtered and evaporated on a rotary evaporator at 40°C. The crude product (0.41 g) was dissolved in H_2O (20 mL) and washed with $CHCl_3$ (5 × 20 mL). The product in the H_2O phase was purified using a weak cation exchanger Amberlite® CG50 (6 mL, NH_4^+ form). The elution solutions were successively H_2O and 1.4 M NH_4HCO_3 aq. solution. Fractions containing pure product were evaporated on a rotary evaporator at 50°C. The residue (0.18 g) was suspended in MeOH (5 mL), filtered, neutralized with 1 M HCl, and evaporated on a rotary evaporator at 50°C. The product (0.21 g) was dissolved in H_2O (4 mL) and freeze-dried. The product was obtained as an orange solid in a 53% yield (0.15 g). **IR(DRIFT)**: 3440, 3019, 2956, 2920, 2875, 1682, 1640, 1583, 1491, 1359, 1290, 1248, 1195, 1108 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 8.79 (d, *J* = 8.3 Hz, 1H, H-12), 8.43 (d, *J* = 7.2 Hz, 1H, H-6), 8.30 (s, 1H, H-24), 8.25 (d, *J* = 8.5 Hz, 1H, H-10), 7.67 (dd, *J* = 8.4, 7.3 Hz, 1H, H-11), 6.84 (d, *J* = 8.7 Hz, 1H, H-7), 4.66 (s, 2H, H-26), 4.50 (t, *J* = 5.2 Hz, 2H, H-23), 4.43 (s, 2H, H-27), 4.27 (s, 6H, H-29), 3.97 (t, *J* = 5.9 Hz, 2H, H-3), 3.80 (t, *J* = 5.2 Hz, 2H, H-22), 3.72 (t, *J* = 5.8 Hz, 2H, H-17), 3.62 – 3.45 (m, 10H, H-16, H-18, H-19, H-20, H-21), 3.39 (s, 27H, H-30), 1.62 (h, *J* = 7.4 Hz, 2H, H-2), 0.90 (t, *J* = 7.4 Hz, 3H, H-1) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 163.75 (C-15), 162.93 (C-4), 150.67 (C-8), 141.79 (C-25), 134.19 (C-10), 130.70 (C-6), 129.41 (C-13), 128.80 (C-12), 125.11 (C-24), 124.30 (C-11), 121.83 (C-14), 120.12 (C-9), 107.65 (C-5), 103.42 (C-7), 69.80 – 69.49 (C-18, C-19, C-20, C-21), 68.96 (C-27), 68.64 (C-29), 68.60 (C-22), 68.15 (C-17), 63.15 (C-26), 56.04 (C-30), 51.48 (C-28), 49.40 (C-23), 42.58 (C-16), 40.74 (C-3), 20.97 (C-2), 11.40 (C-1) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 203.6, λ_{max2}, nm: 259.2, λ_{max3}, nm: 282.0, λ_{max4}, nm: 438.1, $2*10^{-5}$ M. **ESI MS**: for $C_{40}H_{67}N_8O_6^{3+}$ calcd: *m/z* 251.8 (for $[M^{3+}+Cl^-]^{2+}$ calcd: *m/z* 395.2), found 395.6 $[M^{3+}+Cl^-]^{2+}$. **HRMS**: for $C_{40}H_{67}N_8O_6^{3+}$ calcd: *m/z* 825.4555 (for $[M^{3+}+2\times Cl^-]^+$ calcd: *m/z* 825.4555), found 825.4524 $[M^{3+}+2\times Cl^-]^+$, Δ 3.7 ppm.

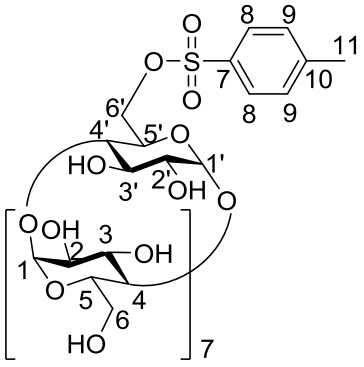
6.8.6 Azido amino oligo(ethylene glycols) cyclodexrins

General procedure for preparation of 6^A-*O*-*p*-Toluenesulfonyl-*α*- and γ-CDs (GP2).

Compounds mono(6-*O*-Ts)-*α*-CD **55** and mono(6-*O*-Ts)-γ-CD **57** were prepared according to the previously published procedure[105], with some modifications of the purification process. CD was suspended in dry pyridine, and the mixture was cooled to 0°C. TsCl (0.9 eq.) solution in dry pyridine was added dropwise over 1 hour. The resulting solution was warmed up to room temperature and stirred for 24 hours. The reaction mixture was monitored by TLC using PrOH/H_2O/EtOAc/conc. NH_3 aq. solution 6/3/1/1 mixture. Spots were detected by the method M5. Pyridine was distilled from the reaction mixture under reduced pressure. The sirup-like crude product was precipitated from acetone and isolated by filtration. The solid crude product was purified by a C18 RP column chromatography using H_2O and 5-25% w/w MeOH aq. solution for elution. Fractions with the pure product were evaporated on a rotary evaporator at 40°C. The product was dissolved in H_2O and freeze-dried.

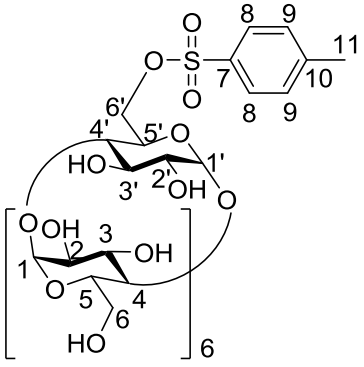
6^A-*O*-*p*-Toluenesulfonyl-*α*-CD (mono(6-*O*-Ts)-*α*-CD **55).** Compound **55** was prepared according to the general procedure (**GP2**). *α*-CD (25.0 g, 26.0 mmol, dried at 90°C for 24 hours) was dissolved in dry pyridine (400 mL). TsCl (4.46 g, 23.4 mmol) in dry pyridine (30 mL) was added. Precipitation was done in acetone (800 mL). C18 RP column chromatography (400 g silica gel) was used for purification. The product (5.9 g) was dissolved in H_2O (130 mL) and freeze-dried. The product was obtained as a white powder in a 10% yield (3.1 g). **$[\alpha]^{25}_D$** = +117.7° (α +0.078, c = 0.34, DMSO). **IR(DRIFT)**: 3351, 2929, 1646, 1595, 1407, 1356, 1290, 1174, 1156, 1084, 1060, 1033 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 7.83 – 7.72 (m, 2H, H-8), 7.45 (m, 2H, H-9), 5.40 (s, 12H, OH), 4.84 – 4.63 (m, 6H, H-1, H-1'), 4.28 (m, 2H, OH), 3.92 – 3.12 (m, 36H, H-2, H-2', H-3, H-3', H-4, H-4', H-5, H-5', H-6, H-6'), 2.41 (s, 3H, H-11) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 144.81 (C-10), 132.45 (C-7), 129.96 (C-9), 127.72 (C-8), 102.28 – 101.51 (C-1, C-1'), 82.43 – 81.63 (C-4, C-4'), 73.44 – 68.74 (C-2, C-2', C-3, C-3', C-5, C-5'), 62.49 (C-6'), 59.90 (C-6), 21.17 (C-11) ppm. **UV-VIS** (H_2O), λ_{max1}, nm: 226.8, λ_{max2}, nm: 262.2, $2*10^{-5}$ M. **ESI MS**: for $C_{43}H_{66}O_{32}S$ calcd: *m/z* 1126.3 (for $[M+H]^+$ calcd: *m/z* 1127.3), found 1127.0 $[M+H]^+$. **HRMS** for $C_{43}H_{66}O_{32}S$ calcd: *m/z* 1126.3258 (for $[M+H]^+$ calcd: *m/z* 1127.3331), found 1127.3321 $[M+H]^+$, Δ 0.9 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[154].

6^{A}-*O*-*p*-Toluenesulfonyl-γ-CD (mono(6-*O*-Ts)-γ-CD **57).** Compound **57** was prepared



according to the general procedure (**GP2**). γ-CD (19.8 g, 15.2 mmol, dried at 90°C for 24 hours) was dissolved in dry pyridine (315 mL). TsCl (2.77 g, 14.5 mmol) in dry pyridine (24 mL) was added. Precipitation was done in acetone (570 mL). C18 RP column chromatography (400 g silica gel) was used for purification. The product (4.5 g) was dissolved in H_2O (95 mL) and freeze-dried. The product was obtained as a white powder in a 15% yield (3.5 g). **[α]^{25}$_D$** = +128.7° (α +0.139, c = 0.54, DMSO). **IR(DRIFT)**: 3324, 2932, 1649, 1598, 1416, 1338, 1296, 1242, 1177, 1156, 1081, 1024 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d$_6$): δ = 7.76 (m, 2H, H-8), 7.45 (m, 2H, H-9), 5.77 (m, 16H, OH), 4.85 (m, 8H, H-1, H-1'), 4.60 – 4.16 (m, 8H, OH, H-6'), 3.82 – 3.20 (m, 47H, H-2, H-2', H-3, H-3', H-4, H-4', H-5, H-5', H-6, H-6', solvent overlay), 2.41 (s, 3H, H-11) ppm. **^{13}C NMR** (101 MHz, DMSO-d$_6$): δ = 144.87 (C-10), 132.56 (C-7), 130.01 (C-9), 127.60 (C-8), 103.44 – 100.76 (C-1, C-1'), 81.50 – 79.74 (C-4, C-4'), 73.47 – 68.60 (C-2, C-2', C-3, C-3', C-5, C-5', C-6'), 60.00 (C-6), 21.16 (C-11) ppm. **UV-VIS** (H_2O), λ_{max1}, nm: 226.7, λ_{max2}, nm: 262.5, 1*10^{-5} M. **ESI MS**: for $C_{55}H_{86}O_{42}S$ calcd: *m/z* 1450.4 (for $[M+Na]^+$ calcd: *m/z* 1473.4), found 1473.4 $[M+Na]^+$. **HRMS** for $C_{55}H_{86}O_{42}S$ calcd: *m/z* 1450.4314 (for $[M+H]^+$ calcd: *m/z* 1451.4387), found 1451.4344 $[M+H]^+$, Δ 3.0 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[154].

6^{A}-*O*-*p*-Toluenesulfonyl-β-CD (mono(6-*O*-Ts)-β-CD **56).** Compound mono(6-*O*-Ts)-β-



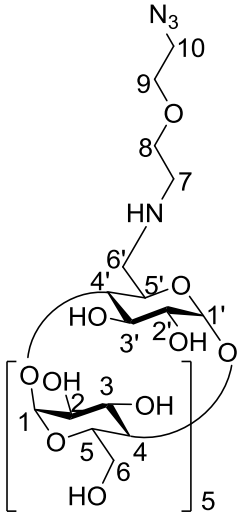
CD **56** was prepared according to the previously published procedure[154]. The suspension of β-CD (58.3 g, 51.4 mmol, not dried) and Ts_2O (25.1 g, 77.0 mmol) in H_2O (1200 ml) was stirred for 2 hours at room temperature. A solution of NaOH (25 g) in H_2O (240 ml) was added. After 10 minutes, unreacted Ts_2O was separated by filtration, the filtrate was neutralized with 10 M HCl, and the solution was put into a fridge for a night. The resulting precipitate was collected by filtration, washed with ice cold H_2O, and dried at 70°C using an oil rotary pump. The crude product was purified by repeated recrystallization from H_2O/MeOH 1/1 mixture. The pure product was dried at 70°C using an oil rotary pump and obtained as a white crystalline solid in an

8% yield (5.6 g). $[\alpha]^{25}_{D}$ = +117.3° (α +0.088, c = 0.38, DMSO). **IR(DRIFT)**: 3303, 2926, 2905, 1649, 1598, 1407, 1362, 1293, 1240, 1174, 1156, 1078, 1033 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 7.75 (m, 2H, H-8), 7.43 (m, 2H, H-9), 5.73 (m, 14H, OH), 4.91 – 4.71 (m, 7H, H-1, H-1'), 4.59 – 4.11 (m, 7H, OH, H-6'), 3.81 – 3.15 (m, 41H, H-2, H-2', H-3, H-3', H-4, H-4', H-5, H-5', H-6, H-6', solvent overlay), 2.43 (s, 3H, H-11) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 144.84 (C-10), 132.69 (C-7), 129.92 (C-9), 127.62 (C-8), 103.20 – 100.85 (C-1, C-1'), 82.06 – 80.36 (C-4, C-4'), 73.69 – 68.31 (C-2, C-2', C-3, C-3', C-5, C-5', C-6'), 60.33 – 58.96 (C-6), 21.24 (C-11) ppm. **UV-VIS** (H_2O), λ_{max1}, nm: 228.0, λ_{max2}, nm: 262.2, $2*10^{-5}$ M. **ESI MS**: for $C_{49}H_{76}O_{37}S$ calcd: *m/z* 1288.4 (for $[M+Na]^+$ calcd: *m/z* 1311.4), found 1311.5 $[M+Na]^+$. **HRMS** for $C_{49}H_{76}O_{37}S$ calcd: *m/z* 1288.3786 (for $[M+H]^+$ calcd: *m/z* 1289.3859), found 1289.3853 $[M+H]^+$, Δ 0.5 ppm. ^{1}H NMR spectrum is in accordance with the literature[400].

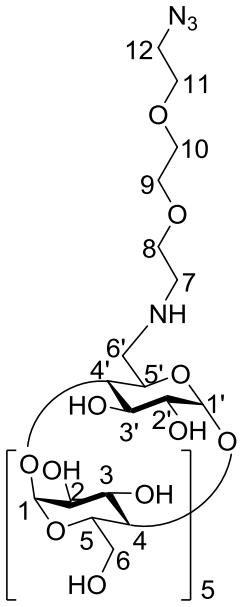
General procedure for preparation of azido amino oligo(ethylene glycols) CDs (GP3).

Mono(6-*O*-Ts)-CD (0.89 mmol for mono(6-*O*-Ts)-α-CD **55**, 0.78 mmol for mono(6-*O*-Ts)-β-CD **56**, and 0.69 mmol for mono(6-*O*-Ts)-γ-CD **57**) was mixed with azido amino oligo(ethylene glycols) NH_2-DEG-N_3 **33**, NH-TrEG-N **34**, or **35** (2.0 mL), and the mixture was stirred for 24 hours at 60°C. The reaction mixture was monitored by TLC using PrOH/H_2O/EtOAc/conc. NH_3 aq. solution 6/3/1/1 mixture. Spots were detected by the method M5. The resulting solution was diluted with H_2O (2 mL), and the reaction mixture was poured into acetone (200 mL). The precipitate was collected by filtration and dried at room temperature for 2 hours using an oil rotary pump. The crude product was dissolved in H_2O (14 mL) and poured into acetone (200 mL). The formed precipitate was collected by centrifugation, dissolved in the lowest possible amount of H_2O, and purified using a strong cation exchanger, Amberlite® IR 120 (160 mL, H^+ form). The side-products eluted with H_2O and the main product eluted with 5% w/w NH_3 aq. solution. The product was collected and evaporated on a rotary evaporator at 50°C. The evaporated product was dissolved in H_2O (14 mL) and freeze-dried.

Mono-(*N*-(2-(2-azidoethoxy)eth-1-yl-6^{A}-amino-6^{A}-deoxy)-α-CD (mono[6-(NH-DEG-N_3)]-α-CD **58).** Compound **58** was prepared according to the general procedure (**GP3**). This product was prepared on a smaller scale (0.61 mmol of mono(6-*O*-Ts)-α-CD **55** and 1.3 mL of NH_2-DEG-N_3 **33**), and thus all solvents, reagents, and sorbents were reduced proportionally. The product was obtained as a white powder in a 59% yield (0.39 g). **[α]$^{25}_{D}$** = +114.8° (α +0.072, c = 0.32, H_2O). **IR(DRIFT)**: 3312, 2928, 2096 ν(azide), 1661, 1410, 1367, 1297, 1153, 1078, 1035 cm^{-1}. **^{1}H NMR** (600 MHz, D_2O, *t*BuOH): δ = 5.05 – 5.04 (m, 6H, H-1, H-1'), 3.99 – 3.59 (m, 37H, H-2, H-2', H-3, H-3', H-4, H-5, H-5', H-6, H-8, H-9), 3.51 (bs, 2H, H-10), 3.43 (t, *J* = 9.3 Hz, 1H, H-4'), 3.12 – 2.79 (m, 4H, H-6', H-7), 1.24 (s, *t*BuOH) ppm. **^{13}C NMR** (151 MHz, D_2O, *t*BuOH): δ = 102.22 – 101.76 (C-1, C-1'), 84.53 – 81.63 (C-4, C-4'), 74.05 – 73.85 (C-3, C-3'), 72.86 – 72.31 (C-2, C-2', C-5), 71.43 (C-5'), 70.37 (*t*BuOH), 70.22 – 69.89 (C-8, C-9), 61.06 – 60.91 (C-6), 51.13 (C-10), 50.06 (C-6'), 48.44 (C-7), 30.29 (*t*BuOH) ppm. **ESI MS**: for $C_{40}H_{68}N_4O_{30}$ calcd: *m/z* 1084.4 (for $[M+H]^+$ calcd: *m/z* 1085.4), found 1085.0 $[M+H]^+$. **HRMS**: for $C_{40}H_{68}N_4O_{30}$ calcd: *m/z* 1084.3918 (for $[M+Na]^+$ calcd: *m/z* 1107.3811), found 1107.3759 $[M+Na]^+$, Δ 4.7 ppm.



Mono-(*N*-(2-(2-(2-azidoethoxy)ethoxy)eth-1-yl-6^{A}-amino-6^{A}-deoxy)-α-CD (mono[6-(NH-TrEG-N_3)]-α-CD **59).** Compound **59** was prepared according to the general procedure (**GP3**). The product was obtained as a white powder in a 75% yield (0.76 g). **[α]$^{25}_{D}$** = +114.8° (α +0.072, c = 0.32, H_2O). **IR(DRIFT)**: 3321, 2927, 2101 ν(azide), 1651, 1413, 1296, 1153, 1078, 1036 cm^{-1}. **^{1}H NMR** (600 MHz, D_2O, *t*BuOH): δ = 5.06 – 5.03 (m, 6H, H-1, H-1'), 3.99 – 3.58 (m, 41H, H-2, H-2', H-3, H-3', H-4, H-5, H-5', H-6, H-8, H-9, H-10, H-11), 3.52 (t, *J* = 4.7 Hz, 2H, H-12), 3.42 (t, *J* = 9.1 Hz, 1H, H-4'),3.11 – 2.74 (m, 4H, H-6', H-7), 1.24 (s, *t*BuOH) ppm. **^{13}C NMR** (151 MHz, D_2O, *t*BuOH): δ = 102.20 – 101.82 (C-1, C-1'), 84.46 – 81.67 (C-4, C-4'), 74.02 – 73.88 (C-3, C-3'), 72.80 – 72.27 (C-2, C-2', C-5), 71.50 (C-5'), 70.31 – 69.87 (C-8, C-9, C-10, C-11), 60.92 – 60.85 (C-6), 50.95 (C-12), 49.96 (C-6'), 48.39 (C-7), 30.29 (*t*BuOH) ppm. **ESI MS**: for $C_{42}H_{72}N_4O_{31}$ calcd: *m/z* 1128.4 (for $[M+H]^+$ calcd: *m/z* 1129.4), found 1129.0 $[M+H]^+$. **HRMS**: for $C_{42}H_{72}N_4O_{31}$



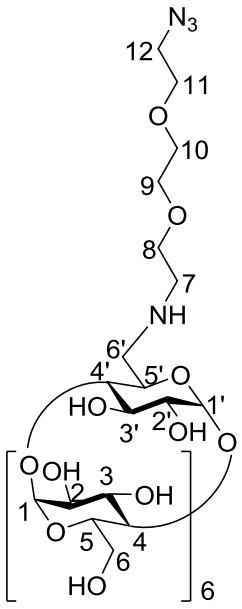
calcd: *m/z* 1128.4181 (for $[M+H]^+$ calcd: *m/z* 1129.4253), found 1129.4260 $[M+H]^+$, Δ 0.6 ppm.

Mono-(*N*-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)eth-1-yl-6^A-amino-6^A-deoxy)-α-CD (mono[6-(NH-TEG-N_3)]-α-CD **60).** Compound **60** was prepared according to the general procedure (**GP3**). The product was obtained as a white powder in a 69% yield (0.71 g). $[\alpha]^{25}_D$ = +106.8° (α +0.063, c = 0.30, H_2O). **IR(DRIFT)**: 3346, 2927, 2100 ν(azide), 1647, 1413, 1362, 1332, 1297, 1152, 1078, 1033 cm^{-1}. **^{1}H NMR** (600 MHz, D_2O, *t*BuOH): δ = 5.06 – 5.04 (m, 6H, H-1, H-1'), 3.99 – 3.59 (m, 45H, H-2, H-2', H-3, H-3', H-4, H-5, H-5', H-6, H-8, H-9, H-10, H-11, H-12, H-13), 3.53 (m, 2H, H-14), 3.43 (t, *J* = 9.1 Hz, 1H, H-4'), 3.12 – 2.75 (m, 4H, H-6', H-7), 1.24 (s, *t*BuOH) ppm. **^{13}C NMR** (151 MHz, D_2O, *t*BuOH): δ = 102.30 – 101.96 (C-1, C-1'), 84.58 – 81.77 (C-4, C-4'), 74.11 –74.01 (C-3, C-3'), 72.80 – 72.33 (C-2, C-2', C-5), 71.66 (C-5'), 70.55 – 69.96 (C-8, C-9, C-10, C-11, C-12, C-13), 60.95 – 60.87 (C-6), 50.87 (C-14), 50.06 (C-6'), 48.44 (C-7), 30.29 (*t*BuOH) ppm. **ESI MS**: for $C_{44}H_{76}N_4O_{32}$ calcd: *m/z* 1172.4 (for $[M+H]^+$ calcd: *m/z* 1173.5), found 1173.0 $[M+H]^+$. **HRMS**: for $C_{44}H_{76}N_4O_{32}$ calcd: *m/z* 1172.4443 (for $[M+H]^+$ calcd: *m/z* 1173.4515), found 1173.4467 $[M+H]^+$, Δ 4.1 ppm.

Mono-(*N*-(2-(2-azidoethoxy)eth-1-yl-6^A-amino-6^A-deoxy)-β-CD (mono[6-(NH-DEG-N_3)]-β-CD **61).** Compound **61** was prepared according to the general procedure (**GP3**). The product was obtained as a white powder in a 94% yield (0.92 g). $[\alpha]^{25}_D$ = +121.4° (α +0.085, c = 0.35, H_2O). **IR(DRIFT)**: 3324, 2932, 2113 ν(azide), 1651, 1455, 1300, 1155, 1079, 1032 cm^{-1}. **^{1}H NMR** (600 MHz, D_2O, *t*BuOH): δ = 5.07 – 5.02 (m, 7H, H-1, H-1'), 3.92 – 3.56 (m, 43H, H-2, H-2', H-3, H-3', H-4, H-5, H-5', H-6, H-8, H-9), 3.42 (t, *J* = 4.8 Hz, 2H, H-10), 3.38 (t, *J* = 9.5 Hz, 1H, H-4'), 3.10 – 2.74 (m, 4H, H-6', H-7), 1.24 (s, *t*BuOH) ppm. **^{13}C NMR** (151 MHz, D_2O, *t*BuOH): δ = 102.62 – 101.98 (C-1, C-1'), 84.46 – 81.12 (C-4, C-4'), 73.71 –73.40 (C-3, C-3'), 72.58 – 72.32 (C-2, C-2', C-5), 70.28 (C-5'), 70.10 – 69.72 (C-8, C-9), 60.62 – 60.45 (C-6), 50.65 (C-10), 49.63 (C-6'), 47.77 (C-7), 30.29 (*t*BuOH) ppm. **ESI MS**: for $C_{46}H_{78}N_4O_{35}$

calcd: *m/z* 1246.4 (for $[M+H]^+$ calcd: *m/z* 1247.5), found 1248.0 $[M+H]^+$. **HRMS**: for $C_{46}H_{78}N_4O_{35}$ calcd: *m/z* 1246.4447 (for $[M+H]^+$ calcd: *m/z* 1247.4519), found 1247.4518 $[M+H]^+$, Δ 0.1 ppm.

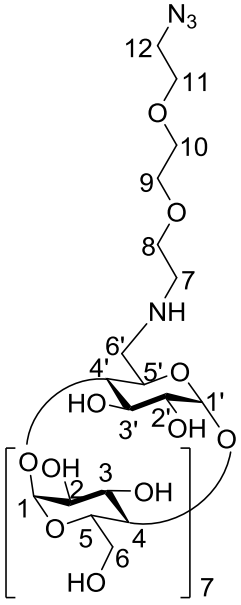
Mono-(*N*-(2-(2-(2-azidoethoxy)ethoxy)eth-1-yl-6^A-amino-6^A-deoxy)-β-CD (mono[6-(NH-TrEG-N_3)]-β-CD **62).** Compound **62** was prepared according to the general procedure (**GP3**). The product was obtained as a white powder in a 90% yield (0.90 g). **$[\alpha]^{25}_D$** = +120.6° (α +0.076, c = 0.35, H_2O). **IR(DRIFT)**: 3343, 2923, 2112 ν(azide), 1654, 1544, 1416, 1369, 1300, 1156, 1079, 1032 cm^{-1}. **^{1}H NMR** (600 MHz, D_2O, *t*BuOH): δ = 5.07 – 5.02 (m, 7H, H-1, H-1'), 3.94 – 3.55 (m, 47H, H-2, H-2', H-3, H-3', H-4, H-5, H-5', H-6, H-8, H-9, H-10, H-11), 3.46 (t, *J* = 4.9 Hz, 2H, H-12), 3.39 (t, *J* = 9.3 Hz, 1H, H-4'), 3.08 – 2.74 (m, 4H, H-6', H-7), 1.24 (s, *t*BuOH) ppm. **^{13}C NMR** (151 MHz, D_2O, *t*BuOH): δ = 102.51 – 101.80 (C-1, C-1'), 84.39 – 81.02 (C-4, C-4'), 73.70 –73.38 (C-3, C-3'), 72.57 – 72.25 (C-2, C-2', C-5), 70.55 (C-5'), 70.28 – 69.78 (C-8, C-9, C-10, C-11), 60.59 – 60.48 (C-6), 50.58 (C-12), 49.42 (C-6'), 47.68 (C-7), 30.29 (*t*BuOH) ppm. **ESI MS**: for $C_{48}H_{82}N_4O_{36}$ calcd: *m/z* 1290.5 (for $[M+H]^+$ calcd: *m/z* 1291.5), found 1291.0 $[M+H]^+$. **HRMS**: for $C_{48}H_{82}N_4O_{36}$ calcd: *m/z* 1290.4709 (for $[M+H]^+$ calcd: *m/z* 1291.4782), found 1291.4749 $[M+H]^+$, Δ 2.6 ppm. ^{13}C NMR and ESI MS spectra are in accordance with the literature[372].



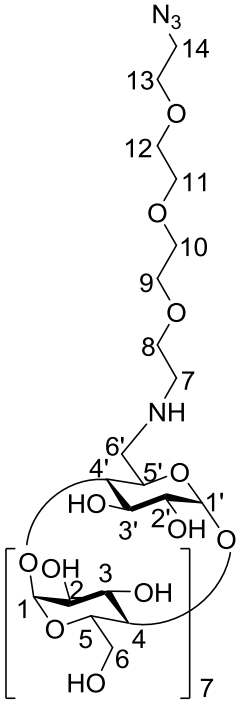
Mono-(*N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)eth-1-yl-6^{A}-amino-6^{A}-deoxy)-β-CD (mono[6-(NH-TEG-N_3)]-β-CD **63).** Compound **63** was prepared according to the general procedure (**GP3**). The product was obtained as a white powder in an 85% yield (0.88 g). **[α]$^{25}_{D}$** = +89.4° (α +0.059, c = 0.33, H_2O). **IR(DRIFT)**: 3361, 2925, 2113 ν(azide), 1645, 1301, 1154, 1080, 1031 cm^{-1}. **^{1}H NMR** (600 MHz, D_2O, *t*BuOH): δ = 5.07 – 5.03 (m, 7H, H-1, H-1'), 3.92 – 3.56 (m, 51H, H-2, H-2', H-3, H-3', H-4, H-5, H-5', H-6, H-8, H-9, H-10, H-11, H-12, H-13), 3.48 (m, 2H, H-14), 3.41 (t, *J* = 9.3 Hz, 1H, H-4'), 3.11 – 2.74 (m, 4H, H-6', H-7), 1.24 (s, *t*BuOH) ppm. **^{13}C NMR** (151 MHz, D_2O, *t*BuOH): δ = 102.46 – 101.78 (C-1, C-1'), 84.35 – 80.99 (C-4, C-4'), 73.60 –73.33 (C-3, C-3'), 72.43 – 72.19 (C-2, C-2', C-5), 70.72 (C-5'), 70.32 – 69.39 (C-8, C-9, C-10, C-11, C-12, C-13), 60.52 – 60.39 (C-6), 50.51 (C-14), 49.43 (C-6'), 47.74 (C-7), 30.29 (*t*BuOH) ppm. **ESI MS**: for $C_{50}H_{86}N_4O_{37}$ calcd: *m/z* 1334.5 (for $[M+H]^+$ calcd: *m/z* 1335.5), found 1336.0 $[M+H]^+$. **HRMS**: for $C_{50}H_{86}N_4O_{37}$ calcd: *m/z* 1334.4971 (for $[M+H]^+$ calcd: *m/z* 1335.5044), found 1335.4979 $[M+H]^+$, Δ 4.8 ppm.

Mono-(*N*-(2-(2-azidoethoxy)eth-1-yl-6^{A}-amino-6^{A}-deoxy)-γ-CD (mono[6-(NH-DEG-N_3)]-γ-CD **65).** Compound **65** was prepared according to the general procedure (**GP3**). The product was obtained as a white powder in an 81% yield (0.79 g). **[α]$^{25}_{D}$** = +117.4° (α +0.101, c = 0.43, H_2O). **IR(DRIFT)**: 3272, 2931, 2112 ν(azide), 1649, 1417, 1335, 1157, 1079, 1028 cm^{-1}. **^{1}H NMR** (600 MHz, D_2O, *t*BuOH): δ = 5.16 – 5.08 (m, 8H, H-1, H-1'), 3.97 – 3.57 (m, 49H, H-2, H-2', H-3, H-3', H-4, H-5, H-5', H-6, H-8, H-9), 3.49 (m, 2H, H-10), 3.44 (t, *J* = 9.5 Hz, 1H, H-4'),3.08 – 2.80 (m, 4H, H-6', H-7), 1.24 (s, *t*BuOH) ppm. **^{13}C NMR** (151 MHz, D_2O, *t*BuOH): δ = 102.54 – 101.33 (C-1, C-1'), 82.96 – 80.32 (C-4, C-4'), 73.73 –73.27 (C-3, C-3'), 73.03 – 72.36 (C-2, C-2', C-5), 71.00 (C-5'), 70.07 – 69.96 (C-8, C-9), 60.95 – 60.74 (C-6), 50.01 (C-10), 49.84 (C-6'), 48.39 (C-7), 30.29 (*t*BuOH) ppm. **ESI MS**: for $C_{52}H_{88}N_4O_{40}$ calcd: *m/z* 1408.5 (for $[M+H]^+$ calcd: *m/z* 1409.5), found 1409.0 $[M+H]^+$. **HRMS**: for $C_{52}H_{88}N_4O_{40}$ calcd: *m/z* 1408.4975 (for $[M+H]^+$ calcd: *m/z* 1409.5048), found 1409.4990 $[M+H]^+$, Δ 4.1 ppm.

Mono-(*N*-(2-(2-(2-azidoethoxy)ethoxy)eth-1-yl-6^{A}-amino-6^{A}-deoxy)-γ-CD (mono[6-(NH-TrEG-N_3)]-γ-CD **66).** Compound **66** was prepared according to the general procedure (**GP3**). This product was prepared on a smaller scale (0.37 mmol of mono(6-*O*-Ts)-γ-CD **57** and 1.0 mL of NH-TrEG-N **34**), and thus all solvents, reagents, and sorbents were reduced proportionally. The product was obtained as a white powder in a 56% yield (0.30 g). **[α]$^{25}_{D}$** = +98.8° (α +0.065, c = 0.33, H_2O). **IR(DRIFT)**: 3332, 2928, 2114 ν(azide), 1652, 1448, 1374, 1299, 1158, 1081, 1030 cm^{-1}. **^{1}H NMR** (600 MHz, D_2O, *t*BuOH): δ = 5.10 – 5.05 (m, 8H, H-1, H-1'), 3.97 – 3.53 (m, 53H, H-2, H-2', H-3, H-3', H-4, H-5, H-5', H-6, H-8, H-9, H-10, H-11), 3.50 (t, *J* = 4.9 Hz, 2H, H-12), 3.37 (t, *J* = 9.5 Hz, 1H, H-4'), 3.07 – 2.74 (m, 4H, H-6', H-7), 1.24 (s, *t*BuOH) ppm. **^{13}C NMR** (151 MHz, D_2O, *t*BuOH): δ = 102.90 – 102.07 (C-1, C-1'), 84.06 – 80.82 (C-4, C-4'), 74.08 –73.17 (C-3, C-3'), 72.66 – 72.46 (C-2, C-2', C-5), 70.98 (C-5'), 70.46 – 70.06 (C-8, C-9, C-10, C-11), 60.95 – 60.76 (C-6), 50.88 (C-12), 49.81 (C-6'), 48.11 (C-7), 30.29 (*t*BuOH) ppm. **ESI MS**: for $C_{54}H_{92}N_4O_{41}$ calcd: *m/z* 1452.5 (for $[M+H]^+$ calcd: *m/z* 1453.5), found 1453.0 $[M+H]^+$. **HRMS**: for $C_{54}H_{92}N_4O_{41}$ calcd: *m/z* 1452.5237 (for $[M+H]^+$ calcd: *m/z* 1453.5310), found 1453.5256 $[M+H]^+$, Δ 3.7 ppm.



Mono-(*N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)eth-1-yl-6^{A}-amino-6^{A}-deoxy)-γ-CD (mono[6-(NH-TEG-N_3)]-γ-CD **67).** Compound **67** was prepared according to the general procedure (**GP3**). The product was obtained as a white powder in a 72% yield (0.74 g). **[α]$^{25}_{D}$** = +84.1° (α +0.58, c = 0.35, H_2O). **IR(DRIFT)**: 3315, 2924, 2113 ν(azide), 1653, 1410, 1366, 1302, 1156, 1080, 1030 cm^{-1}. **^{1}H NMR** (600 MHz, D_2O, *t*BuOH): δ = 5.14 – 5.07 (m, 8H, H-1, H-1'), 3.97 – 3.58 (m, 57H, H-2, H-2', H-3, H-3', H-4, H-5, H-5', H-6, H-8, H-9, H-10, H-11, H-12, H-13), 3.51 (t, *J* = 4.1 Hz, 2H, H-14), 3.41 (t, *J* = 9.5 Hz, 1H, H-4'), 3.08 – 2.75 (m, 4H, H-6', H-7), 1.24 (s, *t*BuOH) ppm. **^{13}C NMR** (151 MHz, D_2O, *t*BuOH): δ = 102.81 – 101.68 (C-1, C-1'), 83.86 – 80.44 (C-4, C-4'), 73.94 –73.37 (C-3, C-3'), 72.12 – 72.44 (C-2, C-2', C-5), 70.89 (C-5'), 70.61 – 69.86 (C-8, C-9, C-10, C-11, C-12, C-13), 60.85 – 60.62 (C-6), 50.89 (C-14), 49.71 (C-6'), 48.01 (C-7),



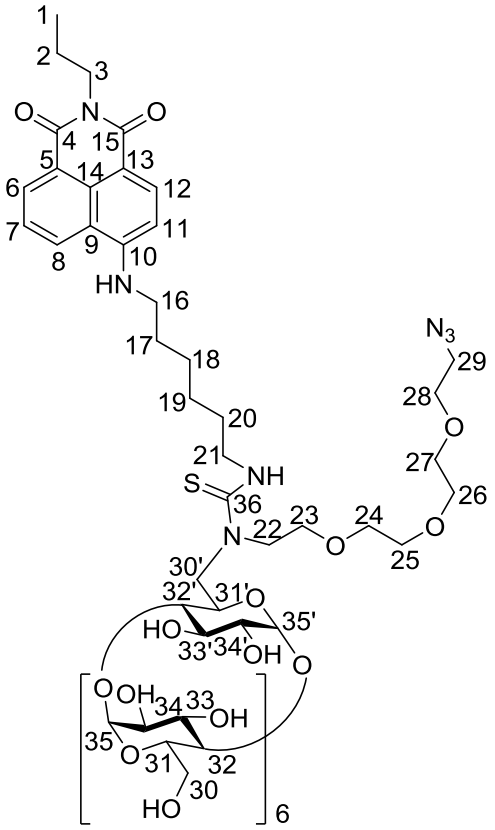
30.29 (*t*BuOH) ppm. **ESI MS**: for $C_{56}H_{96}N_4O_{42}$ calcd: *m/z* 1496.5 (for $[M+H]^+$ calcd: *m/z* 1497.6), found 1498.0 $[M+H]^+$. **HRMS**: for $C_{56}H_{96}N_4O_{42}$ calcd: *m/z* 1496.5499 (for $[M+H]^+$ calcd: *m/z* 1497.5572), found 1497.5567 $[M+H]^+$, Δ 0.3 ppm.

23-Azido-*N*-(6^A-amino-6^A-deoxy-β-CD)-3,6,9,12,15,18,21-heptaoxatricosan-1-amine (mono[6-(NH-OEG-N_3)]-β-CD **64).** Mono(6-*O*-Ts)-β-CD **56** (4.0 g, 3.10 mmol) and NH_2-OEG-N_3 **40** (2.45 g, 6.21 mmol) were dissolved in DMSO (40 mL) and the mixture was stirred for 15 hours at 60°C. The reaction mixture was monitored by TLC using PrOH/H_2O/EtOAc/conc. NH_3 aq. solution 6/4/1/1 mixture. Spots were detected by methods M1 and M5. The reaction was not finished, so the mixture was stirred at 90°C for 22 hours. The reaction mixture was poured into acetone (800 mL). The precipitate was collected by filtration and dried at room temperature for 2 hours using an oil rotary pump. The crude product (6.19 g) was dissolved in H_2O (60 mL) and poured into acetone (800 mL). The formed precipitate was collected by filtration, dissolved in H_2O (70 mL), and purified using a strong cation exchanger, DOWEX® 50W-X8 (90 mL, H^+ form). The side-products eluted with H_2O, and the main product eluted with 10% w/w NH_3 aq. solution. The product was collected and evaporated on a rotary evaporator at 50°C. The evaporated product (1.73 g) was dissolved in H_2O (20 mL) and freeze-dried. The product was obtained as a light brown powder in a 34% yield (1.73 g). **$[\alpha]^{25}_D$** = +117.2° (α +0.068, c = 0.29, H_2O). **IR(DRIFT)**: 3327, 2929, 2899, 2875, 2113 ν(azide), 1643, 1422, 1353, 1302, 1153, 1087, 1030 cm^{-1}. **^{1}H NMR** (600 MHz, D_2O, *t*BuOH): δ = 5.07 (bs, 7H, H-1, H-1'), 3.94 – 3.51 (m, 67H, H-2, H-2', H-3, H-3', H-4, H-5, H-5', H-6, H-8, H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16, H-17, H-18, H-19, H-20, H-21), 3.49 (t, *J* = 4.9 Hz, 2H, H-22), 3.43 (t, *J* = 9.3 Hz, 1H, H-4'), 3.12 – 2.80 (m, 4H, H-6', H-7), 1.24 (s, *t*BuOH) ppm. **^{13}C NMR** (151 MHz, D_2O, *t*BuOH): δ = 102.66 – 101.32 (C-1, C-1'), 84.38 – 81.56 (C-4, C-4'), 73.76 – 69.85 (C-2, C-2', C-3, C-3', C-5, C-5', C-8, C-9, C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-17, C-18, C-19, C-20, C-21), 70.23 (*t*BuOH), 60.79 – 60.62 (C-6), 50.71 (C-22), 49.72 (C-6'), 48.31 (C-7), 30.29 (*t*BuOH) ppm. **ESI MS**: for $C_{58}H_{102}N_4O_{41}$ calcd: *m/z* 1510.6 (for $[M+H]^+$ calcd: *m/z* 1511.6), found 1512.1 $[M+H]^+$. **HRMS**: for

$C_{58}H_{102}N_4O_{41}$ calcd: *m/z* 1510.6019 (for $[M+H]^+$ calcd: *m/z* 1511.6092), found 1511.6048 $[M+H]^+$, Δ 2.9 ppm.

