CHARLES UNIVERSITY IN PRAGUE

Study programme: Organic chemistry

Mgr. Martin Popr

SYNTHESIS OF CYCLODEXTRIN DERIVATIVES FOR PRACTICAL APPLICATIONS

PhD thesis

Supervisor: doc. RNDr. Jindřich Jindřich, CSc.

Prague 2016

Statement:

I declare, that I worked up this PhD thesis individually, under the supervision of doc. RNDr. Jindřich Jindřich, CSc., and that all the literature sources have been cited properly. I am aware of the fact, that potential use of the results published in this work, outside of the Charles University in Prague, is possible only with the official permission of the University. Neither this work, nor any of its substantial parts have ever been used for acquiring of another academic degree.

In Prague, 4 October 2016.

Synthesis of cyclodextrin derivatives for practical applications

Abstract

The first part of this PhD thesis is focused on the synthesis of a series of monosubstituted tetraalkylammonium cyclodextrin (CD) derivatives. The emphasis was placed on the possible applicability of the synthetic process to multigram or even industrial scale. Monotosylation of the native cyclodextrins (α-, β-, γ-) on the primary side of the macrocycle afforded the starting materials. Derivatives with one cationic group were prepared by the reaction with aqueous trimethylamine. The reaction of the mono-Ts-CD with neat *N,N,N'*-trimethylethane-1,2-diamine or *N,N,N'*-trimethylpropane-1,3-diamine and subsequent methylation led to derivatives with the substituent bearing two cationic groups (PEMEDA- and PEMPDA-β-CD). Analogs bearing a moiety with three tetraalkylammonium sites were synthesized by reaction of mono-Ts-CD with bis(3-aminopropyl)amine with subsequent methylation. 1,3-Dipolar cycloaddition of mono-6-azido-β-CD with diaminoacetylenes followed by methylation led to analogs with a avariable distance of the charged substituent from the CD core. Majority of the presented reactions are straightforward, relatively high-yielding and the workup does not require chromatographic steps.

The second part of the work is dealing with the determination of properties of the selected prepared derivatives. Thermal stability of the two charged analogs PEMEDA-β-CD and PEMPDA-β-CD, which differ in the substituent linker length (ethylene and propylene respectively) was studied by ^{1}H NMR experiment. The measured kinetics of the Hofmann degradation proved PEMPDA-β-CD to have higher thermal stability and was selected as the most suitable host molecule for further measurements. The inclusion capabilities of permanently charged PEMPDA-β-CD in aqueous solution with three model aromatic guests (salicylic acid, *p*-methoxyphenol and *p*-nitroaniline) were determined by isothermal titration microcalorimetry (ITC) and the obtained values of stability constants (K_s) compared to the ones of native β-CD. Permanently charged cationic CD derivatives were successfully deposited on the anionic solid surface of polymeric Nafion® 117 membrane via electrostatic interactions. Deposition kinetics and coverage of the surface were determined by ELSD. The ability of the ionic assembly on the solid surface to encapsulate aromatic compounds from aqueous solution was measured by UV/Vis spectrometry.

The results indicate that prepared cationic derivatives maintain the ability to form inclusion complexes with a suitable guest in solution and also when deposited on an anionic surface.

Keywords: Cyclodextrins, Tetraalkylammonium derivatives, Cationic, Monosubstitution, Regioselectivity, Host-guest complex, Thermal stability, Solid surface, Inclusion properties

Syntéza cyklodextrinových derivátů pro praktické aplikace

Abstrakt

První část této disertační práce je zaměřena na syntézu série monosubstituovaných tetraalkylamoniových derivátů cyklodextrinů (CD). Důraz byl kladen především na možné rozšíření syntetického procesu na přípravu látek v multigramovém, případně i průmyslovém měřítku. Základní výchozí látky byly připraveny monotosylací přírodních cyklodextrinů (α-, β-, γ-) na primárním okraji makrocyklu. Deriváty nesoucí substituent s jednou kationickou skupinou byly připraveny reakcí s vodným roztokem trimethylaminu. Reakce mono-Ts-CD s *N,N,N'*-trimethylethan-1,2-diaminem nebo *N,N,N'*-trimethylpropan-1,3-diaminem následovaná methylací vedla k derivátům se substituentem nesoucím dvě kationické skupiny (PEMEDA- a PEMPDA-β-CD). Analoga se substituentem opatřeným třemi tetraalkylamoniovými funkčními skupinami byly syntetizovány pomocí reakce mono-Ts-CD s bis(3-aminopropyl)aminem následovanou kvarternizací methyljodidem. 1,3-Dipolární cykloadice mono-6-azido-β-CD s diaminoacetyleny, následovaná methylací poskytla deriváty s variabilní vzdáleností permanentně nabitého substituentu od makrocyklu CD. Většina reakcí vykazovala relativně vysoké výtěžky produktů, bez nutnosti použití chromatografických separačních postupů, k jejich přečištění.

Druhá část práce se zabývá určováním vlastností vybraných připravených látek. Nejprve byla studována teplotní stabilita dvou vybraných analogů (PEMEDA-β-CD a PEMPDA-β-CD), které se liší délkou linkeru spojujícího nabité atomy dusíku v substituentu. Měření kinetiky Hofmannovy eliminace ukázalo, že PEMPDA-β-CD, mající propylenový linker, je stabilnější než PEMEDA-β-CD s ethylenovým linkerem, a proto byla vybrána jako vhodná hostitelská molekula pro další experimenty. Komplexační vlastnosti nabitého derivátu PEMPDA-β-CD byly ověřeny ve vodném prostředí se třemi modelovými aromatickými hosty (kyselinou salicylovou, *p*-methoxyfenolem and *p*-nitroanilinem). Pomocí izotermální titrační mikrokalorimetrie (ITC) byly získány hodnoty vazebných konstant (K_s) a byly porovnány s hodnotami naměřenými pro nesubstituovaný β-CD. Trvale nabité kationické deriváty CD byly úspěšně ukotveny na pevnou fázi anionického polymeru (Nafion® 117) pomocí elektrostatických interakcí. Kinetika depozice na povrch a stupeň pokrytí byly určeny pomocí

ELSD. Schopnost iontového seskupení na pevném povrchu enkapsulovat aromatické hosty z vodného roztoku byla zkoumána pomocí UV/Vis spektrometrie.

Získané výsledky naznačují, že připravené kationické deriváty CD si zachovávají schopnost komplexovat vhodné aromatické molekuly v roztoku i ukotvené na pevnou fázi.

Klíčová slova: Cyklodextriny, Tetraalkylamoniové deriváty, Kationické látky, Monosubstituce, Regioselektivita, Host-guest komplexace, Termální stabilita, Pevný povrch, Inkluzní komplexy

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

CD	cyclodextrin
COSY	correlated spectroscopy
CuAAC	Cu(I)-catalyzed azide-alkyne cycloaddition
DBU	1,8-diazabicycloundec-7-ene
DEPT	distortionless enhancement by polarization transfer
DLS	dynamic light scattering
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
ELSD	evaporative light scattering detector
Eq	equivalent
FTIR	Fourier transform infrared spectroscopy
GC	gas chromatography
HMBC	heteronuclear multiple bond coherence
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
ITC	isothermal titration microcalorimetry
NMR	nuclear magnetic resonance
MALDI	matrix-assisted laser desorption ionization
Me	methyl

MS	mass spectrometry
NOESY	nuclear Overhauser effect spectroscopy
PEMEDA	*N*,*N*,*N'*,*N'*,*N'*-pentamethylethane-1,2-diammonium
PEMPDA	*N*,*N*,*N'*,*N'*,*N'*-pentamethylpropane-1,3-diammonium
ROESY	rotating frame nuclear Overhauser effect spectroscopy
rt	room temperature
SAM	self-assembled monolayer
SPE	solid phase extraction
TEA	triethylamine
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
Ts	*p*-toleuenesulfonyl, tosyl

1. INTRODUCTION

Naturally occurring and chemically modified cyclodextrins (CDs) are a very interesting group of organic compounds, which were first reported by Williers in 1891[1]. They have a molecular structure of cyclic oligosaccharides and are composed of α-(1→4)-linked D-glucopyranose units forming a macrocycle. In three-dimensional space, they occupy the shape of a hollow truncated cone, which has a hydrophilic surface, while the cavity is relatively lipophilic. These features indicate the main area of cyclodextrin utilization, which is based on their ability to form noncovalent inclusion complexes with a large variety of organic and inorganic guests.

Historically, there have been three main stages in the development of CD technology[2,3]. The first discovery period (1891 - 1930s) included mostly studies of converting starch to mixtures of cyclic oligosaccharides using different bacterial strains, like *Bacillus macerans*[4] and their separation. The second period (1930s - 1970s) covered systematic research on CDs and their inclusion complexes. In 1936 the cyclic structure of CDs was first proposed along with the introduction of procedures for obtaining pure fractions[5]. The last period (1970s – present) can be marked as a time of industrial production and utilization of CDs. Many patents and CD-related publications have been released through past few decades and a mass production and utilization has been enabled.

Recently the annual production of cyclodextrins is exceeding 10,000 tons a year, and the average price is 5 USD per kilogram[6]. Today we can meet with CDs in our everyday life, because they are often used for drug formulations[7], as food additives[8], in cosmetics industry[9] or separation science[10] and many other areas[11].

To expand and support further options of utilization of CDs, new methods for preparation of their derivatives and novel methods for modern application need to be introduced. Routes for the synthesis of new chemically modified CDs, which show specific properties, need to be explored. Such derivatives may be then employed in the construction of "smart" materials (gels[12], polymers[13], assemblies[14]), that can be designed for various practical purposes, such as transdermal delivery of drugs[15,16].

2. OBJECTIVES

The main goal of this thesis was to propose and execute the synthesis of a series of novel cyclodextrin derivatives monosubstituted with substituent bearing one or more permanently charged tetraalkylammonium functional groups. In the next phase, the prepared derivatives were to be deposited on an anionic solid surface via simple ionic interactions. Such assembly was to be designed to encapsulate proper therapeutic agents and finally serve as a potential drug carrier for transdermal drug delivery.

The synthetic process can be summarized into following steps:

- Monotosylation of α-, β- and γ- CDs in position 6
- Substitution of the p-toluenesulfonate group with an amine nucleophile
- Quaternization by methyl iodide (MeI)
- Preparation of analogs bearing azidoethane function
- Preparation of analogs with variable length of the linker, which connects the charged substituent to the CD core via “click” chemistry

The determination of the properties of prepared compounds can be summarized into following steps:

- NMR measurement of thermal degradation kinetics of derivative with two charges separated by ethylene linker vs. derivative with propylene linker
- Determination of inclusion properties of cationic CD derivatives in solution with model guest compounds
- Immobilization of cationic derivatives on solid anionic surface
- Inclusion of model guest molecules from the solution in the cavities of cationic CD derivatives anchored on a solid surface

3. THEORETICAL OVERVIEW

3.1 Cyclodextrins

Nowadays, native cyclodextrins are produced by enzymatic degradation of starch or starch derivatives, which are widely available and inexpensive starting material[17]. The enzyme involved is called cyclodextrin glycosyltransferase (CGTase, EC 2.4.1.19). The product of the conversion is a mixture of compounds, in which α-, β- and γ- CDs are the most abundant substances. Due to its relatively low aqueous solubility, the β-CD is the easiest to separate from the mixture of cyclic oligosaccharides, and it is the reason for its low price and highest utilization and marketing potential[18].

Cyclodextrins can be described as all-purpose molecular containers, which are capable of accommodating in their cavity organic, inorganic, organometallic compounds, that may be neutral, cationic, anionic or even radical[19]. They are classified as supramolecular host molecules, similarly to calixarenes, cucurbiturils, porphyrins, crown ethers, cryptophanes etc. Among the listed groups of compounds, CDs possess some unique properties which open the door to a very broad area of utilization. On the first place, there is definitely the presence of two types of multiple hydroxyl groups, due to which the possibility of chemical modification is almost unlimited. Other positive aspects are low toxicity, good availability, and easy biodegradability. Owing to these features CDs gained a great attention in the chemical research society over the years. As a proof, we can mention the existence of a European and international conferences on cyclodextrins which are organized annually in alternating matter and attract wide audiences from various branches of industry and academia.

3.1.1 Properties and structure

Cyclodextrins are cyclic oligosaccharides composed of connected α-D-glucopyranose units forming a macrocycle. Mutually interconnected glucose units occupy $^4C_1$ conformation and are linked via α-(1→4) glycosidic bonds. For this reason, they can be classified as non-reducing sugars sometimes also referred to as cyclomaltoses, cycloamyloses,

cycloglucopyranoses or Schardinger dextrins[11]. The most common naturally occurring CDs are named α-, β- and γ- which are formed by 6, 7 and 8 glucose units respectively (Figure 1).

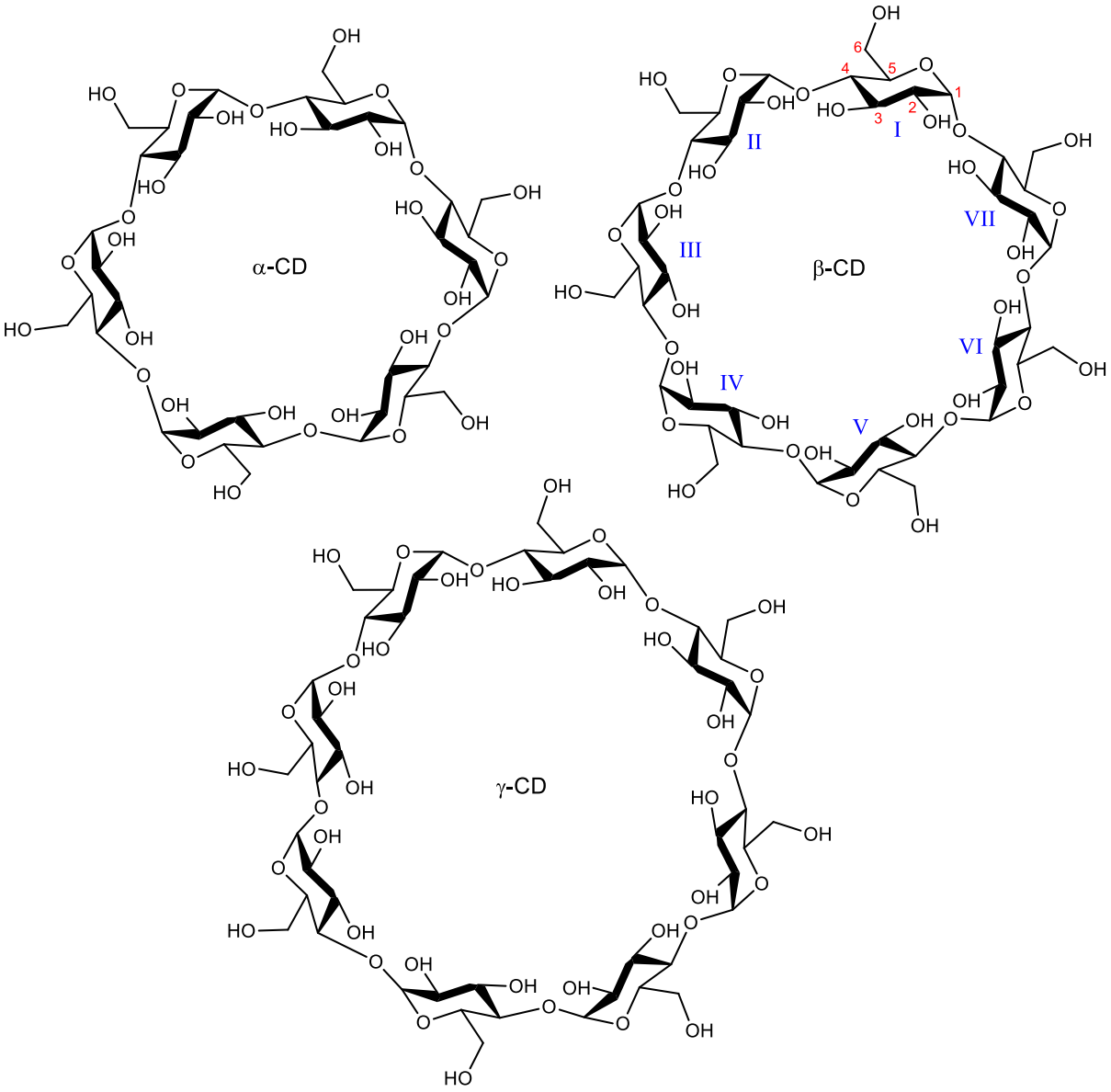


Figure 1. The molecular structures of α-, β- and γ-cyclodextrins with example of glucose atom numbering (red Arabic numbers) and glucose unit numbering (blue Greek numbers).

Other CDs with a lower number of glucose units (pre-α-CD with 5 monomeric units)[20] as well as higher homologs with 9, 10, 11, 12 and D-glucopyranose units, which are referred to as δ-, ε-, ξ-, η-, and θ-CDs respectively, were reported[21]. Although cyclic α-1,4-glucans with degree of polymerization up to 60 are known[22], these compounds are produced in the early stage of CGTase action, but are rather rare, and the potential of their utilization is low,

especially due to their poor accessibility in connection with difficult preparation and purification. A large number of synthetic analogs of cyclic oligosaccharides which were prepared by methods of total synthesis (usually involving the strategy of sequential coupling of protected monomeric carbohydrate units) with various structural properties has been reported[21]. Various cycloglycosylation approaches lead to the creation of products with all possible glycosidic bond linkages (1→4, 1→3, 1→2 and 1→6) which give rise to the cyclic oligosaccharides formed by linked oligopyranosides[21].Preparation of such analogs most often requires multiple synthesis steps with relatively low yields. It is the reason for their limited utilization possibilities when compared to natural CDs, which can be quite easily obtained by enzymatic processes from widely available starch.

Cyclodextrins have a shape of a hollow truncated cone (conical cylinder) with the inner cavity being relatively lipophilic (presence of C-H bonds and glycosidic oxygen bridges) while the surface of the molecule is mostly hydrophilic (presence of the hydroxyl groups). Secondary hydroxyls on carbons C2 and C3 are located on the wider end of the molecule which is also called the secondary face, while the primary hydroxyls on carbons C6 are located on the narrower side of the macrocycle, called the primary face (Figure 2). These unique structural properties are the core requirements for the CD molecule to act as a molecular container. Due to the outer hydrophilic character, the CDs show relatively good solubility in polar solvents as water, DMF, DMSO, etc. On the secondary face, the belts of hydrogen bonds between neighboring C2 and C3 are formed. This effect was observed to be the strongest in the case of β-CD where a complete belt of hydrogen bonds is formed, which is the possible reason for the β-CD to exhibit the lowest aqueous solubility of the three natural CDs. On the other side, the belt of hydrogen bonds in hydrogen α-CD is incomplete, and the compound shows higher solubility in H_2O. The largest analog γ-CD is not as rigid and its glucose units are not coplanar. The flexibility of the macrocycle ring causes the highest aqueous solubility of the three CDs.

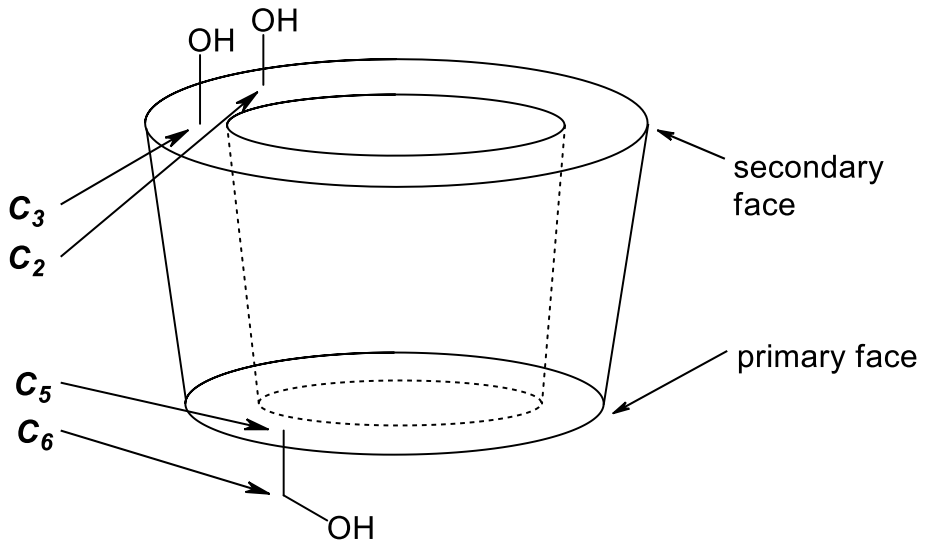


Figure 2. Schematic representation of CD with distribution of the hydroxyl groups.

Unmodified CDs show relatively high stability under common experimental conditions. They are stable up to pH values of 12.1, at higher pH their hydroxyls start to deprotonate. Also, their resistance to acid hydrolysis in acidic media is advantageous. CDs typically start to hydrolyze at pH lower than 3 with a concurrent effect of elevated temperature.[23] Table 1 summarizes other selected physical and chemical properties of the native CDs.

Table 1. Selected properties of native cyclodextrins.[2]

	α-CD	β-CD	γ-CD
number of glucose units	6	7	8
molecular weight (g mol^{-1})	973	1135	1297
cavity diameter (Å)	4.7-5.3	6.0-6.5	7.5-8.3
height of torus (Å)	7.9±0.1	7.9±0.1	7.9±0.1
outer diameter (Å)	14.6±0.4	15.4±0.4	17.5±0.4
cavity volume ($Å^3$)	174	262	427
cavity volume (ml/1 g)	0.10	0.14	0.20
$[\alpha]^{25}_D$	150 ± 0.5	162,5 ± 0.5	177,4 ± 0.5
crystal water, wt %	10.2	13.2-14.5	8.3-17.7
aqueous solubility (mg/ml) at 25 °C	145	18.5	232
melting point (°C)	255-260	255-265	240-245
number of water molecules in cavity	6	11	17
pK_a at 25 °C (by potentiometry)	12.33	12.20	12.08

Concerning the toxicity of native CDs, they can be claimed as practically non-toxic compounds, especially due to the lack of absorption from the gastrointestinal tract. Hydrophilic CDs permeate the biological membranes with difficulty and even somewhat more lipophilic randomly methylated CD does not readily permeate through a biological barrier.[24,25] Number of safety studies have shown that γ-CD, 2-hydroxypropyl-β-CD, sulfobutylether-β-CD and sulfated β-CD are safe even when introduced parenterally.[11] Administered CDs are almost exclusively excreted by kidneys without any significant metabolization.[26] The LD50 value of acute CD toxicity in mice after per oral admission is more than 12.5 g/kg, which can be considered as practically non-toxic.[27]

3.1.2 Formation of inclusion complexes

The most useful feature of CDs is their ability to form inclusion complexes with a very wide range of solid, liquid or gaseous compounds. Supramolecular complexes of this type are

often referred to as host-guest complexes. Complex formation is a dimensional fit between the species of host (CD) and guest molecules. The CD cavity provides a microenvironment for the guest, which needs to be relatively non-polar, have an appropriate size and molecular shape to form a stable complex.[28] It can be generalized, that α-CD forms the most stable complexes with molecules having aliphatic chain (e.g. decanol), β-CD with compounds containing an aromatic functional group (e.g. toluene, naphthalenesufonate or adamantane), and γ-CD includes preferentially larger molecules (e.g. pyrene, fullerene). The strength of resulting interaction is dependent mostly on the sterical match between host and guest. Such a phenomenon can also be named as molecular recognition.[29] Noncovalent bonds are broken or formed during the complexation process.[30]

In the aqueous solutions, the cavity of CD is occupied by high energy water molecules, which have high enthalpy and their presence in the cavity is energetically disfavored. Because of the cavity environment restriction, the H_2O molecules cannot form hydrogen bonds in a fashion which is standard in normal liquid. The main driving force of the inclusion is the exchange of the high energy water molecules for the guest molecule (Figure 3).[31] Water molecules are displaced from the cavity by hydrophobic guest, which is present in the solution to give rise to the apolar-apolar interaction and lowering the CD ring strain resulting in a more stable lower energy state.[2]

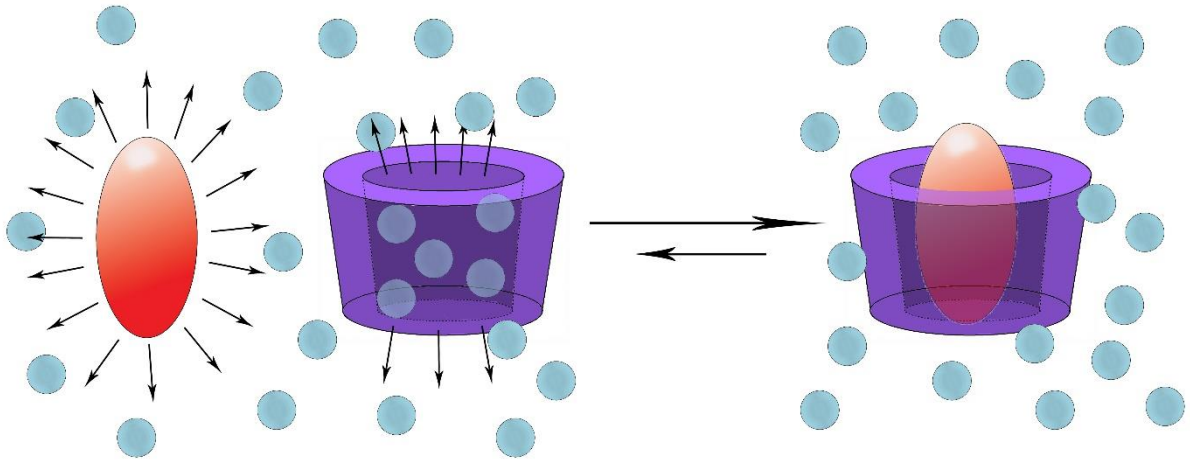


Figure 3. Schematic representation of the complex formation process.

The inclusion of a guest into the cyclodextrin host is not static, but rather a dynamic equilibrium process. The non-covalent bonds between host and guest are continually being formed and disarranged. The other driving forces, which are employed during the

complexation process, are hydrophobic interactions, hydrogen bonding, van der Waal's forces, electrostatic interactions, changes in solvent-surface tensions, and the release of a ring strain in CD molecule.[11,32]

It is not surprising that the most commonly claimed stoichiometric ratio for CD complexes is 1:1, although other ratios have also been reported - inclusion complexes with host-guest ratios of 2:1 (α-CD with methyl orange)[33], 1:2 (α-CD with long chain surfactants)[34]. Three-component complexes of CD are also known. Ternary complexes with the ratio of 1:1:1, composed of CD host and two different guest molecules have been proved.[35,36] Simple alcohols are often employed in the encapsulation process as the third component, having a "space-regulating" function by optimizing the fit of the guest in CD cavity.

Most often, the complexes are formed as a result of bimolecular process, so following thermodynamic equilibrium can be assumed:

$$[CD]+[guest] \rightleftharpoons [CD\ldots guest]$$

The formed inclusion complexes can be isolated as crystalline species. When dissolved in water, an equilibrium between dissociated and associated species is established. Its value can be expressed by the stability constant K_s, (also referred to as association, binding or complexation constant), which is defined by equation (1).

$$K_s = \frac{[CD...guest]}{[CD][guest]} \quad (1)$$

The important task in the stability constant determination is a concentration scale setting. The usual practice is to measure the concentrations in molar units, which affords the K_s in M^{-1} units. As a result of the complex formation, the physicochemical properties of the two participating species are significantly changed. The increase of the guest concentration in solution (solubilization) together with the host concentration decrease are among the most apparent and also analytically important expressions. Spectral characteristics of the host and guest are also altered. NMR shifts of the anisotropically shielded protons are modified as well as the maximal absorbance and position of the maxima in the UV/Vis spectra. Fluorescence of the guest is often strongly enhanced upon inclusion because of the move of the molecule from the aqueous surrounding into the apolar cavity. Additive properties that can be titrated are

used for K_s determination include:[37] aqueous solubility,[38,39] chemical reactivity,[40] molar absorptivity and other optical properties,[41,42] phase solubility measurements,[43] NMR shifts,[44] calorimetric titrations,[45] pH-metric methods or LC chromatographic retention times.[46]

3.2 Methods for determination of stability constants of CD complexes

In this section, a mini-review of most commonly used methods for describing properties of the inclusion complexes, especially determination of stoichiometry and values of stability constants by various physicochemical methods will be given. The aim is to provide an overview of possible approaches with the advantages and restrictions characteristic for each of them. Particular details, such as specific mathematical equations associated with each method, will be omitted, because it exceeds the scope of this thesis and can be easily looked up in the cited literature if needed. In the section "Results and discussion", the methods which we have used for the determination of binding properties will be described more in detail.

3.2.1 Solid state complexes

Solid state complexes of CDs and guest compounds can be characterized by numerous methods. Among the simplest ones is thermal analysis. Thermoanalytical techniques are routinely used for investigation the CD complexes. Thermogravimetry (TG) coupled with other supplementary methods as TG-FTIR, TG-mass spectrometry (TG-MS) or TG-Powder X-ray diffractometry (TG-PXRD) are recently gaining attention.[47] These instrumental methods allow determination of mass and heat flow changes along with structural characterization of the sample or its decomposition products. The principle of the thermoanalytical methods in the determination of the CD complex properties is monitoring of the undergoing changes in the properties of the guest substance, before the decomposition of the CD occurs. Typical setup requires the comparison of analysis of the physical mixture of the two components and the solid inclusion complex. The presence or absence of endothermic peaks in one of the thermograms is considered as an evidence of the complex formation. The key role in the final affinity between the host and guest plays the complex preparation process. Physical methods as kneading, co-precipitation, neutralization, co-grinding, or spray-drying are known to afford complexes with varying values of stability constants.[48]

The properties of solid CD complexes can also be investigated by **microscopic techniques**. Recent technological advancements in the field of electron microscopy allow the

usage of instruments with very high zooming capabilities and resolution, thus objects with size less than 1 nm up to several micrometers can be visualized. Modern microscopy tools as Scanning tunneling microscopy (STM), Atomic force microscopy (AFM), Scanning electron microscopy (SEM), Transmission electron microscopy (TEM) have been employed in the characterization of native and modified CD, CD aggregates and CD rotaxanes or polyrotaxanes.[49] The presence of an inclusion complex between CD and guest is indicated by monitoring of the crystallization state of individual components, physical mixture and the inclusion complex prepared by co-precipitation or evaporation. SEM image analysis was performed to investigate different morphologies of ketoprofen, β-CD, hydroxypropyl-β-CD, their physical mixtures, and aggregates obtained by co-evaporation.[50] The differences in the shapes and sizes of the crystals provide solid evidence of the formation of solid complex between ketoprofen and hydroxypropyl-β-CD.

The formation of a solid inclusion complex can be indicated by submitting the samples to the **X-ray powder diffraction or single crystal X-ray analysis**.[51,52] The complex identification is based on the comparison of the diffraction pattern of the free guest molecule and the inclusion complex. When the guest compound is a solid substance, diffractograms of the assumed complex and the mechanical mixture of the guest and CD has to be made.[53] The prerequisite for comparing the diffractograms is using the identical conditions for treating both CD and guest molecule, because the preparation process (such as freeze-drying, co-precipitation, or grinding) is known to affect the resulting diffraction patterns substantially. The diffraction pattern of the physical mixture most often appears as the sum of those of individual components, while the complex formation leads to the appearance of new peaks, peak sharpening or shifting. The X-ray analysis of the solid inclusion complex in the form of single crystal affords detailed description of the structure, mutual geometrical properties and positioning information.

Fourier transform infrared spectroscopy (FTIR) can be used for a detection of solid inclusion complexes of CD and a proper guest, but its importance is lower compared to the methods mentioned above. The use of the FTIR is limited to the guests having at least one or more functional groups, which strongly absorb in the infrared region. The complex formation can lead to the shifts or splitting of the absorption bands. Valuable information is gained when observation of the bands of the hydrogen-containing functional groups participating in the hydrogen bonding is possible. It has been observed, that the cleavage of

the hydrogen bonding due to the complex formation leads to the band shift toward the higher frequencies. Bilensoy et al. have confirmed the complexation of 5-fluorouracyl gel formulation containing either β-CD or hydroxypropyl-β-CD by FTIR by comparing the spectra of separate components and the final complex-containing drug formulation.[54]

Another methods, such as wettability studies[55] can be used for determination of inclusion properties of solid complexes. In most of the cases, a combination of two or more methods mentioned above is used for unambiguous evidence and description of the solid CD complexes. The calculation of exact values of K_s is possible only in the liquid phase. Concerning the solid state complexes, we have to be satisfied even with the information about presence or absence of the complex and at the most with estimating the order of magnitude of the K_s. On the other side, the investigation of solid complexes, which are often the actual drug formulations, can lead to the discovery of important structural properties of the host-guest interactions.

3.2.2 Inclusion complexes in solution

Complexes of CDs in solution can be characterized by a large number of different methods. The main groups are: a) electrochemical methods, b) spectroscopic methods and c) calorimetric methods.[53] Each approach can be used in specific cases and usually the nature and properties of guest and host molecules play the key role in the choice of the most suitable method. Typically the process consists of a rough estimation of stability constant by titrating the solutions of guest and host in the concentration range of several orders of magnitude, with the concentration of one component being constant (its properties are monitored) and concentration of the second component being gradually increased. From such preliminary study, the optimal concentration range (usually the area of the biggest changes in the observed property) of both components can be determined. In the second step, the stoichiometry of the complex is evaluated.[56] The standard and most widely used tool is the continuous variation method, which requires graphical treatment of the data obtained from measurement of a series of solutions containing both host and guest in varying portions. The complete range of concentration ratios is sampled ($0<=[H]_0/([H]_0+[G]_0)<=1$) and total concentration $[H]_0+[G]_0$ is kept constant. The data are plotted in the form $X_g\Delta Y$ (where X_g is the guest mole fraction and Y is the measured property affected by the complex formation) versus X_g).[57] The

graphical representation is referred to as the Job plot (Figure 4) and the actual stoichiometry is determined from the curve maxima, where the value of X_g = 0.5 describes 1:1 inclusion complex.

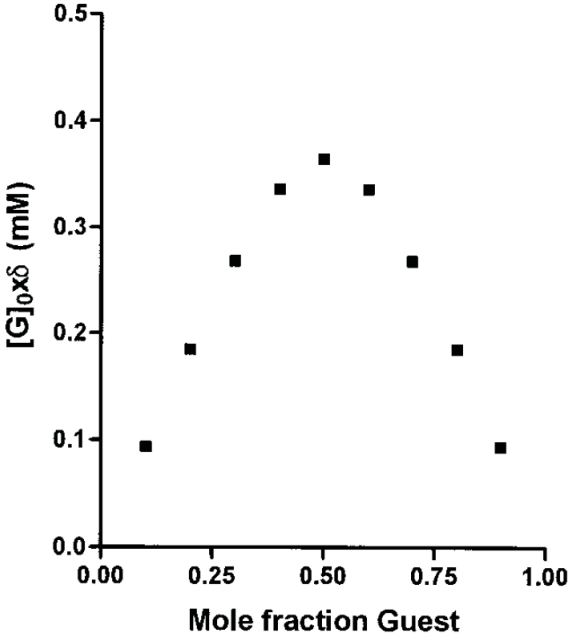


Figure 4. An example of the Job plot, used for complex stoichiometry determination. (from ref. [57])

A separate experiment must be carried out in order to determine the value of K_s. Usually a measurement of the physical quantity which is affected by the complex formation takes place. The data are collected for a series of samples with varying concentration of both or only one of the participating components. Different approaches can be chosen for the data treatment and K_s calculation. Graphical (also called linearization) methods produce a linear relationship between the observed physical quantity and K_s. Among the most commonly used are Benesi-Hildebrand[58] method or Rose-Drago[59] method. Limitation for the use of graphical methods is the conditional complex stoichiometry 1:1. Second possible approach is the use of the curve fitting methods, which should provide the most reliable and accurate data. Such methods require the use of modern computational algorithms in order to find the best fit between the observed and calculated data.

a) Electrochemical methods:

Measurement of electrochemical properties, which are affected by the complex formation can also be carried out. Among the most popular electrochemical methods for

characterization of CD complexes, the cyclic voltammetry, polarography, coulometry or measurements of redox potential can be classified.[60,61] Electrochemical methods afford superior results especially when an electroactive guest molecule is employed. The advantageous fact is, that information about the substrate as well as about the electrogenerated species formed as a result of electron transfer reaction can be gained concurrently.

b) Spectroscopic methods:

Due to the recent availability of spectroscopic instruments, much higher attention is given to the determination of the binding properties by measuring spectral changes as a result of complex formation. The most useful and direct evidence of the inclusion of guest into cyclodextrin cavity can be obtained from the NMR spectroscopy.[44,57,62] The signals in 1H and 13C NMR spectra of the CD show significant upfield shifts upon the complexation. Especially the H-3 and H-5 protons which are directed toward the CD interior cavity show markable shifts. The NMR spectra of a guest may also exhibit shifting of the signals of atoms entering the cavity. From this observation, the information about the direction of penetration of the guest into CD cavity can be obtained as well as positioning of the molecule inside the cavity. Employing titration series, data for determination of stoichiometry and value of stability constant can be obtained from the NMR experiment with high precision. Often experienced drawback of the NMR measurements is the need for considerably high aqueous concentration of most often lipophilic guest, which may not be accomplished due to the solubility issues. The advanced 2D NMR techniques as NOESY, DOSY or ROESY can afford further information about the detailed structure of the complex. Very detailed description of different options of probing the CD complexes by NMR techniques along with the mathematical and physical models are described in review articles.[56,57,63]

Ultraviolet/visible spectroscopy (UV/Vis) can be counted among the valuable spectroscopic methods for the description of CD complexes. The main requirement for the use of UV/Vis spectroscopy for determination of the host-guest equilibrium is the presence of chromophore in one of the species (most commonly in the guest molecule). As a result of the complexation, the chromophore is transferred from the aqueous environment into the lipophilic cavity of CD. Hypsochromic or bathochromic shift, as well as increased absorption, are the commonly reported effects of the complex formation. Thy typical setup of the experiment leading toward the K_s determination consists of titration of the guest solution (with keeping its concentration constant) by the solution of CD. The described procedure was

used for the study of inclusion complexes of genistein with different β-CD derivatives.[64] The increasing concentration of the host in the solution caused the increase or decrease of the absorbance of the guest in the absorption maxima (Figure 5). The restriction for employing the UV/Vis method is that the absorbance of the host molecule must be equal to zero in the area of the absorption maxima of the guest. The inclusion and complexation equilibrium of fluorescent guests as naphthalene or pyrene can be probed by fluorescence spectroscopy.[65] Complexation process has usually has a strong influence on the fluorescence and leads to changes in excitation and emission wavelength or fluorescence intensity.

