Weihua Tang
Siu-Choon Ng
Dongping Sun *Editors*

Modified Cyclodextrins for Chiral Separation

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Editors
Weihua Tang
Key Laboratory of Soft Chemistry
and Functional Materials
Ministry of Education
Nanjing University of Science
and Technology
Nanjing, People's Republic of China

Dongping Sun
Key Laboratory of Soft Chemistry
and Functional Materials
Ministry of Education
Nanjing University of Science
and Technology
Nanjing, People's Republic of China

Siu-Choon Ng
Division of Chemical and Biomedical
Engineering
School of Chemical and Biomedical
Engineering
Nanyang Technological University
Singapore

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Preface

Chiral separation has attracted considerable attention in both academia and industry, particularly in biological, pharmaceutical, and agrochemical fields. Such interests can be attributed largely to a heightened awareness that many compounds of biological and pharmaceutical interest are chiral and that their enantiomers often exhibit divergent biological properties in terms of, for example, pharmacodynamics, pharmacokinetics and toxicity. The demand for single enantiomers in developing chiral drugs has led to burgeoning development of new strategies toward asymmetric synthesis on the one hand and new methods of chiral resolution on the other. As such, various chromatographic and electromigration techniques have been developed to meet the need for characterizing chiral compounds to greater extents as well as with greater accuracy and precision.

Successful chiral separation in various analytical techniques is largely dependent on the chiral selectors used in either stationary phases or mobile phases. Among all chiral selectors explored in literature, cyclodextrins (CDs) and their derivatives are the most widely used ones. CDs are torus-shapedcyclic D-GLUCO-OLIGOSACCHARIDES, comprising 6, 7, and 8 glucopyranose units connected through glycosidic α-(1,4) linkages. CDs are chiral in nature and feature a hydrophobic interior cavity and hydrophilic edges due to the presence of hydroxyl groups. The hydrophobic cavity endows CDs with their unique inclusion complexation capability with organic guest molecules, which serves as the foundation of their applications in enantioseparation of racemic compounds. In tailoring for chiral separation in various analytical techniques, natural CDs have been extensively modified to increase their stereoselectivity for guest enantiomers, CDs' solubility in desired solvents, and chemical bonding to supporting materials like silica gel.

Modified Cyclodextrins for Chiral Separation focuses on the strategies in cyclodextrin modifications for chiral separation in various chromatographic and electroseparation techniques on an analytical scale. It is not the aim of this book to give a comprehensive overview of the principles for all chiral separation and methods development in various analytical techniques, since there are already many reviews and series of specialized books on this topic. However, hitherto there is no specific book on CD's modification targeting their applications as chiral selectors in either stationary phases or mobile phases for the various analytical techniques.

Modified Cyclodextrins for Chiral Separation begins with an introduction to cyclodextrin and the general strategies for CDs' modification (Chap. 1). Chapters 2, 3, and 4 summarize functionalized cyclodextrins based chiral stationary phases for the chiral separations using gas chromatography, high-performance liquid chromatography, and supercritical fluid chromatography. Chapters 5, 6, and 7 review the neutral and charged CDs including both anionic and cationic ones for the chiral separation using capillary electrophoresis. These chapters differ from conventional articles because primary emphasis is set on giving overview of different modified CDs for specific analytical techniques.

Ten experienced researchers from both Tang's and Ng's research laboratories have contributed to *Modified Cyclodextrins for Chiral Separation*. We want to express our thanks to all of our authors for making their expertise and knowledge available to those who are not already versed in this area.

This book should be helpful to analytical chemists, pharmaceutical chemists, organic chemists, and pharmacologists, both in research institutions and in industry.

Nanjing, People's Republic of China	Weihua Tang
Nanyang Technological University, Singapore	Siu-Choon Ng
Nanjing, People's Republic of China	Dongping Sun

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Weihua Tang
Siu-Choon Ng
Dongping Sun

Contents

Chapter 1
Modification of Cyclodextrin

Jian Tang and Weihua Tang

Abstract The general information of cyclodextrins is firstly provided in this chapter. The efficient strategies for selective modification of cyclodextrins are then summarized for the development of new chiral selectors for different chromatography and electromigration analytical techniques. The modification strategies here are specially tailored for the interest of researchers in the field of applying cyclodextrin chemistry to chiral separation.

Abbreviations

CD	Cyclodextrin
Mess-N$_3$-CD	6^A-Azido-6^C-mesitylenesulfonyl-β-cyclodextrin
per-6-NH2-β-CD	Heptakis(6-amino-6-deoxy)-β-cyclodextrin
β-CD-EA	Heptakis(6-hydroxyethylamino-6-deoxy)-β-cyclodextrin
β-CD-OMe (VII)	Heptakis(6-methoxyethylamine-6-deoxy)-β-cyclodextrin
TBDMS	*tert*-Butyldimethylsilyl

1.1 Introduction

Cyclodextrins (CDs) are also known as Schardinger dextrins, cycloamyloses, and cycloglucoamyloses, which comprise a family of cyclic oligosaccharides obtained from starch by enzymatic degradation [1]. They were discovered in 1891 by Villiers

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J. Tang • W. Tang (✉)
Key Laboratory of Soft Chemistry and Functional Materials (Ministry of Education of China), Nanjing University of Science and Technology, Nanjing 210094, People's Republic of China
e-mail: whtang@mail.njust.edu.cn

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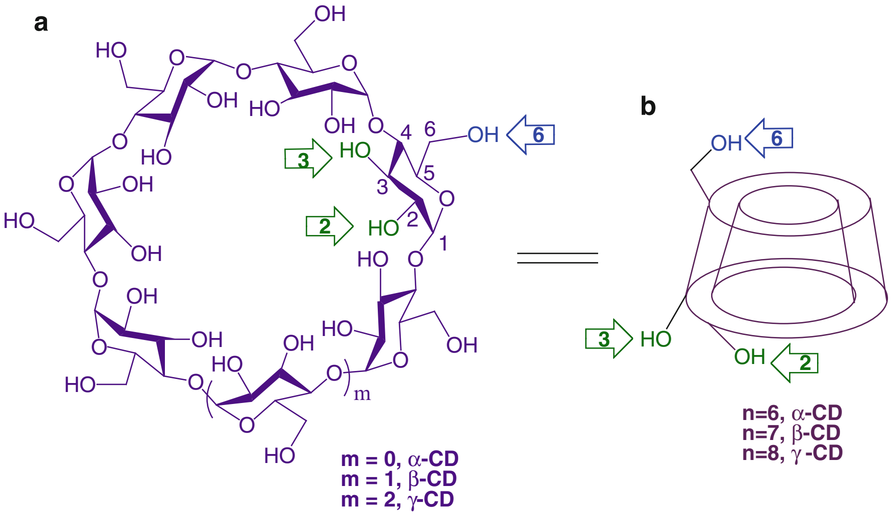


Fig. 1.1 Schematic illustration of α-, β-, and γ-CD (**a**) and their equivalent truncated cone structures; (**b**) 6-hydroxyl groups at the primary rim and the 2- and 3-hydroxyl groups at the secondary rim of CD construct a hydrophilic exterior surface

[2], but the first detailed description of the preparation process and isolation was made in 1903 by Schardinger [3]. In the preparation process, the starch is treated with a group of amylases called cyclodextrinases. The starch helix is hydrolyzed off, and its ends are joined together through α-1,4 linkages [4, 5]. Since these enzymes are not very specific as to the site of hydrolysis, the product contains α-, β-, and γ-cyclodextrins together with small amounts of higher analogues consisting of up to 13 glucose units [6–8].

