Preparation of Di-altro-β-cyclodextrins with Two Altrosides as the Macrocyclic Units through Selective Synthesis of Regioisomers of Di(2-*O*-mesitylenesulfonyl)-β-cyclodextrin

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Received October 31, 1996; accepted December 17, 1996

Regioisomers of di(2-*O*-mesitylenesulfonyl)-β-cyclodextrin 2—4 were synthesized in 3, 4 times larger yields compared to those of the reported method using dibutyltin oxide/triethylamine/*p*-toluenesulfonyl chloride. Regioisomers of di-mannoepoxy-β-cyclodextrin 5—7 were prepared from 2—4 and converted to the corresponding di-altro-β-cyclodextrins (8—10) with two altrosides as macrocyclic units, respectively. Conformations of the altrose parts of di-altro-β-cyclodextrins were investigated by NMR, and the altroside was found to change its conformation depending on whether or not it is a member of the macrocyclic ring and its position in the ring.

Key words β-cyclodextrin; di(2-*O*-mesitylenesulfonyl)-β-cyclodextrin; di-altro-β-cyclodextrin; altroside; conformation

Cyclodextrins (CDs) are torus-shaped oligosaccharides consisting of D-glucoses which are linked by α-1,4 bonds. CDs can include various guest molecules into their hydrophobic cavities in water. Therefore, they have been widely used in studies on molecular recognition.[1] Recently, for the purpose of widening the recognition ability of CDs, various modifications of the shapes of CD's cavities have been undertaken: $3^A,2^B$-anhydration,[2] 3,6-anhydration,[3] and 2,3-epoxidation.[4] However, the deformed cavities generally lack flexibility. If the cavities can be made flexible, the scope of molecular recognition would be extended.

α-D-Altropyranose is an interesting sugar with two conformers, $^1C_4$ and $^4C_1$, of almost equal conformational energy, 3.85 and 3.65 kcal/mol, respectively.[5] Previously we reported a general method for selective production of altropyranosides from 2,3-epoxy-mannopyranosides without protecting the hydroxyls.[6] We applied the method to the preparation of altro-β-CD with one altropyranoside as a constituent of the macrocycle from 2,3-mannoepoxy-β-CD in a high yield (80%).[6] The predominant conformation of the altrose part in altro-β-CD is $^1C_4$ rather than $^4C_1$, which is preferred in the case of methyl α-D-altropyranoside. This conformational change is attributed to the small difference in energy between the two conformations. This unique property prompted us to prepare di-altro-β-CDs regioselectively and to investigate the conformations of the altrose parts. To obtain the regioisomers of di-altro-β-CD (**8**—**10**) selectively, the selective preparation of the regioisomers of di(2-*O*-arenesulfonyl)-β-CD is required (Chart 1). However, our method for preparing di(2-*O*-tosyl)-β-CD,[7] which is the only reported method for the di(2-*O*)-disulfonylation, is unsatisfactory in this respect.

We describe herein i) selective synthesis of di(2-*O*-mesitylenesulfonyl)-β-CDs (**2**—**4**) by a modification of the method reported by Rong and D'Souza,[8] ii) preparations of di-altro-β-CDs **8**—**10** from **2**—**4**, and iii) the conformations of the altrose parts of di-altro-β-CDs.

Results and Discussion

Preparations of $2^A,2^D$-, $2^A,2^C$-, and $2^A,2^B$-Di-(*O*-mesitylenesulfonyl)-β-CDs (2—4) Rong and D'Souza[8] reported the preparation of 2-*O*-tosyl-β-CD through the activation

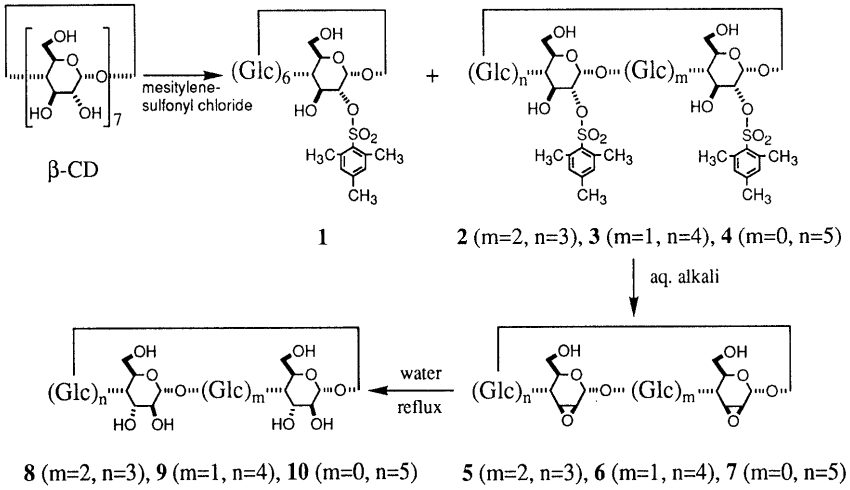

Chart 1. Synthesis of Di-altro-β-CDs

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Table 1. Products Obtained by the Reaction of β-CD with Sulfonyl Chloride