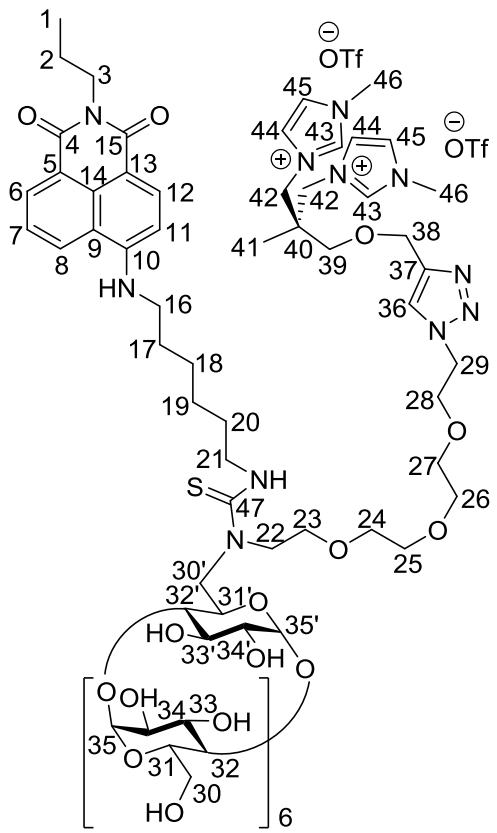
6.8.7 Charged fluorescent cyclodexrin derivatives

1-(2-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)ethyl)-3-(6-((1,3-dioxo-2-propyl-2,3-dihydro-1*H*-benzo[de]isoquinolin-6-yl)amino)hexyl)-1-(6^A-amino-6^A-deoxy-β-CD)thiourea (mono{6-[TU-HDA-NPNI-(TEG-N_3)]}-β-CD **68).** Mono[6-(NH-TEG-N_3)]-β-CD **63** (0.47 g, 0.35 mmol) was dissolved in DMF (18 mL), NPNI-HDA-ITC **43** (0.21 g, 0.53 mmol) and DIPEA (70 μL, 0.40 mmol) were added and the reaction mixture was stirred at room temperature for 6 hours. The reaction was monitored by TLC using PrOH/H_2O/EtOAc/conc. NH_3 aq. solution 6/1/1/1 mixture. Spots were detected by methods M1 and M5. The solvent was distilled off at 80°C using an oil rotary pump. The residue (0.87 g) was dissolved in MeOH, C18 RP silica gel (4.5 g) was added, and the suspension was evaporated at 40°C on a rotary evaporator. The adsorbed product was purified by a C18 RP column chromatography (18 g silica gel) eluting with 50% w/w MeOH aq. solution. Fractions containing the product were evaporated at 55°C on a rotary evaporator. The product was dried at 60°C using an oil rotary pump and obtained as a yellow bulk solid in a 64% yield (0.40 g). **$[\alpha]^{25}_D$** = +98.1° (α +0.058, c = 0.31, DMSO). **IR(DRIFT)**: 3411, 2923, 2851, 2113 ν(azide), 1643, 1616, 1577, 1362, 1326, 1248, 1159, 1030 cm^{-1}. **^{1}H NMR** (600 MHz, DMSO-d_6): δ = 8.71 (d, *J* = 8.4 Hz, 1H, H-6), 8.43 (d, *J* = 7.2 Hz, 1H, H-12), 8.27 (d, *J* = 8.5 Hz, 1H, H-8), 7.74 (t, *J* = 5.5 Hz, 1H, NH1), 7.67 (dd, *J* = 8.4, 7.3 Hz, 1H, H-7), 7.24 (d, *J* = 37.7 Hz, 1H, NH2), 6.79 (d, *J* = 8.6 Hz, 1H, H-11), 6.00 – 5.63 (m, 14H, sec. OH), 4.89 – 4.80 (m, 7H, H-35, H-35'), 4.49 – 4.24 (m, 6H, prim. OH),4.17 – 4.13 (m, H-31'), 3.97 (t, *J* = 7.4 Hz, 2H, H-3), 3.88 – 3.25 (m, 62H, H-16, H-21, H-22, H-23, H-24, H-25, H-26, H-27, H-28, H-29, H-30, H-30', H-31, H-32, H-33, H-33', H-34, H-34'), 3.16 (t, *J* = 9.4 Hz, 1H, H-32'), 1.79 – 1.30 (m, 8H, H-17, H-18, H-19, H-20),



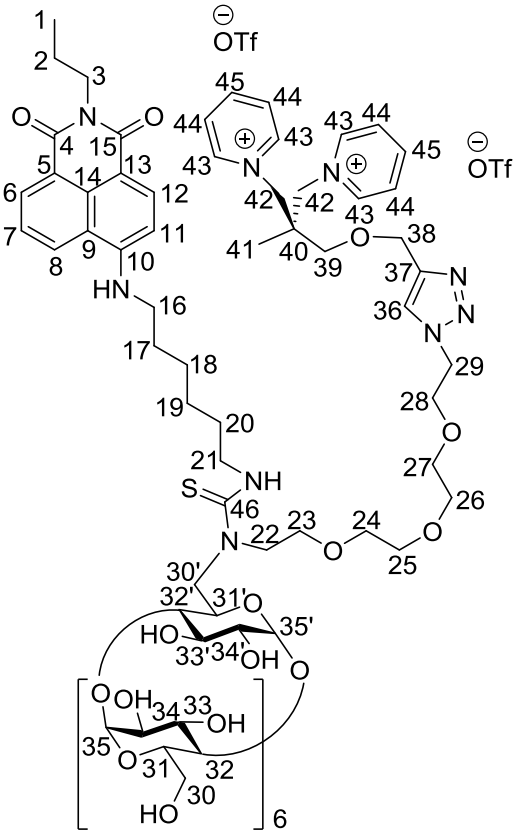
1.62 (h, J = 7.4 Hz, 2H, H-2), 0.90 (t, J = 7.4 Hz, 3H, H-1) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ = 181.81 (C-36), 163.79 – 162.95 (C-4, C-15), 150.68 (C-10), 134.37 (C-8), 130.65 (C-12), 129.46 (C-5), 128.61 (C-6), 124.21 (C-7), 121.85 (C-14), 120.11 (C-9), 107.45 (C-13), 103.81 (C-11), 102.55 – 101.55 (C-35, C-35'), 84.68 – 80.84 (C-32, C-32'), 73.09 – 71.79 (C-31, C-33, C-33', C-34, C-34'), 69.96 – 68.32 (C-23, C-24, C-25, C-26, C-27, C-28, C-31'), 59.97 – 59.20 (C-30, C-30'), 49.96 (C-29), 45.96 – 40.06 (C-16, C-21, C-22), 40.73 (C-3), 28.57 – 26.34 (C-17, C-18, C-19, C-20), 20.98 (C-2), 11.40 (C-1) ppm. **UV-VIS** (H_2O), λ_{max1}, nm: 204.0, λ_{max2}, nm: 256.5, λ_{max3}, nm: 283.5, λ_{max4}, nm: 450.0, $2*10^{-5}$ M. **ESI MS**: for $C_{72}H_{111}N_7O_{39}S$ calcd: *m/z* 1729.7 (for $[M+Na]^+$ calcd: *m/z* 1752.7), found 1753.2 $[M+Na]^+$. **HRMS**: for $C_{72}H_{111}N_7O_{39}S$ calcd: *m/z* 1729.6638 (for $[M+H]^+$ calcd: *m/z* 1730.6711), found 1730.6688 $[M+H]^+$, Δ 1.3 ppm.

3,3'-(2-(((1-(20-((1,3-Dioxo-2-propyl-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)amino))-12-(6^A-amino-6^A-deoxy-β-CD)-13-thioxo-3,6,9-trioxa-12,14-diazaicosyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2-methylpropan-1,3-diyl)bis(1-methyl-1*H*-imidazol-3-ium) bis(trifluoromethanesulfonate) (mono{6-[TU-HDA-NPNI-(TEG-MTZ-O-MIM2)]}-β-CD **69).** Mono{6-[TU-HDA-NPNI-(TEG-N_3)]}-β-CD **68** (0.35 g, 0.21 mmol) and Prg-O-MIM2 bis(trifluoromethanesulfonate) **4** (97 mg, 0.17 mmol) were dissolved in H_2O/MeOH 1/1 mixture (10 mL) and bubbled with argon for 30 minutes. CuI (32 mg, 0.17 mmol) was then added, and the reaction mixture was stirred at room temperature for 12 hours. The reaction was monitored by TLC and RP-18 TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by methods M1 and M5. The starting CD compound was detected, so the temperature was raised to 65°C. The reaction was completed in 2 hours. The reaction mixture was evaporated at 55°C on a rotary evaporator. The residue (0.75 g) was dissolved in the smallest amount of H_2O and purified by a C18 RP column chromatography (8 g silica gel) eluting with 20% w/w



MeOH aq. solution. Fractions containing the product were evaporated at 50°C on a rotary evaporator. PrOH was added to the product solution during evaporation to give a solid substance. The product was dried at 70°C using an oil rotary pump and obtained as a yellow bulk solid in a 65% yield (0.25 g). $[\alpha]^{25}_{D}$ = +67.3° (α +0.035, c = 0.26, DMSO). **IR(DRIFT)**: 3336, 2932, 2869, 1679, 1634, 1580, 1553, 1281, 1251, 1159, 1033 cm^{-1}. **^{1}H NMR** (600 MHz, DMSO-d_6): δ = 9.00 (s, 2H, H-43), 8.70 (dd, *J* = 8.8, 2.3 Hz, 1H, H-6), 8.43 (d, *J* = 7.3 Hz, 1H, H-12), 8.27 (d, *J* = 8.5 Hz, 1H, H-8), 8.11 (s, 1H, H-36), 7.73 (s, 3H, NH1, H-45), 7.68 (t, *J* = 7.8 Hz, 1H, H-7), 7.58 (s, 2H, H-44), 7.22 (d, *J* = 64.1 Hz, 1H, NH2), 6.78 (dd, *J* = 8.7, 3.4 Hz, 1H, H-11), 6.89 – 5.65 (m, 14H, sec. OH), 4.84 – 4.82 (m, 7H, H-35, H-35'), 4.58 – 4.38 (m, 10H, prim. OH, H-29, H-38), 4.29 (d, *J* = 14.0 Hz, 2H, H-42), 4.16 (d, *J* = 14.0 Hz, 2H, H-42), 3.97 (t, *J* = 7.6 Hz, 2H, H-3), 3.86 (s, 6H, H-46), 3.81 (d, *J* = 5.2 Hz, 2H, H-28), 3.74 – 3.26 (m, 58H, H-16, H-21, H-22, H-23, H-24, H-25, H-26, H-27, H-30, H-30', H-31, H-31', H-32, H-33, H-33', H-34, H-34'), 3.19 (bs, 1H, H-32'), 3.09 (s, 2H, H-39), 1.73 – 1.28 (m, 8H, H-17, H-18, H-19, H-20), 1.63 (h, *J* = 7.3 Hz, 2H, H-2), 0.89 (t, *J* = 6.7 Hz, 3H, H-1), 0.83 (s, 3H, H-41) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ = 181.61 (C-47), 163.76 (C-4), 162.94 (C-15), 150.64 (C-10), 142.90 (C-37), 137.37 (C-43), 134.07 (C-8), 130.39 (C-12), 129.43 (C-5), 128.29 (C-6), 124.33 (C-36), 123.95 (C-7), 123.40 (C-44), 123.19 (C-45), 121.56 (C-14), 120.37 (q, *J* = 322.3 Hz, CF_3), 119.80 (C-9), 107.44 (C-13), 103.51 (C-11), 102.23 – 101.21 (C-35, C-35'), 84.31 – 80.54 (C-32, C-32'), 72.79 – 71.54 (C-31, C-31', C-33, C-33', C-34, C-34'), 70.09 – 68.37 (C-23, C-24, C-25, C-26, C-27, C-28, C-39), 63.08 (C-38), 59.72 – 59.00 (C-30, C-30'), 52.25 (C-42), 49.11 (C-29), 45.10 – 42.61 (C-16, C-21, C-22), 40.45 (C-3), 39.52 (C-40, solvent overlay), 35.66 (C-46), 28.26 – 26.02 (C-17, C-18, C-19, C-20), 20.70 (C-2), 17.02 (C-41), 11.12 (C-1) ppm. **UV-VIS** (H_2O), λ_{max1}, nm: 204.5, λ_{max2}, nm: 256.5, λ_{max3}, nm: 283.5, λ_{max4}, nm: 450.0, $1*10^{-5}$ M. **ESI MS**: for $C_{88}H_{135}N_{11}O_{40}S^{2+}$ calcd: *m/z* 1008.9, found 1009.3 [M^{2+}]. **HRMS**: for $C_{88}H_{135}N_{11}O_{40}S^{2+}$ calcd: *m/z* 1008.9289, found 1008.9256 [M^{2+}], Δ 3.3 ppm.

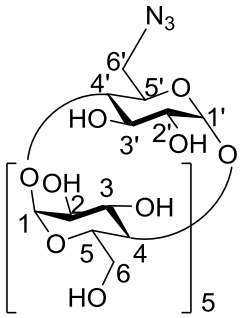
1,1'-(2-(((1-(20-((1,3-Dioxo-2-propyl-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)amino)-12-(6^{A}-amino-6^{A}-deoxy-β-CD)-13-thioxo-3,6,9-trioxa-12,14-diazaicosyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2-methylpropane-1,3-diyl)bis(pyridin-1-ium) bis(trifluoromethanesulfonate) (mono{6-[TU-HDA-NPNI-(TEG-MTZ-O-PYR2)]}-β-CD **70).** Mono{6-[TU-HDA-NPNI-(TEG-N_3)]}-β-CD **68** (0.71 g, 0.41 mmol) and Prg-O-PYR2 bis(trifluoromethanesulfonate) **5** (0.19 g, 0.33 mmol) were dissolved in H_2O/PrOH 1/1 mixture (70 mL) and bubbled with argon for 30 minutes. CuI (62 mg, 0.33 mmol) was then added, and the reaction mixture was stirred at 60°C for 3 hours. The reaction was monitored by TLC and RP-18 TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by methods M1 and M5. The starting CD compound was detected, so CuI (62 mg, 0.33 mmol) was added, and the reaction was stirred for 15 hours. The reaction was still not finished, so another CuI (0.24 g, 0.132 mol) was added, and the reaction was stirred for 24 hours. The reaction mixture was filtered through celite and evaporated at 50°C on a rotary evaporator. The residue (0.88 g) was dissolved in the smallest amount of H_2O and purified by a C18 RP column chromatography (9 g silica gel) eluting with 20% w/w MeOH aq. solution. Fractions containing the product were evaporated at 50°C on a rotary evaporator. PrOH was added to the product solution during evaporation to give a solid substance. The product was dried at 60°C using an oil rotary pump and obtained as a yellow glassy solid in a 46% yield (0.35 g). **$[\alpha]^{25}_{D}$** = +68.8° (α +0.044, c = 0.32, DMSO). **IR(DRIFT)**: 3387, 2929, 2875, 1679, 1640, 1610, 1551, 1398, 1359, 1248, 1225, 1156, 1027 cm^{-1}. **^{1}H NMR** (600 MHz, DMSO-d_6): δ = 8.87 (s, 4H, H-43), 8.68 (m, 3H, H-6, H-45), 8.43 (s, 1H, H-12), 8.27 (d, J = 8.4 Hz, 1H, H-8), 8.15 – 8.09 (m, 5H, H-36, H-44), 7.74 (s, 1H, NH), 7.67 (s, 1H, H-7), 7.16 – 7.00 (m, 1H, NH), 6.78 (d, J = 9.3 Hz, 1H, H-11), 6.01 – 5.69 (m, 14H, sec. OH), 4.93 – 4.71 (m, 11H, H-35, H-35', H-42), 4.60 – 4.38 (m, 10H, prim. OH, H-29, H-38), 3.98 (t, J = 7.5 Hz, 2H, H-3), 3.85 – 3.82 (m, 2H, H-28), 3.65 – 3.33 (m, 58H,



H-16, H-21, H-22, H-23, H-24, H-25, H-26, H-27, H-30, H-30', H-31, H-31', H-32, H-32', H-33, H-33', H-34, H-34'), 3.16 (s, 2H, H-39), 1.71 – 1.29 (m, 8H, H-17, H-18, H-19, H-20), 1.61 (t, J = 7.6 Hz, 2H, H-2), 0.90 (bs, 6H, H-1, H-41) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ = 181.74 (C-46), 163.78 (C-4), 162.95 (C-15), 150.64 (C-10), 146.20 (C-45), 145.73 (C-43), 142.40 (C-37), 134.07 (C-8), 130.42 (C-12), 129.45 (C-5), 128.33 (C-6), 127.80 (C-44), 124.55 (C-36), 124.00 (C-7), 121.86 (C-14), 120.68 (q, J = 322.2 Hz, CF_3), 120.10 (C-9), 107.51 (C-13), 103.53 (C-11), 102.25 – 101.56 (C-35, C-35'), 84.19 – 80.56 (C-32, C-32'), 72.97 – 71.75 (C-31, C-31', C-33, C-33', C-34, C-34'), 69.36 – 68.42 (C-23, C-24, C-25, C-26, C-27, C-28, C-39), 63.42 (C-42), 62.99 (C-38), 60.01 – 59.61 (C-30, C-30'), 49.13 (C-29), 45.16 – 42.54 (C-16, C-21, C-22), 40.49 (C-3), 39.52 (C-40, solvent overlay), 27.65 – 25.93 (C-17, C-18, C-19, C-20), 20.73 (C-2), 16.35 (C-41), 11.15 (C-1) ppm. **^{19}F NMR** (376 MHz, DMSO-d_6, C_6F_6): δ = -80.06, -164.90 (C_6F_6) ppm. **UV-VIS** (H_2O), λ_{max1}, nm: 200.5, λ_{max2}, nm: 259.0, λ_{max3}, nm: 283.0, λ_{max4}, nm: 450.0, 1*10^{-4} M. **ESI MS**: for $C_{90}H_{133}N_9O_{40}S^{2+}$ calcd: *m/z* 1005.9, found 1006.6 [M^{2+}]. **HRMS**: for $C_{90}H_{133}N_9O_{40}S^{2+}$ calcd: *m/z* 1005.9180, found 1005.9152 [M^{2+}], Δ 2.8 ppm.

6.8.8 Charged cyclodexrin derivatives

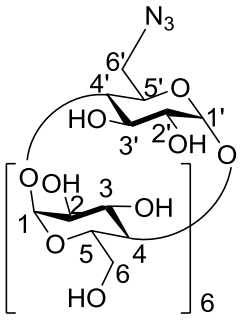
6^A-Azido-6^A-deoxy-*a*-CD (mono(6-N_3)-*a*-CD **71).** Compound mono(6-N_3)-*a*-CD **71**



was prepared according to the previously published procedure[375]. Mono(6-*O*-Ts)-*a*-CD **55** (1.15 g, 1.02 mmol) was dissolved in dry DMF (23 mL), and NaN_3 (0.66 g, 10.2 mmol) was added. The mixture was heated to 140°C and stirred for 2 hours. The reaction mixture was monitored by TLC using PrOH/H_2O/EtOAc/conc. NH_3 aq. solution 6/3/1/1 mixture. Spots were detected by the method M5. DMF was distilled off at 80°C using an oil rotary pump. The crude product (2.3 g) was purified by column chromatography (80 g silica gel) eluting with CH_3CN/H_2O 4/1. After purification, fractions with product were evaporated on a rotary evaporator at 50°C. The solid (1.24 g) was dissolved in the smallest volume of H_2O and poured into acetone (500 mL). The precipitated product was filtered and washed with acetone (3 × 100 mL). The product was dried at 70°C using an oil rotary pump and obtained as a beige solid in an 83% yield (0.85 g). **[α]$^{25}_D$** = +106.9° (α +0.062, c = 0.29, DMSO). **IR(DRIFT)**: 3345, 2925, 2097, 2038, 1643, 1416, 1328, 1303, 1156, 1076, 1030 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 5.51 (bs, 12H, OH), 4.87 – 4.74 (m, 6H, H-1, H-1'), 4.52 (bs, 5H, OH), 3.82 – 3.73 (m, 6H, H-3, H-3'), 3.70 – 3.53 (m, 18H, H-5, H-5', H-6, H-6'), 3.45 – 3.22

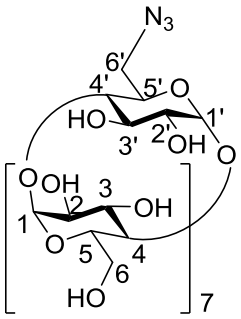
(m, 12H, H-2, H-2', H-4, H-4') ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 101.88 – 101.47 (C-1, C-1'), 83.52 – 80.40 (C-4, C-4'), 72.99 – 72.89 (C-3, C-3'), 72.05 – 69.99 (C-2, C-2', C-5, C-5'), 59.95 – 59.68 (C-6), 50.95 (C-6') ppm. **ESI MS**: for $C_{36}H_{59}N_3O_{29}$ calcd: *m/z* 997.3 (for $[M+Na]^+$ calcd: *m/z* 1020.3), found 1020 $[M+Na]^+$. **HRMS**: for $C_{36}H_{59}N_3O_{29}$ calcd: *m/z* 997.3234 (for $[M+Na]^+$ calcd: *m/z* 1020.3126), found 1020.3133 $[M+Na]^+$, Δ 0.6 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[137].

6^A-Azido-6^A-deoxy-β-CD (mono(6-N_3)-β-CD **72).** Compound mono(6-N_3)-β-CD **72**



was prepared according to the previously published procedure[401], with some modifications including lower temperature and prolonged reaction time. Mono(6-*O*-Ts)-β-CD **56** (6.0 g, 4.65 mmol) was dissolved in dry DMF (60 mL), and NaN_3 (0.61 g, 9.31 mmol) was added. The mixture was heated to 80°C and stirred for 24 hours. The reaction mixture was monitored by TLC using PrOH/H_2O/EtOAc/conc. NH_3 aq. solution 6/3/1/1 mixture. Spots were detected by the method M5. The reaction mixture was poured into acetone (1000 mL), the resulting precipitate was isolated by filtration, and the solid was washed with acetone (3 × 100 mL). The solid (5.95 g) was dissolved in H_2O (70 mL) and poured into acetone (1000 mL). The precipitated product was filtered and washed with acetone (3 × 100 mL). The product was dried at 70°C using an oil rotary pump and obtained as a white solid in a 95% yield (5.17 g). **$[\alpha]^{25}_D$** = +135.2° (α +0.123, c = 0.46, DMSO). **IR(DRIFT)**: 3324, 2923, 2107, 1649, 1413, 1365, 1326, 1293, 1237, 1201, 1159, 1081, 1036 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 5.71 (bs, 14H, OH), 4.91 – 4.77 (m, 7H, H-1, H-1'), 4.62 – 4.33 (bs, 6H, OH), 3.83 – 3.15 (m, 42H, H-2, H-2', H-3, H-3', H-4, H-4', H-5, H-5', H-6, H-6', solvent overlay) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 103.29 – 101.15 (C-1, C-1'), 83.85 – 80.91 (C-4, C-4'), 74.07 – 69.69 (C-2, C-2', C-3, C-3', C-5, C-5'), 60.18 – 59.78 (C-6), 51.13 (C-6') ppm. **ESI MS**: for $C_{42}H_{69}N_3O_{34}$ calcd: *m/z* 1159.4 (for $[M+Na]^+$ calcd: *m/z* 1182.4), found 1182 $[M+Na]^+$. **HRMS**: for $C_{42}H_{69}N_3O_{34}$ calcd: *m/z* 1159.3763 (for $[M+H]^+$ calcd: *m/z* 1160.3835), found 1160.3802 $[M+H]^+$, Δ 2.8 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[402].

6^A-Azido-6^A-deoxy-γ-CD (mono(6-N_3)-γ-CD **73).** Compound mono(6-N_3)-γ-CD **73** was



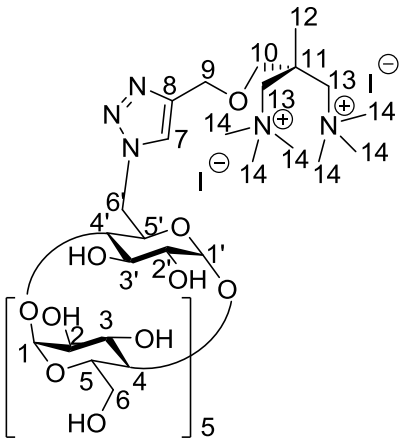
prepared according to the previously published procedure[401], with some modifications including lower temperature and prolonged reaction time. Mono(6-*O*-Ts)-γ-CD **57** (0.50 g, 0.35 mmol) was dissolved in dry DMF (25 mL), and NaN_3 (44.9 mg, 0.70 mmol) was added. The mixture was heated to 80°C and stirred for 24 hours. The reaction mixture was monitored by TLC using PrOH/H_2O/EtOAc/conc. NH_3 aq. solution 6/3/1/1 mixture. Spots were detected by the method M5. DMF was distilled off at 80°C using an oil rotary pump, the solid (0.79 g) was dissolved in the smallest volume of H_2O. The crude product was purified by a C18 RP column chromatography (32.0 g silica gel) using H_2O and 5-10% w/w MeOH aq. solution for elution. Fractions with the pure product were evaporated on a rotary evaporator at 40°C. The purified product (0.39 g) was dissolved in H_2O (8 mL) and freeze-dried. The product was obtained as a white amorphous powder in an 84% yield (0.38 g). $[\alpha]^{25}_D$ = +141.8° (α +0.095, c = 0.34, DMSO). **IR(DRIFT)**: 3336, 2923, 2101, 1646, 1413, 1365, 1335, 1296, 1237, 1201, 1156, 1081, 1033 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 5.78 (bs, 16H, OH), 4.97 – 4.82 (m, 8H, H-1, H-1'), 4.59 – 4.48 (bs, 7H, OH), 3.80 – 3.20 (m, 48H, H-2, H-2', H-3, H-3', H-4, H-4', H-5, H-5', H-6, H-6', solvent overlay) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 103.36 – 100.65 (C-1, C-1'), 83.42 – 79.87 (C-4, C-4'), 74.15 – 69.94 (C-2, C-2', C-3, C-3', C-5, C-5'), 60.22 – 59.84 (C-6), 51.04 (C-6') ppm. **ESI MS**: for $C_{48}H_{79}N_3O_{39}$ calcd: *m/z* 1321.4 (for $[M+Na]^+$ calcd: *m/z* 1344.4), found 1344 $[M+Na]^+$. **HRMS**: for $C_{48}H_{79}N_3O_{39}$ calcd: *m/z* 1321.4291 (for $[M+H]^+$ calcd: *m/z* 1322.4363), found 1322.4304 $[M+H]^+$, Δ 4.5 ppm. ^{1}H NMR spectrum is in accordance with the literature[403].

General procedure for preparation of mono[6-(MTZ-O-TMA2)]-CDs (GP4).

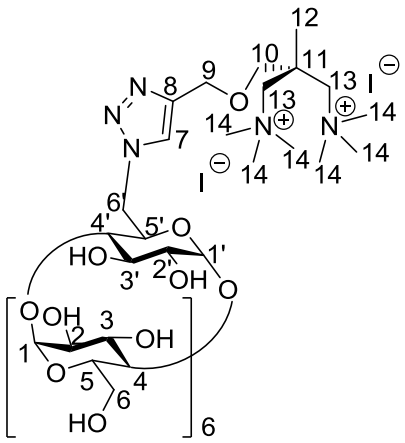
Mono(6-N_3)-CD (0.12 g, 0.12 mmol for *α*-CD derivative **71**, 0.12 g, 0.10 mmol for β-CD derivative **72**, 0.27 g, 0.20 mmol for γ-CD derivative **73**) and Prg-O-TMA2 diiodide **6** (1.0 eq.) were dissolved in H_2O. A metal Cu (30-50 eq.) was added, and the mixture was heated to 80°C and stirred for several hours. The reaction mixture was monitored by TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture for the product and PrOH/H_2O/EtOAc/conc. NH_3 aq. solution 6/3/1/1 for the starting compound. Spots were detected by the method M5. The reaction mixture was filtered through celite, and the solution was evaporated on a rotary evaporator at 40°C. The residue was dissolved in the

smallest amount of H_2O and purified by a C18 RP column chromatography eluting with H_2O. Fractions containing the product were evaporated at 50°C on a rotary evaporator. The solid was dissolved in H_2O and freeze-dried.

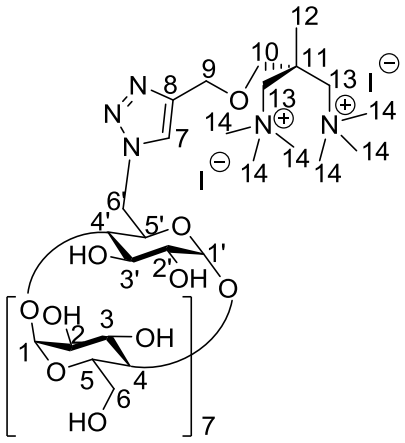
2-(((1-(6^A-Deoxy-α-CD)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-N^1,N^1,N^1,N^3,N^3,N^3,2-heptamethylpropane-1,3-diaminium diiodide (MTZ-OTMA2-α-CD **74).** Compound **74** was prepared according to the general procedure (**GP4**). The product was prepared using Prg-O-TMA2 diiodide **6** (59.6 mg, 0.12 mmol) in H_2O (11 mL). A metal Cu (0.40 g, 6.02 mmol) was added, and the mixture was heated to 80°C and stirred for 20 hours. For 0.19 g of the crude product, 4 g of silica gel were utilized for a C18 RP column chromatography. The purified product was dissolved in H_2O (3 mL) and freeze-dried. The product was obtained as a white powder in a 73% yield (0.133 g). **[α]$^{25}_D$** = +86.1° (α +0.062, c = 0.36, H_2O). **IR(DRIFT)**: 3300, 2926, 2032, 1643, 1479, 1332, 1230, 1150, 1072, 1027 cm^{-1}. **^{1}H NMR** (600 MHz, D_2O, *t*BuOH): δ = 8.27 (s, 1H, H-7), 5.16 – 4.67 (m, 8H, H-1, H-1', H-6'), 4.75 (s, 2H, H-9), 4.32 (td, *J* = 9.4, 2.3 Hz, 1H, H-5'), 4.16 – 2.88 (m, 37H, H-2, H-2', H-3, H-3', H-4, H-4', H-5, H-6, H-13), 3.81 (s, 2H, H-10), 3.27 (s, 18H, H-14), 1.50 (s, 3H, H-12), 1.24 (s, *t*BuOH) ppm. **^{13}C NMR** (151 MHz, D_2O, *t*BuOH): δ = 143.43 (C-8), 127.78 (C-7), 102.25 – 101.76 (C-1, C-1'), 83.72 – 81.46 (C-4, C-4'), 73.97 –72.09 (C-2, C-2', C-3, C-3', C-5, C-10, C-13), 71.13 (C-5'), 63.41 (C-9), 61.21 – 59.90 (C-6), 56.49 (C-14), 52.00 (C-6'), 43.81 (C-11), 30.29 (*t*BuOH), 21.53 (C-12) ppm. **ESI MS**: for $C_{50}H_{88}N_5O_{30}^{2+}$ calcd: *m/z* 619.3, found 620.0 [M^{2+}]. **HRMS**: for $C_{50}H_{88}N_5O_{30}^{2+}$ calcd: *m/z* 619.7791, found 619.7798 [M^{2+}], Δ 1.1 ppm.



2-(((1-(6^{A}-Deoxy-β-CD)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-N^{1},N^{1},N^{1},N^{3},N^{3},N^{3},2-heptamethylpropane-1,3-diaminium diiodide (MTZ-OTMA2-β-CD **75).** Compound **75** was prepared according to the general procedure (**GP4**). The product was prepared using Prg-O-TMA2 diiodide **6** (46.0 mg, 0.10 mmol) in H_2O (11 mL). A metal Cu (0.30 g, 4.64 mmol) was added, and the mixture was heated to 80°C and stirred for 5 hours. For 0.14 g of the crude product, 3 g of silica gel were utilized for a C18 RP column chromatography. The purified product was dissolved in H_2O (3 mL) and freeze-dried. The product was obtained as a white powder in a 70% yield (0.108 g). **[α]^{25}$_{D}$** = +108.8° (α +0.074, c = 0.34, DMSO). **IR(DRIFT)**: 3300, 2929, 1649, 1482, 1335, 1236, 1162, 1075, 1030 cm^{-1}. **^{1}H NMR** (600 MHz, D_2O, *t*BuOH): δ = 8.18 (s, 1H, H-7), 5.15 – 4.62 (m, 9H, H-1, H-1', H-6'), 4.79 (s, 2H, HDO overlay, H-9), 4.18 (bs, 1H, H-5'), 4.02 – 2.82 (m, 43H, H-2, H-2', H-3, H-3', H-4, H-4', H-5, H-6, H-13), 3.77 (s, 2H, H-10), 3.24 (s, 18H, H-14), 1.46 (s, 3H, H-12), 1.24 (s, *t*BuOH) ppm. **^{13}C NMR** (151 MHz, D_2O, *t*BuOH): δ = 143.11 (C-8), 127.24 (C-7), 102.34 – 101.74 (C-1, C-1'), 83.39 – 81.02 (C-4, C-4'), 73.35 –71.71 (C-2, C-2', C-3, C-3', C-5, C-10, C-13), 70.79 (C-5'), 62.99 (C-9), 60.75 – 59.40 (C-6), 56.14 (C-14), 51.55 (C-6'), 43.46 (C-11), 30.29 (*t*BuOH), 21.18 (C-12) ppm. **ESI MS**: for $C_{56}H_{99}N_5O_{35}^{2+}$ calcd: *m/z* 700.8, found 701.0 [M^{2+}]. **HRMS**: for $C_{56}H_{99}N_5O_{35}^{2+}$ calcd: *m/z* 700.8055, found 700.8057 [M^{2+}], Δ 0.3 ppm.

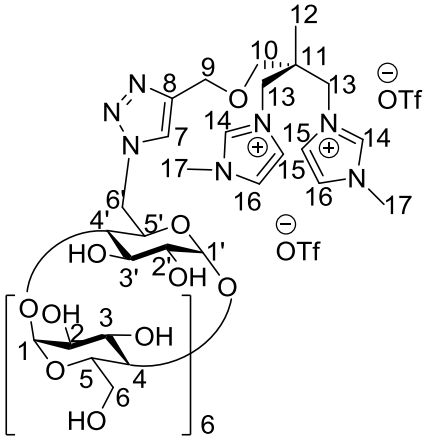


2-(((1-(6^{A}-Deoxy-γ-CD)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-N^{1},N^{1},N^{1},N^{3},N^{3},N^{3},2-heptamethylpropane-1,3-diaminium diiodide (MTZ-OTMA2-γ-CD **76).** Compound **76** was prepared according to the general procedure (**GP4**). The product was prepared using Prg-O-TMA2 diiodide **6** (0.10 g, 0.20 mmol) in H_2O (25 mL). A metal Cu (0.65 g, 10.2 mmol) was added, and the mixture was heated to 80°C and stirred for 5 hours. For 0.39 g of the crude product, 15 g of silica gel were utilized for a C18 RP column chromatography. The purified product was dissolved in H_2O (5 mL) and freeze-dried. The product was obtained



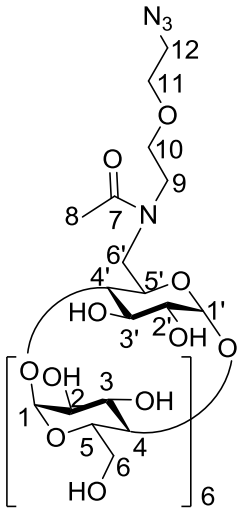
as a white powder in a 73% yield (0.27 g). $[\alpha]^{25}_{D}$ = +115.1° (α +0.061, c = 0.27, H_2O). **IR(DRIFT)**: 3330, 2932, 1634, 1482, 1368, 1335, 1239, 1153, 1078, 1027 cm^{-1}. **^{1}H NMR** (600 MHz, D_2O, *t*BuOH): δ = 8.19 (s, 1H, H-7), 5.21 – 4.66 (m, 10H, H-1, H-1', H-6'), 4.75 (s, 2H, HDO overlay, H-9), 4.27 – 4.21 (m, 1H, H-5'), 4.05 – 2.91 (m, 51H, H-2, H-2', H-3, H-3', H-4, H-4', H-5, H-6, H-10, H-13), 3.28 (s, 18H, H-14), 1.50 (s, 3H, H-12), 1.24 (s, *t*BuOH) ppm. **^{13}C NMR** (151 MHz, D_2O, *t*BuOH): δ = 143.49 (C-8), 127.57 (C-7), 102.62 – 101.03 (C-1, C-1'), 83.25 – 81.08 (C-4, C-4'), 73.58 –71.19 (C-2, C-2', C-3, C-3', C-5, C-10, C-13), 70.97 (C-5'), 63.44 (C-9), 61.14 – 60.02 (C-6), 56.56 (C-14), 51.90 (C-6'), 43.88 (C-11), 30.29 (*t*BuOH), 21.61 (C-12) ppm. **ESI MS**: for $C_{62}H_{109}N_5O_{40}^{2+}$ calcd: *m/z* 781.8, found 782.0 [M^{2+}]. **HRMS**: for $C_{62}H_{109}N_5O_{40}^{2+}$ calcd: *m/z* 781.8319, found 781.8311 [M^{2+}], Δ 1.0 ppm.

3,3'-(2-(((1-(6^A-Deoxy-β-CD)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2-methylpropane-1,3-diyl)bis(1-methyl-1*H*-imidazol-3-ium) bis(trifluoromethanesulfonate) (mono[6-(MTZ-O-MIM2)]-β-CD **77).** Prg-O-MIM2 bis(trifluoromethanesulfonate) **4** (0.84 g, 1.43 mmol) and mono(6-N_3)-β-CD **72** (2.0 g, 1.72 mmol) were dissolved in H_2O (40 mL). The mixture was bubbled with nitrogen for 30 minutes. A metal Cu (2.7 g, 43.0 mmol) was added, and the reaction mixture was stirred for 20 hours at room temperature. The reaction was monitored by TLC and RP-18 TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by methods M1, M2, and M5. The reaction mixture was filtered through celite, and the aqueous solution was purified by a C18 RP column chromatography (50 g silica gel) eluting with H_2O and 5% w/w MeOH aq. solution. Fractions containing the product were evaporated at 50°C on a rotary evaporator. The solid (2.32 g) was dissolved in H_2O (50 mL) and freeze-dried. The product was obtained as a white amorphous solid in an 87% yield (2.18 g). $[\alpha]^{25}_{D}$ = +92.1° (α +0.058, c = 0.32, H_2O). **IR(DRIFT)**: 3352, 3159, 2933, 2887, 1643, 1564, 1452, 1334, 1250, 1153, 1078, 1026 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 9.02 (d, *J* = 2.0 Hz, 2H, H-14), 8.10 (s, 1H, H-7), 7.73 – 7.62 (m, 4H, H-15, H-16), 5.98 – 5.60 (m, 14H, sec.OH), 5.11 – 4.73 (m, 9H, H-1, H-1', H-6'), 4.61 – 4.40 (m, 10H, H-6', H-9, prim.OH), 4.36 – 4.11 (m, 4H, H-13), 4.01 (t, *J* = 10.1 Hz, 1H, H-5'), 3.87 (s, 6H, H-17), 3.79 – 3.49 (m, 25H, H-3, H-



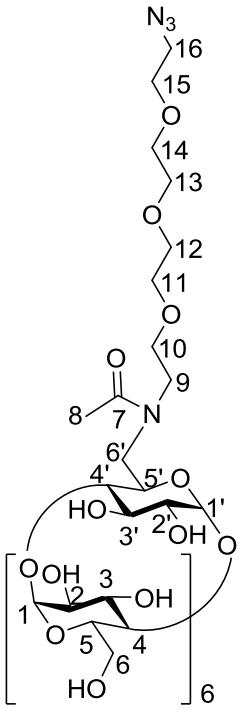
3', H-5, H-6), 3.44 – 3.23 (m, 14H, HDO overlay, H-2, H-2', H-4, H-4') , 3.18 – 3.09 (m, 2H, H-10), 3.01 (d, J = 9.8 Hz, 1H), 2.79 (t, J = 9.3 Hz, 1H), 0.85 (s, 3H, H-12) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 143.06 (C-8), 137.72 (C-14), 124.90 (C-7), 123.75 – 123.54 (C-15, C-16), 102.85 – 100.57 (C-1, C-1'), 84.01 – 80.24 (C-4, C-4'), 73.51 – 71.57 (C-2, C-2', C-3, C-3', C-5), 70.52 (C-10), 70.05 (C-5'), 63.34 (C-9), 60.56 – 58.77 (C-6), 52.57 (C-13), 50.58 (C-6'), 39.52 (C-11, solvent overlay), 35.95 (C-17), 17.35 (C-12) ppm. **^{19}F NMR** (376 MHz, DMSO-d_6, C_6F_6): δ = -80.04, -164.90 (C_6F_6) ppm. **UV-VIS** (H_2O), λ_{max}, nm: 213.0, 1*10^{-4} M. **ESI MS**: for $C_{58}H_{93}N_7O_{35}^{2+}$ calcd: *m/z* 723.8, found 724.0 [M^{2+}]. **HRMS**: for $C_{58}H_{93}N_7O_{35}^{2+}$ calcd: *m/z* 723.7851, found 723.7860 [M^{2+}], Δ 1.2 ppm.

Mono-(*N*-acetyl-*N*-(2-(2-azidoethoxy)eth-1-yl-6^A-amino-6^A-deoxy)-β-CD (mono[6-(NAc-DEG-N_3)]-β-CD **78).** Mono[6-(NH-DEG-N_3)]-β-CD **61** (1.0 g, 0.802 mmol) was dissolved in H_2O (22 mL). A solution of NaOH (128 mg in 2.4 mL of H_2O) was added. Then, Ac_2O (0.30 mL, 3.21 mmol) was added, and the mixture was stirred at room temperature for 3 hours. The reaction mixture was monitored by TLC using $CHCl_3$/MeOH/H_2O 5/5/1 mixture. Spots were detected by the method M5. No starting compound was detected. Subsequently, another solution of NaOH (256 mg in 4.8 mL of H_2O) was poured into the reaction mixture, which was stirred at room temperature for 19 hours. The reaction mixture was monitored by TLC using $CHCl_3$/MeOH/H_2O 5/5/1 mixture again. Spots were detected by the method M5. No overacetylated side-products were detected. The mixture was neutralized with DOWEX® 50W-X8 in H^+ form and diluted with MeOH (30 mL). The heterogeneous mixture was poured into a DOWEX® 50W-X8 (40 mL, H^+ form), and the product was eluted with 50% w/w MeOH aq. solution. Fractions with the product were evaporated on a rotary evaporator at 50°C. The product was dried at 70°C using an oil rotary pump. The product was obtained as a white powder in an 89% yield (0.92 g). **[α]$^{25}_D$** = +137.3° (α +0.070, c = 0.26, DMSO). **IR(DRIFT)**: 3378, 2926, 2113 ν(azide), 1622 ν(C=O), 1419, 1251, 1156, 1078, 1039 cm^{-1}. **^{1}H NMR** (600 MHz, DMSO-d_6): δ = 5.97 – 5.66 (m, 14H, 2,3-OH), 4.88 – 4.80 (m, 7H, H-1, H-1'), 4.53 – 4.20 (m, 6.5H, 6-OH, H-6'), 3.87 – 3.78 (m, 1H, H-5'), 3.74 – 3.26 (m, 47H, H-2, H-2', H-3, H-3', H-4, H-5, H-6, H-6', H-9, H-10, H-11, H-12), 3.24 – 3.08 (m, 1H, H-4'), 3.02 – 2.99 (m, 0.5H, H-6'), 2.02 (s, 1.8H, H-8),



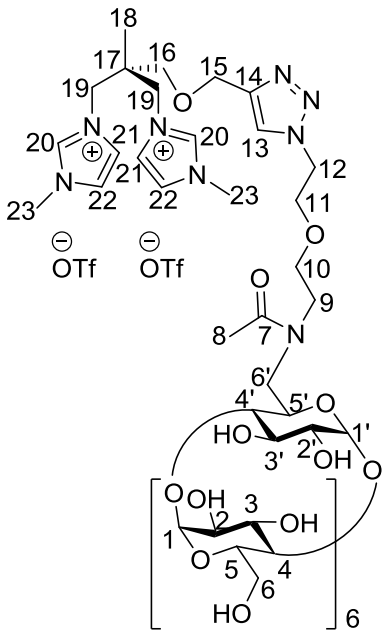
1.97 (s, 1.2H, H-8) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ = 170.35 (C-7), 170.01 (C-7), 102.42 – 101.55 (C-1, C-1'), 84.70 – 84.16 (C-4'), 81.88 – 81.17 (C-4), 73.06 – 71.78 (C-2, C-2', C-3, C-3', C-5),70.02 – 69.92 (C-5'), 69.45 – 67.55 (C-10, C-11), 59.99 – 59.15 (C-6), 50.22 – 48.83 (C-9, C-12), 45.91 – 45.76 (C-6'), 21.51 – 21.28 (C-8) ppm. **ESI MS**: for $C_{48}H_{80}N_4O_{36}$ calcd: *m/z* 1288.5 (for $[M+H]^+$ calcd: *m/z* 1289.5, for $[M+Na]^+$ calcd: *m/z* 1311.4), found 1289.0 $[M+H]^+$, 1311.0 $[M+Na]^+$. **HRMS**: for $C_{48}H_{80}N_4O_{36}$ calcd: *m/z* 1288.4552 (for $[M+H]^+$ calcd: *m/z* 1289.4625), found 1289.4636 $[M+H]^+$, Δ 0.9 ppm.

