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Nonaqueous synthesis of a selectively modified, highly anionic sulfopropyl ether derivative of cyclomaltoheptaose (β-cyclodextrin) in the presence of 18-crown-6

Daniel L. Kirschner[a] and Thomas K. Green[a,b,*]

[a]*Department of Chemistry and Biochemistry, University of Alaska, Fairbanks, AK 99775-5940, USA*
[b]*Institute of Arctic Biology, University of Alaska, Fairbanks, AK 99775-5940, USA*

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Abstract—A highly anionic cyclomaltooligosaccharide (cyclodextrin, CD) derivative containing sulfopropyl functional groups on the primary face of the CD was synthesized. Heptakis(2,3-di-*O*-methyl)cyclomaltoheptaose [heptakis(2,3-di-*O*-methyl)-β-cyclodextrin] was reacted with 1,3-propane sultone and potassium hydride (KH) in anhydrous tetrahydrofuran in the presence of 18-crown-6 to yield highly substituted potassium heptakis(2,3-di-*O*-methyl-6-*O*-sulfopropyl)cyclomaltoheptaose [heptakis(KSPDM)-β-CD] with an average degree of substitution (DS_{CE}) of 6.9 as determined by inverse detection capillary electrophoresis (CE). The principal species in the product is the fully substituted heptakis(KSPDM)-β-CD. Complete NMR assignments of the hydrogen and carbon atoms are made using a combination of gCOSY and gHSQC. In the absence of 18-crown-6, the reaction generates a mixture of multiply charged derivatives with average DS_{CE} of 4.1. The possible roles of the crown ether in the reaction are discussed. The ROESY NMR spectrum of the inclusion complex that forms between heptakis(KSPDM)-β-CD and 2-naphthoic acid in D_2O reveals that 2-naphthoic acid inserts with the carboxyl group toward the derivatized primary rim of the cyclodextrin.

Keywords: Cyclomaltoheptaose; β-Cyclodextrin; 18-Crown-6; Propylsulfonation

1. Introduction

The enantioselectivity inherent in native and modified cyclomaltooligosaccharides (cyclodextrins, CDs) has lead to their wide use as running buffer additives in capillary electrophoresis (CE).[1–3] Both natural and neutrally derivatized CDs are limited to the separation of charged enantiomers. CDs derivatized with multiply charged functional groups have been shown to be highly effective in separating a wide variety of neutral and charged enantiomers.[4–6]

A major challenge for synthetic chemists derivatizing CDs is the similar reactivity of the 21 hydroxyl groups. Reactions involving CDs often yield products that are mixtures of both regional isomers (nonselective substitution at the 2,3, or 6-OH sites) and positional isomers (nonselective substitution pattern on the glucose units). One method to partially overcome this problem is the use of bulky protecting groups selective to primary hydroxyl groups. Once protected, the secondary hydroxyl groups can be reacted, and several procedures have yielded full regioselective modification. After deprotection, the primary hydroxyl groups can be fully reacted to yield single-isomer CDs.

Vigh and co-workers have demonstrated this approach with the synthesis of three families of single isomer, highly anionic sulfated cyclodextrins.[4,7–15] Modification of the primary face of the CDs with sulfato functional groups gave moderately hydrophobic, single-isomer charged cyclodextrins, which are able to separate a variety of analytes.

In this study, we undertook the project of synthesizing a highly anionic CD that exhibits sulfopropyl functional

* Corresponding author. Tel.: +1 907 474 1559; fax: +1 907 474 5640; e-mail: fftkg@uaf.edu

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groups on its primary face. Sulfoalkyl ether-β-CDs (SAE-β-CDs) were first introduced in 1990 by Rajewski[16] and have since been widely studied and applied in drug delivery[17,18] and capillary electrophoresis.[19,20] Anionic sulfoalkyl-β-CDs differ from sulfated CDs in that the lipophylic cavity is separated from the charged sulfonate group by alkyl spacer groups. Zia et al. have hypothesized that increasing the distance between the charged sulfonates and the CD torus should increase the binding potential.[21] Observed binding potentials of some nonpolar steroids with SAE-β-CDs are consistent with this hypothesis. In addition, the bulky, hydrated, anionic sulfonate groups near the entrance of the cavity may inhibit the approach of a hydrophobic molecule. By placing the sulfonate groups on the less open primary face, it may allow for more favorable interaction with the cyclodextrin cavity.