Naturally occurring α-, β-, and γ-cyclodextrins are cyclic oligosaccharides consisting six, seven, and eight α-1,4-D-glucopyranose units, respectively. Built of glucopyranoside units in the $^4C_1$ conformation, CDs are pictured as a shallow truncated cone with a cavity lined with H3 and H5 protons and lone pairs of glycosidic oxygen atoms lying in a plane thus endowing the cavity with hydrophobic character, while the bases formed by the primary and secondary OH groups bestow a hydrophilic character (Fig. 1.1) [9]. Due to the hydrophilic cavity exteriors, native CDs are water-soluble, while the hydrophobic cavity interiors endow CDs with the capability to encapsulate hydrophobic moieties of a wide range of guest molecules either completely or partially inside the cavity to form "host-guest" inclusion complexes. As the host molecules, cyclodextrins are "all-purpose molecular containers for organic, inorganic, organometallic, and metallo-organic compounds that may be neutral, cationic, anionic, or even radical" [11, 12]. The principal factors involved in the inclusion complexation are van der Waals forces and hydrophobic interactions, while hydrogen bonding and steric effect may play certain roles [12, 13]. The inclusion complexation ability is of great significance to CDs in research and applications including pharmaceutical [14–16], food [17–19], the chemical industry, and, to the greatest extent, separation science [1, 17–20].

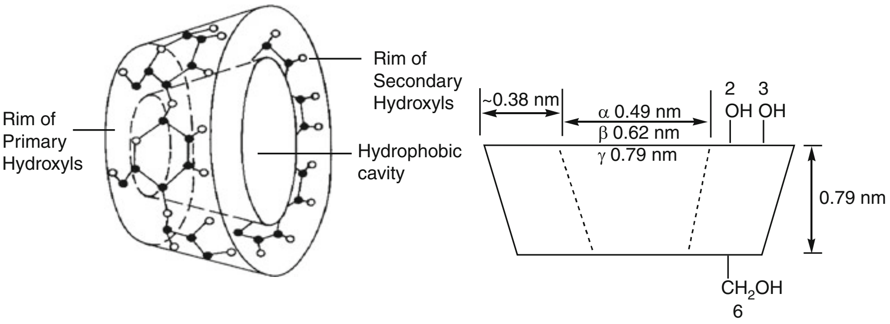


Fig. 1.2 Schematic representation of the cyclodextrin torus

Table 1.1 Some physical and chemical properties of native cyclodextrins

	α-cyclodextrin	β-cyclodextrin	γ-cyclodextrin
Number of glucose units	6	7	8
Formula (anhydrous)	$C_{36}H_{60}O_{30}$	$C_{42}H_{70}O_{35}$	$C_{48}H_{80}O_{40}$
Molecular weight	972.85	1134.99	1297.12
Approximate cavity volume (nm^3)	1.74	2.62	4.27
α_D (deg.)	+150.5	+162.0	+177.4
p*K*a (25°)	12.33	12.20	12.08
Solubility (g/100 ml water, 25 °C)	14.5	1.85	23.2

In general, a cyclodextrin molecule can be briefly described as a torus but is somewhat more realistically pictured as a shallow truncated cone possessing multiple stereogenic centers with a partially blocked base, a hydrophobic interior cavity, and hydrophilic edges due to the presence of hydroxyl groups. All the glucose units in this toroidal structure are in their chair conformation. The interior of the CD cavity is relatively hydrophobic, while the outside rim is more hydrophilic [21, 22]. The rim on the wider side of the CD cavity contains the chiral secondary hydroxyl groups, while the opposite smaller opening is occupied by achiral primary hydroxyl groups. The dimensions are schematically shown in Fig. 1.2.

The sizes of the hydrophobic cavities are different for different types of cyclodextrin; the α-CD can accommodate a single phenyl ring, while β-CD and γ-CD can accommodate substituted single and multiple ring systems. This inclusion alone is not enough for chiral recognition: interactions between substituents on the asymmetric center of the analyte and the hydroxyl groups on the CD rim are responsible for chiral recognition. Some physical properties of these three CDs are quite different (as shown in Table 1.1) [23–27].

CDs are able to be regarded as “hosts” for “guest” molecules capable of entering (in whole or in part) the cavity and forming noncovalent host-guest inclusion complexes. Almost all applications of CDs involve complexation. The mechanism of inclusion complexation between CD-host and molecule-guest is schematically shown in Fig. 1.3. A combination of weak forces such as hydrophobic interaction, electrostatic interaction, van der Waals interaction, hydrogen bonding, and dipole-dipole

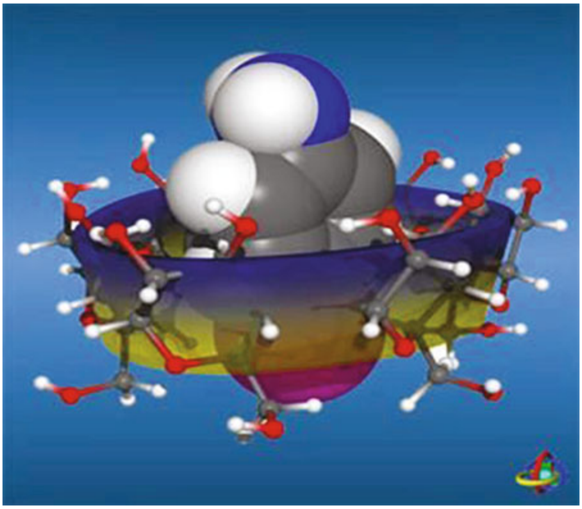


Fig. 1.3 Inclusion complexation between CD-host and molecule-guest

interaction cooperatively governs the inclusion complexation behavior of the cyclodextrin hosts. These weak interactions between CDs and guest molecules result in a selectivity-structure correlation, and this correlation forms the basis for chiral separation and other applications using CDs. An important feature of this technique is that CDs introduce a shape-selective effect that is beneficial for the separation of structural, geometrical, and optical isomers. The inclusion complex between the CDs and solutes depends on the size and shape of molecules, as well as the interior size of the CDs. The attractive properties of CDs have led many researchers to develop novel CD derivatives. The hydroxyl groups present on the rim of the CDs can be easily modified by chemical reactions. Modified CDs may have very different properties compared with native CDs. These include increased solubility, possibility for different secondary bonds, different hydrophobicity of the cavity, and potentiality for the analysis of highly hydrophobic and uncharged compounds.

When CDs selectively form inclusion complexes with enantiomeric guest, chiral recognition occurs. CDs and their derivatives are the most widely used chiral selectors for enantiomeric separation of racemic guest molecules, especially those with biological, pharmaceutical, and agrochemical interest [28, 29]. There are literally thousands of cyclodextrin derivatives that have variable ring size and random or site-specific chemical functionalization [14]. The modified CDs harvest better solubility in certain solvents and improved chiral selectivities towards specific guest molecules in various practical applications.

Despite the numerous CD derivatives were investigated in the past decades for the purpose of chiral separation, a great need still exists for further effort in synthetic chemistry aiming at the development of novel CDs with improved chiral recognition ability towards a large pool of racemic analytes with shorter separation time and greater analysis accuracy.

