Chapter 2

β-Cyclodextrin based Monomers

2.1. Monofunctionalization of β-Cyclodextrin on primary side and their applications

2.1.1 Brief Review

Chemically modified CDs are synthesized so as to vary their solubility behavior, to modify their complexation properties (i.e. stability constant, guest selectivity) and to introduce functional groups that can achieve specific functions. There are generally two common ways in which the CD hydroxyls groups can be functionalized.

i. monofunctionalization - functionalizing of only one hydroxyl group.
ii. per-functionalization – functionalizing of an entire set of hydroxyl groups.

Although di- and tri-functionalizations exist[1], they have not been well investigated and are difficult to perform. Monofunctionalization of the CDs have, however, been well studied in the functionalization of these starch derivatives[2]. These monofunctionalizations can be achieved by a reaction of the hydroxyl groups with an electrophile. The large number of hydroxyl groups at the three different positions of CDs makes modification at a single desired place complicated[3]. Although selective monofunctionalization at a desired position is a challenging task, the differences in the chemical properties and reactivities among these sites can be exploited to yield a specific product.

The most popular pathway for monomodification at the 6-position of β-CDs is to exploit mono- 6-tosylate β-CD as starting materials (II, III, IV and VI in Figure2.1.1) although some of monosubstitutions can be obtained straightforward from the native β-CD (V and IX in Figure 2.1.1). Monotosylation of β-CD (I in Figure 2.1.2) has been carried by reacting one equivalent of *p*-toluenesulfonyl chloride with freeze-dried CD in pyridine[4]. Pyridine tends to protect β-CD from decomposition in the presence of a strong acid[5], and also to direct the reaction to the 6-position.

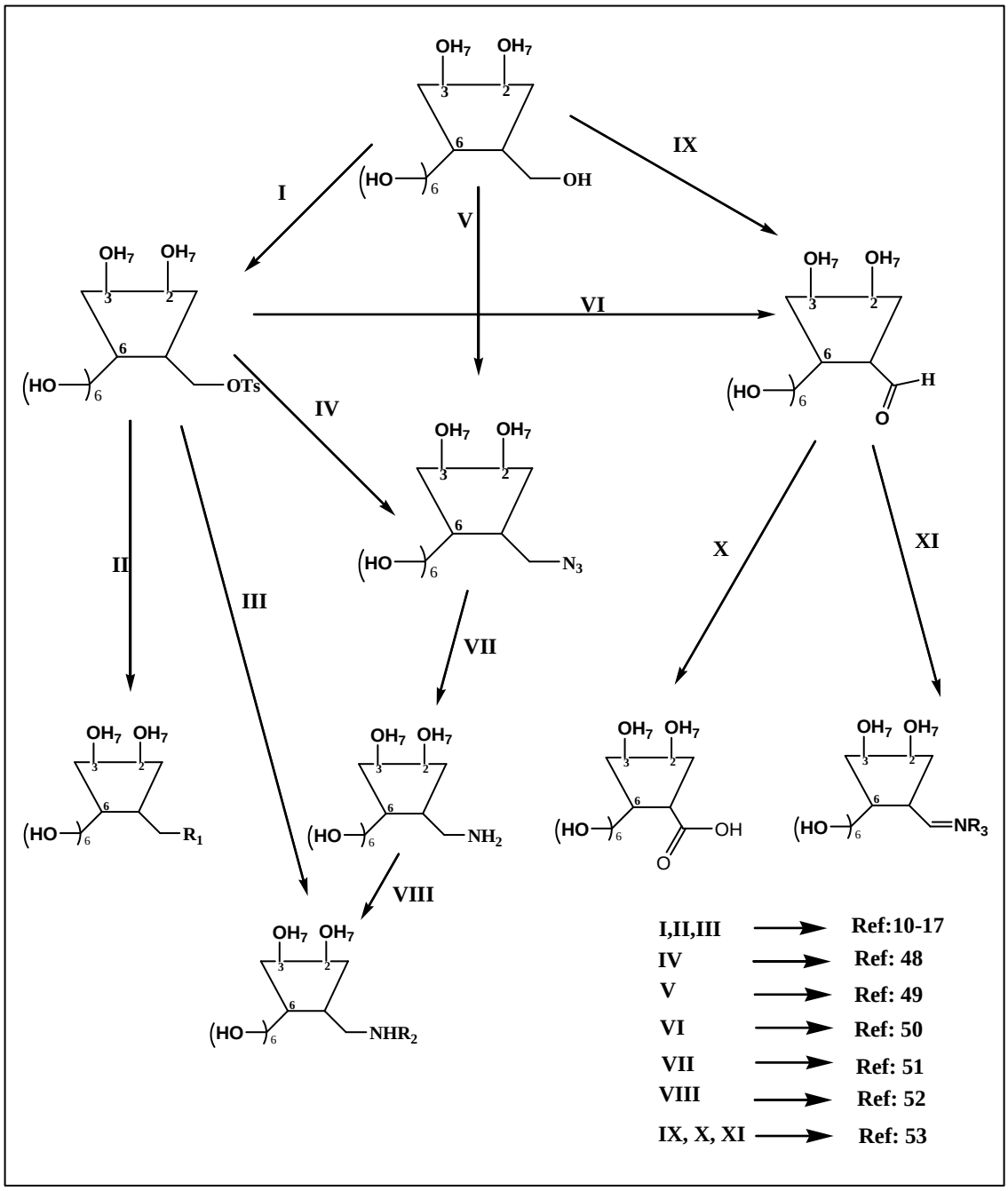


Figure 2.1.1 Overview of strategies for monosubstitution at the 6-position of β-CD

R1 halogens (Br, I), azide, alkyl and polyalkyl amine, alcohol, hydroxylamine, heterocycles, carboxilc acid, thiols, metals;

R2 aldehyde, isocyante, acyl chloride, carboxylic acid;

R3 hydroxylamine, hydrazine, alkyl amine.