No.	Sulfonylating reagent (eq)	[NaH] (eq)	Activation time of OH (h)	Reaction with sulfonyl chloride		Yield of sulfonate (%)				
						Mono-sulfonate		Di-sulfonate		
				temp. (°C)	time (h)	2-	3-	$2^A,2^D$-	$2^A,2^C$-	$2^A,2^B$-
1	MesCl (2.2)	2.4	26.0	r.t.	2.0	29.4		3.9	4.5	5.8
2	MesCl (2.0)	3.1	13.0	0	2.0	27.4		3.6	3.9	2.8
3	MesCl (2.0)	3.0	14.5	r.t.	2.0	27.1		4.0	5.5	3.7
4	MesCl (3.1)	3.9	24.0	r.t.	2.0	26.7		5.7	5.9	7.6
6	MesCl (2.5)	3.2	44.5	r.t.	3.0	28.3		5.8	7.4	11.8
7	TsCl (2.0)	3.0	14.0	0	3.0	19.3		3.1	4.6	9.3
8	2-NsCl (2.1)	3.0	19.5	0	4.0	15.1	15.3	(Total 16.2)[a]		
9	1-NsCl (2.2)	2.9	18.5	0	3.0	22.6		(Total 24.2)[a]		
10	MbsCl (2.0)	2.8	23.5	r.t.	3.5	24.7		(Total 15.9)[b]		
11	MtbCl (2.6)	3.3	44.0	r.t.	3.0	28.0		(Total 9.9)[b]		

MesCl = mesitylenesulfonyl chloride, TsCl = *p*-toluenesulfonyl chloride, 2- or 1-NsCl = 2- or 1-naphthalenesulfonyl chloride, MbsCl = methoxybenzenesulfonyl chloride, MtbCl = 2,4,6-tri-methoxybenzenesulfonyl chloride.[10] *a*) Regioisomers of di-sulfonylated β-cyclodextrins were not separable. *b*) Structures of the regioisomers were not determined.

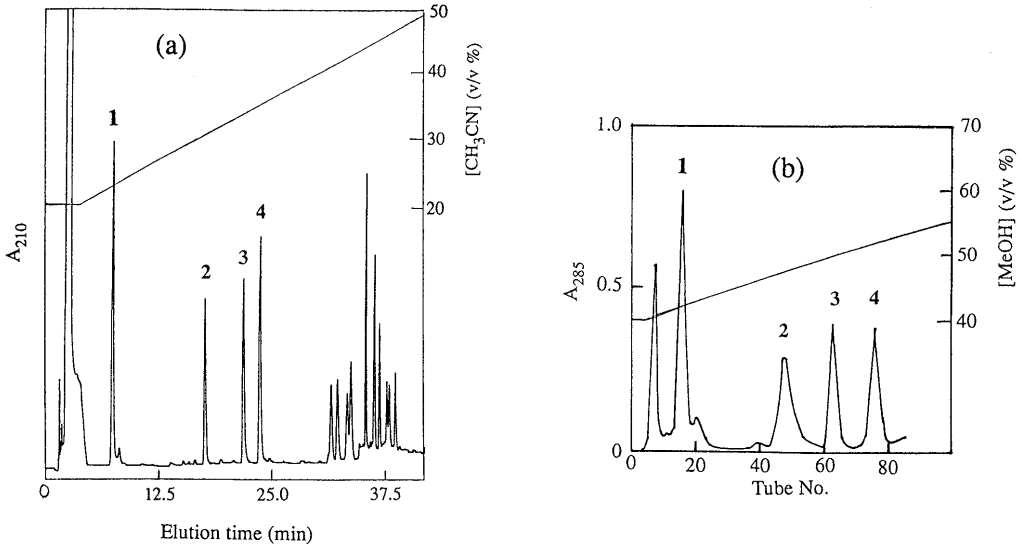


Fig. 1. Reversed-Phase HPLC (Column, TSK ODS 80Tm) (a) and Reversed-Phase Column Chromatography (Column, Merck Lobar Column Rp 18 B-type) (b) of the Mixture Obtained by the Reaction of β-CD with Mesitylenesulfonyl Chloride