Strategies for selective CD modification have been well reviewed in other literature for wide applications such as analytical, catalytic, biological, and pharmaceutical [30, 31]. This chapter provides a specific overview of the modification strategies of various CD derivatives as chiral selectors for analytical chromatographic techniques and capillary electrophoresis.

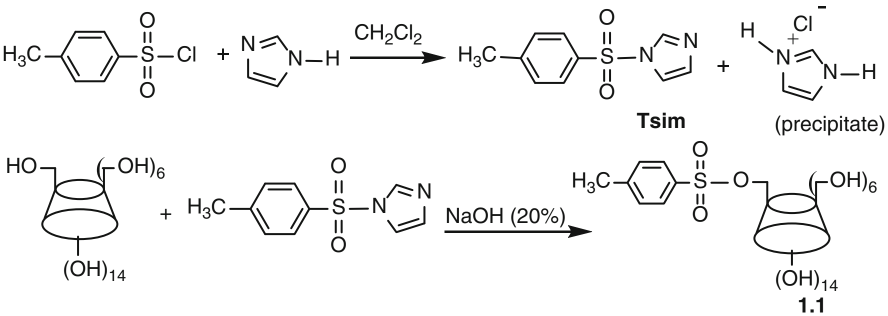
1.2 Cyclodextrin Modification

There are two primary factors needed to be considered in the chemistry of cyclodextrins for their modification: the nucleophilicity of the hydroxyl groups and the ability of cyclodextrins to form complexes with the reagents used [31]. All modifications of cyclodextrins take place at the hydroxyl groups. Since hydroxyl groups are nucleophilic in nature, the initial reaction, which directs the regioselectivity and the extent of modification (mono, di, tri, per, etc.) of all subsequent reactions, is an electrophilic attack on these positions.

The best method to provide cyclodextrins of any size, shape, and most importantly containing any functional groups is to selectively convert the hydroxyl groups to other desired functionalities. The modification of cyclodextrins offers chemists both enormous opportunities and challenges. Hydroxyl groups present at the 2-, 3-, and 6-positions compete for the reagent used, which makes selective modification extremely difficult. Of the three types of hydroxyl groups present in CD rims, the most basic (and often most nucleophilic) are those at the 6-position, the most acidic are those at the 2-position, and the most inaccessible are those at the 3-position [32, 33]. Thus, under normal circumstances, the 6-position is easily attacked by an electrophilic reagent (Fig. 1.1). The less reactive reagents will attack the hydroxyl groups more selectively. Thus, more reactive reagents will not only react with hydroxyl groups at the 6-positions but also with those on the secondary side; whereas, less reactive reagents will react more selectively with the 6-position hydroxyl groups. For instance, the less reactive reagent *tert*-butyldimethylsilyl chloride (TBDMSCl) will react selectively with hydroxyl groups at the 6-positions [34], while the more reactive reagent trimethylsilyl chloride (TMSCl) will indiscriminately react with all hydroxyl groups in CD rims [35].

1.2.1 Monosubstitution at the 6-Position of Cyclodextrins

As discussed above, the primary hydroxyl groups of the CD are the most nucleophilic; this may be selectively modified by reaction with electrophilic species. For example, cyclodextrins react with *p*-toluenesulfonyl chloride in pyridine or DMF containing a base, to give a mixture of products arising from sulfonation of these groups [36, 37]. The corresponding C(6) CD monotosylates may then be obtained by separation from the mixtures via chromatography in the case of α-CD, and



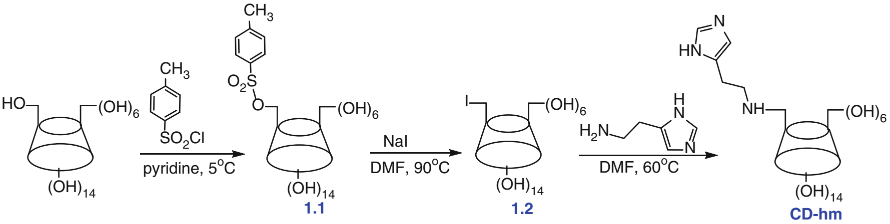
1.1

Scheme 1.1 Synthesis of mono-6-tosyl-β-cyclodextrin

through recrystallization from water for the β-CD derivative. As a nonuser-friendly solvent of choice for this reaction, pyridine forms a pyridinium complex with the cavity and complicates the work-up process. However, the major advantage of this solvent is its ability to direct the reaction to the 6-position as compared to DMF where sulfonation occurs on both faces of cyclodextrin. The method of choice [38] for the synthesis of monotosylcyclodextrin is to react cyclodextrin with tosyl chloride in 1:1 equivalent ratio in aqueous alkaline medium for a short time to give the mono-6-tosylate in fairly good yield. The product is obtained in a reasonable purity either by repeated crystallization from water or by chromatography on a charcoal column [38].

The synthesis of mono-6-tosyl-β-cyclodextrin was recently improved by taking advantage of CD's complexation property to introduce one tosyl group specifically to 6-position or 2-position. Facile synthesis of mono-6-tosyl-β-CD is achieved by preparing the key intermediate compound, 1-(*p*-toluenesulfonyl)imidazole (Tsim), for better yield and easier handling. As shown in Scheme 1.1, a two-step protocol was used to eliminate the drawbacks of the previously reported approach using pyridine [39, 40]. With tosyl imidazole as key intermediate compound, mono-6-tosyl-β-cyclodextrin **1.1** can be synthesized in aqueous base solution in an overall yield of 36 %. This approach features easy work-up and selective modification with good yield.

With the readily obtained mono-6-tosyl-cyclodextrins at hand, a further nucleophilic attack of mono-6-sulfonylcyclodextrin with a reagent containing the appropriate group has thus been developed into the most popular method for monomodifications at the 6-position of cyclodextrins. Mono-6-tosyl-cyclodextrins are important precursors for a variety of modified cyclodextrins because a nucleophile can attack the electrophilic carbon atom at the 6-position to produce a corresponding functionality. A nucleophilic displacement of the tosyl group may be realized by using suitable nucleophiles such as iodide, azide, thioacetate, hydroxylamine, alkyl, or poly- (alkylamines) to afford monoiodo- [31], ^{0}azido- [41], thio- [42, 43], (hydroxylamino)- [44], or (alkylamino)cyclodextrins [45].