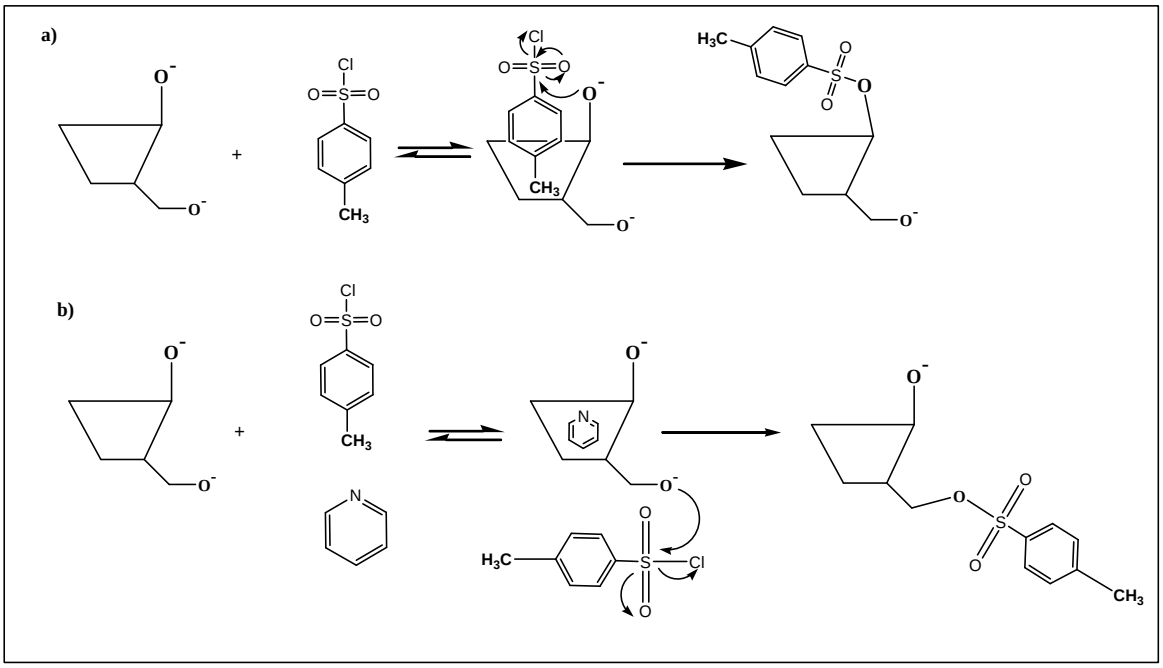


Figure 2.1.2 Monotosylation of β-CD: (a) without pyridine, the secondary face is promoted or (b) with pyridine, the primary face is promoted.

Latter pyridine, a non-user-friendly solvent, was replaced by an aqueous alkaline medium without noticing any change in terms of selectivity or yield[6]. These methods turned out to have time-consuming processes, low yields and poor reproducibility. New synthetic methods have been developed to overcome these drawbacks. Using 1-(*p*-toluenesulfonyl) imidazole instead of *p*-toluenesulfonyl chloride to react with CD (1:1 equivalent ratio) in aqueous alkaline medium for a short period allows to obtain the mono-6-tosyl in fairly good yield[7]. Recently, Trotta et al. have shown that an ultrasound-promoted monotosylation proved to be very advantageous in terms of yield, reaction times and product purity[8, 9]. The selectivity of all these processes depends on the reaction time: the substitution rate increases with the reaction time. In all cases, repeated crystallizations from water will be necessary to obtain the product in a reasonable purity.

6-Tosyl CDs are important precursors for a variety of modified CDs because a nucleophile can attack the electrophile carbon atom at the 6-position to produce a corresponding functionality. Suitable nucleophiles such as iodide, bromine, azide, hydroxylamine, alkyl, poly (alkylamine) or diamine can react with 6- tosyl CDs to yield monoiodo[10], bromo[11], azido[12], hydroxylamino[13], alkylamino[14, 15] and diamino-CDs[16, 17]. These CDs are used as chemical sensor[18], chiral selector[19], protein carrier system[20] and in catalyst[21]. A variety of other CD-derivatives have been prepared by displacing the tosyl group from the 6-position of CD. An acetylenic group like propargyl alcohol has been connected to CD to provide a building block for the construction of CDs dimers and trimers with a core junction which is potentially electron conducting[22]. Tang *et al* preapred ethylene diamine linked β-CD dimer to provide building block for encapsulation of non-polar molecules[23]. Ma *et al.* anchored an azobenzene group to obtain a CD-based rotaxane[24]. In the meantime, Casas-Solvas *et al.* used it to afford a photoswitchable molecular receptor by conjugation with molecules involved in molecular processes[25]. Efficient fluorescent sensors for molecular recognition of bile salts have been synthesized by attaching 1-naphthol and 8-hydroxyquinoline[26]. The tosylate group has also been replaced by a heterocycle such as imidazole. The cationic CD synthesized provides base-line enantioseparation of appropriately sized aromatic carboxylic acid by capillary electrophoresis[27]. β-CD hemicyanine dyes compounds have been synthesized by reacting 4-methyl pyridine to 6-tosyl CDs[28]. These compounds exhibit a higher photostability than free hemicyanine.

6-Tosyl CDs have also been used to link compounds containing a carboxylic acid function with an ester bond. Catechol-type ligands have been introduced on CDs by reacting 2, 3- and 3, 4-dibenzyloxybenzoic acid with 6-tosyl CDs: the chiral catalytic activity of their Mo^{V} and Cu^{II} complex have been examined in the asymmetric oxidation of aromatic sulfides using hydrogen peroxide in water[29]. Carboxylates could be also applied: naproxen, sulindac, Flurbiprofen[30] and etodolac[31] salts have been coupled to CDs to produce colon targeted delivery systems for treatment of inflammatory bowel diseases. A variety of mercapto CD derivatives have been obtained from monotosylated CDs and have been used to study *e.g.* the catalytic activity a phosphine-modified CD and its corresponding rhodium complex in desymmetrization[32], immobilized films on a gold surface[33], novel fluorinated amphiphilic CD derivatives as delivery systems[34], the stabilisation effect on free radicals for electron paramagnetic resonance detection[35]. 6-Tosyl CDs have also been bridged through a sulfur link to give potential tumor pretargeting agents[36] or a molecular carrier of ferrocene[37].

Amino acids such as *N*-dansylcysteine or L- and D-tryptophan have also been attached to the primary side of CD to control the geometry around the cavity of the CD[38] or to study binding ability and selectivity of these CD derivatives towards a series of bile acids[39]. Metals have also been introduced on the primary face of CD. Alkyl and aryltellurides were grafted on 6-tosyl CD to provide enzyme mimicking (glutathione peroxidase) compounds and inhibitors of thioredoxin reductase[40].Monoamino derivatives of CD have also been reported[41]. These are conveniently obtained from monoazides of CDs by reduction with triphenylphosphine in the presence of ammonia. Monoazides of CDs are indirectly obtained by heating the monotosylate with sodium or lithium azide salt containing triphenylphosphine in DMF[42]. Monoamines show greater solubility in organic solvents and react with isocyanates without the need to protect the primary hydroxyl groups to produce isocyanato CDs.