Elution was done with a linear gradient of CH_3CN (a) or MeOH (b) was applied.

of β-CD with NaH in dimethylformamide (DMF) followed by reaction with *p*-toluenesulfonyl chloride. For the purpose of obtaining di(2-*O*)-disulfonylated β-CD, we examined their method with respect to the amount of NaH, the activation time, the amount and kind of sulfonylating reagent, and the time and temperature of the reaction with the sulfonylating reagent. The results are summarized in Table 1. Consequently, the method based on entry No. 6 in Table 1 was employed. The yields of di(2-*O*-mesitylenesulfonyl)-β-CDs are 3, 4 times better than those of our previous method using dibutyltin oxide/triethylamine/*p*-toluenesulfonyl chloride in DMF.[7] Reversed-phase HPLC of the reaction mixture was conducted, as shown in Fig. 1a. The products (**2**—**4**) were well separated and purified by gradient elution with aqueous CH_3OH (Fig. 1b).

The 2-*O* and 3-*O* monosulfonates were obtained in comparable yield by the reaction of β-CD with 2-naphthalenesulfonyl chloride in DMF (entry No. 8). It is interesting to note that, in the direct reaction with 2-naphthalenesulfonyl chloride in aqueous solution (30% aqueous CH_3CN solution (pH 12) at 40 °C), the 3-*O* sulfonate was exclusively produced owing to formation of the inclusion complex prior to the sulfonylation.[9]

Structure Determination of $2^A,2^D$-, $2^A,2^C$-, and $2^A,2^B$-Di-(*O*-mesitylenesulfonyl)-β-CDs 2—4 and Their Conversion to the Mannoepoxides The double 2-*O*-sulfonylation in **2**—**4** was confirmed by the FAB-MS and NMR spectra (Figs. 2, 3). The FAB-MS showed the molecular ion peak at *m/z* 1499 ($M+H^+$). The ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were measured at 40 °C in dimethyl sulfoxide (DMSO)-d_6. The proton and carbon signals were assigned by the use of H–H and C–H correlation spectroscopy (COSY). All ^{13}C-NMR spectra showed large downfield shifts for two C-2, and small upfield shifts for two C-3 and two C-1 compared with those for β-CD. The ^1H-NMR spectra, in all cases, presented extreme downfield shifts of two H-2 as compared with those of β-CD. These results indicate the occurrence of double 2-*O*-mesitylenesulfonylation in **2**—**4**.