Mono-(*N*-acetyl-*N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)eth-1-yl-6^A-amino-6^A-deoxy)-β-CD (mono[6-(NAc-TEG-N_3)]-β-CD **79).** Mono[6-(NH-TEG-N_3)]-β-CD **63** (0.80 g, 0.60 mmol) was dissolved in H_2O (18 mL). A solution of NaOH (96 mg in 1.8 mL of H_2O) was added. Then, Ac_2O (0.23 mL, 2.40 mmol) was added, and the mixture was stirred at room temperature for 3 hours. The reaction mixture was monitored by TLC using $CHCl_3$/MeOH/H_2O 5/5/1 mixture. Spots were detected by the method M5. No starting compound was detected. Another solution of NaOH (192 mg in 3.6 mL of H_2O) was poured into the reaction mixture, which was then stirred at room temperature for 19 hours. The reaction mixture was monitored by TLC using $CHCl_3$/MeOH/H_2O 5/5/1 mixture again. Spots were detected by the method M5. No overacetylated side-products were detected. The mixture was neutralized with DOWEX® 50W-X8 in H^+ form. The heterogeneous mixture was poured into a DOWEX® 50W-X8 (20 mL, H^+ form), and the product was eluted with H_2O. Fractions containing the product were evaporated on a rotary evaporator at 50°C. The product was dried at 60°C using an oil rotary pump. The product was obtained as a white powder in a 95% yield (0.78 g). **$[\alpha]^{25}_D$** = +133.3° (α +0.096, c = 0.36, H_2O). **IR(DRIFT)**: 3351, 2920, 2116 ν(azide), 1622 ν(C=O), 1413, 1251, 1159, 1081, 1036 cm^{-1}. **^{1}H NMR** (600 MHz, DMSO-d_6): δ = 5.95 – 5.68 (m, 14H, 2,3-OH), 4.89 – 4.80 (m, 7H, H-1, H-1'), 4.51 – 4.23 (m, 6.5H, 6-OH, H-6'), 3.81 – 3.77 (m, 1H, H-5'), 3.74 – 3.29 (m, 55H, H-2, H-2', H-3, H-3', H-4, H-5, H-6, H-6', H-9, H-10, H-11, H-12, H-13, H-14, H-15, H-16), 3.25 – 3.10 (m, 1H, H-4'), 2.95 – 2.92 (m, 0.5H, H-6'), 2.03 (s, 2H, H-8), 1.98 (s, 1H, H-8) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ = 170.24 (C-7), 170.10 (C-7), 102.40 – 101.64 (C-1, C-1'), 84.63 – 83.99



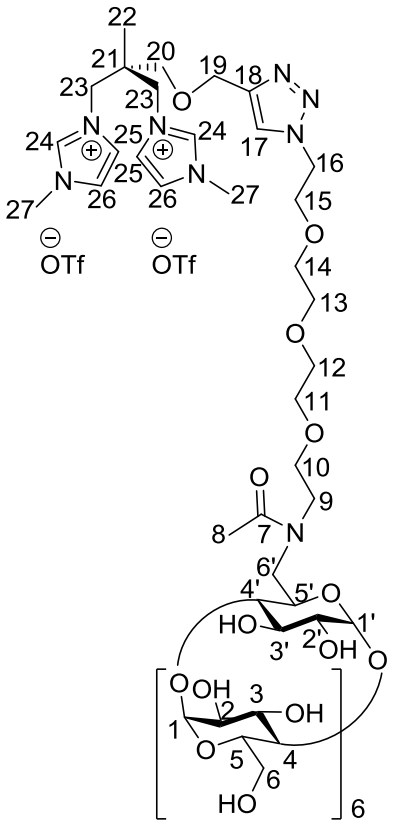
(C-4'), 81.93 – 81.17 (C-4), 73.04 – 71.76 (C-2, C-2', C-3, C-3', C-5), 70.23 (C-5'), 69.98 – 67.69 (C-10, C-11, C-12, C-13, C-14, C-15), 60.01 – 59.14 (C-6), 49.03 – 45.86 (C-9, C-16, C-6'), 21.48 – 21.28 (C-8) ppm. **ESI MS**: for $C_{52}H_{88}N_4O_{38}$ calcd: *m/z* 1376.5 (for $[M+K]^+$ calcd: *m/z* 1415.5), found 1415.0 $[M+K]^+$. **HRMS**: for $C_{52}H_{88}N_4O_{38}$ calcd: *m/z* 1376.5077 (for $[M+H]^+$ calcd: *m/z* 1377.5149), found 1377.5114 $[M+H]^+$, Δ 2.5 ppm.

3,3'-(2-(((1-(2-(3-(*N*-(6^A-deoxy-β-CD)acetamido)propoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2-methylpropane-1,3-diyl)bis(1-methyl-1*H*-imidazol-3-ium) bis(trifluoromethanesulfonate) (mono[6-(NAc-DEG-MTZ-O-MIM2)]-β-CD **80).** Mono[6-(NAc-DEG-N_3)]-β-CD **78** (0.90 g, 0.699 mmol) and Prg-O-MIM2 bis(trifluoromethanesulfonate) **4** (0.33 g, 0.559 mmol) were dissolved in H_2O/MeCN 1/1 mixture (60 mL), and the solution was bubbled with argon for 20 minutes. Then, CuI (0.106 g, 0.559 mmol) was added, and the mixture was stirred at 50°C for 18 hours. The reaction mixture was monitored by TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture Spots were detected by methods M1 and M5. The reaction was not finished, but the reaction mixture was slowly evaporated on a rotary evaporator at 50°C, and the reaction was completed during evaporation. The resulting solid was suspended in H_2O (50 mL), and insoluble CuI was filtered off using celite. The product in a aq. solution was purified by a C18 RP column chromatography (20 g silica gel). The product eluted with 5% w/w MeOH aq. solution. Fractions with the product were evaporated on a rotary evaporator at 50°C. The product (1.06 g) was dissolved in H_2O (30 mL) and freeze-dried. The product was obtained as a white powder in a 91% yield (0.96 g). $[\alpha]^{25}_D$ = +90.0° (α +0.072, c = 0.4, H_2O). **IR(DRIFT)**: 3306, 2929, 1619 ν(C=O), 1422, 1281, 1153, 1081, 1036 cm^{-1}. **^{1}H NMR** (600 MHz, DMSO-d_6): δ = 9.04 – 9.03 (m, 2H, H-20), 8.09 (s, 0,4H, H-13), 8.06 (s, 0,6H, H-13), 7.74 – 7.60 (m, 4H, H-21, H-22), 6.12 – 5.76 (m, 14H, 2,3-OH), 4.91 – 4.78 (m, 7H, H-1, H-1'), 4.58 – 4.49 (m, 10H, 6-OH, H-12, H-15), 4.31 – 4.13 (m, 5H, H-6', H-19), 3.88 (s, 6H, H-23), 3.82 – 3.31 (m, 46H, H-2, H-2', H-3, H-3', H-4, H-5, H-5', H-6, H-6', H-9, H-10, H-11), 3.25 – 3.22 (m, 0.5H, H-4') 3.11 – (m, 2.5H, H-4', H-16), 2.97 – 2.93 (m, 0.5H, H-6'), 1.96 (s, 1.2H, H-8), 1.91 (s, 1.8H, H-8), 0.85 (m, 3H, H-18) ppm.



^{13}C NMR (151 MHz, DMSO-d_6): δ = 170.42 – 170.17 (C-7), 142.96 (C-14), 137.74 (C-20), 124.64 – 124.56 (C-13), 123.72 – 123.50 (C-21, C-22), 120.69 (q, J = 322.3 Hz, CF_3), 102.03 – 101.67 (C-1, C-1'), 84.59 – 83.95 (C-4'), 81.99 – 81.19 (C-4), 73.53 – 72.28 (C-2, C-2', C-3, C-3', C-5), 70.82 (C-16), 70.51 (C-5'), 69.32 – 67.77 (C-10, C-11), 63.46 – 63.42 (C-15), 60.10 – 59.29 (C-6), 52.57 (C-19), 50.17 –49.00 (C-9, C-12), 45.99 – 45.82 (C-6'), 39.52 (C-17, solvent overlay), 35.96 (C-23), 21.53 – 21.26 (C-8), 17.35 – 17.32 (C-18) ppm. **^{19}F NMR** (282 MHz, DMSO-d_6, C_6F_6): δ = -80.07, -164.90 (C_6F_6) ppm. **UV-VIS** (H_2O), λ_{max}, nm: 209.0, 7*10^{-5} M. **ESI MS**: for $C_{64}H_{104}N_8O_{37}^{2+}$ calcd: *m/z* 788.3, found 788.2 [M^{2+}]. **HRMS**: for $C_{64}H_{104}N_8O_{37}^{2+}$ calcd: *m/z* 788.3246, found 788.3221 [M^{2+}], Δ 3.2 ppm.

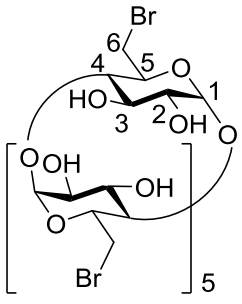
3,3'-(2-(((1-(3-(6^A-Deoxy-β-CD)-2-oxo-6,9,12-trioxa-3-azatetradecan-14-yl)-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-2-methylpropane-1,3-diyl)bis(1-methyl-1*H*-imidazol-3-ium) bis(trifluoromethanesulfonate) (mono[6-(NAc-TEG-MTZ-O-MIM2)]-β-CD **81)** Mono[6-(NAc-TEG-N_3)]-β-CD **79** (0.82 g, 0.596 mmol) and Prg-O-MIM2 bis(trifluoromethanesulfonate) **4** (0.28 g, 0.477 mmol) were dissolved in H_2O/MeCN 1/1 mixture (55 mL), and the solution was bubbled with argon for 20 minutes. Then, CuI (91 mg, 0.477 mmol) was added, and the mixture was stirred at 50°C for 18 hours. The reaction mixture was monitored by TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture Spots were detected by methods M1 and M5. The reaction was not finished, but the reaction mixture was slowly evaporated on a rotary evaporator at 50°C, and the reaction was completed during evaporation. The resulting solid was suspended in H_2O (50 mL), and insoluble CuI was filtered off using celite. The product in a aq. solution was purified by a C18 RP column chromatography (20 g silica gel). The product eluted with 5% w/w MeOH aq. solution. Fractions with the product were evaporated on a rotary evaporator at 50°C. The product (0.79 g) was dissolved in H_2O (30 mL) and freeze-dried. The product was obtained as a white powder in an 82% yield (0.78 g). **[α]$^{25}_D$** = +95.9° (α +0.070, c = 0.37, H_2O). **IR(DRIFT)**: 3357,



2935, 1622 ν(C=O), 1425, 1254, 1159, 1081, 1030 cm^{-1}. **^{1}H NMR** (600 MHz, DMSO-d_6): δ = 9.02 (s, 2H, H-24), 8.13 (s, 1H, H-17), 7.74 – 7.59 (m, 4H, H-25, H-26), 5.99 – 5.69 (m, 14H, 2,3-OH), 4.85 – 4.80 (m, 7H, H-1, H-1'), 4.58 – 4.40 (m, 10H, 6-OH, H-16, H-19), 4.31 – 4.15 (m, 5H, H-6', H-23), 3.87 (s, 6H, H-27), 3.85 (t, *J* = 5.2 Hz, 1H, H-15), 3.79 – 3.29 (m, 52H, H-2, H-2', H-3, H-3', H-4, H-5, H-5', H-6, H-6', H-9, H-10, H-11, H-12, H-13, H-14), 3.26 – 3.09 (m, 3H, H-4', H-20), 2.96 – 2.93 (m, 0.5H, H-6'), 2.02 (s, 1.8H, H-8), 1.97 (s, 1.2H, H-8), 0.85 (s, 3H, H-22) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ = 170.30 – 170.14 (C-7), 142.89 (C-18), 137.71 (C-24), 124.67 – 124.63 (C-17), 123.70 – 123.48 (C-25, C-26), 120.68 (q, *J* = 322.3 Hz, CF_3), 102.38 – 101.65 (C-1, C-1'), 84.57 – 83.96 (C-4'), 81.96 – 81.16 (C-4), 73.07 – 71.79 (C-2, C-2', C-3, C-3', C-5), 70.79 (C-20), 70.66 (C-5'), 70.41 – 67.91 (C-10, C-11, C-12, C-13, C-14, C-15), 63.39 (C-19), 60.11 – 59.24 (C-6), 52.56 (C-23), 50.31 – 49.39 (C-9, C-16), 46.22 (C-6'), 39.52 (C-21, solvent overlay), 35.95 (C-27), 21.50 – 21.30 (C-8), 17.33 (C-22) ppm. **^{19}F NMR** (282 MHz, DMSO-d_6, C_6F_6): δ = -80.07, -164.90 (C_6F_6) ppm. **UV-VIS** (H_2O), λ_{max}, nm: 209.0, 8*10^{-5} M. **ESI MS**: for $C_{68}H_{112}N_8O_{39}^{2+}$ calcd: *m/z* 832.4, found 832.5 [M^{2+}]. **HRMS**: for $C_{68}H_{112}N_8O_{39}^{2+}$ calcd: *m/z* 832.3508, found 832.3476 [M^{2+}], Δ 3.8 ppm.

6.8.9 Fluorescent multiply charged and multiply charged cyclodextrins

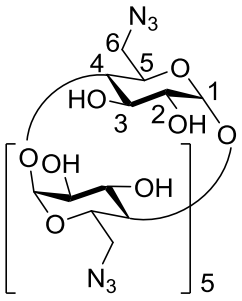
Per(6-bromo-6-deoxy)-α-CD (per(6-Br)-α-CD **82).** Compound per(6-Br)-α-CD **82** was prepared according to the previously published procedure[124]. PPh_3 (192 g, 0.73 mol) was



dissolved in dry DMF (250 mL) in a one-liter, three-necked flask equipped with an argon inlet, a drying tube, and a thermometer. The solution was cooled to approximately 18°C, and NBS (137 g, 0.77 mol) was added in small portions in a way that the temperature of the solution did not exceed 30°C. Dried α-CD (38.9 g, 0.04 mol) was then added to the obtained brown solution, and the mixture self-heated to 40°C. The temperature was raised to 70°C, and the reaction mixture was stirred for 8 hours. The reaction was monitored by TLC using dioxane/PrOH/conc. NH_3 aq. solution 10/3/7 mixture. Spots were detected by methods M1 and M5. The reaction mixture was diluted with MeOH (200 mL) and poured into MeOH (2000 mL). A 25% w/w solution of NaOMe in MeOH (300 mL) was added, the pH was raised to 9, and a precipitate formed. The precipitate dissolved again. Another 25% w/w solution of NaOMe in MeOH (100 mL) was added, and the suspension was stirred for 1 hour. The

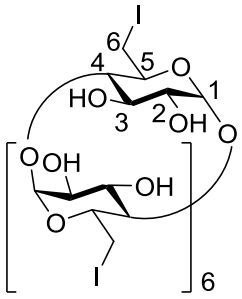
precipitate was collected by filtration, washed with MeOH (4 × 250 mL). The solid was suspended in H_2O (800 mL) and neutralized with conc. AcOH. The neutral suspension was collected by filtration, washed with H_2O (3 × 500 mL) and MeOH (500 mL). The product was air-dried and then evacuated at 70°C for 10 days. The product was obtained as a beige bulk solid in a 78% yield (42.1 g). $[\alpha]^{25}_D$ = +96.4° (α +0.132, c = 0.69, DMSO). **IR(DRIFT)**: 3345, 2914, 2866, 1649, 1257, 1153, 1051 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 5.70 (bs, 12H, OH), 4.94 (s, 6H, H-1), 3.93 – 3.72 (m, 24H, H-3, H-5, H-6), 3.44 – 3.34 (m, 12H, HDO overlay, H-2, H-4) ppm. **^{13}C NMR** (100 MHz, DMSO-d_6): δ = 101.87 (C-1), 84.72 (C-4), 72.50 (C-3), 71.64 (C-2), 70.63 (C-5), 34.83 (C-6) ppm. **ESI MS**: for $C_{36}H_{54}Br_6O_{24}$ calcd: *m/z* 1349.8 (for $[M+Na]^+$ calcd: *m/z* 1372.8), found 1373 $[M+Na]^+$. **HRMS**: for $C_{36}H_{54}Br_6O_{24}$ calcd: *m/z* 1349.8044 (for $[M+Na]^+$ calcd: *m/z* 1372.7936), found 1372.7952 $[M+Na]^+$, Δ 1.1 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[404].

Per(6-azido-6-deoxy)-α-CD (per(6-N_3)-α-CD **83).** Compound per(6-N_3)-α-CD **83** was prepared according to the previously published procedure[119]. Per(6-Br)-α-CD **82** (20.0 g, 14.9 mmol) was dissolved in DMF (300 mL) in a 1 L three-necked flask equipped with an argon inlet, a drying tube, and a thermometer. NaN_3 (20.3 g, 0.312 mol) was added, and the reaction mixture was heated to 70°C and stirred at this temperature for 5 hours. The reaction was monitored by TLC using dioxane/PrOH/conc. NH_3 aq. solution 10/3/7. Spots were detected by methods M1, M3, and M5. The reaction mixture was cooled to room temperature and stirred overnight. An excess of NaN_3 was removed by filtration. The solvent was removed on a rotary evaporator at 65°C. H_2O (300 mL) was added to the oily residue, and the resulting precipitate was collected by filtration. The product was washed with H_2O (3 × 200 mL) and acetone (200 mL) and air-dried for 15 days. The product was obtained as a brownish bulk solid in a 94% yield (15.9 g). $[\alpha]^{25}_D$ = +75.5° (α +0.071, c = 0.47, DMSO). **IR(DRIFT)**: 3351, 2920, 2107 ν(azide), 1640, 1293, 1153, 1054 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 5.65 – 5.48 (m, 12H, OH), 4.88 (s, 6H, H-1), 3.82 – 3.57 (m, 24H, H-3, H-5, H-6), 3.40 – 3.33 (m, 12H, HDO overlay, H-2, H-4) ppm. **^{13}C NMR** (100 MHz, DMSO-d_6): δ = 101.81 (C-1), 83.44 (C-4), 72.76 (C-3), 71.61 (C-2), 70.06 (C-5), 51.40 (C-6) ppm. **ESI MS**: for $C_{36}H_{54}N_{18}O_{24}$ calcd: *m/z* 1122.4 (for $[M+Na]^+$ calcd: *m/z* 1145.3), found 1145 $[M+Na]^+$. **HRMS**: for $C_{36}H_{54}N_{18}O_{24}$ calcd: *m/z* 1122.3558 (for $[M+Na]^+$ calcd: *m/z* 1145.3451),



found 1145.3468 $[M+Na]^+$, Δ 1.4 ppm. ^1H and ^{13}C NMR spectra are in accordance with the literature[405].

Per(6-deoxy-6-iodo)-β-CD (per(6-I)-β-CD **84).** Compound per(6-I)-β-CD **84** was



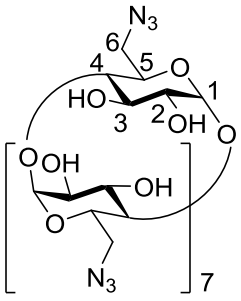
prepared according to the previously published procedure[119]. PPh_3 (210 g, 0.8 mol) was dissolved in dry DMF (340 mL) in a one-liter, three-necked flask equipped with an argon inlet, a drying tube, and a thermometer. The solution was cooled to approximately 18°C, and I_2 (216 g, 0.85 mol) was added in small portions in a way that the temperature of the solution did not exceed 30°C. Dried β-CD (56 g, 0.05 mol) was then added to the obtained brown solution, and the mixture self-heated to 50°C. The temperature was raised to 70°C, and the reaction mixture was stirred for 3 hours. The reaction was monitored by TLC using dioxane/PrOH/conc. NH_3 aq. solution 10/3/7 mixture. Spots were detected by methods M1 and M5. The reaction mixture was diluted with MeOH (250 mL) and poured into MeOH (2250 mL). A 25% w/w solution of NaOMe in MeOH (200 mL) was added, the pH was raised to 9, and a precipitate formed. The suspension was stirred overnight. Another 25% w/w solution of NaOMe in MeOH (50 mL) was added, and the suspension was stirred for 1 hour. The precipitate was collected by filtration, washed with MeOH (4 × 500 mL), H_2O to neutrality (2 × 500 mL), and again with MeOH (500 mL). The product was air-dried and then evacuated at 70°C for 3 days. The product was obtained as a beige bulk solid in a 90% yield (86 g). $\mathbf{[\alpha]^{25}_D}$ = +80.5° (α +0.140, c = 0.87, DMSO). **IR(DRIFT)**: 3348, 2905, 1216, 1150, 1042 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 6.03 – 5.92 (m, 14H, OH), 4.99 (s, 7H, H-1), 3.81 – 3.79 (m, 7H, H-6), 3.67 – 3.57 (m, 14H, H-3, H-5), 3.46 – 3.26 (m, 21H, HDO overlay, H-2, H-4, H-6) ppm. **^{13}C NMR** (100 MHz, DMSO-d_6): δ = 102.12 (C-1), 85.94 (C-4), 72.16 – 71.91 (C-2, C-3), 70.94 (C-5), 9.48 (C-6) ppm. **ESI MS**: for $C_{42}H_{63}I_7O_{28}$ calcd: *m/z* 1903.7 (for $[M+Na]^+$ calcd: *m/z* 1926.7), found 1927 $[M+Na]^+$. **HRMS**: for $C_{42}H_{63}I_7O_{28}$ calcd: *m/z* 1903.6819 (for $[M+Na]^+$ calcd: *m/z* 1926.6711), found 1926.6686 $[M+Na]^+$, Δ 1.2 ppm. ^1H and ^{13}C NMR spectra are in accordance with the literature[405].

Per(6-azido-6-deoxy)-β-CD (per(6-N_3)-β-CD **85).** Compound per(6-N_3)-β-CD **85** was prepared according to the previously published procedure[119]. Per(6-I)-β-CD **84** (70.0 g, 0.037 mol) was dissolved in DMF (700 mL) in a 2 L three-necked flask equipped with an argon inlet, a drying tube, and a thermometer. NaN_3 (50 g, 0.772 mol) was added, and the reaction mixture was heated to 70°C and stirred at this temperature for 3 hours. The reaction was monitored by TLC using dioxane/PrOH/conc. NH_3 aq. solution 10/3/7. Spots were detected by methods M1, M3, and M5. The reaction mixture was cooled to room temperature and stirred overnight. An excess of NaN_3 was removed by filtration. The solvent was removed on a rotary evaporator at 65°C. H_2O (400 mL) was added to the oily residue, and the resulting precipitate was collected by filtration. The product was washed with H_2O (200 mL) and air-dried. The product was then dried for one week in an evacuated oven at 70°C. The product was obtained as a brownish bulk solid in a 99% yield (47 g). $[\alpha]^{25}_D$ = +81.3° (α +0.135, c = 0.83, DMSO). **IR(DRIFT)**: 3315, 2920, 2110 ν(azide), 1661, 1284, 1159, 1054 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 5.90 – 5.76 (m, 14H, OH), 4.91 (d, J = 3.6 Hz, 7H, H-1), 3.79 – 3.71 (m, 14H, H-5, H-6), 3.62 – 3.56 (m, 14H, H-3, H-6), 3.39 – 3.31 (m, 14H, HDO overlay, H-2, H-4) ppm. **^{13}C NMR** (100 MHz, DMSO-d_6): δ = 102.05 (C-1), 83.19 (C-4), 72.58 (C-3), 72.00 (C-2), 70.32 (C-5), 51.33 (C-6) ppm. **ESI MS**: for $C_{42}H_{63}N_{21}O_{28}$ calcd: *m/z* 1309.4 (for $[M+Na]^+$ calcd: *m/z* 1332.4), found 1332 $[M+Na]^+$. **HRMS**: for $C_{42}H_{63}N_{21}O_{28}$ calcd: *m/z* 1309.4151 (for $[M+Na]^+$ calcd: *m/z* 1332.4044), found 1332.4046 $[M+Na]^+$, Δ 0.1 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[405].

Per(6-chloro-6-deoxy)-γ-CD (per(6-Cl)-γ-CD **86).** Compound per(6-Cl)-γ-CD **86** was prepared according to the previously published procedure describing the synthesis of β-CD analog[125]. PPh_3 (192 g, 0.73 mol) was dissolved in dry DMF (250 mL) in a one-liter, three-necked flask equipped with an argon inlet, a drying tube, and a thermometer. The solution was cooled to approximately 18°C, and TsCl (146.8 g, 0.77 mol) was added in small portions in a way that the temperature of the solution did not exceed 30°C. Dried γ-CD (52.0 g, 0.04 mol) was then added to the obtained yellow solution, and the mixture self-heated to 35°C. The temperature was raised to 70°C, and the reaction mixture was stirred for 4 hours. The reaction was monitored by

TLC using dioxane/PrOH/conc. NH_3 aq. solution 10/3/7 mixture. Spots were detected by methods M1 and M5. The reaction mixture was cooled to room temperature and stayed at this temperature overnight. The resulting crystals of TsCl were separated by filtration, and the homogeneous reaction mixture was poured into MeOH (2000 mL). A 25% w/w solution of NaOMe in MeOH (200 mL) was added, the pH was raised to 9, and a precipitate formed. The precipitate dissolved again. Another 25% w/w solution of NaOMe in MeOH (100 mL) was added, and the suspension was stirred for 2 hours. The precipitate was collected by filtration, washed with MeOH (3 × 200 mL). The product was air-dried and then evacuated at 70°C for 7 days. The product was obtained as a beige bulk solid in an 82% yield (47.0 g). $[\alpha]^{25}_D$ = +140.4° (α +0.125, c = 0.45, DMSO). **IR(DRIFT)**: 3315, 2923, 1634, 1299, 1159, 1042 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 6.28 (bs, 16H, OH), 4.95 (s, 8H, H-1), 4.01 – 3.83 (m, 24H, H-5, H-6), 3.62 (bs, 8H, H-3), 3.36 (bs, 16H, H-2, H-4) ppm. **^{13}C NMR** (100 MHz, DMSO-d_6): δ = 102.25 (C-1), 83.08 (C-4), 72.63 – 72.53 (C-2, C-3), 71.09 (C-5), 45.09 (C-6) ppm. **ESI MS**: for $C_{48}H_{72}Cl_8O_{32}$ calcd: *m/z* 1444.2 (for $[M+Na]^+$ calcd: *m/z* 1467.1), found 1467 $[M+Na]^+$. **HRMS**: for $C_{48}H_{72}Cl_8O_{32}$ calcd: *m/z* 1444.1456 (for $[M+Na]^+$ calcd: *m/z* 1467.1348), found 1467.1437 $[M+Na]^+$, Δ 6.1 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[406].

Per(6-azido-6-deoxy)-γ-CD (per(6-N_3)-γ-CD **87).** Compound per(6-N_3)-γ-CD **87** was prepared according to the previously published procedure[119]. Per(6-Cl)-γ-CD **86** (5.0 g, 3.46 mmol) was dissolved in DMF (200 mL) in a 500 mL flask. NaN_3 (4.72 g, 72.7 mmol) was added, and the reaction mixture was heated to 70°C and stirred at this temperature for 65 hours. The reaction was monitored by TLC using PrOH/H_2O/EtOAc/conc. NH_3 aq. solution 6/1/1/1 mixture. Spots were detected by methods M3 and M5. The reaction mixture was cooled to room temperature. An excess of NaN_3 was removed by filtration. The solvent was distilled from the reaction mixture under reduced pressure at 70°C. H_2O (100 mL) was added to the residue, and the resulting precipitate was collected by filtration. The product was washed with H_2O (3 × 50 mL) and MeOH (2 × 50 mL) and dried at 70°C using an oil rotary pump. The product was obtained as a brownish bulk solid in an 84% yield (4.4 g). $[\alpha]^{25}_D$ = +106.8° (α +0.078, c = 0.37, DMSO). **IR(DRIFT)**: 3333, 2923, 2107 ν(azide), 1658, 1287, 1153, 1045 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 5.92 – 5.86 (m, 16H, OH),



4.94 (s, 8H, H-1), 3.75 – 3.54 (m, 32H, H-3, H-5, H-6), 3.39 – 3.33 (m, 16H, HDO overlay, H-2, H-4) ppm. **^{13}C NMR** (100 MHz, DMSO-d_6): δ = 102.04 (C-1), 82.66 (C-4), 72.46 (C-3), 72.24 (C-2), 70.46 (C-5), 51.15 (C-6) ppm. **ESI MS**: for $C_{48}H_{72}N_{24}O_{32}$ calcd: *m/z* 1496.5 (for $[M+Na]^+$ calcd: *m/z* 1519.5), found 1519 $[M+Na]^+$. **HRMS**: for $C_{48}H_{72}N_{24}O_{32}$ calcd: *m/z* 1496.4745 (for $[M+Na]^+$ calcd: *m/z* 1519.4637), found 1519.4664 $[M+Na]^+$, Δ 1.7 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[405].

Per(6-azido-6-deoxy)-mono-2^A-*O*-(2-(Boc-amino)ethyl)-β-CD **(**mono[2-*O*-(2-(Boc-amino)ethyl)]-per(6-N_3)-β-CD **88).** Per(6-N_3)-β-CD **85** (2.0 g, 1.53 mmol, dried at 60°C for 2 hours) was dissolved in dry DMSO (40 mL). NaH (0.37 g, 9.16 mmol, 60% dispersion in oil) was added, and the mixture was stirred for 1 hour at room temperature. Then, 15-crown-5 (0.9 mL, 4.58 mmol) and TBAI (0.28 g, 0.76 mmol) were added. At last, 2-(Boc-amino)ethyl bromide (0.41 g, 1.83 mmol) was added, and the reaction mixture was stirred at room temperature for 17 hours. The reaction mixture was monitored by TLC using PrOH/H_2O/EtOAc/conc. NH_3 aq. solution 6/1/1/1 mixture. Spots were detected by the method M5. A new portion of 2-(Boc-amino)ethyl bromide (0.41 g, 1.83 mmol) was added, and the reaction mixture was stirred at room temperature for another 23 hours. The reaction mixture was added dropwise to H_2O (200 mL), and the resulting precipitate was collected by filtration. The crude product (8.2 g) was dissolved in the smallest possible amount of dioxane, silica gel (10 g) was added, and the mixture was evaporated on a rotary evaporator at 50°C. The adsorbed crude product was purified by column chromatography (40 g silica gel) eluting with PrOH/H_2O/conc. NH_3 aq. solution 36/1/6. After purification, fractions with product were evaporated on a rotary evaporator at 50°C. The product was dried at 70°C using an oil rotary pump. The final product was obtained as a white solid in a 30% yield (0.67 g). **$[\alpha]^{25}_D$** = +92.2° (α +0.059, c = 0.32, DMSO). **IR(DRIFT)**: 3339, 2917, 2107 ν(azide), 1682 ν(C=O), 1278, 1156, 1051 cm^{-1}. **^{1}H NMR** (600 MHz, DMSO-d_6): δ = 6.78 (t, *J* = 5.6 Hz, 1H, NH), 6.01 – 5.72 (m, 13H, OH), 5.08 (s, 1H, H-1'), 4.91 (s, 6H, H-1), 3.86 – 3.53 (m, 30H, H-3', H-3, H-5', H-5, H-6', H-6, H-7), 3.45 – 3.29 (m, 14H, HDO overlay, H-2', H-2, H-4', H-4,), 3.13 (dt, *J* = 12.5, 6.6 Hz, 1H, H-8), 3.05 (dt, *J* = 13.5, 6.5 Hz, 1H, H-8), 1.38 (s, 9H, H-11).ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ = 155.62 (C-9), 102.13 – 101.93 (C-1),

100.24 (C-1'), 83.39 – 83.10 (C-4', C-4), 79.99 (C-2'), 77.83 (C-10), 72.57 – 71.81 (C-2, C-3', C-3), 70.59 (C-7), 70.32 – 70.02 (C-5', C-5), 51.24 (C-6', C-6), 39.52 (C-8, solvent overlay), 28.22 (C-11) ppm. **ESI MS**: for $C_{49}H_{76}N_{22}O_{30}$ calcd: *m/z* 1452.5 (for $[M+Na]^+$ calcd: *m/z* 1475.5), found 1476.2 $[M+Na]^+$. **HRMS**: for $C_{49}H_{76}N_{22}O_{30}$ calcd: *m/z* 1452.5098 (for $[M+Na]^+$ calcd: *m/z* 1475.4990), found 1475.4953 $[M+Na]^+$, Δ 2.5 ppm.

Per(6-azido-6-deoxy)-mono-2^A-*O*-(2-aminoethyl)-β-CD 2,2,2-trifluoroacetate (mono[2-*O*-(2-aminoethyl)]-per(6-N_3)-β-CD **89).** Mono[2-*O*-(2-(Boc-amino)ethyl)]-per(6-N_3)-β-CD **88** (0.57 g, 0.39 mmol) was dissolved in TFA (17 mL, 0.22 mol) and the solution was stirred at room temperature for 1 hour. The reaction mixture was monitored by TLC using PrOH/H_2O/EtOAc/conc. NH_3 aq. solution 6/1/1/1 mixture. Spots were detected by the method M5. The TFA was distilled from the reaction mixture under reduced pressure at 45°C. The solid crude product was suspended in H_2O (10 mL), and H_2O was later evaporated on a rotary evaporator at 50°C. The product was dried at 70°C using an oil rotary pump. The final product was obtained as a white solid in a 99% yield (0.57 g). $[\alpha]^{25}_D$ = +79.7° (α +0.051, c = 0.32, DMSO). **IR(DRIFT)**: 3300, 2929, 2113 ν(azide), 1670 ν(C=O), 1284, 1156, 1048 cm^{-1}. **^{1}H NMR** (600 MHz, DMSO-d_6): δ = 7.66 (s, 3H, NH), 6.17 – 5.68 (m, 13H, OH), 5.13 (d, *J* = 3.6 Hz, 1H, H-1'), 4.93 (m, 6H, H-1), 3.83 (t, *J* = 5.5 Hz, 2H, H-7), 3.82 – 3.54 (m, 28H, H-3', H-3, H-5', H-5, H-6', H-6), 3.45 – 3.30 (m, 14H, H-2', H-2, H-4', H-4), 3.01 (dt, *J* = 13.1, 5.2 Hz, 1H, H-8), 2.96 (dt, *J* = 13.2, 5.0 Hz, 1H, H-8) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ = 102.09 – 101.92 (C-1), 99.81 (C-1'), 83.22 – 82.97 (C-4', C-4), 79.91 (C-2'), 72.64 – 72.51 (C-3', C-3), 72.08 – 71.49 (C-2), 70.35 – 69.86 (C-5', C-5), 67.47 (C-7), 51.29 (C-6), 51.17 (C-6'), 38.85 (C-8) ppm. **^{19}F NMR** (282 MHz, DMSO-d_6, C_6F_6): δ = -76.01, -164.90 (C_6F_6) ppm. **ESI MS**: for $C_{44}H_{69}N_{22}O_{28}^+$ calcd: *m/z* 1353.5, found 1354.2 $[M^+]$. **HRMS**: for $C_{44}H_{69}N_{22}O_{28}^+$ calcd: *m/z* 1353.4646, found 1353.4631 $[M^+]$, Δ 1.1 ppm.

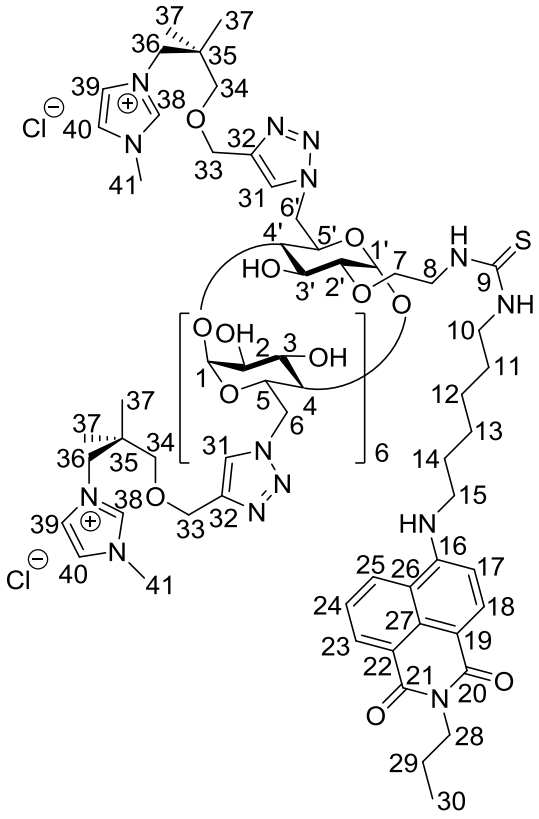
1-(6-((1,3-Dioxo-2-propyl-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-6-yl)amino)hexyl)-3-(per(6-azido-6-deoxy)-mono-2^{A}-*O*-ethyl-β-CD)thiourea (mono[2-*O*-(2-(NPNI-HDA-TU)ethyl)]-per(6-N_3)-β-CD **90).** Mono[2-*O*-(2-aminoethyl)]-per(6-N_3)-β-CD 2,2,2-trifluoroacetate **89** (0.57 g, 0.39 mmol) was dissolved in dry DMSO (20 mL) and DIPEA (0.27 mL, 1.55 mmol) was added. Then, NPNI-HDA-ITC **43** (0.23 g, 0.58 mmol) was added and the reaction mixture was stirred at room temperature for 15 hours. The reaction mixture was monitored by TLC using PrOH/H_2O/EtOAc/conc. NH_3 aq. solution 6/1/1/1 mixture. Spots were detected by methods M1 and M5. The solvent and DIPEA were distilled from the reaction mixture under reduced pressure at 90°C. The solid crude product was dissolved in THF (8 mL), and the product was precipitated by adding EtOAc (60 mL). The product was filtered, washed with EtOAc (30 mL), and dried at 70°C using an oil rotary pump. The final product was obtained as a light brown solid in a 76% yield (0.52 g). **[α]$^{25}_{D}$** = +69.2° (α +0.045, c = 0.33, DMSO). **IR(DRIFT)**: 3330, 2926, 2860, 2104 ν(azide), 1643, 1580, 1284, 1245, 1153, 1051 cm^{-1}. **^{1}H NMR** (600 MHz, DMSO-d_6): δ = 8.71 (dd, J = 8.4 Hz, J = 1.2 Hz, 1H, H-23), 8.43 (dd, J = 7.3 Hz, J = 1.0 Hz, 1H, H-18), 8.27 (d, J = 8.5 Hz, 1H, H-25), 7.75 (t, J = 5.5 Hz, 1H, NH), 7.68 (dd, J = 8.4, 7.3 Hz, 1H, H-24), 7.54 (bs, 1H, NH), 7.27 (bs, 1H, NH), 6.78 (d, J = 8.7 Hz, 1H, H-17), 6.15 – 5.70 (m, 13H, OH), 5.12 (d, J = 3.6 Hz, 1H, H-1'), 4.92 – 4.80 (m, 6H, H-1), 4.00 – 3.95 (m, 2H, H-28), 3.84 – 3.54 (m, 30H, H-3', H-3, H-5', H-5, H-6', H-6, H-7), 3.44 – 3.28 (m, 20H, HDO overlay, H-2', H-2, H-4', H-4, H-8, H-10, H-15), 1.79 – 1.32 (m, 8H, H-11, H-12, H-13, H-14), 1.61 (h, J = 7.5 Hz, 2H, 29), 0.90 (t, J = 7.4 Hz, 3H, H-30) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ = 163.80 – 162.96 (C-20, C-21), 150.67 (C-16), 134.32 (C-25), 130.67 (C-18), 129.47 (C-22), 128.61 (C-23), 124.23 (C-24), 121.87 (C-27), 120.12 (C-26), 107.48 (C-19), 103.78 (C-17). 102.47 – 101.61 (C-1), 100.20 (C-1'), 83.85 – 82.86 (C-4', C-4). 80.11 (C-2'), 73.55 – 69.65 (C-2, C-3', C-3, C-5', C-5), 51.31 – 51.24 (C-6', C-6), 42.85 (C-8), 40.74 (C-28), 28.22 – 25.89 (C-11, C-12, C-13, C-14), 20.98 (C-29), 11.40 (C-30) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 205.2, λ_{max2}, nm: 258.9, λ_{max3}, nm: 283.3, λ_{max4}, nm: 442.2, 3*10^{-5} M. **ESI MS**: for $C_{66}H_{93}N_{25}O_{30}S$ calcd: *m/z* 1747.6 (for $[M+Na]^+$ calcd: *m/z* 1770.6), found 1770 $[M+Na]^+$.

HRMS: for $C_{66}H_{93}N_{25}O_{30}S$ calcd: *m/z* 1747.6241 (for $[M+H]^+$ calcd: *m/z* 1748.6314), found 1748.6273 $[M+H]^+$, Δ 2.3 ppm.