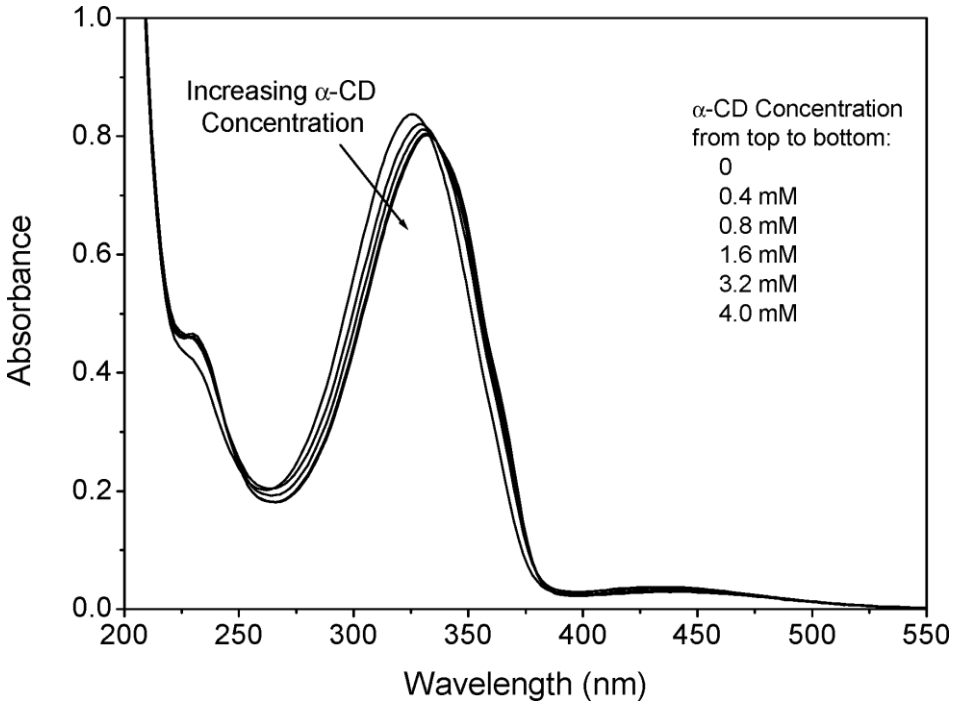


Figure 5. An example of the effect of the inclusion complex formation on the UV/Vis absorption spectra of the guest molecule with the increasing concentration of CD in the solution. (from ref. [45])

The polarimetric measurement can also be classified among spectroscopic methods because the signal is obtained as a result of the interaction of matter with electromagnetic radiation. Changes of specific rotation [α] are recorded as a result of complex formation.[66] CD is an optically active molecule, and supramolecular complexation of the guest molecule leads to the changes in its specific rotation.

c) Calorimetric methods:

Microcalorimetric methods as Isothermal titration microcalorimetry (ITC) are used as a tool for monitoring of thermodynamic changes in the solution, which arise due to the complexation. ITC is versatile technique suitable for monitoring of any reaction which is initiated by the addition of the binding component. Due to the complex formation, heat is either generated or absorbed, and measurement of this heat allows accurate determination of K_S, stoichiometry, enthalpy (ΔH) and entropy (ΔS) change.[45] These thermodynamic changes are associated with the behavior of the water molecules within the cavity of CD, their removal from the cavity or changes in their distribution around the guest molecule.[53] Although the ITC was primarily developed to study the biomolecular interactions, it has proved itself to have high usability for a description of supramolecular complexes, especially because it affords complete thermodynamic profile in a single experiment. In some publications, it is even referred to as the best method for studying of the binding thermodynamics of supramolecular systems.[45] Among the most important advantages, which make it superior over the previously described methods, can be listed: high throughput, low needed concentrations and volumes of both components, precise and reliable results and unattended automated operation. As an example of use, the ITC of differently substituted benzoic acids into the solution of heptakis-(2,6-dimethyl)-β-CD can be mentioned.[67] The ITC measurement affords an enthalpogram, which displays the temperature changes after each titration point (Figure 6). The obtained points are then subjected to mathematical curve fitting analysis, which affords the thermodynamical properties of the resulting inclusion complex (Figure 7).

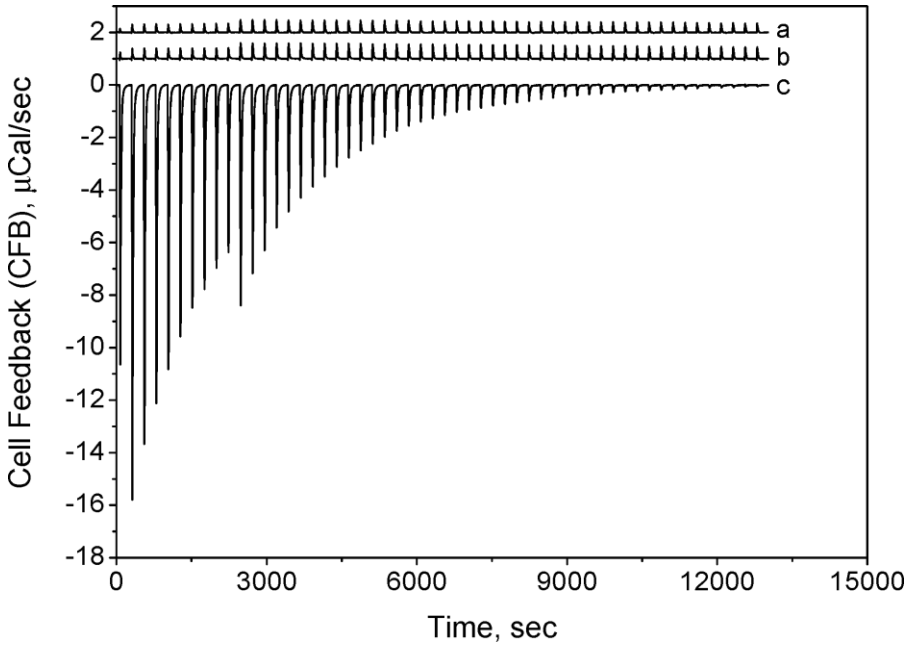


Figure 6. An example of enthalpograms obtained for titration of α-CD into a) water, b) hydroxypropyl methylcellulose (HPMC) and c) azobenzene-substituted hydroxypropyl methylcellulose (azo-HPMC) at 25 °C. (from ref.[45])

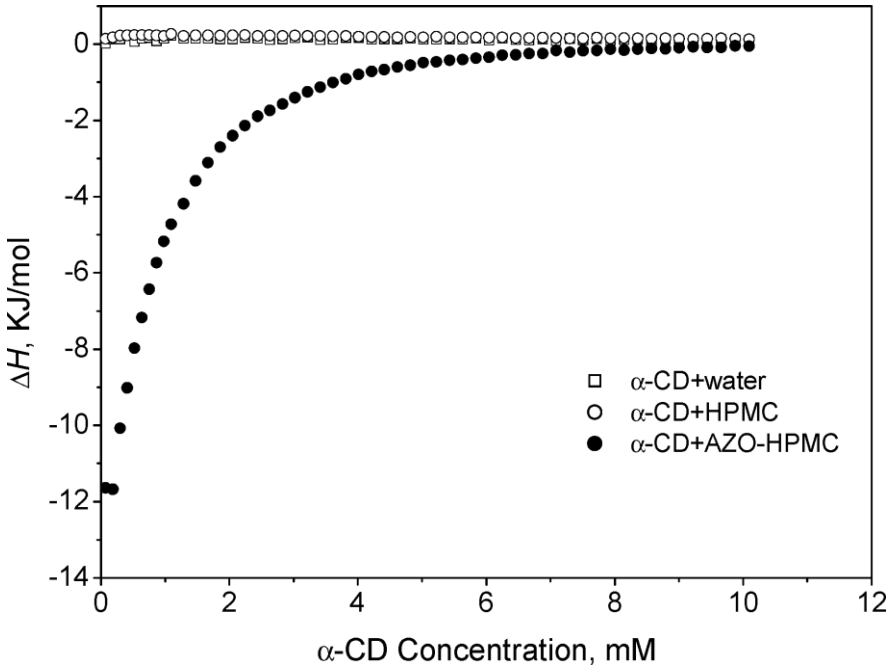


Figure 7. Binding curves obtained for titrating aqueous solution of a-CD into water, HPMC and azo-HPMC. (from ref.[45])

3.3 Chemically modified cyclodextrins

Natural cyclodextrins have a limited solubility in a majority of the commonly used solvents and also, they contain only one type of functional group. For maximizing their utilization possibilities, chemical modification of one or more of the hydroxyl groups is necessary. For practical purposes, the CD derivatives can be classified according to their intended use as: carriers for substances with biological activity (solubilizers or stabilizers), separating agents in chromatography, catalysts, enzyme models and various additives as detergents or viscosity modifiers.[2] Another reason for the preparation of new CD derivatives is a need for achieving specific binding behavior.[68] Attachment of one or more substituents on the macrocycle backbone can significantly modify the strength of non-covalent interaction with specific guest. Due to the highly-functionalized and symmetrical nature, selective modification of CD remains very challenging task.[69,70] The preparation of selectively substituted CD derivatives often involves application extensive purification procedures to recover pure single isomer compound. This is indisputable justification for the effort put in developing new synthetic strategies which would allow synthesis of pure derivatives in reasonable yields.

For derivatization of hydroxyl groups, common approaches known from carbohydrate chemistry can be applied. Among the most wildly used CDs are different randomly substituted cyclodextrins with varying degree of substitution. Randomly methylated β-CD (RAMEB), heptakis(2,6-dimethyl)-β-CD (DIMEB), heptakis(2,3,6-trimethyl)-β-CD (TRIMEB), hydroxypropyl-CD (HP–CD), peracetylated β-CD (per-*O*-Ac-β-CD), sulfobutylether-CD (SBE-β-CD), sulfated CD as well as various CD polymers are industrially produced in ton amounts every year. Above listed products are most often statistical mixtures with varying degree of substitution. All of them show higher water solubility than their parent analogs (Table 2), are commercially available and are utilized in many pharmaceutical applications for improving solubility of FDA approved drugs.

Table 2. Examples of commercially available CD derivatives and their solubility in water.[28,71]

CD derivative	substitution[a]	MW ($g.mol^{-1}$)	solubility in water(mg/ml)[b]
RAMEB	1.8	1312	>500
HP-α–CD	0.65	1199	>500
HP-β–CD	0.65	1400	>600
HP-γ–CD	0.6	1576	>600
SBE- β-CD	0.9	2163	>500

[a] average number of substituents per glucose unit

[b] at 25 °C

Despite the previously listed complications, few synthetically useful methods leading to pure substituted CDs in reasonable yields have been published. Among those, the substitutions of all hydroxyl groups, such as peracetylation[72], permethylation[73], perbenzylation[74], perallylation[75] and persilylation[76] are worth mentioning. A common approach for the preparation of per-substituted derivatives is a use of an excess of derivatization agent. Fully substituted products are easily accessible in high yields and can be used in pharmaceutical formulations or as starting materials for further transformations. Another example of selective modification is perfacial substitution of all primary hydroxyls by iodine.[77] The formed per-6-iodo-β-CD can be then transformed by the reaction with a nucleophilic reagent to a wide variety of per-6-substituted analogs.

3.4 Monosubstituted cyclodextrin derivatives

Generally speaking, the monosubstituted CD derivatives are the ones having only one hydroxyl substituted by a different functional group. The successful monomodification is typically hindered by the number of possible isomers, which can result from the reaction. Common problem, which must be faced while attempting to prepare monomodified CDs, is the formation of mixtures of products with a different degree of substitution, which have to be separated chromatographically. Although the selective synthesis of desired monosubstituted isomers functionalized at the desired position (2, 3 and 6) is a challenging task, a number of research groups has invested a considerable amount of effort to achieve this goal. Most of the published procedures are taking advantage of the different reactivity of the hydroxyl groups at positions 2, 3 and 6 to achieve regioselectivity. More complicated approaches consisting of several protection and deprotection steps can be used in order to receive selectivity for a specific position, but the drawback of such procedures lies in the number of reaction steps and low overall yields. In the following paragraphs the basic approaches toward monosubstituted products in different positions will be covered, with the stress on substitution in position 6, which is the most easily accessible and widely utilized.[78]

Secondary hydroxyls at position 2 are the most acidic. This property can be used with advantage for the monosubstitution by employing one equivalent of a base, which predominantly deprotonates one hydroxyl group at position 2.[79] The formed alkoxide is more nucleophilic than the remaining hydroxyls, which are not deprotonated, and reacts with electrophile preferentially. The situation can be complicated by the proton transfer between the positions 2 and 6 which can result in the mixture of regioisomers. It has been observed, that addition of the electrophilic reagent in one portion as a solid, after the deprotonation by NaH, reduces the formation of C6-substituted sideproduct.[80] Even though some selectivity is reached by using mentioned approaches, the overall yields of 2-*O*-monosubstituted products are very poor (in the range of units or maximum of few tens of percent).[80,81]

Secondary hydroxyls at the position 3 are the most sterically hindered and therefore hardly accessible. Methods that employ specific electrophilic reagent, which can form an inclusion complex with CD, along with directing the reactive part of the molecule toward the position 3, is a “smart” solution to this problem. Such approach is the most convenient way

toward preparation of CD derivatives regioselectively substituted at the position 3 giving the product in decent yield. As an example of this approach, the sulfonylation of β-CD using 2-naphthalenesulfonyl chloride or 3-nitrobenzenesufonyl chloride can be given.[82,83] As a follow-up on these original works, the highly regioselective monocinnamylation of β-CD using cinnamyl bromide and NaOH in aqueous solution was introduced.[69] The selectivity seems to be driven mainly by the stability and orientation of the inclusion complex formed between the CD and the electrophilic reagent, which is reasonably high in the aqueous environment. The formation of 3-*O*-cinnamyl-β-CD can be considered as a regiospecific (no other regioisomers were detected in the reaction mixture) reaction which affords the product in 30 % overall yield.

Of the three types of hydroxyl groups present on CD molecule, the hydroxyls at the position 6 are the most basic, the most nucleophilic and also considerably sterically accessible. It means that, under standard conditions, the electrophile will attack this position predominantly, unless it is a highly reactive one. As an example, the reaction of β-CD with *tert*-butyldimethylsilyl chloride can be given, which shows selectivity toward the position 6,[84] while the more reactive trimethylsilyl chloride reacts with all hydroxyls indiscriminately.[80] The vast majority of all of the 6-monosubstituted derivatives is prepared via tosylation (using *p*-toluenesulfonyl chloride with suitable base).[85] The regioselectivity toward the position is achieved by the use of reagent with relatively low reactivity and the selectivity for monomodification is achieved by using one or more equivalents of the reagent. The graphical review of methods for preparation selectively substituted CD derivatives is given below (Figure 8).

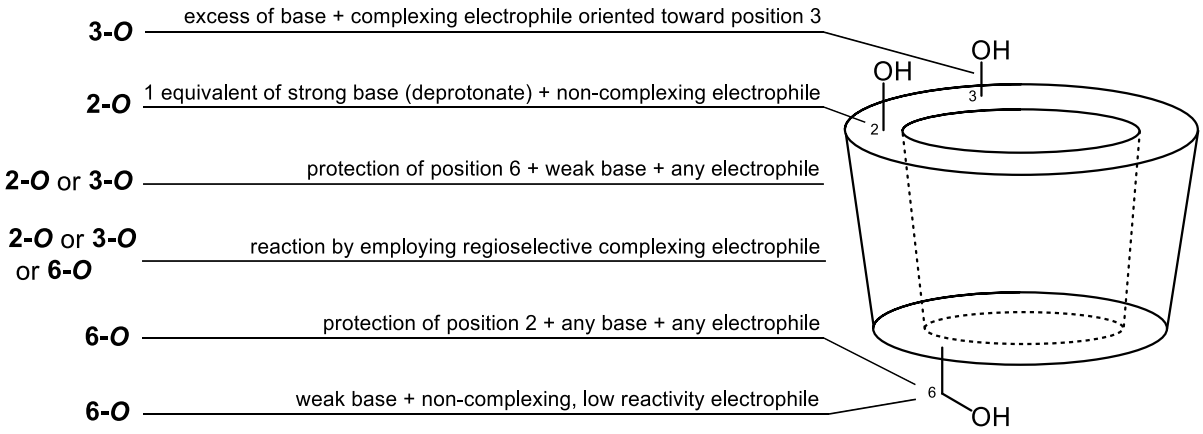
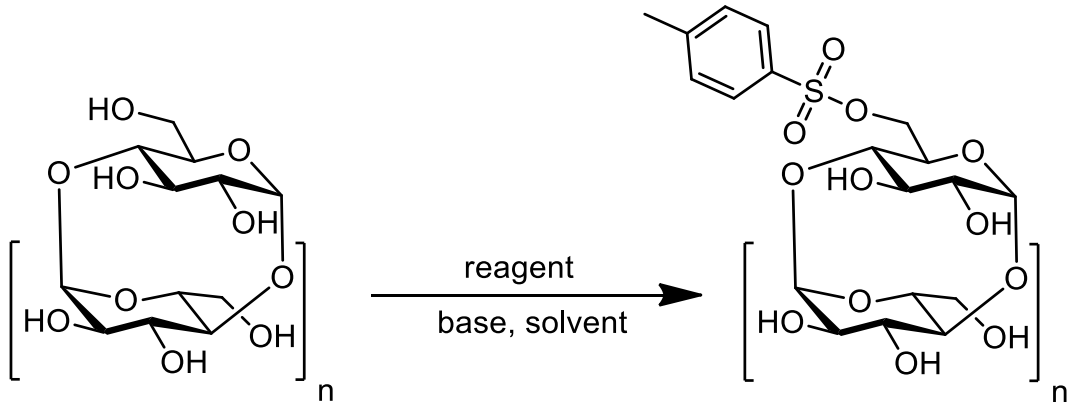


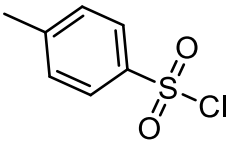
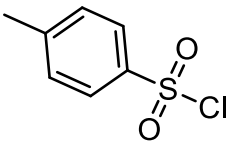
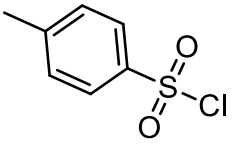
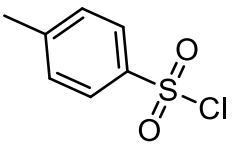
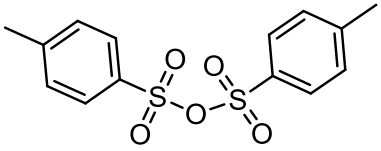
Figure 8. Overview of the different approaches used for synthesis of selectively mono-*O*-substituted CDs.[80] By "weak base" it is meant a base, not strong enough to deprotonate CD hydroxyls (e.g. pyridine), while "strong base" deprotonates the CD hydroxyls readily.

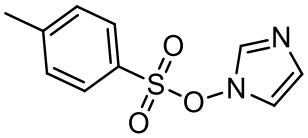
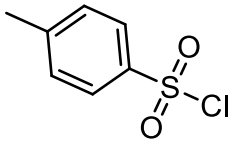
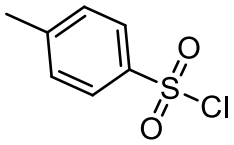
Monotosylation of unmodified CDs (α, β, γ) in the position 6 is a typical starting reaction leading to a reactive CD intermediate, which upon a reaction with appropriate nucleophile can be easily converted to a broad range of useful CD derivatives. Owing to the straightforward preparation process and relatively high yield (compared to other monosubstituted CDs), there is a large number of publications reporting the synthesis and further transformations of the 6-*O*-monotosylated CD. According to SciFinder®, there were 55 references for 6^{I}-*O*-toluenesulfonyl-α-CD, 819 for 6^{I}-*O*-toluenesulfonyl-β-CD and 37 for 6^{I}-*O*-toluenesulfonyl-γ-CD, to date of April, 2015. In the Scheme 1, the general reaction scheme is displayed, and Table 3 offers the overview of different conditions used for the synthesis of monotosylated CDs along with the reported yields. The examples of literature were selected according to the yields, to demonstrate the enormous variability of reported results and according to the number of citing articles.



Scheme 1. General reaction scheme for monotosylation of CD.

Table 3. Overview of conditions for monotosylation of CD.

CD	reagent	base	solvent	yield	references
α–		pyridine	pyridine	20 %	86, 87
α–		NaOH	H_2O	2 %	88
β–		NaOH	H_2O	25-53 %	89, 90, 91, 92
β–		-	pyridine	3-88 %	93, 94, 95
β–		NaOH	H_2O	26-61 %	96, 97, 98

β–		NaOH	H_2O	21-45 %	99, 100, 101
γ–		-	pyridine	31 %	102, 103
γ–		triethylamine	DMF	4 %	104

From the yields given above it can be assumed that monotosylated CDs can be prepared in yields up to 88 %, but the reality proves to be quite different. The most crucial step of the reaction is the extensive separation of the mixture of isomers with various degrees of substitution as well as the ability to determine the purity of the product. From the long-time experience of our research group it follows that, regardless the method used, yields of pure monotosylated products around 30 % can be considered as a great success. This topic will be discussed in the results and discussion section more in detail.

It is worth mentioning that the choice of the solvent has an enormous effect on the outcome of the tosylation. Pyridine forms a pyridinium complex with the CD cavity and directs the sulfonylation reagent toward the position 6. On the other side, it is a toxic solvent and the relatively stable complex complicates the workup process.[80] In the case of β-CD, the most convenient way toward the preparation of monotosylate in position 6 is using water as the solvent and NaOH as the base. These conditions favor the formation of the inclusion complex between the CD and sulfonylating reagent, which is directed facing the reactive site toward the position 6. This factor causes high regioselectivity and almost exclusive substitution in position 6. Yields of 6^{I}-*O*-toluenesulfonyl-β-CD can be improved when using less reactive electrophilic reagent, such as 1-(*p*-toluenesulfonyl)imidazole or *p*-toluenesulfonic anhydride. Although the regioselectivity can be handled quite efficiently by choice of the reagent, solvent, and base, the formation of more or less statistical mixture of

products with a different degree of substitution cannot be avoided. The β-CD and its derivatives have remarkably lower aqueous solubility, which can be used to separate the monosubstituted product from the mixture by repeated crystallization or by chromatography on charcoal.[105] For α–and γ–CD the employment of chromatographic methods for isolation of pure single isomer is unavoidable. Tosylated CDs are very important building blocks, which upon the reaction with nucleophile as iodide, azide, alkylamine or alkylsulfanyl, afford monoiodo-[106], azido-[107], amino-[108] and sulfanyl-[109] cyclodextrins. *O*-Alkylated CD cannot be prepared in reasonable yields by direct substitution of tosylates with alcoholates, due to the preferential formation of 3,6-anhydro-CDs.[110]

3.4.1 Permanently charged cationic CD derivatives

Cationic CD derivatives have one or more hydroxyl groups of the macrocycle backbone substituted by one or more substituents bearing the positive charge. Typically this is realized by attachment of different quaternary ammonium, phosphonium or sulfonium[111] salts. Either mono- or poly-substituted cationic CD derivatives proved to have the ability to resolve racemic mixtures of various compounds. For this reason, many publications describe them as outstanding chiral selectors utilized mostly in capillary zone electrophoresis (CZE),[112,.,113,114,115] where they are added into the separation buffer. CZE is a versatile separation technique which is orthogonal to chromatographic methods as HPLC, GC or SPE, which are based on the partition of analytes between stationary and mobile phase. The selector-analyte interactions in CZE are mimicking the receptor-ligand interactions, which take place in free solution in contrast to the chromatographic methods, where the selector is immobilized on a solid phase. The advantage of charged chiral selectors used in CZE, specifically the single isomer cationic CD derivatives, is their enhanced aqueous solubility (compared to the unmodified CDs) which allows the preparation of highly concentrated solutions of separation electrolyte. Native CDs can be used in chiral CZE as well, but the undisputable benefit of the charged derivatives is the possibility of chiral discrimination of charged but also neutral analytes. The ionic interaction between charged CD and oppositely charged analyte significantly increases the stability of the inclusion complex and alters the selectivity of the host-guest interaction.[116]

Another field of the utilization of cationic CDs is the catalysis of chemical reactions. Matsui et al. investigated the catalytic effects of charged CD derivatives (mono(6-trimethylammonio-6-deoxy)- (**a**) and mono[6-(1-pyridinio)-6-deoxy]-β-cyclodextrins (**b**) on the alkaline hydrolyses of *o*-, *m*-, and *p*-acetoxybenzoic acids[117,118]. The differences of catalytic properties of cationic CDs and native CD were attributed to the different electrostatic, steric, and hydrophobic interactions between the hosts and guests. It has been published that amphiphilic polycationic CD derivatives can be used for the preparation of self-assembled cationic vesicles, with the ability to condens the plasmid DNA and transfect it into targeted cells.[119] Stable spherical nanoparticles composed of anionic polymer containing cell-specific ligand for targeting to cancer cells and cationic sulfanylether derivative of β-CD were loaded with 1,4-dihydroxyanthraquinone guest and served as model for targeted cancer therapy.[120]

Publications reporting the detailed synthetic procedures for preparation of single isomer monosubstituted cationic CD derivatives, along with the complete spectral characterization of the resulting material are very sparse. For example, permanently charged 6^{I}-(*N*,*N*,*N*-trimethylammonio)-6^{I}-deoxy-β-cyclodextrin (**a**) was reported as having catalytical capabilities.[118] Muderawan et al. reported the preparation of different monosubstituted pyridinium (**b**), imidazolium (**c**) and quaternary ammonium (**d**) CD tosylates and chlorides for separation of anionic dansyl racemates by CZE.[121] The majority of the articles describing the preparation have been published in journals focused on analytical chemistry and the procedures are often lacking substantial data. The synthesis of monosubstituted dicationic CD derivative 6^{I}-(*N*,*N*,*N'*,*N'*,*N'*-pentamethylethane-1,2-diammonio)-6^{I}-deoxy-β-cyclodextrin (**e**) has been described by Nzeadibe et al..[112] The most relevant examples of previously published monosubstituted cationic CD derivatives are displayed in the Figure 9. Other CDs bearing cationic substituent with more than one tetraalkyl ammonium groups, as well as α- and γ-CD analogs, were first reported by our group within the scope of this PhD thesis.[98] Therefore there exists a need for the development of preparation processes, which could lead toward complete sets (α-, β-, γ-) of single-isomer monosubstituted quaternary ammonium CD derivatives with various numbers of permanent positive charges, suitable for high-scale production.

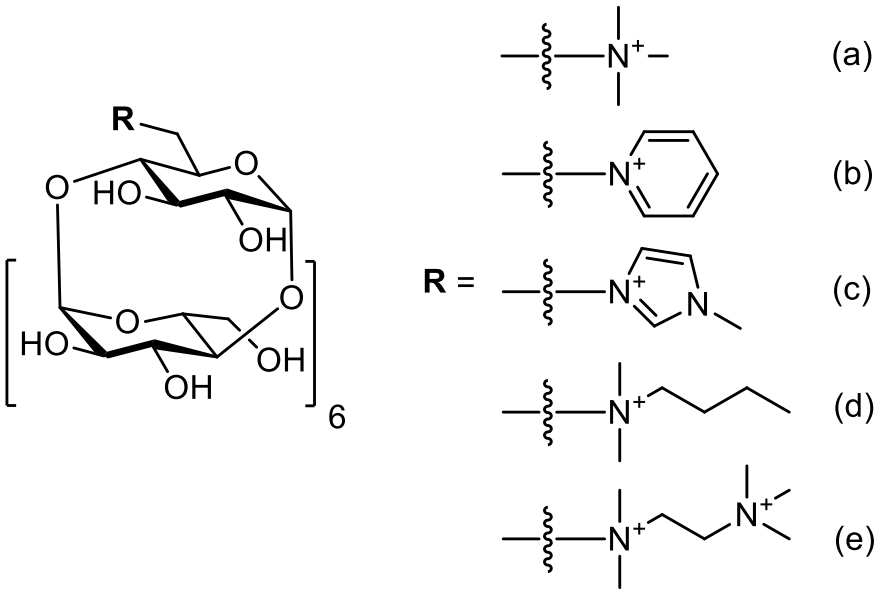


Figure 9. Examples of mono-6-deoxy- substituted tetraalkylammonium CD derivatives, which have been previously described in the literature.

3.5 Deposition of modified CDs on a solid support

One of the main goals of our work was to prepare series of cationic CD derivatives, which could be immobilized on a solid surface via simple electrostatic interaction between oppositely charged CD and the solid surface. We were first to describe the concept of depositing permanently charged cationic single isomer CD derivatives onto the surface of an anionic polymeric membrane.[122] There are three major research areas dealing with the immobilization of CD derivatives on a solid support: 1) binding of alkylsulfanyl CD derivatives onto the surface of gold, 2) deposition of CD-siloxanes on the glass surface and 3) immobilization of various ionic cross-linked CD polymers or CD containing nanoparticles.

Nelles et al. described the immobilization of different sulfanyl-CDs on the golden surfaces via chemisorption process.[123] It has been proved that orientation of the CD moieties deposited as a film on the gold surface can be controlled by using either CD derivatives monosulfanylated at position 6 with different linker lengths or CDs multisulfanylated on primary face with 2-4 sulfanyl groups. The formation of the films was investigated by several analytical methods as Fourier transform infrared (FTIR) spectroscopy, time-of-flight mass spectrometry, contact angle measurements, plasmon surface polariton (PSP) spectroscopy, and cyclic voltammetry. Finally, the results were used to create models of the orientation of the CD films (Figure 10).

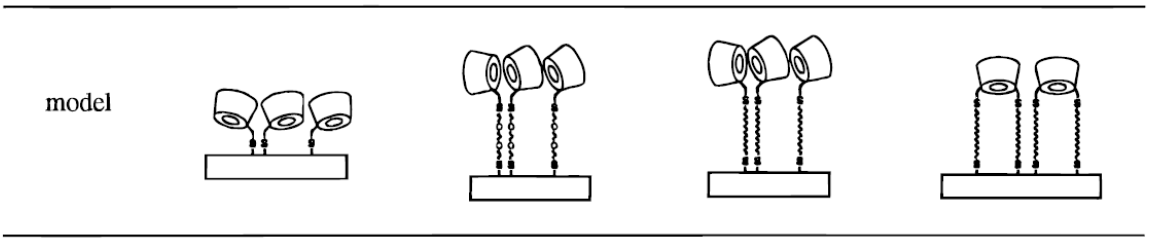


Figure 10. Models of the film structures of chemisorbed sulfanyl CD on the gold support. (from ref. [123])

Weisser et al. have investigated the immobilization kinetics of sulfanyl-CDs at gold surfaces.[124] Four β-CD derivatives containing one or more sulfanyl functional groups were employed, namely: mono(6-deoxy-6-sulfanyl)-β-CD, mono(6-deoxy-6-[(sulfanyldecamethylene)sulfanyl])-β-CD, mono(6-deoxy-6-

[[[(sulfanylethoxy)ethoxy]ethyl]sulfanyl])-β-CD and heptakis(2,3-O-dimethyl)oligo[6-deoxy-6-[(sulfanyldecamethylene)sulfanyl]]-β-CD. The formed CD films were characterized by physicochemical methods as plasmon surface polariton spectroscopy, cyclic voltammetry, contact angle measurement, and atomic force microscopy. The results indicate that the immobilization is a multistep process involving activation energy. Adsorption kinetics can be described as a three-step process, consisting of a physisorption step, binding and orientation step, and adlayer formation step. The following work of Weisser et al. was focused on guest-host interactions with immobilized CDs on gold.[125] Four guest molecules were taken into account: the redox couple ferro-/ferricenecarboxylic acid, 4-*tert*-butylbenzoic acid, cyclohexanol and the electro-optically active dye methyl orange. The host-guest interactions were detected by surface plasmon measurement, cyclic voltammetry, and electro-optical experiment. After the measurements in the presence and absence of cyclohexanol, the authors conclude the host-guest interactions with immobilized CDs are possible. Absorbed methyl orange molecules were observed to form aligned conformation, and 4-*tert*-butylbenzoic acid envinces 1:1 stoichiometry.

The supramolecular interactions of self-assembled monolayers of monofunctionalized CDs onto gold, analyzed by MALDI mass spectrometry and impedance measurement were reported by Henke et al.[126] High packing density with a surface coverage of 99.6 % was determined by the impedance spectroscopy as well. The inclusion process of the anilinonaphthalenesulfonates into the cavity of CD was verified by impedance spectroscopy. Another field of utilization of the CD SAMs on golden electrodes is the construction of electrochemical sensors which be used for sensing of molecules by electrochemical quartz crystal microbalance experiments.[127]

The second most reported strategy for anchoring the CD derivatives onto the solid surface is using CDs with one or more silane functions, which can be immobilized on glass surface via siloxane bonding. Cyclodextrin-siloxane coatings on surface acoustic wave (SAW) devices have been studied by Yang et al., and used as supramolecular sensors for volatile organic compounds (VOCs).[128] *Tert*-butyldimethylsilylated β-CD was deposited as a film on the surface of glass separatory column for fused chromatography, and successful chiral separation of enantiomers was reported.[129] Similarly, capillary columns with immobilized CD coating proved to be useful for resolving enantiomeric mixtures.[130] An optical measurement on integrated Mach-Zender interferometer, which was functionalized by

per-6-silylated β-CD derivative was described.[131] The results of measurements of guest interactions (adamantanecarboxylic acid, 4-*tert*-butylbenzoic acid, and methyl orange) in the restricted two-dimensional environment show, the stability constants to be one-third to one-half lower when compared to the values obtained in a three-dimensional solution environment.

The reports of deposition of CD derivatives onto solid a support via ionic interactions has been almost exclusively limited to the randomly cross-linked CD polymers. A novel system for separation of gases, based on cationic CD pyridinium polymer immobilized on Nafion® polymer membrane, was reported by Grossi and coworkers.[132] The authors observed decreased transport of gasses and water across the β-CD-modified ionomer membrane when compared to the unmodified Nafion®. Kusumocahyo and coworkers have reported the synthesis of ultrathin polyion complex membrane, composed of cationic allyl amine-CD copolymer.[133] This layer was formed on the solid surface of Nafion® membrane containing negatively charged ions via ion-ion interactions (Figure 11**)**. The assembly was tested for separation of isomeric mixtures of butanols. Due to the presence of CD moieties in the membrane, the system exhibited a good selectivity toward butanol isomers, while the unmodified Nafion® showed almost no separation selectivity.

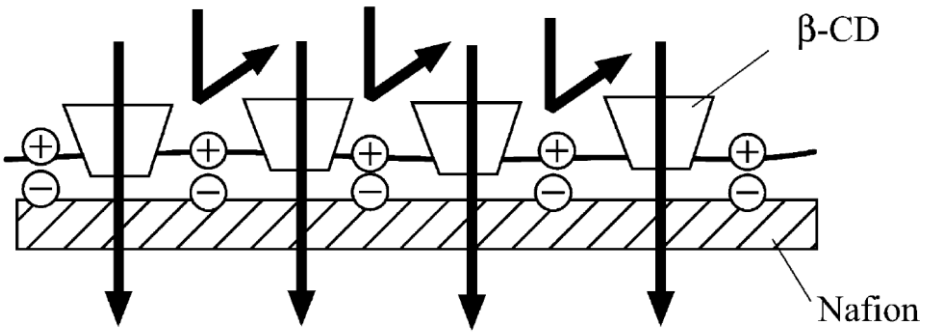


Figure 11. Structural model of the polyion complex membrane. (from ref. [133])

Another example of ionic deposition of CD derivatives with polyelectrolytes (charged polymers) is a preparation of multilayered films on a suitable solid support. Multilayered molecular films can be prepared by layer-by-layer deposition strategy, which is based on repeated adsorption of polyelectrolyte/CD mixtures from aqueous solution onto the surface of quartz or silicon. These multilayered assemblies can be utilized as chemical sensors,[134]

membranes with a size-selective transport of aromatic compounds[135] or as chiral membranes for separation of enantiomeric mixtures of amino acids[136].

3.6 CDs in transdermal drug transportation

It has been generally observed, that bioavailability of topically administered drugs is considerably low. This fact has led to the formation of research areas which are investigating possible ways to enhance the topical availability of drugs. Physical as well as chemical enhancers of transdermal drug transport have been introduced, but their application often leads to unwanted physiochemical or metabolic changes within the biological barrier (skin).

The smart option of transdermal delivery of drugs can be achieved by employing supramolecular carriers, which are able to form vehicles allowing more effective and gentle ways of topical adsorption of bioactive compounds. Cyclodextrins and their derivatives can be used to improve the solubility or stability of drugs in local and systemic dermal delivery, to enhance the transdermal absorption of drugs, to modulate the pharmacokinetic and pharmacodynamic behavior of the drugs and to avoid the undesirable side effects as irritancy of topically applied drugs.[137] When compared to conventional chemical penetration enhancers, such as unsaturated fatty acids, fatty alcohols and glycerol monoethers which can temporarily alter or damage the skin barrier, the CDs are considerably safer. These standard enhancers increase the skin permeability by disrupting the lamellar structure of the biolayers in stratum corneum, leading to fluidization of the intracellular medium.[138] Hydrophilic complexes of CDs can permeate the skin only with considerable difficulty. The studies have shown, that some CD derivatives such as randomly methylated β-CD or 2-hydroxypropyl β-CD can extract a majority of the lipid classes from isolated stratum corneum of rats, especially cholesterol, cholesterol esters, and triglycerides.[139] The positive effects of drug absorption enhancement caused by CDs can be in part attributed to the changes in a barrier function of the skin by extraction of the skin lipid components.

The rate of the export and bioavailability of a lipophilic drug from its complex with CD can be modulated by various factors (Figure 12). One of the factors that strongly influence the absorption of topically administered drugs is the amount of encapsulating agent (CD). Because only the free, uncomplexed form of the drug is able to cross the lipophilic barrier and eventually enter the blood stream, the maximum absorption is reached when just enough CD is used which is requisite to solubilize the entire drug. A higher quantity of the CD causes lower bioavailability due to the reduction of the free fraction of the drug. Another option of

accelerating the transport rate is the use of competing agent with the ability to form complexes with CD. Two types of competitors exist: a) the pharmaceutical excipients, which are often present in topical drug formulations and b) endogenous substances present at the absorption site (glycerides etc.). The overall process of a drug transport in the presence of a competing agent is depicted in Figure 13. The process starts with the dissolution of the solid inclusion complex described by dissolution rate constant (k_d), latter is followed by the complex dissociation described by stability constant (K_s). K_i is the stability constant of the CD-competing agent complex, and k_a is the absorption rate constant of the drug. High dissolution rates together with the relative stability of the complexes (K_s<Ki) give rise to the high concentration of free drug which causes enhanced absorption. Cooperative mechanism of absorption enhancement comes due to the extraction of some components of the biological barrier by the dissociated CD, which causes drug passage through the skin to be more facile (especially for hydrophilic drugs).[140]

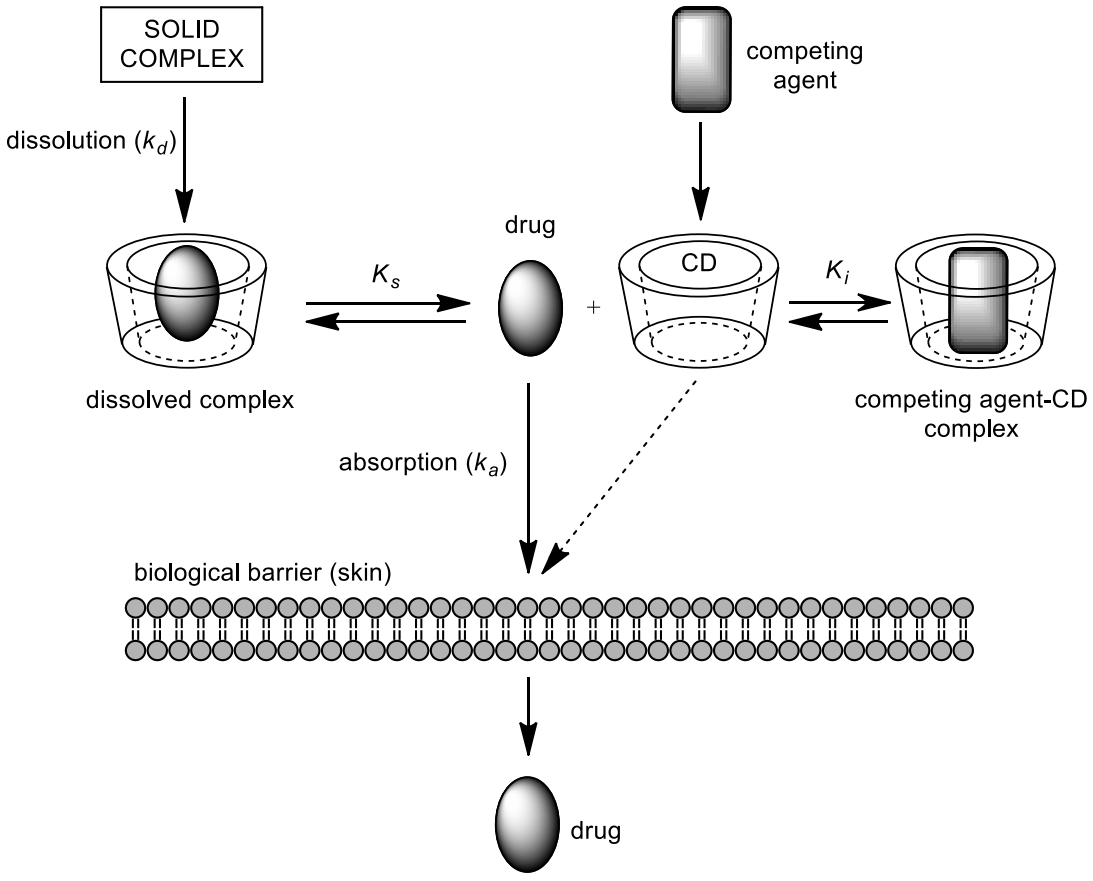


Figure 13. Graphical representation of the drug absorption from the CD complex across the biomembrane with the influence of a competing agent.[137]

Although there are many papers reporting the novel methods of controlled transdermal drug delivery, such as electrically assisted transport, use of vesicles and particles, stratum corneum bypassing or removing,[141,142] the need for new drug formulations which would allow precise control over the drug transport is still pending. The promising solutions are offered by manufacturing advanced plasters, which are very comfortable for the patients and allow sustained release of the drug for up to several weeks.[143,144] To our best knowledge, there has not been any reports on the concept of using single isomer CD derivative anchored to the solid phase as a drug carrier system for the construction of plasters with precisely controlled release and transdermal transport of the cargo up today. Construction of such a system, consisting of suitable solid anionic support, such as plasma-treated nanofabric, enabling immobilization of cationic CD derivatives, should allow the production of advanced plasters with desired functionality.

