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Synthesis and analytical characterization of the sodium salt of heptakis(2-*O*-methyl-3,6-di-*O*-sulfo)cyclomaltoheptaose, a chiral resolving agent candidate for capillary electrophoresis

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Abstract

A pure, single isomer, strong electrolyte chiral resolving agent candidate for capillary electrophoresis, the sodium salt of heptakis(2-*O*-methyl-3,6-di-*O*-sulfo)cyclomaltoheptaose has been synthesized on the 100-g scale, in greater than 97% purity, through heptakis(2,6-di-*O*-*tert*-butyldimethylsilyl)cyclomaltoheptaose, heptakis(2-*O*-methyl-3,6-di-*O*-*tert*-butyldimethylsilyl)cyclomaltoheptaose and heptakis(2-*O*-methyl)cyclomaltoheptaose intermediates. The structural identity of each intermediate and the final product was conclusively established by high-resolution MALDI-TOF mass spectrometry, variable-temperature ^{1}H and ^{13}C NMR spectroscopy and X-ray crystallography. The purity of each intermediate and the final product was determined by gradient high-performance liquid chromatography (HPLC) and indirect UV detection capillary electrophoresis.

Keywords: Heptakis(2-*O*-methyl-3,6-di-*O*-sulfo)cyclomaltoheptaose; Heptakis(2-*O*-methyl-3,6-di-*O*-sulfo)-β-cyclodextrin; Cyclodextrin sulfates; Single isomer chiral resolving agent; Capillary electrophoresis

1. Introduction

In the last decade, capillary electrophoresis (CE) has become a very powerful, widely applied technique for the analysis of water-soluble enantiomers. Currently, cyclomaltoheptaose (β-cyclodextrin, β-CD) and its derivatives are the most frequently used chiral resolving agents in CE [1–4]. In order to create a wide range of intermolecular interactions that could facilitate enantiorecognition, nonionic, weak electrolyte and strong electrolyte CD derivatives have been tested as resolving agents [2]. Unfortunately, the majority of the weak and strong electrolyte CD derivatives used so far are complex mixtures of CD isomers with different degrees and loci of substitution [2]. Although they do afford enantiomer separations, these randomly substituted CDs cannot be used to spectroscopically elucidate, at the molecular level, the processes that are responsible for enantiorecognition. Also, the inherent, batch-to-batch variations in the compositions of these randomly substituted CDs represent a serious liability when one is trying to develop rugged, reproducible analytical methods. Recently, we described four well-characterized, single iso-

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mer, pure, 6-*O*-sulfo CDs [5–8] and made them commercially available [9]. A single isomer cationic β-CD derivative has also been described [10,11], but it is not yet commercially available.