General procedure for preparation of fluorescent multiply charged CDs and multiply charged CDs (GP5).

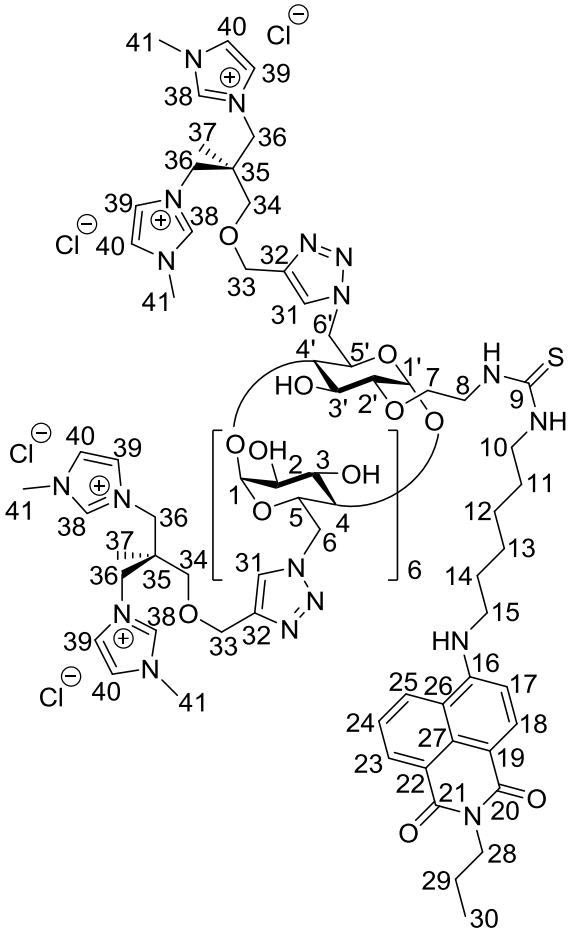
Mono[2-*O*-(2-(NPNI-HDA-TU)ethyl)]-per(6-N_3)-β-CD **90**, per(6-N_3)-α-CD **83**, per(6-N_3)-β-CD **85**, or per(6-N_3)-γ-CD **87** and Prg-O-MIM1 trifluoromethanesulfonate **1**, Prg-O-MIM2 bis(trifluoromethanesulfonate) **4**, Prg-O-MIM3 trichloride **7**, or Prg-O-PYR2 bis(trifluoromethanesulfonate) **5** (1.1 eq. per glucose unit) were dissolved in dry DMF. The solution was bubbled with argon for 30 minutes. $CuSO_4 \cdot 5H_2O$ (1.0 eq.) and sodium ascorbate (2.0 eq.) were added, the reaction mixture was heated to 60°C, and stirred for several hours. The reaction mixture was monitored by TLC using PrOH/H_2O/EtOAc/conc. NH_3 aq. solution 6/1/1/1 mixture for the starting compound and MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture for the product. Spots were detected by methods M1 and M5. The solvent was distilled from the reaction mixture under reduced pressure at 60°C. The solid crude product was dissolved in 0.01 M NH_4HCO_3 aq. solution (4 mL) and purified by column chromatography (basic alumina) using a gradient elution method (0.01 M, 0.1 M, and 1 M NH_4HCO_3 aq. solution). Fractions with the pure product were evaporated on a rotary evaporator at 50°C. The solid was dissolved in MeOH and neutralized with 1 M HCl. The pure product was evaporated on a rotary evaporator at 50°C again, dissolved in H_2O, and freeze-dried.

Mono[2-*O*-(2-(NPNI-HDA-TU)ethyl)]-per[6-(MTZ-O-MIM1)]-β-CD heptachloride (91) Compound **91** was prepared according to the general procedure (**GP5**). Mono[2-*O*-(2-(NPNI-HDA-TU)ethyl)]-per(6-N_3)-β-CD **90** (0.1 g, 57.2 μmol) and Prg-O-MIM1 trifluoromethanesulfonate **1** (0.16 g, 0.44 mmol), $CuSO_4 \cdot 5H_2O$ (14.0 mg, 57.2 μmol), and sodium ascorbate (22.7 mg, 0.114 mmol) were dissolved in 5 mL of DMF. The reaction mixture was stirred for 14 hours. Basic alumina (9 g) was used for column chromatography. The product was obtained as a yellow powder in a 76% yield (0.15 g) after freeze-drying from 4 mL of H_2O. $[\alpha]^{25}_D$ = +11.6° (α +0.008, c = 0.35, DMSO). **IR(DRIFT)**: 3297, 3126, 2959, 2854, 1637, 1577, 1335, 1228, 1162, 1087, 1045 cm^{-1}. **UV-VIS** (H_2O), λ_{max}, nm: 450.0, $2*10^{-4}$ M.

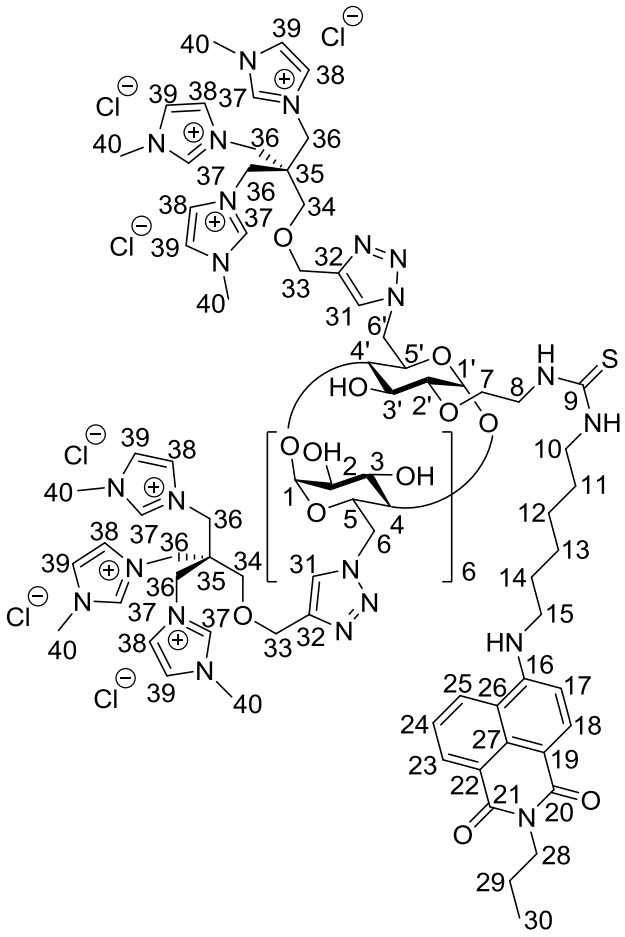


HRMS: for $C_{150}H_{226}N_{39}O_{37}S^{7+}$ calcd: *m/z* 456.9531 (for $[M^{7+}+4\times Cl^-]^{3+}$ calcd: *m/z* 1113.5155, for $[M^{7+}+3\times Cl^-]^{4+}$ calcd: *m/z* 826.3943, for $[M^{7+}+2\times Cl^-]^{5+}$ calcd: *m/z* 653.9228), found 1113.5343 $[M^{7+}+4\times Cl^-]^{3+}$, 826.4085 $[M^{7+}+3\times Cl^-]^{4+}$, 653.9329 $[M^{7+}+2\times Cl^-]^{5+}$.

Mono[2-*O*-(2-(NPNI-HDA-TU)ethyl)]-per[6-(MTZ-O-MIM2)]-β-CD tetradecachloride (92). Compound **92** was prepared according to the general procedure (**GP5**). Mono[2-*O*-(2-(NPNI-HDA-TU)ethyl)]-per(6-N_3)-β-CD **90** (0.09 g, 51.5 μmol) and Prg-O-MIM2 bis(trifluoromethanesulfonate) **4** (0.23 g, 0.40 mmol), $CuSO_4 \cdot 5H_2O$ (13.0 mg, 51.5 μmol), and sodium ascorbate (20.0 mg, 0.103 mmol) were dissolved in 5 mL of DMF. The reaction mixture was stirred for 14 hours. Basic alumina (10 g) was used for column chromatography. The product was obtained as a yellow powder in a 71% yield (0.16 g) after freeze-drying from 7 mL of H_2O. $[\alpha]^{25}_D$ = +3.3° (α +0.002, c = 0.31, MeOH). **IR(DRIFT)**: 3132, 3028, 1676, 1637, 1574, 1407, 1159, 1084, 1045 cm^{-1}. **UV-VIS** (H_2O), λ_{max}, nm: 450.0, $2*10^{-4}$ M. **HRMS**: for $C_{178}H_{261}N_{53}O_{37}S^{14+}$ calcd: *m/z* 268.9989 (for $[M^{14+}+9\times Cl^-]^{5+}$ calcd: *m/z* 817.1414, for $[M^{14+}+8\times Cl^-]^{6+}$ calcd: *m/z* 675.1229), found 817.1521 $[M^{14+}+9\times Cl^-]^{5+}$, 675.1319 $[M^{14+}+8\times Cl^-]^{6+}$.

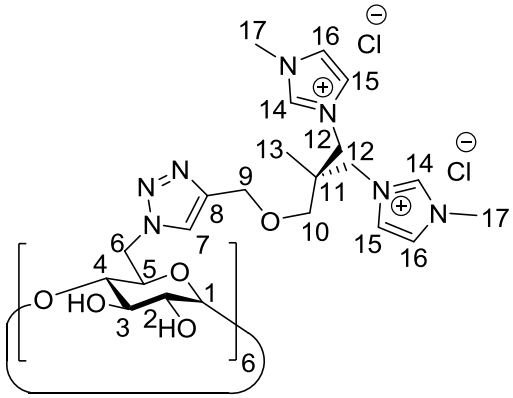


Mono[2-*O*-(2-(NPNI-HDA-TU)ethyl)]-per[6-(MTZ-O-MIM3)]-β-CD henicosachloride (93). Compound **93** was prepared according to the general procedure (**GP5**). Mono[2-*O*-(2-(NPNI-HDA-TU)ethyl)]-per(6-N_3)-β-CD **90** (0.1 g, 57.2 μmol) and Prg-O-MIM3 trichloride **7** (0.21 g, 0.44 mmol), $CuSO_4 \cdot 5H_2O$ (14.0 mg, 57.2 μmol),



and sodium ascorbate (22.7 mg, 0.114 mmol) were dissolved in 5 mL of DMF. The reaction mixture was stirred for 14 hours. The reaction was not finished, so MeOH (4 mL) was added, and the reaction mixture was stirred for another 25 hours. Basic alumina (13 g) was used for column chromatography. The product was obtained as a yellow powder in a 58% yield (0.17 g). $[\alpha]^{25}_{D}$ = +10.3° (α +0.006, c = 0.29, H_2O). **IR(DRIFT)**: 3363, 3079, 2956, 2869, 1643, 1577, 1344, 1231, 1168, 1090, 1048 cm^{-1}. **UV-VIS** (H_2O), λ_{max}, nm: 450.0, $2*10^{-4}$ M. **HRMS**: for $C_{206}H_{296}N_{67}O_{37}S^{21+}$ calcd: *m/z* 206.3953 (for $[M^{21+}+17\times Cl^-]^{4+}$ calcd: *m/z* 1234.1923, for $[M^{21+}+16\times Cl^-]^{5+}$ calcd: *m/z* 980.3600, for $[M^{21+}+15\times Cl^-]^{6+}$ calcd: *m/z* 810.8056), found 1234.2061 $[M^{21+}+17\times Cl^-]^{4+}$, 980.3710 $[M^{21+}+16\times Cl^-]^{5+}$, 810.9812 $[M^{21+}+15\times Cl^-]^{6+}$.

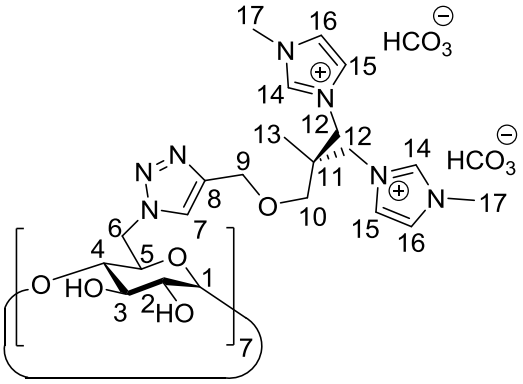
Per[6-(MTZ-O-MIM2)]-α-CD dodecachloride (94). Compound **94** was prepared according to the general procedure (**GP5**). Per(6-N_3)-α-CD **83** (0.3 g, 0.27 mmol) and Prg-O-MIM2 bis(trifluoromethanesulfonate) **4** (1.03 g, 1.76 mmol), $CuSO_4\cdot 5H_2O$ (66.7 mg, 0.27 mmol), and sodium ascorbate (0.106 mg, 0.53 mmol) were dissolved in 15 mL



of DMF. The reaction mixture was stirred for 17 hours. Basic alumina (30 g) was used for column chromatography. The product was obtained as a brown solid in a 40% yield (0.35 g) after freeze-drying from 6 mL of H_2O. $[\alpha]^{25}_{D}$ = +13.1° (α +0.008, c = 0.31, H_2O). **IR(DRIFT)**: 3363, 2962, 2929, 2875, 1799, 1664, 1580, 1260, 1231, 1162, 1036 cm^{-1}. **^{1}H**

NMR (600 MHz, D_2O, *t*BuOH): δ = 8.80 (d, *J* = 10.1 Hz, 12H, H-14), 8.26 (s, 6H, H-7), 7.51 – 7.41 (m, 24H, H-15, H-16), 5.19 (d, *J* = 3.3 Hz, 6H, H-1), 4.68 – 4.49 (m, 24H, H-6, H-9), 4.44 – 4.16 (m, 30H, H-5, H-12), 4.09 (t, *J* = 9.3 Hz, 6H, H-3), 3.92 (d, *J* = 9.1 Hz, 36H, H-17), 3.45 (dd, *J* = 10.0, 3.2 Hz, 6H, H-2), 3.29 – 3.10 (m, 18H, H-4, H-10), 1.24 (*t*BuOH), 0.92 (s, 18H, H-13) ppm. **^{13}C NMR** (151 MHz, D_2O, *t*BuOH): δ = 144.42 (C-8), 137.85 (C-14), 128.14 (C-7), 124.47 – 124.35 (C-15, C-16), 101.99 (C-1), 82.59 (C-4), 73.04 (C-3), 72.10 (C-2), 71.04 (C-10), 70.63 (C-5), 63.68 (C-9), 53.64 – 53.44 (C-12), 51.20 (C-6), 40.57 (C-11), 36.67 (C-17), 30.29 (*t*BuOH), 17.54 (C-13) ppm. **UV-VIS** (H_2O), λ_{max}, nm: 210.3, 9*10^{-7} M. **HRMS**: for $C_{132}H_{198}N_{42}O_{30}^{12+}$ calcd: *m/z* 237.7102 (for $[M^{12+}+9\times Cl^-]^{3+}$ calcd: *m/z* 1057.0805, for $[M^{12+}+8\times Cl^-]^{4+}$ calcd: *m/z* 784.0680), found 1057.4140 $[M^{12+}+9\times Cl^-]^{3+}$, 784.0686 $[M^{12+}+8\times Cl^-]^{4+}$.

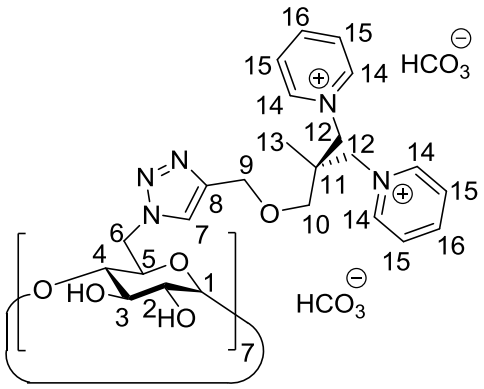
Per[6-(MTZ-O-MIM2)]-β-CD tetradecabicarbonate (95). Compound **95** was prepared according to the general procedure (**GP5**). Per(6-N_3)-β-CD **85** (1.0 g, 0.76 mmol) and Prg-O-MIM2 bis(trifluoromethanesulfonate) **4** (3.4 g, 5.88 mmol), $CuSO_4 \cdot 5H_2O$ (0.19 g, 0.76 mmol), and sodium ascorbate (0.30 g, 1.53 mmol) were dissolved in 50 mL of DMF. The reaction mixture was stirred for 19 hours. Basic alumina (180 g) was used for column chromatography. This time, the product after chromatography was just dissolved in MeOH without any neutralization, filtered, and evaporated. The product was obtained as a brown solid in an 84% yield (2.7 g) after freeze-drying from 50 mL of H_2O. **$[\alpha]^{25}_D$** = +15.9° (α +0.010, c = 0.32, H_2O). **IR(DRIFT)**: 3360, 3076, 2869, 1646, 1580, 1422, 1338, 1260, 1231, 1156, 1087, 1042 cm^{-1}. **^{1}H NMR** (600 MHz, D_2O, *t*BuOH): δ = 8.78 (d, *J* = 8.7 Hz, 14H, H-14), 8.19 (s, 7H, H-7), 7.48 – 7.39 (m, 28H, H-15, H-16), 5.17 (s, 7H, H-1), 4.70 – 4.67 (m, 7H, H-6), 4.54 – 4.49 (m, 21H, H-6, H-9), 4.34 – 4.15 (m, 35H, H-5, H-12), 4.03 (t, *J* = 9.3 Hz, 7H, H-3), 3.89 (d, *J* = 7.8 Hz, 42H, H-17), 3.44 (d, *J* = 10.4 Hz, 7H, H-2), 3.26 (t, *J* = 9.8 Hz, 7H, H-4), 3.23 – 3.08 (m, 14H, H-10), 1.24 (*t*BuOH), 0.90 (s, 21H, H-13) ppm. **^{13}C NMR** (150 MHz, D_2O, *t*BuOH): δ = 144.24 (C-8), 137.81 – 137.78 (C-14), .128.02 (C-7), 124.40 – 124.30 (C-15, C-16), 102.33 (C-1), 82.48 (C-4), 72.86 (C-3), 72.46 (C-2), 71.07 (C-10), 70.45 (C-5), 70.33 (*t*BuOH), 63.66 (C-9), 53.59 – 53.41 (C-12), 50.86 (C-6),



40.51 (C-11), 36.62 (C-17), 30.29 (*t*BuOH), 17.50 (C-13) ppm. **UV-VIS** (H_2O), λ_{max}, nm: 212.8, $2*10^{-6}$ M. **HRMS**: for $C_{154}H_{231}N_{49}O_{35}^{14+}$ calcd: *m/z* 237.6983 (for $[M^{14+}+10\times Cl^-]^{4+}$ calcd: *m/z* 920.3660, for $[M^{14+}+9\times Cl^-]^{5+}$ calcd: *m/z* 729.4996), found 920.6254 $[M^{14+}+10\times Cl^-]^{4+}$, 729.5065 $[M^{14+}+9\times Cl^-]^{5+}$.

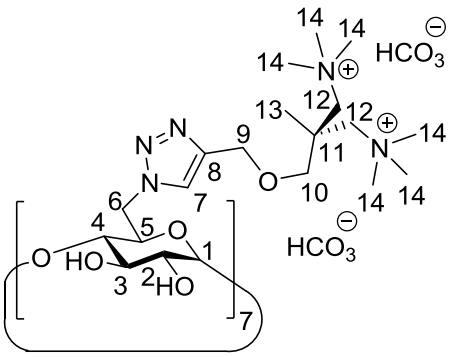
Per[6-(MTZ-O-MIM2)]-γ-CD hexadecachloride (96). Compound **96** was prepared according to the general procedure (**GP5**). Per(6-N_3)-γ-CD **87** (0.3 g, 0.20 mmol) and Prg-O-MIM2 bis(trifluoromethanesulfonate) **4** (1.03 g, 1.76 mmol), $CuSO_4\cdot 5H_2O$ (50.0 mg, 0.20 mmol), and sodium ascorbate (80.0 mg, 0.40 mmol) were dissolved in 15 mL of DMF. The reaction mixture was stirred for 20 hours. Basic alumina (50 g) was used for column chromatography. The product was obtained as a brown solid in a 49% yield (0.43 g) after freeze-drying from 8 mL of H_2O. $[\alpha]^{25}_D$ = +23.4° (α +0.015, c = 0.32, H_2O). **IR(DRIFT)**: 3351, 3079, 2884, 2101, 1649, 1574, 1338, 1231, 1159, 1084, 1042 cm^{-1}. **^{1}H NMR** (600 MHz, D_2O, *t*BuOH): δ = 8.78 – 8.65 (m, 16H, H-14), 8.11 (s, 8H, H-7), 7.43 – 7.31 (m, 32H, H-15, H-16), 5.17 (s, 8H, H-1), 4.61 – 4.40 (m, 32H, H-6, H-9), 4.39 – 4.06 (m, 40H, H-5, H-12), 4.00 – 3.78 (m, 56H, H-3, H-17), 3.41 (s, 8H, H-2), 3.26 (s, 8H, H-4), 3.19 – 3.02 (m, 16H, H-10), 1.24 (*t*BuOH), 0.85 (s, 24H, H-13) ppm. **^{13}C NMR** (151 MHz, D_2O, *t*BuOH): δ = 144.29 (C-8), 137.88 (C-14), 127.90 (C-7), 124.49 – 124.43 (C-15, C-16), 102.04 (C-1), 82.08 (C-4), 72.78 – 72.58 (C-3, C-2), 71.17 (C-10), 70.43 (*t*BuOH), 70.22 (C-5), 63.79 (C-9), 53.67 – 53.56 (C-12), 50.97 (C-6), 40.62 (C-11), 36.71 (C-17), 30.29 (*t*BuOH), 17.63 (C-13) ppm. **UV-VIS** (H_2O), λ_{max}, nm: 212.2, $2*10^{-7}$ M. **HRMS**: for $C_{176}H_{264}N_{56}O_{40}^{16+}$ calcd: *m/z* 237.6893 (for $[M^{16+}+11\times Cl^-]^{5+}$ calcd: *m/z* 838.7367, for $[M^{16+}+10\times Cl^-]^{6+}$ calcd: *m/z* 729.4996), found 692.9534 $[M^{16+}+11\times Cl^-]^{5+}$, 692.9532 $[M^{16+}+10\times Cl^-]^{6+}$.

Per[6-(MTZ-O-PYR2)]-β-CD tetradecabicarbonate (97). Compound **97** was prepared according to the general procedure (**GP5**). Per(6-N_3)-β-CD **85** (0.3 g, 0.23 mmol) and



Prg-O-PYR2 bis(trifluoromethanesulfonate) **5** (1.02 g, 1.76 mmol), $CuSO_4 \cdot 5H_2O$ (57.0 mg, 0.23 mmol), and sodium ascorbate (91.0 mg, 0.46 mmol) were dissolved in 15 mL of DMF. The reaction mixture was stirred for 20 hours. Basic alumina (60 g) was used for column chromatography. This time, the product after chromatography was just dissolved in MeOH without any neutralization, filtered, and evaporated. The product was obtained as a dark purple glassy powder in a 65% yield (0.63 g) after freeze-drying from 10 mL of H_2O. **$[\alpha]^{25}_D$** = +22.7° (α +0.017, c = 0.38, H_2O). **IR(DRIFT)**: 3363, 3055, 2899, 2606, 1631, 1251, 1213, 1183, 1156, 1096, 1045 cm^{-1}. **^{1}H NMR** (400 MHz, D_2O, *t*BuOH): δ = 8.76 – 8.71 (m, 28H, H-14), 8.60 – 8.55 (m, 14H, H-16), 8.18 – 7.95 (m, 35H, H-7, H-15), 5.16 (s, 7H, H-1), 4.88 (d, *J* = 13.3 Hz, 14H, H-12), 4.70 – 4.58 (m, 28H, H-10, H-12, solvent overlay), 4.52 – 4.39 (m, 21H, H-5, H-9), 4.03 (s, 7H, H-3), 3.34 (s, 7H, H-2), 3.23 – 3.06 (m, 21H, H-4, H-6), 1.24 (s, *t*BuOH), 0.94 (s, 21H, H-13) ppm. **^{13}C NMR** (101 MHz, D_2O, *t*BuOH): δ = 147.19 – 147.07 (C-16), 145.89 (C-14), 143.32 (C-8), 128.66 – 128.59 (C-15), 127.79 (C-7), 101.75 (C-1), 81.75 (C-4), 72.41 (C-3), 72.07 (C-2), 69.81 (C-5), 69.20 (C-6), 64.48 – 64.24 (C-12), 62.99 (C-9), 50.38 (C-10), 41.69 (C-11), 30.29 (*t*BuOH), 16.59 (C-13) ppm. **UV-VIS** (H_2O), λ_{max1}, nm: 216.0, λ_{max2}, nm: 260.0, $3*10^{-6}$ M. **HRMS**: for $C_{168}H_{217}N_{35}O_{35}^{14+}$ calcd: *m/z* 234.6874 (for $[M^{14+}+11\times Cl^-]^{3+}$ calcd: *m/z* 1225.4260, for $[M^{14+}+10\times Cl^-]^{4+}$ calcd: *m/z* 910.3271, for $[M^{14+}+9\times Cl^-]^{5+}$ calcd: *m/z* 720.8684), found 1225.0559 $[M^{14+}+11\times Cl^-]^{3+}$, 910.0495 $[M^{14+}+10\times Cl^-]^{4+}$, 721.0454 $[M^{14+}+9\times Cl^-]^{5+}$.

Per[6-(MTZ-O-TMA2)]-β-CD tetradecabicarbonate (98). Per(6-N_3)-β-CD **85** (0.15 g, 0.11 mmol) and Prg-O-TMA2 diiodide **6** (0.68 g, 1.37 mmol) were dissolved in dry DMF (8 mL). The solution was bubbled with argon for 30 minutes. $CuSO_4 \cdot 5H_2O$ (57.0 mg, 0.23 mmol) and sodium ascorbate (91.0 mg, 0.46 mmol) were added, and the reaction mixture was heated to 80°C and stirred for 3 days. The reaction mixture was monitored by TLC using PrOH/H_2O/EtOAc/conc. NH_3 aq. solution 6/1/1/1 mixture for the starting compound and MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture for the product. Spots were detected by the method M5. The solvent was distilled from the reaction mixture under reduced pressure at 80°C. The solid crude product was dissolved in 0.01 M NH_4HCO_3 aq. solution (10 mL) and purified by column chromatography (20 g basic alumina) using a gradient elution method (0.01 M, 0.1 M, and 1 M NH_4HCO_3 aq. solution). Fractions with the pure product were evaporated on a rotary evaporator at 50°C. The solid (0.56 g) was dissolved in MeOH (30 mL) and separated from insoluble NH_4HCO_3 solution impurities by filtration. The pure product was evaporated on a rotary evaporator at 50°C again, dissolved in H_2O (10 mL), and freeze-dried. The product was obtained as a brown glassy solid in a 70% yield (0.32 g). **$[\alpha]^{25}_D$** = +13.8° (α +0.008, c = 0.29, H_2O). **IR(DRIFT)**: 3369, 3022, 2902, 2612, 2107, 1637, 1482, 1338, 1225, 1153, 1090, 1051 cm^{-1}. **^{1}H NMR** (600 MHz, D_2O, *t*BuOH): δ = 8.24 (s, 7H, H-7), 5.21 (s, 7H, H-1), 4.62 (s, 28H, H-9, H-10), 4.37 (s, 7H, H-5), 4.06 (s, 7H, H-3), 3.78 (s, 28H, H-12), 3.69 – 3.47 (m, 14H, H-6), 3.28 (s, 133H, H-4, H-14), 1.49 (s, 21H, H-13), 1.27 (s, *t*BuOH) ppm. **^{13}C NMR** (151 MHz, D_2O, *t*BuOH): δ = 143.79 (C-8), 128.45 (C-7), 102.20 (C-1), 82.27 (C-4), 73.17 – 72.00 (C-2, C-3, C-5, C-12), 70.27 (C-6), 63.31 (C-9), 56.39 (C-14), 50.77 (C-10), 43.70 (C-11), 30.29 (*t*BuOH), 21.43 (C-13) ppm. **HRMS**: for $C_{140}H_{273}N_{35}O_{35}^{14+}$ calcd: *m/z* 214.7187 (for $[M^{14+}+10\times Cl^-]^{4+}$ calcd: *m/z* 840.4367, for $[M^{14+}+9\times Cl^-]^{5+}$ calcd: *m/z* 664.9560), found 840.1952 $[M^{14+}+10\times Cl^-]^{4+}$, 665.1632 $[M^{14+}+9\times Cl^-]^{5+}$.



6.8.10 Secondary rim modification

Per[2,3-phenylcarbamoyl-6-(MTZ-O-MIM2)]-β-CD (99)

Method A. Per[6-(MTZ-O-MIM2)]-β-CD tetradecabicarbonate **95** (0.2 g, 47.6 μmol) was dissolved in MeOH (10 mL), neutralized with 1 M TfOH in MeOH. The solution was evaporated on a rotary evaporator at 40°C, and the solid was dried at 70°C for 2 hours using an oil rotary pump. The compound was dissolved in dry pyridine (4 mL), and phenyl isocyanate (0.1 mL, 1.0 mmol) was added. The mixture was heated to 80°C and stirred for 18 hours. The reaction mixture was monitored by TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture. Spots were detected by methods M1 and M2. The reaction mixture was poured into Et_2O (200 mL). The formed precipitate was filtered and washed with Et_2O (5 × 50 mL). The product was dried at 50°C using an oil rotary pump. The final product was obtained as a grey solid in a 91% yield (0.31 g).

Method B. Per(2,3-phenylcarbamoyl-6-N_3)-β-CD **100** (0.2 g, 67.0 μmol) and Prg-O-MIM2 bis(trifluoromethanesulfonate) **4** (0.27 g, 0.47 mmol) were dissolved in DMF (10 mL). The solution was bubbled with argon for 30 minutes. $CuSO_4 \cdot 5H_2O$ (17.0 mg, 67.0 μmol) and sodium ascorbate (26.0 mg, 0.13 mmol) were added, and the reaction mixture was heated to 60°C and stirred for 19 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 2/1 mixture for the starting compound and MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture for the product. Spots were detected by methods M1 and M2. The solvent was distilled from the reaction mixture under reduced pressure at 70°C. The solid crude product (0.64 g) was dissolved in 0.01 M NH_4HCO_3 aq. solution (12 mL) and purified by column chromatography (15 g basic alumina) eluting with 0.01 M NH_4HCO_3 aq. solution. Fractions with the pure product were evaporated on a rotary evaporator at 40°C. The solid (0.67 g) was dissolved in MeOH (10 mL) and poured into Et_2O (150 mL). The formed precipitate was filtered and washed with Et_2O (2 × 30 mL). The product was dried at room temperature using an oil rotary pump. The final product was obtained as a beige solid in a 55% yield (0.26 g). $[\alpha]^{25}_D$ = +12.3° (α +0.007, c = 0.29, MeOH). **IR(DRIFT)**: 3535, 3400, 3292, 3151, 3116, 2964, 2873, 1736 ν(C=O), 1603, 1545, 1446, 1254, 1225, 1157, 1088, 1028 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 9.01 (bs, 14H, H-14), 8.18 (bs, 7H, H-7), 7.69 – 7.55 (m, 28H, H-15, H-16), 7.24 – 6.64

(m, 70H, H-20, H-21, H-22), 5.63 – 5.38 (m, 21H, H-1, NH), 4.83 – 4.03 (m, 70H, H-3, H-5, H-6, H-9, H-12), 3.81 (bs, 42H, H-17), 3.15 (bs, 28H, H_2O overlay, H-2, H-4, H-10), 0.80 (bs, 21H, H-13) ppm. **^{19}F NMR** (376 MHz, DMSO-d_6, C_6F_6): δ = -80.09, -164.90 (C_6F_6) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 228.0, λ_{max2}, nm: 272.0, 3*10^{-6} M. **HRMS**: for $C_{252}H_{301}N_{63}O_{49}^{14+}$ calcd: *m/z* 356.807 (for $[M^{14+}+13\times TfO^-]^+$ calcd: *m/z* 6931.682, $[M^{14+}+12\times TfO^-]^{2+}$ calcd: *m/z* 3391.364), found 6929.261 $[M^{14+}+13\times TfO^-]^+$, 3390.489 $[M^{14+}+12\times TfO^-]^{2+}$.

Per(6-azido-6-deoxy-2,3-di-*O*-phenylcarbamoyl)-β-CD (per(2,3-phenylcarbamoyl-6-N_3)-β-CD **100).** Compound **100** was prepared according to the previously published procedure[278]. Per(6-N_3)-β-CD **85** (1.0 g, 0.76 mmol) was dissolved in dry pyridine (20 mL). Phenyl isocyanate (1.8 mL, 16.0 mmol) was added, the reaction mixture was heated to 80°C and stirred for 16 hours. The reaction mixture was monitored by TLC using PrOH/H_2O/EtOAc/conc. NH_3 aq. solution 6/1/1/1 mixture for the starting compound and hexane/EtOAc 2/1 mixture for the product. Spots were detected by methods M1 and M5. The solvent was distilled from the reaction mixture under reduced pressure at 80°C. The solid crude product was dissolved in $CHCl_3$ (30 mL), silica gel (10 g) was added, and the mixture was evaporated on a rotary evaporator at 40°C. The adsorbed crude product was purified by column chromatography (60 g silica gel) eluting with hexane/EtOAc 5/1 and 1/1. Fractions with the pure product were evaporated on a rotary evaporator at 50°C. The product was dried at room temperature using an oil rotary pump. The final product was obtained as a slightly yellow solid in an 81% yield (1.86 g). **$[\alpha]^{25}_D$** = +100.0° (α +0.063, c = 0.32, $CHCl_3$). **IR(DRIFT)**: 3393, 3061, 2953, 2923, 2104 ν(azide), 1736 ν(C=O), 1601, 1536, 1443, 1311, 1225, 1165, 1084, 1057 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.17 – 6.74 (m, 84H, H-9, H-10, H-11, NH), 5.54 (dd, *J* = 10.3, 8.9 Hz, 7H, H-3), 5.24 (d, *J* = 3.7 Hz, 7H, H-1), 5.10 (dd, *J* = 10.4, 3.6 Hz, 7H, H-2), 4.30 – 4.17 (m, 7H, H-5), 3.99 (t, *J* = 9.3 Hz, 7H, H-4), 3.89 (dd, *J* = 13.5, 2.1 Hz, 7H, H-6), 3.76 (dd, *J* = 13.5, 5.3 Hz, 7H, H-6) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 153.00 – 152.66 (C-7), 137.13 – 136.83 (C-8), 129.17 – 119.03 (C-9, C-10, C-11), 98.56 (C-1), 78.55 (C-4), 73.10 (C-3), 71.47 (C-5), 71.24 (C-2), 51,53 (C-6) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 201.4, λ_{max2}, nm: 229.7, 4*10^{-6} M. **HRMS**: for $C_{140}H_{133}N_{35}O_{42}$ calcd: *m/z* 2976.9381 (for

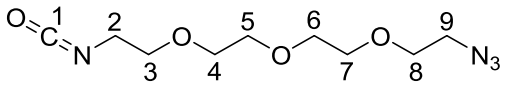
$[M+2\times Na]^{2+}$ calcd: *m/z* 1511.4583), found 1511.4579 $[M+2\times Na]^{2+}$, Δ 0.2 ppm. ^{1}H spectrum is in accordance with the literature[278].

1-Isocyanatooctadecane (101). Compound **101** was prepared according to the previously published procedure[380]. Octadecan-1-amine (6.0 g, 22.3 mmol) was partially dissolved in CH_2Cl_2 (180 mL). A saturated Na_2CO_3 aq. solution (180 mL) was added, and the mixture was vigorously stirred for 5 minutes. Then, the mixture was cooled to 0°C, and triphosgene (3.31 g, 11.2 mmol) in CH_2Cl_2 (170 mL) was added. The white suspension was formed after addition which slowly dissolved during the process. The mixture was stirred at room temperature for 2 hours. The reaction mixture was monitored by TLC using hexane. Spots were detected by the method M2. The aqueous phase was separated, and the organic phase was washed with 1 M HCl (200 mL). The organic phase was dried with $MgSO_4$ (3 g), filtered, and organic solvents were removed on a rotary evaporator at 30°C. The product was dried at room temperature using an oil rotary pump. The final product was obtained as colorless oil in a 90% yield (5.93 g). **IR(DRIFT)**: 2920, 2848, 2274 ν(isocyanate), 1778, 1679, 1467, 1359 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 3.28 (t, *J* = 6.7 Hz, 2H, H-2), 1.68 – 1.50 (m, 2H, H-3), 1.26 (m, 30H, H-4 – H-18), 0.87 (t, *J* = 7.0 Hz, 3H, H-19) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 121.85 (C-1), 43.15 (C-2), 31.47 (C-3), 32.08 – 22.85 (C-4 – C-18), 14.27 (C-19) ppm.

Per[2,3-octadecylcarbamoyl-6-(MTZ-O-MIM2)]-β-CD (102). Per[6-(MTZ-O-MIM2)]-β-CD tetradecabicarbonate **95** (0.1 g, 23.8 μmol) was dissolved in MeOH (2 mL), neutralized with 3 M TfOH in MeOH. The solution was evaporated on a rotary evaporator at 40°C, and the solid was dried at 70°C for 2 hours using an oil rotary pump. The compound was dissolved in dry DMF (2 mL). 1-Isocyanatooctadecane **101** (0.15 g, 0.5 mmol) and DBTDL (14 μL, 23.8 μmol) were added, the solution was heated

to 50°C, and stirred for 24 hours. The reaction mixture was monitored by TLC using EtOAc for the starting compound and MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture for the product. Spots were detected by the method M7. The reaction mixture was poured into EtOAc (30 mL), forming the precipitate. The precipitated product was filtered, washed with EtOAc (2 × 30 mL). The product was dried at 50°C using an oil rotary pump. The final product was obtained as a white solid in a 70% yield (0.16 g). **IR(DRIFT)**: 3340, 3153, 3114, 2954, 2916, 1716 ν(C=O), 1612, 1570, 1468, 1254, 1225, 1159, 1030 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 8.88 (bs, 14H, H-14), 7.97 (bs, 7H, H-7), 7.42 (bs, 28H, H-15, H-16), 5.24 (bs, 21H, H-1, NH), 4.32 (bs, 70H, H-3, H-5, H-6, H-9, H-12), 3.90 (bs, 42H, H-17), 3.15 (bs, 42H, H-2, H-4, H-10, H-19), 1.46 – 1.23 (m, 448H, H-20 – H-35), 0.90 – 0.87 (m, 63H, H-13, H-36) ppm. **^{19}F NMR** (282 MHz, DMSO-d_6, C_6F_6): δ = -80.15, -164.90 (C_6F_6) ppm. **HRMS**: for $C_{420}H_{749}N_{63}O_{49}^{14+}$ calcd: *m/z* 533.201 (for $[M^{14+}+13\times TfO^- - C_{19}H_{37}NO]^+$ calcd: *m/z* 9108.314, $[M^{14+}+13\times TfO^- - 2\times C_{19}H_{37}NO]^+$ calcd: *m/z* 8812.803, $[M^{14+}+13\times TfO^- - 3\times C_{19}H_{37}NO]^+$ calcd: *m/z* 8517.292, $[M^{14+}+13\times TfO^- - 4\times C_{19}H_{37}NO]^+$ calcd: *m/z* 8221.781, $[M^{14+}+13\times TfO^- - 5\times C_{19}H_{37}NO]^+$ calcd: *m/z* 7926.270), $[M^{14+}+13\times TfO^- - 6\times C_{19}H_{37}NO]^+$ calcd: *m/z* 7630.759), found 9108.555 $[M^{14+}+13\times TfO^- - C_{19}H_{37}NO]^+$, 8813.277 $[M^{14+}+13\times TfO^- - 2\times C_{19}H_{37}NO]^+$, 8517.734 $[M^{14+}+13\times TfO^- - 3\times C_{19}H_{37}NO]^+$, 8221.877 $[M^{14+}+13\times TfO^- - 4\times C_{19}H_{37}NO]^+$, 7926.877 $[M^{14+}+13\times TfO^- - 5\times C_{19}H_{37}NO]^+$, 7630.613 $[M^{14+}+13\times TfO^- - 6\times C_{19}H_{37}NO]^+$.

1-Azido-2-(2-(2-isocyanatoethoxy)ethoxy)ethane (110). Compound **110**



was prepared according to the previously published procedure describing the synthesis of compound **101**[380]. **35** (6.0 g, 27.5 mmol) was dissolved in CH_2Cl_2 (120 mL). A saturated Na_2CO_3 aq. solution (120 mL) was added, the mixture was vigorously stirred for 5 minutes, and cooled to 0°C. Then, triphosgene (4.1 g, 13.7 mmol) in CH_2Cl_2 (120 mL) was added. The mixture was stirred at room temperature for 2 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 1/2. Spots were detected by the method M2. The mixture was diluted with H_2O (100 mL), the aqueous phase was separated, and the organic phase was washed with 1 M HCl (150 mL). The organic phase was dried with $MgSO_4$ (3 g), filtered, and organic solvents were removed on a rotary evaporator at 30°C. The product was dried at room temperature using an oil rotary pump. The final product was obtained as an orange oil in a 95% yield (6.4 g). **IR(DRIFT)**: 3088, 2875, 2753, 2277 ν(isocyanate), 2223, 2098, 1772, 1733, 1440, 1347, 1287, 1248, 1120 cm^{-1}. **^{1}H NMR**

(400 MHz, $CDCl_3$): δ = 3.72 – 3.64 (m, 10H, H-4, H-5, H-6, H-7, H-8), 3.62 (d, J = 5.4 Hz, 2H, H-3), 3.41 (d, J = 5.4 Hz, 2H, H-2), 3.37 (d, J = 5.0 Hz, 2H, H-9) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 125.06 (C-1), 71.10 – 69.71 (C-3, C-4, C-5, C-6, C-7, C-8), 50.81 (C-9), 43.30 (C-2) ppm.