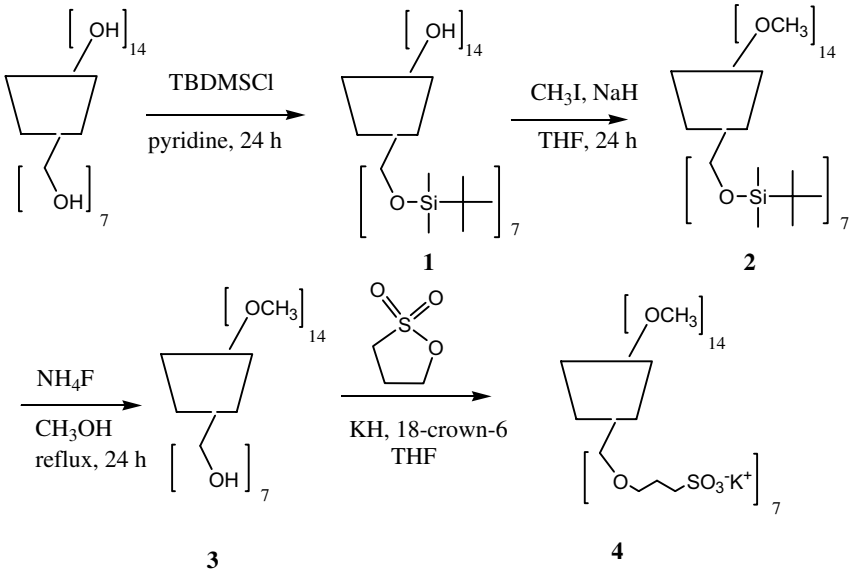
The sulfoalkyl CDs described thus far in the literature do not utilize any selective modification techniques, and as a result they give multiply charged mixtures containing regional and positional isomers. The reactions are generally carried out in an aqueous environment under highly basic conditions. Our preliminary investigations in aqueous sulfoalkyl synthesis of our title compound proved to be unsuccessful, yielding a mixture of charged CDs with an average degree of substitution ~4 based on inverse detection CE. The incomplete reaction of the primary hydroxyl groups may be due in part to a high rate of 1,3-propane sultone decomposition to produce hydroxyalkylsulfonate by NaOH[22] or by a low reactivity of the increasingly ionic product.

Alternatively, we chose to investigate the nonaqueous synthesis of potassium heptakis(2,3-di-*O*-methyl-6-*O*-sulfopropyl)cyclomaltoheptaose [heptakis(KSPDM)-β-CD] using tetrahydrofuran (THF) and potassium hydride (KH) as base. Reactions were conducted with and without the presence of 18-crown-6 to promote reactivity. Nearly complete reaction of the primary hydroxyl groups (>95%) with 1,3-propanesultone was achieved in the presence of 18-crown-6 as determined by inverse detection CE. The high symmetry of the product allowed complete NMR assignments of the carbon and hydrogen resonances, which further allowed an interpretation of the 2D ROESY spectra obtained on an inclusion complex with 2-naphthoic acid.