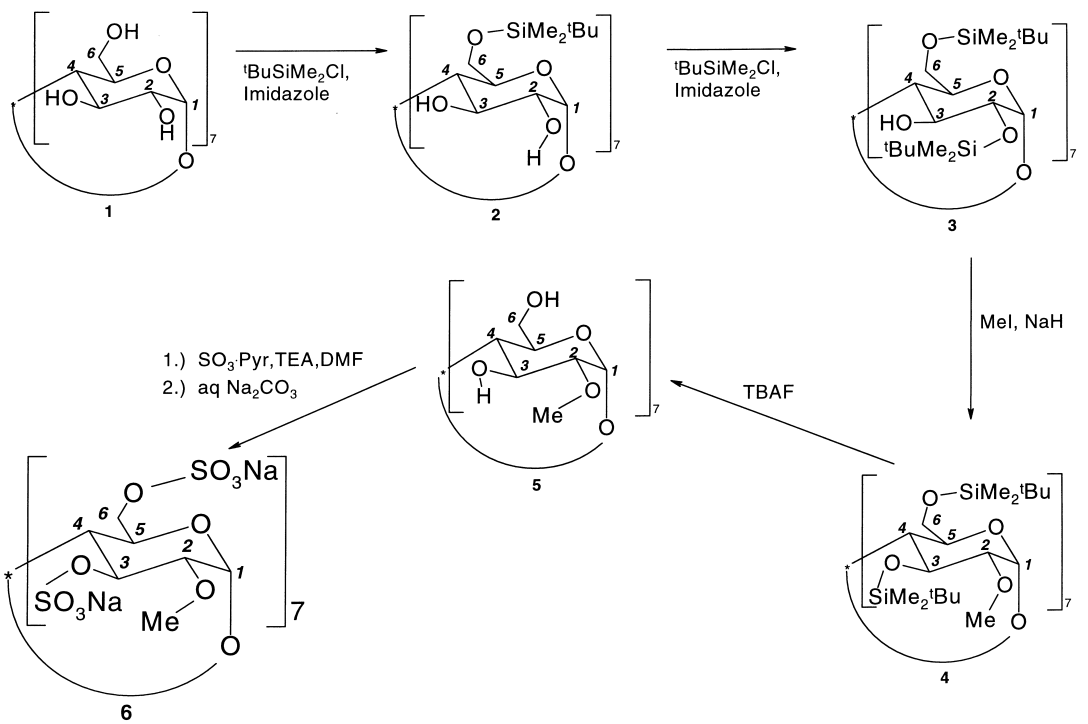
In order to explore the separation selectivities that other types of CD substitution patterns would offer in CE, our group is interested in synthesizing additional, single isomer sulfated CDs. This manuscript describes the synthesis and detailed analytical characterization of the sodium salt of heptakis(2-*O*-methyl-3,6-di-*O*-sulfo)cyclomaltoheptaose.

2. Results and discussion

The synthesis scheme is shown in Scheme 1. Although the synthesis relies on known reactions, several of the steps were modified to simplify the respective byproduct distributions and to create a synthetic methodology that can be readily scaled up. The synthesis of **2** and **3** is straightforward and requires little modification [12,13]. Nonaqueous reversed-phase high-performance liquid chromatography (HPLC) and MALDI-TOF-MS indicate that the isomeric purity of **2** and **3** is about 99 and 98%, respectively.

The first crucial step in the synthesis is the conversion of **3** to **4**, which involves simultaneous, quantitative migration of the *tert*-butyldimethylsilyl group from the 2-*O*-position to the 3-*O*-position and methylation at the 2-*O*-position. Complete migration of the 2-*O*-*tert*-butyldimethylsilyl groups has been postulated on the basis of detailed NMR studies [14,15] and has been subsequently corroborated by X-ray crystallography [16]. The conversion of intermediate **4** into **5** by removal of all 3-*O*-*tert*-butyldimethylsilyl groups using tetrabutylammonium fluoride is once again straightforward [12]. The structure of **5** has also been proven by X-ray crystallography [16].

The second crucial point in the synthetic scheme is the introduction of 14 sulfate groups onto **5**. While several highly sulfated β-CD preparations are available commercially (and have been used for the capillary electrophoretic separation of enantiomers [17]), all of them proved to be complex mixtures [18] of many isomers. By the combined use of corroborating evidence derived from high-resolution UV-MALDI-TOF-MS, NMR spectroscopy



Scheme 1. Schematic of the synthetic process.

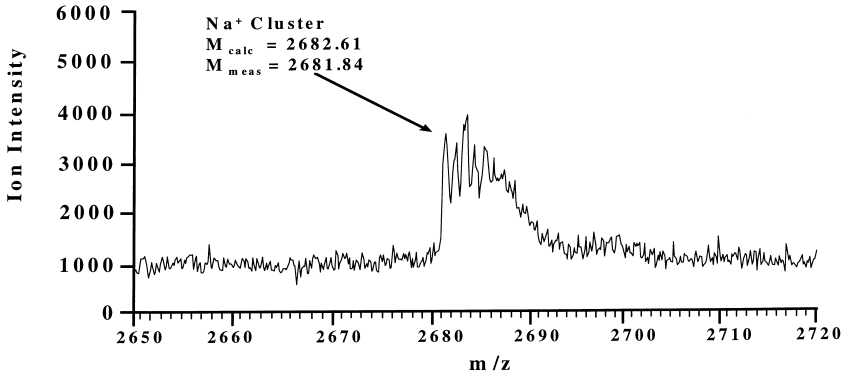


Fig. 1. The $[M + Na]^+$ region of the high-resolution MALDI-TOF mass spectrum of **6**.