4. RESULTS AND DISCUSSION

4.1 Preparation of the target compounds

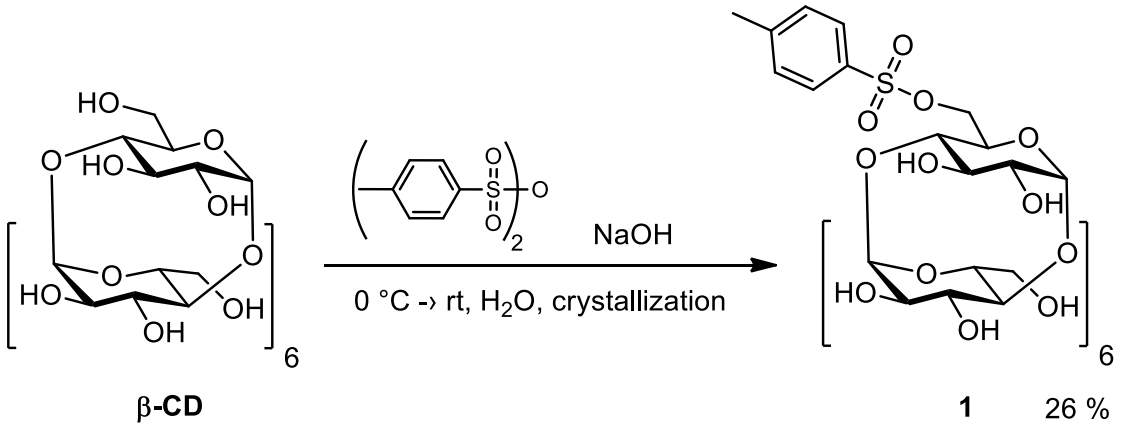
Some of the findings which will be introduced in the chapters describing the preparation of target compounds are part of the manuscript published by our group.[98]

4.1.1 Synthesis of 6-*O*-tosyl- monosubstituted CD derivatives

As the first reaction step in the sequence leading toward the tetraalkyl ammonium-substituted CD derivatives, the regioselective monotosylation of native CDs in position 6 was performed. Preparation of the suitable starting material by introduction of the toluenesulfonyl leaving group on one of the hydroxyls on the primary face of native CDs is the most critical and scope-limiting step, taking in account the fact, that most of the subsequent modifications afford products in nearly quantitative yields.

6^{I}-*O*-*p*-Toluenesulfonyl-β-cyclodextrin **1** was prepared according to the published procedure[97] by the reaction of β-CD with *p*-toluenesulfonic anhydride (Ts_2O) in the aqueous base (Scheme 2). This procedure proved to give the highest yields of the monosubstituted product. Comparable results can also be achieved by employing the reagent 1-(*p*-toluenesulfonyl)imidazole[100], which has dramatically shorter shelf life and requires its immediate use in the reaction. Ts_2O was easily prepared by the reaction of *p*-toluenesulfonyl chloride (TsCl) with p-toluenesulfonic acid monohydrate. The preferential formation of the monosubstituted product can be reasoned by two effects: a) lower reactivity of Ts_2O (compared to the TsCl) limits the formation of multiply substituted side products and b) the formation of the inclusion complex of the reagent and β-CD in the aqueous environments drives the substitution toward the position 6. Still, the reaction produces a mixture of monosubstituted product **1**, unreacted β-CD, and some highly-substituted derivatives, which needed to be separated to receive the pure tosylate **1**. In the original literature[97], the product is separated from the mixture by crystallization from water. When trying to reproduce the procedure, we failed to obtain the pure monosubstituted isomer. This imperfection is actually

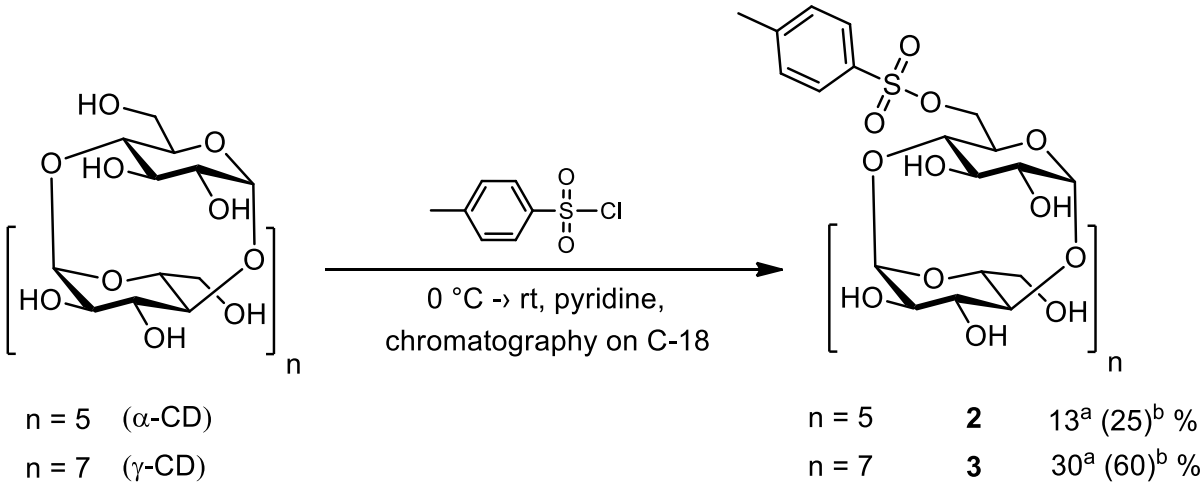
quite frequent in the literature dealing with the synthesis of monosubstituted derivatives, most probably caused by the inability of the researchers to fully determine the purity (or better unpurity of their final products). The same reason also explains the unrealistically high yields which are often reported. For this reason, we developed a modification of the purification step by repeated crystallization in MeOH/H_2O (1:1 v/v) mixture which gives pure **1** after the third run in the satisfying overall yield of 26 %. TLC and detection by carbonization proved to be a very simple yet very sensitive method to follow the purity of the tosylates, able to detect even slightest traces of the side products in the resulting material.



Scheme 2. Synthesis of mono-Ts-β-CD.

The α- and γ- CDs have significantly higher aqueous solubility when compared to β-CD. The use of aqueous solvent is not applicable, as the products of the reaction cannot be isolated by precipitation, and the regioselectivity is lower as well.[145] The monotosylates of α- and γ- CDs have been prepared by the modification of the conventional method employing TsCl in pyridine[146] (Scheme 3). Similarly as in the case of β-CD mentioned above, this reaction provided a mixture of products with several degrees of substitution. Different separation techniques for isolation of the pure single isomers of **2** or **3** were tested. Either crystallization from water[145] nor purification on the column packed with activated charcoal[147] has led to the pure monosubstituted derivatives. We found the most convenient way to obtain pure **2** and **3** to be the flash chromatography on reverse phase C-18 using step gradient. This approach is taking advantage of the different lipophilicity of individual components of the mixture. Using this method about 40-50 % of the starting α- or γ-CD can be recovered by flushing the column with 10 % MeOH and used again as the starting material. The pure monotosylated product is then eluted by 20-30 % MeOH. After complete elution of the

product, the column was washed with 50 % MeOH (to elute the highly substituted side products) and the column was reused several times. This strategy affords compounds **2** and **3** in very high purity and sufficient 13 % and 30 % yield respectively. After subtracting the amounts of the recovered native CD, which is ready to be used repeatedly, the yields of **2** and **3** can be corrected to 25 % and 60 % respectively.



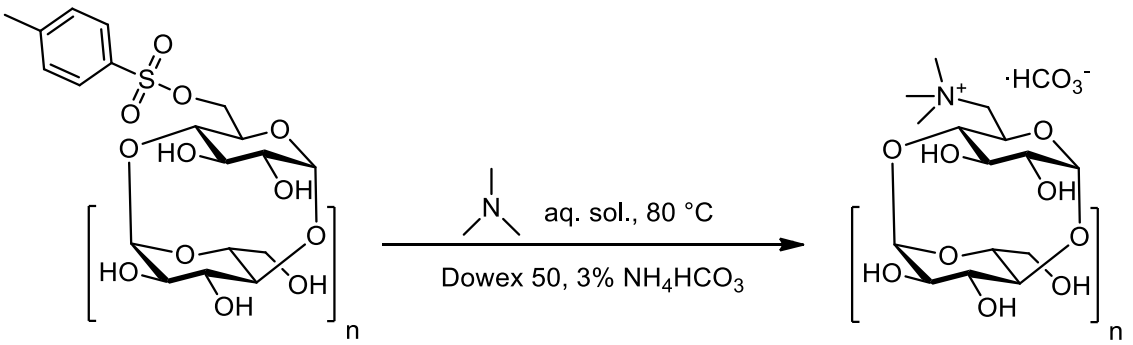
Scheme 3. Synthesis mono-Ts-α– and γ-CDs. [a] Regular yields of monotosylates. [b] Corrected yields, when the amount of recovered starting material is taken into account.

The monotosylated CDs **1**, **2** and **3** were used as general-purpose starting materials and were usually prepared in multiple tens of grams. We experienced some unwanted degradation of the products by hydrolysis when storing the products for extended period. To avoid hydrolysis, the crucial step is the final drying of the compounds. Drying the samples for 8 h at 80 °C and vacuum (1 Pa) proved to be efficient enough to remove all residual water and storing under argon, to prevent atmospheric moisture, at room temperature prolonged the shelf life of the monotosylated CDs to several years without any noticeable (TLC) signs of decomposition.

4.1.2 Synthesis of monotrimethylammonio CD derivatives

The next step in the reaction sequence toward the complete series of tetraalkyl ammonium CD derivatives was the preparation of the simplest 6^{I}-(*N*,*N*,*N*-trimethylammonio)-6^{I}-deoxy-β-cyclodextrin bicarbonate **4**. The research of the literature revealed the procedure

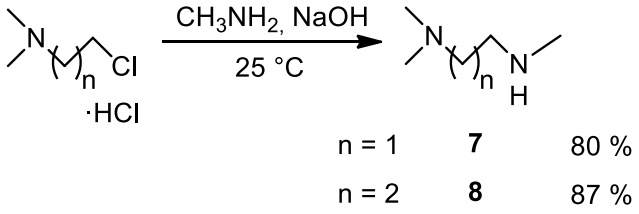
by Matsui et al.[117], which describes the reaction of mono-Ts-β-CD with trimethylamine solution in DMF. Their procedure afforded the product **4** in only 42 % yield, which may have been caused by the high amount of byproducts formed possibly by decomposition of DMF or by formylation side reactions. Authors obtained pure **4** after lengthy chromatographic separation on carboxymethylcellulose column, involving collection of 150 fractions. No synthetic procedures for synthesis of analogs **5** and **6** have ever been published before. Since we aimed at synthetic protocols to be applicable in a high scale, potentially suitable for industrial scale production, we decided to tune the conditions of the described procedure to achieve higher yields and to avoid the chromatographic separation, if possible. 45 % v/v aqueous solution of trimethylamine, which is cheap and commercially available, was used (Scheme 4). The reaction was carried out at 80 °C in sealed thick-wall glass ampoule to prevent evaporation of the volatile amine reagent. Only two compounds were produced as the result of the reaction – product **4** and byproduct 3,6-anhydro-β-cyclodextrin. The product **4** was easily separated on a short column of strong cation-exchange resin in H^+ form by flushing the column firstly with H_2O to wash out 3,6-anhydro-β-cyclodextrin and then with aqueous NH_4HCO_3 to elute the product. The hydrogen carbonate salt was then decomposed at 50 °C in vacuo to yield 71 % of pure **4**. Derivatives of α- and γ- cyclodextrin (**5** and **6**) with one tetraalkylammonium group were prepared by the identical procedure in reasonable 58 % and 54 % yields respectively (Scheme 4).



Scheme 4. Synthesis of monotrimethylammonio- series of CD derivatives.

4.1.3 Synthesis of CD derivatives monosubstituted with quaternary diamine

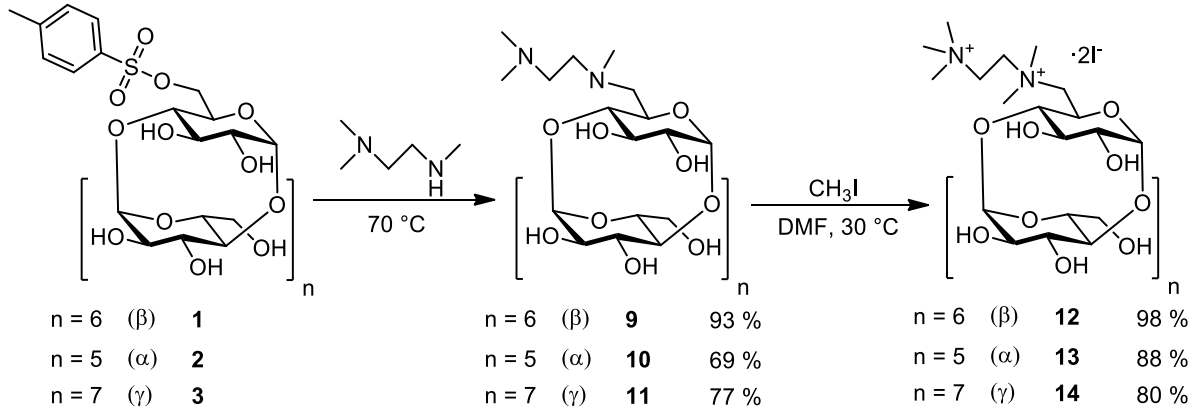
The series of CD derivatives bearing a substituent with two tetraalkylammonium groups separated by variable alkyl chain were prepared in the two-step procedure. The only example of the CD compound with two permanent positive charges in the literature was found to be 6^I-(*N,N,N',N',N'*-pentamethylethane-1,2-diammonio)-6^I-deoxy-β-cyclodextrin diiodide (PEMEDA-β-CD, **12**) published by Nzeadibe et al[112]. The paper is focused on the CZE and lacks some substantial data as full NMR assignment of the intermediates and product and the yields of the reactions as well. In the first step the *N,N,N'*-trimethylethane-1,2-diamine **7** was prepared from inexpensive and commercially available *N*-(2-chloroethyl)-dimethylamine hydrochloride and $MeNH_2$ (40 % solution in H_2O) (Scheme 5) by the published procedure[148]. Diamines **7** and **8** can be alternatively purchased from common commercial suppliers, but the price for 1 gram of the substance is after approximate cost calculation about 10 × more expensive. Therefore all the following reactions were prepared with diamines **7** and **8** prepared in our laboratory.



Scheme 5. Preparation of diamines **7** and **8** as reagents for further synthesis.[148]

Since **7** is a liquid at rt, it is convenient to use it as a solvent in the reaction with the monotosylate to yield intermediate 6^I-((2-(dimethylamino)ethyl)-1-(methyl)amino)-6^I-deoxy-β-cyclodextrin **9** (Scheme 6). This modification brings two improvements: a) the absence of the solvent avoids the unwanted side reactions and b) unreacted **7** can be efficiently distilled from the reaction mixture and reused. Pure intermediate **9** was then obtained by simple precipitation of reaction mixture from propan-1-ol, in 93 % yield. The side product (tosylate salt of the diamine **7**) was conveniently removed with the mother liquor during precipitation as it has a good solubility in the propan-1-ol, so the product **9** was obtained as free amine. The final reaction step toward PEMEDA-β-CD is the quaternization of the tertiary amine

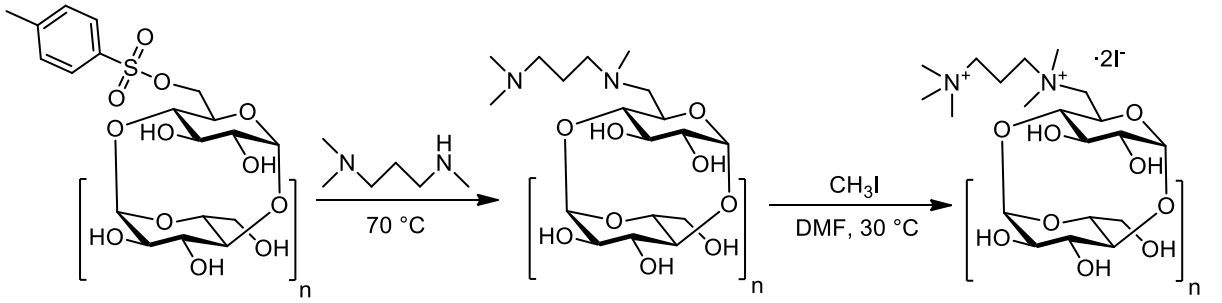
intermediate **9** by methyl iodide (MeI). Pure **12** was obtained after acetone precipitation of the reaction mixture in nearly quantitative yield. The whole sequence of conversion of the tosylate to **12** does not require any chromatographic separation. PEMEDA analogs of α- and γ- CDs were prepared by the procedure analogical to the preparation of PEMEDA-β-CD (Scheme 6). The only deviation from the described process was the purification of intermediates **10** and **11**. The precipitation of the crude product from the reaction mixture by PrOH showed to be unsatisfactory because it led to gum-like precipitate, which was impossible to filter. An alternative workup procedure consisted of precipitation of the evaporated reaction mixture from acetone with subsequent separation of byproduct (*p*-toluenesulfonic acid) on a short column of strong cation exchanger. This may also be the possible explanation of the lower yields of the compounds **10** and **11**, when compared to **9**.



Scheme 6. Synthesis of PEMEDA CD derivatives.

While handling the final compounds **12**, **13** and **14** we came across some partial decomposition of the material. Especially when dried at higher temperatures or when subjected to aqueous bases, the TLC revealed new spots with higher R_f than has the according product. Quaternary ammonium salts are known to be liable toward Hofmann elimination[149], which results in the formation of olefin decomposition product via the E2 mechanism. Following some precautions as avoiding temperatures above 40 °C, avoiding contact with basic aqueous solutions, limiting the stand time in aqueous solution and long-term storing as a dry powder under argon atmosphere enabled us to prolong the shelf-life of PEMEDA analogs to several months. The extensive study of the decomposition products kinetics of PEMEDA-β-CD diiodide will be discussed in section 4.2.1.

Due to the lower stability of the final products of the PEMEDA- series we decided to prepare new complete series of derivatives, where the two tetraalkylammonium groups are separated by the propylene linker instead of the ethylene one. The idea behind the proposal to use the longer linker between the charged nitrogen atoms was introduced to induce higher stability of the products by generating lower electrostatic repulsion and disfavor the Hofmann elimination. The identical synthetic protocol, as for preparation of **12** and its α-, and γ-analogs, was employed, with the only modification of using *N,N,N'*-trimethylpropane-1,3-diamine **8** as the nucleophilic reagent (Scheme 7). Products **18**, **19** and **20** with one methylene longer linker between the charged nitrogen atoms (PEMPDA-series) proved to be more stable and resistant toward Hofmann elimination. The comparison of the thermal stabilities of PEMEDA-β-CD and PEMPDA-β-CD will be again discussed in detail in the section 4.2.1. Yield of the corresponding intermediates and products of the PEMPDA- series were also very satisfactory and in few cases even higher than for the compounds in the PEMEDA- series.



n = 6	(β)	**1**		n = 6	(β)	**15**	99 %	n = 6	(β)	**18**	98 %
n = 5	(α)	**2**		n = 5	(α)	**16**	86 %	n = 5	(α)	**19**	83 %
n = 7	(γ)	**3**		n = 7	(γ)	**17**	78 %	n = 7	(γ)	**20**	80 %

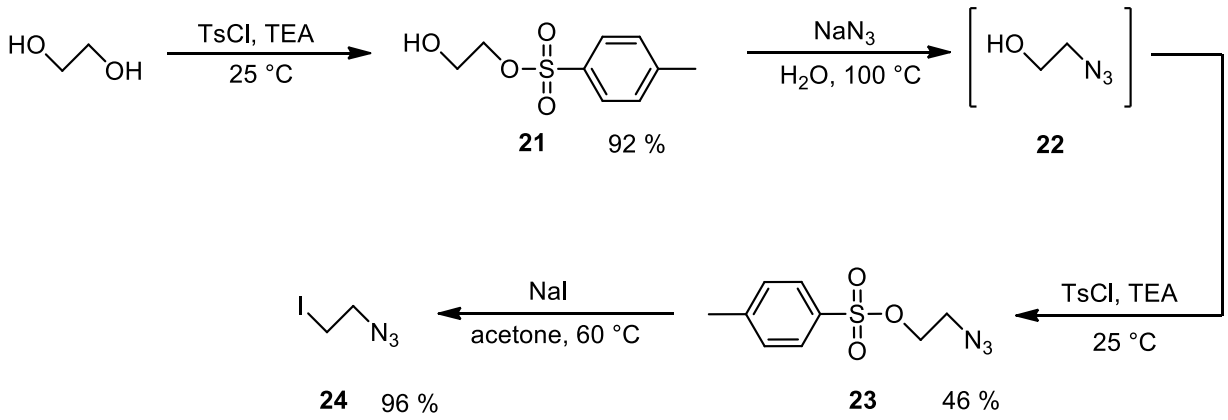
Scheme 7. Synthesis of PEMPDA CD derivatives.

4.1.4 Synthesis of CD derivatives monosubstituted with quaternary diamine bearing azidoethane function

In the next part of the synthetic work, a novel series of permanently charged CD derivatives bearing an azide function were prepared. There are two main reasons for the choice of incorporating an azide functional group: a) it is a relatively stable function which allows the attachment of various substituted acetylenes via a robust Cu(I)-catalyzed azide-

alkyne cycloaddition (CuAAC), sometimes also referred to as "click" reaction[150] and b) azide group exhibits very distinctive and intensive absorption band in the infrared (IR) spectra, which could be potentially used to monitor the deposition of the derivative on a solid support.

The strategy of introducing the azide function by preparing suitable alkylation agent to be used for the quaternization of intermediates **9** and **15** in the place of MeI was proposed. Firstly, the 1-azido-2-iodoethane **24** was prepared by simple reaction sequence which consisted of 4 reaction steps (Scheme 8), which were previously published in the literature. In the first step, one hydroxyl of ethylene glycol was substituted for tosylate to yield 91 % of 2-hydroxyethyl-4-methylbenzenesulfonate **21**. Next, 2-azidoethanol **22** was prepared by nucleophilic substitution with sodium azide[151], the product was not isolated due to its possible explosive character and was directly subjected to the tosylation to yield 2-azidoethyl-4-methylbenzensulfonate **23**[151]. Finally compound **24** was prepared by Finkelstein reaction in 96 % yield[152].

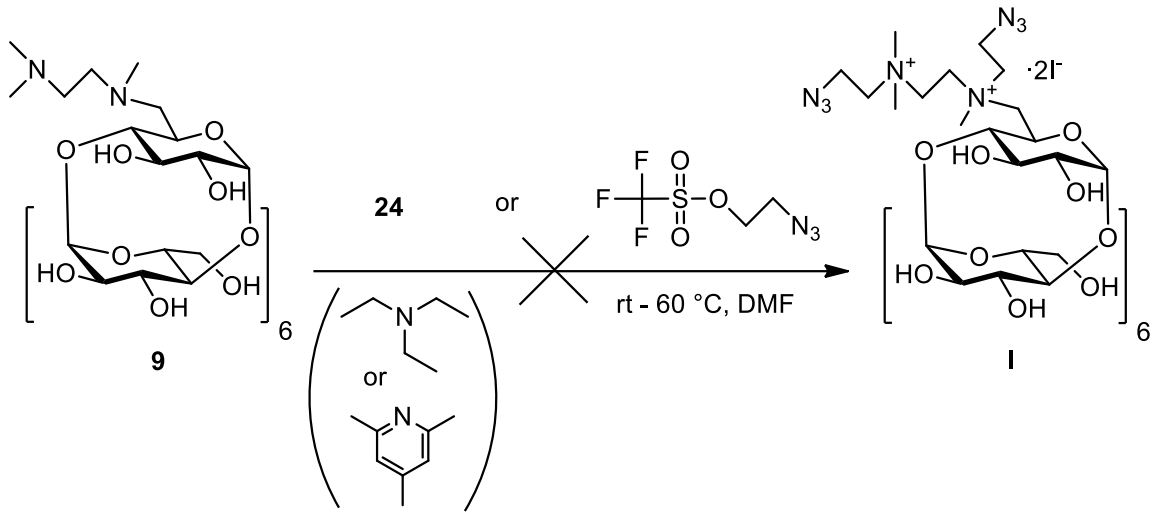


Scheme 8. Synthesis of 1-azido-2-iodoethane.

We supposed the quaternization of intermediates **9** by alkylation reagent **24** in dry DMF would lead to product **I**[a] (Scheme 9). Unfortunately, MS spectra revealed that only one nitrogen atom gets substituted. Different conditions were tested to achieve the desired attachment of two azidoethane groups. Up to 50 equivalents of **24**, or even more reactive 2-azidoethyl trifluoromethanesulfonate, different sterically hindered bases (TEA, collidine)

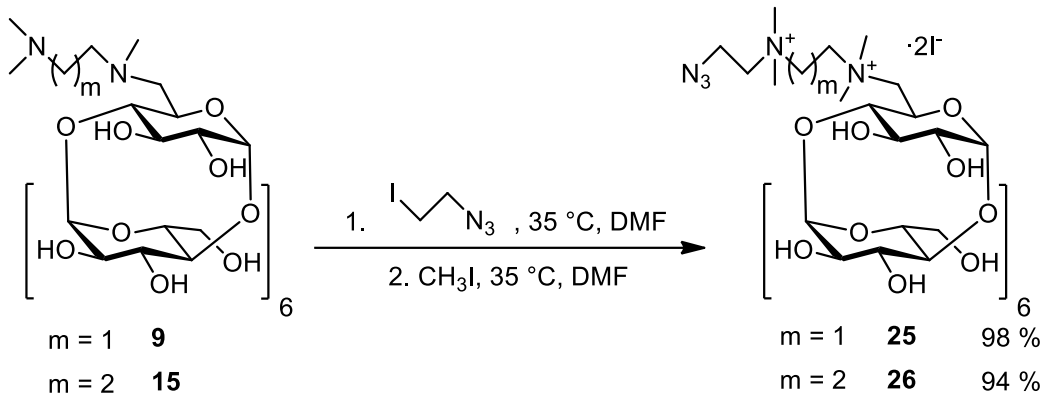
[a] Greek numbering is applied for labeling of products which could not be prepared.

and temperatures up to 60 °C were attempted. No conditions afforded even traces of the desired product **I**.



Scheme 9. Attempts to prepare analog of PEMEDA-β-CD with two azidoethane functional groups.

A new strategy for the preparation of azide-equipped PEMEDA- and PEMPDA-β-CD consisted of a two-step procedure, which involved attachment of **24** in the first step and quaternization by MeI in the second step, without the need of isolation of the intermediate (Scheme 10). This approach afforded compounds **25** and **26** in 98 % and 94 % yields respectively. The structure of the compounds **25** and **26** (especially the location of the substituent and the position of the azidoethane function on the terminal nitrogen were confirmed by a combination of 2D NMR techniques. The assignment is discussed in a special section within the experimental part.

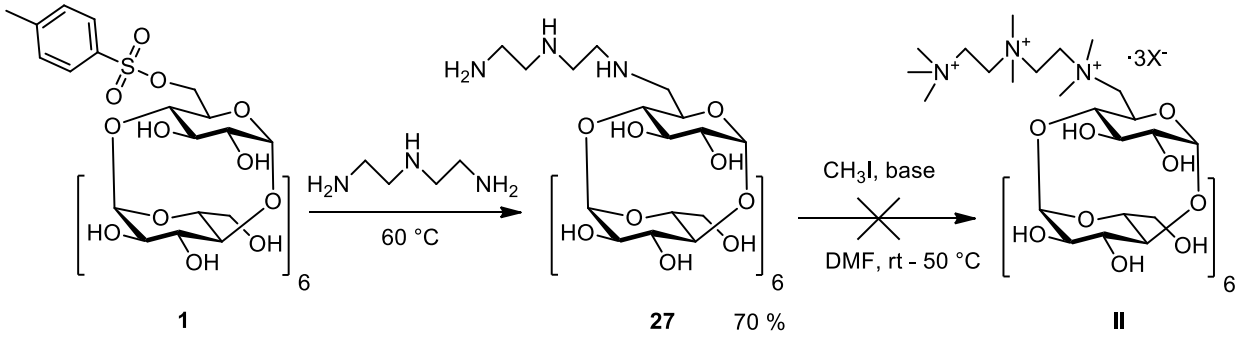


Scheme 10. Synthesis of azidoethane-containing derivatives of PEMEDA and PEMPDA-β-CD.

4.1.5 Synthesis of CD derivatives monosubstituted with quaternary triamine

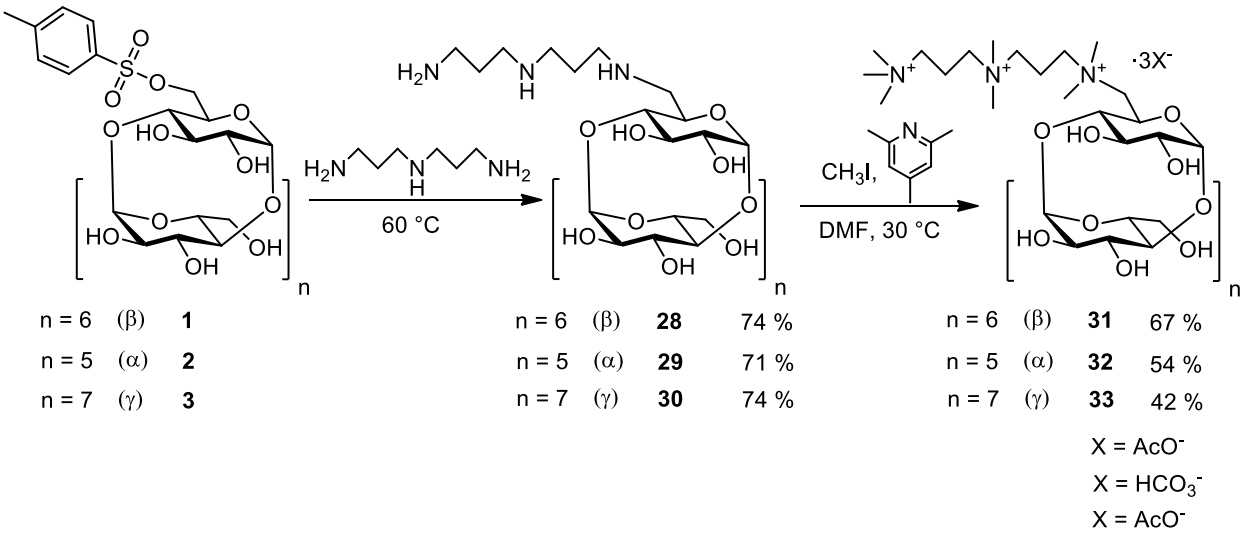
To finish the series of positively charged monosubstituted CD derivatives, the analogs bearing substituent with three tetraalkylammonium groups in position 6 were prepared. These derivatives were expected to have the highest affinity for the anionic solid surface and would form stable assemblies. The following strategy for the synthesis was selected: in the first step the according CD tosylate reacts with triamine, the formed intermediate is then quaternized by MeI to afford the desired product. The dry mono-Ts-β-CD **1** reacted with the diethylenetriamine to yield 70 % of 6^I-((2-((2-aminoethyl)amino)ethyl)amino)-6^I-deoxy-β-cyclodextrin **27** (Scheme 11). Again, solvent-free reaction conditions, where liquid triamine serves as a solvent, were employed to prevent undesired side reaction. Similar conditions for synthesis of **27** were published by Tabushi (for β-CD only) in year 1977.[153] Unreacted triamine was removed from the reaction by vacuum distillation and recycled. Product **27** was purified on a column of strong cation exchanger in H^+ form. Next, the methylation of **27** was carried out, employing MeI and sterically hindered base, but unfortunately, the reaction did not give product **II** (Scheme 11). Different amounts of MeI along with low nucleophilic / sterically hindered bases (K_2CO_3; 2,6-lutidine; 2,4,6-collidine and DBU) were tested as well as higher temperatures (up to 50 °C). But only partially methylated products along with products of Hofmann elimination of **II** were detected. The negative outcome of this conversion was reasoned by the sterical hindrance and proximity of the charged nitrogens atoms, causing an electrostatic repulsion which probably prevents completion of the product

formation. Similarly, as in the case of PEMEDA- derivatives, **27** is vulnerable toward decomposition via Hofmann elimination.



Scheme 11. Unsuccessful attempt to synthesize β-CD derivative bearing methylated diethylenetriamine.

The lack of success with preparing the PEMEDA- analog with three permanent positive charges (**II**) guided us toward the strategy employing more suitable triamine bis(3-aminopropyl)-amine (*N'*-(3-aminopropyl)propane-1,3-diamine, norspermidine). The reaction of starting tosylates with bis(3-aminopropyl)-amine **1**-**3** gave intermediates **29**-**30** in reasonable yields ranging from 71 to 74 % (Scheme 12). Product **30** contained some traces of impurities. Purification on silica gel column gave the pure compound **30** in the form of triacetate salt. The methylation of intermediates **29**-**30** (which contain nitrogen heteroatoms separated by longer propylene linker) proved to be more efficient and afforded the products **31**-**33** in good yields (Scheme 12). Full conversion was reached after 20 h at 30 °C when 100 eq of MeI and 60 eq of 2,4,6-collidine were used. The product **31** was efficiently purified on a column packed with weak cation exchanger resin, containing carboxylic groups. α- and γ-CD analogs **32** and **33** were obtained as triacetates, for they had to be purified on a short column of silica gel with HOAc containing eluent. Structures of the compounds bearing triamine moiety (especially to which nitrogen atom is the C6 CD atom attached) were confirmed by a combination of 2D NMR techniques. From the measurement, it follows, that contrary to the higher reactivity of secondary amine, the substituent is attached by the terminal nitrogen atom, most probably due to the steric factors. The assignment is discussed in a special section within Experimental part 6.1.

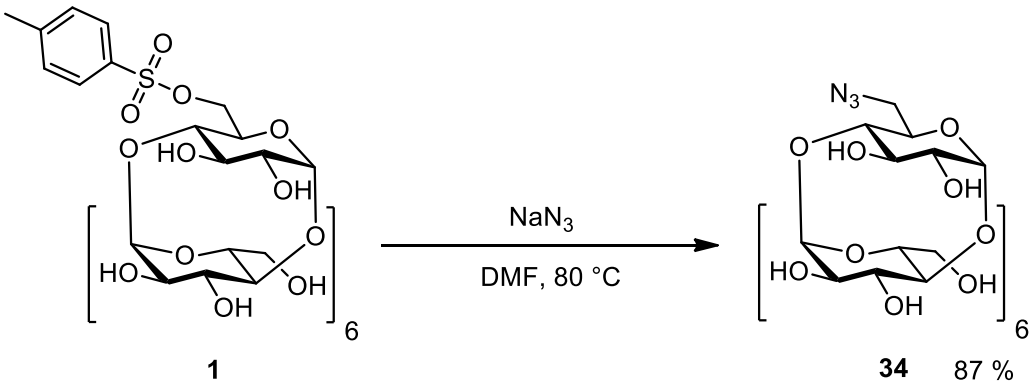


Scheme 12. Synthesis of CD derivatives monosubstituted with quaternary triamine moiety.

4.1.6 Synthesis of tetraalkylammonium CD derivatives with variable linker length by 1,3-dipolar cycloaddition

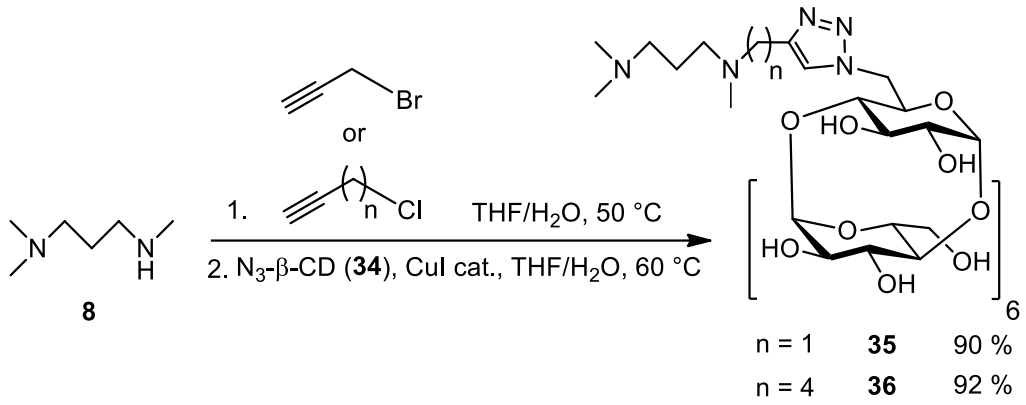
Since the PEMPDA-β-CD proved to be the most stable and easiest to prepare derivative, we decided to synthesize novel CD derivatives with PEMPDA- moiety attached to the CD core by a linker with variable length. This variability in the distance of the permanently charged dicationic functional group from the CD macrocycle should assure different distance of the CD backbone from the solid support, which might be important for the resulting topology of the final assembly and its complexation properties. The synthetic strategy which was proposed involved an attachment of the acetylenic diamine to the monosubstituted CD azide via CuAAC, which is a copper(I)-catalyzed modification of the 1,3-dipolar Huisgen cycloaddition, also referred to as "click" reaction.[150]

In the first reaction, the 6^I-azido-6^I-deoxy-β-CD **34** (N_3-β-CD) was prepared by the modification of the published procedure[154] from mono-Ts-β-CD **1** and sodium azide (Scheme 13). The improvement of the procedure resides in the purification step. The product **34** was precipitated, directly from the reaction mixture by pouring it into 70 % acetone-water mixture, filtered, and washed by 70 % acetone-water. This improvement assures no residual NaN_3 to be present in the final product. Pure compound **34** was obtained in 87 % yield, without a need of chromatographic purification.