2. Materials and methods

2.1. General methods

All chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI). THF was freshly distilled over 4 Å sieves. The synthesis of KSPDM-β-CD (**4**) is shown in Scheme 1. Heptakis(6-*O*-*tert*-butyldimethylsilyl)cyclomaltoheptaose (**1**) was synthesized according to the procedure described by Fügedi[23] with the exception that 10.5 equiv of *tert*-butylchlorodimethylsilane was used instead of 7.7 equiv. Heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)cyclomaltoheptaose (**2**) was synthesized according to the procedure described by Takeo and Dazuhiko.[24] Heptakis(2,3-di-*O*-methyl)cyclomaltoheptaose (**3**) was synthesized from **2** according to the procedure described by Takeo and Dazuhiko,[24] with the exception that ammonium fluoride was used in place of tetrabutylammonium fluoride. Purification of **3** was accomplished by preparative column chromatography on silica gel using a 4:1 chloroform–MeOH mobile phase. All intermediates were characterized by their ^{1}H and ^{13}C NMR spectra that matched the literature as cited.



Scheme 1. Synthesis of KSPDM-β-CD.

2.2. NMR spectral analysis

^{1}H, ^{13}C, gCOSY, gHSQC, and ROESY spectra were acquired with a Varian Mercury 300 MHz FT-NMR instrument. The ROESY spectrum was acquired with 16 acquisitions in f2 and 200 increments in f1 with a mixing time of 300 ms. Assignments were made by gCOSY and gHSQC techniques. Chemical shifts are reported relative to internal sodium 3-(trimethylsilyl)-1-propanesulfonic acid (TSP).

2.3. Potassium heptakis(2,3-di-*O*-methyl-6-*O*-sulfopropyl)cyclo-maltoheptaose (KSPDM-β-CD, 4) (DS_{CE} 6.9, DS_{EA} 6.7)

Compound **3** (0.200 g, 0.152 mmol) was added to 35 mL of anhyd THF under nitrogen, followed by the slow addition of 0.300 g (7.48 mmol) of KH. After stirring at room temperature for 15 min, 1.02 g (3.86 mmol) of 18-crown-6 was slowly added, and the reaction was allowed to stir for 30 min. 1,3-Propanesultone (0.520 g, 4.26 mmol) was dissolved in 4 mL of anhyd THF and introduced dropwise over 30 min. The reaction was stirred at room temperature for 24 h. Excess KH was decomposed by cooling the reaction to 0 °C and slowly adding MeOH. The solvents were removed by rotary evaporation at 70 °C, and the brown oil was dried in a vacuum oven at 70 °C for 1 h. Dry acetone (80 mL) was added to the oil, and the precipitate that formed was transferred to centrifuge tubes where successive acetone washes, followed by centrifugation removed all 18-crown-6 yielding an off-white solid. The solid was blown dry with N_2 gas, dissolved in water, and neutralized with 1 M HCl. Compound **4** was purified by ultrafiltration through an Amicon ultrafiltration cell with a 1000 MWCO RC membrane to give, after concentration, 0.24 g (0.098 mmol, 61%) of **4.** ^{1}H NMR (300 MHz, D_2O): δ 5.27 (d, J 3.3 Hz, 1H, H-1), 3.95 (d, J 10.5 Hz, 1H, H-6a), 3.73 (d, 1H, H-6b), 3.85 (m, 1H, H-5), 3.85 (m, 1H, H-4), 3.71 (m, 1H, H-3), 3.66 (m, 2H, OCH_2), 3.63 (s, 3H, OCH_3), 3.53 (s, 3H, OCH_3), 3.36 (dd, J 3.3, 9.9 Hz, 1H, H-2), 2.99 (m, 2H, CH_2S), 2.05 (m, 2H CH_2CH_2S). ^{13}C NMR (75 MHz, D_2O): δ 100.5 (C-1), 83.6 (C-3), 83.0 (C-2), 80.7 (C-4), 73.6 (C-5), 72.4 (OCH_2), 71.6 (C-6), 62.7 (OCH_3), 60.9 (63), 51.1 (CH_2S), 27.5 (CH_2CH_2S). Anal. Calcd for DS_{EA} 6.7 $C_{76.1}H_{131.5}K_{6.7}O_{55.1}S_{6.7}\cdot 6.7H_2O$: C, 36.20; H, 5.78; S, 8.50. Found: C, 36.08; H, 5.45; S, 8.52.