Per[2,3-(N_3-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD (111). Per[6-(MTZ-O-MIM2)]-β-CD tetradecabicarbonate **95** (0.8 g, 0.19 mmol) was dissolved in MeOH (20 mL), neutralized with 3 M TfOH in MeOH. The solution was evaporated on a rotary evaporator at 40°C, and the solid was dried at 70°C for 2 hours using an oil rotary pump. The compound was dissolved in dry DMF (20 mL). 1-Azido-2-(2-(2-(2-isocyanatoethoxy)ethoxy)ethoxy)ethane **110** (0.98 g, 4.0 mmol) and DBTDL (1.1 mL, 2.0 mmol) were added, the solution was heated to 70°C, and stirred for 20 hours. The reaction mixture was monitored by TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture for the product. Spots were detected by the method M7. The sample was measured by NMR and it was obvious the reaction was not finished. 1-Azido-2-(2-(2-(2-isocyanatoethoxy)ethoxy)ethoxy)ethane **110** (0.98 g, 4.0 mmol) was added and the reaction was stirred for another 20 hours at 70°C. The reaction mixture was poured into Et_2O (1 L), forming the precipitate. The precipitated product was filtered, washed with Et_2O (3 × 150 mL). The product was dissolved in MeOH (150 mL) and evaporated on a rotary evaporator at 40°C. The product (1.19 g) was dissolved in H_2O (35 mL) and freeze-dried. The final product was obtained as a beige amorphous solid in a 67% yield (1.14 g). **$[\alpha]^{25}_D$** = +9.3° (α +0.005, c = 0.27, MeOH). **IR(DRIFT)**: 3564, 3323, 3153, 3114, 2939, 2873, 2114 ν(azide), 1722 ν(C=O), 1545, 1464, 1346, 1259, 1157, 1097, 1032 cm^{-1}. **^{1}H NMR** (400 MHz, MeOD): δ = 8.88 (bs, 14H, H-14), 8.12 (bs, 7H, H-7), 7.61 – 7.45 (m, 28H, H-15, H-16), 5.55 – 5.21 (m, 7H, H-1), 4.76 – 4.05 (m, 70H, H-3, H-5, H-6, H-9, H-12), 3.95 (bs, 42H, H-17), 3.73 – 3.03 (m, 252H, solvent overlay, H-2, H-4, H-10, H-19 – H-26), 0.94 (bs, 21H, H-13) ppm. **^{19}F NMR** (282 MHz, DMSO-d_6, C_6F_6): δ = -80.08, -164.90 (C_6F_6) ppm. **UV-VIS** (H_2O), λ_{max}, nm: 207.0, 5*10^{-6} M.

(S)-3,5-Dinitro-N-(2-oxo-1-phenyl-2-(prop-2-yn-1-ylamino)ethyl)benzamide (PrgPA **118).** DNB-PhGly **112a** (3.0 g, 8.7 mmol) was suspended in TBME (75 mL). DCC (1.97 g, 9.6 mmol) and NHS (1.1 g, 9.6 mmol) were added, and the mixture was stirred for 10 minutes at room temperature. Then the reaction mixture was cooled to 0°C, $PrgNH_2$ (0.6 mL, 9.6 mmol) and DMAP (1.17 g, 9.6 mmol) were added. The mixture was warmed up to room temperature and stirred for 17 hours. The reaction mixture was monitored by TLC using hexane/EtOAc 3/1 mixture for the product and $CHCl_3$/MeOH 5/1 for the starting compound. Spots were detected by the method M1. The reaction was not completed. Another DCC (1.97 g, 9.6 mmol) and $PrgNH_2$ (0.6 mL, 9.6 mmol) were added, and the mixture was stirred for 24 hours. The heterogeneous mixture was dissolved in hot MeOH (500 mL) and poured into hexane (3 L). The precipitated mixture was left to stand in a fridge for 2 hours. The precipitated product was filtered and washed with hexane (2 × 200 mL). The product was dried at room temperature using an oil rotary pump. The final product was obtained as a pink powder in a 51% yield (1.7 g). $\mathbf{[\alpha]^{25}_D}$ = +38.9° (α +0.021, c = 0.27, DMSO). **IR(DRIFT)**: 3327, 3307, 3086, 3062, 3035, 2987, 2927, 2850, 1660, 1635, 1585, 1537, 1346, 1264, 1225, 1192, 1099, 1082 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 9.81 (d, J = 7.5 Hz, 1H, NH), 9.13 (d, J = 2.2 Hz, 2H, H-9), 8.96 (t, J = 2.1 Hz, 1H, H-11), 8.85 (t, J = 5.5 Hz, 1H, NH), 7.64 – 7.21 (m, 5H, H-4, H-5, H-6), 5.73 (d, J = 7.5 Hz, 1H, H-2), 3.92 (td, J = 5.7, 2.6 Hz, 2H, H-12), 3.14 (t, J = 2.5 Hz, 1H, H-14) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 169.15 (C-1), 162.47 (C-7), 147.97 (C-10), 137.49 (C-3), 136.41 (C-8), 128.39 – 128.28 (C-4, C-5), 127.91 (C-9), 121.02 (C-11), 80.72 (C-13), 73.33 (C-14), 57.43 (C-2), 28.17 (C-12) ppm. **UV-VIS** (MeOH), λ_{max}, nm: 202.0, $1*10^{-7}$ M. **ESI MS**: for $C_{18}H_{14}N_4O_6$ calcd: *m/z* 382.1, found 405 $[M+Na]^+$. **HRMS**: for $C_{18}H_{14}N_4O_6$ calcd: *m/z* 382.0913 (for $[M-H^+]^-$ calcd: *m/z* 381.0841), found 381.0841 $[M-H^+]^-$, Δ 0.0 ppm.

Per[2,3-(PA-MTZ-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD (119). Per[2,3-(N_3-TEG-carbamoyl)-6-(MTZ-O-MIM2)]-β-CD **111** (0.35 g, 39.6 μmol) and PrgPA **118** (0.24 g, 0.63 mmol) were dissolved in DMF (16 mL), and the solution was bubbled with nitrogen for 30 minutes. A metal Cu (80 mg, 1.2 mmol) was added, and the reaction mixture was stirred for 24 hours at room temperature. The reaction mixture was monitored by TLC using MeOH/conc. AcOH/1% NH_4OAc aq. sol. 10/1/9 mixture for the product and hexane/EtOAc 3/1 mixture for the starting amide **118**. Spots were detected by methods M1 and M2. The sample was measured by IR, and it was obvious the reaction was finished. The reaction mixture was filtered through celite, and the solution was poured into Et_2O (600 mL). The precipitated product was filtered and washed with an excess of Et_2O. The crude product (0.53 g) was dissolved in hot H_2O (200 mL), and the solution was washed with $CHCl_3$ (2 × 40 mL). The product in an aqueous solution was purified by column chromatography (16 g basic alumina) eluting with 0.01 M NH_4HCO_3 aq. solution. Fractions with the pure product were evaporated on a rotary evaporator at 40°C. The product (0.41 g) was dissolved in H_2O (50 mL) and poured into a strong anion exchanger column DOWEX® 1-8 (20 mL, OH^- form). The product was eluted with H_2O. The solution was neutralized with 1% w/w HPF_6 aq. solution. The neutralized solution was evaporated on a rotary evaporator at 40°C. The product was dried at 60°C using an oil rotary pump. The final product was obtained as a light brown amorphous solid in a 62% yield (0.35 g). $[\alpha]^{25}_D$ = +25.8° (α +0.017, c = 0.33, DMSO). **IR(DRIFT)**: 3294, 3149, 3101, 2941, 2871, 1722, 1668, 1543, 1346, 1275, 1257, 1165, 1097, 1032 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 9.81 (bs, 14H, NH), 9.32 – 8.93 (m, 56H, H-14, H-34, H-36), 8.25 – 7.24 (m, 119H, H-7, H-15, H-16, H-27, H-38 – H-40), 6.40 (bs, 14H, NH), 5.73 – 5.64 (m, 14H, H-31),

5.31 – 2.94 (m, 392H, H-2 – H-6, H-9, H-10, H-12, H-17, H-19 – H-26, H-29), 0.80 (bs, 21H, H-13) ppm. **^{19}F NMR** (282 MHz, DMSO-d_6, C_6F_6): δ = -71.21, -73.73, -164.90 (C_6F_6) ppm. **UV-VIS** (MeCN/H_2O 1/1), λ_{max1}, nm: 218.6, λ_{max2}, nm: 255.9, 8*10^{-7} M.

6.8.11 Chiral analytes

General procedure for dansylation of amino acids (GP6).

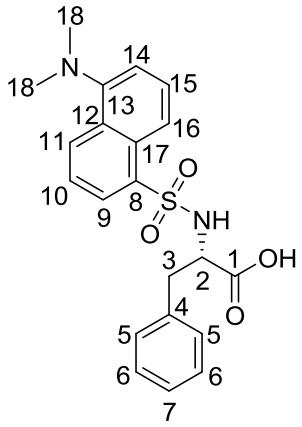
Compounds **103** – **105** were prepared according to the previously published procedure[382,383]. The amino acid was dissolved in 0.1 M $NaHCO_3$ aq. solution (0.5% w/v solution). Dansyl chloride (DNSCl) (1.0 eq.) in acetone (1.6% w/v solution) was added. The resulting yellow suspension was stirred for 2 hours at room temperature and then poured into $CHCl_3$/H_2O 1/1 mixture (6-times more volume than 0.1 M $NaHCO_3$ aq. solution). The reaction mixture was monitored by TLC using $CHCl_3$/MeOH 5/1 mixture. Spots were detected by methods M1 and M4. The aqueous phase was neutralized with 1 M HCl, and the product was extracted to $CHCl_3$ (3-times more volume than 0.1 M $NaHCO_3$ aq. solution). The organic phase was dried with $MgSO_4$, filtered, and evaporated on a rotary evaporator at 40°C. The crude product was purified by column chromatography (30-times more silica gel than crude product weight) eluting with $CHCl_3$/MeOH 3/1 if necessary. After purification, fractions with product were evaporated on a rotary evaporator at 40°C. The product was dried at room temperature using an oil rotary pump.

(S)-2-((5-(Dimethylamino)naphthalene)-1-sulfonamido)-2-phenylacetic acid (DNS-L-PhGly **103a).** Compound **103a** was prepared according to the general procedure (**GP6**). L-Phenylglycine (0.1 g, 0.66 mmol) was dissolved in 0.1 M $NaHCO_3$ aq. solution (20 mL). DNSCl(0.18 g, 0.66 mmol) in acetone (10 mL) was added. The product was purified by column chromatography and obtained as a yellow-green glassy compound in a 53% yield (0.14 g). **[α]$^{25}_D$** = +45.9° (α +0.028, c = 0.31, MeOH). **IR(ATR)**: 3265, 3062, 3030, 2941, 2868, 2833, 2787, 1716, 1610, 1587, 1574, 1454, 1319, 1142, 1092, 1061 cm^{-1}. **^{1}H NMR** (400 MHz, MeOD): δ = 8.43 (dt, *J* = 8.5, 1.1 Hz, 1H, H-10), 8.35 (dt, *J* = 8.7, 0.9 Hz, 1H, H-15), 8.11 (dd, *J* = 7.3, 1.3 Hz, 1H, H-8), 7.90 (s, 1H, NH), 7.52 (dd, *J* = 8.7, 7.5 Hz, 1H, H-13), 7.43 (dd, *J* = 8.6, 7.3 Hz, 1H, H-9), 7.21 (dd, *J* = 7.6, 1.0 Hz, 1H, H-14), 7.08 – 6.93 (m, 5H, H-4, H-5, H-6), 4.91 (s, 1H, H-2), 2.84 (s, 6H, H-17) ppm. **^{13}C NMR** (101 MHz, MeOD): δ = 172.89 (C-1), 152.86 (C-12), 137.38 (C-3),

137.16 (C-11), 131.15 (C-10), 131.01 – 130.95 (C-7, C-16), 130.27 (C-8), 129.09 – 128.22 (C-4, C-5, C-6), 128.89 (C-13), 124.04 (C-9), 120.90 (C-15), 116.27 (C-14), 61.08 (C-2), 45.82 (C-17) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 217.0, λ_{max2}, nm: 252.0, λ_{max3}, nm: 340.0, $1*10^{-5}$ M. **ESI MS**: for $C_{20}H_{20}N_2O_4S$ calcd: *m/z* 384.1 (for $[M+Na]^+$ calcd: *m/z* 407.1), found 407.0 $[M+Na]^+$. **HRMS**: for $C_{20}H_{20}N_2O_4S$ calcd: *m/z* 384.1144 (for $[M+H]^+$ calcd: *m/z* 385.1217), found 385.1227 $[M+H]^+$, Δ 2.6 ppm.

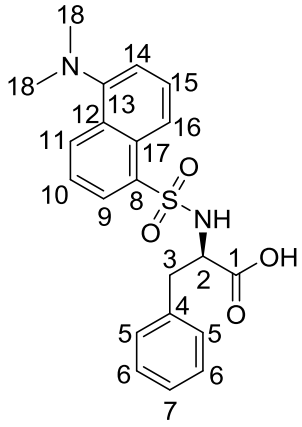
(R)-2-((5-(Dimethylamino)naphthalene)-1-sulfonamido)-2-phenylacetic acid (DNS-D-PhGly **103b).** Compound **103b** was prepared according to the general procedure (**GP6**). D-Phenylglycine (0.1 g, 0.66 mmol) was dissolved in 0.1 M $NaHCO_3$ aq. solution (20 mL). DNSCl (0.18 g, 0.66 mmol) in acetone (10 mL) was added. The product was purified by column chromatography and obtained as a yellow-green glassy compound in a 60% yield (0.15 g). **$[\alpha]^{25}_D$** = -43.9° (α -0.025, c = 0.29, MeOH). **IR(ATR)**: 3269, 3064, 3030, 2941, 2868, 2833, 2787, 1716, 1587, 1574, 1454, 1319, 1142, 1092, 1061 cm^{-1}. **^{1}H NMR** (400 MHz, MeOD): δ = 8.43 (dd, *J* = 8.5, 1.1 Hz, 1H, H-10), 8.35 (d, *J* = 8.7 Hz, 1H, H-15), 8.11 (dd, *J* = 7.3, 1.3 Hz, 1H, H-8), 7.90 (s, 1H, NH), 7.53 (dd, *J* = 8.7, 7.6 Hz, 1H, H-13), 7.43 (dd, *J* = 8.6, 7.3 Hz, 1H, H-9), 7.22 (dd, *J* = 7.6, 0.9 Hz, 1H, H-14), 7.09 – 6.94 (m, 5H, H-4, H-5, H-6), 4.91 (s, 1H, H-2), 2.85 (s, 6H, H-17) ppm. **^{13}C NMR** (101 MHz, MeOD): δ = 172.77 (C-1), 152.86 (C-12), 137.37 (C-3), 137.16 (C-11), 131.15 (C-10), 131.01 – 130.95 (C-7, C-16), 130.27 (C-8), 129.08 – 128.22 (C-4, C-5, C-6), 128.89 (C-13), 124.04 (C-9), 120.91 (C-15), 116.27 (C-14), 60.93 (C-2), 45.82 (C-17) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 217.5, λ_{max2}, nm: 251.5, λ_{max3}, nm: 340.0, $1*10^{-5}$ M. **ESI MS**: for $C_{20}H_{20}N_2O_4S$ calcd: *m/z* 384.1 (for $[M+Na]^+$ calcd: *m/z* 407.1), found 407.0 $[M+Na]^+$. **HRMS**: for $C_{20}H_{20}N_2O_4S$ calcd: *m/z* 384.1144 (for $[M+H]^+$ calcd: *m/z* 385.1217), found 385.1198 $[M+H]^+$, Δ 4.9 ppm.

((5-(Dimethylamino)naphthalen-1-yl)sulfonyl)-L-phenylalanine (DNS-L-Phe **104a).**



Compound **104a** was prepared according to the general procedure (**GP6**). L-Phenylalanine (0.1 g, 0.61 mmol) was dissolved in 0.1 M $NaHCO_3$ aq. solution (20 mL). DNSCl (0.16 g, 0.61 mmol) in acetone (10 mL) was added. The product was obtained as a light green glassy compound in a 68% yield (0.17 g). $[\alpha]^{25}_D$ = -44.4° (α -0.024, c = 0.27, MeOH). **IR(DRIFT)**: 3298, 3105, 3028, 3005, 2968, 2939, 2860, 2472, 1942, 1878, 1805, 1705, 1568, 1462, 1415, 1342, 1288, 1151, 1090 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 8.48 (d, *J* = 8.5 Hz, 1H, H-11), 8.23 – 8.12 (m, 2H, H-9, H-16), 7.50 (dd, *J* = 8.7, 7.5 Hz, 1H, H-14), 7.44 (dd, *J* = 8.5, 7.3 Hz, 1H, H-10), 7.21 – 7.17 (m, 1H, H-15), 7.10 – 6.86 (m, 5H, H-5, H-6, H-7), 5.31 (d, *J* = 8.2 Hz, 1H, NH), 4.27 – 4.08 (m, 1H, H-2), 3.01 – 2.92 (m, 2H, H-3), 2.89 (s, 6H, H-18) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 174.19 (C-1), 151.08 (C-13), 134.73 (C-4), 134.48 (C-12), 130.77 (C-11), 129.71 (C-9), 129.65 (C-8, C-17), 129.36 – 128.51 (C-5, C-6, C-7), 127.29 (C-14), 123.38 (C-10), 119.50 (C-16), 115.62 (C-15), 56.84 (C-2), 45.68 (C-18), 38.76 (C-3) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 217.0, λ_{max2}, nm: 252.0, λ_{max3}, nm: 335.0, $1*10^{-5}$ M. **ESI MS**: for $C_{21}H_{22}N_2O_4S$ calcd: *m/z* 398.1 (for $[M+Na]^+$ calcd: *m/z* 421.1), found 421.0 $[M+Na]^+$. **HRMS**: for $C_{21}H_{22}N_2O_4S$ calcd: *m/z* 398.1300 (for $[M+H]^+$ calcd: *m/z* 399.1373), found 399.1378 $[M+H]^+$, Δ 1.3 ppm. ^{1}H NMR spectrum is in accordance with the literature[407].

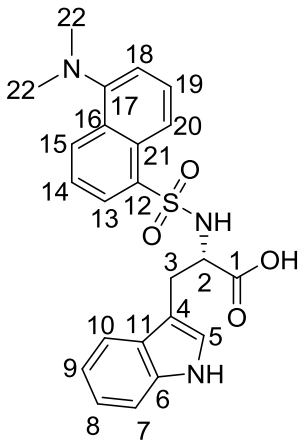
((5-(Dimethylamino)naphthalen-1-yl)sulfonyl)-D-phenylalanine (DNS-D-Phe **104b).**



Compound **104b** was prepared according to the general procedure (**GP6**). D-Phenylalanine (0.1 g, 0.61 mmol) was dissolved in 0.1 M $NaHCO_3$ aq. solution (20 mL). DNSCl (0.16 g, 0.61 mmol) in acetone (10 mL) was added. The product was obtained as a light green glassy compound in a 41% yield (0.10 g). $[\alpha]^{25}_D$ = +53.6° (α +0.030, c = 0.28, MeOH). **IR(DRIFT)**: 3298, 3105, 3026, 3006, 2968, 2941, 2858, 2790, 2440, 1942, 1878, 1805, 1709, 1568, 1462, 1415, 1342, 1288, 1151, 1090 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 8.48 (d, *J* = 8.5 Hz, 1H, H-11), 8.26 – 8.11 (m, 2H, H-9, H-16), 7.50 (dd, *J* = 8.7, 7.6 Hz, 1H, H-14), 7.44 (dd, *J* = 8.6, 7.3 Hz, 1H, H-10), 7.19 (d, *J* = 7.5 Hz, 1H, H-15), 7.08 – 6.88 (m, 5H, H-5, H-6, H-7), 5.33 (d, *J* = 8.6

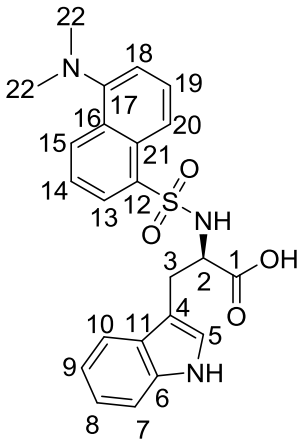
Hz, 1H, NH), 4.17 (d, *J* = 7.5 Hz, 1H, H-2), 2.95 (dd, *J* = 14.2, 8.9 Hz, 2H, H-3), 2.89 (s, 6H, H-18) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 174.30 (C-1), 151.06 (C-13), 134.77 (C-4), 134.52 (C-12), 130.70 (C-11), 129.69 (C-9), 129.64 (C-8, C-17), 129.34 – 128.49 (C-5, C-6, C-7), 127.27 (C-14), 123.41 (C-10), 119.62 (C-16), 115.66 (C-15), 56.91 (C-2), 45.69 (C-18), 38.74 (C-3) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 217.0, λ_{max2}, nm: 252.0, λ_{max3}, nm: 336.0, 1*10^{-5} M. **ESI MS**: for $C_{21}H_{22}N_2O_4S$ calcd: *m/z* 398.1 (for $[M+Na]^+$ calcd: *m/z* 421.1), found 421.0 $[M+Na]^+$. **HRMS**: for $C_{21}H_{22}N_2O_4S$ calcd: *m/z* 398.1300 (for $[M+H]^+$ calcd: *m/z* 399.1373), found 399.1382 $[M+H]^+$, Δ 2.3 ppm. ^{1}H NMR spectrum is in accordance with the literature[407].

((5-(Dimethylamino)naphthalen-1-yl)sulfonyl)-L-tryptophan (DNS-L-Trp **105a).**



Compound **105a** was prepared according to the general procedure (**GP6**). L-Tryptophan (0.1 g, 0.49 mmol) was dissolved in 0.1 M $NaHCO_3$ aq. solution (20 mL). DNSCl (0.13 g, 0.49 mmol) in acetone (10 mL) was added. The product was purified by column chromatography and obtained as a brown-green glassy compound in a 17% yield (0.04 g). **$[\alpha]^{25}_D$** = -35.2° (α -0.019, c = 0.27, MeOH). **IR(ATR)**: 3404, 3276, 3080, 3055, 2985, 2939, 2866, 2831, 2785, 1712, 1587, 1576, 1456, 1394, 1302, 1230, 1140, 1092, 1061 cm^{-1}. **^{1}H NMR** (400 MHz, MeOD): δ = 8.38 (dd, *J* = 8.6, 1.1 Hz, 1H, H-15), 8.17 (d, *J* = 8.7 Hz, 1H, H-20), 7.98 (dd, *J* = 7.3, 1.3 Hz, 1H, H-13), 7.43 (dd, *J* = 8.7, 7.5 Hz, 1H, H-18), 7.36 – 7.28 (m, 2H, H-10, H-14), 7.18 (dd, *J* = 7.6, 0.9 Hz, 1H, H-19), 7.16 – 7.11 (m, 1H, H-7), 6.96 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H, H-8), 6.87 – 6.80 (m, 2H, H-5, H-9), 3.94 (dd, *J* = 7.2, 5.1 Hz, 1H, H-2), 3.14 (ddd, *J* = 14.4, 5.2, 0.9 Hz, 1H, H-3), 2.96 (ddd, *J* = 14.4, 7.2, 0.7 Hz, 1H, H-3), 2.86 (s, 6H, H-22) ppm. **^{13}C NMR** (101 MHz, MeOD): δ = 177.27 (C-1), 152.81 (C-17), 137.68 (C-6), 136.84 (C-16), 131.04 – 130.76 (C-12, C-21), 130.89 (C-15), 129.80 (C-13), 128.83 (C-11), 128.69 (C-18), 124.70 (C-5), 123.93 (C-14), 121.89 (C-8), 120.77 (C-20), 119.47 – 119.38 (C-9, C-10), 116.23 (C-19), 111.89 (C-7), 110.95 (C-4), 59.84 (C-2), 45.83 (C-22), 30.34 (C-3) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 218.0, λ_{max2}, nm: 253.0, λ_{max3}, nm: 340.0, 1*10^{-5} M. **ESI MS**: for $C_{23}H_{23}N_3O_4S$ calcd: *m/z* 437.1 (for $[M+Na]^+$ calcd: *m/z* 460.1), found 460.0 $[M+Na]^+$. **HRMS**: for $C_{23}H_{23}N_3O_4S$ calcd: *m/z* 437.1409 (for $[M+H]^+$ calcd: *m/z* 438.1482), found 438.1485 $[M+H]^+$, Δ 0.7 ppm.

((5-(Dimethylamino)naphthalen-1-yl)sulfonyl)-D-tryptophan (DNS-D-Trp **105b).**



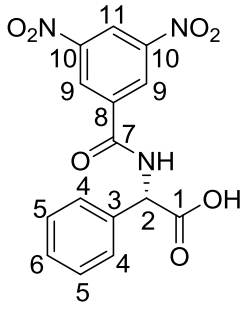
Compound **105b** was prepared according to the general procedure (**GP6**). D-Tryptophan (0.1 g, 0.49 mmol) was dissolved in 0.1 M $NaHCO_3$ aq. solution (20 mL). DNSCl (0.13 g, 0.49 mmol) in acetone (10 mL) was added. The product was obtained as a yellow-green glassy compound in a 66% yield (0.14 g). $[\alpha]^{25}_D$ = +58.5° (α +0.031, c = 0.27, MeOH). **IR(ATR)**: 3394, 3288, 3053, 2939, 2868, 2833, 2785, 1720, 1587, 1574, 1456, 1394, 1311, 1230, 1140, 1092, 1061 cm^{-1}. **^{1}H NMR** (400 MHz, MeOD): δ = 8.37 (dt, J = 8.6, 1.1 Hz, 1H, H-15), 8.23 – 8.18 (m, 1H, H-20), 7.94 (dd, J = 7.3, 1.3 Hz, 1H, H-13), 7.44 (dd, J = 8.7, 7.5 Hz, 1H, H-18), 7.27 (dd, J = 8.6, 7.3 Hz, 1H, H-14), 7.22 – 7.12 (m, 3H, H-7, H-10, H-19), 6.97 (ddd, J = 8.1, 7.0, 1.2 Hz, 1H, H-8), 6.87 – 6.79 (m, 2H, H-5, H-9), 4.05 (dd, J = 8.1, 5.8 Hz, 1H, H-2), 3.12 (ddd, J = 14.4, 5.8, 0.8 Hz, 1H, H-3), 2.90 (ddd, J = 14.4, 8.1, 0.6 Hz, 1H, H-3), 2.85 (s, 6H, H-22) ppm. **^{13}C NMR** (101 MHz, MeOD): δ = 175.34 (C-1), 152.79 (C-17), 137.74 (C-6), 136.68 (C-16), 131.06 (C-15), 130.99 – 130.70 (C-12, C-21), 129.90 (C-13), 128.68 (C-11), 128.18 (C-18), 124.77 (C-5), 123.86 (C-14), 122.15 (C-8), 120.75 (C-20), 119.61 – 118.77 (C-9, C-10), 116.25 (C-19), 112.15 (C-7), 109.98 (C-4), 58.13 (C-2), 45.83 (C-22), 29.86 (C-3) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 218.5, λ_{max2}, nm: 252.5, λ_{max3}, nm: 340.0, $1*10^{-5}$ M. **ESI MS**: for $C_{23}H_{23}N_3O_4S$ calcd: *m/z* 437.1 (for $[M+Na]^+$ calcd: *m/z* 460.1), found 460.0 $[M+Na]^+$. **HRMS**: for $C_{23}H_{23}N_3O_4S$ calcd: *m/z* 437.1409 (for $[M+H]^+$ calcd: *m/z* 438.1482), found 438.1486 $[M+H]^+$, Δ 0.9 ppm.

General procedure for dinitrobenzoylation of amino acids (GP7).

Compounds **112 – 114** were prepared according to the previously published procedure[296]. The amino acid and 3,5-dinitrobenzoyl chloride (DNBCl) (1.0 eq.) were suspended in dry THF (10% w/v solution, calculated for the amino acid). The mixture was stirred for 4 days at room temperature. The mixture was filtered, the solid was washed with THF, and the filtrate was evaporated on a rotary evaporator at 40°C. The solid was suspended in a saturated $NaHCO_3$ aq. solution and washed with Et_2O. The extraction efficiency was monitored by TLC using $CHCl_3$/MeOH 10/1 or 5/1 (for tryptophane derivatives) mixture. Spots were detected by method M1. The saturated $NaHCO_3$ aq. solution was acidified to pH 4-5 by conc. HCl and the product was extracted to Et_2O. After each extraction, the

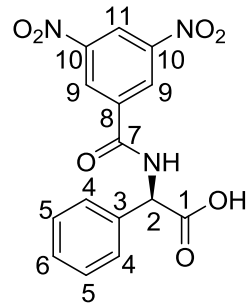
aqueous phase was acidified to pH 4-5 again. Organic extracts were dried with $MgSO_4$, filtered, and organic solvents were removed on a rotary evaporator at 30°C. The final isolation differs based on the final compound.

(S)-2-(3,5-Dinitrobenzamido)-2-phenylacetic acid (DNB-L-PhGly **112a).** Compound



112a was prepared according to the general procedure (**GP7**). L-phenylglycine (5.0 g, 33.0 mmol) and DNBCl (7.6 g, 33.0 mmol) were suspended in dry THF (50 mL). The solid was suspended in a saturated $NaHCO_3$ aq. solution (400 mL) and washed with Et_2O (2 × 400 mL). After acidification, the product was extracted to Et_2O (6 × 400 mL). Organic extracts were dried with $MgSO_4$ (11 g), filtered, and organic solvents were removed on a rotary evaporator at 30°C. The solid was dissolved in hot MeOH (140 mL) and poured into H_2O (760 mL). The precipitated product was filtered and washed with MeOH (2 × 150 mL). The product was dried at 70°C using an oil rotary pump. The final product was obtained as a white solid in a 74% yield (8.6 g). $[\alpha]^{25}_D$ = +96.1° (α +0.073, c = 0.38, THF). **IR(DRIFT)**: 3369, 3095, 2968, 2881, 1730 ν(C=O), 1699, 1646 ν(C=O), 1630, 1539, 1387, 1344, 1217, 1190, 1076 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 13.10 (bs, 1H, OH), 9.88 (d, J = 7.0 Hz, 1H, NH), 9.13 (d, J = 2.1 Hz, 2H, H-9), 8.97 (t, J = 2.1 Hz, 1H, H-11), 7.59 – 7.35 (m, 5H, H-4, H-5, H-6), 5.64 (d, J = 7.0 Hz, 1H, H-2) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 171.42 (C-1), 162.41 (C-7), 148.13 (C-10), 136.31 (C-3), 136.15 (C-8), 128.59 – 128.30 (C-4, C-5), 128.24 (C-6), 128.06 (C-9), 121.14 (C-11), 57.43 (C-2) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 202.4, λ_{max2}, nm: 249.0, 2*10^{-6} M. **ESI MS**: for $C_{15}H_{11}N_3O_7$ calcd: *m/z* 345.1 (for $[M+Na]^+$ calcd: *m/z* 368.0), found 368.2 $[M+Na]^+$. **HRMS**: for $C_{15}H_{11}N_3O_7$ calcd: *m/z* 345.0597 (for $[M+Na]^+$ calcd: *m/z* 368.0489), found 368.0495 $[M+Na]^+$, Δ 1.6 ppm. ^{1}H NMR spectrum is in accordance with the literature[296].

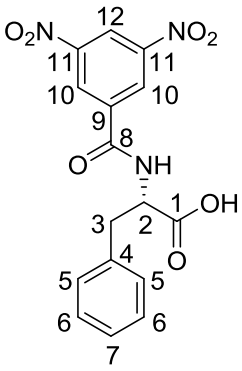
(R)-2-(3,5-Dinitrobenzamido)-2-phenylacetic acid (DNB-D-PhGly **112b).** Compound



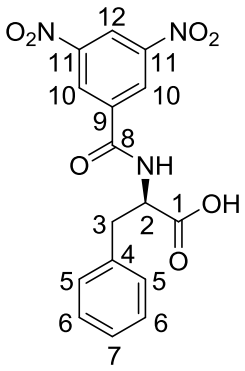
112b was prepared according to the general procedure (**GP7**). D-phenylglycine (0.2 g, 1.3 mmol) and DNBCl (0.3 g, 1.3 mmol) were suspended in dry THF (2 mL). The solid was suspended in a saturated $NaHCO_3$ aq. solution (20 mL) and washed with Et_2O (2 × 20 mL). After acidification, the product was extracted to Et_2O (2 × 20 mL). Organic extracts were dried with $MgSO_4$ (1.6 g), filtered, and organic solvents were removed on a rotary evaporator at 30°C. The product was dried

at 70°C using an oil rotary pump. The final product was obtained as a white solid in a 21% yield (98 mg). **[α]$^{25}_{D}$** = -84.6° (α -0.044, c = 0.26, THF). **IR(ATR)**: 3338, 3089, 3033, 2952, 2924, 2881, 2850, 1736, 1647, 1628, 1533, 1456, 1437, 1344, 1281, 1215, 1194, 1176, 1078 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d$_6$):*δ* = 9.98 (d, *J* = 6.6 Hz, 1H, NH), 9.13 (t, *J* = 1.8 Hz, 2H, H-9), 8.97 (q, *J* = 2.2 Hz, 1H, H-11), 7.57 – 7.34 (m, 5H, H-4, H-5, H-6), 5.73 (d, *J* = 6.6 Hz, 1H, H-2) ppm. **^{13}C NMR** (101 MHz, DMSO-d$_6$):*δ* = 170.59 (C-1), 162.57 (C-7), 148.16 (C-10), 135.84 (C-3), 135.41 (C-8), 129.24 – 127.72 (C-4, C-5, C-6, C-9), 121.25 (C-11), 57.41 (C-2) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 206.0, λ_{max2}, nm: 248.4, 3*10^{-6} M. **ESI MS**: for $C_{15}H_{11}N_3O_7$calcd: *m/z* 345.1 (for $[M+Na]^+$ calcd: *m/z* 368.0), found 368 $[M+Na]^+$. **HRMS**: for $C_{15}H_{11}N_3O_7$calcd: *m/z* 345.0597 (for $[M+Na]^+$ calcd: *m/z* 368.0489), found 368.0478 $[M+Na]^+$, Δ 3.0 ppm.

(3,5-Dinitrobenzoyl)-L-phenylalanine (DNB-L-Phe **113a).** Compound **113a** was prepared according to the general procedure (**GP7**). L-phenylalanine (0.2 g, 1.2 mmol) and DNBCl (0.28 g, 1.2 mmol) were suspended in dry THF (2 mL). The solid was suspended in a saturated $NaHCO_3$ aq. solution (20 mL) and washed with Et_2O (2 × 20 mL). After acidification, the product was extracted to Et_2O (2 × 20 mL). Organic extracts were dried with $MgSO_4$ (1.0 g), filtered, and organic solvents were removed on a rotary evaporator at 30°C. The solid (0.2 g) was dissolved in MeOH (5 mL), precipitated from H_2O (30 mL), and evaporated again on a rotary evaporator at 50°C. The product was dried at 70°C using an oil rotary pump. The final product was obtained as a white solid in a 40% yield (175 mg). **[α]$^{25}_{D}$** = -20.4° (α -0.011, c = 0.27, THF). **IR(ATR)**: 3388, 3305, 3093, 3032, 2925, 2856, 2625, 1716, 1651, 1630, 1533, 1464, 1342, 1217, 1180, 1078 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d$_6$):*δ* = 9.53 (d, *J* = 8.1 Hz, 1H, NH), 9.01 (d, *J* = 2.1 Hz, 2H, H-10), 8.94 (t, *J* = 2.1 Hz, 1H, H-12), 7.34 – 7.15 (m, 5H, H-5, H-6, H-7), 4.73 (ddd, *J* = 10.5, 8.1, 4.6 Hz, 1H, H-2), 3.33 – 3.03 (m, 2H, H-3, solvent overlay) ppm. **^{13}C NMR** (101 MHz, DMSO-d$_6$):*δ* = 172.58 (C-1), 162.31 (C-8), 148.24 (C-11), 137.77 (C-4), 136.23 (C-9), 129.00 – 126.48 (C-5, C-6, C-7), 127.55 (C-9), 121.10 (C-12), 54.55 (C-2), 36.23 (C-3) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 205.2, λ_{max2}, nm: 233.3, 2*10^{-5} M. **ESI MS**: for $C_{16}H_{13}N_3O_7$calcd: *m/z* 359.1 (for $[M+Na]^+$ calcd: *m/z* 382.1), found 382 $[M+Na]^+$. **HRMS**: for $C_{16}H_{13}N_3O_7$calcd: *m/z* 359.0754 (for $[M+Na]^+$ calcd: *m/z* 382.0646), found 382.0630 $[M+Na]^+$, Δ 4.2 ppm.

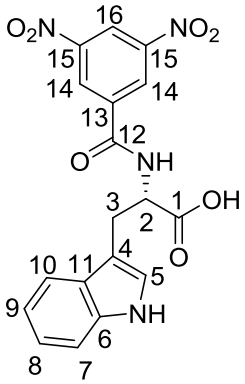


(3,5-Dinitrobenzoyl)-D-phenylalanine (DNB-D-Phe **113b).** Compound **113b** was



prepared according to the general procedure (**GP7**). D-phenylalanine (0.2 g, 1.2 mmol) and DNBCl (0.28 g, 1.2 mmol) were suspended in dry THF (2 mL). The solid was suspended in a saturated $NaHCO_3$ aq. solution (20 mL) and washed with Et_2O (2 × 20 mL). After acidification, the product was extracted to Et_2O (2 × 20 mL). Organic extracts were dried with $MgSO_4$ (1.2 g), filtered, and organic solvents were removed on a rotary evaporator at 30°C. The product was dried at 70°C using an oil rotary pump. The final product was obtained as a pale yellow glassy solid in a 88% yield (385 mg). **$[\alpha]^{25}_D$** = +22.6° (α +0.012, c = 0.27, THF). **IR(ATR)**: 3392, 3305, 3093, 3032, 2937, 2885, 2632, 1720, 1651, 1630, 1537, 1456, 1342, 1217, 1180, 1078 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6):δ = 9.53 (d, *J* = 8.1 Hz, 1H, NH), 9.01 (d, *J* = 2.1 Hz, 2H, H-10), 8.94 (t, *J* = 2.1 Hz, 1H, H-12), 7.36 – 7.15 (m, 5H, H-5, H-6, H-7), 4.73 (ddd, *J* = 10.5, 8.1, 4.6 Hz, 1H, H-2), 3.40 – 2.98 (m, 2H, H-3, solvent overlay) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6):δ = 172.57 (C-1), 162.31 (C-8), 148.24 (C-11), 137.75 (C-4), 136.22 (C-9), 129.00 – 126.49 (C-5, C-6, C-7), 127.55 (C-9), 121.11 (C-12), 54.53 (C-2), 36.23 (C-3) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 202.1, λ_{max2}, nm: 248.5, $3*10^{-5}$ M. **ESI MS**: for $C_{16}H_{13}N_3O_7$calcd: *m/z* 359.1 (for $[M+Na]^+$ calcd: *m/z* 382.1), found 382 $[M+Na]^+$. **HRMS**: for $C_{16}H_{13}N_3O_7$calcd: *m/z* 359.0754 (for $[M+Na]^+$ calcd: *m/z* 382.0646), found 382.0619 $[M+Na]^+$, Δ 4.2 ppm.

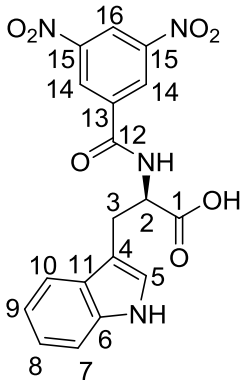
(3,5-Dinitrobenzoyl)-L-tryptophan (DNB-L-Trp **114a).** Compound **114a** was prepared



according to the general procedure (**GP7**). L-tryptophan (0.2 g, 0.98 mmol) and DNBCl (0.23 g, 0.98 mmol) were suspended in dry THF (2 mL). The solid was suspended in a saturated $NaHCO_3$ aq. solution (20 mL) and washed with Et_2O (2 × 20 mL). After acidification, the product was extracted to Et_2O (2 × 20 mL). Organic extracts were dried with $MgSO_4$ (2.0 g), filtered, and organic solvents were removed on a rotary evaporator at 30°C. The solid (0.11 g) was dissolved in MeOH (2 mL), precipitated from H_2O (10 mL), and the resulting solid was filtered and washed with H_2O (2 × 2 mL). The product was dried at 70°C using an oil rotary pump. The final product was obtained as an orange solid in a 15% yield (59 mg). **$[\alpha]^{25}_D$** = -5.4° (α -0.003, c = 0.28, THF). **IR(ATR)**:

3408, 3383, 3101, 2954, 2920, 2858, 2580, 1711, 1674, 1628, 1537, 1522, 1458, 1344, 1294, 1234, 1180, 1093, 1074 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6):δ = 12.93 (bs, 1H, OH), 10.82 (d, *J* = 2.5 Hz, 1H, NH), 9.52 (d, *J* = 7.8 Hz, 1H, NH), 9.03 (d, *J* = 2.2 Hz, 2H, H-14), 8.98 – 8.89 (m, 1H, H-16), 7.61 (d, *J* = 7.8 Hz, 1H, H-7), 7.31 (d, *J* = 8.0 Hz, 1H, H-10), 7.20 (d, *J* = 2.4 Hz, 1H, H-5), 7.05 (ddd, *J* = 8.2, 7.0, 1.3 Hz, 1H, H-8), 6.97 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H, H-9), 4.76 (ddd, *J* = 9.9, 7.7, 4.6 Hz, 1H, H-2), 3.42 – 3.20 (m, 2H, H-3, solvent overlay) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6):δ = 172.93 (C-1), 162.37 (C-12), 148.17 (C-15), 136.35 (C-13), 136.10 (C-6), 127.65 (C-14), 127.09 (C-11), 123.54 (C-5), 121.02 – 120.98 (C-8, C-16), 118.42 (C-9), 118.10 (C-7), 111.44 (C-10), 110.11 (C-4), 54.19 (C-2), 26.65 (C-3) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 202.2, λ_{max2}, nm: 220.3, $1*10^{-5}$ M. **ESI MS**: for $C_{18}H_{14}N_4O_7$calcd: *m/z* 398.1 (for $[M+Na]^+$ calcd: *m/z* 421.1), found 421 $[M+Na]^+$. **HRMS**: for $C_{18}H_{14}N_4O_7$calcd: *m/z* 398.0863 (for $[M+H]^+$ calcd: *m/z* 399.0935), found 399.0920 $[M+H]^+$, Δ 3.8 ppm.