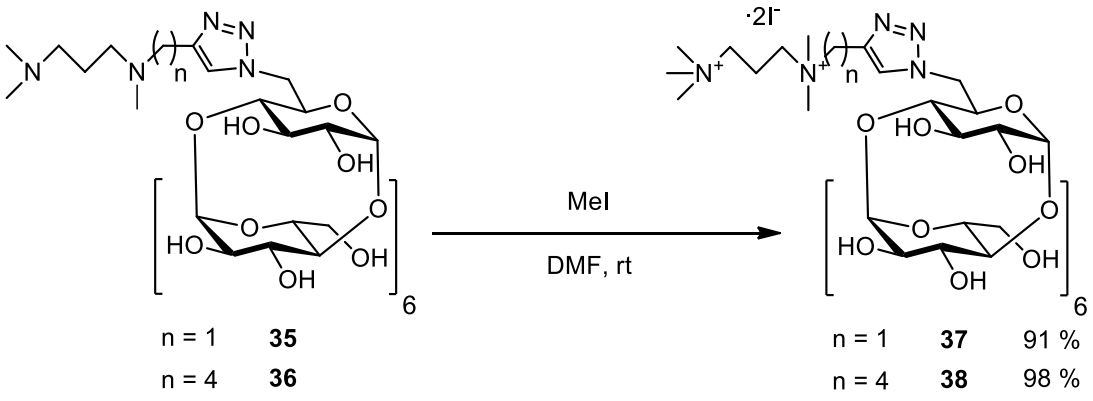
Scheme 13. Synthesis of N_3-β-CD.

The subsequent conversion was carried out as two-step one-pot reaction. In the first step either propargyl bromide or 6-chloro-1-hexyne is used as an electrophilic reagent to react with the diamine **8** to yield acetylenic diamine intermediate (Scheme 14). This product was not isolated but used directly in the second step, which is the copper (I) catalyzed click reaction with **34**. After some optimization of the reaction conditions, as thorough degassing of the THF/water solvent by freeze-pump-thaw method and fine tuning of the reagent ratios, the full conversion was reached overnight with only 10 molar % of the CuI catalyst. The reaction workup consisted of precipitation of the product from acetone, purification on the column of strong cation exchanger resin in H^+ form. There are several advantages of the separation on cation exchanger column. All non-basic byproducts can be removed by washing the column with water; the product is eluted by elution mixtures containing increasing concentration of aqueous ammonia and ammonium hydrogencarbonate. The faith of blue-colored copper catalyst can be conveniently monitored by naked eye, and usually it resides on the beginning of the column. After the separation, the resin can be recycled and used repeatedly. Pure products **35** and **36** were obtained in high yields of 90 % and 92 % respectively.



Scheme 14. Preparation of the tertiary diamine intermediates via one-pot reaction.

The final reaction toward the PEMPDA- analogs with variable linker length is the quaternization using MeI (Scheme 15). Similarly, as in the before-mentioned cases, the reaction was carried out in DMF, at rt. Product **35** contains methylene linker and was obtained in 91 % yield in the form of diiodide. Analog **36** has the PEMPDA- moiety attached by butylene linker and was obtained in diiodide form, in nearly quantitative yield of 98 %. Described reaction pathways for compounds **35** and **36** proved to be very efficient and serve as examples for the preparation of permanently charged cationic derivatives of CDs with a modifiable distance of the substituent from the CD scaffold. By employing alkyl halides with different alkyl chain length, derivatives with variable linker length can be prepared.



Scheme 15. Final quaternization of intermediate tertiary amine derivatives of CD with variable linker lenght using MeI.

4.2 Properties of the prepared derivatives

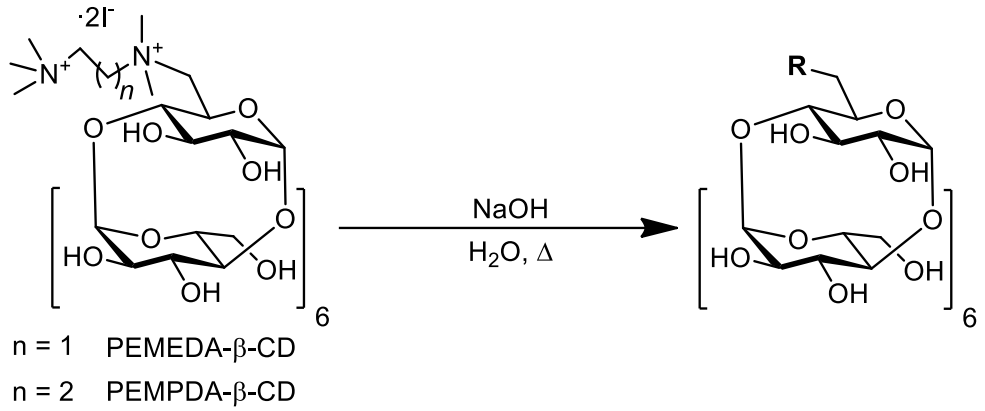
The following parts of the thesis will be focused on the determination of important properties of some of the prepared monosubstituted tetraalkylammonium CD derivatives. The results of the analyses should serve us as a valuable indication about the feasibility of the proposed utilization of the derivatives, for the construction of advanced systems for transdermal administration of topical drugs. Some of the findings which will be introduced in the following chapters are part of the manuscript published by our group.[122]

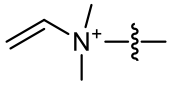
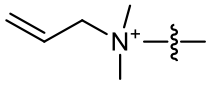
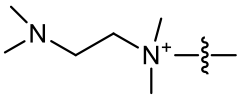
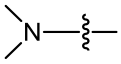
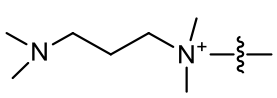
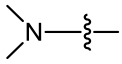
4.2.1 Thermal stability of PEMEDA- and PEMPDA-β-CD

In section 4.1.3 it was mentioned, that we have encountered a partial decomposition of our final products with two permanent positive charges, when subjected to temperatures above 50 °C, which was attributed to the decomposition via Hofmann elimination. We decided to investigate the stability of two selected analogs PEMEDA- and PEMPDA-β-CD. These two species were selected as the most favorable candidates for future utilization, because of the availability of the starting material (β-CD has the lowest price out of the three native CDs), due to the highest yields and chromatography-free purification methods.

The first experiment was designed to estimate the difference in the stability of the two analogs and to isolate, separate and characterize the degradation products. The preliminary experiment setup consisted of heating the aqueous solution of both isomers to 80 °C with 1 equivalent of NaOH. The decomposition process was monitored by TLC and revealed higher stability of PEMPDA-β-CD derivative. The TLC after 20 h showed a spot of the starting compound in the case of PEMPDA-β-CD, while the degradation of PEMEDA-β-CD was already complete at that time. This experiment gave us the information about the difference in the thermal stability of the two derivatives. The higher resistance of the PEMPDA-β-CD was presumed, due to the larger distance between the positively charged nitrogen centers. In the next step, we performed total decomposition of the two analogs in higher scale to identify the decomposition products. We managed to separate the degradation products on the silica gel column and identify them by ESI-MS (Scheme 16). Major products of the thermal

degradation were the corresponding olefins, which confirmed the main pathway to be the Hofmann elimination.

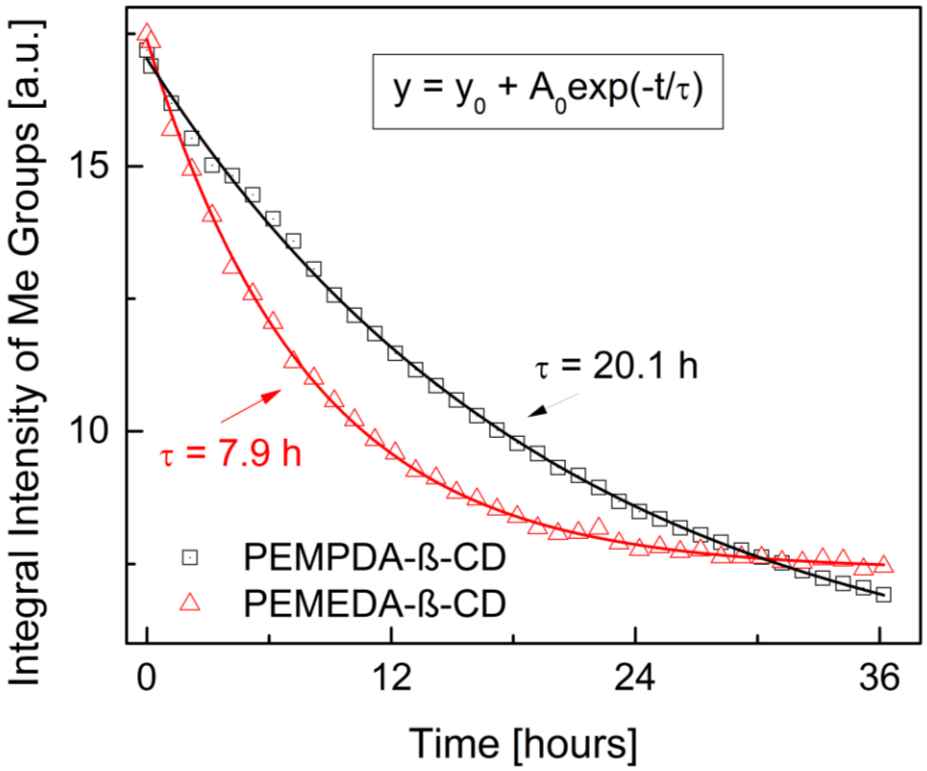


R	n = 1	n = 2
major		
minor	 	 

Scheme 16. Thermal decomposition of PEMEDA- and PEMPDA-β-CD with the decomposition products as characterized by MS.

We decided to investigate further the kinetics of the thermal decomposition of PEMEDA- and PEMPDA-β-CD diiodides determine half-lifes of the two compounds. ^{1}H NMR experiment at elevated temperature (50 °C) was set up. Spectra of the sample solutions in D_2O, with 20 equivalents of NaOH, were acquired every hour in the course of 36 hours. The decreasing values of the integral intensity of CH_3 protons of the substituent were plotted against time and kinetic curves for each derivative were obtained (Graph 1). The resulting kinetic profiles show clearly the PEMPDA-β-CD to be the more stable derivative. The values of decomposition half-life time constants were calculated by fitting the experimental data by monoexponential function and are 7.9 h and 20.1 h for the PEMEDA-β-CD and PEMPDA-β-CD, respectively. The detailed experimental setting of the NMR experiment along with the collected spectra is given in section 6.3. The PEMPDA-β-CD was selected as a suitable

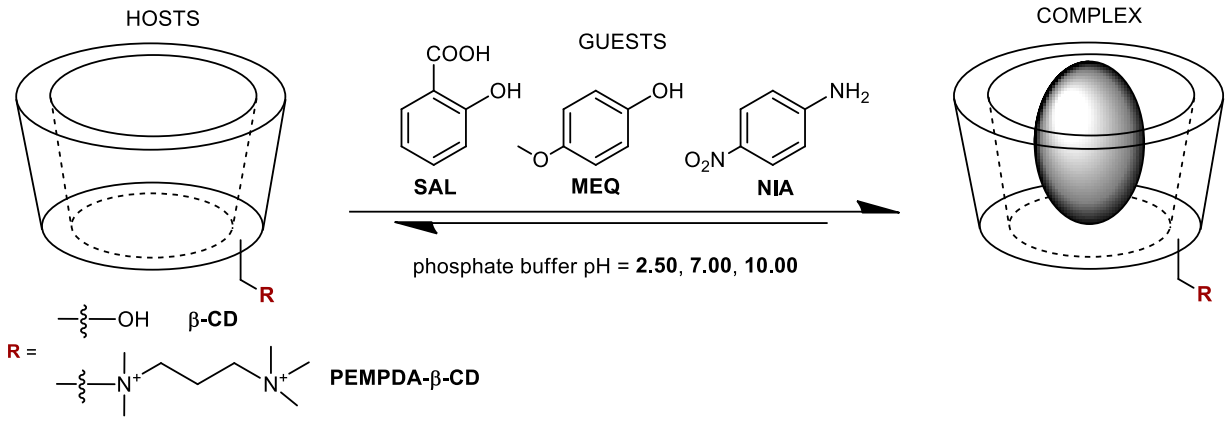
candidate for further measurements due to its decomposition half-life to be more than double when compared to the PEMEDA- analog.



Graph 1. Decomposition kinetics of PEMEDA- and PEMPDA-β-CD at 50°C as determined by ^{1}H NMR thermal experiment.

4.2.2 Inclusion properties of PEMPDA-β-CD in solution

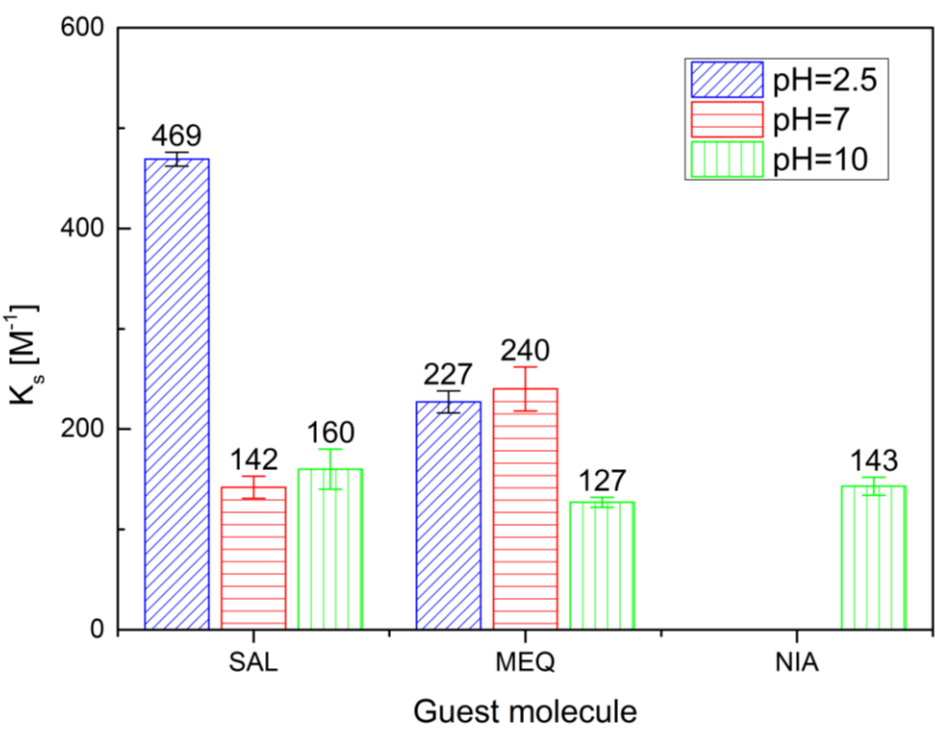
Next experiment was about to reveal whether the permanently charged cationic CD derivatives, represented by PEMPDA-β-CD diiodide, maintain the ability to form inclusion complexes with organic aromatic compounds. The values of stability constants (K_s) of PEMPDA-β-CD with series of three aromatic guest molecules (salicylic acid – SAL, *p*-methoxyphenol – MEQ, *p*-nitroaniline – NIA) at three different pH values (2.50, 7.00, 10.00) were measured and compared to the ones obtained for native β-CD (Scheme 17).



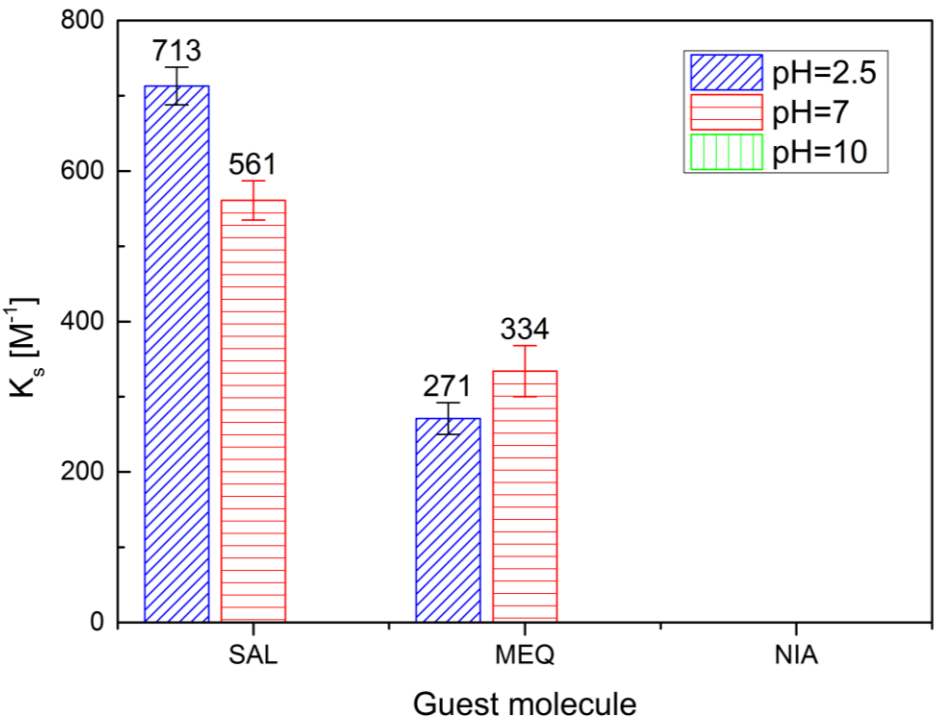
Scheme 17. Host and guest molecules employed in K_s determination in solution at different pH.

Various techniques for determination of the K_s were tested before the most suitable method was found. The most easily accessible method was the UV/Vis spectrometry. The procedure as described in section 2.2.3.b) was employed. The concentration of the guest molecule was kept constant, while the CD concentration was gradually increased. Increasing absorbance in the absorption maxima of the guest indicated the complex formation. Typical setup consisted of three experiments – 1) rough estimation of the K_s to set the concentration ranges, 2) stoichiometry determination by the Job method and 3) actual titration of the guest by CD solution. The first problem we came across was the fact, that there was some slight absorbance of the host molecule in the range of the absorption maxima of guest, which makes it impossible to proceed. In the case of β-CD we managed to avoid the absorbance by extensive heating of the aqueous solution with subsequent recrystallization. The principle of this purification method is a steam distillation of the impurities absorbed by CD from atmosphere but in the case of PEMPDA-β-CD we were not successful. Even though the absorbance of the host was very low, it disabled the measurement of K_s by UV/Vis spectrometry. The second attempted method was the ^{1}H NMR spectroscopy, which proved to be inefficient as well. The experimental setup also consisted from the three-step procedure as in the case of UV/Vis. The main hindrance proved to be the low aqueous solubility of the aromatic guest molecules. The sensitivity of the NMR is rather low and considerably higher concentrations of the titrated solutions were needed. In our case it was not possible to achieve appreciable signal to noise ratio, to be able to determine the changes in the chemical shifts of the signals of some guests to calculate the K_s with sufficient accuracy.

The solution for a suitable procedure was found to be the microcalorimetric method ITC. This automated method is sensitive enough to work with very low concentrations of aqueous solution of individual components. It also provides full thermodynamic profile along with the complex stoichiometry and K_s values in a single run, which takes about 45 minutes. The detailed experimental conditions can be found in section 6.3. The K_s values for β-CD and PEMPDA-β-CD diiodide from ITC measurements are summarized in Graph 2 and 3 respectively. Stoichiometries of all of the employed complexes were found to be 1:1. The K_s calculated for β-CD are in agreement with the literature[155,156,157,158]. The most stable complex of β-CD was obtained with SAL at pH = 2.50 (K_s = 469±7 M^{-1}), while no association was detected with SAL and MEQ at pH = 10.00. NIA was complexed by β-CD only at pH = 10.00 (Graph 2). The collected data comply with the concept, that uncharged neutral molecules show the largest affinity toward the CD's lipophilic inner cavity and form the most stable inclusion complexes. In the case of positively charged PEMPDA-β-CD, again the highest K_s value was received for SAL at pH = 2.50 (Graph 3). This may be explained by the contribution of the ion-dipole interaction between the positively charged substituent of the host and carboxylic group of SAL, similarly as described in the literature[159]. Also, the values of K_s of the PEMPDA-β-CD with MEQ in acidic and neutral solutions were higher than those obtained for the β-CD. Complexation of NIA with PEMPDA-β-CD was not observed as well as binding of SAL and MEQ at basic pH. In conclusion, we can state that monosubstituted derivative PEMPDA-β-CD is able to form inclusion complexes with SAL and MEQ at acidic and neutral pH, whose stabilities are superior to those of the native β-CD.



Graph 2. Stability constants for β-CD with SAL, MEQ and NIA obtained by ITC measurements.

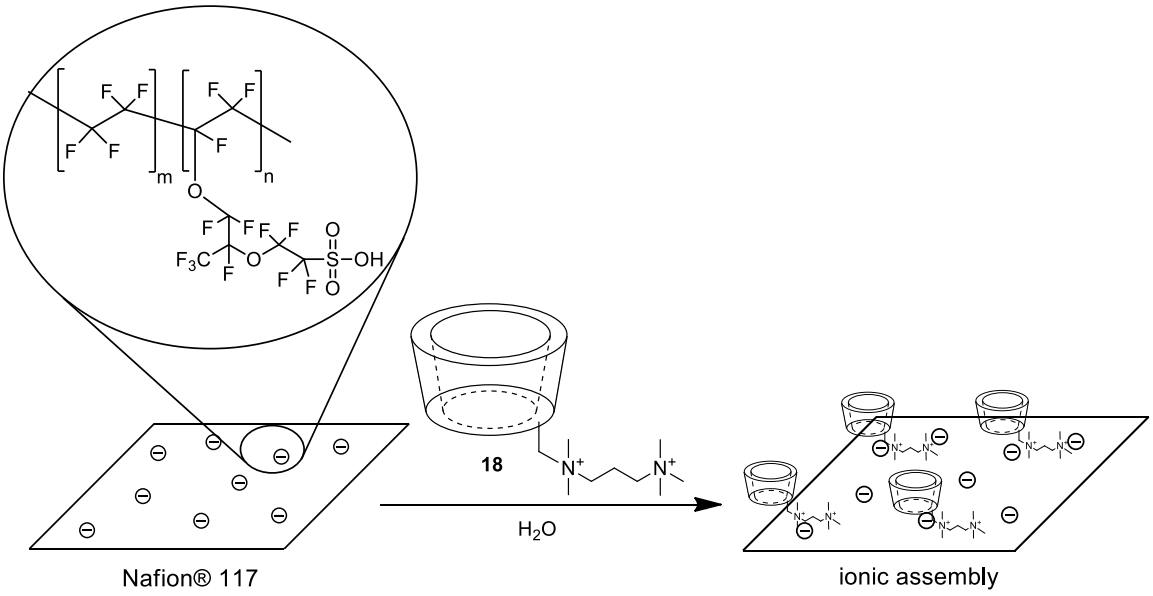


Graph 3. Stability constants for PEMPDA-β-CD with SAL, MEQ and NIA obtained by ITC measurements.

4.2.3 Immobilization of PEMPDA-β-CD on anionic surface via ionic self-assembly

We have verified, that cationic PEMPDA-β-CD maintains its complexation abilities and can form relatively stable inclusion complexes with model aromatic compounds in acidic and neutral solutions. The next task was to deposit the macrocyclic host onto a solid support via ionic interactions (Scheme 18). As the model solid surface, the Nafion® 117 was selected. Nafion® (E.I. DuPont De Nemours and Company) is a sulfonated tetrafluoroethylene fluoropolymer-copolymer, especially favorable for our purpose because of its defined structure, with a known number of $-SO_3H$ groups (equivalent weight – EW = 1100 $g.mol^{-1}$) and the absence of aromatic groups, which could be encapsulated by CD cavity and negatively influence complexation properties of the deposited CD.

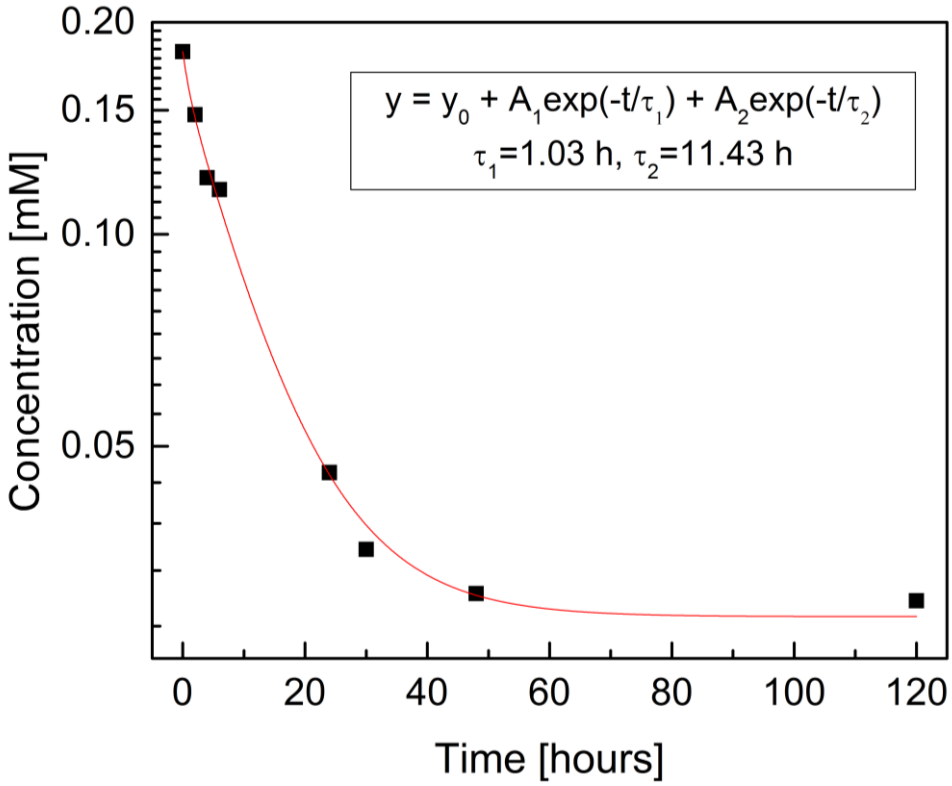
Preliminary results were obtained from the deposition of PEMPDA-β-CD on strong cation exchange resin (Dowex® 50), which has a chemical constitution of sulfonated polystyrene. It showed high deposition of cationic PEMPDA-β-CD, with a remarkable stability of the assembly. We were not able to desorb the deposited derivative from the resin by any possible means. The hindrance of using the Dowex® 50 resin as the model solid support was the observation that native β-CD is absorbed as well. This can be reasoned by the formation of the inclusion complex with the aromatic sulfonated polystyrene moieties present on the resin. At this point, we switched for the Nafion® 117 with aliphatic backbone. First experiments consisted of stirring the cut-out of the foil (100 mm^2, 35 mg) in H^+ form in the aqueous solution of the PEMPDA-β-CD and provisional data were collected by monitoring the decrease of the concentration by TLC and gravimetry (details in section 6.3). TLC indicated completion of the immobilization after 48 h and the amount of deposited PEMPDA-β-CD diiodide determined by gravimetry was 5.0 mg.



Scheme 18. Deposition of PEMPDA-β-CD onto solid surface (Nafion® 117).

To obtain more reliable and precise information about the amount of immobilized PEMPDA-β-CD together with deposition kinetics, we decided to monitor the concentration decay by direct injection of the reaction mixture into the Evaporative light scattering detector (ELSD). The initial concentration of PEMPDA-β-CD was 0.2 mM (5.0 mg dissolved in 16.5 ml H_2O). Nafion®117 H^+ foil (100 mm^2) immersed in the solution with continuous stirring and the deposition rate was monitored by injection of the reaction mixture in the ELSD input. Measured peak areas (area under curve - AUC) were converted to the concentrations (using a linear calibration) and plotted against time to obtain the deposition kinetics (Graph 4). From the log *c* vs. *t* plot, it follows, that the deposition kinetics is governed by a two-exponential process. The deposition time constants were calculated by fitting the experimental data by two exponential decay functions. Two time constants – τ_1 = 1.03 h and τ_2 = 11.43 h were obtained. The residual equilibrium concentration was attributed to the signal caused by nascent HI in the solution. The hydroiodic acid is formed as the result of the deposition process. This fact was supported by the additional experiment which consisted of injecting the standard solution of HI in the ELSD. Overall 10 mol % of available $–SO_3H$ groups were saturated by cationic CD derivative (calculated with assumed 1:1 stoichiometry, i.e. one CD molecule interacts with one SO_3H group). If we have presumed stoichiometry of the $–SO_3H$ groups:CD to be 2:1, which would mean both tetraalkylammonium groups of PEMPDA-β-CD interact with $–SO_3H$ groups of the solid support, the coverage would be 20 mol %. These results were

confirmed by repeated experiments and composition of the assembly was reproducible, also when using Nafion®117 in NH_4^+ form.

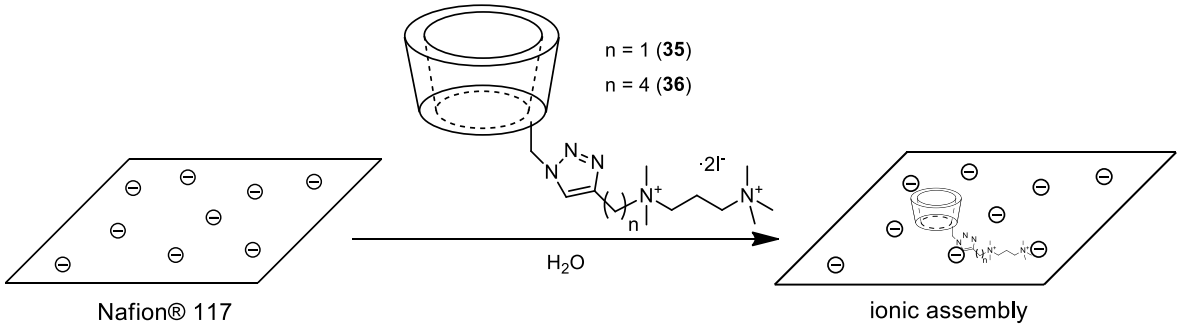


Graph 4. Deposition kinetics of PEMPDA-β-CD onto Nafion® 117 as obtained from ELSD detection of the decreasing concentration in the solution.

Pilot studies which included the attempts of deposition of PEMPDA-β-CD on the practicable solid support, such as anionic nanofabric, were not successful. Different batches of plasma-treated electro-spun nanofabrics based on polyurethane or acetylcellulose were tested. The novel anionic nanofabric materials were prepared by the Spur Company in Zlín. One of the uneasy tasks was to validate the presence of negative charges on the polymeric material, which was also not stable in time. Further research in this area was beyond the scope of this thesis and would require more time to be properly investigated.

4.2.4 Immobilization of tetraalkylammonium CD derivatives with variable linker length

After the successful anchoring of the PEMPDA-β-CD on the solid support of Nafion® 117 we decided to apply the identical procedure to the analogs **35** and **36**, which were prepared by the "click" reaction and had variable linker length (Figure 14). We were hoping to discover higher surface coverage, especially for compound **36**, due to the enhanced spatial accessibility and flexibility of the cationic anchor.



Scheme 19. Deposition of tetraalkylammonium CD derivatives with variable linker length onto solid surface (Nafion® 117).

To our disappointment, by gravimetric and TLC monitoring of the immobilization process, we discovered that the deposition ability of **35** and **36** is significantly lower when compared to PEMPDA-β-CD. The procedure for deposition of **18** onto the surface of Nafion® 117 using gravimetric quantification of immobilized material (as described in section 4.2.3) was followed with hosts **35** and **36**. In the case of PEMPDA- analog with methylene linker **35**, the TLC showed only traces of immobilized host after 48 h. Second analog with butylene linker **36** appeared to have lost the anchoring ability via electrostatic interactions completely. Possible explanation for the different behavior of cationic CD host with more flexible linker is a certain hindrance of the charged part of the substituent by CD cavity. This effect may be caused by self-inclusion process where either intramolecular complexes or intermolecular oligomers are formed (Figure 15). The only weak point of this hypothesis is the fact that self-inclusion complexes have been reported mostly for derivatives bearing a lipophilic moiety such as aliphatic or aromatic hydrocarbons.[160]

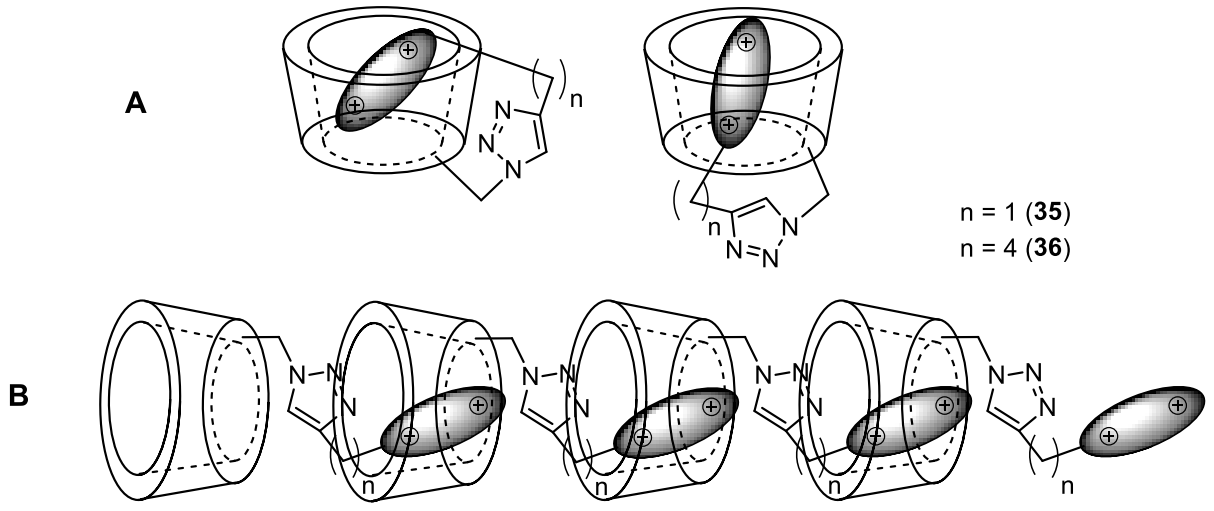


Figure 14. Plausible structures of A) Intramolecular and B) Intermolecular self-inclusion complexes of cationic hosts **35** and **36**.

As a first step toward gathering the evidence to support our theory of the formation of self-inclusion complexes was an introduction of a competitive guest. Competitive guest is a molecule which forms a stable supramolecular complex and has the ability to displace a weaker guest (in our case the cationic moiety) from the cavity. Sodium 1-adamantanecarboxylate (AdCOONa) was a compound of the first choice, due to its large K_s with the β-CD cavity and concurrently reasonable aqueous solubility. Next, the anchoring experiment was carried out in parallel with hosts **18**, **35** and **36** with the addition of AdCOONa in solution (10 eq). TLC monitoring of the reaction mixtures showed complete immobilization of **18** after 2 days, which is in agreement with previous results. An additional step was added to the standard protocol, which assures the extraction of encapsulated AdCOONa from the host's cavities (refluxing in MeOH for 2 h). Host **35** was completely adsorbed after 7 days, which clearly indicates the functionality of the competitive guest. This is an evident improvement from previous measurements, where only a minute amount of **35** got anchored in the absence of AdCOONa. Interestingly, in the case of **36**, again none of the material was adsorbed onto the solid support after 7 days. Experiments were repeated several times with identical results. From the obtained results, we can deduce certain trends. Both of the PEMPDA-β-CD analogs with longer linker length cannot be deposited on the anionic surface without using a competitive guest. Host **35** gets anchored after significantly longer time when compared to **18** (7 d vs. 48 h respectively) and does so only in the presence of the

strong competitive guest. Binding of derivative with longest butylene linker **36** was not detected at all.

To support our theory of formation self-inclusion complexes of **35** and **36,** we decided to submit aqueous solutions of a guest to Dynamic Light Scattering analysis (DLS). This method allows detection of particle size in solution and should reveal a presence of some larger aggregates, which would be a confirmation of intermolecular host linkage. Surprisingly, the results of the DLS measurement summarized in Table 4 reject the presence of aggregates of larger size. The majority of found particles have a size of 1-2 nm, which corresponds to hosts in the form of monomers.

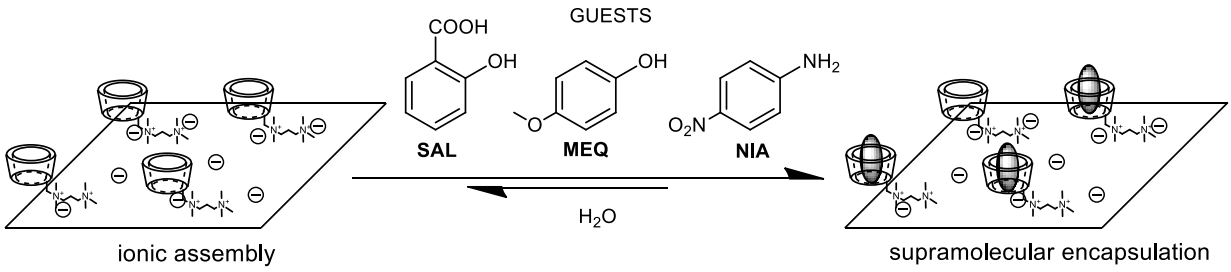
Table 4. Size distribution of particles in aqueous solutions of **35** and **36** measured by DLS.

host	*c* (host) mM	size distribution by intensity (*d*, nm)	size distribution by volume (*d,* nm)
35	10	2.7 (92 %), 2681 (8 %)	1.4 (100 %)
35	50	1.8 (91 %), 981 (9 %)	1.1 (100 %)
36	10	1.6 (51 %), 626 (49 %)	1.0 (100 %)
36	50	4.5 (100 %)	1.5 (100 %)

2D NMR techniques were employed to confirm the intramolecular complex formation. Rotating frame nuclear Overhauser effect spectroscopy (ROESY) spectra of **35** and **36** were acquired, to check for spatial contacts of C-H groups of the inner CD cavity with any proton of the positively charged substituent. Interestingly, no such correlations have been found. Nuclear Overhauser effect spectroscopy (NOESY) spectra of **35** and **36** were measured to provide complete information. Again, no contacts indicating a shielding of the cationic moiety by the CD cavity have been found. These results have left us with some unanswered questions about the unexpected behavior of the CD derivatives with a longer linker attached via triazole moiety.

4.2.5 Inclusion of model guest molecules from the solution into the cavities of Nafion®-bound PEMPDA-β-CD

Finally, the ability of the ionic assembly of PEMPDA-β-CD anchored on the surface of Nafion®117 to accommodate a small series of simple aromatic guest molecules was studied. The same three model guest molecules (SAL, MEQ, and NIA) were employed as in the complexation study in solution, described in the section 4.2.2. This made results comparable with the K_s values obtained from ITC measurement in solution. The cut-outs of Nafion® 117 foil in NH_4^+ form (100 mm^2, 35 mg) with known amount of deposited PEMPDA-β-CD (5 mg) were stirred in the aqueous solution of the guest for 20 h, to reach equilibrium inclusion (Figure 16). In the next step, the assembly was washed by H_2O (5 × 3 ml) to remove the unspecifically bound guest and then the included guest was extracted from cavities by MeOH (1 × 3ml). The amount of complexed guest was quantified by UV/Vis spectrometry of the MeOH extracts against the blank sample which consisted of Nafion®117 with no deposited PEMPDA-β-CD. This method proved to be very useful and simple tool for determination of the amount of included guests in the cavities of CD anchored to the surface.