In this chapter a short synthesis of mono-6-isothiocyanato-6-deoxy-β-Cyclodextrin monomer is described. Cyclodextrins containing isothiocyanato functionality were very useful precursors in the synthesis of thiourea CD derivatives[43]. It is well known that compounds containing the urea or thiourea functionality are of extended biological interest in several classes of drugs[44]. Reported literature says in order to

synthesis of isothiocyanto CD derivatives, the first and most important step is to synthesize of tosylated CD derivative (CD-OTs). Garacia and et al synthesized oligosacharadiyal isothiocyanate at the 6-primary alcohol position from amines by reaction with thiophosgene[45]. After that Hiramatsu et al synthesized isothiocyanated CD derivatives by converting tosyl CD into azide CD, azide CD into amine CD and finally into isothiocyanated CD derivatives by adopting long synthetic routes[46]. Thereafter Marsura et al synthesized isothiocyantes of disaccharides and cyclodextrins by using phosphine imide reaction[47]. From all these reported literature the processes involving the synthesize of isothiocyanated derivatives are long synthetic methodologies, time consuming, not economical and using toxic chemicals. Now coming to our synthetic methodology the first step was to synthesis of tosylated CD derivative i.e. mono-6-(p-toluenesulfonyl)-6-deoxy-β-cyclodextrin **1,** (Scheme 2.1.1) which was common for both reported and our present work. After synthesizing tosylated CD derivatives isothiocyanated CD derivatives were synthesized by single one pot reaction by the elimination of the long synthetic methodology which was reported in the literature. So the following isothiocyanted CD monomer was synthesized by simply adding potassiumthiocyanate to the reaction mixture containing tosylated CD. The isothiocyanato CD monomer **2,** (Scheme 2.1.2) was very useful precursor in the synthesis of thiourea CD derivative. This synthetic methodology can be applied for other cyclodextrins (α, γ) and also compounds containing hydroxyl functionality (i.e. who wishes to convert $–CH_2OH$ to $–CH_2NCS$).

To synthesize the disubstituted β-cyclodextrin monomers, biphenyl 4, 4'-disulfonyl chloride was used as the disulfonating agent[48]. The structural dimension of this molecule is shown in Figure 2.2.0. The distance between the two terminal chlorine atoms in this molecule is 12.3 Å and therefore it can form a bridge where the two hydroxyl groups are separated by comparable distance. The disubstituted biphenyl 4,4'-disulfonylbridged-β-cyclodextrin can formed after the structural distortion and the orientation of the reactive chlorine groups towards the AD and AE hydroxyl groups at the 6-position of the β-cyclodextrin. This is shown in Figure 2.2.0. Though biphenyl forms inclusion complex with β-cyclodextrin, the sulfonyl chloride groups attached to the biphenyl molecule prevents it from doing so. Thus, the biphenyl always will form a bridge between the hydroxyl groups of the same cyclodextrin molecule.

In this chapter, a synthetic method for the synthesis of selective diamino β-cyclodextrin monomer by oxido reductive substitution reaction and 6^A, 6^D-diisothiocyanto 6^A, 6^D-dideoxy β-cyclodextrin monomer by nucleophillic addition reaction of NCS group on electrophiilic carbons without protection and destruction of the CDs cavity. One of the key steps of the present synthesis was the reaction of β-cyclodextrin with bulky biphenyl disulphonyl chloride to form a bisulphonate ester on the smaller primary rim of β-cyclodextrin. The bisulphonate ester was further reacted with potassium iodide to give 6^A,6^D-diiodo 6^A,6^D-dideoxy β-cyclodextrin. The obtained 6^A,6^D-diiodo 6^A,6^D-dideoxy β-cyclodextrin further react with sodium azide to give 6^A,6^D-diazido 6^A,6^D-dideoxy β-cyclodextrin followed by reduction to form 6^A,6^D-diamino 6^A,6^D-dideoxy β-cyclodextrin. If the bisulphonate ester was reacted with potassium thiocyanate to afford 6^A, 6^D-diisothiocynato 6^A, 6^D-dideoxy β-cyclodextrin. The synthesized isothiocyanated monomer can further be used to synthesis CD based polymers of well-known electrophillic addition reaction on isothiocynated carbon to form thiourea[49]. It is well known that compounds containing urea or thiourea functionality are of extended biological interest in several classes of drugs[50].

2.1.2 Experimental

2.1.2.1 Materials

β-Cyclodextrin was obtained from Signet Chemical Corporation, Mumbai as a gift sample and dried overnight under a vacuum oven at 60^{o}C, then used without further purification. Para toluene sulfonyl chloride, potassium thiocyantae were purchased from Sigma-Aldrich. *N,N'*-dimethylformamide (DMF) and pyridine, were purchased from Merck (Germany) and dried over molecular sieves for 1 day then distilled under reduced pressure before use. All other chemicals were procured locally and used without further purifications.

2.1.2.2 Measurements

The FTIR spectra of the compounds were recorded on a Shimadzu 8400S Fourier Transform Infrared Spectrometer by KBr pellet method at 10^{-4} resolution and 30 scan

at room temperature. AR grade KBr was used for the preparation of pellet. ^{13}C NMR spectra were recorded on a Bruker spectrometer operating at 400 MHz at room temperature where tetra-methylsilane (TMS) was used as internal standard. The chemical shifts are given in δ ppm from TMS signal. TLC was carried out on silica gel plates (Merck 60-F254).

2.1.2.3 Synthesis of Mono-6-(p-toluenesulfonyl)-6-deoxy-β-cyclodextrin (1)

β-CD (2.86 g, 2.53 mmol) and *p*-toluene sulfonic anhydride (1.24 g, 3.80 mmol) in distilled water (62.5 mL) was stirred under inert atmosphere for 2 hours at ambient temperature. A solution of NaOH (1.25 g, 31.3 mmol) in water (12.5 mL) was then added. After 10 minutes unreacted *p*-toluene sulfonic anhydride was removed by filtration through silica gel. The filtrate was adjusted to pH = 8 by the addition of ammonium chloride (3.36 g). Cooling overnight in the refrigerator followed by drying under vacuum afforded **1** as a white fine powder. The reaction scheme is shown in Scheme 2.1.1.