2.4. Synthesis of potassium heptakis(2,3-di-*O*-methyl-6-*O*-sulfopropyl)cyclomaltoheptaose (KSPDM-β-CD, 4) (DS_{CE} 4.1, DS_{EA} 3.8)

The same procedure was followed as above except that 18-crown-6 was omitted from the reaction. The product was worked up in an identical fashion. Yield 69%. Anal. Calcd for avg DS_{EA} 3.8 $C_{67.4}H_{117}K_{3.8}O_{46.4}S_{3.8}\cdot 3.8H_2O$: C, 40.31; H, 6.25; S, 6.07. Found: C, 40.50; H, 6.12; S, 6.10.

2.5. Indirect UV detection CE

Indirect UV detection CE electropherograms of KSPDM-β-CD (**4**) were obtained with a Beckman P/ACE 2000 instrument. CE conditions are as follows: buffer, 30 mM benzoic acid titrated to pH 6.0 with 0.10 M Tris base; wavelength, 254 nm; applied potential, 25 kV; injection, 22 mg/mL, 0.5 psi—6 s injection. The analyses were performed in a bare, fused-silica capillary of dimensions 50 × 59.5 cm × 50 μm.

3. Results and discussion

3.1. Characterization of products

Inverse detection CE[25] was employed to monitor the reaction as well as assess the average degree of substitution of the final product. Using this method, Luna et al. have demonstrated that the molar response factor of sulfobutyl ether-β-CDs increases with degree of substitution (DS_{CE}).[26] Uncorrected integration of the CD bands therefore overestimated DS for an SBE-β-CD (DS_{CE} 4.7 from CE compared to DS_{EA} 3.6 from elemental analysis). However, our results show much better agreement between DS_{CE} and DS_{EA} for these selectively modified cyclodextrins (vide infra).

Typical inverse detection CE electropherograms of the reaction product obtained without 18-crown-6 show a mixture of multiply charged derivatives centered on DS 4 (DS_{CE} 4.1) with 8% of the product containing CDs with DS 5 (Fig. 1). Elemental analyses (C, H, S) are consistent with a DS_{EA} of 3.8. In comparison, the electropherogram of the product from the reaction

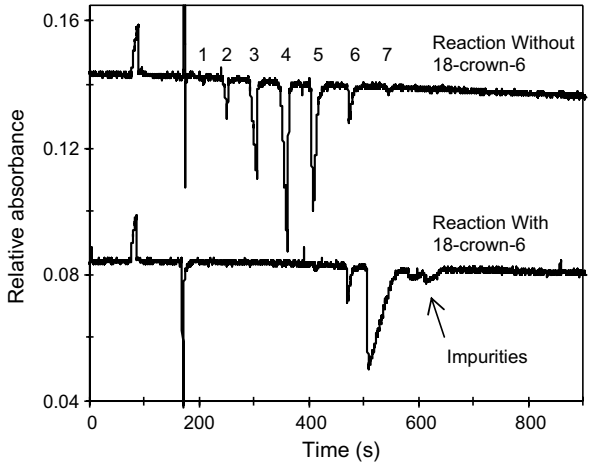


Figure 1. Indirect UV detection electropherograms of KSPDM-β-CD (**4**) synthesized with and without 18-crown-6 present. See Section 2.5.