Scheme 20. Inclusion of three model guests into the cavities of immobilized PEMPDA-β-CD.

To be able to quantify the obtained results in a way, which clearly displays the differences of inclusion of the three guests, we used a unit of measure, which describes the extent of inclusion of the guest in Nafion®-bound PEMPDA-β-CD. *ROC* (ratio of occupied cavities) is defined as a molar percentage of the cavities forming inclusion complex (Equation 1). In other words, the final number describes the percentage of the cavities saturated by the guest molecule.

$$ROC = \frac{n(\text{extr. guest}) - n(\text{extr. guest blank})}{n(\text{PEMPDA} - \beta - \text{CD})}$$

Equation 1. Definition of ROC, where *n*(extr. guest) is the number of moles of guest extracted by MeOH from the Nafion®-bound PEMPDA-β-CD, *n*(extr. guest blank) is the number of moles of guest extracted by MeOH from the unmodified Nafion® and *n*(PEMPDA-β-CD) is the number of moles of deposited PEMPDA-β-CD.

The calculated values are summarized in Table 5. The highest *ROC* was obtained for SAL (34.5 %) which corresponds very well with the data from the measurement of complexation in the solution. *ROC* of MEQ (17.5 %) is proportionally lower and also correlates nicely with the data from solution. The maximal solubility of NIA is lower than the ones of SAL and MEQ; for this reason, we had to use a lower concentration of the incubation solution. When using the concentration of 3.20 mM we received the value of 10.3 %. It is apparent that *ROC* depends strongly on the initial concentration of the incubation solution. Binding in the solution with one-tenth of the maximum concentration of SAL or MEQ results in the *ROC* about ten times lower. In the case of NIA, the dependence of the inclusion on the guest concentration is even higher.

Table 5. Results of the inclusion of different guests on the Nafion®117 and PEMPDA-β-CD ionic assembly at neutral pH.

guest	*c* (guest) M	*n* (included guest) mol	*n* (CD on surface)	***ROC* %**
SAL	1.40E-02	1.14E-06	3.30E-06	**34.5**
SAL	1.40E-03	1.05E-07	3.30E-06	**3.2**
MEQ	1.40E-02	5.76E-07	3.30E-06	**17.5**
MEQ	1.40E-03	4.55E-08	3.30E-06	**1.4**
NIA	3.20E-03	3.40E-07	3.30E-06	**10.3**
NIA	3.20E-04	1.40E-08	3.30E-06	**0.4**

5. CONCLUSION

In the synthetic part of the thesis, a complete (α-, β-, γ-) series of positively charged monosubstituted CD derivatives was successfully prepared. Previously described procedures for synthesis of key starting materials (6-*O*-tosyl- monosubstituted derivatives of α-, β- and γ-CDs) were optimized to afford products in highest possible yields and purity. Reactions of monotosylates with tertiary or secondary amines, diamines, and triamines, followed by quaternization with MeI, afforded CD derivatives bearing substituent with one, two, or three tetraalkylammonium groups, respectively. Additionally, cationic derivatives equipped with azidoethane function and two analogs of PEMPDA-β-CD with variable linker length (attached by CuAAC) were prepared. The majority of the prepared compounds were obtained in multigram scale by straightforward reaction sequences from commercially available and inexpensive starting materials. Most of the substitutions of monotosylated CDs by amine nucleophiles with subsequent methylation showed yields above 90 % without the need for any chromatographic purification steps. Prepared target compounds can be used as versatile hosts with variable cavity size and number of cationic sites, having the ability to be immobilized on an anionic surface. The possibility of using the cationic CD derivatives for preparation of novel chiral stationary phase for HPLC,[161] and as chiral selectors in CZE,[162] has been investigated.

The second part of the thesis is focused on the determination of properties of selected compounds. Thermal stability of two most promising monosubstituted bis(tetraalkylammonium) CD derivatives PEMEDA- and PEMPDA-β-CD was studied by ^{1}H NMR experiment at elevated temperature. Resulting kinetic profiles of thermal degradation revealed that the analog with longer propylene linker PEMPDA-β-CD is less vulnerable toward thermal decomposition caused by Hofmann elimination. The ability of PEMPDA-β-CD to form supramolecular complexes was studied by ITC using three model guest molecules (salicylic acid – SAL, *p*-methoxyphenol – MEQ, *p*-nitroaniline – NIA) at three pH values (2.50, 7.00 and 10.00). We discovered that PEMPDA-β-CD can encapsulate SAL and MEQ at acidic and neutral pH. Values of the calculated complex stability constants indicate the formation of complexes with comparable stabilities to the ones of native β-CD. A method for immobilization of PEMPDA-β-CD onto the solid surface of Nafion® 117, along

with the determination of the surface coverage and deposition kinetics by ELSD, was developed. Finally, the ability of PEMPDA-β-CD deposited on an anionic surface to encapsulate the three model guests was investigated. A straightforward method for quantification of the inclusion extent, using UV/Vis spectrometric measurement of MeOH washes, was introduced. Best results were obtained for SAL, where 34.5 % of the available cavities of the PEMPDA-β-CD were in the form of inclusion complexes. The amount of included guests in the PEMPDA-β-CD anchored onto a solid surface correlates quite nicely with the stability constants of the corresponding complexes in solution. Presented conclusions are promising for the intended application of supramolecular ionic assemblies of positively charged CD derivatives as drug delivery systems for controlled release of suitable bioactive compounds.

6. EXPERIMENTAL PART

6.1 Instruments, general methods and materials

^{1}H NMR spectra were acquired on Varian VNMRS 300 at 300 MHz and ^{13}C at 75 MHz. For determination of the substituent position, DEPT and 2D NMR measurements (H,H-COSY, HSQC, and HMBC) were performed on Bruker AVANCE III at 600 MHz (^{1}H) and 125 MHz (^{13}C). Samples were dissolved in D_2O with a drop of *tert*-butanol or DMSO-d_6 with few drops of CD_3COOD. All chemical shift values (δ) are reported in ppm and coupling constants in Hz. Signals of tetramethylsilane (for ^{1}H NMR) and $CDCl_3$ (for ^{13}C NMR, δ = 77.0 ppm) served as internal standards. Atoms of the CD glucose rings and substituents were labeled according to the established standards used in the publications regarding CD chemistry (Figure 16).

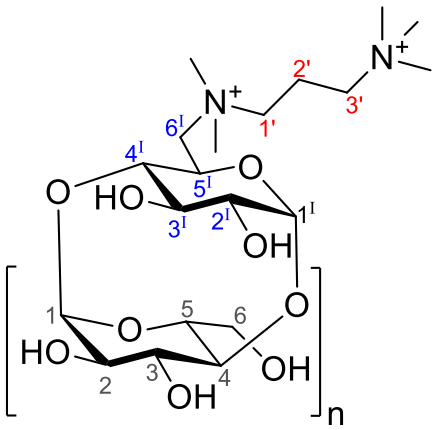


Figure 15. An example of the atom numbering in the NMR data listing

Mass spectra were obtained on Bruker ESQUIRE 3000 ES-ion trap instrument with electrospray ionization (ESI) in positive mode. All samples were dissolved in methanol.

Infrared spectroscopy was performed on Thermo Nicolet AVATAR 370 FT-IR instrument. Samples were prepared as a suspension with KBr and measured via DRIFT method. HRMS ESI spectra were acquired on Thermo Fisher Scientific LTQ Orbitrap XL instrument. Specific optical rotation was measured on Rudolph Research AUTOPOL III polarimeter at 589 nm (sodium D line) and values of $[\alpha]^{25}_{D}$ are reported together with used concentration (c, g/100

ml) and solvent. Melting points were determined on Kofler apparatus NAGEMA-RAPIDO with temperature gradient 4 °C/min. Thin layer chromatography (TLC) was performed on silica gel coated aluminum sheets DC-Alufolien Keisegel 60 F_{265} (Merck, Darmstadt, Germany). Dipping in 50 % H_2SO_4 with subsequent carbonization by a heat gun was used for spot detection for all CD derivatives. TLC detection by dipping in basic $KMnO_4$, which was prepared by dissolving of $KMnO_4$ (1.5 g), K_2CO_3 (10 g), 10 % NaOH (1.25 ml) in H_2O (100 ml), was used for all non-CD derivatives. Silica gel 60 (40-63 μm; Fluka, Neu-Ulm, Switzerland) was used to perform preparative flash column chromatography. Anhydrous DMF was prepared by distillation with P_2O_5 at reduced pressure and was stored over molecular sieves 3 Å under argon atmosphere. Anhydrous pyridine was prepared by standing over KOH for several days and subsequent distillation from CaH_2 under atmosphere of Ar. Molecular sieves were activated at 280 °C for 8 h under reduced pressure (1 Pa). Organic solvents were distilled before use. β- and γ-CD were purchased from WAKO Chemicals (Germany). α-CD was purchased from Wacker-chemie. Other reagents were purchased from common commercial sources and used without further purification unless otherwise noted.

For determination of the inclusion properties of prepared derivatives and binding to the solid surface, the following instruments and materials were used. ITC measurements were carried out on GE MicroCal^{TM} iTC_{200}. ELSD measurements were performed using Shimadzu LC-20AD HPLC pump and evaporative light scattering detector Alltech® 3300 ELSD. UV/Vis spectra were acquired on THERMO Spectronic Heλios Gamma. The hydrodynamic radius of particles, R_h, and the scattering intensity, I_s, were measured at a scattering angle of θ = 173º with a Zetasizer Nano-ZS instrument, Model ZEN3600 (Malvern Instruments, UK). H_2O and MeOH washes of the Nafion® were obtained using shaker Scientific Industries Vortex-Genie 2 at 1500 RPM. β-CD was purchased from WAKO Chemicals (Germany) and used without further purification. For UV/Vis measurements the β-CD was recrystallized from water before use (10 g of β-CD from 100 ml of H_2O). Salicylic acid (SAL), *p*-methoxyphenol (MEQ), *p*-nitroaniline (NIA) were purchased from Sigma-Aldrich. Their identity and purity was checked by ^{1}H NMR and were used without further purification. Nafion® 117 membrane was obtained from Ion Power, Munich, Germany.

6.2 Synthesis of compounds

6^{I}-*O*-*p*-Toluenesulfonyl-β-cyclodextrin (1). Compound **1** was prepared according to the published procedure[97] which was modified. The reaction of β-CD hydrate (23.00 g, 20.26 mmol) with Ts_2O (9.80 g, 30.12 mmol) and NaOH (10.00 g, 250.00 mmol) yielded mixture a of isomers (unsubstituted β-CD, product **1**, and multiply substituted isomers). Pure **1** (6.76 g, 5.24 mmol) was obtained after repeated (3 ×) crystallization in 10-fold excess (m/v) of 50 % MeOH. The overall yield of the reaction was 26 % of pure monotosylated isomer **1**.

m.p. 170 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +127.2° (c = 0.29, DMSO);

IR (KBr): 3342 ν(O-H), 2933 ν(C-H), 1402 δ(C-H), 1369 δ(C-H), 1155 ν(C-O), 1026 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.75 (d, J = 8.1 Hz, 1H, H-2'), 7.43 (d, J = 8.1 Hz, 1H, H-3'), 5.81 – 5.63 (m, 14H, 7 × OH-2, 7 × OH-3), 4.84 - 4.76 (m, 7H, 7 × H-1), 4.49 – 4.34 (m, 6H, 6 × OH-6), 4.43 (d, J = 10.3 Hz, 1H, H-6a^{I}), 4.19 (dd, J_1 = 11.0 Hz, J_2 = 6.4 Hz, 1H, H-6b^{I}), 3.71 – 3.20 (m, 40H, 7 × H-2, 7 × H-3, 7 × H-4, 7 × H-5, 12 × H-6), 2.43 (s, 3H, 3 × H-5') ppm.

^{13}C NMR (125 MHz, DMSO-d_6): δ = 144.78 (C-4'), 132.65 (C-1'), 129.86 (2 × C-3'), 127.56 (2 × C-2'), 102.21 – 101.26 (7 × C-1), 81.48 – 80.75 (m, 7 × C-4), 73.03 – 71.85 (m, 7 × C-2, 7 × C-3, 7 × C-5), 69.68 (C-6^{I}), 68.88 (C-5^{I}), 59.88 – 59.24 (6 × C-6), 21.18 (C-5') ppm.

HRMS: for $C_{49}H_{76}O_{37}S$ calcd: *m/z* 1288.3786 (for $[M + Na]^+$ calcd 1311.3678), found 1311.3672 $[M + Na]^+$, Δ -0.44 ppm.

6^{I}-*O*-*p*-Toluenesulfonyl-α-cyclodextrin (2). Compound **2** was prepared according to the published procedure[146] which was modified. The reaction of dry α-CD (10.13 g, 10.42 mmol) with TsCl (2.19 g, 11.47 mmol) yielded a mixture of isomers (unsubstituted α-CD, product **2**, and multiply substituted isomers). Pure **2** was obtained after column chromatography on reverse phase C18 silica gel, with step gradient elution (MeOH/H_2O mixtures). The column was first washed by H_2O to remove salts. Next, elution by 10 % MeOH afforded 49 % of unreacted α-CD (5.00 g, 5.14 mmol), and fractions containing pure monosubstituted **2** were

eluted by 20-30 % MeOH. The reaction yielded 13 % of **2** (1.55 g, 1.37 mmol). When calculating the amount of recovered starting material, the corrected yield of **2** is 25 %.

m.p. 140 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +116.1° (c = 0.36, DMSO);

IR (KBr): 3324 ν(O-H), 2926 ν(C-H), 1413 δ(C-H), 1362 δ(C-H), 1159 ν(C-O), 1039 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.77 (d, J = 8.8 Hz, 2H, H-2'), 7. 45 (d, J = 8.2 Hz, 2H, H-3'), 5.44 (m, 16 H, 8 × OH-2, 8 × OH-3), 4.80 – 4.79 (4 × H-1), 4.72 (d, J = 3.1 Hz, 1H, H-1^{I}), 4.66 (d, J = 3.1 Hz, 1H, H-1),4.56 – 4.13 (*br* s, 7H, 7 × OH-6), 4.28 (m, 2H, 2 × H-6^{I}), 3.86 (m, 1H, H-5^{I}), 3.79 – 3.25 (m, 32H, 6 × H-2, 6 × H-3, 5 × H-4, 5 × H-5, 10 × H-6), 3.24 (dd, J_1 = 9.8 Hz, J_2 = 3.00 Hz, 1H, H-4^{I}), 3.17 (dd, J_1 = 10.00 Hz, J_2 = 3.3 Hz, 1H, H-2^{I}), 2.41 (s, 3H, H-5') ppm.

^{13}C NMR (125 MHz, DMSO-d_6): δ = 144.77 (C-4'), 132.42 (C-1'), 129.91 (C-3'), 127.67 (C-2'), 102.05 – 101.89 (5 × C-1), 101.50 (C-1^{I}), 82.07 – 81.71 (5 × C-4), 81.69 (C-4^{I}), 73.22 – 71.59 (6 × C-2, 6 × C-3, 5 × C-5), 69.63 (C-6^{I}), 68.87 (C-5^{I}), 59.94 – 59.76 (5 × C-6), 21.12 (C-5') ppm.

HRMS: for $C_{43}H_{66}O_{32}S$ calcd: *m/z* 1126.3258 (for $[M + Na]^{+}$ calcd 1149.3150) , found 1149.3145 $[M + Na]^{+}$, Δ -0.42 ppm.

6^{I}-*O*-*p*-Toluenesulfonyl-γ-cyclodextrin (3). The compound was prepared according to the procedure described for the synthesis of **2**. The reaction of dry γ-CD (9.46 g, 7.29 mmol) with TsCl (1.53 g, 8.02 mmol) yielded 30 % of **3** (3.38 g, 2.33 mmol). When calculating the amount of recovered starting material γ-CD (4.70 g, 3.62 mmol) the corrected yield of **3** is 60 %.

m.p. 160 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +134.3° (c = 0.72, DMSO);

IR (KBr): 3297 ν(O-H), 2932 ν(C-H), 2905 ν(C-H), 2833 ν(C-H), 1416 , δ(C-H), 1353 δ(C-H), 1156 ν(C-O), 1081 ν(C-O), 1024 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, DMSO-d_6): δ = 7.77 (bd, J = 7.7 Hz, 2H, H-2'), 7.45 (bd, J = 8.5 Hz, 2H, H-3'), 5.74 (m, 16 H, 8 × OH-2, 8 × OH-3), 4.90 – 4.88 (*br* s, 6H, 6 × H-1), 4.81 (d, J = 4.6 Hz, 1H, H-1), 4.80 (d, J = 4.4 Hz, 1H, H-1), 4.51 (*br* s, 7H, 7 × OH-6), 4.30 (d, J = 9.7 Hz, 1H, H-6^I), 4.20 (dd, J_1 = 11.9 Hz, J_2 = 4.4 Hz, 1H, H-6^I), 3.75 – 3.72 (m, 1H, H-5^I), 3.63 – 3.24 (m, 45H, 8 × H-2, 8 × H-3, 8 × H-4,7 × H-5, 14 × H-6), 2.42 (s, 3H, H-5') ppm.

^{13}C NMR (125 MHz, DMSO-d_6): δ = 144.80 (C-4'), 132.53 (C-1'), 129.95 (C-3'), 127.55 (C-2'), 102.20 (C-1), 101.67 – 101.52 (6 × C-1), 101.09 (C-1), 81.08 (C-4), 80.95 (C-4), 80.95 (C-4), 80.92 (C-4), 80.82 (C-4), 80.78 (C-4), 80.69 (C-4), 80.09 (C-4), 72.71 – 72.04 (8 × C-2, 8 × C-3, 7 × C-5), 69.44 (C-6^I), 68.96 (C-5^I), 59.95 (5 × C-6), 59.69 (C-6), 59.35 (C-6), 21.57 (C-5') ppm.

HRMS: for $C_{55}H_{86}O_{42}S$ calcd: *m/z* 1450.4314 (for $[M + Na]^+$ calcd 1473.4207), found 1473.4203 $[M + Na]^+$, Δ -0.22 ppm.

***N,N,N'*-Trimethylethane-1,2-diamine (7).** Compound **7** was prepared according to the published procedure[148], using *N*-(2-chloroethyl)-dimethylamine hydrochloride (5.00 g, 34.72 mmol) and $MeNH_2$ (16.1 g, 520.80 mmol) as 40 % solution of $MeNH_2$ in H_2O (45 ml). Pure **7** was obtained by distillation of $CHCl_3$ extracts. Yield of the reaction 80 %. For $C_5H_{14}N_2$ calcd: *m/z* 102.1, found ESI MS: 102,9 $[M]^+$. NMR spectra are in agreement with the literature[148].

***N,N,N'*-Trimethylpropane-1,3-diamine (8).** The compound was prepared according to the published procedure[163], using *N*-(2-chlorpropyl)-dimethylamine hydrochloride (60.00 g, 379.75 mmol) and $MeNH_2$ (177.00 g, 5.71 mol) as 40 % solution of $MeNH_2$ in H_2O (492 ml). Pure **8** was obtained by distillation of $CHCl_3$ extracts. Yield of the reaction 87 %. For $C_6H_{16}N_2$ calcd: *m/z* 116.1, found ESI MS: 116.2 $[M]^+$. NMR spectra are in agreement with the literature[163].

6^I-Deoxy-6^I-(*N,N,N*-trimethylammonio)-β-cyclodextrin bicarbonate (4). Dry 6^I-*O*-*p*-toluenesulfonyl-β-cyclodextrin **1** (0.50 g, 0.39 mmol) was placed in a sealed tube with an aqueous solution of trimethylamine (45 % wt.) (3.80 ml, 31.20 mmol). The mixture was stirred at 80 °C overnight. TLC of the reaction mixture showed two spots, one corresponding to the product and second which was assigned to 3,6-anhydro-β-cyclodextrin. The mixture was easily separated on a column (15 ml) of strong cation exchange resin in hydrogen form (Dowex 50, mesh 20-50). The column was firstly washed with H_2O (200 ml) to remove the

byproduct 3,6-anhydro-β-cyclodextrin and then with aqueous NH_4HCO_3 3 % wt. (300 ml) to elute the product. Salts were removed by thermal decomposition realized by repeated vacuum evaporation with H_2O (4 × 50 ml). Pure product **4** with bicarbonate anion was obtained by precipitation from acetone as a fine white powder in 71 % yield (0.32 g).

m.p. 220 °C (starts to decompose);

$[\alpha]^{25}_D$ +118.9° (c = 0.30, H_2O);

IR (KBr): 3303 ν(O-H), 2926 ν(C-H), 2911 ν(C-H), 2833 ν(C-H), 1416 , δ (C-H), 1362 δ(C-H), 1299 δ(C-H), 1156 ν(C-O), 1081 ν(C-O), 1033 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.11 (d, J = 3.6 Hz, 1H, H-1), 5.09 (d, J = 4.3 Hz, 1H, H-1), 5.08 (d, J = 3.8 Hz, 1H, H-1), 5.07 (d, J = 3.8 Hz, 1H, H-1), 5.04 (d, J = 3.6 Hz, 1H, H-1), 5.03 (d, J = 3.8 Hz, 1H, H-1), 5.02 (d, J = 3.8 Hz, 1H, H-1), 4.38 (t, J = 8.8 Hz, 1H, H-5I), 4.04 (bt, J = 9.7 Hz, 1H, H-3I), 4.02– 3.45 (m, 39H, 7 × H-2, 6 × H-3, 6 × H-4, 6 × H-5, 14 × H-6), 3.51 (bt, J = 9.1 Hz, 1H, H-4I), 3.20 (s, 9H, 3 × N+-CH3) ppm.

^{13}C NMR (125 MHz, D_2O): δ = 103.85 (C-1), 103.57 (C-1), 103.50 (C-1), 103.50 (C-1), 103.45 (C-1), 103.27 (C-1), 102.13 (C-1), 84.76 (C-4^I), 83.47 (C-4), 83.05 (C-4), 82.95 (C-4), 82.68 (C-4), 82.54 (C-4), 80.99 (C-4), 74.87 – 73.26 (7 × C-2, 7 × C-3, 6 × C-5), 68.91 (C-5^I), 68.12 (C-6^I), 62.59 (C-6), 62.16 (C-6), 62.02 (C-6), 61.97 (C-6), 61.85 (C-6), 61.83 (C-6), 56.04 (3 × N^+-CH_3) ppm.

HRMS: for $C_{45}H_{78}O_{34}N$ calcd: *m/z* 1176.4400, found 1176.4395 $[M]^+$, Δ -0.39 ppm.

6^I-Deoxy-6^I-(*N,N,N*-trimethylammonio)-α-cyclodextrin bicarbonate (5). Compound **5** was prepared by the procedure described for the synthesis of **4**. The reaction of starting compound **2** (0.50 g, 0.44 mmol) with aqueous trimethylamine, 45 % wt. (4.42 ml, 35.20 mmol) gave the product **5** (0.26 g) as a white powder in 58 % yield.

m.p. 190 °C (starts to decompose);

$[\alpha]^{25}_D$ +121.3° (c = 0.37, H_2O);

IR (KBr): 3303 ν(O-H), 2929 ν(C-H), 1476 δ (C-H), 1404 δ(C-H), 1362 δ(C-H), 1293 δ(C-H) + δ(O-H), 1150 ν(C-O), 1081 ν(C-O), 1042 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.14 (d, J = 2.9 Hz, 1H, H-1^{I}), 5.10 (d, J = 2.1 Hz, 1H, H-1), 5.10 (d, J = 2.7 Hz, 1H, H-1), 5.08 (d, J = 3.3 Hz, 1H, H-1), 5.05 (d, J = 3.4 Hz, 1H, H-1), 5.03 (d, J = 3.4 Hz, 1H, H-1), 4.42 (dt, J_1 = 9.1Hz, J_2 = 2.6Hz, 1H, H-5^{I}), 4.10 – 3.56 (m, 33H, 6 × H-2, 6 × H-3, 4 × H-4, 5 × H-5, 12 × H-6), 3.53 (t, J = 8.9 Hz, 1H, H-4^{I}), 3.51 (t, J = 9.3 Hz, 1H, H-4), 3.24 (s, 9H, 3 × N-CH_3) ppm.

^{13}C NMR (125 MHz, D_2O): δ = 103.71 (C-1), 103.44 (C-1), 103.32 (C-1), 103.27 (C-1), 103.17 (C-1), 102.95 (C-1), 85.32 (C-4), 83.65 (C-4), 83.38 (C-4), 83.19 (C-4), 83.12 (C-4), 82.79 (C-4), 75.41 – 73.20 (6 × C-2, 6 × C-3, 5 × C-5), 69.92 (C-5^{I}), 68.45 (C-6^{I}), 63.03 (C-6), 62.79 (C-6), 62.67 (C-6), 62.46 (C-6), 62.30 (C-6), 56.49 (3 × N-CH_3) ppm.

HRMS: for $C_{39}H_{68}O_{29}N$ calcd: *m/z* 1014.3872, found 1014.3869 $[M]^+$, Δ -0.24 ppm.

6^{I}-Deoxy-6^{I}-(*N,N,N*-trimethylammonio)-γ-cyclodextrin bicarbonate (6). Compound **6** was prepared by the procedure described for the synthesis of **4**. The reaction of starting compound **3** (0.50 g, 0.34 mmol) with aqueous trimethylamine, 45 % wt. (3.40 ml, 27.20 mmol), gave the product **5** (0.25 g) as a white powder in 54 % yield.

m.p. 200 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +134.9° (c = 0.52, H_2O);

IR (KBr): 3318 ν(O-H), 2932 ν(C-H), 1476 , δ (C-H), 1410 δ(C-H), 1374 δ(C-H), 1293 δ(C-H)+ δ(O-H), 1159 ν(C-O), 1081 ν(C-O), 1030 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.17 (d, J = 3.3 Hz, 1H, H-1), 5.16 (d, J = 3.5 Hz, 1H, H-1), 5.15 (d, J = 3.9 Hz, 1H, H-1), 5.14 (d, J = 4.1 Hz, 1H, H-1), 5.13 (d, J = 4.4 Hz, 1H, H-1), 5.11 (d, J = 3.5 Hz, 2H,2 × H-1), 5.10 (d, J = 3.4 Hz, 1H, H-1), 4.51 (ddd, J_1 = 9.9 Hz, J_2 = 6.6 Hz, J_3 = 3.5 Hz, 1H, H-5^{I}), 4.06 (t, J = 9.3 Hz, 1H, H-3^{I}), 3.99 – 3.57 (m, 44H, 8 × H-2. 7 × H-3, 6 × H-4, 7 × H-5, 16 × H-6), 3.53 (t, J = 9.2 Hz, 1H, H-4^{I}), 3.50 (t, J = 9.8 Hz, 1H, H-4), 3.23 (s, 9H, 3 × N-CH_3) ppm.

^{13}C NMR (125 MHz, D_2O): δ = 104.01 (C-1), 103.84 (C-1), 103.67 (2 × C-1), 103.62 (C-1), 103.42 (C-1), 103.36 (C-1^{I}), 101.61 (C-1^{I}), 84.47 (C-4^{I}), 83.34 (C-4), 82.71 (C-4), 82.47 (C-4), 82.37 (C-4), 82.32 (C-4), 82.10 (C-4), 79.81 (C-4), 75.13 – 73.57 (8 × C-2, 8 × C-3, 7 × C-5), 68.83 (C-5^{I}), 68.62 (C-6^{I}), 63.00 (C-6), 62.39 (2 × C-6), 62.24 (C-6), 62.17 (C-6), 62.13 (C-6), 62.03 (C-6), 56,32 (3 × N-CH_3) ppm.

HRMS: for $C_{51}H_{88}O_{39}N$ calcd: *m/z* 1338.4928, found 1338.4923 $[M]^+$, Δ -0.39 ppm.

6^I-Deoxy-6^I-((2-(dimethylamino)ethyl)-1-(methyl)amino)-β-cyclodextrin (9). The compound was prepared according to the published procedure[112] which was modified. Dry monotosylate **1** (1.50 g, 1.165 mmol) was dissolved in neat **7** (3.57 g, 4.5 ml, 34.950 mmol) with stirring, under the inert atmosphere of argon. After complete dissolution (10 min) the reaction mixture was heated to 70 °C and stirred overnight under a weak stream of argon. The mixture changed color to dark brown after completion. Unreacted **7** was then recovered by vacuum distillation at rt. The foamy brown residue was dissolved in minimum amount of H_2O (4 ml) and was precipitated by dropwise addition to *n*-propanol (60 ml). The suspension was then refluxed for 90 min, cooled and vacuum filtered on a glass frit. The fine white precipitate was dried at rt overnight and finally in vacuo at 70 °C. The reaction yielded 1.31 g (93 %) of **9** as a white powder.

m.p. 230 °C (starts to decompose);

$[\alpha]^{25}_D$ +134.2° (c = 0.46, DMSO);

IR (KBr): 3283 ν(O-H), 2930 ν(C-H), 2824 ν(C-H), 2786 ν(C-H), 1457 δ(C-H), 1364 δ(C-H), 1154 ν(C-O), 1076 ν(C-O), 1026 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, DMSO-d_6): δ = 5.94 – 5.64 (m, 14H, 7 × OH-2, 7 × OH-3), 4.87 – 4.79 (m, 7H, 7 × H-1), 4.72 – 4.43 (m, 6H, 6 × OH-6), 3.75 – 3.28 (m, 39H, 7 × H-2, 7 × H-3, 6 × H- 4, 7 × H-5, 12 × H-6), 3.17 (bt, J = 9.4 Hz, 1H, H-4^I), 2.69 (m, 1H, H-$6a^I$), 2.45 (m, 2H, H-1a', H-$6b^I$), 2.38 (m, 1H, H-1b'), 2.26 (m, 2H, H-2'), 2.17 (s, 3H, N-CH_3), 2.07 (s, 6H, 2 × N-CH_3) ppm.

^{13}C NMR (125 MHz, DMSO-d_6): δ = 102.14 – 101.54 (7 × C-1), 84.11 (C-4^I), 81.53 – 79.16 (7 × C-2, 7 × C-3, 6 × C-4, 7 × C-5), 70.28 (C-5^I), 59.83 – 59.70 (6 × C-6), 58.36 (C-6^I), 56.96 (C-2'), 55.89 (C-1'), 45.39 (2 × N-CH_3), 43.22 (N-CH_3) ppm.

HRMS: for $C_{47}H_{82}O_{34}N_2$ calcd: *m/z* 1218.4749 (for $[M + H]^+$ calcd 1219.4822), found 1219.4818 $[M + H]^+$, Δ -0.31 ppm.

6^I-Deoxy-6^I-((2-(dimethylamino)ethyl)-1-(methyl)amino)-α-cyclodextrin (10). Compound **10** was prepared by the procedure for preparation of **9** which was modified. Dry monotosylate **2** (0.20 g, 0.178 mmol) was dissolved in neat **7** (0.62 g, 0,8 ml, 5.32 mmol) with

stirring, under the inert atmosphere of argon. After complete dissolution (10 min) the reaction mixture was heated to 70 °C and stirred overnight under a weak stream of argon. The mixture changed color to dark brown after completion. Unreacted **7** was then recovered by vacuum distillation at rt. The foamy brown residue was dissolved in minimum amount of H_2O (1,0 ml) and was precipitated by dropwise addition to acetone (30 ml). The mixture was then refluxed for 1 h, cooled and vacuum-filtered on a glass frit. The crude white solid was superficially dried, dissolved in H_2O (20 ml) and applied to a column (10 ml) of strong cation exchanger resin in hydrogen form (Dowex 50, mesh 20-50). The column was firstly washed with H_2O (100 ml) to remove TsOH and then with 1M NH_4OH (100 ml) to elute the product. This solution was evaporated under reduced pressure. The solid residue was dissolved in minimum amount of H_2O and precipitated by addition of acetone (30 ml). The white precipitate was collected on a glass frit by vacuum filtration and was dried at rt overnight and finally in vacuo at 70 °C. The reaction yielded 0.14 g of **10** as a fine white powder in 69 % yield

m.p. 210 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +114.2° (c = 0.27, H_2O);

IR (KBr): 3312 ν(O-H), 2935 ν(C-H), 2899 ν(C-H), 2830 ν(C-H), 2794 ν(C-H), 1455 δ(C-H), 1416 δ(C-H), 1368 δ(C-H), 1332 δ(C-H), 1150 ν(C-O), 1081 ν(C-O), 1039 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.06 – 5.03 (m, 6H, 6 × H-1), 3.99 – 3.80 (m, 22H, 6 × H-3, 6 × H-5, 10 × H-6), 3.64 – 3.56 (m, 11H, 6 × H-2, 5 × H-4), 3.40 (bt, J = 9.2 Hz, 1H, H-4^I), 2.90 (d, J = 13.0 Hz, 1H, H-6^I), 2.71 – 2.58 (m, 5H, H-6^I, 2 × H-1', 2 × H-2'), 2.29 (s, 6H, 2 × N-CH_3), 2.28 (s, 3H, N-CH_3) ppm.

^{13}C NMR (125 MHz, D_2O): δ = 103.21 (C-1), 103.16 (C-1), 103.14 (C-1), 103.11 (C-1), 103.05 (C-1), 102.94 (C-1), 85.82 (C-4^I), 82.92 (C-4), 82.92 (C-4), 82.83 (C-4), 82.78 (C-4), 82.67 (C-4), 75.06 – 73.34 (6 × C-2, 6 × C-3, 5 × C-5), 71.61 (C-5^I), 62.18 – 62.02 (5 × C-6), 59.47 (C-6^I), 56.35 (C-1'), 55.77 (C-2'), 45.83 (2 × N-CH_3), 43.93 (N-CH_3) ppm.

HRMS: for $C_{41}H_{73}O_{29}N_2$ calcd: *m/z* 1057.4294, found 1057.4291 $[M]^+$, Δ-0.25 ppm.

6^I-Deoxy-6^I-((2-(dimethylamino)ethyl)-1-(methyl)amino)-γ-cyclodextrin (11). Compound **11** was prepared by the procedure described for the synthesis of **10**. Reaction of starting compound **3** (0.20 g, 0.138 mmol) with diamine **7** (0.42 g, 0.54 ml, 4.13 mmol) gave the product **11** (0.15 g) as white powder in 77 % yield.

m.p. 220 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +139.3° (c = 0.32, H_2O);

IR (KBr): 3309 ν(O-H), 2929 ν(C-H), 2824 ν(C-H), 2779 ν(C-H), 1458 δ(C-H), 1422 δ(C-H), 1365 δ(C-H), 1156 ν(C-O), 1078 ν(C-O), 1033 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.20 (d, J = 3.9 Hz, 1H, H-1), 5.15 (d, J = 3.9 Hz, 1H, H-1), 5.14 (d, J = 3.9 Hz, 1H, H-1), 5.12 (d, J = 3.6 Hz, 2H, 2 × H-1), 5.11 – 5.10 (m, 3H, 3 × H-1), 4.04 (t, J = 9.6 Hz, 1H, H-5^I), 3.98 – 3.82 (m, 29H, 8 × H-3, 7 × H-5, 14 × H-6), 3.68 – 3.58 (m, 15H, 8 × H-2, 7 × H-4), 3.40 (t, J = 10.0 Hz, 1H, H-4^I), 2.89 (d, J = 13.6 Hz, 1H, H-6^I), 2.72 – 2.59 (m, 5H, H-6^I, 2 × H-1', 2 × H-2'), 2.34 (s, 6H, 2 × N-CH_3), 2.32 (s, 3H, N-CH_3) ppm.

^{13}C NMR (125 MHz, D_2O): δ = 103.65 (C-1), 103.65 (C-1), 103.61 (C-1),103.53 (C-1), 103.53 (C-1), 103.28 (C-1), 103.12 (C-1), 101.96 (C-1), 83.96 (C-4^I), 82.43 (C-4), 82.43 (C-4), 82.37 (C-4), 82.30 (C-4), 82.19 (C-4), 82.03 (C-4), 80.66 (C-4), 75.30 – 73.70 (8 × C-2, 8 × C-3, 7 × C-5), 71.11 (C-5^I), 62.24 - 61.96 (7 × C-6), 59.44 (C-6^I), 56.91 (C-2'), 55.35 (C-1'), 46.20 (2 × N-CH_3) , 44.49 (N-CH_3) ppm.

HRMS: for $C_{53}H_{93}O_{39}N_2$ calcd: *m/z* 1381.5350, found 1381.5344 $[M]^+$, Δ-0.41 ppm.

6^I-Deoxy-6^I-((3-(dimethylamino)propyl)-1-(methyl)amino)-β-cyclodextrin (15). Compound **15** was prepared by the procedure described for the synthesis of **9**. The reaction of starting compound **1** (2.00 g, 1.55 mmol) with diamine **8** (4.80 g, 6.0 ml, 41.4 mmol) gave the product **15** (2.18 g) as a white powder in 99 % yield.

m.p. 220 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +127.3° (c = 0.47, DMSO);

IR (KBr): 3303 ν(O-H), 2935 ν(C-H), 2797 ν(C-H), 1414 δ(C-H), 1332 δ(C-H), 1155 ν(C-O), 1079 ν(C-O), 1029 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, DMSO-d_6): δ = 5.93 – 5.64 (m, 14H, 7 × OH-2, 7 × OH-3), 4.88 – 4.80 (m, 7H, 7 × H-1), 4.52 – 4.46 (m, 6H, 6 × OH-6), 3.81 – 3.30 (m, 39H, 7 × H-2, 7 × H-3, 6 × H-4, 7 × H-5, 12 × H-6) 3.19 (bt, J-9.2 Hz, 1H, H-4^I), 2.63 (m, 1H, H-$6a^I$), 2.44 (m, 1H, H-

6b^{I}), 2. 39 (m, 1H, H-1a'), 2.22 (m, 1H, H-1b'), 2.16 (s, 3H, N-CH_3), 2.14 (m, 2H, 2 × H-3'), 2.06 (s, 6H, 2 × N-CH_3) 1.45 (m, 2H, 2 × H-2') ppm.

^{13}C NMR (125 MHz, DMSO-d_6): δ = 102.15 – 101.54 (7 × C-1), 84.12 (C-4^{I}), 81.53 – 79.15 (6 × C-4), 73.30 – 71.83 (7 × C-2, 7 × C-3, 6 × C-5), 70.43 (C-5^{I}), 59.79 – 59.56 (6 × C-6), 58.01 (C-6^{I}), 57.07 (C-3'), 55.80 (C-1'), 45.15 (N-CH_3), 45.15 (N-CH_3), 43.20 (N-CH_3), 24.91 (C-2') ppm.

HRMS: for $C_{48}H_{85}O_{34}N_2$ calcd: *m/z* 1232.4905 (for $[M + H]^+$ calcd 1233.4978), found 1233.4974 $[M + H]^+$, Δ -0.34 ppm.

6^{I}-((3-(Dimethylamino)propyl)-1-(methyl)amino)-6^{I}-deoxy-α-cyclodextrin (16). Compound **16** was prepared by the procedure described for the synthesis of **10**. The reaction of starting compound **2** (0.20 g, 0.178 mmol) with diamine **8** (0.69 g, 0.78 ml, 5.32 mmol) gave the product **16** (0.16 g) as a white powder in 86 % yield.

m.p. 210 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +90.9° (c = 0.33, H_2O);

IR (KBr): 3291 ν(O-H), 2923 ν(C-H), 2830 ν(C-H), 1449 δ(C-H), 1329 δ(C-H), 1293 δ(C-H)+ δ(O-H), 1159 ν(C-O), 1036 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.07 – 5.04 (m, 6H, 6 × H-1), 4.01 – 3.82 (m, 21H, 6 × H-3, 5 × H-5, 10 × H-6), 3.65 – 3.58 (m, 11H, 6 × H-2, 5 × H-4), 3.40 (bt, J = 9.4 Hz, 1H, H-4^{I}), 2.85 (m, 1H, H-6^{I}), 2.72 (dd, J_1 = 14.1, J_2 = 9.4Hz, 1H, H-6^{I}), 2.55 (bt, J = 7.6 Hz, 2H, H-3'), 2.49 (m, 2H, H-1'), 2.38 (s, 6H, 2 × N-CH_3), 2.26 (s, 3H, N-CH_3), 1.74 (m, 2H, H-2') ppm.