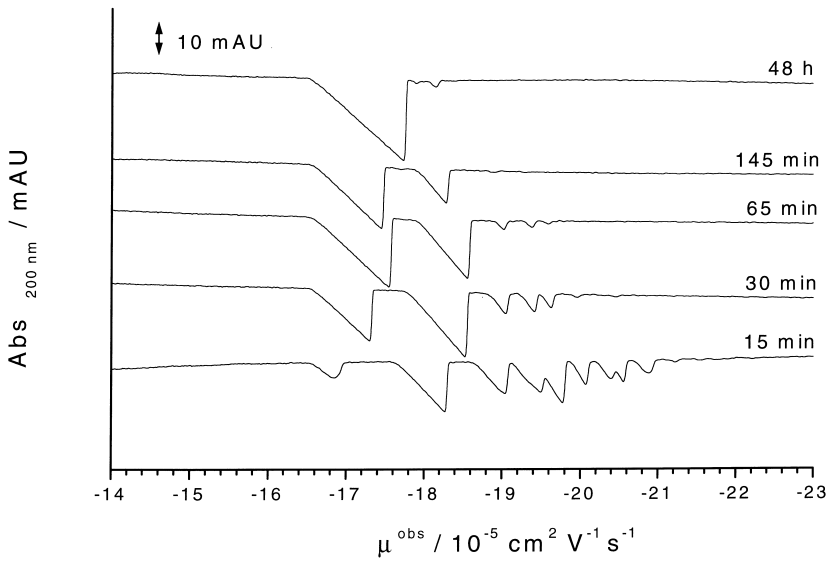


Fig. 2. Indirect UV detection electropherograms of samples taken during the sulfation reaction of **5**.

and indirect UV detection capillary electrophoresis, we could demonstrate, unequivocally, the identity and purity of the target compound, **6**. The Na^+ adduct region of the high-resolution MALDI-TOF mass spectrum of **6** is shown in Fig. 1. The calculated and measured m/z values agree very well, indicating that the highest degree of sulfation is 14 in compound **6**.

Typically, indirect UV detection capillary electrophoresis is used to assay the purity of anionic cyclodextrins that do not absorb light in the 200–300 nm range [19]. Fig. 2 shows the electropherograms for a series of samples taken from the sulfation reaction mixture after 15, 30, 65, 145, and 2880 min. The time axis in these electropherograms is replaced by the physically more meaningful observed mobility scale (analogous to the m/z scale in TOF-MS). The first few sulfate groups are introduced onto intermediate **5** very rapidly. As the sulfation time increases, the peaks that correspond to CDs with lower degrees of substitution disappear, and the size of the peak that corresponds to the target product **6** increases. Only a single major peak and two minor peaks can be seen in the sample taken after 48 h; the major peak represents over 97.5% of the total, sulfated CD-related peak area. In the high-resolution MALDI-TOF mass spectrum of this material, the highest measured m/z is 2681.84, which agrees very well with the calculated m/z for the Na^+ adduct of the base isotope peak of **6**, m/z 2682.61. Furthermore, Fig. 3 shows the electropherogram of the methanolic mother liquor obtained after the sodium sulfate removal step (see next paragraph). The mother liquor re-

tains a number of contaminants that can be separated by indirect UV detection CE, contaminants which are clearly absent in the final product (top trace in Fig. 3). Thus, indirect UV detection capillary electrophoresis can be used to detect the presence of the undersulfated CDs and demonstrate the purity of the final product **6**.

The next crucial step is the complete removal of excess Na_2SO_4 from the quenched reaction mixture. Again, indirect UV detection CE can be used to monitor the progress of the sulfate removal step. The residual Na_2SO_4 content is $< 0.5\%$ after the methanolic precipitation (see Section 3). Fig. 4 shows the electropherogram of the final product **6**. The combined impurity peaks represent only 2% of the area of the target peak.