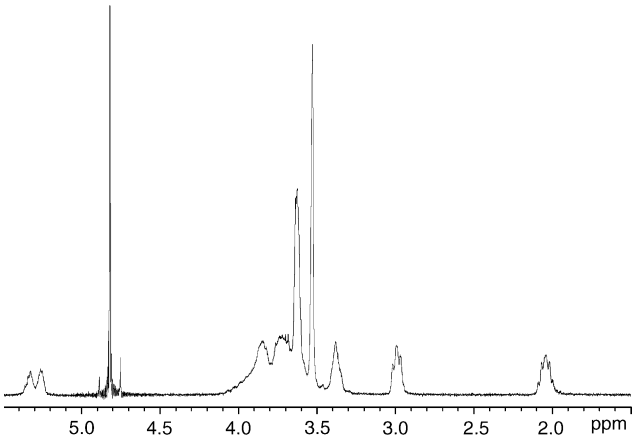


Figure 2. ^{1}H NMR spectrum of KSPDM-β-CD (**4**), DS_{CE} 4.1 in D_2O.

incorporating 18-crown-6 indicates a predominance of DS 7 (DS_{CE} 6.9), with less than 10% of the mixture containing DS <7. Elemental analysis is consistent with a DS_{EA} of 6.7 in comparison.

The ^{1}H NMR spectrum of the product obtained without 18-crown-6 (DS_{CE} 4.1), shown in Figure 2, reveals a series of poorly resolved peaks in the 3.3–4.0 ppm region and two broad peaks near 5.3 ppm, attributed to the anomeric H-1 hydrogens. The poor resolution observed for this spectrum is undoubtedly due to the mixture of multiply charged anionic cyclodextrins that result from incomplete substitution of the primary hydroxyl groups. Additionally, a variety of positional isomers are possible for degrees of substitution 2–5, which further adds to the heterogeneity of the product.

In contrast, the ^{1}H NMR spectrum of the product obtained with the use of 18-crown-6 (DS_{CE} 6.9), shown in Figure 3, reveals a high degree of symmetry; especially sharp peaks with well-defined couplings are observed for H-1 at 5.27 ppm (d, *J* 3.3 Hz) and H-2 at 3.36 ppm (dd *J* 3.3, 9.9 Hz). From CE analysis, this product consists of ~93% the highly symmetric heptakis-(KSPDM)-β-CD (**4**) and ~7% of hexakis product. ^{1}H NMR spectroscopy is a useful diagnostic tool for determining the success of the sulfopropylation reaction. Invariably, poorly resolved H-1 and H-2 signals are indicative of significantly incomplete substitution at the primary hydroxyl groups.

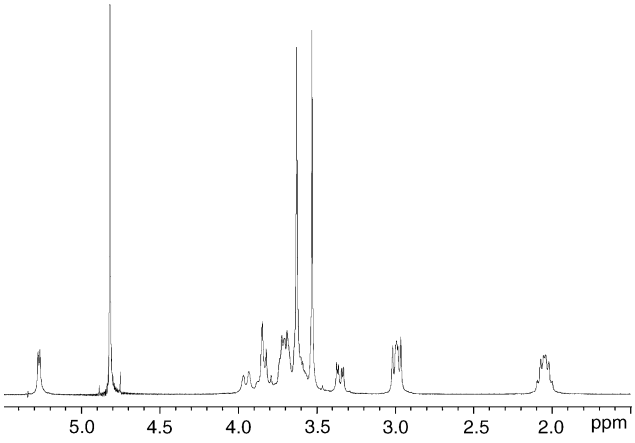


Figure 3. ^{1}H NMR spectrum of KSPDM-β-CD (**4**), DS_{CE} 6.9 in D_2O.

^{1}H NMR spectroscopy with DMSO-d_6 as solvent is also useful for detecting the presence of unreacted primary hydroxyl groups. The spectrum of a sulfopropylated product obtained with 2.4 equiv 18-crown-6 (DS_{CE} 6.7) is shown in Figure 4. This spectrum reveals well-resolved H-1 and H-2 resonances, but the weak triplet at 4.3 ppm is indicative of the presence of unsubstituted primary hydroxyl. This triplet remained undetected in the product with DS_{CE} 6.9.