Scheme 1.2 Synthesis of CD-hm (Reprinted with permission from Ref. [46]. Copyright 1991 American Chemical Society)

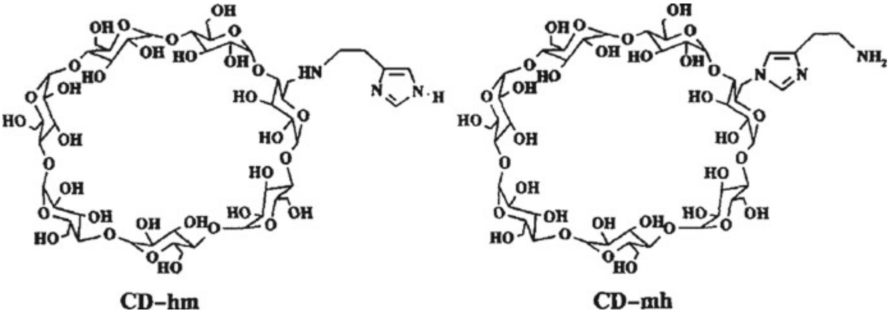
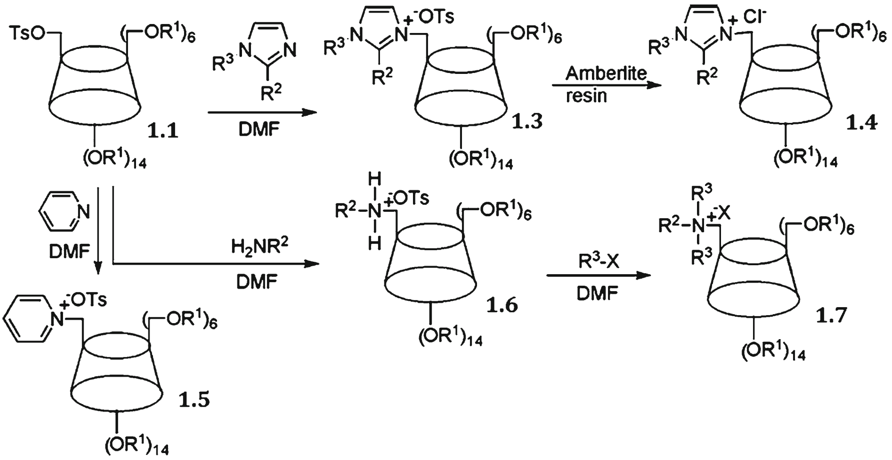


Fig. 1.4 The structure of CD-hm and CD-mh (Reproduced from Ref. [47] by permission of John Wiley & Son Ltd.)

An example of the using of nucleophilic displacement of mono-6-tosyl-cyclodextrin with NaI to prepare monoiodocyclodextrins and further histamine-substituted β-CD [6-Deoxy-6-(N-histamino)-β-cyclodextrin, CD-hm] is shown in Scheme 1.2 [46–48].

By linking the histamine group onto the upper rim of CD linked by the imidazole nitrogen, Marchelli and his coworkers also developed the analogue of CD-hm, 6-deoxy-6-[4-(2-aminoethyl)imidazolyl]-β-cyclodextrin (CD-mh) (structure in Fig. 1.4) [47]. Good enantiomeric separation for dansylated amino acids was obtained by using low concentrations of the selectors (1–3 mM). In order to modulate the number and the position of the positive charges, the electrolyte pH was increased from 5 to 7.5, where the chiral discrimination decreased along with the deprotonation of the imidazolyl moiety when pH increased. The results showed that CD-mh presented better chiral resolution ability than CD-hm under the same analytical conditions, indicating the type and position of cation groups are crucial for the chiral separation using electrostatic interactions as the driving forces besides inclusion complexation.

A library of cationic β-CDs substituted at the 6-position with imidazolium (**1.3**, **1.4**), pyridinium (**1.5**), and quaternary ammonium (**1.6**, **1.7**) moieties (Scheme 1.3) were firstly reported in Ng's group [40, 49]. Starting from mono-6-tosyl-β-CD, a nucleophilic substitution with alkylimidazole, pyridine, or alkylamine and a further

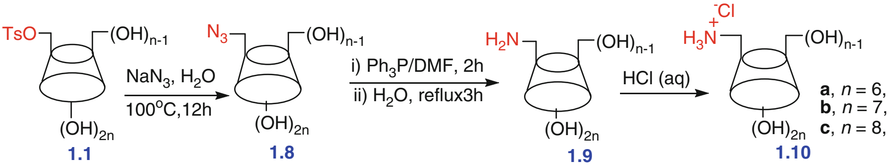


Scheme 1.3 Synthesis of mono-6-alkylimidazolium-, pyridinium-, and quaternary ammonium-substituted β-CDs (Reprinted from Ref. [49], Copyright 2005, with permission from Elsevier)

Table 1.2 Yields and melting point data for cationic β-CDs [49]

CDs	R^1	R^2	R^3	X^-	Yields (%)	Melting point (°C)
1.3a	H	H	CH_3	OTs	99	257–259 (dec)
1.3b	H	H	n-C_4H_9	OTs	97	254–256 (dec)
1.3c	H	H	n-C_3H_{17}	OTs	98	256–258 (dec)
1.3d	H	CH_3	CH_3	OTs	62	257–258 (dec)
1.4a	H	H	CH_3	Cl	93	233–235 (dec)
1.4b	H	H	n-C_4H_9	Cl	93	249–250 (dec)
1.4c	H	CH_3	CH_3	Cl	82	199–200 (dec)
1.3e	Ac	H	CH_3	OTs	64	129–130
1.3f	Ac	H	n-C_4H_9	OTs	76	115–116
1.3g	Ac	CH_3	CH_3	OTs	62	125–126
1.5	H	H	–	OTs	85	239–241 (dec)
1.6a	H	C_3H_5	–	OTs	92	249–250 (dec)
1.6b	H	n-C_3H_7	–	OTs	98	260–261 (dec)
1.6c	H	n-C_4H_9	–	OTs	97	263–264 (dec)
1.6d	H	n-C_5H_{11}	–	OTs	96	266–267 (dec)
1.7	H	n-C_3H_7	CH_3	I^-	89	241–243 (dec)

anion exchange afford the monosubstituted alkylimidazolium β-CDs, pyridinium, and alkylammonium β-CDs with varied alkyl chain length. For CDs **1.3** and **1.4**, a general methodology involves the heating of mono-6-tosyl-β-CD with alkylimidazoles in DMF at 90 °C for 2 days. Furthermore, the tosylate anion was converted into chloride by ion exchange through Amberlite 900 (Cl) resin (Scheme 1.3). The CDs **1.5** was prepared by refluxing mono-6-tosyl-β-CD with pyridine at 90 °C for 2 days. For CDs **1.6** and **1.7**, similar methodology is adopted by refluxing alkylamine together with mono-6-tosyl-β-CD **1.1** in DMF for 5 h. While the melting points of these compounds are too high (115–267 °C) (Table 1.2) for ionic liquid



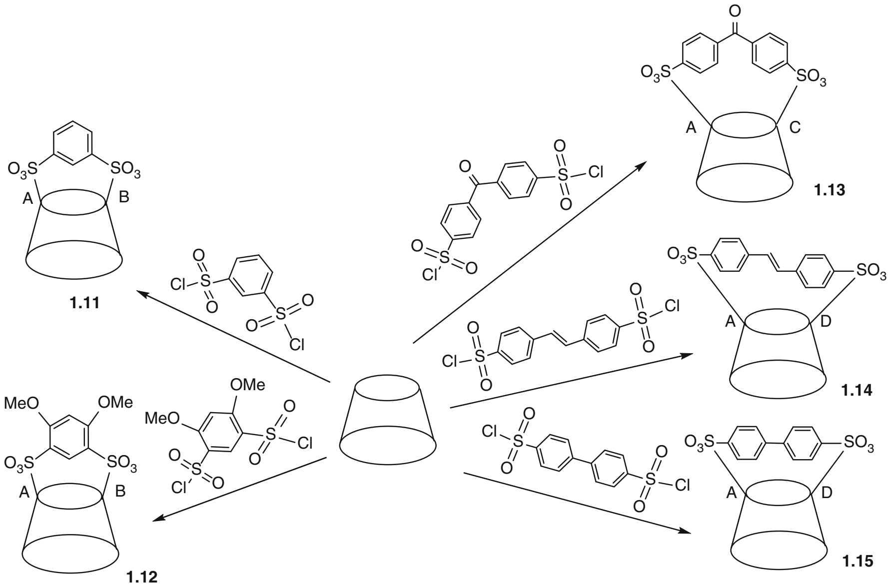
Scheme 1.4 Synthetic approach to mono-6-ammonium CDs

applications, these cationic cyclodextrins are proved highly successful stationary phases for the enantioseparation of aromatic carboxylic acids at low concentrations (3–10 mM). This is presumably due to the strong electrostatic interaction between cationic CD and the anionic analytes [50–52].