Fig. 5 shows the ^{13}C NMR spectra of **6** at 8, 22 and 80 °C. The line widths of the C-6 and C-3 signals decrease quickly as the temperature is increased from 8 through 22 to 80 °C, indicating that **6** is present in deuterium oxide in a number of different conformations that rapidly interconvert as the temperature is increased to 80 °C. The ^1H NMR spectrum of **6** was also obtained and the integral values corroborate the identity of **6**. The chemical shifts,

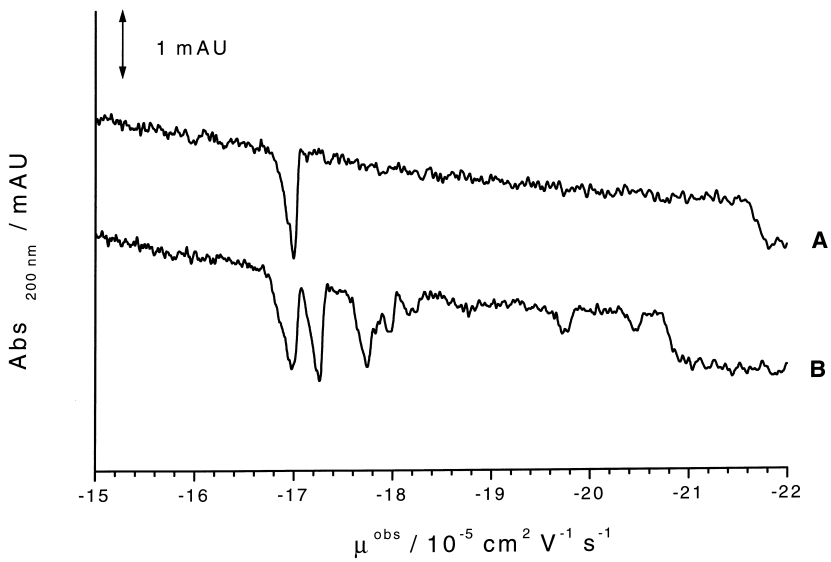


Fig. 3. Indirect UV detection electropherogram of **6** precipitated from methanol (A) and the methanolic mother liquor (B).

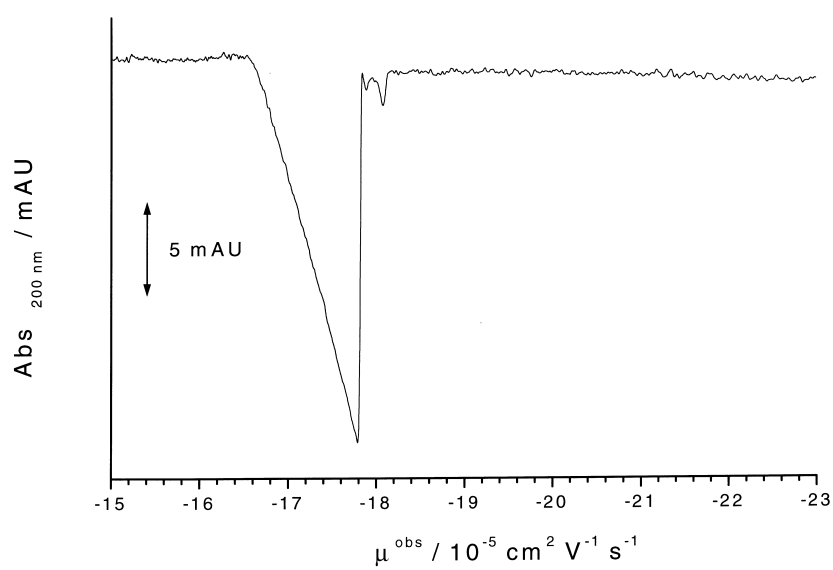


Fig. 4. Indirect UV detection electropherograms of the final product **6**.