3.2. Role of 18-crown-6

In our reaction, increasing amounts of 18-crown-6 were required to achieve a high degree of substitution as shown in Table 1. More than 3 equiv of crown ether, based on the number OH groups, is required to achieve a DS_{CE} of 6.9 in 24 h at room temperature. From a practical standpoint, this amount of 18-crown-6 seems excessive, but the crown ether is recovered from the acetone wash, and can possibly be purified and reused if necessary.

We also note that 18-crown-6 seems to be much more critical than time to the success of the reaction. CE analysis of aliquots taken during the course of the reaction indicates the reaction is essentially complete within 1-h reaction time, with no detectable change during the next 23 h. Addition of more crown ether after 24 h then results in a rapid increase in the degree of substitution. This feature, along with the ability to monitor the reaction with inverse detection CE, allows us to a fine tune the reaction to achieve a desired average degree of substitution.

18-Crown-6 is known to effectively complex to potassium ion in THF. By doing so, the solubility of the increasingly anionic KSPDM-β-CD (**4**) species (DS 1→7) in THF may be enhanced during the course of the reaction, leading to a more substituted product. The 18-crown-6–potassium complex may also result in a more reactive cyclodextrin oxyanion, especially if the crown ether induces some degree of potassium cation–oxyanion separation in THF. For some nucleophilic ring-opening reactions, crown ether–metal complexes also stabilize the developing negative charge on departing leaving group.[27–30] The precise role of the 18-crown-6 in our reaction requires further investigation.

3.3. ROESY 2D spectra of inclusion complexes

Chankvetadze and co-workers have conducted NMR studies on inclusion complexes of single-isomer anionic cyclodextrins with chiral analytes.[31,32] Heptakis(2,3-di-

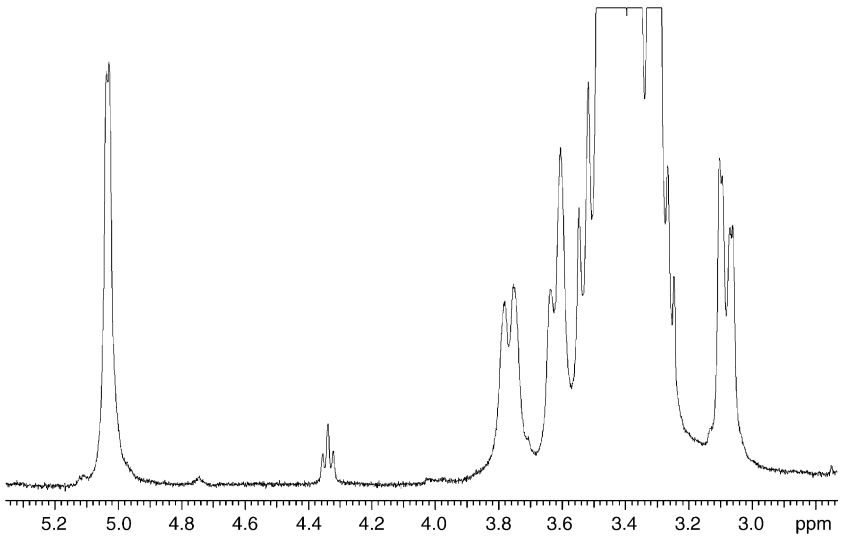


Figure 4. ^{1}H NMR spectrum of KSPDM-β-CD (**4**) (DS_{CE} 6.7) in DMSO-d_6 obtained from reaction using 2.4 equiv of 18-crown-6. The triplet at 4.34 ppm is attributed to 6-OH of the hexakis(KSPDM)-β-CD (**4**), which constitutes 29% of the product as determined from inverse detection CE analysis.