(3,5-Dinitrobenzoyl)-D-tryptophan (DNB-D-Trp **114b).** Compound **114b** was prepared



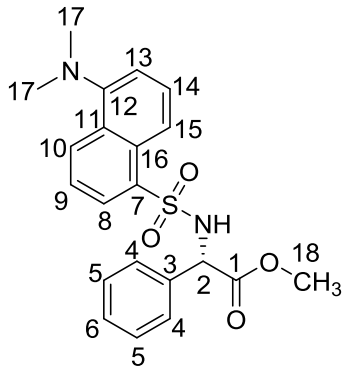
according to the general procedure (**GP7**). D-tryptophan (0.2 g, 0.98 mmol) and DNBCl (0.23 g, 0.98 mmol) were suspended in dry THF (2 mL). The solid was suspended in a saturated $NaHCO_3$ aq. solution (20 mL) and washed with Et_2O (2 × 20 mL). After acidification, the product was extracted to Et_2O (2 × 20 mL). Organic extracts were dried with $MgSO_4$ (1.3 g), filtered, and organic solvents were removed on a rotary evaporator at 30°C. The solid (0.16 g) was dissolved in MeOH (2 mL), precipitated from H_2O (10 mL), and the resulting solid was filtered and washed with H_2O (2 × 2 mL). The product was dried at 70°C using an oil rotary pump. The final product was obtained as an orange solid in a 22% yield (88 mg). **$[\alpha]^{25}_D$** = +4.8° (α +0.003, c = 0.31, THF). **IR(ATR)**: 3408, 3384, 3101, 2956, 2920, 2858, 2565, 1712, 1674, 1628, 1539, 1522, 1458, 1342, 1294, 1234, 1180, 1093, 1074 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6):δ = 12.94 (bs, 1H, OH), 10.82 (d, *J* = 2.5 Hz, 1H, NH), 9.52 (d, *J* = 7.8 Hz, 1H, NH), 9.03 (d, *J* = 2.2 Hz, 2H, H-14), 8.94 (t, *J* = 2.1 Hz, 1H, H-16), 7.61 (d, *J* = 7.8 Hz, 1H, H-7), 7.31 (d, *J* = 8.0 Hz, 1H, H-10), 7.20 (d, *J* = 2.4 Hz, 1H, H-5), 7.08 – 7.02 (m, 1H, H-8), 7.00 – 6.95 (m, 1H, H-9), 4.76 (ddd, *J* = 9.9, 7.8, 4.7 Hz, 1H, H-2), 3.39 – 3.18 (m, 2H, H-3, solvent overlay) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6):δ = 172.93 (C-1), 162.37 (C-12), 148.17 (C-15), 136.35 (C-13), 136.10 (C-6), 127.65 (C-14), 127.09 (C-11), 123.54 (C-5), 121.02

– 120.98 (C-8, C-16), 118.42 (C-9), 118.10 (C-7), 111.43 (C-10), 110.11 (C-4), 54.19 (C-2), 26.65 (C-3) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 202.4, λ_{max2}, nm: 221.0, $3*10^{-6}$ M. **ESI MS**: for $C_{18}H_{14}N_4O_7$calcd: *m/z* 398.1 (for $[M+Na]^+$ calcd: *m/z* 421.1), found 421 $[M+Na]^+$. **HRMS**: for $C_{18}H_{14}N_4O_7$calcd: *m/z* 398.0863 (for $[M+H]^+$ calcd: *m/z* 399.0935), found 399.0918 $[M+H]^+$, Δ 4.3 ppm.

General procedure for methylation of dansylated amino acids, mandelic acid, and dinitrobenzylated amino acids (GP8).

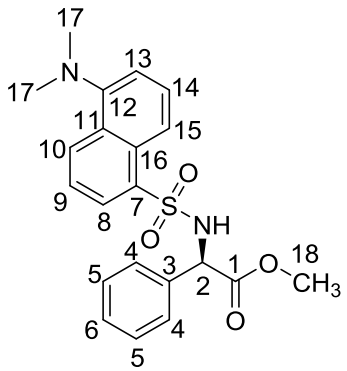
Compounds **106** – **108**, **109**, and **115** – **117** were prepared according to the previously published procedure[385]. The acid was dissolved in Et_2O/MeOH 10/1 or 5/1 mixture (2% w/v solution). The freshly prepared CH_2N_2 in Et_2O[384] was added dropwise until color changed from colorless to light yellow. The reaction mixture was monitored by TLC using $CHCl_3$ or $CHCl_3$/MeOH 30/1 or 20/1 mixture. Spots were detected by the method M1. The reaction mixture was washed with a saturated $NaHCO_3$ aq. solution (3-times more volume than Et_2O/MeOH mixture), dried with $MgSO_4$, filtered and evaporated on a rotary evaporator at 40°C. The crude product was purified by column chromatography (30-times more silica gel than crude product weight) eluting with $CHCl_3$ and $CHCl_3$/MeOH 200/1 to 100/1 if necessary. The product was dried at room temperature using an oil rotary pump or dissolved in benzene and freeze-dried.

Methyl (S)-2-((5-(dimethylamino)naphthalene)-1-sulfonamido)-2-phenylacetate (DNS-L-PhGly-Me **106a).** Compound **106a** was prepared according to the general procedure (**GP8**). DNS-L-PhGly **103a** (82.0 mg, 0.21 mmol) was dissolved in Et_2O/MeOH 5/1 mixture (7.2 mL). The product was dissolved in benzene (6 mL), freeze-dried, and obtained as a light green amorphous solid in a 63% yield (54.2 mg). $[\alpha]^{25}_D$ = +50.8° (α +0.031, c = 0.31, $CHCl_3$). **IR(ATR)**: 3288, 3062, 3032, 2949, 2868, 2833, 2787, 1736, 1612, 1587, 1574, 1454, 1329, 1259, 1201, 1161, 1144, 1093, 1061 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 9.16 (d, *J* = 9.1 Hz, 1H, NH), 8.39 (dt, *J* = 8.5, 1.1 Hz, 1H, H-10), 8.32 (d, *J* = 8.7 Hz, 1H, H-15), 8.08 (dd, *J* = 7.3, 1.3 Hz, 1H, H-8), 7.54 (ddd, *J* = 8.5, 7.4, 4.2 Hz, 2H, H-9, H-13), 7.21 (dd, *J* = 7.6, 1.0 Hz, 1H, H-14), 7.18 – 7.12 (m, 5H, H-4, H-5, H-6), 5.00 (d, *J* = 8.9 Hz, 1H, H-2), 3.30 (s, 3H, H-18), 2.80 (s, 6H, H-17) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 170.05 (C-1), 151.12 (C-12), 135.90 (C-3), 135.44 (C-11), 129.59 (C-10), 129.01 – 128.85 (C-7, C-16), 128.52



(C-8), 128.18 – 127.14 (C-4, C-5, C-6), 127.68 (C-13), 123.29 (C-9), 119.38 (C-15), 114.97 (C-14), 59.26 (C-2), 52.09 (C-18), 45.06 (C-17) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 217.0, λ_{max2}, nm: 251.0, λ_{max3}, nm: 340.0, $1*10^{-5}$ M. **ESI MS**: for $C_{21}H_{22}N_2O_4S$ calcd: *m/z* 398.1 (for $[M+Na]^+$ calcd: *m/z* 421.1), found 421.0 $[M+Na]^+$. **HRMS**: for $C_{21}H_{22}N_2O_4S$ calcd: *m/z* 398.1300 (for $[M+H]^+$ calcd: *m/z* 399.1373), found 399.1360 $[M+H]^+$, Δ 3.2 ppm.

Methyl (R)-2-((5-(dimethylamino)naphthalene)-1-sulfonamido)-2-phenylacetate (DNS-D-PhGly-Me **106b).** Compound **106b** was prepared according to the general procedure (**GP8**). DNS-D-PhGly **103b** (0.10 g, 0.26 mmol) was dissolved in Et_2O/MeOH 5/1 mixture (8.5 mL). The product was dissolved in benzene (6 mL), freeze-dried, and obtained as a light green amorphous solid in a 76% yield (79.1 mg). $[\alpha]^{25}_D$ = -43.1° (α -0.028, c = 0.33, $CHCl_3$). **IR(ATR)**: 3280, 3062, 3032, 2987, 2949, 2868, 2833, 2787, 1738, 1612, 1574, 1454, 1329, 1259, 1201, 1161, 1142, 1093, 1061 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 9.16 (d, *J* = 9.4 Hz, 1H, NH), 8.39 (dd, *J* = 8.5, 1.1 Hz, 1H, H-10), 8.35 – 8.30 (m, 1H, H-15), 8.08 (dd, *J* = 7.3, 1.3 Hz, 1H, H-8), 7.54 (ddd, *J* = 8.5, 7.4, 4.1 Hz, 2H, H-9, H-13), 7.21 (dd, *J* = 7.7, 0.9 Hz, 1H, H-14), 7.17 – 7.12 (m, 5H, H-4, H-5, H-6), 5.00 (d, *J* = 9.3 Hz, 1H, H-2), 3.30 (s, 3H, H-18), 2.80 (s, 6H, H-17) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 170.05 (C-1), 151.12 (C-12), 135.89 (C-3), 135.44 (C-11), 129.59 (C-10), 129.01 – 128.85 (C-7, C-16), 128.52 (C-8), 128.18 – 127.14 (C-4, C-5, C-6), 127.68 (C-13), 123.29 (C-9), 119.38 (C-15), 114.97 (C-14), 59.26 (C-2), 52.10 (C-18), 45.06 (C-17) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 218.0, λ_{max2}, nm: 252.0, λ_{max3}, nm: 340.0, $1*10^{-5}$ M. **ESI MS**: for $C_{21}H_{22}N_2O_4S$ calcd: *m/z* 398.1 (for $[M+Na]^+$ calcd: *m/z* 421.1), found 421.0 $[M+Na]^+$. **HRMS**: for $C_{21}H_{22}N_2O_4S$ calcd: *m/z* 398.1300 (for $[M+H]^+$ calcd: *m/z* 399.1373), found 399.1366 $[M+H]^+$, Δ 1.8 ppm.

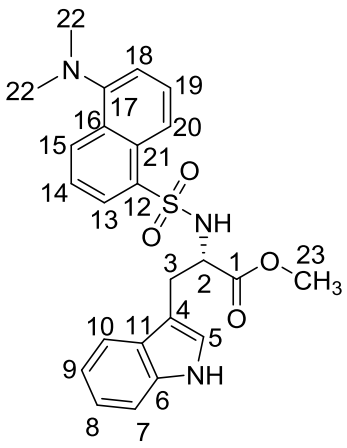


Methyl ((5-(dimethylamino)naphthalen-1-yl)sulfonyl)-L-phenylalaninate (DNS-L-Phe-Me **107a).** Compound **107a** was prepared according to the general procedure (**GP8**). DNS-L-Phe-Me **104a** (0.02 g, 50.1 μmol) was dissolved in Et_2O/MeOH 5/1 mixture (1.8 mL). The product was dissolved in benzene (2 mL), freeze-dried, and obtained as a light green amorphous solid in a 92% yield (19.5 mg). $[\alpha]^{25}_D$ = +48.0° (α +0.024, c = 0.25, $CHCl_3$). **IR(DRIFT)**: 3323, 3062, 3024, 2999, 2949, 2925, 2852, 2789, 1948, 1755, 1610, 1587, 1576, 1454, 1435, 1410, 1329, 1284, 1221, 1200, 1163, 1146, 1093, 1076, 1063 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 8.76 (d, J = 7.3 Hz, 1H, NH), 8.42 – 8.35 (m, 1H, H-11), 8.19 (d, J = 8.7 Hz, 1H, H-16), 7.87 (dd, J = 7.3, 1.3 Hz, 1H, H-9), 7.53 (dd, J = 8.7, 7.5 Hz, 1H, H-14), 7.46 (dd, J = 8.5, 7.3 Hz, 1H, H-10), 7.23 (dd, J = 7.6, 0.9 Hz, 1H, H-15), 7.05 – 6.96 (m, 5H, H-5, H-6, H-7), 3.90 (d, J = 7.6 Hz, 1H, H-2), 3.18 (s, 3H, H-19), 2.87 (dd, J = 13.7, 6.0 Hz, 1H, H-3), 2.82 (s, 6H, H-18), 2.73 (dd, J = 13.7, 9.2 Hz, 1H, H-3) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 171.24 (C-1), 151.14 (C-13), 136.14 (C-4), 135.83 (C-12), 129.42 (C-11), 128.96 – 128.90 (C-8, C-17), 128.09 (C-9), 128.82 – 126.42 (C-5, C-6, C-7), 127.60 (C-14), 123.22 (C-10), 119.35 (C-16), 114.95 (C-15), 57.51 (C-2), 51.50 (C-19), 45.08 (C-18), 37.39 (C-3) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 217.0, λ_{max2}, nm: 252.0, λ_{max3}, nm: 340.0, $1*10^{-5}$ M. **ESI MS**: for $C_{22}H_{24}N_2O_4S$ calcd: *m/z* 412.1 (for $[M+Na]^+$ calcd: *m/z* 435.1), found 435.0 $[M+Na]^+$. **HRMS**: for $C_{22}H_{24}N_2O_4S$ calcd: *m/z* 412.1457 (for $[M+H]^+$ calcd: *m/z* 413.1530), found 413.1539 $[M+H]^+$, Δ 2.2 ppm.

Methyl ((5-(dimethylamino)naphthalen-1-yl)sulfonyl)-D-phenylalaninate (DNS-D-Phe-Me **107b).** Compound **107b** was prepared according to the general procedure (**GP8**). DNS-D-Phe **104b** (0.057 g, 0.14 mmol) was dissolved in Et_2O/MeOH 5/1 mixture (5.3 mL). The product was dissolved in benzene (6 mL), freeze-dried, and obtained as a light green amorphous solid in an 88% yield (52.0 mg). $[\alpha]^{25}_D$ = -39.6° (α -0.021, c = 0.27, $CHCl_3$). **IR(DRIFT)**: 3286, 3064, 3026, 2925, 2852, 2789, 1747, 1612, 1587, 1576, 1456, 1435, 1410, 1331, 1284, 1219, 1201, 1163, 1146, 1095, 1063 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 8.76 (d, J = 6.2 Hz, 1H, NH), 8.38 (dt, J = 8.5, 1.1 Hz, 1H, H-11), 8.19 (dt, J = 8.7, 1.0 Hz, 1H,

H-16), 7.87 (dd, *J* = 7.3, 1.3 Hz, 1H, H-9), 7.53 (dd, *J* = 8.7, 7.5 Hz, 1H, H-14), 7.46 (dd, *J* = 8.5, 7.3 Hz, 1H, H-10), 7.23 (dd, *J* = 7.6, 0.9 Hz, 1H, H-15), 7.07 – 6.95 (m, 5H, H-5, H-6, H-7), 3.90 (d, *J* = 8.5 Hz, 1H, H-2), 3.18 (s, 3H, H-19), 2.87 (dd, *J* = 13.7, 6.1 Hz, 1H, H-3), 2.82 (s, 6H, H-18), 2.73 (dd, *J* = 13.7, 9.2 Hz, 1H, H-3) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 171.24 (C-1), 151.14 (C-13), 136.14 (C-4), 135.82 (C-12), 129.42 (C-11), 128.96 – 128.90 (C-8, C-17), 128.09 (C-9), 128.82 – 126.42 (C-5, C-6, C-7), 127.60 (C-14), 123.22 (C-10), 119.35 (C-16), 114.95 (C-15), 57.51 (C-2), 51.50 (C-19), 45.08 (C-18), 37.39 (C-3) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 217.0, λ_{max2}, nm: 252.0, λ_{max3}, nm: 340.0, 1*10^{-5} M. **ESI MS**: for $C_{22}H_{24}N_2O_4S$ calcd: *m/z* 412.1 (for $[M+Na]^+$ calcd: *m/z* 435.1), found 435.0 $[M+Na]^+$. **HRMS**: for $C_{22}H_{24}N_2O_4S$ calcd: *m/z* 412.1457 (for $[M+H]^+$ calcd: *m/z* 413.1530), found 413.1546 $[M+H]^+$, Δ 3.9 ppm.

Methyl ((5-(dimethylamino)naphthalen-1-yl)sulfonyl)-L-tryptophanate (DNS-L-Trp-Me **108a).** Compound **108a** was prepared according to the general procedure (**GP8**). DNS-L-Trp **105a** (0.13 g, 0.31 mmol) was dissolved in Et_2O/MeOH 5/1 mixture (11 mL). The product was purified by column chromatography. The product was dissolved in benzene (6 mL), freeze-dried, and obtained as a light green amorphous solid in 28% yield (39.0 mg). **$[\alpha]^{25}_D$** = +11.5° (α +0.006, c = 0.26, $CHCl_3$). **IR(ATR)**: 3396, 3284, 3057, 2985, 2947, 2868, 2833, 2787, 1738, 1612, 1587, 1574, 1456, 1435, 1329, 1201, 1161, 1144, 1093, 1063 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 10.76 (d, *J* = 2.5 Hz, 1H, NH), 8.77 (d, *J* = 8.7 Hz, 1H, NH), 8.41 (dt, *J* = 8.6, 1.1 Hz, 1H, H-15), 8.27 (dt, *J* = 8.6, 0.9 Hz, 1H, H-20), 7.93 (dd, *J* = 7.3, 1.2 Hz, 1H, H-13), 7.56 (dd, *J* = 8.7, 7.5 Hz, 1H, H-18), 7.45 (dd, *J* = 8.6, 7.3 Hz, 1H, H-14), 7.31 – 7.22 (m, 2H, H-7, H-19), 7.07 – 7.00 (m, 3H, H-5, H-8, H-10), 6.87 (ddd, *J* = 7.9, 7.0, 1.0 Hz, 1H, H-9), 3.92 (q, *J* = 7.7 Hz, 1H, H-2), 3.09 (s, 3H, H-23), 3.05 – 2.97 (m, 1H, H-3), 2.87 – 2.83 (m, 1H, H-3), 2.81 (s, 6H, H-22) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 171.31 (C-1), 151.19 (C-17), 135.98 (C-6), 135.66 (C-16), 129.52 (C-15), 128.96 – 128.91 (C-12, C-21), 128.45 (C-13), 127.71 (C-18), 126.62 (C-11), 123.95 (C-5), 123.25 (C-14), 120.93 (C-8), 119.20 (C-20), 118.38 – 117.46 (C-9, C-10), 115.03 (C-19), 111.43 (C-7), 108.28 (C-4), 56.64 (C-2), 51.34 (C-23), 45.06 (C-22), 28.10 (C-3) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 219.0, λ_{max2}, nm: 252.0, λ_{max3}, nm: 340.0, 1*10^{-5} M. **ESI MS**: for $C_{24}H_{25}N_3O_4S$ calcd: *m/z* 451.2 (for $[M+Na]^+$



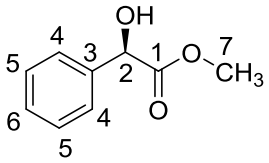
calcd: *m/z* 474.1), found 474.0 $[M+Na]^+$. **HRMS**: for $C_{24}H_{25}N_3O_4S$ calcd: *m/z* 451.1566 (for $[M+H]^+$ calcd: *m/z* 452.1639), found 452.1618 $[M+H]^+$, Δ 4.6 ppm. ^1H NMR spectrum is in accordance with the literature[408].

Methyl ((5-(dimethylamino)naphthalen-1-yl)sulfonyl)-D-tryptophanate (DNS-D-Trp-Me **108b).** Compound **108b** was prepared according to the general procedure (**GP8**). DNS-D-Trp **105b** (82.0 mg, 0.19 mmol) was dissolved in Et_2O/MeOH 5/1 mixture (7.2 mL). The product was dissolved in benzene (6 mL), freeze-dried, and obtained as a light green amorphous solid in a 98% yield (84.0 mg). $[\alpha]^{25}_D$ = -40.0° (α -0.026, c = 0.33, $CHCl_3$). **IR(ATR)**: 3406, 3288, 3055, 2947, 2924, 2866, 2833, 2787, 1736, 1612, 1587, 1574, 1456, 1433, 1331, 1201, 1161, 1144, 1093, 1063 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6): δ = 10.76 (d, *J* = 2.5 Hz, 1H, NH), 8.77 (d, *J* = 8.4 Hz, 1H, NH), 8.41 (dt, *J* = 8.5, 1.1 Hz, 1H, H-15), 8.27 (dt, *J* = 8.6, 0.9 Hz, 1H, H-20), 7.93 (dd, *J* = 7.3, 1.2 Hz, 1H, H-13), 7.56 (dd, *J* = 8.7, 7.6 Hz, 1H, H-18), 7.45 (dd, *J* = 8.6, 7.3 Hz, 1H, H-14), 7.25 (ddd, *J* = 11.2, 7.9, 0.9 Hz, 2H, H-7, H-19), 7.09 – 6.99 (m, 3H, H-5, H-8, H-10), 6.87 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H, H-9), 3.92 (q, *J* = 7.7 Hz, 1H, H-2), 3.09 (s, 3H, H-23), 3.02 (dd, *J* = 14.4, 7.8 Hz, 1H, H-3), 2.86 (m, 1H, H-3), 2.81 (s, 6H, H-22) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6): δ = 171.31 (C-1), 151.20 (C-17), 135.98 (C-6), 135.66 (C-16), 129.53 (C-15), 128.96 – 128.91 (C-12, C-21), 128.45 (C-13), 127.71 (C-18), 126.62 (C-11), 123.94 (C-5), 123.25 (C-14), 120.93 (C-8), 119.19 (C-20), 118.38 – 117.46 (C-9, C-10), 115.02 (C-19), 111.42 (C-7), 108.28 (C-4), 56.64 (C-2), 51.34 (C-23), 45.06 (C-22), 28.10 (C-3) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 218.0, λ_{max2}, nm: 252.5, λ_{max3}, nm: 340.0, 1*10^{-5} M. **ESI MS**: for $C_{24}H_{25}N_3O_4S$ calcd: *m/z* 451.2 (for $[M+Na]^+$ calcd: *m/z* 474.1), found 474.0 $[M+Na]^+$. **HRMS**: for $C_{24}H_{25}N_3O_4S$ calcd: *m/z* 451.1566 (for $[M+H]^+$ calcd: *m/z* 452.1639), found 452.1625 $[M+H]^+$, Δ 3.1 ppm. ^1H NMR spectrum is in accordance with the literature[408].

Methyl (S)-2-hydroxy-2-phenylacetate (109a). Compound **109a** was prepared according to the general procedure (**GP8**). (S)-Mandelic acid (0.1 g, 0.66 mmol) was dissolved in Et_2O/MeOH 10/1 mixture (5.5 mL). The product was evaporated on a rotary evaporator at 40°C and obtained as a white amorphous solid in a 99% yield (0.11 g).

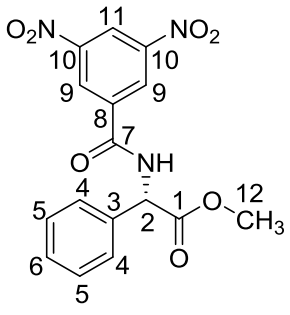
$[\alpha]^{25}_{D}$ = +148.1° (α +0.077, c = 0.26, MeOH). **IR(ATR)**: 3435, 3087, 3060, 3033, 3003, 2954, 2848, 1736, 1491, 1454, 1433, 1385, 1267, 1203, 1188, 1092, 1065 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.46 – 7.30 (m, 5H, H-4, H-5, H-6), 5.18 (s, 1H, H-2), 3.77 (s, 3H, H-7).ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 174.31 (C-1), 138.38 (C-3), 128.79 – 126.75 (C-4, C-5, C-6), 73.04 (C-2), 53.22 (C-7) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 203.0, λ_{max2}, nm: 258.0, $5*10^{-5}$ M. **ESI MS**: for $C_9H_{10}O_3$ calcd: *m/z* 166.1 (for $[M+Na]^+$ calcd: *m/z* 189.1), found 189.0 $[M+Na]^+$. **HRMS**: for $C_9H_{10}O_3$ calcd: *m/z* 166.0630 (for $[M+Na]^+$ calcd: *m/z* 189.0522), found 189.0523 $[M+Na]^+$, Δ 0.5 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[409].

Methyl (R)-2-hydroxy-2-phenylacetate (109b). Compound **109b** was prepared according to the general procedure (**GP8**). (R)-Mandelic acid (0.1 g, 0.66 mmol) was dissolved in Et_2O/MeOH 10/1 mixture (5.5 mL). The product was evaporated on a rotary evaporator at 40°C and obtained as a white amorphous solid in a 62% yield (68.1 mg).



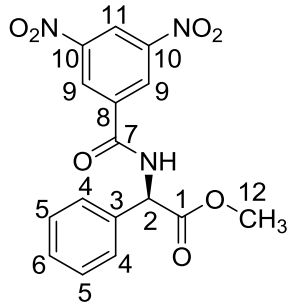
$[\alpha]^{25}_{D}$ = -122.7° (α -0.081, c = 0.33, MeOH). **IR(ATR)**: 3444, 3064, 3033, 2954, 2848, 2601, 1728, 1495, 1454, 1439, 1219, 1186, 1092, 1065 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.47 – 7.32 (m, 5H, H-4, H-5, H-6), 5.18 (s, 1H, H-2), 3.77 (s, 3H, H-7) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$): δ = 174.29 (C-1), 138.38 (C-3), 128.78 – 126.74 (C-4, C-5, C-6), 73.03 (C-2), 53.21 (C-7) ppm. **ESI MS**: for $C_9H_{10}O_3$ calcd: *m/z* 166.1 (for $[M+Na]^+$ calcd: *m/z* 189.1), found 189.0 $[M+Na]^+$. **HRMS**: for $C_9H_{10}O_3$ calcd: *m/z* 166.0630 (for $[M+Na]^+$ calcd: *m/z* 189.0522), found 189.0520 $[M+Na]^+$, Δ 1.1 ppm. ^{1}H and ^{13}C NMR spectra are in accordance with the literature[409].

Methyl (S)-2-(3,5-dinitrobenzamido)-2-phenylacetate (DNB-L-PhGly-Me **115a).** Compound **115a** was prepared according to the general procedure (**GP8**). DNB-L-PhGly **112a** (35.0 mg, 0.1 mmol) was dissolved in Et_2O/MeOH 10/1 mixture (2.0 mL). The product was evaporated on a rotary evaporator at 40°C and obtained as a white amorphous solid in a 80% yield (29 mg). $[\alpha]^{25}_{D}$ = +75.0° (α +0.042, c = 0.28, $CHCl_3$). **IR(ATR)**: 3342, 3089, 3033, 2952, 2924, 2883, 2850, 1738, 1645, 1533, 1496, 1437, 1346, 1317, 1281, 1217, 1196, 1176, 1082 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$):δ = 9.18 (t, *J* = 2.1 Hz, 1H, H-11), 8.97 (d, *J* = 2.1 Hz, 2H, H-9), 7.45 – 7.36 (m, 6H, NH, H-4, H-5, H-6), 5.78 (d, *J* = 6.8 Hz, 1H, H-2), 3.81 (s, 3H, H-12) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$):δ = 171.15



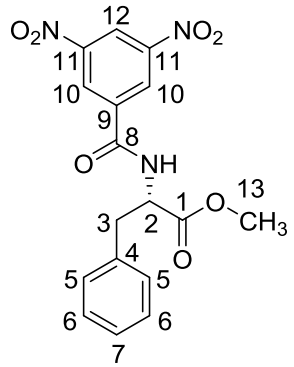
(C-1), 162.09 (C-7), 148.83 (C-10), 137.16 (C-8), 135.62 (C-3), 129.44 – 127.50 (C-4, C-5, C-6, C-9), 121.58 (C-11), 57.46 (C-2), 53.46 (C-12) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 202.9, λ_{max2}, nm: 240.3, 8*10^{-7} M. **ESI MS**: for $C_{16}H_{13}N_3O_7$calcd: *m/z* 359.1 (for [M-H$^+$]$^-$ calcd: *m/z* 358.1), found 358.0 [M-H$^+$]$^-$. **HRMS**: for $C_{16}H_{13}N_3O_7$calcd: *m/z* 359.0754 (for [M+H]$^+$ calcd: *m/z* 360.0826), found 360.0823 [M+H]$^+$, Δ 1.0 ppm.

Methyl (R)-2-(3,5-dinitrobenzamido)-2-phenylacetate (DNB-D-PhGly-Me **115b).**



Compound **115b** was prepared according to the general procedure (**GP8**). DNB-D-PhGly **112b** (84.0 mg, 0.24 mmol) was dissolved in Et_2O/MeOH 10/1 mixture (5.0 mL). The product was evaporated on a rotary evaporator at 40°C and obtained as a white amorphous solid in a 89% yield (78 mg). **[α]$^{25}_D$** = -71.2° (α -0.042, c = 0.30, $CHCl_3$). **IR(ATR)**: 3344, 3089, 3032, 2952, 2924, 2848, 1736, 1647, 1533, 1489, 1435, 1350, 1321, 1279, 1214, 1196, 1176, 1081 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$):*δ* = 9.18 (t, *J* = 2.1 Hz, 1H, H-11), 8.97 (d, *J* = 2.1 Hz, 2H, H-9), 7.52 – 7.33 (m, 6H, NH, H-4, H-5, H-6), 5.78 (d, *J* = 6.9 Hz, 1H, H-2), 3.81 (s, 3H, H-12) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$):*δ* = 171.13 (C-1), 162.09 (C-7), 148.84 (C-10), 137.18 (C-8), 135.62 (C-3), 129.45 – 127.50 (C-4, C-5, C-6, C-9), 121.58 (C-11), 57.47 (C-2), 53.46 (C-12) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 203.0, λ_{max2}, nm: 241.1, 8*10^{-7} M. **ESI MS**: for $C_{16}H_{13}N_3O_7$calcd: *m/z* 359.1 (for [M-H$^+$]$^-$ calcd: *m/z* 358.1), found 358.0 [M-H$^+$]$^-$. **HRMS**: for $C_{16}H_{13}N_3O_7$calcd: *m/z* 359.0754 (for [M+H]$^+$ calcd: *m/z* 360.0826), found 360.0825 [M+H]$^+$, Δ 0.5 ppm.

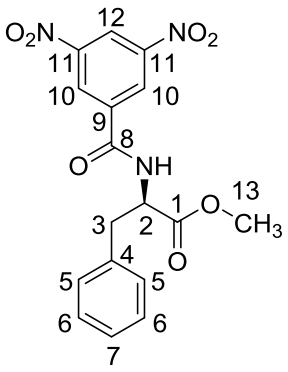
Methyl (3,5-dinitrobenzoyl)-L-phenylalaninate (DNB-L-Phe-Me **116a).** Compound



116a was prepared according to the general procedure (**GP8**). DNB-L-Phe **113a** (88.0 mg, 0.24 mmol) was dissolved in Et_2O/MeOH 10/1 mixture (5.0 mL). The product was evaporated on a rotary evaporator at 40°C and obtained as a light orange glassy solid in a 89% yield (81 mg). **[α]$^{25}_D$** = +59.0° (α +0.036, c = 0.31, $CHCl_3$). **IR(ATR)**: 3334, 3095, 3032, 2956, 2925, 2852, 1732, 1653, 1630, 1537, 1454, 1437, 1342, 1282, 1217, 1195, 1182, 1093, 1074 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$):*δ* = 9.16 (t, *J* = 2.1 Hz, 1H, H-12), 8.83 (d, *J* = 2.1 Hz, 2H, H-10), 7.36 – 7.11 (m, 5H, H-5, H-6, H-7), 6.82 (d, *J* = 7.7 Hz, 1H, NH), 5.11 (dt, *J* = 7.7, 5.8 Hz, 1H, H-2), 3.84 (s, 3H, H-13), 3.40 – 3.20 (m, 2H, H-3) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$):*δ* = 171.74 (C-1), 162.43 (C-8), 148.82 (C-

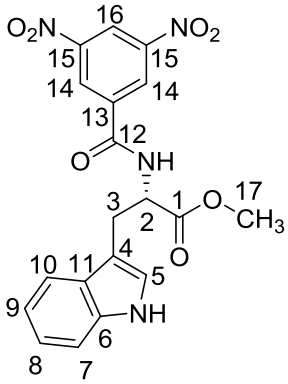
11), 137.47 (C-9), 135.32 (C-4), 129.34, – 127.35 (C-5, C-6, C-7, C-10), 121.48 (C-12), 54.07 (C-2), 53.02 (C-13), 37.84 (C-3) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 203.4, λ_{max2}, nm: 241.3, $8*10^{-7}$ M. **ESI MS**: for $C_{17}H_{15}N_3O_7$calcd: *m/z* 373.1 (for $[M-H^+]^-$ calcd: *m/z* 372.1), found 372.0 $[M-H^+]^-$. **HRMS**: for $C_{17}H_{15}N_3O_7$calcd: *m/z* 373.0910 (for $[M+H]^+$ calcd: *m/z* 374.0983), found 374.0983 $[M+H]^+$, Δ 0.1 ppm.

Methyl (3,5-dinitrobenzoyl)-D-phenylalaninate (DNB-D-Phe-Me **116b).** Compound



116b was prepared according to the general procedure (**GP8**). DNB-D-Phe **113b** (100.0 mg, 0.28 mmol) was dissolved in Et_2O/MeOH 10/1 mixture (5.0 mL). The product was evaporated on a rotary evaporator at 40°C and obtained as a light yellow glassy solid in a 98% yield (103 mg). $[\alpha]^{25}_D$ = -57.7° (α -0.030, c = 0.26, $CHCl_3$). **IR(ATR)**: 3336, 3086, 3028, 2952, 2939, 2850, 1734, 1649, 1628, 1529, 1441, 1344, 1302, 1250, 1221, 1200, 1103, 1078 cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$):*δ* = 9.16 (t, *J* = 2.1 Hz, 1H, H-12), 8.83 (d, *J* = 2.1 Hz, 2H, H-10), 7.39 – 7.09 (m, 5H, H-5, H-6, H-7), 6.81 (d, *J* = 7.7 Hz, 1H, NH), 5.11 (dt, *J* = 7.7, 5.8 Hz, 1H, H-2), 3.84 (s, 3H, H-13), 3.39 – 3.20 (m, 2H, H-3) ppm. **^{13}C NMR** (101 MHz, $CDCl_3$):*δ* = 171.74 (C-1), 162.43 (C-8), 148.82 (C-11), 137.47 (C-9), 135.32 (C-4), 129.34 – 127.35 (C-5, C-6, C-7, C-10), 121.49 (C-12), 54.07 (C-2), 53.02 (C-13), 37.85 (C-3) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 202.8, λ_{max2}, nm: 240.3, $7*10^{-7}$ M. **ESI MS**: for $C_{17}H_{15}N_3O_7$calcd: *m/z* 373.1 (for $[M-H^+]^-$ calcd: *m/z* 372.1), found 372.0 $[M-H^+]^-$. **HRMS**: for $C_{17}H_{15}N_3O_7$calcd: *m/z* 373.0910 (for $[M+H]^+$ calcd: *m/z* 374.0983), found 374.0983 $[M+H]^+$, Δ 0.1 ppm.

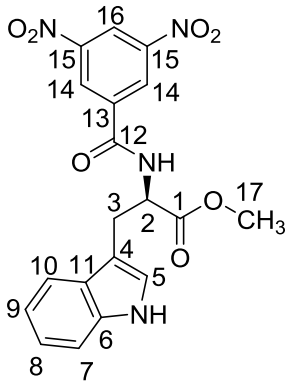
Methyl (3,5-dinitrobenzoyl)-L-tryptophanate (DNB-L-Trp-Me **117a).** Compound



117a was prepared according to the general procedure (**GP8**). DNB-L-Trp **114a** (46.0 mg, 0.12 mmol) was dissolved in Et_2O/MeOH 5/1 mixture (2.5 mL). The product was isolated by filtration and drying of the solid at 70°C. The product was obtained as an orange solid in a 45% yield (21 mg). $[\alpha]^{25}_D$ = -19.0° (α -0.011, c = 0.29, DMSO). **IR(ATR)**: 3440, 3379, 3114, 3091, 3059, 3003, 2954, 2935, 2856, 1714, 1668, 1628, 1530, 1515, 1444, 1340, 1255, 1227, 1182, 1099, 1074 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6):*δ* = 10.86 (d, *J* = 2.4 Hz, 1H, NH), 9.65 (d, *J* = 7.5 Hz, 1H, NH), 9.04 (d, *J* = 2.1 Hz, 2H, H-14), 8.95 (t, *J* = 2.1 Hz, 1H, H-16), 7.58 (d, *J* = 7.8 Hz, 1H, H-

7), 7.32 (d, *J* = 8.0 Hz, 1H, H-10), 7.21 (d, *J* = 2.4 Hz, 1H, H-5), 7.06 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H, H-8), 7.01 – 6.92 (m, 1H, H-9), 4.80 (ddd, *J* = 9.2, 7.5, 5.5 Hz, 1H, H-2), 3.66 (s, 3H, H-17), 3.42 – 3.20 (m, 2H, H-3, solvent overlay) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6):*δ* = 171.91 (C-1), 162.43 (C-12), 148.20 (C-15), 136.11 – 136.05 (C-6, C-13), 127.68 (C-14), 127.01 (C-11), 123.69 (C-5), 121.15 – 121.03 (C-8, C-16), 118.48 (C-9), 117.99 (C-7), 111.49 (C-10), 109.60 (C-4), 54.27 (C-2), 52.13 (C-17), 26.62 (C-3) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 201.9, λ_{max2}, nm: 208.5, λ_{max3}, nm: 254.9, 7*10^{-7} M. **ESI MS**: for $C_{19}H_{16}N_4O_7$calcd: *m/z* 412.1 (for $[M+Na]^+$ calcd: *m/z* 435.1), found 435.2 $[M+Na]^+$. **HRMS**: for $C_{19}H_{16}N_4O_7$calcd: *m/z* 412.1019 (for $[M+H]^+$ calcd: *m/z* 413.1092), found 413.1092 $[M+H]^+$, Δ 0.1 ppm.

Methyl (3,5-dinitrobenzoyl)-D-tryptophanate (DNB-D-Trp-Me **117b).** Compound



117b was prepared according to the general procedure (**GP8**). DNB-D-Trp **114b** (77.0 mg, 0.19 mmol) was dissolved in Et_2O/MeOH 5/1 mixture (4.0 mL). The product was isolated by filtration and drying of the solid at 70°C. The product was obtained as an orange solid in a 65% yield (52 mg). **$[\alpha]^{25}_D$** = +118.2° (α +0.065, c = 0.28, DMSO). **IR(ATR)**: 3440, 3379, 3114, 3093, 3057, 3003, 2954, 2933, 2856, 1714, 1668, 1628, 1530, 1442, 1344, 1255, 1227, 1184, 1097, 1074 cm^{-1}. **^{1}H NMR** (400 MHz, DMSO-d_6):*δ* = 10.86 (d, *J* = 2.5 Hz, 1H, NH), 9.65 (d, *J* = 7.5 Hz, 1H, NH), 9.04 (d, *J* = 2.1 Hz, 2H, H-14), 8.95 (t, *J* = 2.1 Hz, 1H, H-16), 7.58 (d, *J* = 8.0 Hz, 1H, H-7), 7.32 (d, *J* = 8.1 Hz, 1H, H-10), 7.21 (d, *J* = 2.4 Hz, 1H, H-5), 7.05 (ddd, *J* = 8.2, 7.0, 1.3 Hz, 1H, H-8), 6.98 (td, *J* = 7.4, 1.1 Hz, 1H, H-9), 4.80 (ddd, *J* = 9.2, 7.4, 5.5 Hz, 1H, H-2), 3.66 (s, 3H, H-17), 3.42 – 3.22 (m, 2H, H-3, solvent overlay) ppm. **^{13}C NMR** (101 MHz, DMSO-d_6):*δ* = 171.91 (C-1), 162.43 (C-12), 148.20 (C-15), 136.11 – 136.06 (C-6, C-13), 127.69 (C-14), 127.01 (C-11), 123.68 (C-5), 121.14 – 121.03 (C-8, C-16), 118.48 (C-9), 117.99 (C-7), 111.49 (C-10), 109.60 (C-4), 54.27 (C-2), 52.13 (C-17), 26.62 (C-3) ppm. **UV-VIS** (MeOH), λ_{max1}, nm: 202.2, λ_{max2}, nm: 209.7, λ_{max3}, nm: 254.9, 7*10^{-7} M. **ESI MS**: for $C_{19}H_{16}N_4O_7$calcd: *m/z* 412.1 (for $[M+Na]^+$ calcd: *m/z* 435.1), found 435.1 $[M+Na]^+$. **HRMS**: for $C_{19}H_{16}N_4O_7$calcd: *m/z* 412.1019 (for $[M+H]^+$ calcd: *m/z* 413.1092), found 413.1085 $[M+H]^+$, Δ 1.7 ppm.

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8 OTHER RESEARCHERS' CONTRIBUTIONS TO THIS THESIS

All NMR spectra acquired on 600MHz Bruker AVANCE III were measured by RNDr. Simona Petrželová, Ph.D., Department of Teaching and Didactics of Chemistry, Faculty of Science, Charles University, Hlavova 8, 128 00, Prague 2, Czech Republic.

Temperature NMR experiments were measured by RNDr. Zdeněk Tošner, Ph.D., Faculty of Science, Charles University, Hlavova 8, 128 00, Prague 2, Czech Republic.

Some HRMS spectra were obtained from RNDr. Martin Štícha, Ph.D., Department of Organic Chemistry, Faculty of Science, Charles University, Hlavova 8, 128 00, Prague 2, Czech Republic.

Preferential sorption and pertraction experiments were done in the laboratory of Ing. Pavel Izák, Ph.D., DSc. at Institute of Chemical Process Fundamentals of the Czech Academy of Sciences, Rozvojová 1, 165 02, Prague 6, Czech Republic.

Initial experiments on the chiral separation capability of the first coated column were performed in the laboratory of prof. RNDr. Zuzana Bosáková, CSc., Department of Analytical Chemistry, Faculty of Science, Charles University, Hlavova 8, 128 00, Prague 2, Czech Republic.

X-ray analysis was done by RNDr. Ivana Císařová, CSc., Department of Inorganic Chemistry, Faculty of Science, Charles University, Hlavova 8, 128 00, Prague 2, Czech Republic.

Mgr. Attila Palágyi reproduced developed protocols and prepared several anchors on a large scale. Some of them were prepared in quantities exceeding 100 grams.

9 AUTHOR'S PUBLICATIONS

Used in this thesis

1. Jindřich, J.; Kasal, P. Compounds for Modification of Negatively Charged Carrier Surface, Method of Their Preparation and Use Thereof. Pat. Appl. PCT/CZ2020/050088, 11 2020.