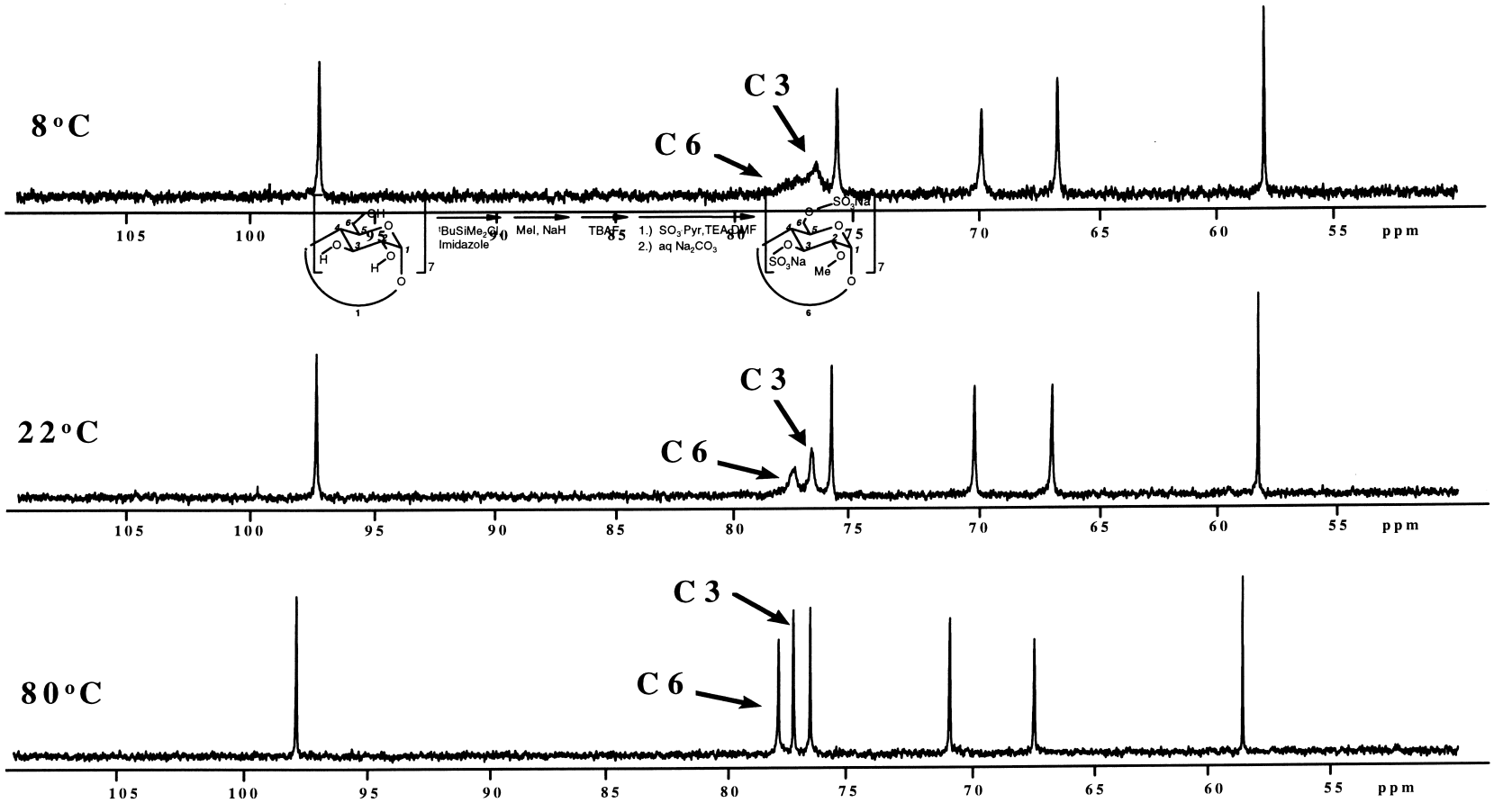


Fig. 5. Variable temperature ^{13}C NMR spectra of **6**.