2.1.2.4 Synthesis of mono-6-isothiocyanato-6-deoxy-β-Cyclodextrin (2)

A solution of precursor **1**, 2 g (0.00138mol) in 15ml DMF and 0.4 g (0.00412mol) of dry KSCN was stirred at 80 ^0C under nitrogen atmosphere for 2h. After cooling to room temperature the solids were separated by centrifugation and supernatant was decanted. The solid precipitates were washed twice with DMF. The residue was dissolved in small amount of water and cooled, before acetone was added with rapid stirring. The precipitate was filtered and dried under vacuum, and dissolved in 50ml of water. Then it was purified by ultra-filtration using MWCO 1K. This affords 75.8% (1.5 g) of mono-6-isothiocyanato-6-deoxy-β-Cyclodextrin as colorless solid.

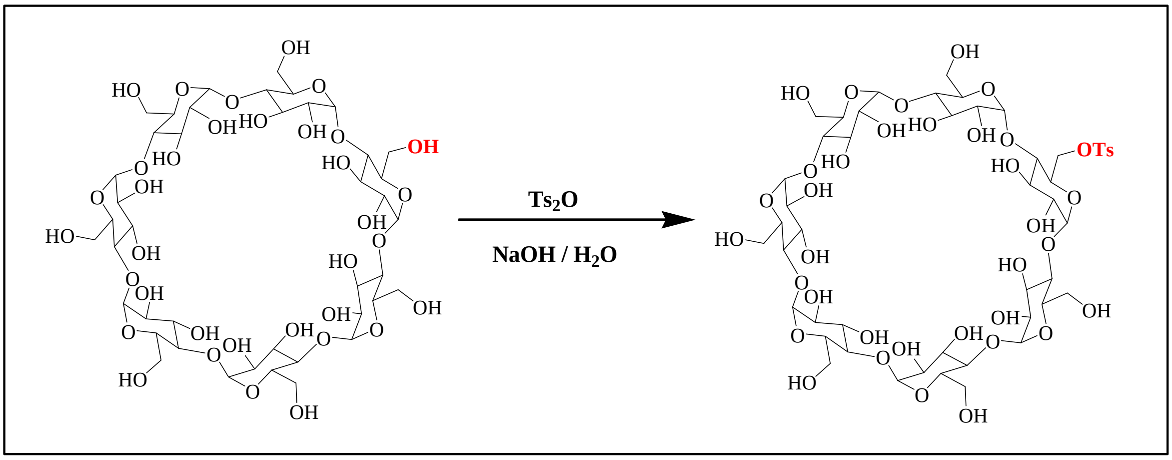
2.1.3 Results and Discussion

2.1.3.1 Mono-6-(p-toluenesulfonyl)-6-deoxy-β-cyclodextrin monomer

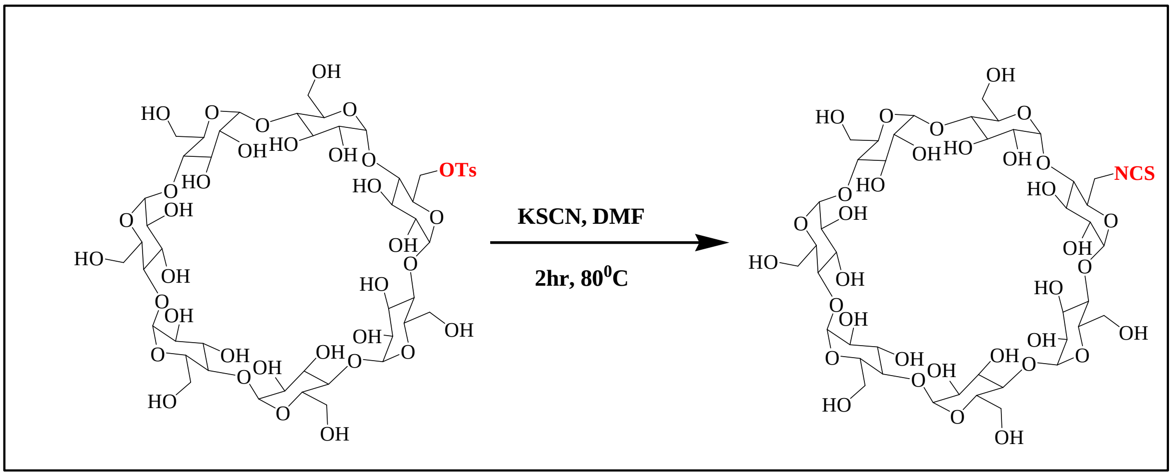
The major bands in IR (KBr) spectrum (Figure 2.1.3) are at ν 3400cm^{-1} (OH, b), 2927cm^{-1} (C-O, s), 1654cm^{-1} (C=C), 1599cm^{-1} (C-C), 1415 cm^{-1} (SO_2 , Assy.), 1157 cm^{-1} (SO_2, Sym.), 1009 cm^{-1} (C-O). The band at 1415cm^{-1} is due to the presence of tosyl group on to the β-cyclodextrin molecule. The ^1H-NMR (DMSO-d_6, 400 MHz)

of mono-6-(p-toluenesulfonyl)-6-deoxy-β-cyclodextrin (Figure.2.1.4) shows different chemical shifts of aliphatic protons and aromatic protons which clearly show the attachment of tosyl group on to CD. The chemical shifts of aliphatic protons of cyclodextrin at δ 5.71-5.52 (m, OH-b, OH-c, 14H), 4.71 (s, H-a, 7H), 4.49-4.52 (m, OH-f, 6H), 3.76 -3.5O (m, H-c, H-e, H-f, 32H), 3.49-3.29 (m, H-b, H-d, overlap with HDO), 2.42 (s, $-CH_3$, 3H) and chemical shifts of aromatic protons at δ 7.76 (d, H-g, 2H), 7.44 (d, H-h, 2H). The ^{13}C-NMR (DMSO-d_6, 400 MHz) of mono-6-(p-toluenesulfonyl)-6-deoxy-β-cyclodextrin (Figure.2.1.5) shows different chemical shifts of aliphatic carbons and aromatic carbons which clearly show the attachment of tosyl group on to the CD.

The chemical shifts of aliphatic carbons of cyclodextrin at δ 102 (C-1), 82 (C-4), 73 (C-3), 72.8 (C-2), 72 (C-5), 60 (C-6), 68.0 (C-6'), and for aromatic carbons of cyclodextrin at δ 138 (C-7), 127 (C-8), 124 (C-9), 121 (C-10) and separate chemical shift for methyl group of toluene at δ 21 ($-CH_3$). The formation of only mono-6-(p-toluenesulfonyl)-6-deoxy-β-cyclodextrin was also confirmed by ESI-MS, which was shown in (Figure 2.1.6). ESI-MS (m/z): calculated for $C_{49}H_{76}O_{37}S$, 1288.4; found, 1311.2 for $[M+Na]^+$. From all these (FTIR, ^{1}H NMR, ^{13}C NMR and ESI-MS) data it was confirmed that tosyalation has taken place on β-cyclodextrin.