2. Kasal, P.; Michel, M.; Gaálová, J.; Cuřínová, P.; Izák, P.; Dian, J.; Jindřich, J. Chiral Nafion Membranes Prepared by Strong Electrostatic Binding of Multiply Positively Charged β-Cyclodextrin Derivatives for Tryptophan Racemic Mixtures' Separation. *Mater. Today Commun.* **2021**, *27*, 102234–102242. https://doi.org/10.1016/j.mtcomm.2021.102234.

3. Gaálová, J.; Michel, M.; Bourassi, M.; Ladewig, B. P.; Kasal, P.; Jindřich, J.; Izák, P. Nafion Membranes Modified by Cationic Cyclodextrin Derivatives for Enantioselective Separation. *Sep. Purif. Technol.* **2021**, *266*, 118538–118547. https://doi.org/10.1016/j.seppur.2021.118538.

4. Kasal, P.; Jindřich, J. Mono-6-Substituted Cyclodextrins—Synthesis and Applications. *Molecules* **2021**, *26* (16), 5065–5116. https://doi.org/10.3390/molecules26165065.

5. Kasal, P.; Jindřich, J. Kinetics of Nucleophilic Substitution of Compounds Containing Multiple Leaving Groups Bound to a Neopentyl Skeleton. *ACS Omega* **2022**, *7* (23), 20137–20144. https://doi.org/10.1021/acsomega.2c01965.

Other publications

6. Seggio, M.; Payamifar, S.; Fraix, A.; Kalydi, E.; Kasal, P.; Catanzano, O.; Conte, C.; Quaglia, F.; Sortino, S. Visible Light-Activatable Cyclodextrin-Conjugates for the Efficient Delivery of Nitric Oxide with Fluorescent Reporter and Their Inclusion Complexes with Betaxolol. *New J. Chem.* **2021**, *45* (19), 8449–8455. https://doi.org/10.1039/D1NJ00039J.

10 SUPPLEMENTAL INFORMATION

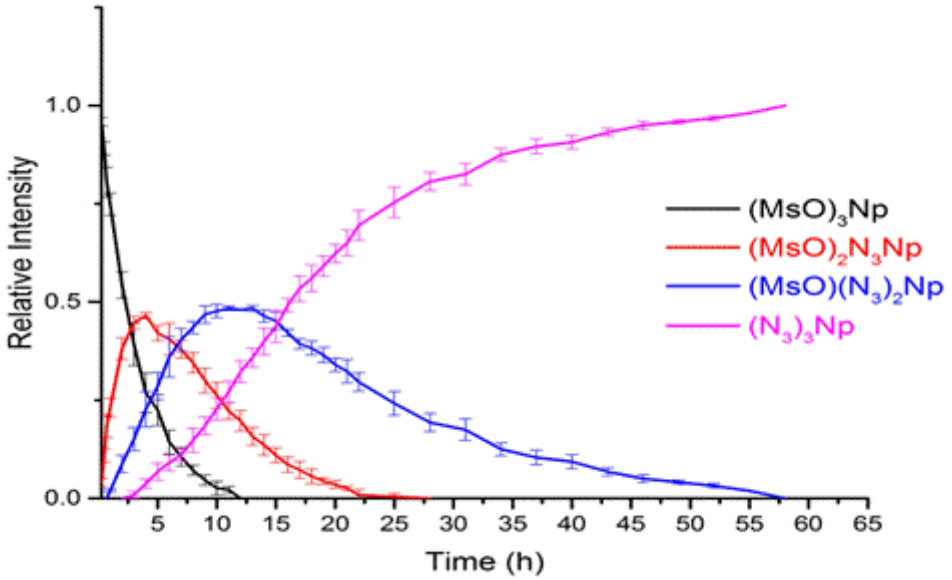
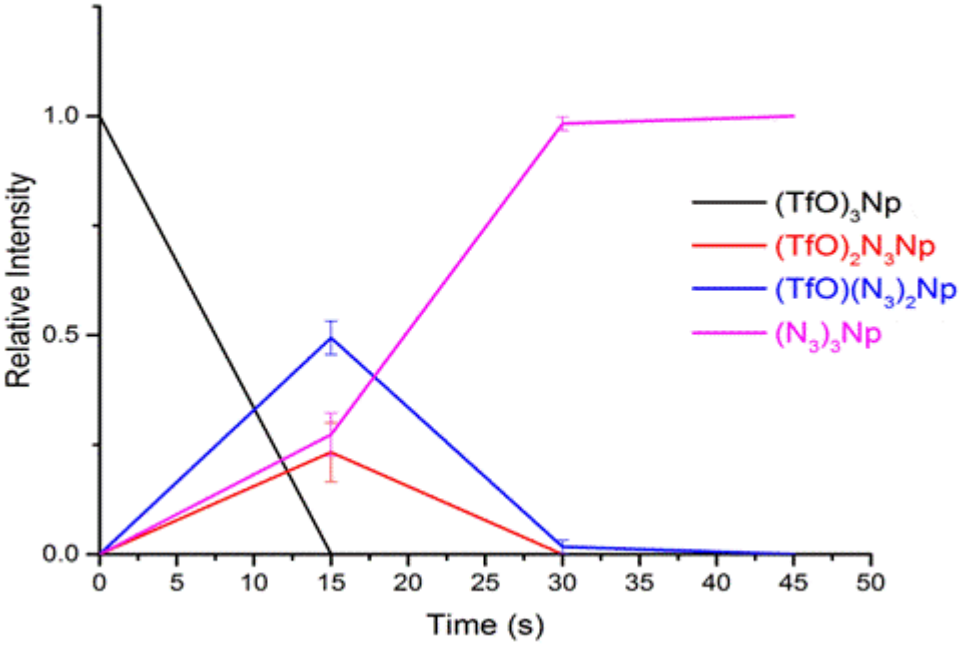


Figure S1. Composition of the reaction mixture in the reaction of $(MsO)_3Np$ **19** with excess of NaN_3 in time.



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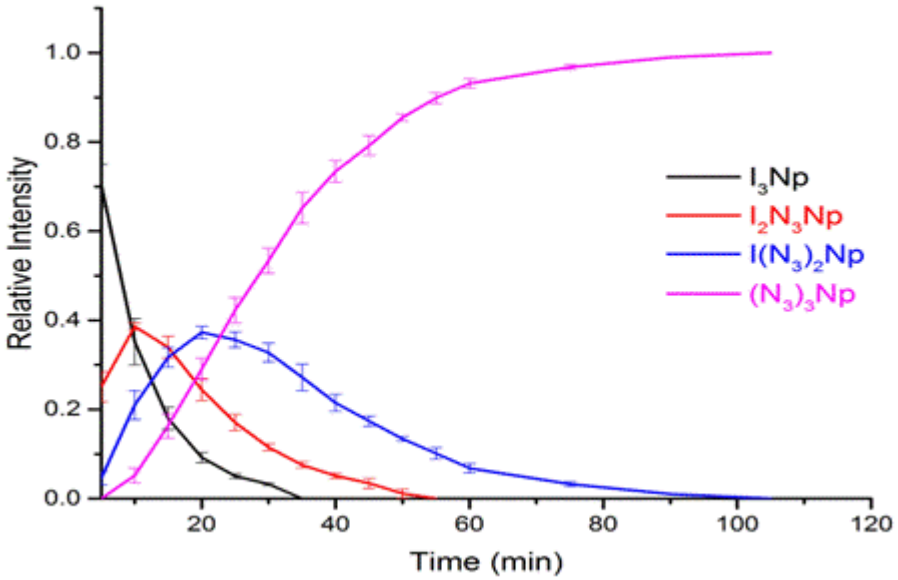


Figure S3. Composition of the reaction mixture in the reaction of I_3Np **22** with excess of NaN_3 in time.

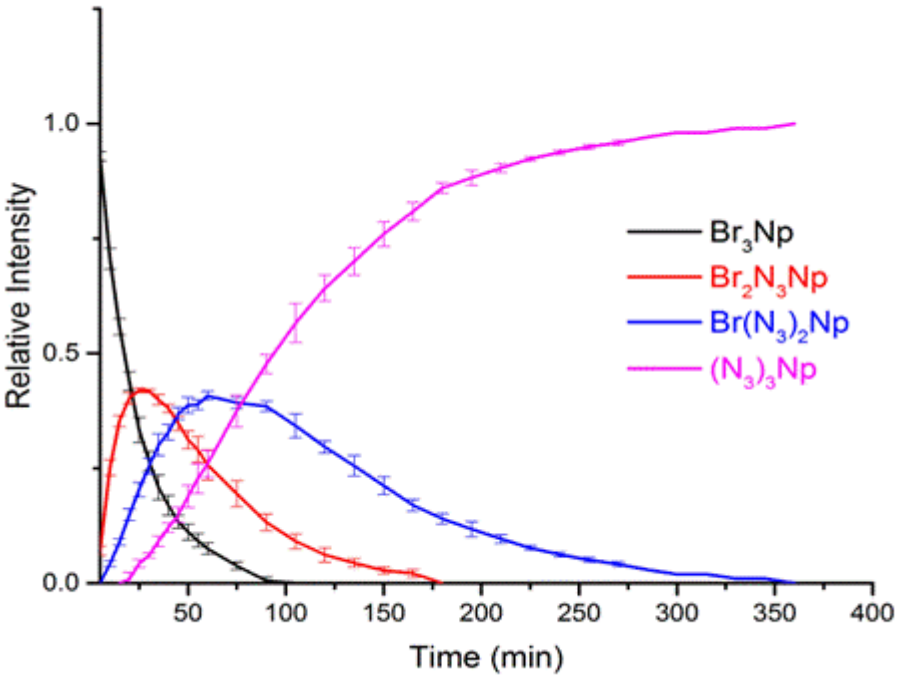


Figure S4. Composition of the reaction mixture in the reaction of Br_3Np **23** with excess of NaN_3 in time.

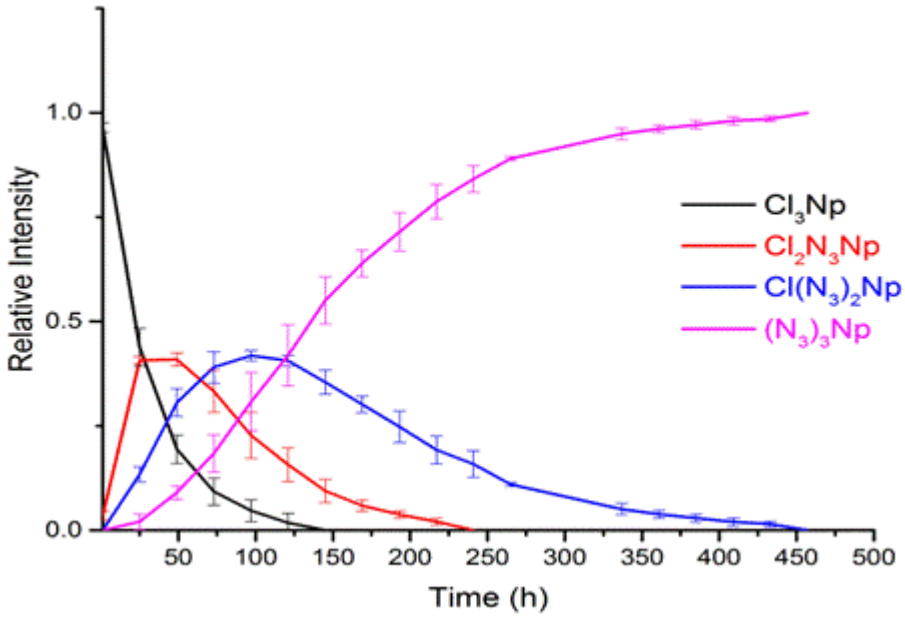


Figure S5. Composition of the reaction mixture in the reaction of Cl_3Np **24** with excess of NaN_3 in time.

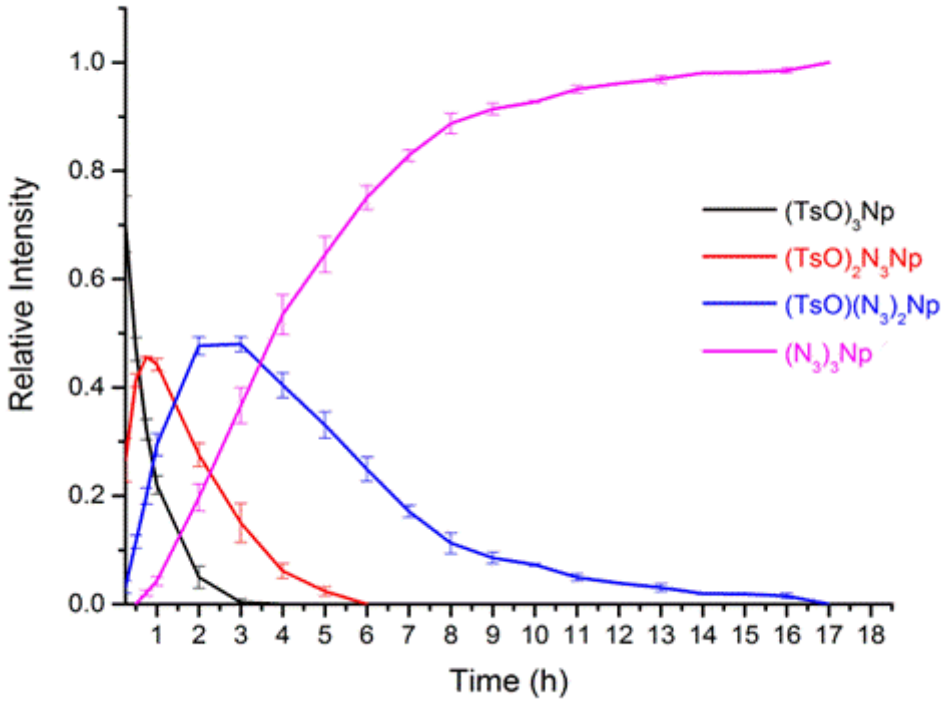


Figure S6. Composition of the reaction mixture in the reaction of $(TsO)_3Np$ **21** with excess of CsN_3 in time.

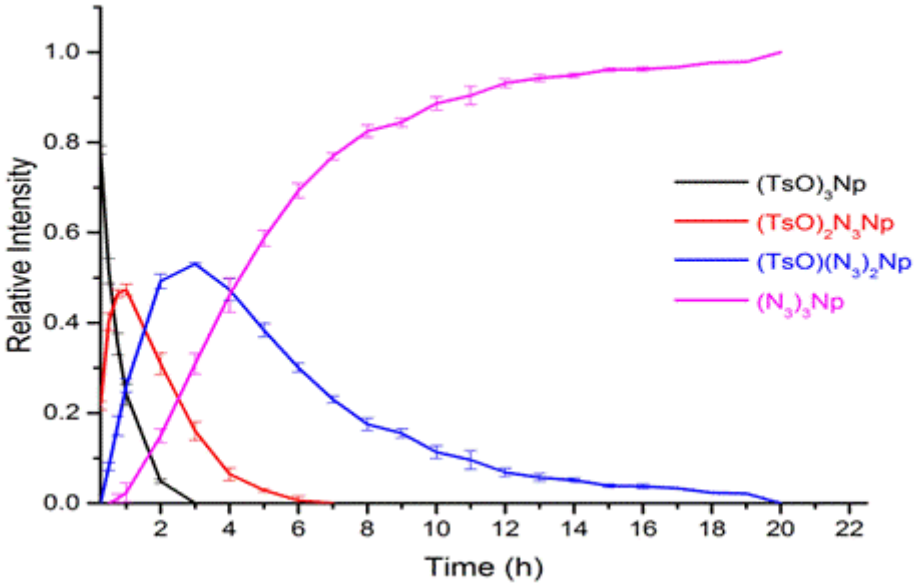


Figure S7. Composition of the reaction mixture in the reaction of $(TsO)_3Np$ **21** with excess of $(Me_4N)N_3$ in time.

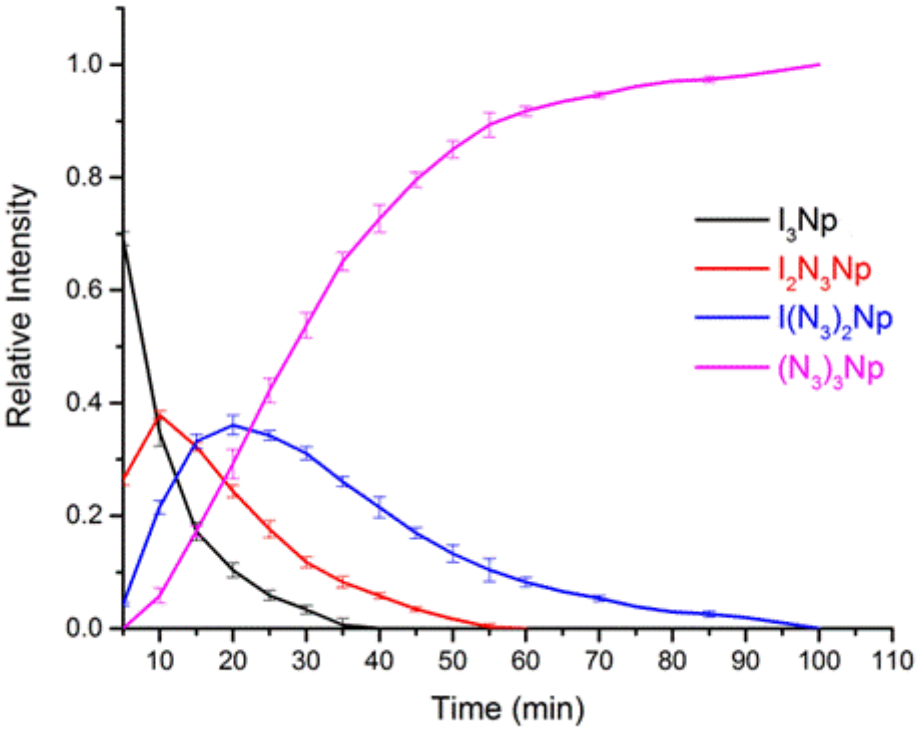


Figure S8. Composition of the reaction mixture in the reaction of I_3Np **22** with excess of CsN_3 in time.

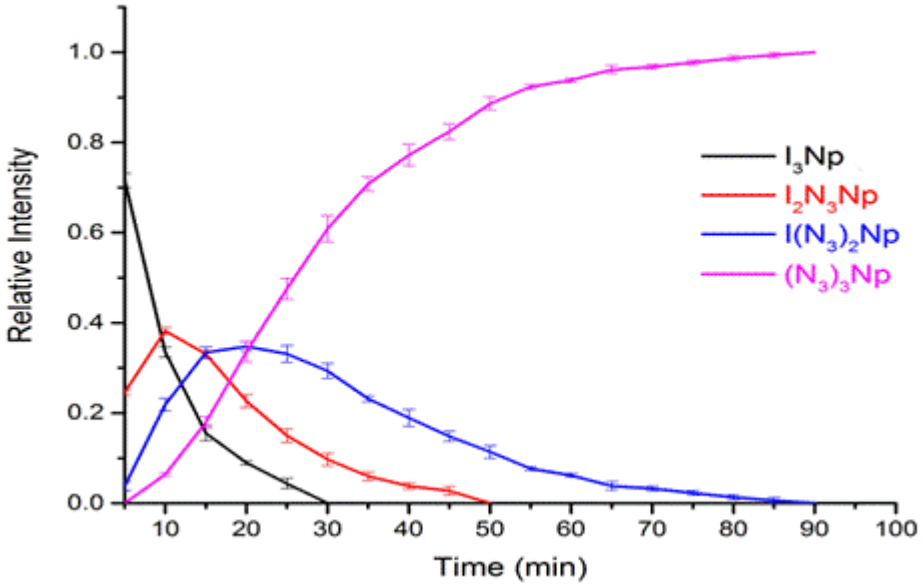


Figure S9. Composition of the reaction mixture in the reaction of I_3Np **22** with excess of $(Me_4N)N_3$ in time.

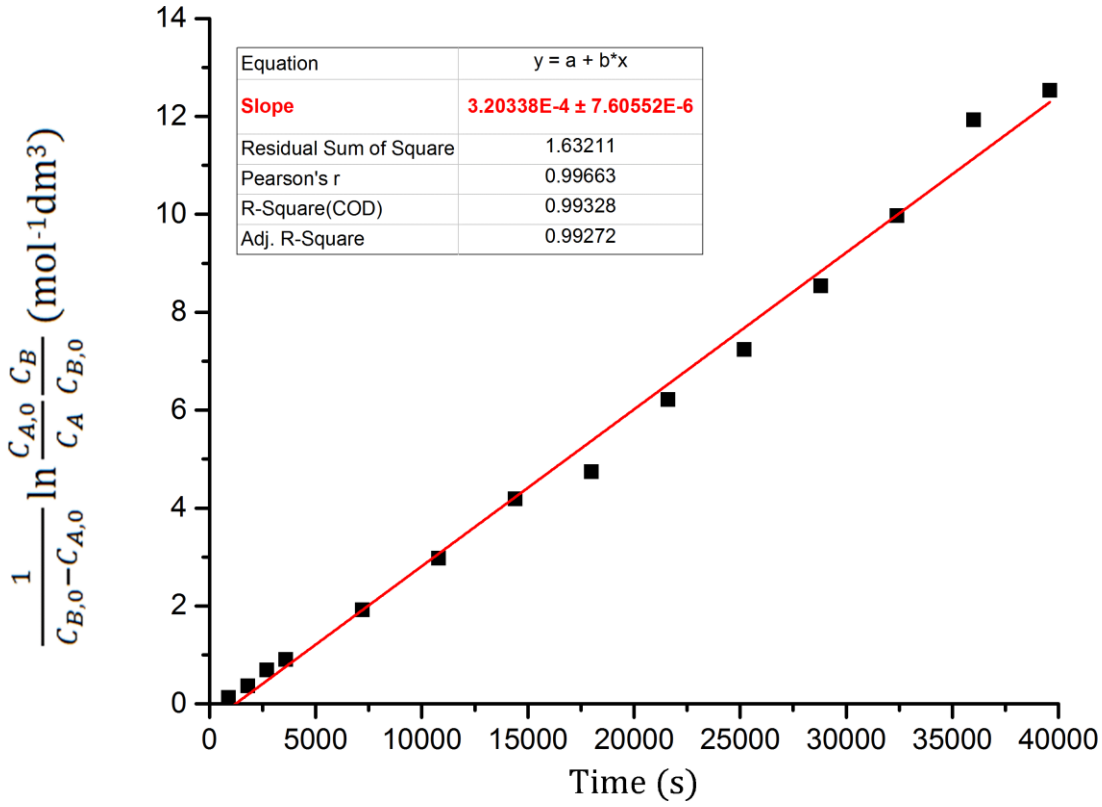


Figure S10. Variation of concentration of $(MsO)_3Np$ **19** and NaN_3 as a function of time.

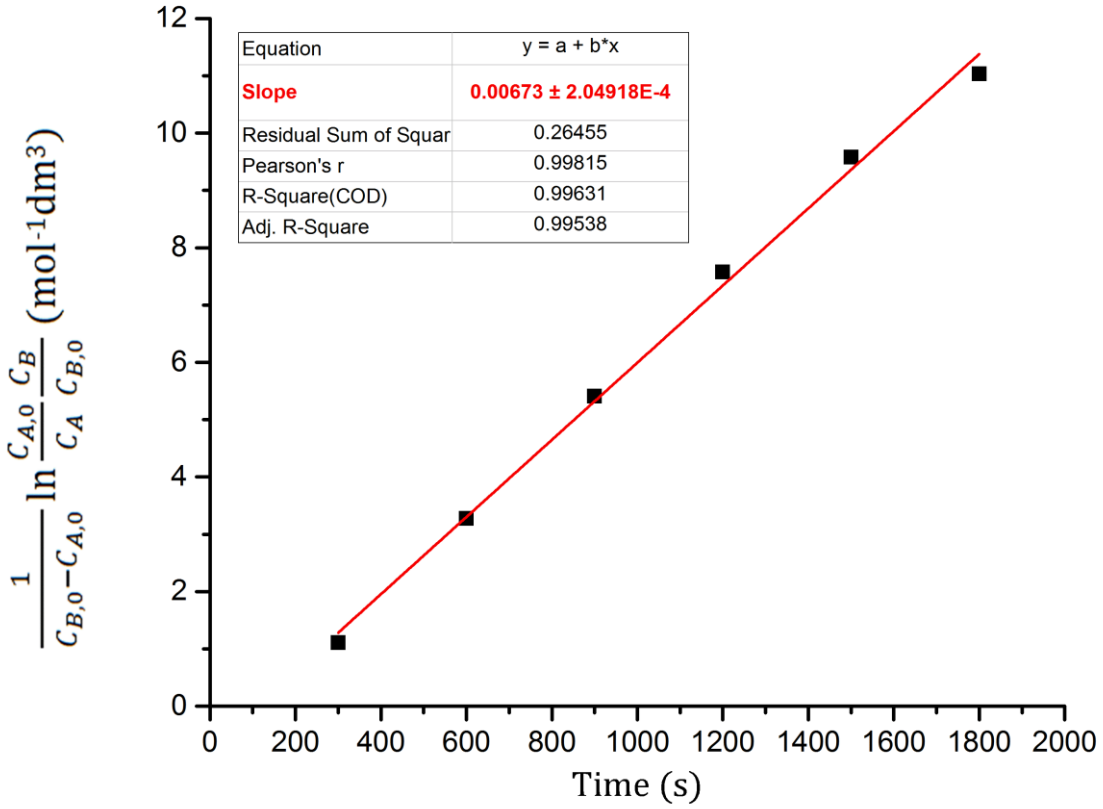


Figure S11. Variation of concentration of I_3Np **22** and NaN_3 as a function of time.

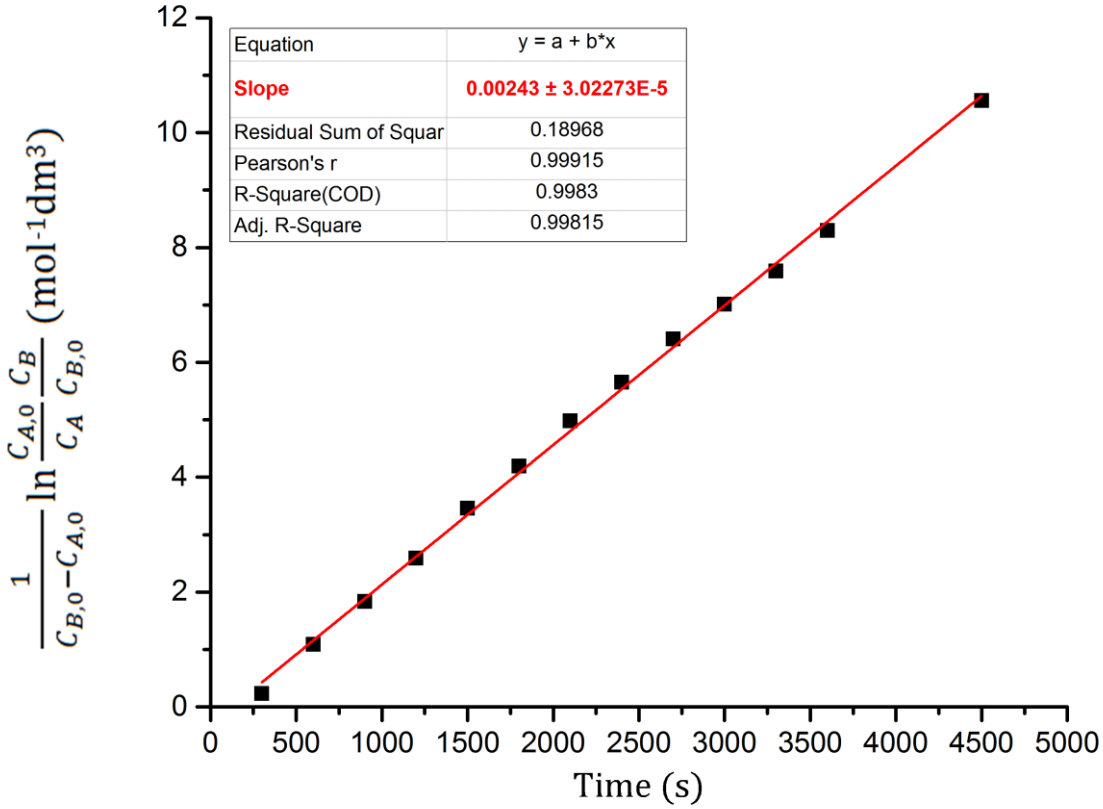


Figure S12. Variation of concentration of Br_3Np **23** and NaN_3 as a function of time.

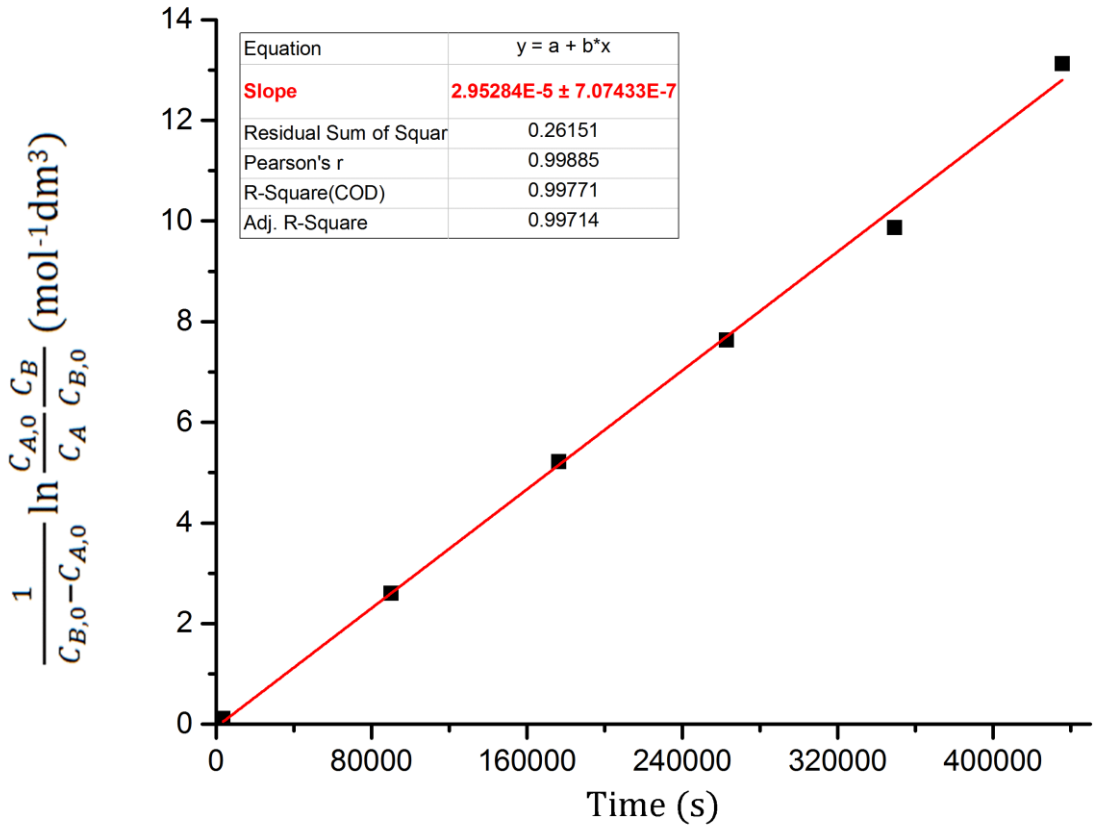


Figure S13. Variation of concentration of Cl_3Np **24** and NaN_3 as a function of time.

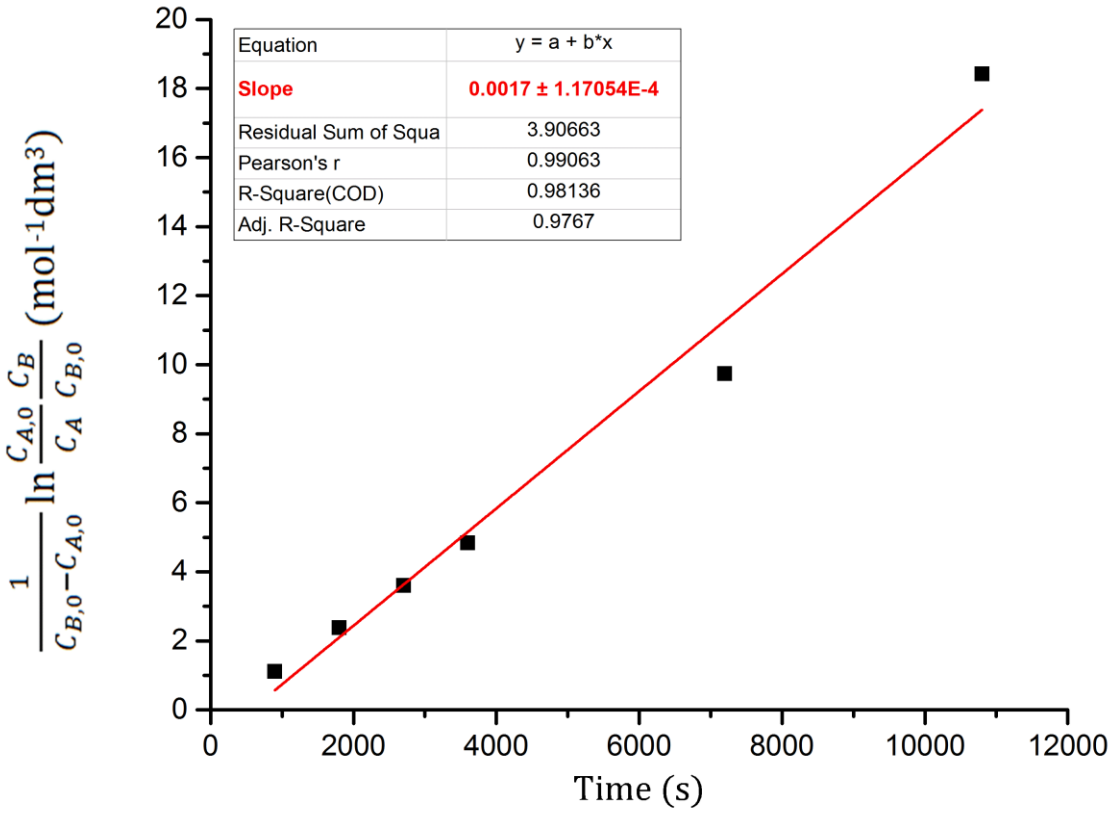


Figure S14. Variation of concentration of $(TsO)_3Np$ **21** and CsN_3 as a function of time.

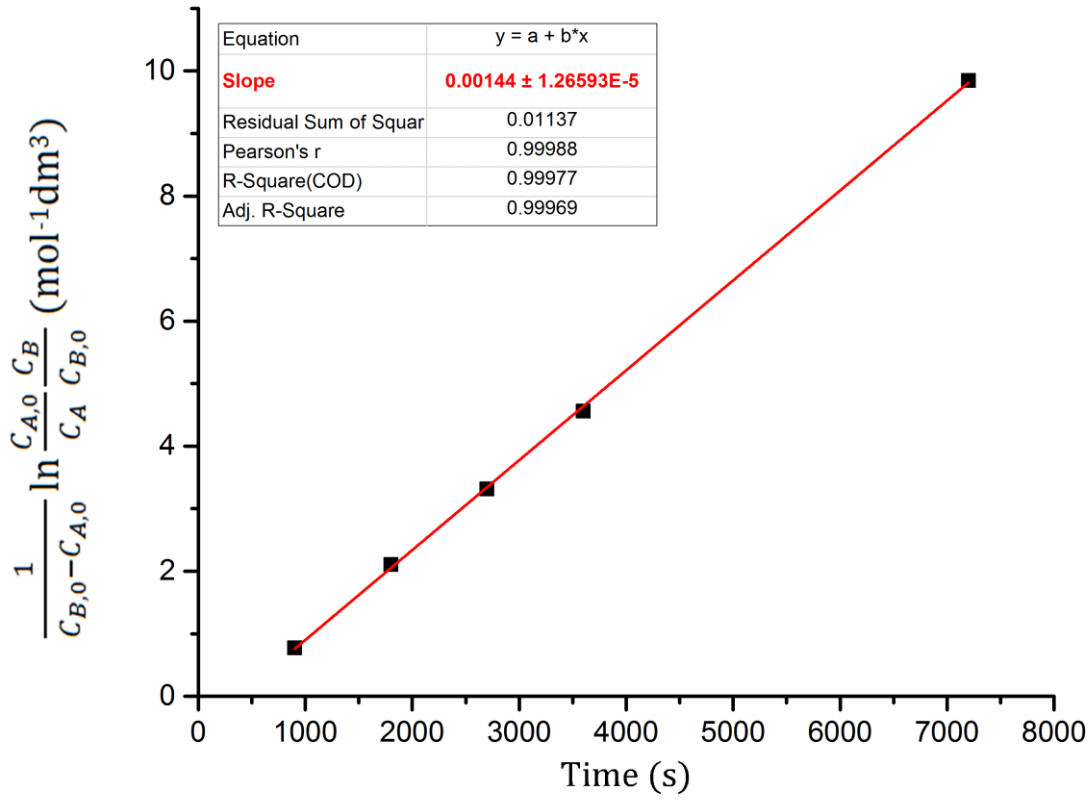


Figure S15. Variation of concentration of $(TsO)_3Np$ **21** and $(Me_4N)N_3$ as a function of time.

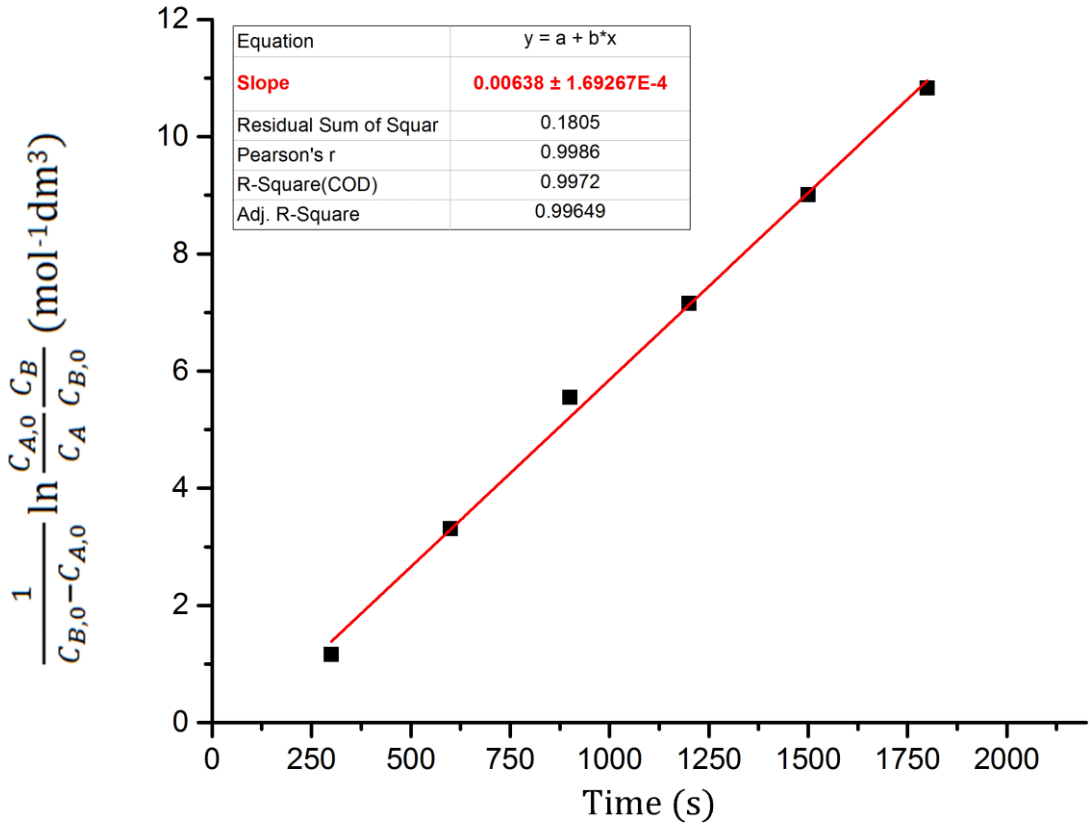


Figure S16. Variation of concentration of I_3Np **22** and CsN_3 as a function of time.