Table 1
^{1}H NMR chemical shifts for compounds **2**–**6**

Derivative	Solvent	Chemical shifts in ppm (J, Hz)																
		H-1	H-2	H-3	H-4	H-4 & H-5	H-5	H-6 A	H-6A & H-6B	H-6B	CH_3–O on C-2	OH on C-3	OH on C-6	$[(CH_3)_3C]$ on C-2	$[(CH_3)_3C]$ on C-3 & C-6	$[(CH_3)_3C]$ on C-6	$[(CH_3)_2Si]$ on C-2	$[(CH_3)_2Si]$ on C-3	$[(CH_3)_2Si]$ on C-6
2 (500 MHz)	$CDCl_3$	4.87 d, 7 H $J_{1,2}$ 3.5	3.62 dd, 7 H	4.02 m, 7 H	3.54 m, 7 H		3.6 m, 7 H	3.88 m, 7 H		3.69 m, 7 H						0.85 s, 63 H			0.01, 0.02 d, 42 H
3 (500 MHz)	$CDCl_3$	4.87 d, 3 H $J_{1,2}$ 3.3	3.62 m, 7 H	3.87 m, 7 H	3.47 m, 7 H		3.62 m, 7 H	3.98 m, 7 H		3.69 m, 7 H		4.45 s, 7 H		0.94 s, 63 H		0.90 s, 63 H	0.17, 0.18 d, 42 H		0.04, 0.05 d, 42 H
4 (300 MHz)	$CDCl_3$	5.25 d, 7 H $J_{1,2}$ 3.5	3.02 dd, 7 H $J_{2,3}$ 8.4	4.10 abq, 7 H $J_{3,4}$ 6.0		3.79 m, 14 H		4.18 dd, 7 H		3.67 m, 7 H	3.33 s, 21 H				0.86 d, 126 H			0.06, 0.08 d, 42 H	0.01 s, 42 H
5 (300 MHz)	Pyridine-d_5	5.53 d, 7 H $J_{1,2}$ 3.6	3.53 dd, 7 H $J_{2,3}$ 9.6	4.62 m, 7 H	4.07 m, 7 H		4.32 m, 7 H		4.42 m, 14 H		3.69 s, 21 H	5.67 s, 7 H	6.50 m, 7 H						
6 (500 MHz)	D_2O	5.27 d, 7 H $J_{1,2}$ 3.9	3.75 m, 7 H	4.73 m, 7 H		4.10 m, 14 H			4.35 m, 14 H		3.47 s, 21 H								

Table 2
^{13}C NMR chemical shifts for compounds **2**–**6**

Derivatives	Solvent	Chemical shifts in ppm																	
		C-1	C-2	C-3	C-4	C-4 and C-5	C-5	C-6	CH_3-O on C-2	$[(CH_3)_3C]$ on C-2	$[(CH_3)_3C]$ on C-3	$[(CH_3)_3C]$ on C-6	$[(CH_3)_3C]$ on C-2	$[(CH_3)_3C]$ on C-3	$[(CH_3)_3C]$ on C-6	$[(CH_3)_3Si]$ on C-2	$[(CH_3)_3Si]$ on C-3	$[(CH_3)_2Si]$ on C-6
2 (125 MHz)	$CDCl_3$	102.10	74.10	73.90	81.90		72.60	61.70				25.90			18.30			
3 (75 MHz)	$CDCl_3$	102.50	74.90	71.90	81.99		72.14	61.95		26.28		25.86	18.88		18.30	−4.51, −4.51		−5.03, −5.25
4 (75 MHz)	$CDCl_3$	96.11	78.03	73.02	81.13		72.14	62.79	57.26		26.26	25.94		18.37	18.28		−3.78, −3.86	−4.76, −5.08
5 (75 MHz)	Pyridine-d_5	102.10	83.30	74.30	84.30		73.20	61.40	60.30									
6 (75 MHz) (at 80 °C) [a]	D_2O	98.30	76.80	77.60		71.0, 67.5		78.20	58.80									

[a] Relative to an external Me_4Si reference.

multiplicities, and coupling constants of the respective H atoms in **6** are listed in Table 1.