Scheme 2.1.1 Synthesis of intermediate Ts-CD via direct monotosylation at primary position.



Scheme 2.1.2 Synthesis of mono-6-isothiocyanato-6-deoxy-β-Cyclodextrin

2.1.3.2 Mono-6-isothiocyanato-6-deoxy-β-Cyclodextrin monomer

The major bands in FTIR (KBr) spectrum (Figure 2.1.7) are at ν 3400cm^{-1} (OH, b), 2927cm^{-1} (C-O, s), 2069cm^{-1} (-NCS, vs). The band at 2069cm^{-1} is due to the presence of isothiocyante (-NCS) group and it indicates the presence of the isothiocyanate group on to the β-cyclodextrin molecule and absence of band above 2100cm^{-1} clearly shows there is no formation of thiocyanto (-SCN) group on β-cyclodextrin molecule. The ^{13}C NMR (DMSO-d_6, 400 MHz) of (Figure.2.1.8) shows chemical shift of aliphatic carbons of cyclodextrin at δ 60.05 (C-6'), δ 60.42 (C-6), δ 72.89 (C-5), δ 73.28 (C-2), 73.90 (C-3), 82.38 (C-4), 102.77 (C-1), 129.39 (N=C=S). It is observed that due to the primary hydroxyl group substitution the signal of the substituted C-6' carbon of the aliphatic cyclodextrin shifts up field from 60.42ppm to 60.05ppm. So from this (FTIR and ^{13}C NMR) data we conclude that the attachment of NCS group has been taken place on cyclodextrin and forms mono-6-isothiocyanato-6-deoxy-β-Cyclodextrin. The formation of only mono-6-isothiocyanato-6-deoxy-β-Cyclodextrin was confirmed from MALDI-TOF which was shown in Figure 2.1.9. The appearance of molecular ion at 1200 $[M+Na]^{+}$ clearly indicates the presence of –NCS group on β-Cyclodextrin.