Monoamino derivatives of cyclodextrin are conveniently obtained by nucleophilic displacement of tosyl group with ammonia under pressure (10^6 N m^{-2}) for 18 h at room temperature [53] or azide substitution of CD tosylate and followed by hydrogenation with Pd/C for 12 h [54]. Both procedures are low- yield and include incomplete reactions, thus undesirable for large-scale production. An innovative methodology was developed by Tang et al. (Scheme 1.4) [55, 56], which involved the reduction of 6-azido CD (**1.8**) by using of triphenylphosphine and a further hydrolysis to afford monoamino CD (**1.9**). A subsequent treatment of monoamino CDs with dilute hydrochloric acid yields the targeted highly water-soluble cationic CD (**1.10**). These steps are almost quantitative with yield higher than 90 %. This approach has been greatly improved with mild reaction conditions and higher yields than previously reported methods.

1.2.2 Disubstitution at the 6-Position of Cyclodextrins

A particularly efficient method to obtain disubstituted sulfonates of cyclodextrins is by reaction of arenedisulfonyl chlorides with cyclodextrins to give AB, AC, and AD isomers [57–59]. Although these disulfonyl chlorides give a mixture of regioisomers, they show distinct regiospecificity based on their structures. An elegant method to control the regiospecificity to produce AB, AC, or AD isomers by using the geometry of the reagents has been described [60]. For example, as shown in Scheme 1.5, *trans*-stilbene and biphenyl-based capping reagents preferentially give AD isomers **1.14** and **1.15** in yields of 20 and 18 % [60, 61]. Benzophenone-based reagents give AC isomers **13** in 40 % yield [58, 60]. 1,3-Benzenedisulfonyl chlorides [62] (especially the electron-rich 4,6-dimethoxybenzene-1,3-disulfonyl chloride [63]) gives the AB isomers **1.11** and **1.12** in 40 and 12 % yields. Anthraquinone-2,6-disulfonyl chloride gives AC and AD isomers in low yield after purification by HPLC [64]. Bis(9,10-dicyanoanthracenesulfonyl) chloride produces two isomers (AD and AC) in a ratio of 3:1, showing some degree of selectivity [65]. Among the three kinds of regioisomers, the $6^A,6^B$-capped CDs are the most stable and less susceptible to hydrolysis [30].



Scheme 1.5 Using the geometry of reagents to direct the regiospecificity in disubstitution of cyclodextrins

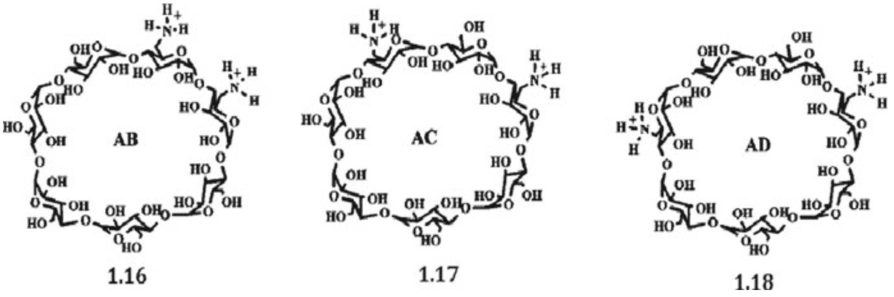
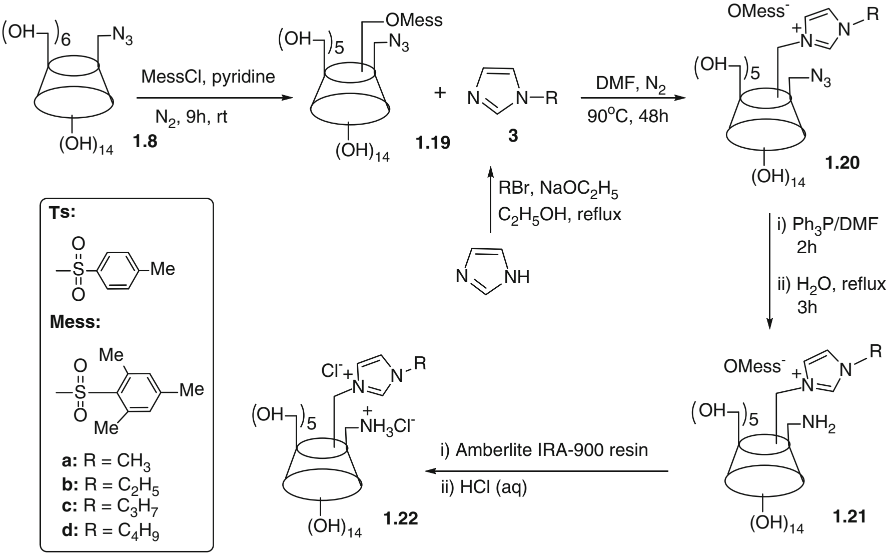


Fig. 1.5 Structure of the three 6,6′-dideoxy-6,6′-diamino-β-cyclodextrins AB (**1.16**), AC (**1.17**), and AD (**1.18**) (Reproduced from Ref. [66] by permission of John Wiley & Sons Ltd.)

The disubstituted sulfonates of cyclodextrins can be used as important precursor for many disubstituted CDs with the same functionalities at 6-positions. For example, selectively modified 6,6′-dideoxy-6,6′-diamino-β-cyclodextrins (AB, AC, AD) was successfully developed by Marchelli and his coworkers [66]. Disulfonated AB, AC, and AD isomers of β-CD were synthesized with high selectivity by using appropriate capping reagents, i.e., 4,6-dimethoxybenzene-1,3-disulfonylchloride, benzophenone-3,3′-disulfonylchloride, and biphenyl-4,4′-disulfonylchloride, respectively [60, 62, 63]. The disulfonated CDs were further nucleophilic substituted with sodium azide [67], followed by reduction with the Staudinger reagents [68] to afford the desired diamino CD derivatives (**1.16, 1.17, 1.18** in Fig. 1.5).



Scheme 1.6 Synthetic route to 6^A-ammonium-6^C-alkylimidazolium-β-cyclodextrin chlorides (Reproduced from Ref. [69] by permission of The Royal Society of Chemistry)

For the case of disubstituted CDs with different functionalities, a facile approach was recently developed in Tang's group [69, 70]. As depicted in Scheme 1.6, the synthetic route for dicationic CDs was established as following: an azide group was first introduced onto C(6A) position of CD [39]; mono-azido-β-CD **1.8** was further nucleophilic substituted with 2-mesitylenesulfonyl chloride to give a mixture of three regioisomers, 6^A-azido-6^X-mesitylenesulfonyl-β-cyclodextrin (X = B, C or D). The mixture was then subjected to a reversed-phase column liquid chromatography [71] to afford the desired AC regioisomer, 6^A-azido-6^C-mesitylenesulfonyl-β-cyclodextrin (Mess-N_3-CD) **1.19**, with a yield of 42 %.