3. Experimental

General methods.—For the intermediates, progress of the reaction was monitored by thin-layer chromatography (TLC) (Silica-60, E.M. Science, Gibbstown, NJ). The CD derivative spots were visualized by dipping the developed TLC plates into an ethanolic α-naphthol solution of H_2SO_4 and heating the plate in an oven at 105 °C for 10 min. To determine the purity of the intermediates, analytical HPLC separations were carried out with a gradient system consisting of a Star 9010 ternary gradient pump (Varian, Walnut Creek, CA), a Rheodyne 7125 injection valve (Rheodyne, Cotati, CA), a DDL-31 evaporative light scattering detector (Bodman Industries, Aston, PA), and an AD 406 data acquisition system operated under Gold 8.1 software control (Beckman-Coulter, Fullerton, CA) running on an IBM 486 DX4 personal computer (Computer Associates, College Station, TX). The separations were obtained on 4.6 mm i.d. × 250 mm columns packed with either Zorbax silica or Zorbax 300 Bidentate C_{18} stationary phase [20]. The isomeric purity values reported here are based on the assumption that the response factors of the evaporative light scattering detector are identical for the closely eluting CD isomers. Indirect UV detection capillary electrophoresis was used to monitor the progress of the sulfation reaction, the extent of excess sodium sulfate removal, and the purity of the final product. The background electrolyte was a 13 mM tetramethylethylenediamine solution whose pH was adjusted to 5.5 with phthalic acid. The electrophoretic separations were carried out with a P/ACE 2100 system (Beckman-Coulter, Fullerton, CA), at 200 nm and 25 °C, with 10 kV as the separation potential, on a 39/46 cm long, 25 μm i.d. bare fused silica capillary column (Polymicro Technologies, Phoenix, AZ). High-resolution UV-MALDI-TOF mass spectral data were obtained as the most reliable indicators of elemental composition. The high-resolution mass spectra were collected with a Voyager Elite XL TOF mass spectrometer, equipped with delayed extraction capability (PerSeptive Biosystems, Framingham, MA), using the following instrument settings: reflectron mode, acceleration voltage 25 kV, 70% grid voltage, 0.035% guide wire voltage, and a delay of 180 μs. The analytes were applied onto the Teflon target stage using the dried droplet method [21]. For the nonionic CD derivatives (**2**–**5**), the matrix was 2,4,6-trihydroxyacetophenone dissolved in acetonitrile. For the ionic final product **6**, the matrix was created by mixing 10 μL of a 28 mg/mL aqueous fructose solution, 1 μL of 33 mg/mL methanolic 2,5-dihydroxybenzoic acid solution, 9 μL of 10 mg/mL methanolic 1-hydroxyisoquinoline solution and 10 μL of a 10 mg/mL solution of **6** dissolved in 0. 1% trifluoroacetic acid. Generally, the mass spectra from 80 laser shots were averaged to achieve an adequate signal-to-noise ratio. ^1H and ^{13}C NMR spectra were obtained either on a UnityPlus 300 or a UnityPlus 500 MHz spectrometer, with a quad nucleus ($^1H/^{19}F/^{31}P/^{13}C$) probe, using Solaris 2.4 and VnmrX 5.3b software. The proton and carbon assignments are based on ^1H–^1H COSY and ^1H–^{13}C HETCOR measurements.

Heptakis(6-O-tert-butyldimethylsilyl)cyclomaltoheptaose (**2**).—β-Cyclodextrin (**1**) was reacted according to the modified [5] procedure of Takeo and co-workers [12] to obtain **2** (2.8 kg, 82%); mp 300 °C (dec); TLC (40:10:1 $CHCl_3$–CH_3OH–water), R_f 0.45; analytical HPLC: ($ZorbaxC_{18}$ column, 25 °C, 1:9 EtOAc–MeOH at 2 mL/min; isomeric purity > 99%); ^1H NMR (500 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (125 MHz, $CDCl_3$,): see Table 2, in excellent agreement with the reported ^{13}C spectra [12]. Calcd for the base isotope peak of $[C_{84}H_{168}O_{35}Si_7 + Na]^+$: m/z 1955.96. Found: m/z 1955.66.