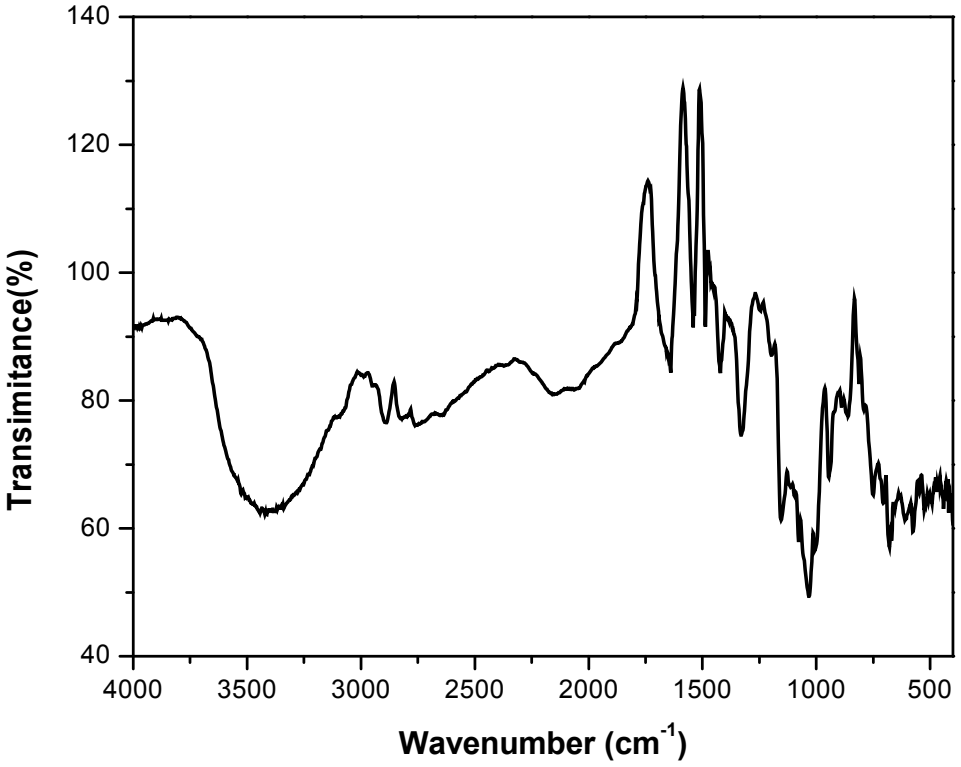


Figure 2.1.3 FTIR of Mono-6-(p-toluenesulfonyl)-6-deoxy-β-cyclodextrin

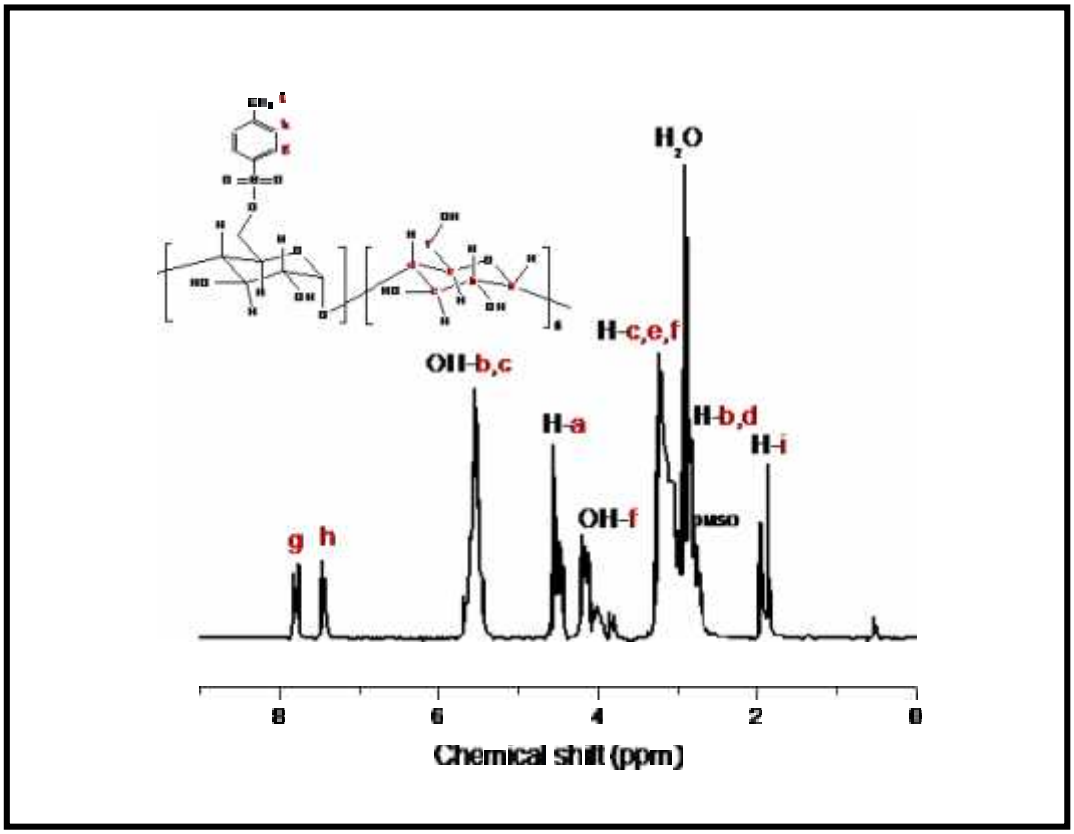


Figure 2.1.4 [1]H NMR of Mono-6-(p-toluenesulfonyl)-6-deoxy-β-cyclodextrin

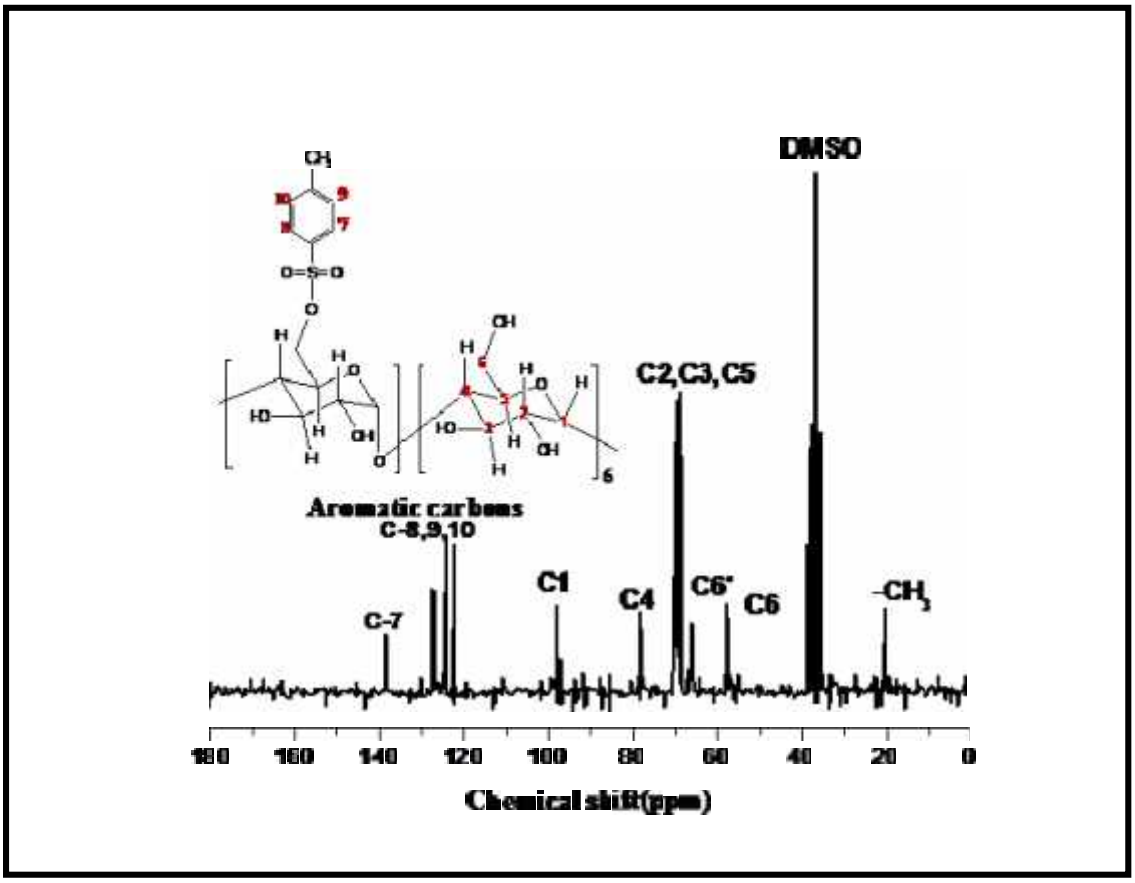


Figure 2.1.5 ^{13}C NMR of Mono-6-(p-toluenesulfonyl)-6-deoxy-β-cyclodextrin