Table 1. Effect of 18-crown-6 on the average degree of substitution of KSPDM-β-CD (**4**)

$\frac{\text{mmol 18-crown-6}}{\text{mmol OH of } \mathbf{3}}$	DS_{CE}	No. of added sulfopropyl groups	Percent in mixture
0.00	4.1	1	0.1
		2	3.9
		3	21.6
		4	37.8
		5	28.6
		6	7.1
		7	0.9
1.20	6.5	5	6.5
		6	40.5
		7	53.0
2.40	6.7	5	1.0
		6	28.9
		7	70.1
3.60	6.9	6	7.0
		7	93.0

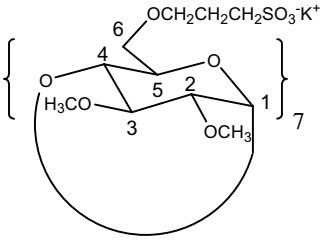
O-acetyl-6-*O*-sulfo)cyclomaltoheptaose (HDMS-β-CD) forms an inclusion complex with clenbuterol although it interacts differently than β-CD.[31] The highly charged heptakis(2-*O*-methyl-3,6-di-*O*-sulfo)cyclomaltoheptaose (HmdiSu-β-CD) forms external complexes, presumably due to structural crowding.[32]

The predominance of the heptakis(KSPDM)-β-CD (**4**) (93%) in the highly substituted sulfopropylated CD allows us to make specific NMR assignments of the hydrogens and carbons using a combination of the ^{1}H, ^{13}C, gCOSY, ROESY, and gHSQC. Assignments are shown in Table 2.

Because the ring hydrogen resonances of the heptakis derivative are confidently assigned in the ^{1}H NMR, its inclusion complexes with small molecules can be studied. Of particular relevance are the H-3, H-5, and H-6 hydrogens, which are directed toward the inside of the toroid of the cyclodextrin and often exhibit strong NOEs with small molecules that form inclusion complexes. For inclusion complexes of medium-sized molecules (~2000 MW) such as cyclodextrins, the 2D rotating frame NOE or ROESY experiment is used to detect NOE effects.[33]

As an initial study, we examined the inclusion complex that heptakis(KSPDM)-β-CD (**4**) makes with

Table 2. Carbon and hydrogen assignments of potassium heptakis(2,3-di-*O*-methyl-6-*O*-sulfopropyl)cyclomaltoheptaose [heptakis(KSPDM)-β-CD, **4**]



Carbon	ppm	Hydrogen	ppm
C-1	100.5	H-1	5.27
C-2	83	H-2	3.36
C-3	83.6	H-3	3.71
C-4	80.7	H-4	3.85
C-5	73.6	H-5	3.85
C-6	71.6	H-6a	3.95
		H-6b	3.73
C-3 OCH_3	62.7	C-3 OCH_3	3.63
C-2 OCH_3	60.9	C-2 OCH_3	3.53
OCH_2	72.4	OCH_2	3.66
CH_2CH_2S	27.5	CH_2CH_2S	2.05
CH_2S	51.1	CH_2S	2.99

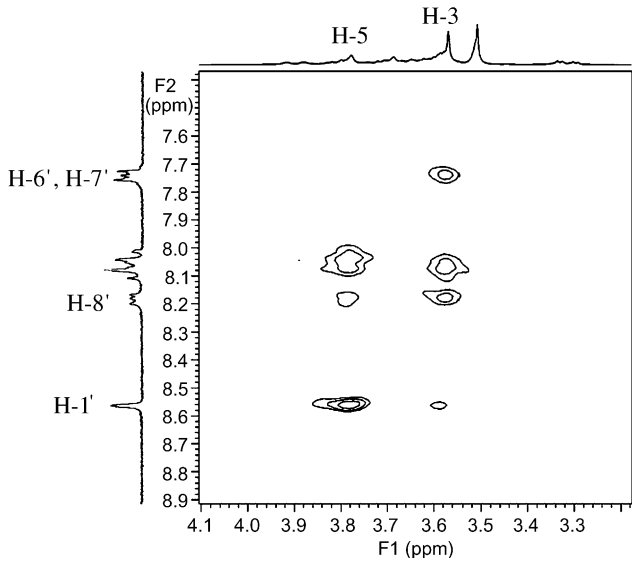


Figure 5. 2D ROESY spectrum of inclusion complex of KSPDM-β-CD: 2-naphthoic acid (molar ratio 2:1). Mixing time 300 ms.