The AC regiochemistry was supported by comparison with the known AC [58], AB [60], and AD [63] regioisomers synthesized with capping of CD. The AC regioisomer structure **1.19** is confirmed by its most characteristic absorptions [101.68 (C1), 81.53 (C4)] in ^{13}C NMR, where the strong peaks centered at 71.82 are assigned to C2, C3, and C5 while peak at 59.67 assigned to C6 adjacent to hydroxyl group on primary rim of CD (Fig. 1.6). The as-prepared Mess-N_3-CD **1.19** was then reacted with 3-alkylimidazoles via nucleophilic addition to introduce imidazolium cation onto C(6C) position of CD. Staudinger reaction was further employed to achieve 6^A-amino-6^C-alkylimidazolium-β-cyclodextrin mesitylene sulfonate **1.21**. The final dicationic CDs were synthesized via protonation with dilute hydrochloric acid and anionic exchange with Amberlite (Cl) resin. An example of the confirmation of the final products is shown in Fig. 1.6b for 6^A-ammonium-6^C-butylimidazolium-β-CD chlorides (**1.22d**) [70].

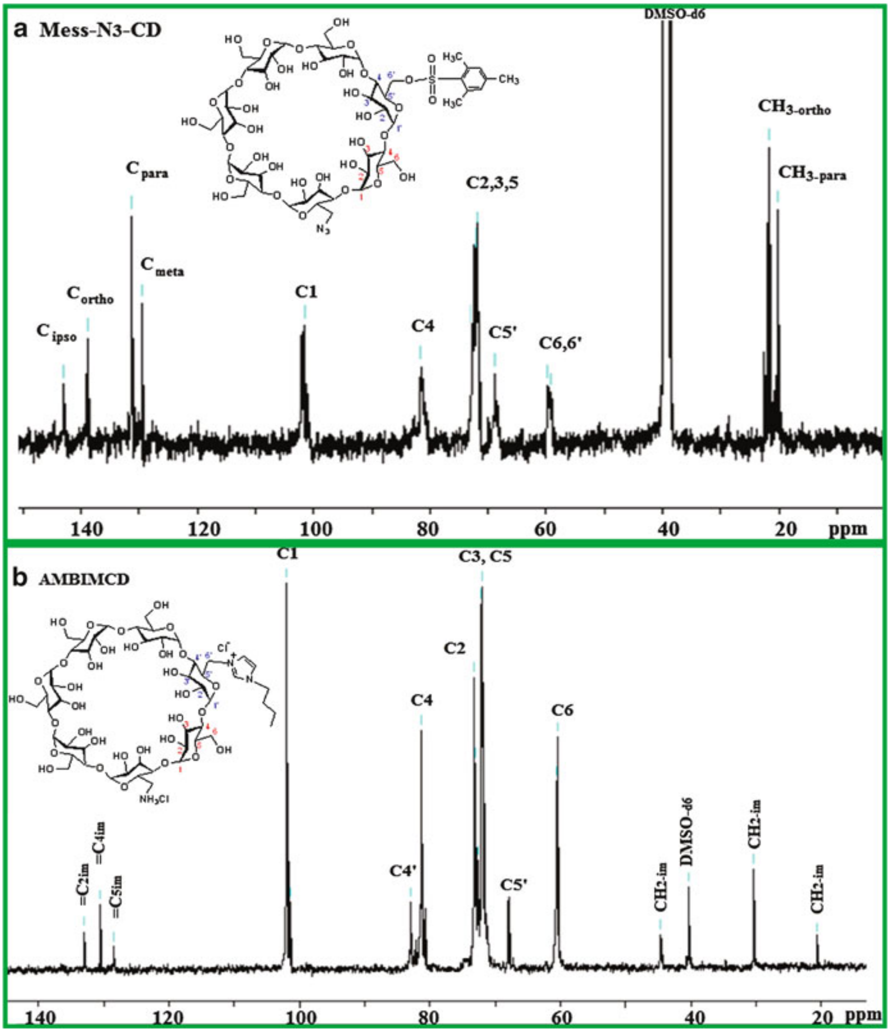
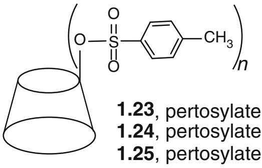


Fig. 1.6 (**a**) 500 MHz ^{13}C NMR spectrum of Mess-N_3-CD **1.19** in DMSO-$_{d6}$, where C_{ipso}, C_{ortho}, C_{para}, C_{meta} refers to the respective carbon atoms in benzyl ring of mesitylenesulfonyl moiety while $CH_{3\text{-}ortho}$ and $CH_{3\text{-}para}$ are assigned to the respective carbon atoms of methyl groups. (**b**) 500 MHz ^{13}C NMR spectrum of **1.22d** in DMSO-$_{d6}$

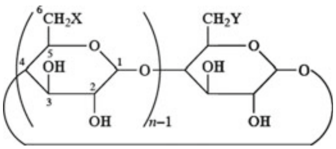
1.2.3 Persubstitution at the 6-Position of Cyclodextrins

According to statistical calculations, permodification of the primary face should give 57 % yield (assuming 91 % yield per reaction; $0.91^6=0.57$) in the case of α-cyclodextrin. However, the actual yield of hexa-6-substituted cyclodextrin is often found to be much lower. Steric crowding, the geometry of the molecule, the

Table 1.3 Structure of 6-persubstituted CDs



1.23, pertosylated α-CD
1.24, pertosylated β-CD
1.25, pertosylated γ-CD



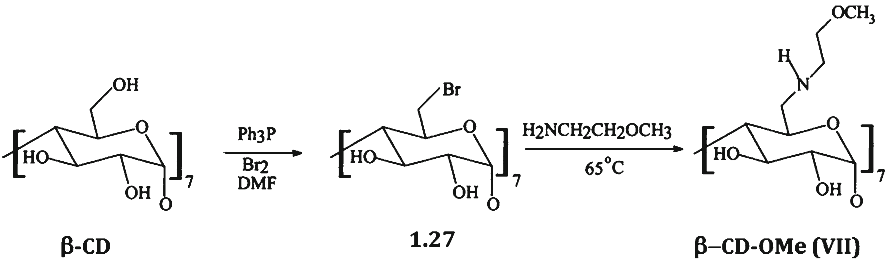
1.26-1.38

	$X = Y$	n	Abbreviation
1.26	Br	6	Ref. [75]
1.27	Br	7	Refs. [75, 76]
1.28	Br	8	Ref. [75]
1.29	I	6	Ref. [77]
1.30	I	7	Ref. [78]
1.31	Cl	7	Ref. [79]
1.32	NH_2	7	per-6-NH2-β-CD [78, 80]
1.33	$NHCH_3$	7	heptamethylamino-β-CD [81, 82]
1.34	$NHCH_2CH_3$	7	heptaethylamino-β-CD [81]
1.35	NH_3^+	6	Per-NH_3^+-α-CD [83]
1.36	NH_3^+	7	Per-NH_3^+-β-CD [83]
1.37	$NHCH_2CH_2OH$	7	β-CD-EA [77, 78]
1.38	$NHCH_2CH_2OCH_3$	7	β-CD-OMe (VII) [79]

type of inclusion complex formed, and positional isomerism decrease the yield of the product as the degree of substitution increases [72].