^{13}C NMR (125 MHz, D_2O): δ = 103. 40 (C-1), 103.37 (C-1), 103.37 (C-1), 103.30 (C-1), 103.20 (C-1), 103.05 (C-1^{I}), 86.09 (C-4^{I}), 83.18 (C-4), 83.11 (C-4), 83.11 (C-4), 82.96 (C-4), 82.91 (C-4), 75.26 – 73.53 (6 × C-2 , 6 × C-3, 5 × C-5), 71.62 (C-5^{I}), 62.51– 62.22 (5 × C-6), 59.64 (C-6^{I}), 58.55 (C-3'), 57.58 (C-1'), 45.49 (2 × N-CH_3), 43.48 (N-CH_3), 24.33 (C-2') ppm.

HRMS: for $C_{42}H_{74}O_{29}N_2$ calcd: *m/z* 1070.4377 (for $[M + H]^+$ calcd 1071.4450), found 1071.4448 $[M + H]^+$, Δ -0,17 ppm.

6^I-((3-(Dimethylamino)propyl)-1-(methyl)amino)-6^I-deoxy-γ-cyclodextrin (17). Compound **17** was prepared by the procedure described for the synthesis of **10**. The reaction of starting compound **3** (0.30 g, 0.207 mmol) with diamine **8** (0.72 g, 0.90 ml, 6.20 mmol) gave the product **17** (0.23 g) as a white powder in 78 % yield.

m.p. 210 °C (starts to decompose);

$[\alpha]^{25}_D$ +132.6° (c = 0.34, H_2O);

IR (KBr): 3318 ν(O-H), 2938 ν(C-H), 2821 ν(C-H), 1458 δ(C-H), 1410 δ(C-H), 1368 δ(C-H), 1329 δ(C-H), 1156 ν(C-O), 1081 ν(C-O), 1030 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.19 (d, J = 3.8 Hz, 1H, H-1), 5.15 – 5.10 (m, 6H, 6 × H-1), 5.08 (d, J = 3.6 Hz, 1H, H-1^I), 4.04 (bt, J = 9.6 Hz, 1H, H-5^I), 3.96 – 3.82 (m, 29H, 8 × H-3, 7 × H-5, 14 × H-6), 3.69 – 3.58 (m, 15H, 8 × H-2, 7 × H-4), 3.40 (bt, J = 9.2 Hz, 1H, H-4^I), 2.88 (d, J = 13.2 Hz, 1H, H-6^I), 2.67 (dd, J_1 = 13.8 Hz, J_2 = 9.9 Hz, 1H, H-6^I), 2.57 – 2.44 (m, 4H, 2 × H-1', 2 × H-3'), 2.33 (s, 6H, 2 × N-CH_3), 2.30 (s, 3H, N-CH_3), 1.70 (m, 2H, 2 × H-2')ppm.

^{13}C NMR (125 MHz, D_2O): δ = 103.70 (C-1), 103.70 (C-1), 103.61 (C-1), 103.52 (C-1), 103.52 (C-1), 103.41 (C-1), 103.37 (C-1), 102.10 (C-1^I), 84.52 (C-4^I), 82.43 (C-4), 82.43 (C-4), 82.43 (C-4), 82.28 (C-4), 82.26 (C-4), 82.21 (C-4), 80.55 (C-4), 75.28 – 73.70 (m, 8 × C-2, 8 × C-3, 7 × C-5), 70.99 (C-5^I), 62.23 (C-6), 62.23 (C-6), 62.17 (C-6), 62.14 (C-6), 62.09 (C-6), 62.09 (C-6), 61.96 (C-6), 58.72 (C-6^I), 58.72 (C-3'), 56.09 (C-1'), 45.88 (2 × N-CH_3), 44.21 (N-CH_3), 24.29 (C-2') ppm..

HRMS: for $C_{54}H_{94}O_{39}N_2$ calcd: *m/z* 1394.5434 (for $[M + H]^+$ calcd 1395.5507), found 1395.5503 $[M + H]^+$, Δ -0.22 ppm.

6^I-Deoxy-6^I-(*N,N,N',N',N'*-pentamethylethane-1,2-diammonio)-β-cyclodextrin iodide (12). Starting compound **9** (0.50 g, 0.41 mmol) was dissolved in dry DMF (5.0 ml) with stirring. MeI (0.87 g, 0.38 ml, 6.16 mmol) was then added dropwise via syringe. The reaction mixture was stirred for 18 h under the inert atmosphere of argon at 30 °C. The reaction mixture changed color from colorless to light yellow. Completeness of the reaction was checked by TLC (MeOH/HOAc/1 % solution of NH_4OAc in H_2O 10/1/9). The solvent was removed in vacuo, the solid residue was dissolved in H_2O (2 ml) and precipitated from

acetone (25 ml). The precipitate was filtered on a glass frit and dried in vacuo. The reaction afforded product **12** (0.60 g) in the diiodide form, as a white powder in excellent yield (98 %).

m.p. 230 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +104.3° (c = 0.41, H_2O);

IR (KBr): 3312 ν(O-H), 2926 ν(C-H), 1419 δ(C-H), 1365 δ(C-H), 1329 δ(C-H), 1296 δ(C-H)+ δ(O-H), 1159 ν(C-O), 1078 ν(C-O), 1030 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.19 (d, J = 2.3 Hz, 1H, H-1^I), 5.14 (d, J = 3.4 Hz, 1H, H-1), 5.10 (d, J = 3.1 Hz, 1H, H-1), 5.08 (d, J = 2.9 Hz, 1H, H-1), 5.06 (d, J = 2.9 Hz, 1H, H-1), 5.04 (d, J = 3.1 Hz, 1H, H-1), 5.03 (d, J = 3.1 Hz, 1H, H-1), 4.39 (m, 1H, H-5^I), 4.16 – 3.56 (m, 44H, 7 × H-2, 7 × H-3, 6 × H-4, 6 × H-5, 14 × H-6, 2 × H-1', 2 × H-2'), 3.47 (bt, J = 9.7 Hz, 1H, H-4), 3.38 (s, 3H, N-CH_3), 3.35 (s, 3H, N-CH_3), 3.29 (s, 9H, 3 × N-CH_3) ppm.

^{13}C NMR (125 MHz, D_2O): δ = 104.15 (C-1), 103.92 (C-1), 103.92 (C-1), 103.85 (C-1), 103.72 (C-1), 103.72 (C-1), 102.86 (C-1^I), 84.98 (C-4^I), 83.73 (C-4), 83.21 (C-4), 83.15 (C-4), 82.99 (C-4), 82.89 (C-4), 81.67 (C-4), 75.18 – 73.31 (7 × C-2 , 7 × C-3, 6 × C-5), 69.63(C-5^I), 66.33 (C-6^I), 63.03– 62.07 (6 × C-6), 60.32 (C-1'), 59.52 (C-2'), 55.83 (3 × N-CH_3), 54.91 (N-CH_3), 54.85 (N-CH_3) s ppm.

HRMS: for $C_{49}H_{88}O_{34}N_2$ calcd: *m/z* 1248.5028 (for $[M]^{2+}$ calcd 624.2604), found 624.2604 $[M]^{2+}$, Δ -0.08 ppm.

6^I-Deoxy-6^I-(*N,N,N',N',N'*-pentamethylethane-1,2-diammonio)-α-cyclodextrin iodide (13). Compound **13** was prepared by the procedure described for the synthesis of **12**. The reaction of starting compound **10** (0.050 g, 0.047 mmol) with MeI (0.11 g, 0.04 ml, 0.710 mmol) gave the product **13** (0.056 g) as white powder in 88 % yield.

m.p. 220 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +92.5° (c = 0.26, H_2O);

IR (KBr): 3336 ν(O-H), 2932 ν(C-H), 1488 δ(C-H), 1416 δ(C-H), 1329 δ(C-H), 1293 δ(C-H)+ δ(O-H), 1156 ν(C-O), 1078 ν(C-O), 1036 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.16 (d, J = 2.9 Hz, 1H, H-1^I), 5.07 (d, J = 3.2 Hz, 1H, H-1), 5.06 (d, J = 3.5 Hz, 1H, H-1), 5.04 (d, J = 3.2 Hz, 1H, H-1), 5.02 (d, J = 3.5 Hz, 1H, H-1),

5.00 (d, J = 3.5 Hz, 1H, H-1), 4.87 (bt, J = 9.4 Hz, 1H, H-5^{I}), 4.34 – 4.02 (m, 16H, 6 × H-3, 5 × H-5, 1 × H-6, 2 × H-1', 2 × H-2'), 3.91 – 3.74 (m, 11H, 9 × H-6, 2 × H-6^{I}), 3.68 – 3.57 (m, 4H, 4 × H-4) , 3.55 (bt, J = 9.2 Hz, 1H, H-4^{I}), 3.47 (bt, J = 9.2 Hz, 1H, H-4), 3.42 (s, 3H, N-CH_3), 3.37 (s, 3H, N-CH_3), 3.30 (s, 9H, 3 × N-CH_3) ppm.

^{13}C NMR (125 MHz, D_2O): δ = 103.67 (C-1), 103.52 (C-1), 103.40 (C-1), 103.40 (C-1), 103.37 (C-1), 103.20 (C-1), 85.32 (C-4^{I}), 83.83 (C-4), 83.21 (C-4), 83.21 (C-4), 83.09 (C-4), 82.94 (C-4), 75.38 – 73.27 (6 × C-2, 6 × C-3, 5 × C-5), 70.25 (C-5^{I}), 66.80 (C-6^{I}), 63.18 - 62.43 (5 × C-6), 60.02 (C-2'), 59.68 (C-1'), 55.94 (N-CH_3), 55.90 (3 × N-CH_3), 55.46 (N-CH_3)ppm.

HRMS: for $C_{43}H_{78}O_{29}N_2$ calcd: *m/z* 1086.4679 (for $[M]^{2+}$ calcd 543.2340), found 543.2340 $[M]^{2+}$, Δ 0.02 ppm.

6^{I}-Deoxy-6^{I}-(*N*,*N*,*N'*,*N'*,*N'*-pentamethylethane-1,2-diammonio)-γ-cyclodextrin iodide (14). Compound **14** was prepared by the procedure described for the synthesis of **12**. The reaction of starting compound **11** (0.117 g, 0.085 mmol) with MeI (0.18 g, 0.08 ml, 1.271 mmol) gave the product **14** (0.112 g) as white powder in 80 % yield.

m.p. 230 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +107.2° (c = 0.23, H_2O);

IR (KBr): 3330 ν(O-H), 2935 ν(C-H), 1410 δ(C-H), 1377 δ(C-H), 1329 δ(C-H), 1299 δ(C-H)+ δ(O-H), 1156 ν(C-O), 1081 ν(C-O), 1027 ν(C-O).

^{1}H NMR (600 MHz, D_2O): δ = 5.25 (d, J = 3.0 Hz, 1H, H-1^{I}), 5.17 (d, J = 3.8 Hz, 1H, H-1), 5.16 (d, J = 4.3 Hz, 1H, H-1), 5.14 (d, J = 3.8 Hz, 1H, H-1), 5.13 (d, J = 3.9 Hz, 1H, H-1), 5.12 (d, J = 3.7 Hz, 1H, H-1), 5.11 (d, J = 3.7 Hz, 1H, H-1), 5.10 (d, J = 3.8 Hz, 1H, H-1), 4.65 (bt, J = 9.6 Hz, 1H, H-5^{I}), 4.15 – 3.60 (m, 49H, 8 × H-2, 8 × H-3, 6 × H-4, 7 × H-5, 16 × H-6, 2 × H-1', 2 × H-2'), 3.59 (bt, J = 9.4 Hz, 1H, H-4^{I}), 3.50 (bt, J = 9.4 Hz, 1H, H-4), 3.37 (s, 3H, N-CH_3), 3.36 (s, 3H, N-CH_3), 3.30 (s, 9H, 3 × N-CH_3) ppm.

^{13}C NMR (125 MHz, D_2O): δ = 103.77 (C-1), 103.64 (C-1), 103.56 (2 × C-1), 103.39 (2 × C-1), 103.35 (C-1), 101.64 (C-1^{I}), 84.36 (C-4^{I}), 82.90 (C-4), 82.37 (C-4), 82.33 (C-4), 82.25 (2 ×C-4), 82.09 (C-4), 79.65 (C-4), 75.10 – 73.45 (8 × C-2, 8 × C-3, 7 × C-5), 68.84 (C-5^{I}),

67.09 (C-6^I), 63.19 (C-6), 62.41 – 62.18 (6 × C-6), 60.25 (C-1'), 59.67 (C-2'), 55.94 (3 × N-CH_3), 54.94 (N-CH_3), 54.49 (N-CH_3) ppm.

HRMS: for $C_{55}H_{98}O_{39}N_2$ calcd: *m/z* 1410.5736 (for $[M]^{2+}$ calcd 705.2868), found 705.2868 $[M]^{2+}$, Δ 0.03 ppm.

6^I-Deoxy-6^I-(*N,N,N',N',N'*-pentamethylpropane-1,3-diammonio)-β-cyclodextrin iodide (18). Compound **18** was prepared by the procedure described for the synthesis of **12**. The reaction of starting compound **15** (5.00 g, 4.053 mmol) with MeI (8.63 g, 3.80 ml, 60.803 mmol) gave the product **18** (6.21 g) as a white powder in 98 % yield.

m.p. 240 °C (starts to decompose);

$[\alpha]^{25}_D$ +106.4° (c = 0.36, H_2O);

IR (KBr): 3326 (O-H), 2926 ν(C-H), 1473 δ(C-H), 1407 δ(C-H), 1332 δ(C-H), 1159 ν(C-O), 1078 ν(C-O), 1033 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.13 (d, J = 3.1 Hz, 1H, H-1^I), 5.12 (d, J = 3.6 Hz, 1H, H-1), 5.10 (d, J = 3.6 Hz, 1H, H-1), 5.08 (d, J = 3.5 Hz, 1H, H-1), 5.05 (d, J = 3.6 Hz, 1H, H-1), 5.04 (d, J = 3.8 Hz, 1H, H-1), 5.03 (d, J = 3.6 Hz, 1H, H-1), 4.47 (m, 1H, H-5^I), 4.08 – 3.35 (m, 45H, 7 × H-2, 7 × H-3, 7 × H-4, 6 × H-5, 14 × H-6, 2 × H-1', 2 × H3'), 3.23 (s, 3H, N-CH_3), 3.23 (s, 3H, N-CH_3), 3.18 (s, 9H, 3 × N-CH_3), 2.39 (m, 2H, H-2') ppm.

^{13}C NMR (125 MHz, D_2O): δ = 103.85 (C-1), 103.55 (C-1), 103.55 (C-1), 103.48 (C-1), 103.35 (C-1), 103.31 (C-1), 102.31 (C-1^I), 84.82 (C-4^I), 83.47 (C-4), 82.91(C-4), 82.91 (C-4), 82.75 (C-4), 82.61 (C-4), 81.12 (C-4), 74.90 – 73.20 (7 × C-2 , 7 × C-3, 6 × C-5), 68.99 (C-5^I), 66.51 (C-6^I), 64.42 (C-1'), 63.98 (C-3'), 62.73– 61.95 (6 × C-6), 54.84 (3 × N-CH_3), 53.70 (N-CH_3), 53.00 (N-CH_3), 18.80 (C-2') ppm.

HRMS: for $C_{50}H_{90}O_{34}N_2$ calcd: *m/z* 1262.5364 (for $[M]^{2+}$ calcd 631.2682), found 631.2682 $[M]^{2+}$, Δ 0.07 ppm.

6^I-Deoxy-6^I-(*N,N,N',N',N'*-pentamethylpropane-1,3-diammonio)-α-cyclodextrin iodide (19). Compound **19** was prepared by the procedure described for the synthesis of **12**. The reaction of starting compound **16** (0.050 g, 0.047 mmol) with MeI (0.10 g, 0.05 ml, 0.700 mmol) gave the product **19** (0.052 g) as a white powder in 83 % yield.

m.p. 240 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +92.3° (c = 0.23, H_2O);

IR (KBr): 3327 ν(O-H), 2926 ν(C-H), 1482 δ(C-H), 1329 δ(C-H), 1293 δ(C-H)+ δ(O-H), 1159 ν(C-O), 1078 ν(C-O), 1030 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O):5.14 (d, J = 2.8 Hz, 1H, H-1^I), 5.08 (d, J = 3.7 Hz, 1H, H-1), 5.07 (d, J = 3.7 Hz, 1H, H-1), 5.04 (d, J = 3.4 Hz, 1H, H-1), 5.03 (d, J = 3.7 Hz, 1H, H-1), 5.00 (d, J = 3.7 Hz, 1H, H-1), 4.77 (overlap with signal of water, 1H, H-5^I), 4.29 – 3.75 (m, 23H, 6 × H-3, 5 × H-5, 12 × H-6), 3.66 – 3.46 (m, 16H, 6 × H-2, 6 × H-4, 2 × H-1', 2 × H-2'), 3.31 (s, 3H, N-CH_3), 3.28 (s, 3H, N-CH_3), 3.20 (s, 9H, 3 × N-CH_3), 2.41 (m, 2H, H-2') ppm.

^{13}C NMR (125 MHz, D_2O): δ = 103.70 (C-1), 103.46 (C-1), 103.37 (C-1), 103.37 (C-1), 103.37 (C-1), 103.23 (C-1), 85.36 (C-4^I), 83.61 (C-4), 83.23 (C-4), 83.15 (C-4), 83.10 (C-4), 83.04 (C-4), 75.42 – 73.33 (6 × C-2, 6 × C-3, 5 × C-5), 70.12 (C-5^I), 67.45 (C-6^I), 64.41 (C-1'), 64.35 (C-3'), 62.98 (C-6), 62.90 (C-6), 62.61 (C-6), 62.49 (C-6), 62.42 (C-6), 55.16 (3 × N-CH_3), 54.67 (N-CH_3), 54.30 (N-CH_3), 19.19 (C-2')ppm.

HRMS: for $C_{44}H_{80}O_{29}N_2$ calcd: *m/z* 1100.4836 (for $[M]^{2+}$ calcd 550.3418), found 550.2418 $[M]^{2+}$, Δ 0.07 ppm.

6^I-Deoxy-6^I-(*N,N,N',N',N'*-pentamethylpropane-1,3-diammonio)-γ-cyclodextrin iodide (20). Compound **20** was prepared by the procedure described for the synthesis of **12**. The reaction of starting compound **17** (0.100 g, 0.072 mmol) with MeI (0.15 g, 0.07 ml, 1.075 mmol) gave the product **20** (0.082 g) as a white powder in 80 % yield.

m.p. 240 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +117.6° (c = 0.32, H_2O);

IR (KBr): 3330 cm^{-1}ν(O-H), 2935 cm^{-1}ν(C-H), 1479 cm^{-1} δ(C-H), 1404 cm^{-1} δ(C-H), 1374 cm^{-1} δ(C-H), 1335 cm^{-1} δ(C-H), 1156 cm^{-1}ν(C-O), 1078 cm^{-1}ν(C-O), 1030 cm^{-1}ν(C-O).

^{1}H NMR (600 MHz, D_2O): δ = 5.20 (d, J = 3.3 Hz, 1H, H-1^I), 5.18 (d, J = 4.0 Hz, 1H, H-1), 5.17 (d, J = 4.0 Hz, 1H, H-1), 5.15 (d, J = 3.7 Hz, 1H, H-1), 5.13 (d, J = 3.8 Hz, 1H, H-1), 5.11 (d, J = 4.0 Hz, 2H, 2 × H-1), 5.10 (d, J = 4.0 Hz, 1H, H-1), 4.59 (t, J = 9.5 Hz, 1H, H-5^I), 4.09 (t, J = 9.3 Hz, 1H, H-3^I), 4.01 – 3.43 (m, 50 H, 8 × H-2. 7 × H-3, 8 × H-4, 7 × H-5, 16 ×

H-6, 2 × H-1', 2 × H-3'). 3.27 (s, 6H, 2 × N-CH_3), 3.22 (s, 9H, 3 × N-CH_3), 2.45 – 2.39 (m, 2H, H-2') ppm.

^{13}C NMR (125 MHz, D_2O): δ = 103.91 (C-1), 103.74 (C-1), 103.64 (C-1), 103.60 (C-1), 103.41 (2 × C-1), 103.34 (C-1), 101.61(C-1^I), 84.49 (C-4^I), 83.11 (C-4), 82.44 (C-4), 82.36 (C-4), 82.28 (C-4), 82.26 (C-4), 82.01 (C-4), 79.58 (C-4), 75.15 – 73.41 (8 × C-2, 8 × C-3, 7 × C-5), 68.76 (C-5^I), 67.47 (C-6^I), 64.39 (C-1'*), 64.33 (C-3'*), 63.10 (C-6), 62.44 (2 × C-6), 62.29 (C-6), 62.24 (C-6), 62.21 (C-6), 62.17 (C-6), 55.27 (3 × N-CH_3), 54.05 (N-CH_3), 53.53 (N-CH_3), 19.24 (C-2') ppm.

Signals tagged with * can be mutually interchanged.

HRMS: for $C_{56}H_{100}O_{39}N_2$ calcd: *m/z* 1424.5892 (for $[M]^{2+}$ calcd 712.2946), found 712.2945 $[M]^{2+}$, Δ -0.13 ppm.

2-Hydroxyethyl *p*-toluenesulfonate (21). *p*-Toluenesulfonyl chloride (10.0g, 0.052 mol) was added to ethylene glycol (180 ml, 3.23 mol) with stirring. The mixture was stirred for 30 min at rt. Triethylamine (7.24 ml, 0.052 mol) was then added dropwise over the course of 5 min, and the reaction mixture's temperature has risen spontaneously to approximately 40 °C. The course of the reaction was monitored by TLC (hexane-EtOAc 1:1) and detected with (UV light 254 nm and subsequent dipping in basic $KMnO_4$ solution and dried with a heat gun). After 1 h the reaction was complete. The mixture was transferred to a separatory funnel with H_2O (400 ml) and $CHCl_3$ (400 ml). After shaking, the organic phase was separated, and H_2O phase was extracted with another 200 ml of H_2O. Organic extracts were merged, dried with anhydrous $MgSO_4$ and evaporated in vacuo. The reaction afforded product **21** (10.04 g) as a clear colorless viscous liquid in 92 % yield.

for $C_9H_{12}O_4S$ calcd: *m/z* 216.1, ESI-MS found 239.0 $[M + Na]^+$.

NMR spectra are in agreement with published data[164]

2-Azidoethanol (22). The compound was prepared according to the procedure published in literature[151]. Compound **21** (11.73 g, 0.054 mol) was suspended in H_2O (30 ml) and sodium azide (4.24 g, 0.065 mol) was added. The reaction mixture was refluxed at 115 °C. After 2 h all the components of the reaction were dissolved. The reaction was complete after 20 h, which was determined by TLC (hexane-ethyl acetate 1:1). The reaction mixture was saturated with anhydrous $MgSO_4$ and the product extracted into CH_2Cl_2 (3 × 30 ml), which was

previously purified by distillation with P_2O_5 (to remove ethanol which is used as stabilizing agent). CH_2Cl_2 extracts were merged and dried with anhydrous $MgSO_4$. The product **22** was obtained as a CH_2Cl_2 solution (110 ml) and was neither further isolated, quantified nor characterized due to its potentially explosive character and was used directly in next reaction step. TLC (hexane-ethyl acetate 1:1) of the solution showed one spot with R_f = 0.5.

2-Azidoethyl-4-methylbenzensulfonate (23). The compound was prepared according to the published procedure[151]. The reaction of the CH_2Cl_2 solution of **22** (110 ml), containing ideally compound **22** (4.30 g, 0.049 mol) as starting material, with TsCl (7.54 g, 0.040 mol) and TEA (5.00 g, 6.85 ml, 0.049 mol). Product **23** was purified on silica gel column with isocratic elution by hexane-ethyl acetate 5:1. The overall yield of the two-step reaction was 60 % of **23** (7.09 g, 0.029 mol). NMR spectra are in agreement with literature[151].

1-Azido-2-iodoethane (24). The compound was prepared by Finkelstein reaction according to the published procedure[152], using compound **23** as starting material. The product was purified on silica gel column with isocratic elution by hexane-ethyl acetate 100:1. Yield of the reaction was 96 %. NMR spectra are in agreement with literature[152].

6^I-(*N'*-(2-Azidoethyl)-*N,N,N',N'*-tetramethylethane-1,2-diammonio)-6^I-deoxy-β-cyclodextrin iodide (25). Compound **9** (0.10 g, 0.082 mmol) was dissolved in dry DMF (1.5 ml) with stirring and 1-azido-2-iodoethane **24** (0.32 g, 0.16 ml, 1.641 mmol) was added dropwise. The mixture was stirred overnight at 35 °C under the inert atmosphere of argon. The reaction mixture changed color to yellow and MeI (0.32 g, 0.08 ml, 1.230 mmol) was added dropwise. The mixture was then stirred at 30 °C for next 20 h. Completeness of the reaction was monitored by TLC (MeOH/HOAc/1 % solution of NH_4OAc in H_2O 10/1/9). The solvent was removed in vacuo and residue codistilled with H_2O (3 × 2 ml). Then the solid residue was dissolved in minimum amount of H_2O-EtOH mixture (1.0 ml) and precipitated from acetone (10 ml). The precipitate was vacuum filtered and dried in desiccator over NaOH. Pure compound **25** (0.11 g) was obtained as a white powder in 98 % yield.

m.p. 200 °C (starts to decompose);

$[\alpha]^{25}_D$ +106.2° (c = 0.32, H_2O);

IR (KBr): 3330 ν(O-H), 2929 ν(C-H), 2107 ν(N_3), 1413 δ(C-H), 1362 δ(C-H), 1329 δ(C-H), 1293 δ(C-H)+ δ(O-H), 1159 ν(C-O), 1078 ν(C-O), 1033 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.18 (d, J = 2.8 Hz, 1H, H-1^{I}), 5.13 (d, J = 3.6 Hz, 1H, H-1), 5.10 (d, J = 3.6 Hz, 1H, H-1), 5.08 (d, J = 3.6 Hz, 1H, H-1), 5.06 (d, J = 3.6 Hz, 1H, H-1), 5.04 (d, J = 3.4 Hz, 1H, H-1), 5.03 (d, J = 3.6 Hz, 1H, H-1), 4.43 (bt, J = 9.5 Hz, 1H, H-5^{I}), 4.12 – 3.57 (m, 48H, 7 × H-2, 7 × H-3, 6 × H-4, 6 × H-5, 14 × H-6, 2 × H-1', 2 × H2', 2 × H3', 2 × H-4'), 3.47 (bt, J = 9.5 Hz, 1H, H-4), 3.38 (s, 3H, N-CH_3), 3.36 (s, 3H, N-CH_3), 3.31 (s, 3H, N-CH_3), 3.30 (s, 3H, N-CH_3) ppm.

^{13}C NMR (125 MHz, D_2O): δ = 104.10 (C-1), 103.85 (C-1), 103.85 (C-1), 103.78 (C-1), 103.64 (C-1), 103.64 (C-1), 102.75 (C-1 ^{I}), 84.95 (C-4 ^{I}), 83.70 (C-4), 83.14 (C-4), 83.08 (C-4), 82.93 (C-4), 82.83 (C-4), 81.57 (C-4), 75.13 – 73.31 (7 × C-2 , 7 × C-3, 6 × C-5), 69.54 (C-5^{I}), 66.53 (C-6^{I}), 65.02 (C-3'), 63.04 – 62.19 (6 × C-6), 60.15 (C-1'), 58.39 (C-2'), 54.82 (N-CH_3), 54.77 (N-CH_3), 54.17 (N-CH_3), 54.06 (N-CH_3), 46.39 (C-4') ppm.

HRMS: for $C_{50}H_{89}O_{34}N_5$ calcd: *m/z* 1303.5383 (for $[M]^{2+}$ calcd 651.7689), found 651.7690 $[M]^{2+}$, Δ 0.07 ppm.

6^{I}-(*N'*-(2-Azidoethyl)-*N,N,N',N'*-pentamethylpropane-1,3-diammonio)-6^{I}-deoxy-β-cyclodextrin iodide (26). Compound **26** was prepared by the procedure described for the synthesis of **25**. The reaction of starting compound **15** (0.50 g, 0.406 mmol) with 1-azido-2-iodoethane **24** (1.60 g, 0.80 ml, 8.114 mmol) and MeI (0.86 g, 0.38 ml, 6.086 mmol) afforded the product **26** (0.604 g) as white powder in 94 % yield.

m.p. 210 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +97.7° (c = 0.31, H_2O);

IR (KBr): 3281 ν(O-H), 2929 ν(C-H), 2110 ν(N_3), 1458 δ(C-H), 1410 δ(C-H), 1387 δ(C-H), 1368 δ(C-H), 1153 ν(C-O), 1077 ν(C-O), 1028 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.15 (d, J = 3.2 Hz, 1H, H-1^{I}), 5.15 (d, J = 3.2 Hz, 1H, H-1), 5.10 (d, J = 3.8 Hz, 1H, H-1), 5.08 (d, J = 3.5 Hz, 1H, H-1), 5.05 (d, J = 3.5 Hz, 1H, H-1), 5.04 (d, J = 3.8 Hz, 1H, H-1), 5.03 (d, J = 3.8 Hz, 1H, H-1), 4.33 (bt, J = 9.8 Hz, 1H, H-5^{I}), 4.07 – 3.57 (m, 48H, 7 × H-2, 7 × H-3, 6 × H-4, 6 × H-5, 14 × H-6, 2 × H-1', 2 × H2', 2 × H3', 2 × H-4', 2 × H-5'), 3.48 (bt, J = 9.7 Hz, 1H, H-4), 3.29 (s, 3H, N-CH_3), 3.27 (s, 3H, N-CH_3), 3.23 (s, 3H, N-CH_3), 3.21 (s, 3H, N-CH_3), 2.42 (m, 2H, H-2') ppm.

^{13}C NMR (125 MHz, D_2O): δ = 104.25 (C-1), 103.95 (C-1), 103.95 (C-1), 103.85 (C-1), 103.82 (C-1), 103.68 (C-1), 102.84 (C-1^{I}), 85.13 (C-4^{I}), 83.74 (C-4), 83.26 (C-4), 83.23 (C-4), 82.95 (C-4), 82.79 (C-4), 81.61 (C-4), 75.20 – 73.43 (7 × C-2 , 7 × C-3, 6 × C-5), 69.47(C-5^{I}), 66.93 (C-6^{I}), 64.57 (C-1'), 64.43 (C-4'), 62.96– 62.22 (C-3', 6 × C-6), 53.95 (N-CH_3), 53.78 (N-CH_3), 53.57 (N-CH_3), 53.52 (N-CH_3), 46.46 (C-5'), 18.91 (C-2') ppm.

HRMS: for $C_{51}H_{91}O_{34}N_5$ calcd: *m/z* 1317.5540 (for $[M]^{2+}$ calcd 658,7767), found 658.7767 $[M]^{2+}$, Δ -0.03 ppm.

6^{I}-((2-((2-Aminoethyl)amino)ethyl)amino)-6^{I}-deoxy-β-cyclodextrin (27). The compound was prepared according to the published procedure[153] which was modified. Dry tosylate **1** (0.30 g, 0.233 mmol) was added portion wise to a stirred diethylenetriamine (3.60 g, 34.938 mmol) over 15 min to let totally dissolve. Then the temperature was raised to 60 °C, and the reaction was stirred under the inert atmosphere of Ar for 18 h. The reaction was worked up by subsequent evaporation of unreacted amine under reduced pressure and by precipitating the solid residue from acetone (30 ml). The precipitate was filtrated in vacuo, superficially dried and introduced to 20 ml column packed with a strong cation exchanger in H^+ form. The column was eluted first with H_2O (100 ml) to remove TsOH, then with 1 % NH_4OH (50 ml) to remove byproducts and then with 3 % NH_4OH (100 ml) to elute the product. The eluate was evaporated in vacuo, dissolved in H_2O (2 ml), added dropwise to EtOH (30 ml) with stirring and refluxed overnight. The mixture was evaporated and precipitated from acetone (30 ml). The precipitate was dried in vacuo to obtain pure **27** (0.20 g) as a white powder in 70 % yield.

m.p. 220 °C (starts to decompose);

$[\alpha]^{25}_D$ +130.9° (c = 0.38, H_2O);

IR (KBr): 3318 ν(O-H), 2926 ν(C-H), 2836 ν(C-H), 1455 δ(C-H), 1419 δ(C-H), 1365 δ(C-H), 1293 δ(C-H)+ δ(O-H), 1156 ν(C-O), 1081 ν(C-O), 1036 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.06 (m, 7H, 7 × H-1), 3.95– 3.82 (m, 26H, 7 × H-3, 7 × H-5, 12 × H-6), 3.65 – 3.55 (m, 13H, 7 × H-2, 6 × H-4), 3.45 (bt, J = 9.2 Hz, 1H, H-4^{I}), 3.07 (m, 1H, H-6^{I}), 2.88 (bt, 2H, J = 6.4 Hz, H-4'), 2.83 (dd, J_1 = 12.8, J_2 = 8.7 Hz, 1H, H-6^{I}), 2.78 – 2.72 (m, 6H, 2 × H-1', 2 × H-2', 2 × H-3') ppm.

^{13}C NMR (125 MHz, D_2O): δ = 103.84 – 103.59 (7 × C-1), 85.48 (C-4^{I}), 83.07 – 82.94 (6 × H-4), 74.97 – 74.82 (7 × C-3), 73.91 – 73.77 (7 × C-2, 6 × C-5), 72.13 (C-5^{I}), 62.11 – 62.02 (6 × C-6), 51.07 (C-6^{I}), 50.43 (C-3'), 49.47 (C-1'), 49.23 (C-2'), 40.92 (C-4') ppm.

HRMS: for $C_{46}H_{82}O_{34}N_3$ calcd: *m/z* 1220.4774, found 1220.4774 $[M]^+$, Δ 0.17 ppm.

6^{I}-((3-((3-Aminopropyl)amino)propyl)amino)-6^{I}-deoxy-β-cyclodextrin (28). Compound **28** was prepared by the procedure described for the synthesis of **27**. The reaction of starting compound **1** (0.50 g, 0.388 mmol) with bis(3-aminopropyl)amine (10.18 g, 10.80 ml, 77.616 mmol) gave the product **28** (0.36 g) as a white powder in 74 % yield.

m.p. 220 °C (starts to decompose);

$[\alpha]^{25}_D$ +119.5° (c = 0.32, H_2O);

IR (KBr): 3312 ν(O-H), 2932 ν(C-H), 1458 δ(C-H), 1419 δ(C-H), 1371 δ(C-H), 1326 δ(C-H), 1156 ν(C-O), 1087 ν(C-O), 1039 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.06 – 5.04 (m,7H, 7 × H-1), 3.95 – 3.80 (m, 26H, 7 × H-3, 7× H-5, 12 × H-6), 3.64 – 3.54 (m, 13H,7 × H-2, 6 × H-4), 3.41 (t, J = 9.4 Hz, 1H, H-4^{I}), 3.05 – 3.03 (m, 1H, H-6^{I}), 2.79 – 2.58 (m, 9H, H-6^{I}, 2 × H-1', 2 × H-3', 2× H-4', 2 × H-6'), 1.71 – 1.65 (m, 4H, 2 × H-2', 2 × H-5') ppm.

^{13}C NMR (125 MHz, D_2O): δ = 103.45 (6 × C-1), 103.11 (C-1^{I}), 85.24 C-4^{I}), 82.75 – 82.72 (5 × C-4), 82.39 (C-4), 74.70 – 73.48 (7 × C-2, 7 × C-3, 6 × C-5), 71.78 (C-5^{I}), 61.82 - 61.67 (6 × C-6), 50.83 (C-6^{I}), 48.20* (C-3'), 48.07* (C-4'), 47.56 (C-1'), 39.93 (C-6'), 31.37 (C-5'), 29.46 (C-2') ppm.

Signals tagged with * can be mutually interchanged.

HRMS: for $C_{46}H_{85}O_{34}N_3$ calcd: *m/z* 1247.5014 (for $[M+H]^+$ calcd 1248.5063), found 1248.5066 $[M+H]^+$, Δ 0.25 ppm.

6^{I}-((3-((3-Aminopropyl)amino)propyl)amino)-6^{I}-deoxy-α-cyclodextrin (29). Compound **29** was prepared by the procedure described for the synthesis of **27**. The reaction of starting compound **2** (0.30 g, 0.266 mmol) with bis(3-aminopropyl)amine (6.99 g, 7.50 ml, 53.239 mmol) gave the product **29** (0.21 g) as a white powder in 71 % yield.

m.p. 220 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +37.9° (c = 0.38, H_2O);

IR (KBr): 3318 ν(O-H), 2923 ν(C-H), 1413 δ(C-H), 1359 δ(C-H), 1329 δ(C-H), 1153 ν(C-O), 1084 ν(C-O), 1033 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.06 – 5.04 (m, 6H, 6 × H-1), 4.00 – 3.85 (m, 22H, 6 × H-3, 6 × H-5, 10 × H-6), 3.64 – 3.56 (m, 11H, 6 × H-2, 5 × H-4) , 3.43 (bt, J = 8.9 Hz, 1H, H-4^I), 3.11 – 3.08 (m, 1H, H-6^I), 2.90 (bt, J = 7.1 Hz, 1H, H-4'), 2.82 – 2.60 (m, 8H, 1 × H-6^I, 2 × H-1', 2 × H-3', H-4', 2 × H-6'), 1.77 – 1.69 (m, 4H, 2 × H-2', 2 × H-5') ppm.

^{13}C NMR (125 MHz, D_2O): δ = 103.35 (2 × C-1), 103.28 (3 × C-1), 103.12 (C-1), 85.71 (C-4^I), 83.15 (4 × C-4), 83.06 (C-4), 75.24 – 73.57 (6 × C-2, 6 × C-3, 5 × C-5), 72.78 (C-5^I), 62.38 – 62.28 (5 × C-6), 51.56 (C-6^I), 48.78* (C-1'), 48.27* (C-3'), 47.75 (C-4'), 40.04 (C-6'), 30.56 (C-5'), 29.53 (C-2') ppm.

Signals tagged with * can be mutually interchanged.

HRMS: for $C_{42}H_{75}O_{29}N_3$ calcd: *m/z* 1085.4486 (for $[M+H]^+$ calcd 1086.4560), found 1086.4547 $[M+H]^+$, Δ -1.09 ppm.