2-naphthoic acid in D_2O. The partial ROESY spectrum, showing appropriate crosspeaks, is shown in Figure 5. Well-resolved peaks of 2-naphthoic acid include H-1′, H-8′ and, taken together, H-6′ and H-7′. H-8′ is assigned at 8.20 ppm due its ROESY correlation (not shown) with H-1′ of the same molecule. H-3 of the heptakis(KSPDM)-β-CD (**4**) resonates at 3.58 ppm (revealed by coupling with H-2 in gCOSY), shifted from 3.71 ppm as a result of complexation with 2-naphthoic acid. H-5 resonates at 3.78 ppm, shifted from 3.85 ppm. A relatively intense crosspeak between H-1′ (8.56 ppm) of naphthoic acid and H-5 (3.78 ppm) of the cyclodextrin is observed. The resonance at 8.20 ppm of 2-naphthoic acid shows crosspeaks with both H-3 and H-5 of the CD. Additionally, a correlation between H-6′ and H-7′ (7.75 ppm) and H-3 of the CD is observed. Volume integration of the crosspeaks, shown in Table 3, gives a semi-quantitative evaluation of the

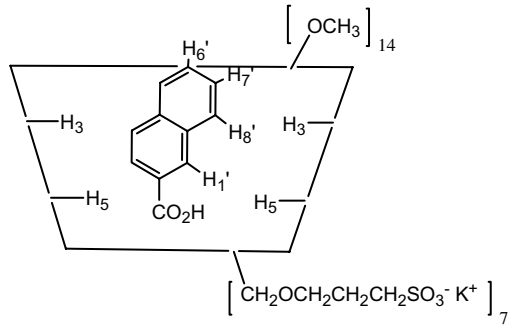


Figure 6. Inclusion complex of heptakis(KSPDM)-β-CD:2-naphthoic acid.

Table 3. Relative ROESY integrals for the inclusion complex between heptakis(KSPDM)-β-CD (**4**) and 2-naphthoic acid in D_2O

Proton	Heptakis(KSPDM)-β-CD	
2-Naphthoic acid	H-5	H-3
H-1′	1.00	0.093
H-8′	0.21	0.45
H6′,H-7′	0	0.43

strengths of the NOE interactions. The absence of a correlation between H-6′/H-7′ and H-5 indicates that greater than 4 Å separates these hydrogens, the generally accepted cutoff distance for the observation of the NOE crosspeaks.[33] The results are consistent with the schematic shown in Figure 6, with the carboxyl group pointing toward the derivatized primary rim.

4. Conclusions

The first selectively modified, highly anionic sulfopropyl ether β-cyclodextrin has been synthesized, with nearly complete substitution (>95%) of the primary hydroxyl groups with sulfopropyl groups. The predominance of the single-isomer heptakis(KSPDM)-β-CD (**4**) (93% from CE) in the product mixture allowed complete assignments of the ^{1}H and ^{13}C NMR resonances, but more importantly, it allows for a reliable interpretation of 2D ROESY spectra of inclusion complexes. The KH/18-crown-6 reagent may prove to be generally useful in the synthesis of other highly substituted sulfoalkyl cyclodextrins.

Future studies will include comparisons of heptakis(KSPDM)-β-CD (**4**) with Vigh's heptakis(2,3-di-*O*-methyl-6-*O*-sulfo)cyclomaltoheptaose,[7] which is an analogue of the CD synthesized here, the difference being that the charged sulfonate group of heptakis(KSPDM)-β-CD (**4**) is separated from the primary rim by three methylene units.

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