Persulfonates are generally prepared directly from cyclodextrins and a large amount of sulfonyl chloride in pyridine. The reaction between CD persulfonates with sodium halides was used to prepare CD polyhalides [73, 74]. More directly, CD polybromides **1.26–1.28** (Table 1.3) were prepared by treatment of native α-, β-, or γ-CD with methanesulfonyl bromide in DMF, followed with sodium methoxide [75]. The treatment of β-CD with triphenylphosphine and iodine afforded polybromides 1.27 in 93 % yield [76]. The α- and β-CD polyiodes **1.29–1.30** were directly prepared with α- or β-CD with triphenylphosphine and iodine in 80 and 88 % yields, respectively [77, 78]. Reaction of β-CD with methanesulfonyl chloride and imidazole in DMF afforded the chloride **1.31** in 90 % yield [79].

With CD persulfonates and CD polyhalides at hand, further nucleophilic substitution can be employed to prepare a large library of 6-persubstituted CD derivatives. For example, a reaction of polyiodine **1.30** with sodium azide, followed by Staudinger reduction, afforded the polyamine **1.32** [78, 80]. Direct substitution of halides such as **1.26–1.30** with alkylamine gives the corresponding per-alkylamino-substituted CDs [84, 85]. Alternatively, the per-methylamino-substituted CD **1.35** and per-ethylamino-substituted CD **1.36** were directly prepared by treated β-CD with methylamine or ethylamine, respectively. The treatment of per-amino-substituted CDs with



Scheme 1.7 Synthesis route for heptakis(6-methoxyethylamine-6-deoxy)-β-cyclodextrin [β-CD-OMe (VII)] (Reprinted from Ref. [87], Copyright 1998, with permission from Elsevier)

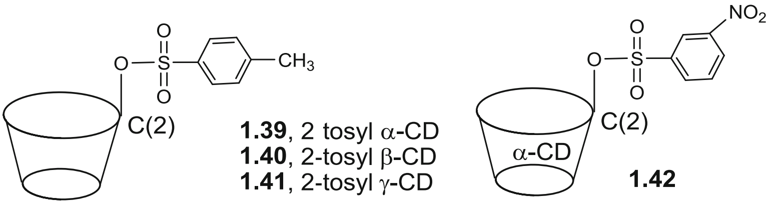
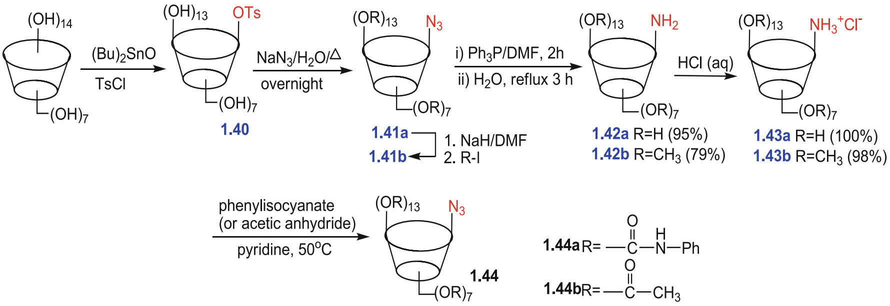


Fig. 1.7 Structure of mono-2-sulfonate CDs

dilute HCl solutions can afford salt form of CDs **1.35** and **1.36** [83]. The heptakis (6-hydroxyethylamine-6-deoxy)-β-cyclodextrin (β-CD-EA) [50, 86] and heptakis (6-methoxyethylamine-6-deoxy)-β-cyclodextrin [β-CD-OMe (VII)] [87] were prepared in 60–65 % yield by direct nucleophilic substitution of polybromide **1.27** with hydroxyethylamine or methoxyethylamine, respectively. The synthetic route for approach β-CD-OMe (VII) is shown as Scheme 1.7.

The secondary hydroxy groups of the CDs are the most acidic, with pKa values near 12.2 [88]. And due to the presence of twice the number of hydroxy groups at the secondary rim of CD than the primary rim, the steric hindrance for substitution at secondary side of CDs is larger than that at the primary side. Moreover, the hydrogen bonding between hydroxy groups at the 2- and 3-positions makes them rigid and less flexible as compared to C-6 hydroxy groups. All these factors make the secondary side less reactive and harder to be selectively functionalized than the primary face. During the course of a reaction, as the degree of substitution increases, the secondary side becomes even more crowded. The increased steric hindrance with the incoming nucleophile forces the attacking group towards the other face, which decreases the selectivity [31].

The C(2) secondary hydroxy groups are the most nucleophilic above pH 10–11, whereas under neutral and acidic condition, it is the primary hydroxy groups [30]. The selective modification of the C(2) secondary hydroxys of CD is thus generally realized through treatment with electrophilic reagents. The preparation of mono-2-tosyl β-CD **1.40** (Fig. 1.7) was achieved by treatment of



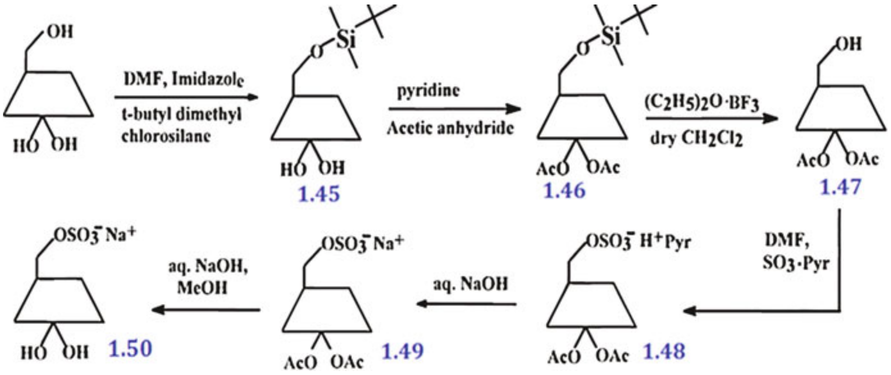
Scheme 1.8 Synthesis route for mono-2-functionalized CDs

β-CD with *p-toluenesulfonyl* chloride and sodium hydride in DMF [89], whereas the corresponding mono-2-tosyl α-CD **1.39** was prepared using *p*-toluenesulfonyl chloride and α-CD in aqueous alkaline solution [90]. The reaction of α-CD with *m*-nitrobenzenesulfonyl chloride at pH 12 afforded C(2) sulfonate **1.42** [91]. The sulfonate **1.40** was also accessible with the reaction of β-CD with *m*-nitrophenyl tosylate in pH 10 aqueous solution [92].