6^I-((3-((3-Aminopropyl)amino)propyl)amino)-6^I-deoxy-γ-cyclodextrin acetate (30). Compound **30** was prepared by the procedure described for the synthesis of **27**. The reaction of starting compound **3** (0.40 g, 0.276 mmol) with bis(3-aminopropyl)amine (5.43 g, 5.80 ml, 41.368 mmol) gave the product **30** (0.29 g) as a white powder in 74 % yield. The product was purified on silica gel column with isocratic elution (MeOH/HOAc/1 % solution of NH_4OAc in H_2O 10/0.1/9). Fractions containing product **30** were merged, evaporated in vacuo and codistilled with H_2O (4 × 5 ml) to eliminate ammonium acetate. Pure **30** was obtained in the form of triacetate salt.

m.p. 210 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +135.8° (c = 0.34, H_2O);

IR (KBr): 3267 ν(O-H), 2929 ν(C-H), 2836 ν(C-H), 1556 ν (C = O acetate) 1407 δ(C-H), 1374 δ(C-H), 1162 ν(C-O), 1081 ν(C-O), 1036 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.19(d, J = 2.9 Hz, 1H, H-1^I),5.13 – 5.11 (m, 7H, 7 × H-1),4.09 (t, J = 9.2 Hz, 1H, H-5^I),3.98 – 3.81(m, 29H, 8 × H-3, 7 × H-5, 14 × H-6), 3.69 – 3.56

(m, 15H, 8 × H-2, 7 × H-4), 3.53 (t, J = 9.9 Hz, 1H, H-4^{I}), 3.45 (d, J = 12.6 Hz, 1H, H-6^{I}), 3.24 (t, J = 9.2 Hz, 1H, H-6^{I}), 3.15 – 3.06 (m, 8H, 2 × H-1', 2 × H-3', 2 × H-4', 2 × H-6'), 2.12 – 2.07 (m, 4H, 2 × H-2', 2 × H-5'), 1.92 (s, 9H, 3 × CH_3COOH) ppm.

^{13}C NMR (125 MHz, D_2O): δ = 183.46(CH_3**C**OOH), 103.62 (2 ×C-1), 103.57 (2 ×C-1), 103.52 (C-1),103.51(C-1), 103.46 (C-1), 102.79 (C-1^{I}), 84.63 (C-4^{I}),82.51 (C-4), 82.47 (C-4), 82.40 (C-4), 82.35 (C-4), 82.33 (C-4), 82.30 (C-4), 81.22 (C-4), 74.88 – 73.69 (8 × C-2, 8 × C-3, 7 × C-5), 70.10 (C-5^{I}), 62.53 – 62.15 (7 × C-6), 50.51 (C-6^{I}), 47.47 (C-1'*), 47.01 (C-3'), 46.71 (C-4'), 38.59 (C-6'), 25.94 (C-2', *), 25.38 (C-4',*), 25.04 (**C**H_3COOH) ppm.

Signals tagged with * can be mutually interchanged.

HRMS: for $C_{54}H_{95}O_{39}N_3$ calcd: *m/z* 1409.5543 (for $[M+H]^+$ calcd 1410.5616), found 1410.5624 $[M+H]^+$, Δ 0.58 ppm.

6^{I}-Deoxy-6^{I}-(*N*,*N*,*N*',*N*'-tetramethyl-*N*'-(3-(trimethylammonio)propyl)propane-1,3-diaminium)-β-cyclodextrin bicarbonate (31). Dry triamine derivative **28** (0.080 g, 0.064 mmol) was dissolved in dry DMF (4 ml) and heated at 60 °C for 30 min to prevent self-inclusion. After cooling down to rt, 2,4,6-collidine (0.411 g, 0.45 ml, 3.848 mmol) and MeI (0.910 g, 0.40 ml, 6.410 mmol) were added. The reaction mixture was stirred for 20 h under argon at 30 °C; its color changed to yellow upon completion. Workup consisted of evaporating the solvent and unreacted reagents in vacuo, precipitating the yellow solid residue from acetone (40 ml). The precipitate was centrifuged (5000 RPM, 5 min) and dried. The product was purified on a column packed with a weak anion exchanger (Amberlite CG 50) in NH_4^+ form, which was firstly washed with H_2O and then the product was eluted with aqueous ammonia (3-10 %) and aqueous NH_4HCO_3 (3-10 %). Fractions containing product **31** were merged, evaporated in vacuo and codistilled with H_2O (4 × 5 ml) to eliminate ammonium bicarbonate. Pure **31** trisbicarbonate (0.058 g) was obtained as a white powder in 67 % yield.

m.p. 200 °C (starts to decompose);

$[\alpha]^{25}_D$ +103.5° (c = 1.43, H_2O);

IR (KBr): 3285 ν(O-H), 2926 ν(C-H), 2836 ν(C-H), 1565 ν (C = O acetate), 1476 δ(C-H), 1404 δ(C-H), 1338 δ(C-H), 1156 ν(C-O), 1078 ν(C-O), 1036 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.13 (d, J = 3.8 Hz, 1H, H-1), 5.12 (d, J = 3.1 Hz, 1H, H-1^I), 5.09 (d, J = 3.1 Hz, 1H, H-1), 5.07 (d, J = 3.5 Hz, 1H, H-1), 5.04 (d, J = 3.3 Hz, 1H, H-1), 5.02 (d, J = 3.8 Hz, 2H, 2 × H-1), 5.01 (d, J = 3.3 Hz, 1H, H-1), 4.52 (t, J = 9.5 Hz, 1H, H-5^I),4.08 (t, J = 9.2 Hz, 1H, H-3^I),4.02 – 3.42 (m, 48 H, 7 × H-2. 6 × H-3, 7 × H-4, 6 × H-5, 14 × H-6, 2 × H-1', 2 × H-3', 2 × H-4', 2 × H-6'), 3.27 (s, 3H, N-CH_3), 3.26 (s, 3H, N-CH_3), 3.21 (s, 6H, 2 × N-CH_3), 3.19 (s, 9H, 3 × N-CH_3), 2.46 – 2.36 (m, 4H, 2 × H-2', 2 × H-5') ppm.

^{13}C NMR (125 MHz, D_2O): δ = 103.78 (C-1), 103.41 (2 × C-1), 103.34 (C-1), 103.19 (C-1), 103.15 (C-1), 102.08 (C-1^I), 84.63 (C-4^I), 83.28 (C-4), 82.61 (C-4), 82.59 (C-4), 82.47 (C-4), 82.33 (C-4), 80.85 (C-4), 74.78 – 73.05 (7 × C-2, 7 × C-3, 6 × C-5), 68.86 (C-5^I), 66.88 (C-6^I), 64.20 C-1'), 63.84 (C-6'), 62.86 (C-3'*), 62.65 (C-4'*, C-6), 62.17 (2 × C-6), 62.03 (C-6), 61.88 (2 × C-6), 54.87 (3 × N-CH_3), 53.73 (N-CH_3), 53.32 (N-CH_3), 52.24 (N-CH_3), 52.20 (N-CH_3), 18.77 (C-2'*), 18.62 (C-5'*) ppm.

Signals tagged with * can be mutually interchanged.

HRMS: for $C_{55}H_{102}O_{34}N_3$ calcd: *m/z* 1348.6328 (for $[M]^{3+}$ calcd 449.5443), found 449.5443 $[M]^{3+}$, Δ 0.11 ppm.

6^I-Deoxy-6^I-(*N*,*N*,*N'*,*N''*-tetramethyl-*N'''*-(3-(trimethylammonio)propyl)propane-1,3-diaminium)-α-cyclodextrin acetate (32). Compound **32** was prepared by the procedure described for the synthesis of **31**, which differs only in the purification step. The product was purified on silica gel column with eluent (MeOH/HOAc/1 % solution of NH_4OAc in H_2O 10/0.1/9). Fractions containing pure **32** were merged, evaporated in vacuo and codistilled with H_2O (4 × 5 ml) to eliminate ammonium acetate. The reaction of starting compound **29** (0.10 g, 0.092 mmol) with 2,4,6-collidine (0.591 g, 0.64 ml, 5.524 mmol) and MeI (1.307 g, 0.57 ml, 9.207 mmol) gave the product **32** triacetate (0.068 g) as white powder in 54 % yield.

m.p. 200 °C (starts to decompose);

$[\alpha]^{25}_D$ +101.2° (c = 0.42, H_2O);

IR (KBr): 3249 ν(O-H), 2926 ν(C-H), 2839 ν(C-H), 1580 ν (C = O acetate), 1488 δ(C-H), 1404 δ(C-H), 1341 δ(C-H), 1156 ν(C-O), 1084 ν(C-O), 1039 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 5.18 (d, J = 2.7 Hz, 1H, H-1), 5.11 (d, J = 2.5 Hz, 1H, H-1), 5.10(d, J = 2.8 Hz, 1H, H-1), 5.08 (d, J = 3.2 Hz, 1H, H-1), 5.05 (d, J = 3.2 Hz, 1H, H-1), 5.03 (d, J = 3.4 Hz, 1H, H-1), 4.49 (dd, J_1 = 9.4 Hz, J_2 = 9.4 Hz, 1H, H-5^{I}), 4.12 – 3.43 (m, 43H, 6 × H-2, 6 × H-3, 6 × H-4, 5 × H-5, 12 × H-6, 2 × H-1', 2 × H-3', 2 × H-4', 2 × H-6'), 3.28 (s, 6H, 2 × N-CH_3), 3.25 (s, 6H, 2 × N-CH_3), 3.20 (s, 9H, 3 × N-CH_3), 2.45 – 2.36 (m, 4H, 2 × H-2', 2 × H-5'), 1.92 (s, 9H, 3 × CH_3COO^-) ppm.

^{13}C NMR (125 MHz, D_2O): δ = 183.29 (CH_3**C**OO^-), 103.69 (C-1), 103.41 (C-1), 103.27 (C-1), 103.22 (2 × C-1), 103.06 (C-1), 85.28 (C-4^{I}), 83.70 (C-4), 83.31 (C-4), 83.24 (C-4), 83.18 (C-4), 82.79 (C-4), 75.39 – 73.17 (6 × C-2, 6 × C-3, 5 × C-5), 69.94 (C-5^{I}), 66.91 – 62.42 (6 × C-6, C-1', C-3', C-4', C-6'), 55.17 (3 × N-CH_3), 53.86 (N-CH_3), 53.56 (N-CH_3), 52.31 (N-CH_3), 52.18 (N-CH_3), 25.18 (**C**H_3COO^-), 18.98 (C-2', *), 18.76 (C-4',*) ppm.

Signals tagged with * can be mutually interchanged.

HRMS: for $C_{49}H_{92}O_{29}N_3$ calcd: *m/z* 1186.5800 (for [M] $^{3+}$ calcd 395.5267), found 395.5267 $[M]^{3+}$, Δ 0.07 ppm.

6^{I}-Deoxy-6^{I}-(*N*,*N*,*N'*,*N''*-tetramethyl-*N'''*-(3-(trimethylammonio)propyl)propane-1,3-diaminium)-γ-cyclodextrin acetate (33). Compound **33** was prepared by the procedure described for the synthesis of **32**. The reaction of starting compound **30** (0.20 g, 0.071 mmol) with 2,4,6-collidine (0.455 g, 0.49 ml, 4.254 mmol) and MeI (1.01 g, 0.44 ml, 7.091 mmol) gave the product **33** triacetate (0.045 g) as a white powder in 42 % yield.

m.p. 190 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +93.1° (c = 0.36, H_2O);

IR (KBr): 3150 ν(O-H), 2929 ν(C-H), 2896 ν(C-H), 2839 ν(C-H), 1556 ν(C = O acetate), 1404 δ(C-H), 1371 δ(C-H), 1329 δ(C-H), 1156 ν(C-O), 1084 ν(C-O), 1030 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O):δ = 5.24 (bs, 1H, H-1^{I}), 5.18 - 5.10 (m, 7H, 7 × H-1), 4.58 (t, J = 9.1 Hz, 1H, H-5^{I}), 4.09 (t, J = 9.4 Hz, 1H, H-3^{I}), 4.01 – 3.44 (m, 54H, 8 × H-2, 7 × H-3, 8 × H-4, 7 × H-5, 16 × H-6, 2 × H-1', 2 × H-3', 2 × H-4', 2 × H-6'), 3.26 (s, 6H, 2 × N-CH_3), 3.21 (bs, 15H, 2 × N-CH_3, 3 × N-CH_3), 2.41 (bs, 4H, 2 × H-2', 2 × H-5'), 1.92 (s, 9H, 3 × CH_3COO^-) ppm.

^{13}C NMR (125 MHz, D_2O): δ = 183.33 (CH_3**C**OO^-), 103.72 (C-1), 103.54 (2 × C-1), 103.47 (C-1), 103.35 (2 × C-1), 103.19 (C-1), 101.54 (C-1^I), 84.38 (C-4^I), 82.79 (C-4), 82.35 (C-4), 82.28 (2 × C-4), 82.18 (C-4), 82.10 (C-4), 79.38 (C-4), 75.09 – 73.44 (8 × C-2, 7 × C-3, 7 × C-5), 68.68 (C-5^I), 66.89 (C-6^I), 64.71 – 62.18 (7 × C-6, C-1', C-3', C-4', C-6'), 55.16 (3 × N-CH_3), 53.97 (N-CH_3), 53.18 (N-CH_3), 52.28 (N-CH_3), 52.18 (N-CH_3), 25.19 (**C**H_3COO^-),18.96 (C-2', *), 18.75 (C-4',*) ppm.

Signals tagged with * can be mutually interchanged.

HRMS: for $C_{61}H_{112}O_{39}N_3$calcd: *m/z* 1510.6857 (for $[M]^{3+}$ calcd 503.5619), found 503.5620 $[M]^{3+}$, Δ 0.07 ppm.

6^I-Azido-6^I-deoxy-β-CD (34). The compound was prepared according to the procedure published in literature[154], directly from compound **1** (8.00 g, 6.221 mmol) and NaN_3 (8.07, 124.22 mmol). The procedure was improved by modification of the purification step, where the crude is dissolved in minimum amount of H_2O and precipitated from 70 % acetone-water mixture (300 ml), with subsequent washing of the precipitate by this solvent mixture (100 ml), was used. This approach ensures no residue NaN_3 to be present in the final product. The reaction provided pure **34** (6.26 g, 5.397 mmol) in 87 % yield as a white powder. NMR spectra are in agreement with literature[154] and ESI-MS is consistent with the structure.

6^I-Deoxy-6^I-(4-(((3-(dimethylamino)propyl)(methyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)-β-cyclodextrin (35). The mixture of THF (4 ml) and H_2O (4 ml) was first carefully degassed by repeating the freeze-pump-thaw method three times. The reaction apparatus which consisted of the reaction flask (25 ml), fitted with small condenser and three-way valve was assembled. Then *N,N,N'*-trimethylpropane-1,3-diamine **8** (1.20 g, 1,50 ml, 10.345 mmol) was added in the reaction flask under the argon. Next the propargyl bromide (0.12 g, 1.034 mmol) as 80 % solution in toluene (0.14 ml) was added dropwise. The first part of the reaction proceeded for 2 h at rt under argon atmosphere. After 2 h the mixture became cloudy and slightly yellow. Then azide **34** (1.00 g, 0.862 mmol) and 0.1 equiv. of metal catalyst CuI (0.016 g, 0.086 mmol) were added promptly under argon, to prevent the oxygen from entering the apparatus. After 10 min, the mixture became clear homogenous solution with a yellowish color. The reaction proceeded overnight at 60 °C under a weak stream of argon and changed color to light green upon completion, which was verified by TLC (MeOH/HOAc/1 % solution of NH_4OAc in H_2O 10/1/9). Next, the mixture was evaporated, the solid residue dissolved in

H_2O, precipitated from acetone (300 ml), vacuum-filtered on a glass frit and dried. The mixture was then purified on a column (25 ml) of strong cation exchange resin in hydrogen form (Dowex 50, mesh 20-50). The column was firstly washed with H_2O (200 ml) and gradient of NH_4OH (1.5-10 %) and aqueous NH_4HCO_3 (3-6 %). Fractions containing pure product were combined, and NH_4HCO_3 was removed by thermal decomposition realized by repeated vacuum evaporation with H_2O (4 × 60 ml). Finally, the solid residue was dissolved in minimum amount of water and precipitated from acetone (160 ml), vacuum-filtered on a glass frit and dried. The reaction afforded pure **35** (1.019 g) as a white powder in 90 % yield.

m.p. 239 °C (starts to decompose);

$[\alpha]^{25}_D$+103.5° (c = 0.34, H_2O);

IR (KBr): 3288 ν(O-H), 2932 ν(C-H), 2905 ν(C-H), 2839 ν(C-H), 1494 δ(C-H), 1419 δ(C-H), 1371 δ(C-H), 1293 δ(C-H)+ δ(O-H), 1156 ν(C-O), 1081 ν(C-O), 1033 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 8.33 (s, 1H, H-1'), 5.17 (d, J = 3.2 Hz, 1H, H-1), 5.05 – 4.97 (m, 7H, 6 × H-1, H-6a^I), 4.74 – 4.69 (m, 1H, H-6b^I), 4.62 – 4.50 (m, 2H, 2 × H-3'), 4.19 (bt, J = 9.2 Hz, 1H, H-5^I), 3.98 – 3.74 (m, 23H, 7 × H-3, 6 × H-5, 10 × H-6), 3.69 – 3.44 (m, 14H, 7 × H-2, 7 × H-4), 3.36 – 3.31 (m, 1H, H-4a'), 3.24 – 3.12 (m, 4H, H-6a, H-4b', 2 × H-6'), 2.94 – 2.89 (m, 10H, H-6b, N_1-CH_3, 2 × N_2-CH_3), 2.30 – 2.22 (m, 2H, 2 × H-5') ppm.

^{13}C NMR (125 MHz, D_2O): δ = 137.33 (C-2'), 130.95 (C-1'), 103.48 – 103.33 (6 × C-1), 103.06 (C-1), 84.40 (C-4^I), 82.80 – 82.48 (6 × C-4), 74.55 – 72.92 (7 × C-2, 7 × C-3, 6 × C-5), 71.85 (C-5^I), 61.88 – 61.77 (5 × C-6), 60.89 (C-6), 55.52 (C-6'), 53.50 – 53.37 (C-4'), 52.83 (C-6^I), 51.22 (C-3'), 44.34 (2 × N_2-CH_3), 41.03 – 41.97 (N_1-CH_3), 21.09 (C-5') ppm.

The NMR spectra for compound **35** were obtained in the form of hydrochloride salt, as it assures protonated amine substituent to remain outside of the CD cavity and provides spectra with higher resolution.

HRMS: for $C_{51}H_{87}O_{34}N_5$calcd: *m/z* 1313.5232 (for $[M+H]^+$ calcd 1314.5305), found 1314.5307 $[M+H]^+$, Δ 0.15 ppm.

6^I-Deoxy-6^I-(4-(((3-(dimethylamino)propyl)(methyl)amino)butyl)-1*H*-1,2,3-triazol-1-yl)-β-cyclodextrin (36). Compound **36** was prepared by the analogical procedure to the one described for the synthesis of **35** with some minor adjustments. The reaction of starting

compound **8** (5.00 g, 6.25 ml, 43.10 mmol) with 6-chloro-1-hexyne (0.603 g, 0.63 ml, 5.172 mmol) was carried out at 50 °C for 18 h. In the second part of the reaction compound **34** (5.00 g, 4.310 mmol) and 0.1 equiv. of CuI (0.082 g, 0.431 mmol) were employed. After the work-up (analogical to the procedure for compound **35**), the reaction provided pure **36** (5.06 g) as a white powder in 92 % yield.

m.p. 202 °C (starts to decompose);

$[\alpha]^{25}_{D}$+111.5° (c = 0.31, H_2O);

IR (KBr): 3291 ν(O-H), 2923 (C-H), 2905 ν(C-H), 1485 δ(C-H), 1458 δ(C-H), 1395 δ(C-H), 1377 δ(C-H), 1156 ν(C-O), 1081 ν(C-O), 1033 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 8.28 (s, 1H, H-1'), 5.16 (d, J = 3.4 Hz, 1H, H-1), 5.13 – 5.10 (m, 1H, H-6a^I), 5.05 – 5.00 (m, 6H, 6 × H-1), 4.87 – 4.78 (m, 1H, H-6b^I, overlapping signal with signal of water), 4.21 (bt, J = 9.4 Hz, 1H, H-5^I), 3.98 – 3.76 (m, 23H, 7 × H-3, 6 × H-5, 10 × H-6), 3.69 – 3.46 (m, 14H, 7 × H-2, 7 × H-4), 3.32 – 3.17 (m, 7H, H-6a, 2 × H-6', 2 × H-7', 2 × H-9'), 3.04 – 3.00 (m, 1H, H-6b), 2.95 – 2.89 (m, 11H, 2 × H-3', N_1-CH_3, 2 × N_2-CH_3), 2.26 – 2.16 (m, 2H, 2 × H-8'), 1.92 – 1.78 (m, 4H, 2 × H-4', 2 × H-5') ppm.

^{13}C NMR (125 MHz, D_2O): δ = 146.17 (C-2'), 129.13 (C-1'), 103.54 – 103.14 (7 × C-1), 84.26 (C-4^I), 82.92 – 82.56 (6 × C-4), 74.63 – 73.14 (7 × C-2, 7 × C-3, 6 × C-5), 71.72 (C-5^I), 61.95 – 61.00 (6 × C-6), 57.38 (C-6'), 55.67 (C-9'), 54.50 (C-6^I), 54.07 (C-7'), 44.42 (2 × N_2-CH_3), 41.19 (N_1-CH_3), 26.26 (C-4'), 24.70 (C-5'), 24.30 (C-3'), 21.04 (C-8') ppm.

The NMR spectra for compound **36** were obtained in the form of hydrochloride salt, as it assures protonated amine substituent to remain outside of the CD cavity and provides spectra with higher resolution.

HRMS: for $C_{54}H_{93}O_{34}N_5$ calcd: *m/z* 1355.5702 (for $[M+H]^+$ calcd 1356.5775), found 1356.5780 $[M+H]^+$, Δ 0.39 ppm.

6^I-Deoxy-6^I-(((*N,N,N,N',N'*-pentamethylpropane-1,3-diaminium)methyl)-1*H*-1,2,3-triazol-1-yl)-β-cyclodextrin diiodide (37). Dry tertiary diamine **35** (1.016 g, 0.773 mmol) was dissolved in dry DMF (15 ml) with stirring. 20 equiv. of MeI (2.196 g, 0.963 ml, 15.463 mmol) were then added dropwise, under argon, using a septum and syringe. The reaction was carried out at rt, under argon, for 20 h. The completion was verified by TLC

(MeOH/HOAc/1 % solution of NH_4OAc in H_2O 10/1/9), showing a single spot of the product of R_f = 0.2. The reaction changed color to yellow upon completion. The work-up consisted of removing the DMF in vacuo at 30 °C, dissolving the gel-like residue in H_2O (15 ml) and precipitation of the product from acetone (300 ml). After drying the reaction gave **37** in the form of diiodide (1.138 g) as a white powder in 91 % yield.

m.p. 220 °C (starts to decompose);

$[\alpha]^{25}_D$ +94.2° (c = 0.29, H_2O);

IR (KBr): 3348 ν(O-H), 2923 ν(C-H), 1470 δ(C-H), 1419 δ(C-H), 1371 δ(C-H), 1332 δ(C-H), 1296 δ(C-H)+ δ(O-H), 1236 δ(C-H)+ δ(O-H), 1153 ν(C-O), 1081 ν(C-O), 1030 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 8.48 (s, 1H, H-1'), 5.16 (d, J = 3.5 Hz, 1H, H-1), 5.06 – 4.97 (m, 7H, 6 × H-1, H-6a^I), 4.81 (d, J = 14.1 Hz, 1H, H-3a'), 4.74 – 4.73 (m, 2H, H-6b^I, H-3b', overlapping signal with signal of water), 4.18 (bt, J = 10.0 Hz, 1H, H-5^I), 4.02 – 3.73 (m, 23H, 7 × H-3, 6 × H-5, 10 × H-6), 3.68 – 3.38 (m, 18H, 7 × H-2, 7 × H-4, 2 × H-4', 2 × H-6'), 3.19 (s, 9H, 3 × N-2 CH_3), 3.15 – 3.11 (m, 7H, H-6a, 2 × N-1 CH_3), 2.88 (dd, J_1 = 12.6 Hz, J_2 = 4.1 Hz, 1H, H-6b), 2.50 – 2.44 (m, 2H, 2 × H-5') ppm.

^{13}C NMR (125 MHz, D_2O): δ = 136.34 (C-2'), 131.93 (C-1'), 103.49 (6 × C-1), 103.07 (C-1), 84.44 (C-4^I), 82.79 – 82.55 (6 × C-4), 74.53 – 72.84 (7 × C-2, 7 × C-3, 6 × C-5), 71.90 (C-5^I), 63.79 (C-6'), 61.97 – 61.81 (5 × C-6), 61.57 (C-4'), 60.88 (C-6), 59.74 (C-3'), 54.73 (3 × N-2 CH_3), 52.93 (C-6^I), 51.93 (N-1 CH_3), 51.71 (N-1 CH_3), 18.68 (C-5') ppm.

HRMS: for $C_{53}H_{93}O_{34}N_5$ calcd: *m/z* 1343.5691 (for $[M]^{2+}$ calcd 671.7846), found 671.7848 $[M]^{2+}$, Δ 0.37 ppm.

6^I-Deoxy-6^I-(((*N*,*N*,*N*,*N'*,*N'*-pentamethylpropane-1,3-diaminium)butyl)-1*H*-1,2,3-triazol-1-yl)-β-cyclodextrin diiodide (38). Compound **38** was prepared by the analogical procedure to the one described for the synthesis of **37** using a shorter reaction time to avoid methylation on the triazole nitrogen. The reaction of starting compound **36** (3.35 g, 2.468 mmol) with MeI (7.011 g, 3.08 ml, 49.370 mmol) was carried out at rt in the course of 75 min. The completion was monitored by TLC (MeOH/HOAc/1 % solution of NH_4OAc in H_2O 10/1/9), showing a single spot of the product having R_f = 0.2.The reaction provided pure **38** (3.981 g) in the form of diiodide as a white powder in 98 % yield.

m.p. 229 °C (starts to decompose);

$[\alpha]^{25}_{D}$ +94.8° (c = 0.49, H_2O);

IR (KBr): 3327 ν(O-H), 2926 ν(C-H), 1485 δ(C-H), 1416 δ(C-H), 1365 δ(C-H), 1335 δ(C-H), 1293 δ(C-H)+ δ(O-H), 1159 ν(C-O), 1078 ν(C-O), 1030 ν(C-O) cm^{-1}.

^{1}H NMR (600 MHz, D_2O): δ = 7.88 (s, H-1'), 5.16 (d, J = 3.6 Hz, 1H, H-1), 5.06 – 5.05 (m, 4H, 4 × H-1), 5.00 – 4.97 (m, 3H, 2 × H-1, H-6^{I}a), 4.57 (dd, J_1 = 14.4, J_2 = 9.6 Hz, 1H, H-6^{I}b), 4.11 (bt, J = 9.9 Hz, 1H, H-5^I), 3.99 – 3.78 (m, 21 H, 6 × H-3, 6 × H-5, 9 × H-6), 3.74 – 3.50 (m, 15 H, 7 × H-2, 7 × H-4, 1 × H-6b), 3.43 – 3.37 (m, 7H, H-3, 2 × H-6', 2 × H-7', 2 × H-9'), 3.19 (s, 9H, 3 × CH_3-N_1), 3.17 – 3.14 (m, 1H, H-6a), 3.12 (s, 6H, 2 × CH_3-N_2), 2.82 – 2.80 (m, 3H, H-6b, 2 × H-3'), 2.35 – 2.30 (m, 2H, 2 × H-8'), 1.88 – 1.83 (m, 2 × H-5'), 1.79 – 1.74 (m, 2H, 2 × H-4') ppm.

^{13}C NMR (125 MHz, D_2O): δ = 149.09 (C-2'), 126.38 (C-1'), 103.91 – 103.14 (m, 7 × C-1), 84.95 (C-4^I), 83.10 – 82.23 (m, 6 × H-4), 74.85 – 73.26 (m, 7 × C-2, 7 × C-3, 6 × C-5), 72.59 (C-5^I), 66.30 (C-6'), 64.13 (C-9'), 62.12 – 61.88 (5 × C-6, C-7'), 60.65 (C-6), 54.93 (3 × CH_3-N_2), 52.83 (C-6^I), 52.29 (2 × CH_3-N_1), 27.11 (C-4'), 25.70 (C-3'), 23.30 (C-5'), 18.75 (C-8') ppm.

HRMS: for $C_{56}H_{99}O_{34}N_5$ calcd: *m/z* 1385.6160 (for $[M]^{2+}$ calcd 692.8080), found 692.8083 $[M]^{2+}$, Δ 0.34 ppm.

General method for the anion exchange of the tetraalkylammonium CD derivatives.

The following general procedure was used to convert the monosubstituted tetraalkylammonium CD iodides to chlorides (or other halide salts) for the needs of analytical measurements (UV/Vis). The corresponding iodide was dissolved in a 20-fold excess of H_2O. This solution was applied on a column of strong anion exchange resin (Dowex 1) in hydroxide form. The volume of the column was identical with the volume of the aqueous solution applied. The column was then washed by H_2O (10-times the volume of the column) and the eluate was directly titrated by 1 % HCl until the pH of 7.0 was reached. Other acids may be used to obtain desired anions. Next, the solution was evaporated in vacuo, the residue

precipitated from acetone and dried to receive the corresponding product as white powder. Overall yields around 90 % were typically achieved.

Characterization of compounds by 2D NMR

Structures of all CD derivatives were unambiguously elucidated using 2D NMR techniques. The combination of COSY, HSQC, HMBC and DEPT led to the assignment of the vast majority of carbon and hydrogen atoms of newly prepared compounds. Especially in the case of compound **25** and **26**, it was necessary to confirm the structure (position of the substituent and location of the azidoethane function on the terminal nitrogen atom). The example of the structural assignment is given in Figure 17 and 18. Using HSQC CH_3 groups on N-1 can be distinguished. In HMBC cross-peaks between hydrogens of C**H**$_3$a and carbons **C**H_3b, C-1' and C-6^I can be observed. Also cross-peaks between hydrogens of C**H**$_3$b and carbons **C**H_3a, C-1' and C-6^I can be observed. The mutual interaction of CH_3a and CH_3b confirms their attachment to the same nitrogen atom. Their correlation with cyclodextrin C-6^I confirms the binding of the substituent to the nitrogen bearing two CH_3 groups. Also both, CH_3a and CH_3b, have cross-peak with C-1' carbon of the substituent. In HMBC we can see the mutual correlation of CH_3 groups attached to N-2 and also with carbons C-3'a C-4'. All these facts confirm the proposed structure **26**. Very similar considerations can be applied for characterization of compounds **31**, **32 or 33** (spectra for **32** attached). The analysis confirmed that the triamine moiety is attached by the terminal nitrogen atom.

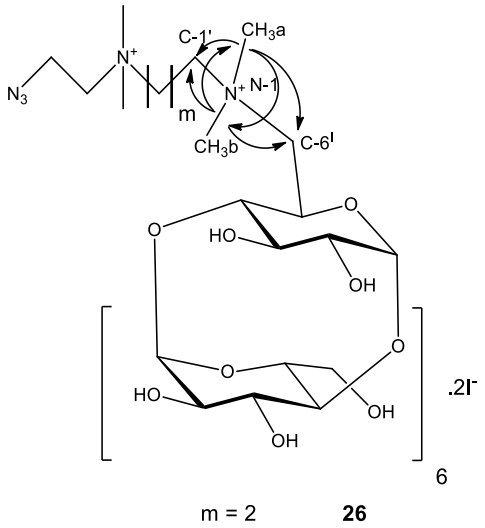


Figure 16. The structure of compound **26**, with the curved arrows indicating the atom contacts obtained from HSQC and HMBC spectra.

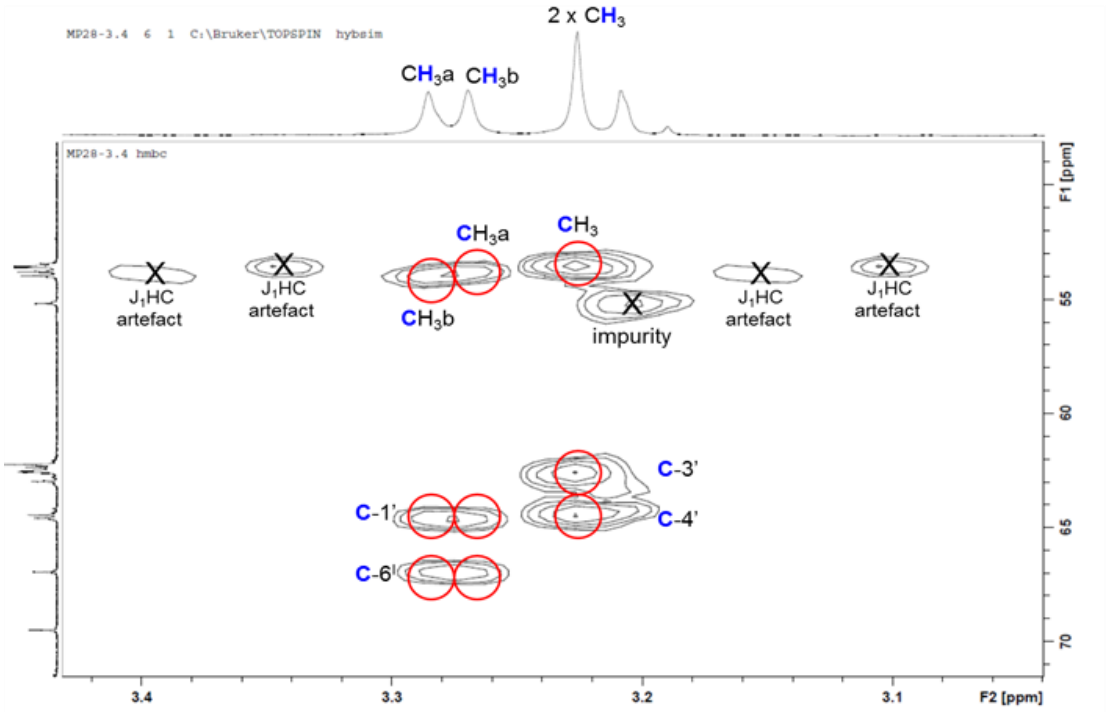


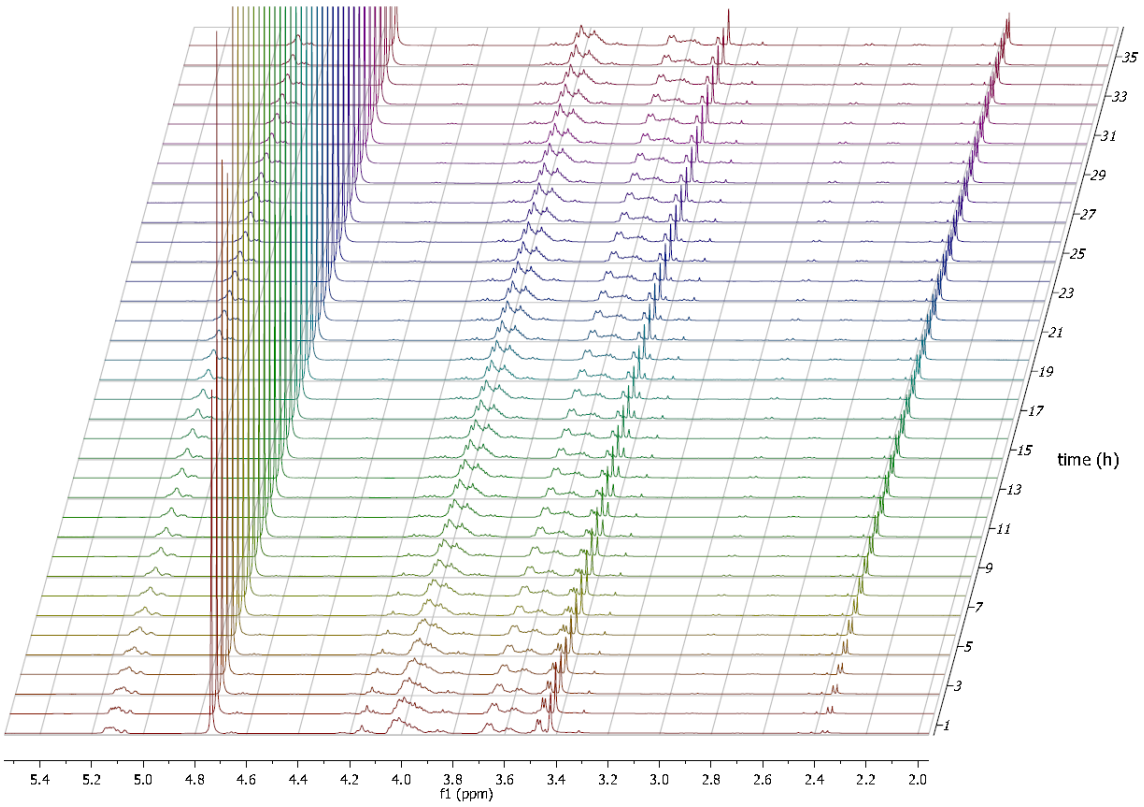
Figure 17. HMBC spectra of compound **26**, used for confirmation of the proposed structure. Analytically useful cross peaks are labeled by red circles.

6.3 Details of analytical measurements

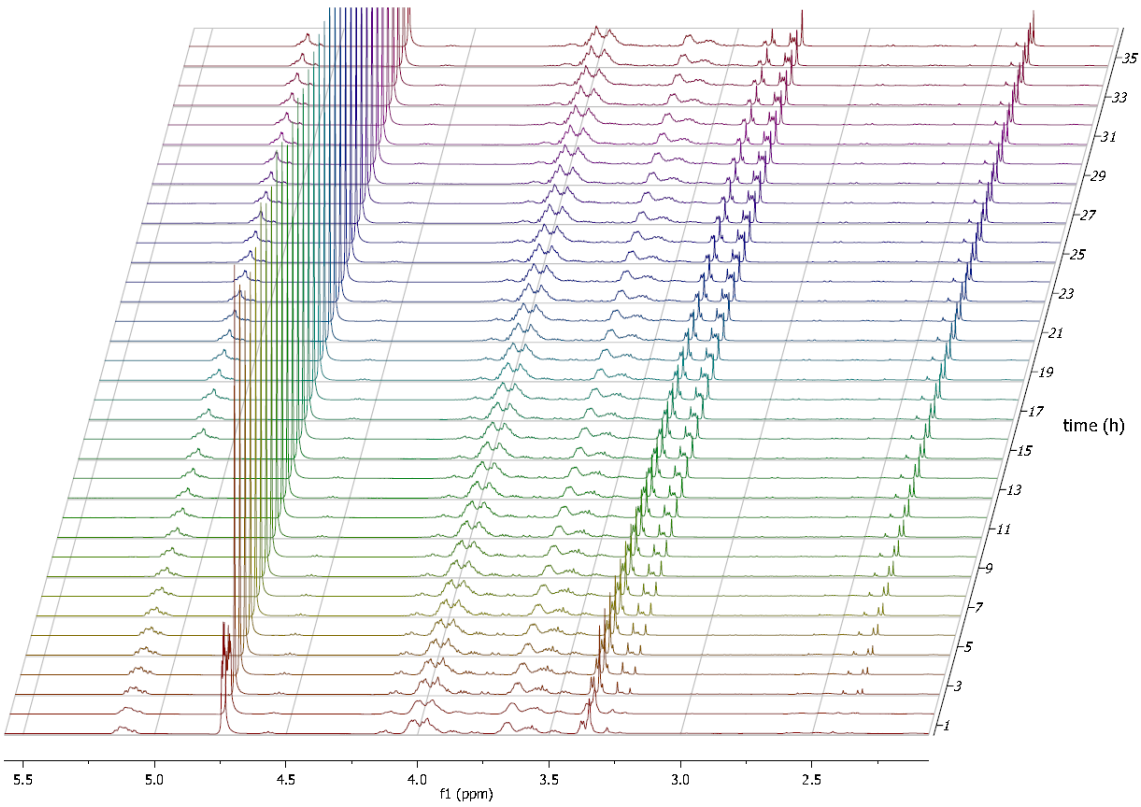
Thermal stability of PEMEDA- and PEMPDA-β-CD

Experiment setup: 20.0 mg of each derivative was dissolved in 0.7 ml of D_2O and transferred to NMR tube. After performing lock and gradient shim, 20 equiv. of NaOH were added (0.2 ml of 7.5 % NaOH in D_2O). First spectrum was acquired immediately after reaching 50 °C in the probe (separately) and then the time-lapse experiment was started. Spectra were acquired after every hour in the course of 36 hours. Each spectrum was individually integrated. The decaying integral intensity of the CH_3 protons (around 3.6 ppm) was related to the integral intensity of H-1 protons (around 5.3 ppm), which remains constant and plotted against time.