Heptakis(2,6-di-O-tert-butyldimethylsilyl)-cyclomaltoheptaose (**3**).—Compound **3** was obtained according to the modified procedure of Fügedi and Nànàsi [13]. Final recrystallization from acetone gave **3** (1207 g, 85%); mp 285 °C; TLC (20:1:0.2 hexane–$CHCl_3$–CH_3OH), R_f 0.73; analytical HPLC: (Zorbax 300 Bidentate C_{18} column, 25 °C, 10:7:3–0:7:3 MeCN–isopropanol–hexane in 30 min at 2

mL/min; isomeric purity >98%); ^{1}H NMR (500 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (75 MHz, $CDCl_3$): see Table 2, in excellent agreement with the reported ^{13}C spectra [15]. Calcd for the base isotope peak of $[C_{126}H_{266}O_{35}Si_{14}+Na]^+$: m/z 2754.57. Found: m/z 2754.49.

Heptakis(2-O-methyl-3,6-di-O-tert-butyldimethylsilyl)cyclomaltoheptaose (**4**).—Compound **4** was obtained according to the modified procedure of Takeo and coworkers [12] and Icheln and co-workers [15]. Final recrystallization from acetone gave **4** (985 g, 87%); mp 226 °C; TLC (24:1:0.1 hexane–$CHCl_3$–CH_3OH), R_f 0.82; analytical HPLC: (Zorbax 300 Bidentate C_{18} column, 25 °C, 10:7:3–0:7:3 MeCN–isopropanol–hexane in 30 min at 2 mL/min; isomeric purity 91%); ^{1}H NMR (300 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (75 MHz, $CDCl_3$): see Table 2, in excellent agreement with the reported ^{13}C spectra [12,15]. Calcd for the base isotope peak of $[C_{133}H_{280}O_{35}Si_{14}+Na]^+$: m/z 2852.68. Found: m/z 2851.84.

Heptakis(2-O-methyl)cyclomaltoheptaose (**5**).—Compound **5** was obtained according to the modified procedure of Takeo and coworkers [12]. Final recrystallization from EtOH gave **5** (325 g, 79%); mp 320 °C (dec); TLC (74:23:3 $CHCl_3$–CH_3OH–water), R_f 0.25; analytical HPLC: (Zorbax silica, 1:4–2:3 MeOH–EtOAc in 15 min at 2 mL/min; isomeric purity >95%); ^{1}H NMR (300 MHz, pyridine-d_5): see Table 1. ^{13}C NMR (75 MHz, pyridine-d_5): see Table 2, in excellent agreement with the reported ^{13}C spectra [22]. Calcd for the base isotope peak of $[C_{49}H_{84}O_{35}+Na]^+$: m/z 1255.47. Found: m/z 1255.52.

Heptakis(2-O-methyl-3,6-di-O-sulfo)cyclomaltoheptaose, sodium salt (**6**).—Compound **6** was obtained according to the modified procedure of Bernstein and coworkers [23] by adding **5** (300 g, 243 mmol), 4-dimethylaminopyridine (31.2 g, 255 mmol) and SO_3·pyridine (1624 g, 10.2 mol) to a mixture of 900 mL of dry DMF and 1020 mL of triethylamine, and reacting the vigorously stirred mixture for 48 h, at room temperature. The reaction was quenched by pouring the mixture into a 1000-mL portion of hot, satd aq Na_2CO_3. Upon cooling, the excess inorganic salt was filtered. The filtrate was concentrated by rotary evaporation and subsequently added to MeOH to precipitate the product **6** (388 g, 60%); mp 214 °C (dec); indirect UV detection capillary electrophoresis: (effective mobility: -16.6×10^{-5} cm^2 V^{-1} s^{-1}; isomeric purity 97.5%); ^{1}H NMR (500 MHz, D_2O): see Table 1; ^{13}C NMR (75 MHz, D_2O): see Table 2; Calcd for the base isotope peak of $[C_{49}H_{70}O_{77}S_{14}Na_{15}]^+$: m/z 2682.61. Found: m/z 2681.84.

Acknowledgements

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