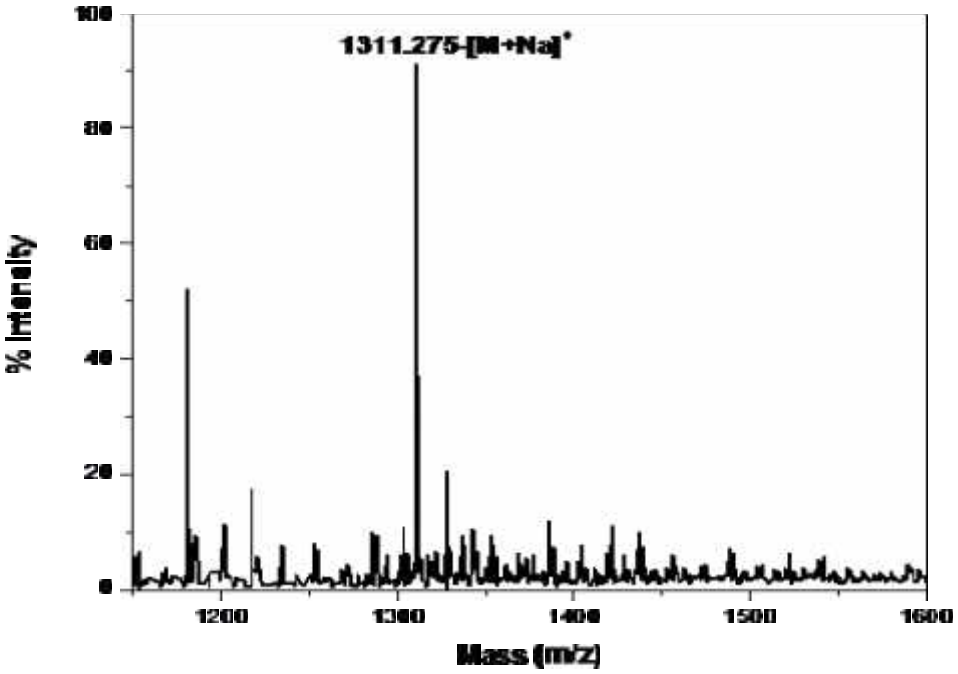


Figure 2.1.6 ESI-MS of Mono-6-(p-toluenesulfonyl)-6-deoxy-β-cyclodextrin

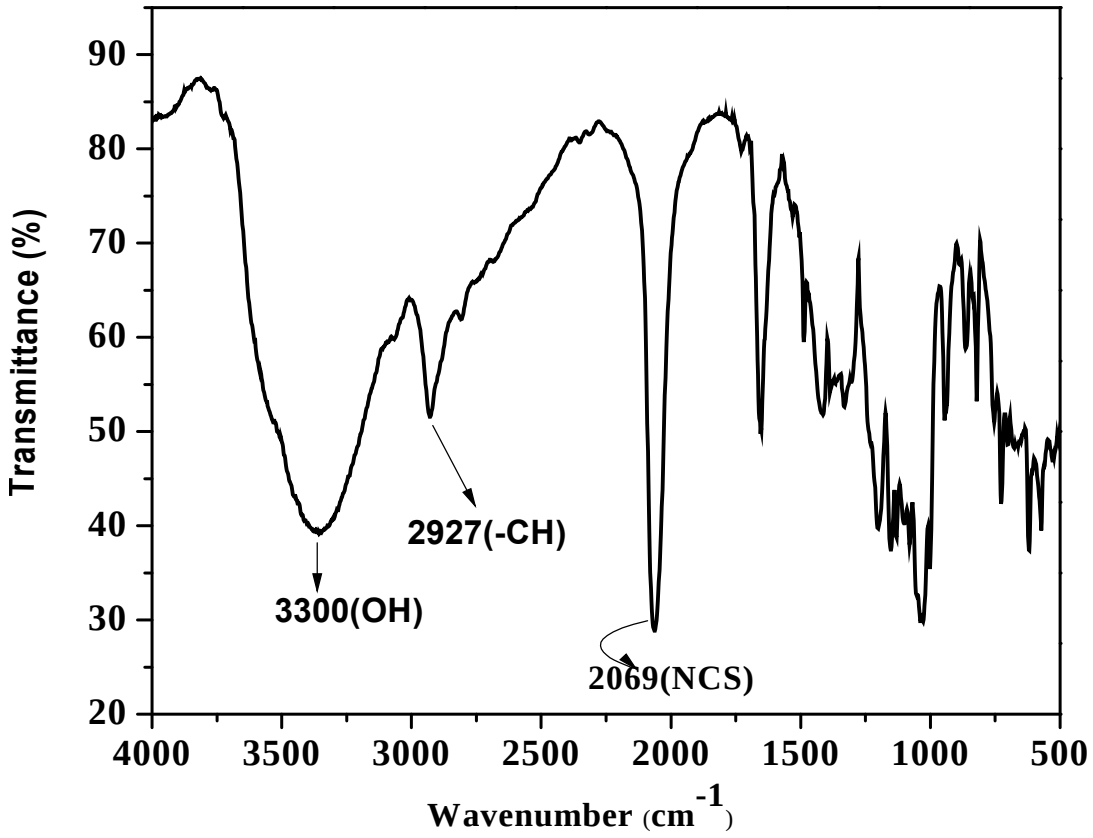


Figure 2.1.7 FTIR of mono-6-isothiocyanato-6-deoxy-β-Cyclodextrin

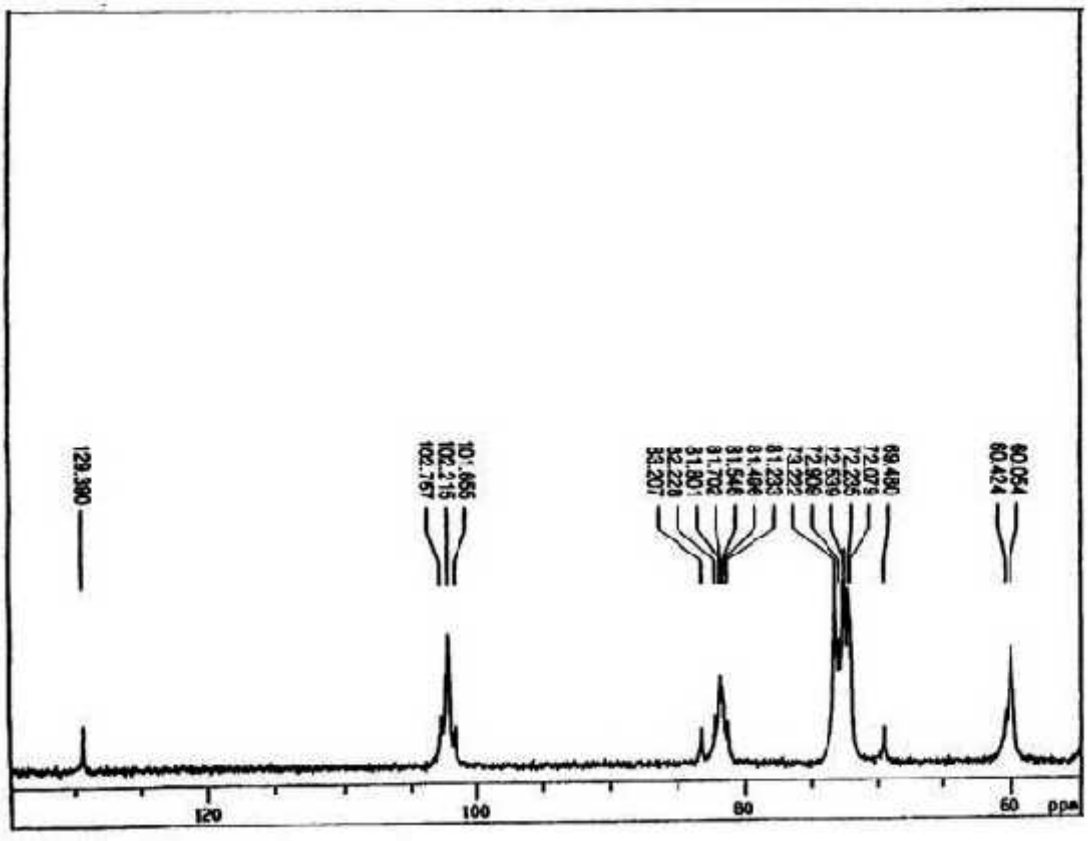