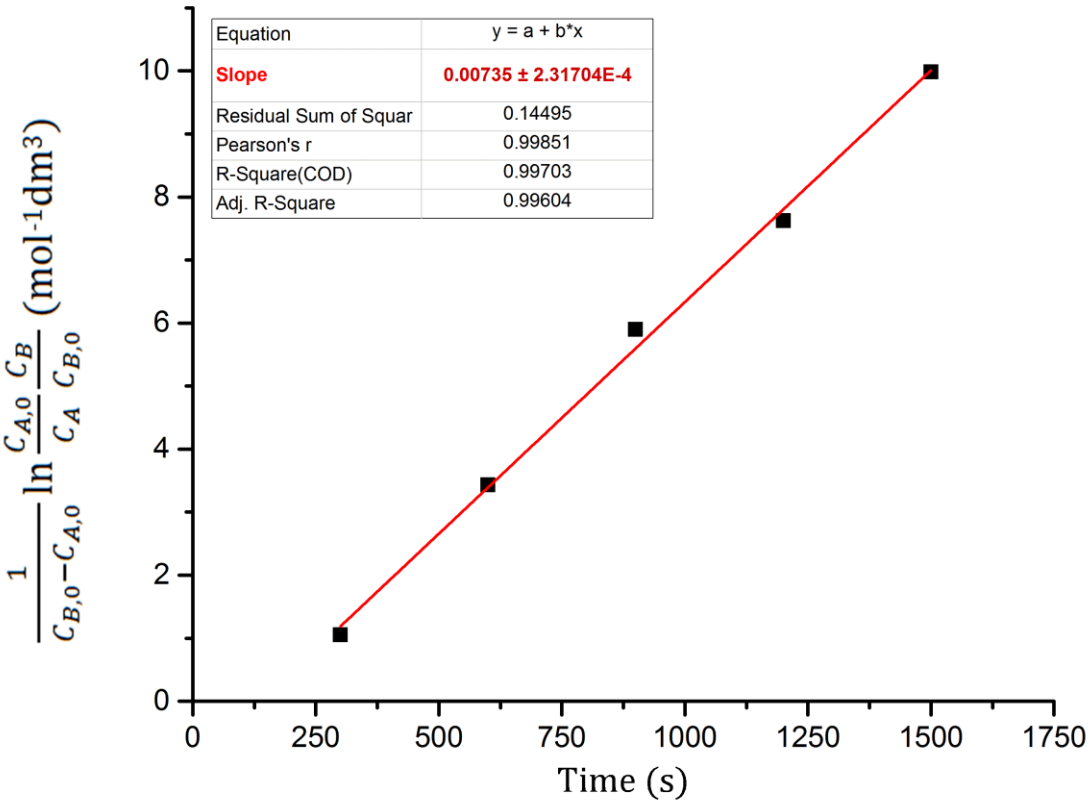


Figure S17. Variation of concentration of I_3Np **22** and $(Me_4N)N_3$ as a function of time

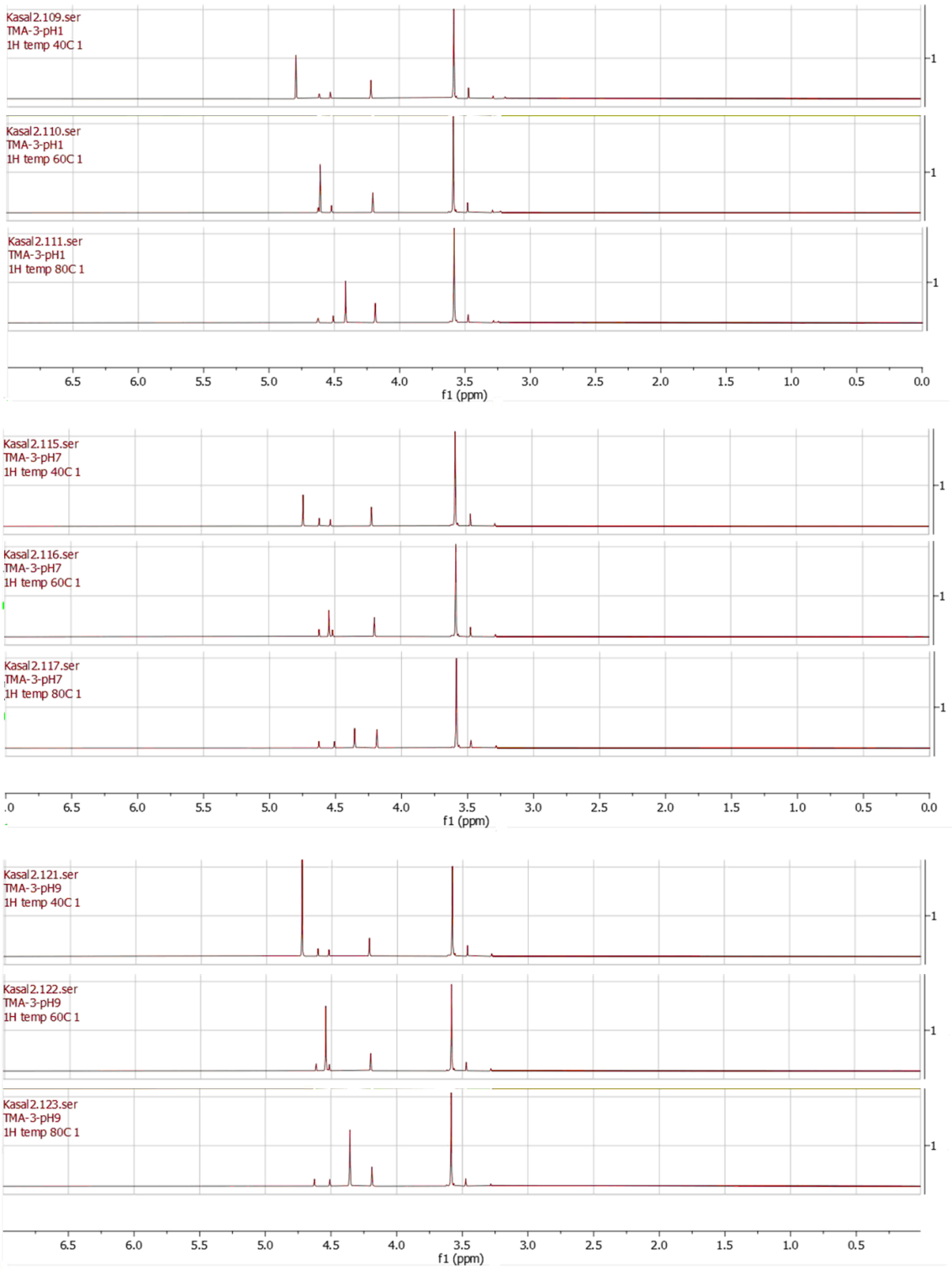


Figure S18. Thermal stability of triply charged trimethylammonium anchor Prg-O-TMA3 **9** under pH 1 and temperature of 40, 60, and 80°C (upper three spectra), pH 7 and temperature of 40, 60, and 80°C (middle three spectra), and pH 9 and temperature of 40, 60, and 80°C (lower three spectra).

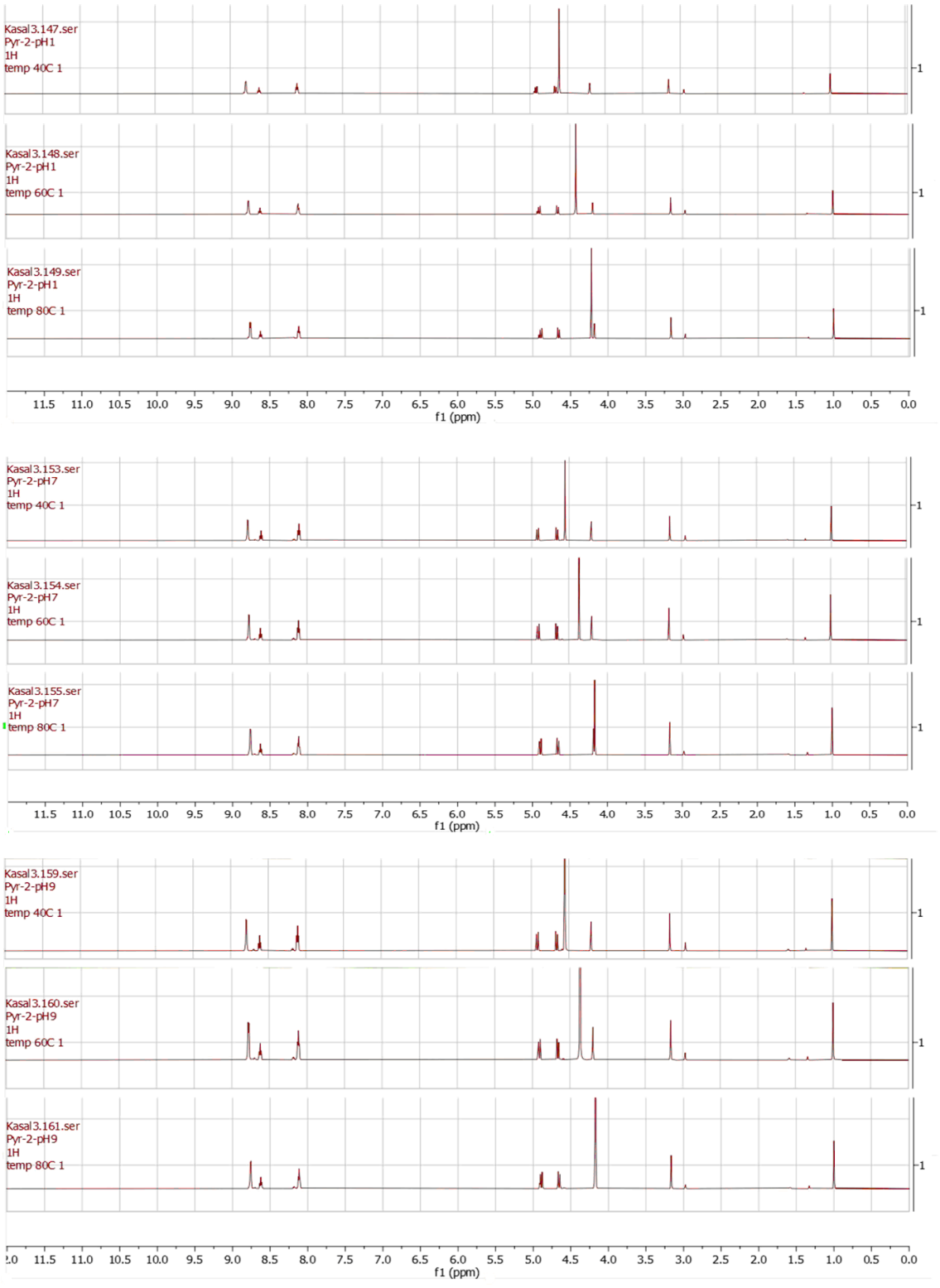


Figure S19. Thermal stability of doubly charged pyridinium anchor Prg-O-PYR2 **5** under pH 1 and temperature of 40, 60, and 80°C (upper three spectra), pH 7 and temperature of 40, 60, and 80°C (middle three spectra), and pH 9 and temperature of 40, 60, and 80°C (lower three spectra).

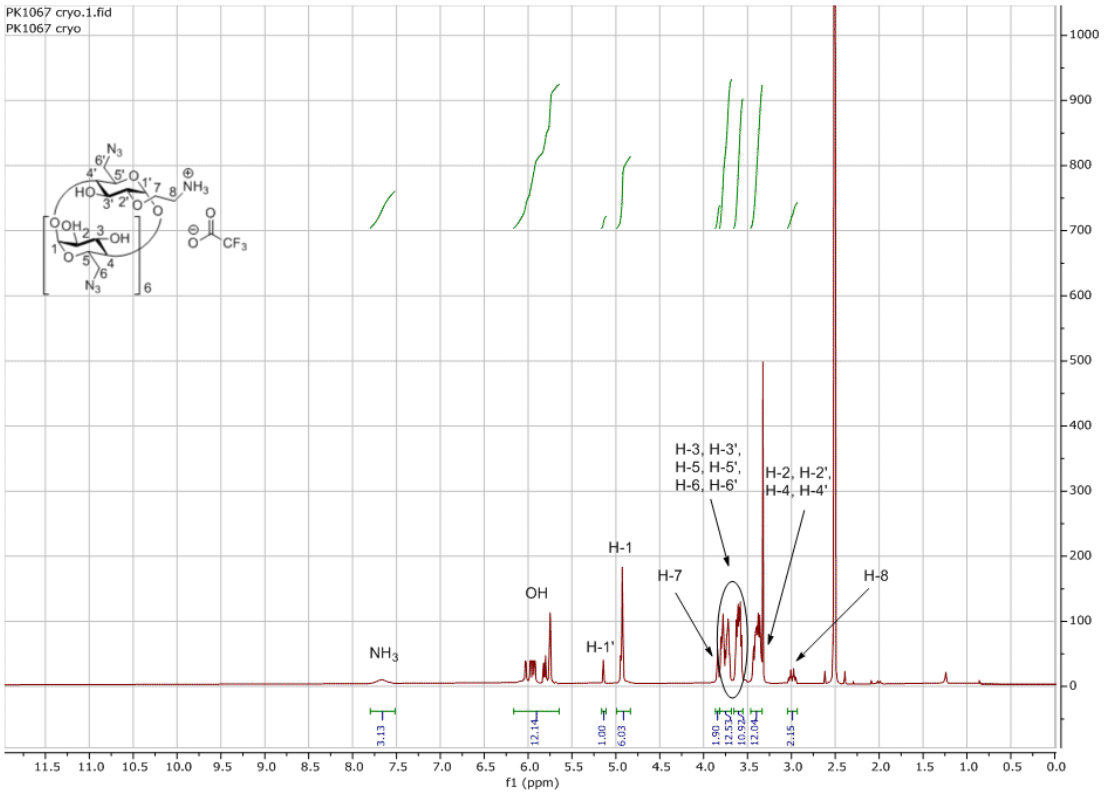


Figure S20. ^{1}H NMR spectrum of mono[2-*O*-(2-aminoethyl)]-per(6-N_3)-β-CD **89**

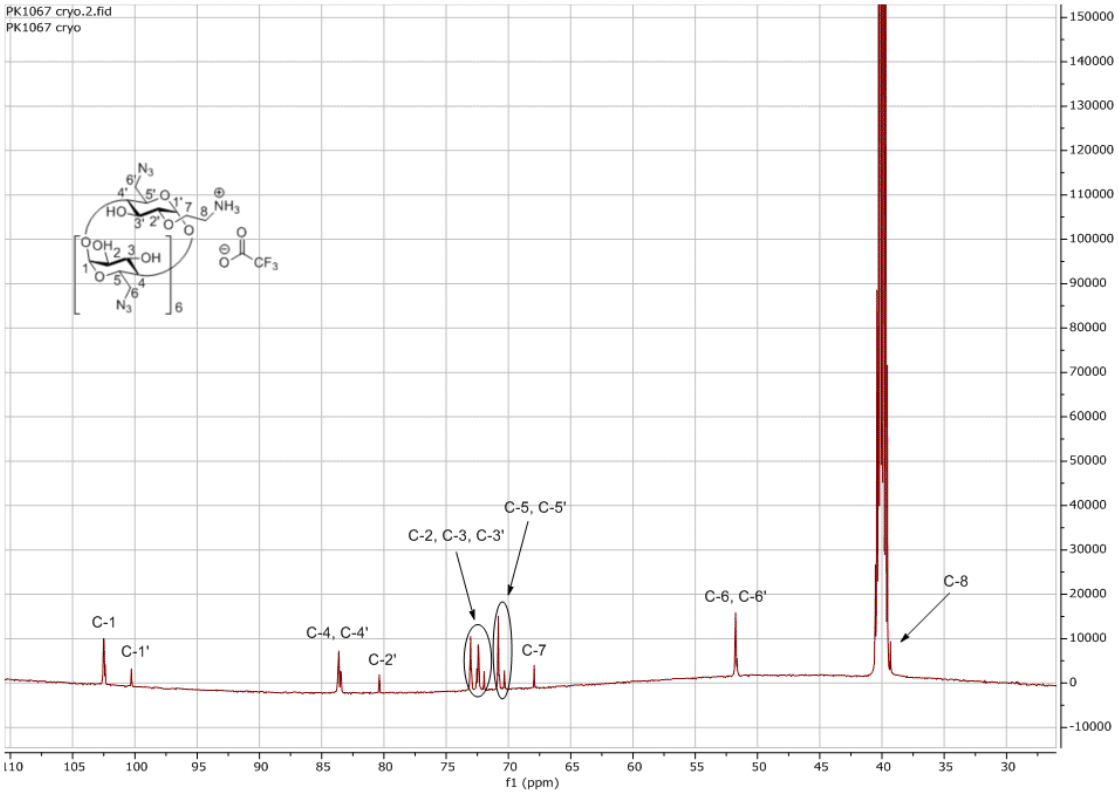


Figure S21. ^{13}C NMR spectrum of mono[2-*O*-(2-aminoethyl)]-per(6-N_3)-β-CD **89**

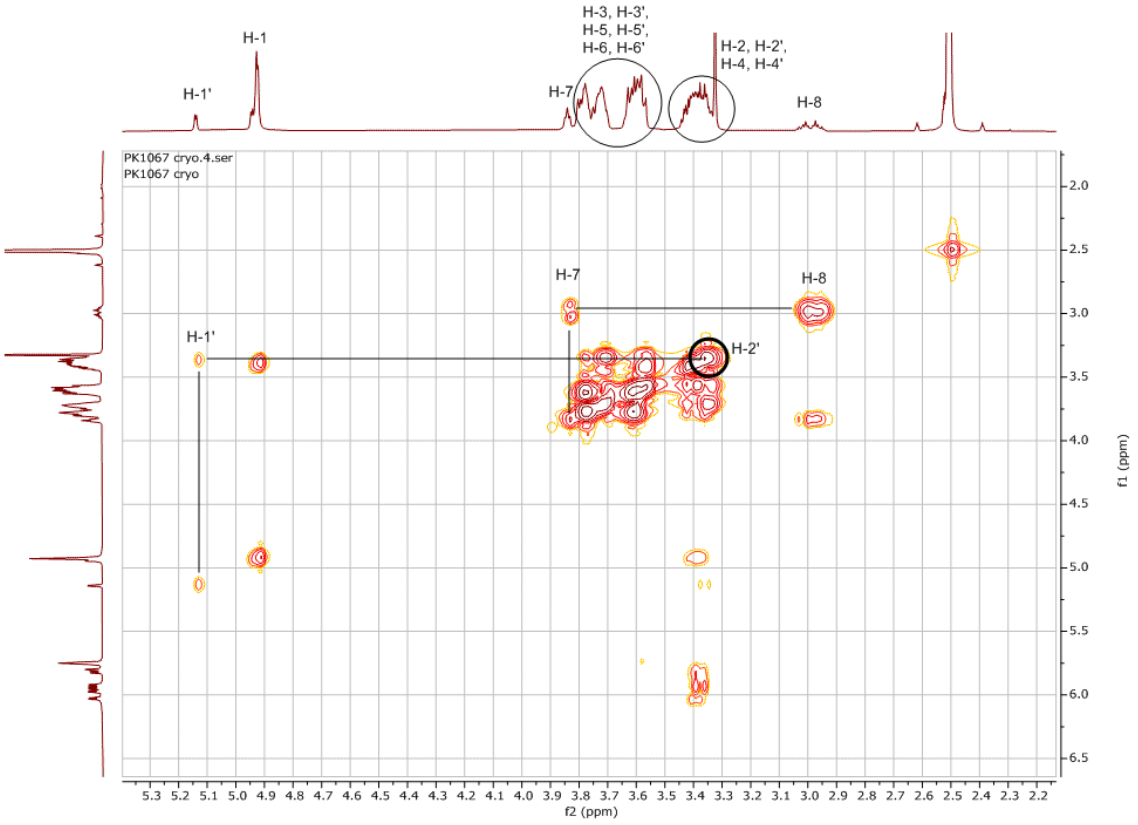


Figure S22. ^{1}H, ^{1}H-COSY NMR spectrum of mono[2-*O*-(2-aminoethyl)]-per(6-N_3)-β-CD **89**

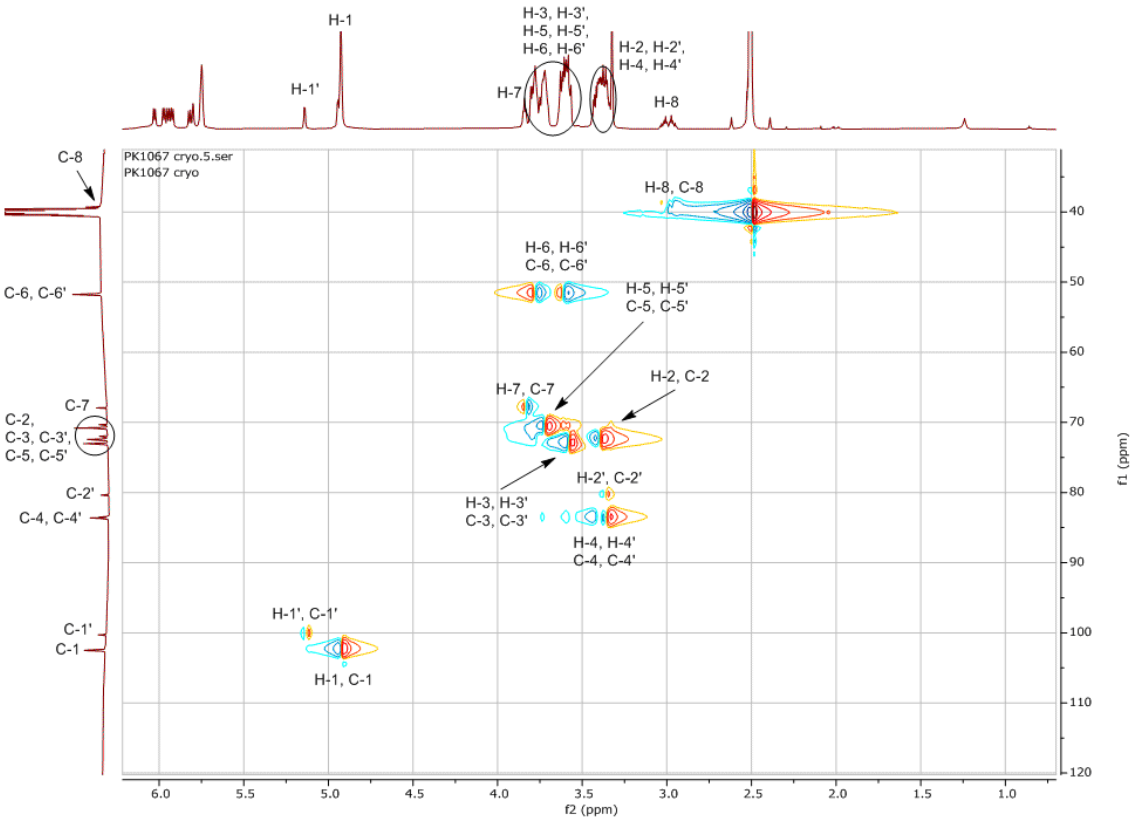


Figure S23. HSQC NMR spectrum of mono[2-*O*-(2-aminoethyl)]-per(6-N_3)-β-CD **89**

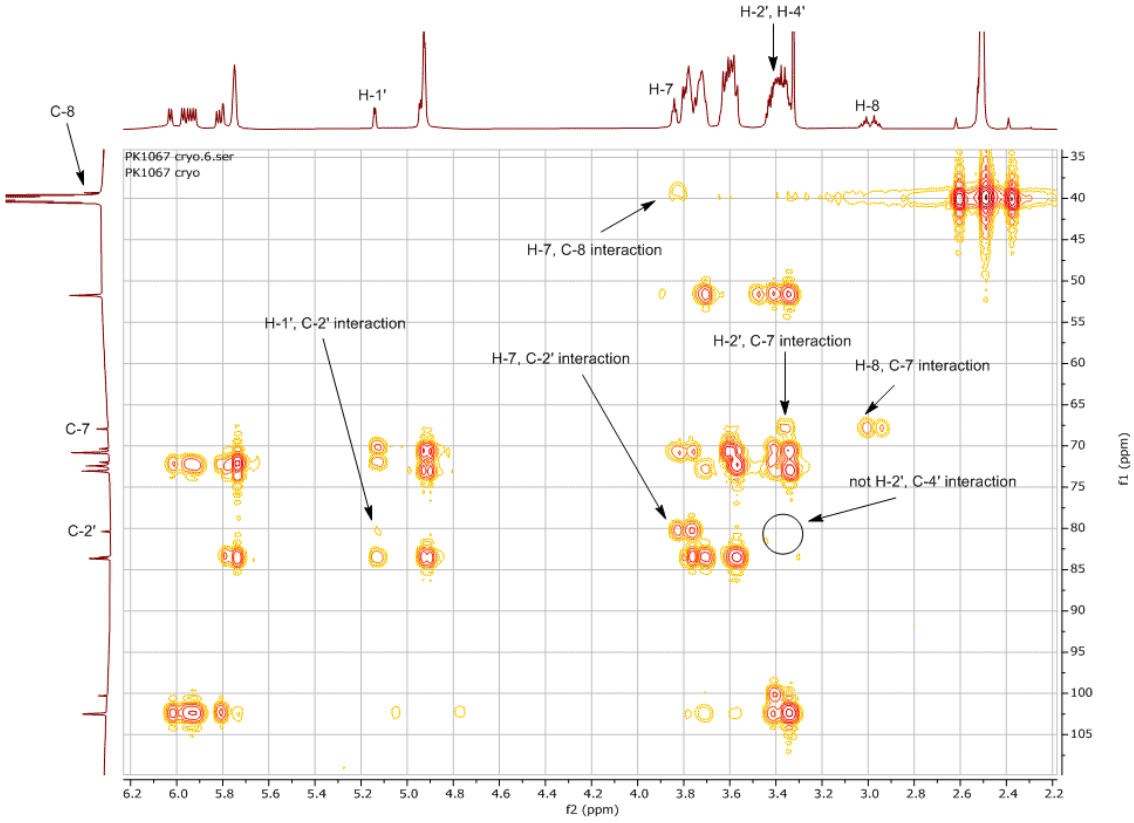
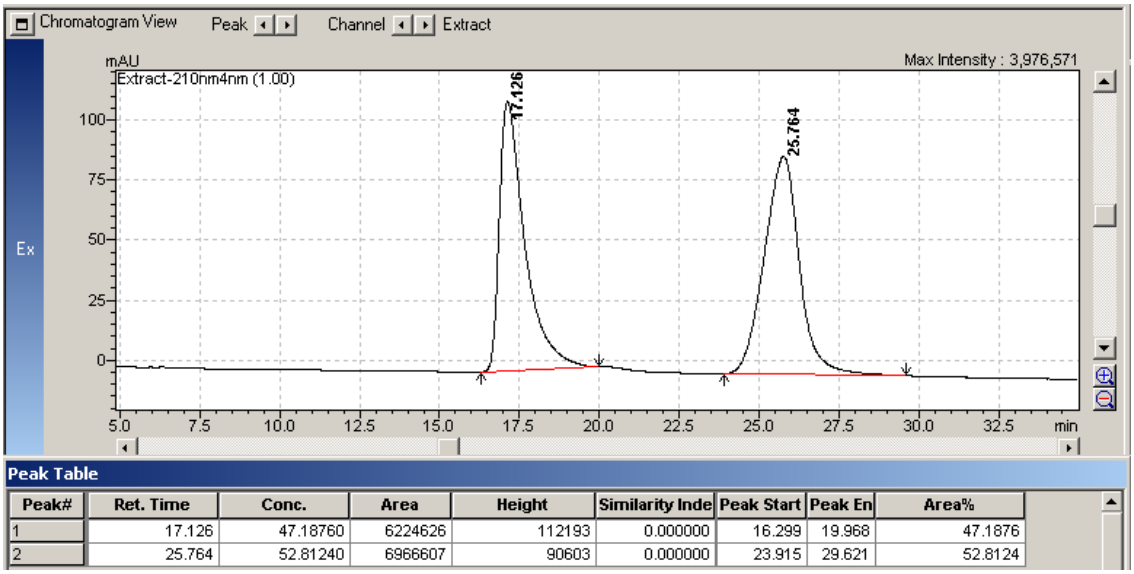
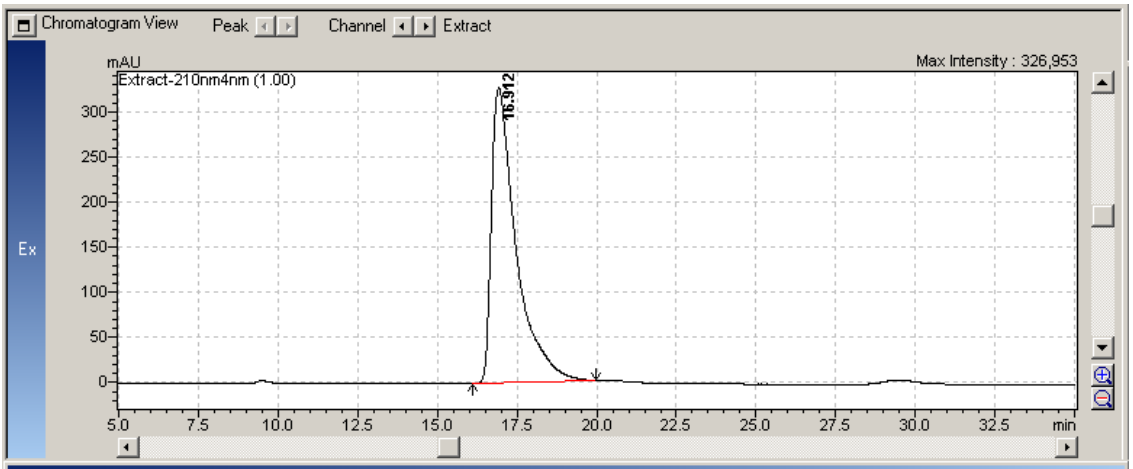


Figure S24. HMBC NMR spectrum of mono[2-*O*-(2-aminoethyl)]-per(6-N_3)-β-CD **89**



Peak Table

Peak#	Ret. Time	Conc.	Area	Height	Similarity Inde	Peak Start	Peak En	Area%
1	17.126	47.18760	6224626	112193	0.000000	16.299	19.968	47.1876
2	25.764	52.81240	6966607	90603	0.000000	23.915	29.621	52.8124



Peak Table

Peak#	Ret. Time	Conc.	Area	Height	Similarity Inde	Peak Start	Peak En	Area%
1	16.912	100.00000	17661623	327380	0.000000	16.085	19.989	100.0000

Figure S25. Optical purity control of PrgPA **118**; Daicel Chiralpak IA HPLC column, heptane/i-PrOH 80/20 mobile phase; flow 1mL/min; temperature 25°C; injection 1 µL; UV (210 nm) detection.

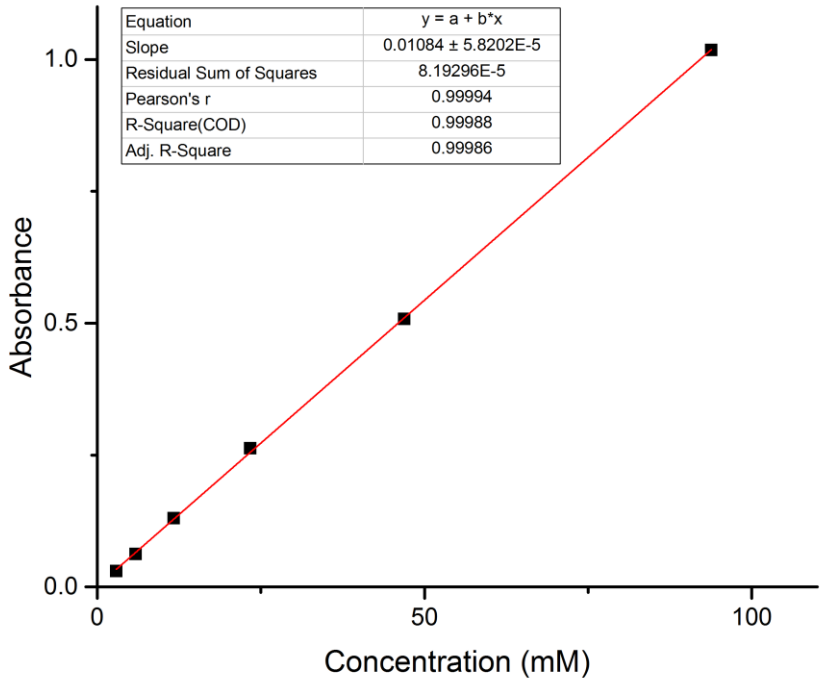


Figure S26. Calibration measurement of NPNI-NH-TEG-MTZ-O-MIM1 **46**.

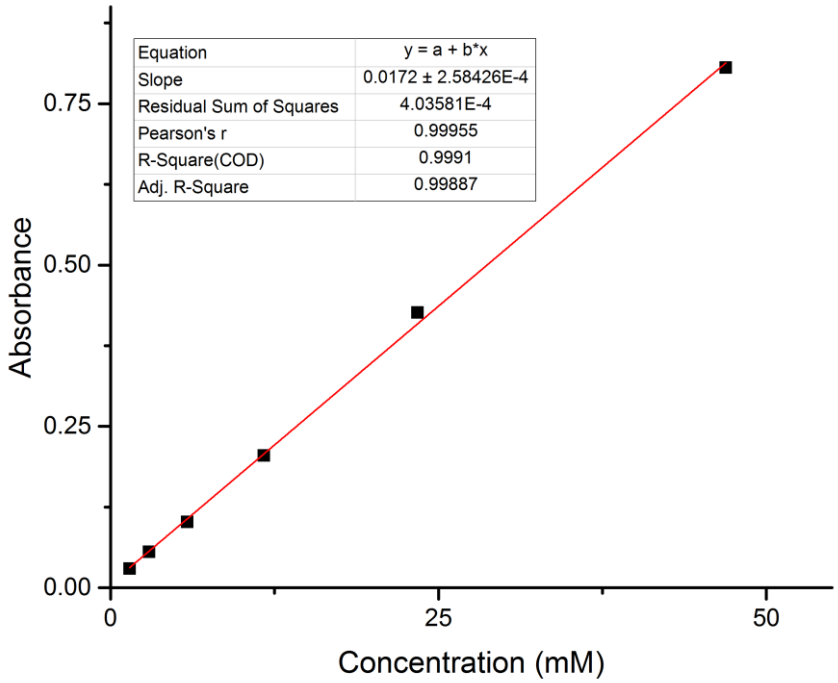


Figure S27. Calibration measurement of NPNI-NH-TEG-MTZ-O-MIM2 **49**.

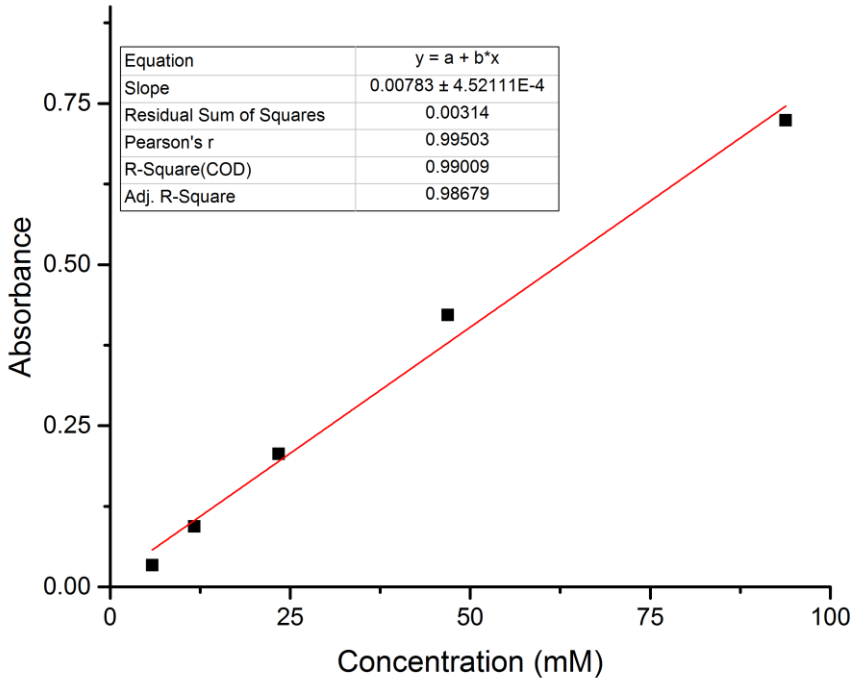


Figure S28. Calibration measurement of NPNI-NH-TEG-MTZ-O-MIM3 **52**.

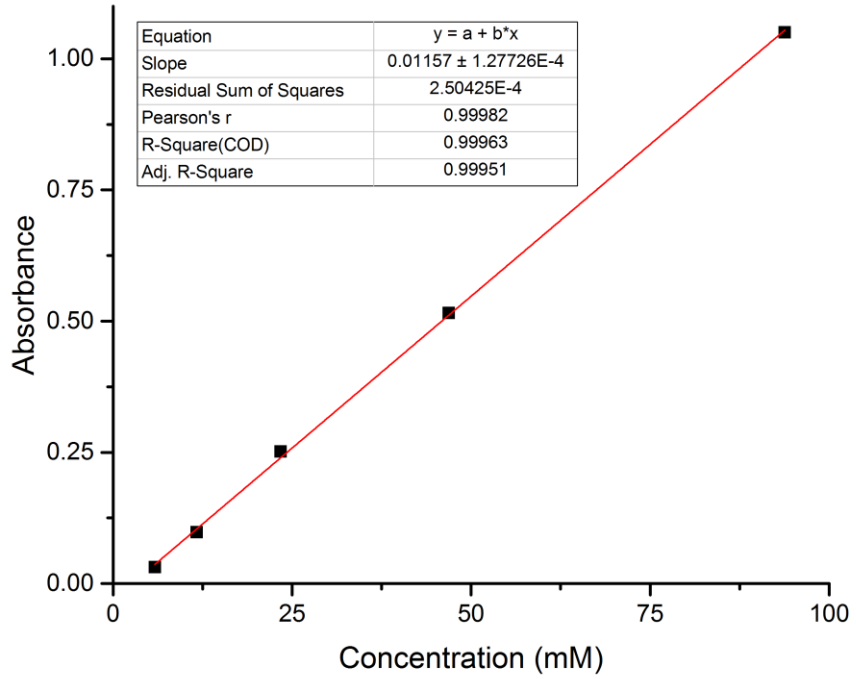


Figure S29. Calibration measurement of mono{6-[TU-HDA-NPNI-(TEG-MTZ-O-MIM2)]}-β-CD **69**.

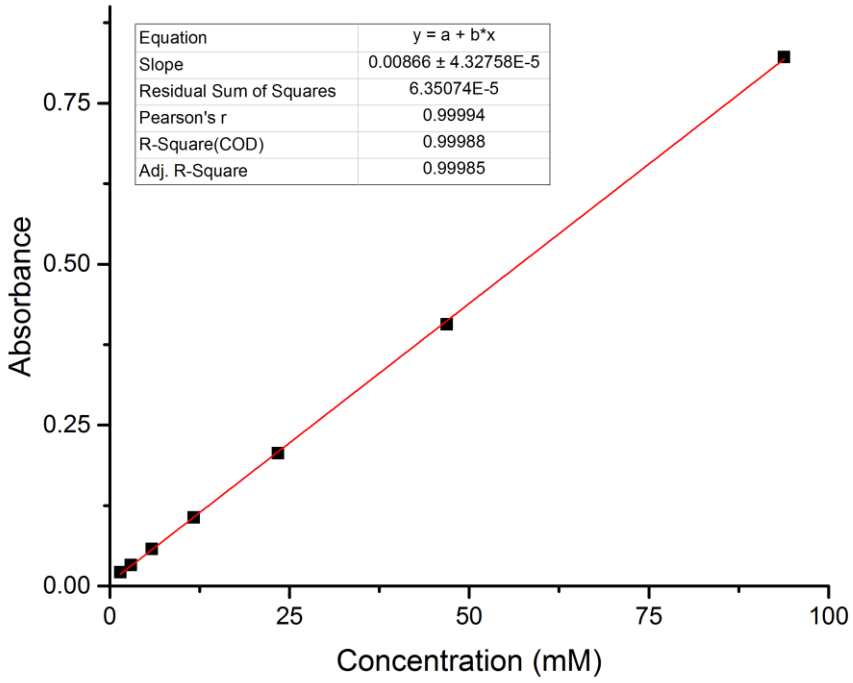


Figure S30. Calibration measurement of mono[2-*O*-(2-(NPNI-HDA-TU)ethyl)]-per[6-(MTZ-O-MIM1)] **91**.

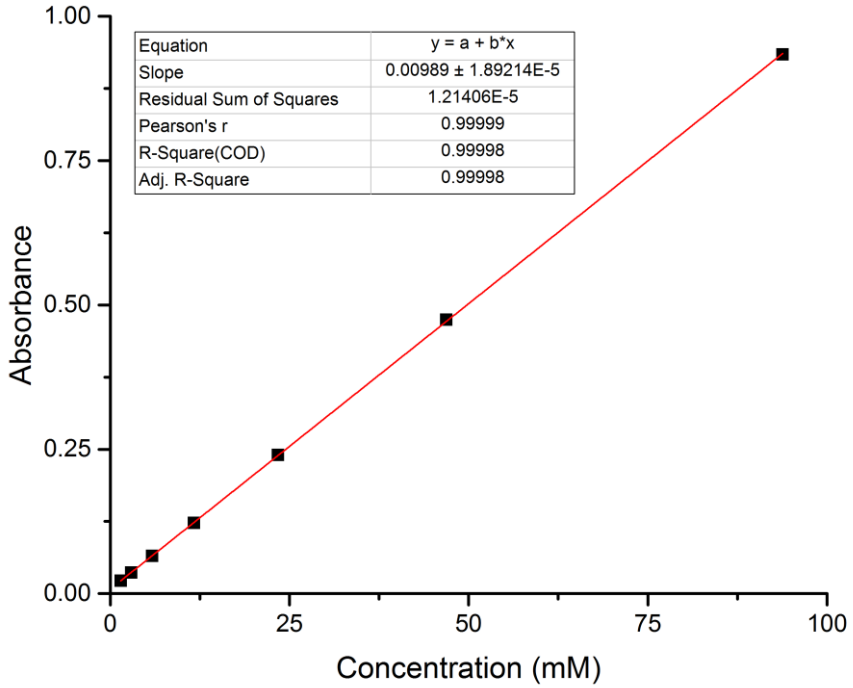


Figure S31. Calibration measurement of mono[2-*O*-(2-(NPNI-HDA-TU)ethyl)]-per[6-(MTZ-O-MIM2)]-β-CD **92**.

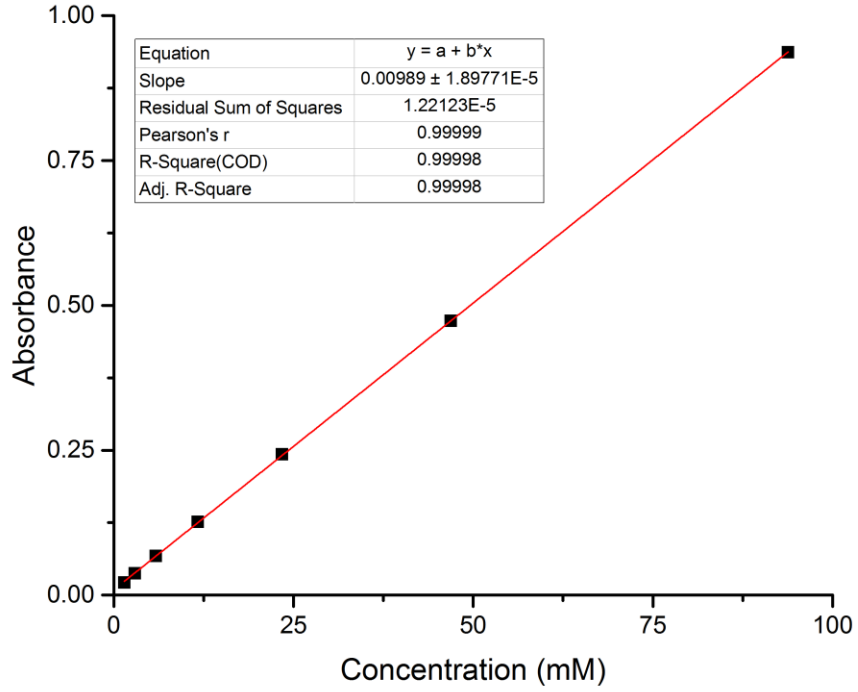


Figure S32. Calibration measurement of mono[2-*O*-(2-(NPNI-HDA-TU)ethyl)]-per[6-(MTZ-O-MIM3)]-β-CD **93**.