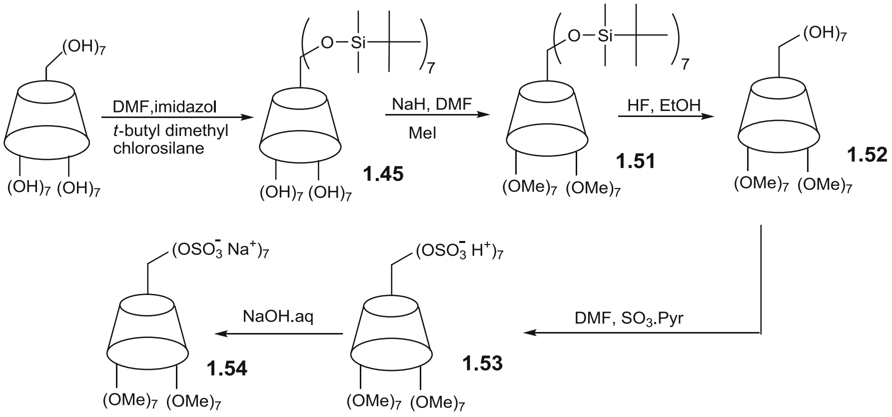
Alternatively, the mono-2-tosylate **1.39–1.41** can be synthesized by using dibutyltin oxide. The C(2) hydroxy group was activated by the reaction of dibutyltin oxide with 1,2-diols at the secondary rim of a CD glucopyranose unit. The mono-2-tosylate **1.39–1.41** can be important precursors for large library of 2-functionalized CDs. As shown in Scheme 1.8, the reaction of mono-2-tosyl β-CD **1.40** with sodium azide afforded mono-2-azide β-CD **1.41** [40]. Followed reduction with triphenylphosphine generated mon-2-amino β-CD **1.42**, which was further transferred into salt form in almost quantitative yields. The rest of hydroxy groups at C(6), C(3), and C(2) positions can be fully substituted with methyl, phenylcarbamate, or acetyl functionalities with nonselective nucleophilic substitution [93].

Besides pH, the formation of an inclusion complex by the cavity of cyclodextrins and the relative orientations of reactive groups in the complexes also play a very prominent role in the regioselectivity of reaction of CDs. An included reagent may react with the hydroxy groups at the 2-, 3-, or 6-positions depending on the nature of the complex [92]. Some of these problems can be overcome by protection of the primary side before modification of the secondary side [31]. For example, per-6-silyl-mono-2-tosylcyclodextrin is synthesized by the reaction of 6-silylated cyclodextrin with tosyl chloride in THF with NaH as a base in 32 % yield after purification [94]. An advantage of this strategy is that the reaction as well as the purification steps can be carried out in organic solvents and the desilylation can be carried out easily to yield the desired tosylate.

The protection strategy was widely used to prepare C(2) and C(3) persubstituted CD derivatives. As shown in Scheme 1.9 [95], heptakis-(2,3-diacetyl-6-sulfato)-*β-cyclodextrin* **1.49** is produced by first reacting β-cyclodextrin with dimethyl-*tert*-butylchlorosilane [96], purifying the intermediate **1.45** with gradient elution preparative column chromatography [97] on silica gel using *n*-hexane/ethyl acetate/ethanol as



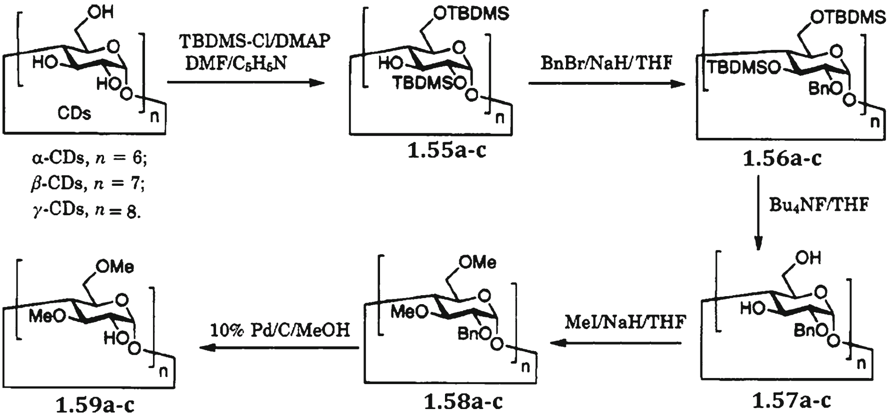
Scheme 1.9 Synthesis scheme for heptakis(2,3-diacetyl-6-sulfato) -β-cyclodextrin (Reprinted with the permission from Ref. [95]. Copyright 1997 American Chemical Society)



Scheme 1.10 Synthesis scheme for heptakis(2,3-dimethyl-6-sulfato)-*β*-cyclodextrin (Reprinted with the permission from Ref. [100]. Copyright 1998 American Chemical Society)

eluent. The purified intermediate was then peracetylated with acetic anhydride. The purified heptakis(2,3-diacetyl-6-dimethyl-*tert*-butylsilyl)-β-cyclodextrin **1.46** was then reacted with boron trifluoride etherate to remove the dimethyl-*tert*butylsilyl-protecting group [96]. Next, the pure heptakis-(2,3-diacetyl)-*β*-cyclodextrin **1.47** was reacted with SO3•pyridine in DMF to completely sulfate the primary hydroxyl groups of the cyclodextrin [98]. By neutralizing the aqueous solution of **1.48** with NaOH, the desired heptakis-(2,3-diacetyl-6-sulfato)-*β*-cyclodextrin **1.49** was obtained. The deacetylation of **1.49** by reacting with 25 % methanol in pH 12 aqueous solution for 12 h afforded sodium salt of hepta-6-sulfato-*β*-cyclodextrin **1.50** [99].

By using similar protection strategy, heptakis(2,3-dimethyl-6-sulfato)-*β*-*cyclodextrin* was prepared from per-6-6-dimethyl-*tert*-butylsilylated *β*-CD. After complete methylation at secondary side of CD, the intermediate **1.51** (Scheme 1.10)



Scheme 1.11 Synthesis of the per(3,6-di-O-methyl)-cyclodextrins (Reprinted with the permission from Ref. [101]. Copyright 1995 American Chemical Society)

was then reacted with HF in ethanol to remove the *tert-butyldimethylsilyl-protecting* group to afford **1.52**. The primary hydroxy groups were further sulfated with pyridinesulfonate in DMF, followed by treatment with aqueous NaOH solution to generate the desired product [100].

The persubstitution of the C(2) hydroxy groups is generally achieved by first protecting the primary and C(3) hydroxy groups, to avoid the competing processes. As illustrated in Scheme 1.11, a convenient synthesis of per-O-benzyl CDs **1.57a-c** is achieved with a first silyl protection step for both C(6) and C(2) positions of CDs and a second migration step of silyl protection groups between C(2) and C(3) hydroxy groups [101]. The migration of silyl protection groups was thought to be an intramolecular process, which exposed the C(2) alkoxides for functionalization. Hence, the treatment of intermediate compound **1.55a-c** with benzyl bromide, followed by desilylation of the product of **1.56a-c** with tetrabutylammonium fluoride, affords the corresponding per-O-benzyl CDs **1.57a-c**. Similarly, the reaction of **1.55a-c** with methyl iodine, followed by desilylation of the resultant product, gives heptakis(2-O-methyl)-β CD.

The mechanism for the migration of silyl group was explained as in Scheme 1.12, where a five-membered ring intermediate **B** containing a pentacovalent silicon atom was most likely involved. The alkylation occurs under kinetic control, i.e., the species **A** is more stable than the isomeric species **C** but **C** is more reactive as a result of the proximity of the 2-OH to the anomeric center [101]. The yields of these reactions are 78 % for β-CD, 76 % for γ-CD, and 49 % for α-CD (49 %). The lower yield of the benzylation of the α-CD derivative is probably a consequence of the higher steric hindrance of the smaller α-CD ring and the bulky substituents, i.e., TBDMS and benzyl groups.

Due to the high inaccessibility of those hydroxy groups at the 3-position of CD, the selective modification of C(3) hydroxy groups is realized by using special reagents to