Figure 2.1.8 ^{13}C NMR of mono-6-isothiocyanato-6-deoxy-β-Cyclodextrin

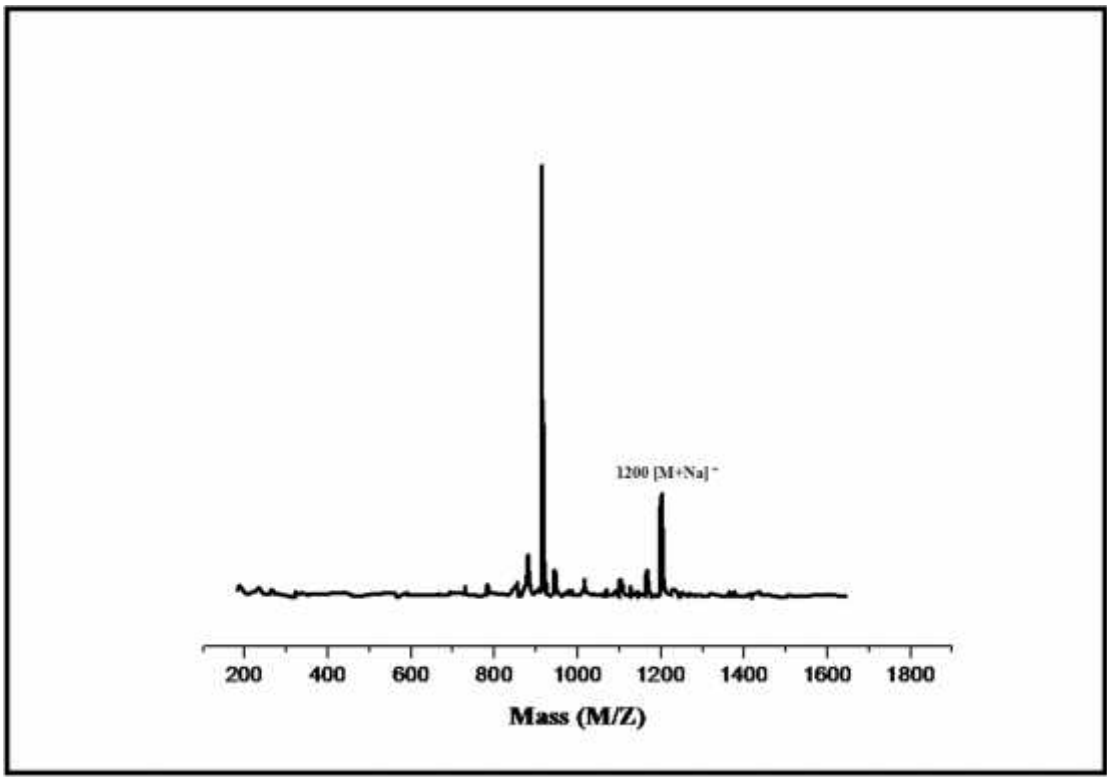


Figure 2.1.9 MALDI-TOF of mono-6-isothiocyanato-6-deoxy-β-Cyclodextrin

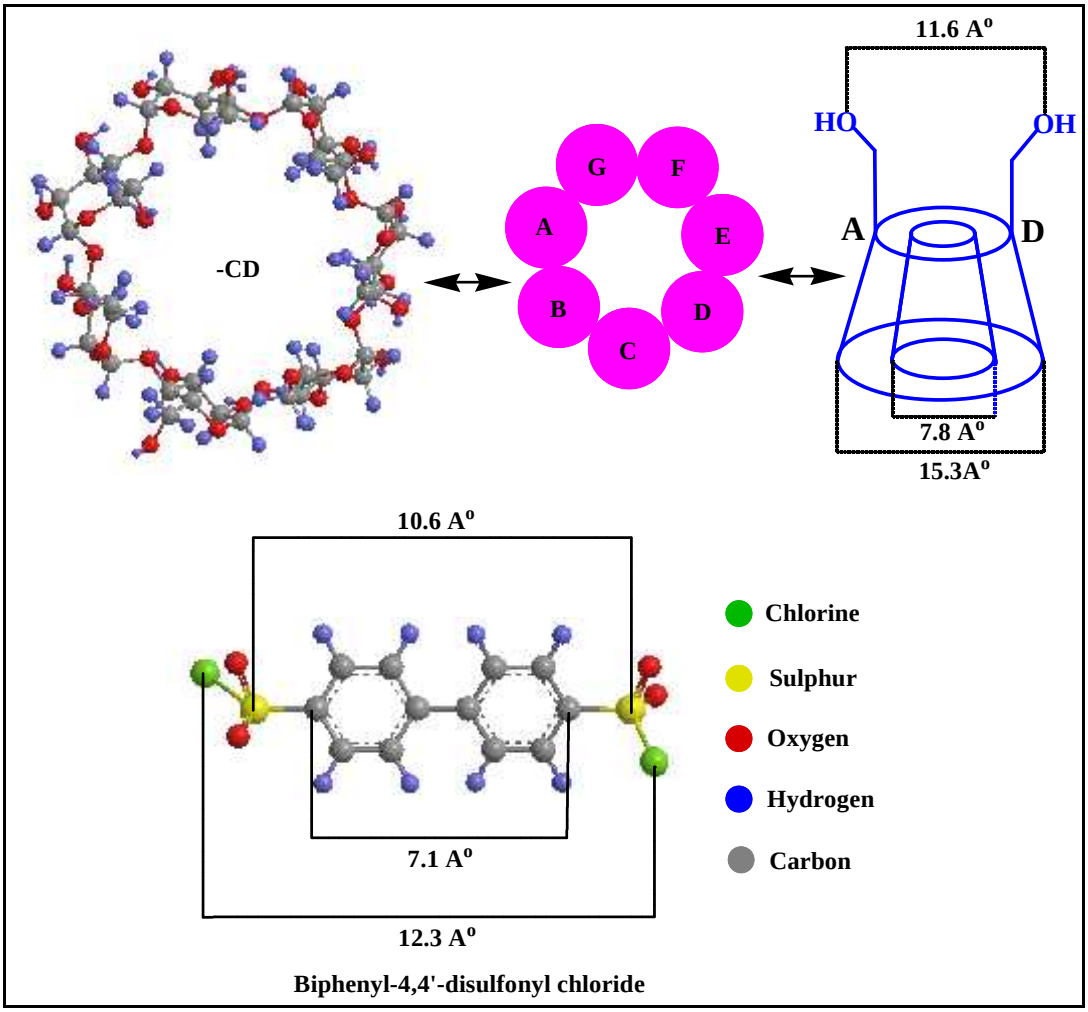


Figure 2.2.0 Structural dimension of the β-cyclodextrin and biphenyl-4,4’-disulfonyl chloride.