Copies of the ^{1}H NMR spectra:



Spectra 1. ^{1}H NMR spectra illustrating the thermal decomposition of PEMEDA-β-CD.



Spectra 2. ^{1}H NMR spectra illustrating the thermal decomposition of PEMPDA-β-CD.

Inclusion properties of PEMPDA-β-CD in solution - ITC

Experiment setup: Both guests and hosts were dissolved in phosphate buffer (c = 0.02 M) of pH 2.50, 7.00 and 10.00. Host concentration was 8.81 mM (for β-CD) and 7.50 mM (for PEMPDA-β-CD diiodide). Guest concentrations were always set ten times lower (0.88 mM and 0.75 mM respectively). The experiment consisted of consecutive injections of the host solution (2 µl) into the calorimeter cell containing the guest solution (280 µl). Temperature of the cell was set to 25 °C. The time between injections was usually 5 min. The data were analyzed using Microcal ORIGIN software. The experimental enthalpy was obtained by integrating the raw data signal. The integrated molar enthalpy change per injection was obtained by dividing the experimentally measured enthalpy by the number of moles of substance added. The final enthalpograms are the plots of the integrated molar enthalpy as a function of total substance concentration in the calorimeter sample cell.

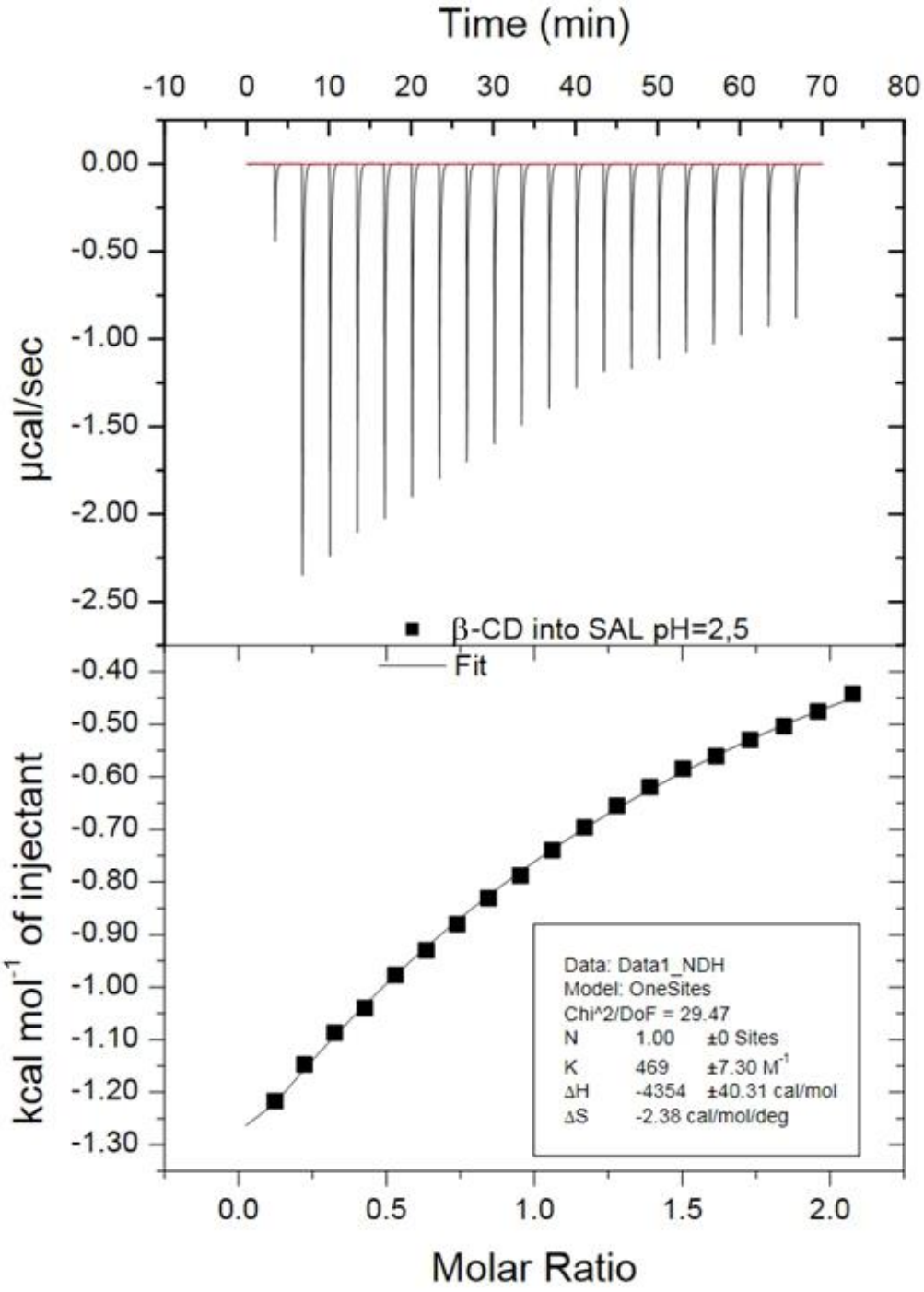


Figure 18. An example of the enthalpogram obtained from the ITC experiment for the titration of β-CD into SAL pH = 2.50.

Immobilization of cationic derivatives on anionic surface via ionic self-assembly

Gravimetric method of quantification of the deposition process consisted of stirring a precisely cut square of Nafion® 117 (100 mm^2, 35 mg, H^+ or HN_4^+ form) in an aqueous

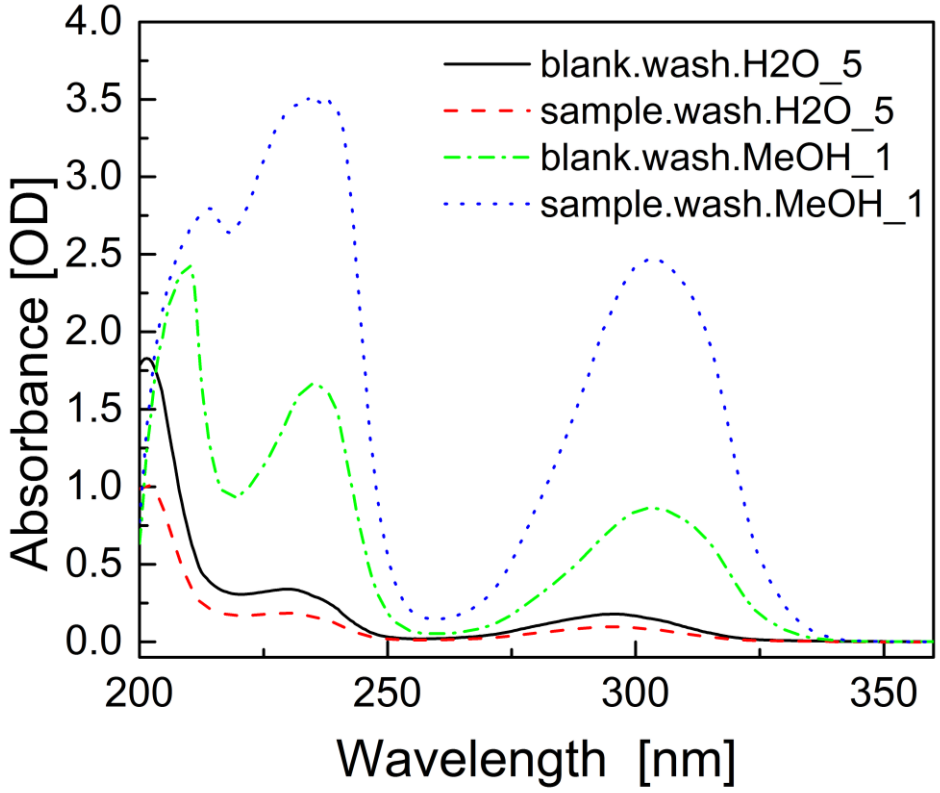
solution of host (1 ml, 5 mg/ml). Immobilization was monitored by TLC. After completion, the Nafion® 117 cut-outs were washed with H_2O (3 × 1 ml) under controlled time (30 sec) and shaking speed of 1500 RPM to assure reproducible and comparable results. The mother liquor was merged with H_2O washes, evaporated to dryness and weighed.

Experimental conditions of the ELSD measurement: Only one compound was present in the solution (PEMPDA-β-CD), so the analysis was carried out without a chromatographic column. The mobile phase was the isocratic mixture of H_2O-MeOH 4:1, flow rate 1 ml/min, pressure about 15 bar. Injected volume was 20 µl. ELDS detector was set to the temperature of 39 °C, gas flow 1.3 l/h, gain 4. Calibration of PEMPDA-β-CD was performed using a set of standard samples with known concentration, prior to the kinetics measurement.

Obtained values of decaying AUC (area under curve) were converted to concentrations using the calibration and plotted against time to receive the kinetics of the deposition on the solid surface. The deposition time constants were calculated by fitting the experimental data by two exponential decay functions using Origin® software.

Inclusion of model guest molecules from the solution in the cavities of Nafion®-bound PEMPDA-β-CD

Experiment setup: Nafion® cut-outs with immobilized PEMPDA-β-CD (5mg) were neutralized by stirring in 3 % NH_4OH (2 ml) for 1 h, prior to the measurement. The washing of the cut-outs after inclusion was performed under controlled time (30 sec) and shaking speed of 1500 RPM to assure reproducible and comparable results. UV/Vis spectra of the H_2O (5 × 3 ml) and MeOH (1 × 3 ml) washes were acquired in the spectral range 200-400 nm. First, calibration of SAL, MEQ and, NIA in H_2O and MeOH were carried out, along with the determination of linearity. Absorbance values were read in the absorption maxima near 300 nm.



Graph 5. Example of UV/Vis spectra of the H_2O (5^{th}) and MeOH washes for inclusion of SAL

WORK AND EXPERIMENTS PERFORMED BY EXTERNAL RESEARCHERS OR AS COLLABORATION

Mathematical processing of the collected data points, especially plotting and curve fitting in sections 4.2.1 and 4.2.3 was realized using Origin software, by doc. RNDr. Juraj Dian, CSc. (Department of Chemical Physics and Optics, Faculty of Mathematics and Physics, Charles University in Prague, Ke Karlovu 3, 121 16, Prague 2, Czech Republic).

ITC and DLS measurements were carried out by Sergey K. Filippov and Nikolai Matushkin (Institute of Macromolecular Chemistry AS CR, v. v. i., Heyrovskeho nam. 22, 16206, Prague 6, Czech Republic).

2D NMR spectra, ^{1}H NMR experiment at elevated temperature (50° C) in section 4.2.1 and spectra acquired on Bruker AVANCE III were measured by RNDr. Simona Hybelbauerová, PhD (Department of Teaching and Didactics of Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 40, Prague 2, Czech Republic)

HRMS spectra of all newly prepared compounds were obtained from Ivan Rosenberg PhD (Institute of Organic Chemistry and Biochemistry ASCR, v.v.i., Flemingovo nam. 2, 166 10 Prague 6, Czech Republic).

ACKNOWLEDGEMENT

First of all, I would like to thank the following subjects for financial support: Ministry of Education, Youth, and Sports of the Czech Republic (project No. MSM0021620857) and by Ministry of Industry and Trade of the Czech Republic (project No. FR-TI3/370).

Great thanks go to my supervisor doc. RNDr. Jindřich Jindřich, CSc. for the flawless leadership of this thesis and great helpfulness and support throughout the whole PhD study period. It was a pleasure to explore and develop some of his ideas.

For characterization of newly prepared compounds, I would like to thank the service laboratories of the Department of organic chemistry. Namely to, RNDr. Simona Hybelbauerová, PhD for measurement of NMR spectra at 600 MHz and also a great help with the interpretation of more complex structures, RNDr. Martin Štícha for acquiring ESI-MS spectra, Mgr. Bohunka Šperlichová for measurement of optical rotations.

For creating of the fruitful research environment and friendly atmosphere, I would like to thank all the colleagues from laboratories 135 and 131, who I have been meeting throughout my PhD studies. They often participated in solving of apparently unsolvable synthetic problems by discussions and hints.

Last but not least I would like to thank my family and girlfriend. Without their endless support it wouldn't be possible for me to cope with all of the obstacles of studying on a prestigious university.

REFERENCES

(1) Villiers, A. *Compt. Rend.* **1891**, *112*, 536–538.

(2) Szejtli, J. *Chem. Rev.* **1998**, *98* (5), 1743–1754.

(3) Crini, G. *Chem. Rev.* **2014**, *114* (21), 10940–10975.

(4) Schardinger, F. *Wien. Klin. Wochenschr.* **1904**, *17*, 207.

(5) Freudenberg, K.; Blomquist, G.; Ewald, L.; Stoff, K. *Ber. Dtsch. Chem. Ges.* **1936**, *69*, 1258.

(6) Xiao, P.; Dudal, Y.; Corvini, P. F.-X.; Pieles, U.; Shahgaldian, P. *Polym. Chem.* **2011**, *2* (6), 1264–1266.

(7) Loftsson, T.; Brewster, M. E. *J. Pharm. Pharmacol.* **2010**, *62* (11), 1607–1621.

(8) Astray, G.; Gonzalez-Barreiro, C.; Mejuto, J. C.; Rial-Otero, R.; Simal-Gándara, *J. Food Hydrocoll.* **2009**, *23* (7), 1631–1640.

(9) H.-J. Buschmann; Schollmeyer, E. *J. Cosm. Sci.* **2002**, *53* (3), 185–191.

(10) Schneiderman, E.; Stalcup, A. M. *J. Chromatogr. B. Biomed. Sci. App.* **2000**, *745* (1), 83–102.

(11) Del Valle, E. M. M. *Process Biochem.* **2004**, *39* (9), 1033–1046.

(12) Rodriguez-Tenreiro, C.; Alvarez-Lorenzo, C.; Rodriguez-Perez, A.; Concheiro, A.; Torres-Labandeira, J. J. *Pharm. Res.* **2006**, *23* (1), 121–130.

(13) van de Manakker, F.; Vermonden, T.; van Nostrum, C. F.; Hennink, W. E. *Biomacromolecules* **2009**, *10* (12), 3157–3175.

(14) Chen, Y.; Liu, Y. *Chem. Soc. Rev.* **2010**, *39* (2), 495–505.

(15) Matsuda, H.; Arima, H. *Adv. Drug Deliv. Rev.* **1999**, *36* (1), 81–99.

(16) Langer, R.; Tirrell, D. A. *Nature* **2004**, *428* (6982), 487–492.

(17) Li, Z.; Wang, M.; Wang, F.; Gu, Z.; Du, G.; Wu, J.; Chen, J. *Appl. Microbiol. Biotechnol.* **2007**, *77* (2), 245–255.

(18) Szejtli, J. *Pure Appl. Chem.* **2004**, *76*, 1825 – 1845.

(19) Dodziuk, H. *Cyclodextrins and Their Complexes: Chemistry, Analytical Methods, Applications*; John Wiley & Sons, 2006.

(20) Lichtenthaler, F. W.; Immel, S. *Tetrahedron Asymmetry* **1994**, *5* (11), 2045–2060.

(21) Gattuso, G.; Nepogodiev, S. A.; Stoddart, J. F. *Chem. Rev.* **1998**, *98* (5), 1919–1958.

(22) Terada, Y.; Yanase, M.; Takata, H.; Takaha, T.; Okada, S. *J. Biol. Chem.* **1997**, *272* (25), 15729–15733.

(23) Smolková-Keulemansová, E.; Krýsl, S. *J. Chromatogr. A* **1980**, *184* (3), 347–361.

(24) Tötterman, A. M.; Schipper, N. G. M.; Thompson, D. O.; Mannermaa, J.-P. *J. Pharm. Pharmacol.* **1997**, *49* (1), 43–48.

(25) Irie, T.; Uekama, K. *J. Pharm. Sci.* **1997**, *86* (2), 147–162.

(26) Stella, V. J.; He, Q. *Toxicol. Pathol.* **2008**, *36* (1), 30–42.

(27) Szejtli, J.; Sebestyén, G. *Starch - Stärke* **1979**, *31* (11), 385–389.

(28) Loftsson, T.; Brewster, M. E. *J. Pharm. Sci.* **1996**, *85* (10), 1017–1025.

(29) Inoue, Y.; Hakushi, T.; Liu, Y.; Tong, L.; Shen, B.; Jin, D. *J. Am. Chem. Soc.* **1993**, *115* (2), 475–481.

(30) Schneiderman, E.; Stalcup, A. M. *J. Chromatogr. B. Biomed. Sci. App.* **2000**, *745* (1), 83–102.

(31) Connors, K. A. *Chem. Rev.* **1997**, *97* (5), 1325–1358.

(32) Bergeron, R. J. *Incl. Compd.* **1984**, *3*, 391–443.

(33) Harata, K. *Bull. Chem. Soc. Jpn.* **1976**, *49* (6), 1493–1501.

(34) Funasaki, N.; Ishikawa, S.; Neya, S. *J. Phys. Chem. B* **2004**, *108* (28), 9593–9598.

(35) Kano, K.; Takenoshita, I.; Ogawa, T. *Chem. Lett.* **1982**, *11* (3), 321–324.

(36) de la Peña, A. M.; Durán-Merás, I.; Salinas, F.; Warner, I. M.; Ndou, T. T. *Anal. Chim. Acta* **1991**, *255* (2), 351–357.

(37) Duchêne, D. *Cyclodextrins and their industrial uses*; Editions de santé, 1987.

(38) Hussain, M. A.; Diluccio, R. C.; Maurin, M. B. *J. Pharm. Sci.* **1993**, *82* (1), 77–79.

(39) Sigurðoardóttir, A. M.; Loftsson, T. *Int. J. Pharm.* **1995**, *126* (1–2), 73–78.

(40) Adachi, H.; Irie, T.; Harayama, F.; Uekame, K. *Chem. Pharm. Bull. (Tokyo)* **1992**, *40* (6), 1586–1591.

(41) Tian-xiang, X.; Anderson, B. D. *Int. J. Pharm.* **1990**, *59* (1), 45–55.

(42) Nishijo, J.; Nagai, M. *J. Pharm. Sci.* **1991**, *80* (1), 58–62.

(43) Liu, F.; Kildsig, D. O.; Mitra, A. K. *Pharm. Res.* **1992**, *9* (12), 1671–1672.

(44) Schneider, H.-J.; Hacket, F.; Rüdiger, V.; Ikeda, H. *Chem. Rev.* **1998**, *98* (5), 1755–1786.

(45) Zheng, P. J.; Wang, C.; Hu, X.; Tam, K. C.; Li, L. *Macromolecules* **2005**, *38* (7), 2859–2864.

(46) *Analytical Methods in Supramolecular Chemistry*, 2nd, Completely Revised and Enlarged Edition edition ed.; Wiley-VCH: Weinheim, 2012.

(47) Giordano, F.; Novak, C.; Moyano, J. R. *Thermochim. Acta* **2001**, *380* (2), 123–151.

(48) Moyano, J. R.; Arias-Blanco, M. J.; Ginés, J. M.; Giordano, F. *Int. J. Pharm.* **1997**, *148* (2), 211–217.

(49) He, Y.; Fu, P.; Shen, X.; Gao, H. *Micron* **2008**, *39* (5), 495–516.

(50) Maestrelli, F.; González-Rodríguez, M. L.; Rabasco, A. M.; Mura, P. *Int. J. Pharm.* **2005**, *298* (1), 55–67.

(51) Baboota, S.; Dhaliwal, M.; Kohli, K.; Ali, J. *Indian J. Pharm. Sci.* **2005**, *67* (2), 226–229.

(52) Harada, A.; Li, J.; Kamachi, M. *Macromolecules* **1993**, *26* (21), 5698–5703.

(53) Singh, R. *J. Pharm. Sci. Technol.* **2010**, *2* (3), 171.

(54) Bilensoy, E.; Çırpanlı, Y.; Şen, M.; Doğan, A. L.; Çalış, S. *J. Incl. Phenom. Macrocycl. Chem.* **2007**, *57* (1–4), 363–370.

(55) Villaverde, J.; Morillo, E.; Pérez-Martínez, J. I.; Ginés, J. M.; Maqueda, C. *J. Agric. Food Chem.* **2004**, *52* (4), 864–869.

(56) Hirose, K. *J. Incl. Phenom. Macrocycl. Chem.* **2001**, *39* (3), 193–209.

(57) Fielding, L. *Tetrahedron* **2000**, *56* (34), 6151–6170.

(58) Benesi, H. A.; Hildebrand, J. H. *J. Am. Chem. Soc.* **1949**, *71* (8), 2703–2707.

(59) Wachter, H. N.; Fried, V. *J. Chem. Educ.* **1974**, *51* (12), 798.

(60) Matsue, T.; Osa, T.; Evans, D. H. *J. Incl. Phenom.* **1984**, *2* (3–4), 547–554.

(61) Bersier, P. M.; Bersier, J.; Klingert, B. *Electroanalysis* **1991**, *3* (6), 443–455.

(62) Wood, D. J.; Hruska, F. E.; Saenger, W. *J. Am. Chem. Soc.* **1977**, *99* (6), 1735–1740.

(63) Pessine, F. B.; Calderini, A.; Alexandrino, G. L. *Review: cyclodextrin inclusion complexes probed by NMR techniques*; INTECH Open Access Publisher, 2012.

(64) Crupi, V.; Ficarra, R.; Guardo, M.; Majolino, D.; Stancanelli, R.; Venuti, V. *J. Pharm. Biomed. Anal.* **2007**, *44* (1), 110–117.

(65) Hashimoto, S.; Thomas, J. K. *J. Am. Chem. Soc.* **1985**, *107* (16), 4655–4662.

(66) Matsui, Y.; Kurita, T.; Yagi, M.; Okayama, T.; Mochida, K.; Date, Y. *Bull. Chem. Soc. Jpn.* **1975**, *48* (7), 2187–2191.

(67) Höfler, T.; Wenz, G. *J. Incl. Phenom. Mol. Recognit. Chem.* **1996**, *25* (1–3), 81–84.

(68) Ashton, P. R.; Boyd, S. E.; Gattuso, G.; Hartwell, E. Y.; Koeniger, R.; Spencer, N.; Stoddart, J. F. *J. Org. Chem.* **1995**, *60* (12), 3898–3903.

(69) Jindřich; Tišlerová, I. *J. Org. Chem.* **2005**, *70* (22), 9054–9055.

(70) Menuel, S.; Doumert, B.; Saitzek, S.; Ponchel, A.; Delevoye, L.; Monflier, E.; Hapiot, F. *J. Org. Chem.* **2015**, *80* (12), 6259–6266.

(71) Loftsson, T.; Duchêne, D. *Int. J. Pharm.* **2007**, *329* (1–2), 1–11.

(72) Freudenberg, K.; Jacobi, R. *Justus Liebigs Ann. Chem.* **1935**, *518* (1), 102–108.

(73) Ciucanu, I.; Kerek, F. *Carbohydr. Res.* **1984**, *131* (2), 209–217.

(74) Sato, T.; Nakamura, H.; Ohno, Y.; Endo, T. *Carbohydr. Res.* **1990**, *199* (1), 31–35.

(75) Kraus, T.; Buděšínský, M.; Závada, J. *J. Org. Chem.* **2001**, *66* (13), 4595–4600.

(76) Harabagiu, V.; Simionescu, B. C.; Pinteala, M.; Merrienne, C.; Mahuteau, J.; Guégan, P.; Cheradame, H. *Carbohydr. Polym.* **2004**, *56* (3), 301–311.

(77) Ashton, P. R.; Koniger, R.; Stoddart, J. F.; Alker, D.; Harding, V. D. *J. Org. Chem.* **1996**, *61* (3), 903–908.

(78) Řezanka, M. *Eur. J. Org. Chem.* **2016**, n/a-n/a.

(79) Rong, D.; D'Souza, V. T. *Tetrahedron Lett.* **1990**, *31* (30), 4275–4278.

(80) Khan, A. R.; Forgo, P.; Stine, K. J.; D'Souza, V. T. *Chem. Rev.* **1998**, *98* (5), 1977–1996.

(81) Jindrich, J.; Pitha, J.; Lindberg, B.; Seffers, P.; Harata, K. *Carbohydr. Res.* **1995**, *266* (1), 75–80.

(82) Fujita, K.; Tahara, T.; Imoto, T.; Koga, T. *J. Am. Chem. Soc.* **1986**, *108* (8), 2030–2034.

(83) Fujita, K.; Chen, W.-H.; Oiwane, K.; Fujioka, T.; Fukudome, M.; Yuan, D.-Q. *Tetrahedron Lett.* **2004**, *45* (37), 6899–6902.

(84) Takeo, K.; Ueraura, K.; Mitoh, H. *J. Carbohydr. Chem.* **1988**, *7* (2), 293–308.

(85) Onozuka, S.; Kojima, M.; Hattori, K.; Toda, F. *Bull. Chem. Soc. Jpn.* **1980**, *53* (11), 3221–3224.

(86) Casati, C.; Franchi, P.; Pievo, R.; Mezzina, E.; Lucarini, M. *J. Am. Chem. Soc.* **2012**, *134* (46), 19108–19117.

(87) Brown, S.; Coates, J.; Coghlan, D.; Easton, C.; Vaneyk, S.; Janowski, W.; Lepore, A.; Lincoln, S.; Luo, Y.; May, B.; Schiesser, D.; Wang, P.; Williams, M. *Aust. J. Chem.* **1993**, *46* (6), 953–958.

(88) Fujita, K.; Nagamura, S.; Imoto, T. *Tetrahedron Lett.* **1984**, *25* (49), 5673–5676.

(89) Tungala, K.; Adhikary, P.; Krishnamoorthi, S. *Carbohydr. Polym.* **2013**, *95* (1), 295–298.

(90) Franchi, P.; Fanì, M.; Mezzina, E.; Lucarini, M. *Org. Lett.* **2008**, *10* (10), 1901–1904.

(91) Jiao, P.; Zhou, H.; Otto, M.; Mu, Q.; Li, L.; Su, G.; Zhang, Y.; Butch, E. R.; Snyder, S. E.; Jiang, G.; Yan, B. *J. Am. Chem. Soc.* **2011**, *133* (35), 13918–13921.

(92) *Org. Synth.* **2000**, *77*, 220.

(93) Ekberg, B.; Andersson, L. I.; Mosbach, K. *Carbohydr. Res.* **1989**, *192*, 111–117.

(94) Capretta, A.; Maharajh, R. B.; Bell, R. A. *Carbohydr. Res.* **1995**, *267* (1), 49–63.

(95) Gao, X.-M.; Tong, L.-H.; Inoue, Y.; Tai, A. *Synth. Commun.* **1995**, *25* (5), 703–710.

(96) Swiech, O.; Mieczkowska, A.; Chmurski, K.; Bilewicz, R. *J. Phys. Chem. B* **2012**, *116* (6), 1765–1771.

(97) Zhong, N.; Byun, H.-S.; Bittman, R. *Tetrahedron Lett.* **1998**, *39* (19), 2919–2920.

(98) Popr, M.; Hybelbauerová, S.; Jindřich, J. *Beilstein J. Org. Chem.* **2014**, *10*, 1390–1396.

(99) Perly, B.; Moutard, S.; Pilard, F. Amphiphilic cyclodextrin derivatives, method for preparation thereof and uses thereof. US2007142324 (A1), June 21, 2007.

(100) Tahir, M. N.; Qamar, R.; Adnan, A.; Cho, E.; Jung, S. *Tetrahedron Lett.* **2013**, *54* (25), 3268–3273.

(101) Kulkarni, A.; DeFrees, K.; Hyun, S.-H.; Thompson, D. H. *J. Am. Chem. Soc.* **2012**, *134* (18), 7596–7599.

(102) Yu, H.; Yuan, D.-Q.; Makino, Y.; Fukudome, M.; Xie, R.-G.; Fujita, K. *Chem. Commun.* **2006**, No. 48, 5057–5059.

(103) Fujita, K.; Tahara, T.; Imoto, T.; Koga, T. *Chem. Lett.* **1988**, *17* (8), 1329–1332.

(104) Pham, D.-T.; Ngo, H. T.; Lincoln, S. F.; May, B. L.; Easton, C. J. *Tetrahedron* **2010**, *66* (15), 2895–2898.

(105) Martin, K. A.; Czarnik, A. W. *Tetrahedron Lett.* **1994**, *35* (37), 6781–6782.

(106) Ueno, A.; Moriwaki, F.; Osa, T.; Hamada, F.; Murai, K. *Tetrahedron* **1987**, *43* (7), 1571–1578.

(107) Hanessian, S.; Benalil, A.; Simard, M.; Bélanger-Gariépy, F. *Tetrahedron* **1995**, *51* (37), 10149–10158.

(108) Petter, R. C.; Salek, J. S.; Sikorski, C. T.; Kumaravel, G.; Lin, F. T. *J. Am. Chem. Soc.* **1990**, *112* (10), 3860–3868.

(109) Velic, D.; Köhler, G. *Chem. Phys. Lett.* **2003**, *371* (3–4), 483–489.

(110) Croft, A. P.; Bartsch, R. A. *Tetrahedron* **1983**, *39* (9), 1417–1474.

(111) Stanley, M. P.; Earle, E. A. J.; Glenn, a H. Cyclodextrin with Cationic Properties. US3453257 (A), July 1, 1969.

(112) Nzeadibe, K.; Vigh, G. *Electrophoresis* **2007**, *28* (15), 2589–2605.

(113) Cucinotta, V.; Contino, A.; Giuffrida, A.; Maccarrone, G.; Messina, M. *J. Chromatogr. A* **2010**, *1217* (7), 953–967.

(114) Wang, F.; Khaledi, M. G. *Electrophoresis* **1998**, *19* (12), 2095–2100.

(115) Bunke, A.; Jira, T. *J. Chromatogr. A* **1998**, *798* (1–2), 275–280.

(116) Chankvetadze, B.; Endresz, G.; Blaschke, G. *Chem. Soc. Rev.* **1996**, *25* (2), 141–153.

(117) Matsui, Y.; Ogawa, K.; Mikami, S.; Yoshimoto, M.; Mochida, K. *Bull. Chem. Soc. Jpn.* **1987**, *60* (4), 1219–1223.

(118) Matsui, Y.; Okimoto, A. *Bull. Chem. Soc. Jpn.* **1978**, *51* (10), 3030–3034.

(119) Cryan, S. A.; Donohue, R.; Ravoo, B. J.; Darcy, R.; O'Driscoll, C. M. *J. Drug Deliv. Sci. Technol.* **2004**, *14* (1), 57–62.

(120) Thiele, C.; Auerbach, D.; Jung, G.; Qiong, L.; Schneider, M.; Wenz, G. *Polym. Chem.* **2011**, *2* (1), 209–215.

(121) Muderawan, I. W.; Ong, T.-T.; Tang, W.; Young, D. J.; Ching, C.-B.; Ng, S.-C. *Tetrahedron Lett.* **2005**, *46* (10), 1747–1749.

(122) Popr, M.; Filippov, K. S.; Matushkin, N.; Dian, J.; Jindřich, J. *Beilstein J. Org. Chem.* **2015**, *11*, 192–199.

(123) Nelles, G.; Weisser, M.; Back, R.; Wohlfart, P.; Wenz, G.; Mittler-Neher, S. *J. Am. Chem. Soc.* **1996**, *118* (21), 5039–5046.

(124) Weisser, M.; Nelles, G.; Wohlfart, P.; Wenz, G.; Mittler-Neher, S. *J. Phys. Chem.* **1996**, *100* (45), 17893–17900.

(125) Weisser, M.; Nelles, G.; Wenz, G.; Mittler-Neher, S. *Sens. Actuators B Chem.* **1997**, *38* (1–3), 58–67.

(126) Henke, C.; Steinem, C.; Janshoff, A.; Steffan, G.; Luftmann, H.; Sieber, M.; Galla, H.-J. *Anal. Chem.* **1996**, *68* (18), 3158–3165.

(127) Lee, J.-Y.; Park, S.-M. *J. Phys. Chem. B* **1998**, *102* (49), 9940–9945.

(128) Yang, X.; Shi, J.; Johnson, S.; Swanson, B. *Sens. Actuators B Chem.* **1997**, *45* (1), 79–84.

(129) Blum, W.; Aichholz, R. *J. High Resolut. Chromatogr.* **1990**, *13* (7), 515–518.

(130) Armstrong, D. W.; Tang, Y.; Ward, T.; Nichols, M. *Anal. Chem.* **1993**, *65* (8), 1114–1117.

(131) Busse, S.; DePaoli, M.; Wenz, G.; Mittler, S. *Sens. Actuators B Chem.* **2001**, *80* (2), 116–124.

(132) Grossi, N.; Espuche, E.; Escoubes, M. *Sep. Purif. Technol.* **2001**, *22–23*, 255–267.

(133) Kusumocahyo, S. P.; Sumaru, K.; Kanamori, T.; Iwatsubo, T.; Shinbo, T. *J. Membr. Sci.* **2004**, *230* (1–2), 171–174.

(134) Yang, X.; Johnson, S.; Shi, J.; Holesinger, T.; Swanson, B. *Sens. Actuators B Chem.* **1997**, *45* (2), 87–92.

(135) Tieke, B.; El-Hashani, A.; Toutianoush, A.; Fendt, A. *Thin Solid Films* **2008**, *516* (24), 8814–8820.

(136) Meng, H.; Li, S.; Xiao, L.; Li, C. *AIChE J.* **2013**, *59* (12), 4772–4779.

(137) Uekama, K.; Hirayama, F.; Irie, T. *Chem. Rev.* **1998**, *98* (5), 2045–2076.

(138) Loftsson MScPharm, P., Thorsteinn; Olafsson MD, P., Jon Hjaltalin. *Int. J. Dermatol.* **1998**, *37* (4), 241–246.

(139) Legendre, J. Y.; Rault, I.; Petit, A.; Luijten, W.; Demuynck, I.; Horvath, S.; Ginot, Y. M.; Cuine, A. *Eur. J. Pharm. Sci.* **1995**, *3* (6), 311–322.

(140) Loftsson, T.; Masson, M. *Int. J. Pharm.* **2001**, *225* (1–2), 15–30.

(141) Barry, B. W. *Eur. J. Pharm. Sci.* **2001**, *14* (2), 101–114.

(142) Naik, A.; Kalia, Y. N.; Guy, R. H. *Pharm. Sci. Technol. Today* **2000**, *3* (9), 318–326.

(143) Benoit, M.-A.; Mousset, B.; Delloye, C.; Bouillet, R.; Gillard, J. *Int. Orthop.* **1998**, *21* (6), 403–408.

(144) Radin, S.; Ducheyne, P.; Kamplain, T.; Tan, B. H. *J. Biomed. Mater. Res.* **2001**, *57* (2), 313–320.

(145) Takahashi, K.; Hattori, K. *J. Incl. Phenom.* **1984**, *2* (3–4), 661–667.

(146) Tang, W.; Ng, S.-C. *Nat. Protoc.* **2008**, *3* (4), 691–697.

(147) Melton, L. D.; Slessor, K. N. *Carbohydr. Res.* **1971**, *18* (1), 29–37.

(148) De Costa, B. R.; Radesca, L.; Di Paolo, L.; Bowen, W. D. *J. Med. Chem.* **1992**, *35* (1), 38–47.

(149) Cope, A. C.; Mehta, A. S. *J. Am. Chem. Soc.* **1963**, *85* (13), 1949–1952.

(150) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2001**, *40* (11), 2004–2021.

(151) Demko, Z. P.; Sharpless, K. B. *Org. Lett.* **2001**, *3* (25), 4091–4094.

(152) Khoukhi, M.; Vaultier, M.; Carrié, R. *Tetrahedron Lett.* **1986**, *27* (9), 1031–1034.

(153) Tabushi, I.; Shimizu, N.; Sugimoto, T.; Shiozuka, M.; Yamamura, K. *J. Am. Chem. Soc.* **1977**, *99* (21), 7100–7102.

(154) Bonnet, V.; Duval, R.; Tran, V.; Rabiller, C. *Eur. J. Org. Chem.* **2003**, *2003* (24), 4810–4818.

(155) Junquera, E.; Peña, L.; Aicart, E. *J. Pharm. Sci.* **1998**, *87* (1), 86–90.

(156) Junquera, E.; Ruiz, D.; Aicart, E. *J. Colloid Interface Sci.* **1999**, *216* (1), 154–160.

(157) Sueishi, Y.; Miyakawa, T. *J. Phys. Org. Chem.* **1999**, *12* (7), 541–546.

(158) Buvári, A.; Barcza, L. *J. Chem. Soc. Perkin Trans. 2* **1988**, No. 4, 543–545.

(159) Kuroda, Y.; Yamada, M.; Tabushi, I. *J. Chem. Soc. Perkin Trans. 2* **1989**, No. 10, 1409–1415.

(160) Corradini, R.; Dossena, A.; Marchelli, R.; Panagia, A.; Sartor, G.; Saviano, M.; Lombardi, A.; Pavone, V. *Chem. – Eur. J.* **1996**, *2* (4), 373–381.

(161) Kučerová, G.; Kalíková, K.; Procházková, H.; Popr, M.; Jindřich, J.; Coufal, P.; Tesařová, E. *Chromatographia* **2016**, *79* (9–10), 529–536.

(162) Havlikova, M.; Bosakova, Z.; Benkovics, G.; Jindrich, J.; Popr, M.; Coufal, P. *Chem. Pap.* **2016**, *70* (9), 1144–1154.

(163) Shawali, A. S. A. S.; Biechler, S. S.; Biechler, S. S. *J. Am. Chem. Soc.* **1967**, *89* (12), 3020–3026.

(164) Choudary, B. M.; Chowdari, N. S.; Kantam, M. L. *Tetrahedron* **2000**, *56* (37), 7291–7298.

List of author's publications

- Popr, M.; Hybelbauerová, S.; Jindřich, J.: Synthesis of 3^{I}-O and 2^{I}-O-monosubstituted derivatives of per-6-azido-β-cyclodextrin—potential molecular scaffolds *Carbohydrate Research* **2012**, *361*, 148–154.
  Journal impact factor (2015): 1.82.

- Popr, M.; Hybelbauerová, S.; Jindřich, J.: A complete series of 6-deoxy-monosubstituted tetraalkylammonium derivatives of α-, β-, and γ-cyclodextrin with 1, 2, and 3 permanent positive charges *Beilstein Journal of Organic Chemistry* **2014**, *10*, 1390–1396.
  Journal impact factor (2015): 2.70.

- Popr, M.; Filippov, K. S.; Matushkin, N.; Dian, J.; Jindřich, J.: Properties of cationic monosubstituted tetraalkylammonium cyclodextrin derivatives – their stability, complexation ability in solution or when deposited on solid anionic surface *Beilstein Journal of Organic Chemistry* **2015**, *11*, 192–199.
  Journal impact factor (2015): 2.70.

- Kalíková, K.; Kučerová, G.; Procházková, H.; Popr, M.; Jindřich, J.; Coufal, P.; Tesařová, T.: Chromatographic Characterization of a New Cationic β-CD Based Stationary Phase Prepared by Dynamic Coating *Chromatographia* **2016**, *79* (9–10), 529–536.
  Journal impact factor (2015): 1.33.

- Havlíková, M.; Bosáková, Z.; Benkovics, G.; Jindřich, J.; Popr, M.; Coufal, P.: Use of 6-O-mono-substituted derivatives of β-cyclodextrin-bearing substituent with two permanent positive charges in capillary electrophoresis *Chemical Papers* **2016**, *70* (9), 1144–1154.
  Journal impact factor (2015): 1.33.