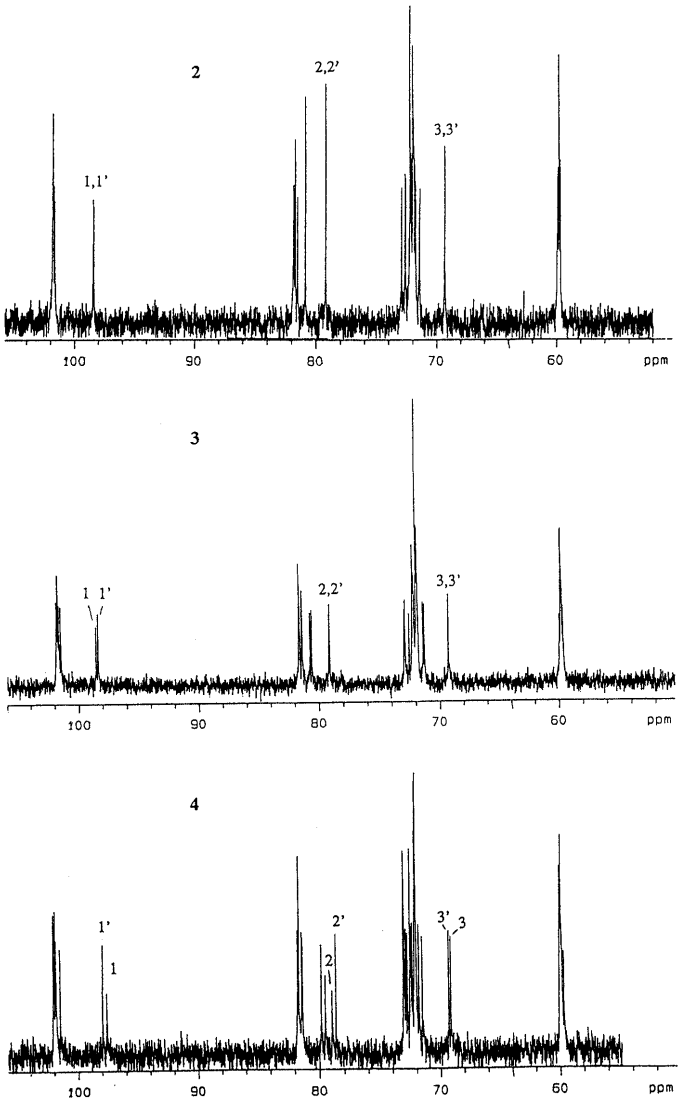


Fig. 2. ^{13}C-NMR Spectra of the CD Moieties of Di(2-*O*-mesitylenesulfonyl)-*β*-CDs **2**—**4** in DMSO-d_6 at 40 °C (125 MHz, Internal Reference: TMS)

The marked signals are those of the 2-*O*-mesitylenesulfonyl glucose moieties.

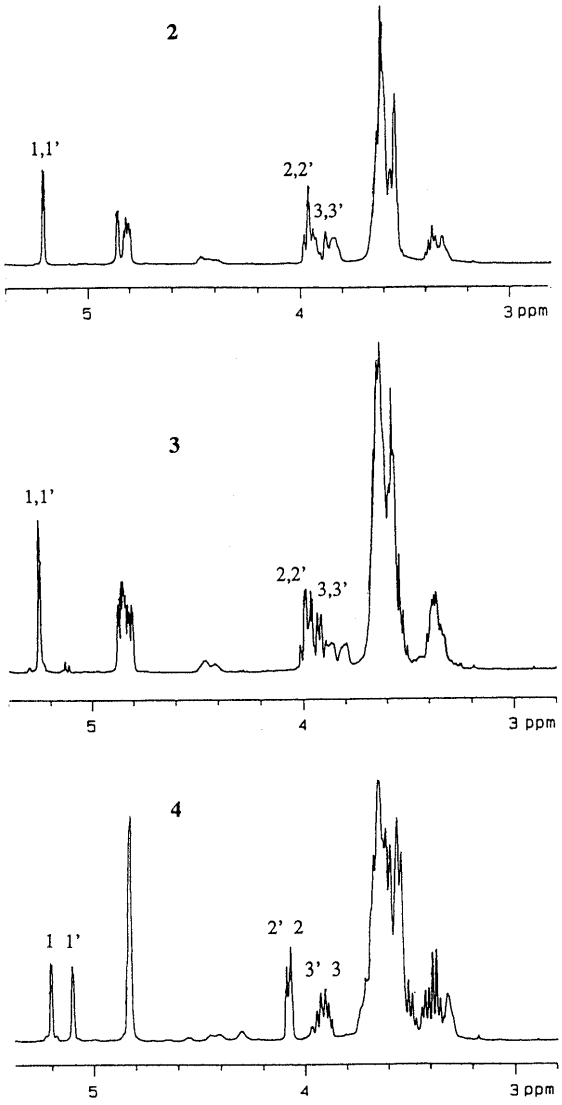


Fig. 3. ^{1}H-NMR Spectra of the CD Moieties of Di(2-*O*-mesitylenesulfonyl)-*β*-CDs **2**—**4** in DMSO-d_6 at 40 °C (500 MHz, Internal Reference: TMS)

The marked signals are those of the 2-*O*-mesitylenesulfonyl glucose moieties.

The positions of the sulfonylated glucosides in **2**—**4** were determined as follows. Compounds **2**—**4** were converted into the corresponding 2,3-mannoepoxides **5**—**7** by treatment with an aqueous solution of NaOH. By comparing the TLC and HPLC behavior, and ^{1}H- and ^{13}C-NMR spectra of **5**—**7** with those of the authentic compounds,[7] **2**—**4** were assigned as $2^A,2^D$-, $2^A,2^C$-, and $2^A,2^B$-di-(*O*-mesitylenesulfonyl)-*β*-CD, respectively.

Conversion of the Mannoepoxides 5—7 to the Altrosides 8—10 A solution of di-mannoepoxy-*β*-CD **5**, **6**, or **7** in water was refluxed for 5, 6 d. The results of TLC and reversed-phase HPLC (TSK ODS 80T$_M$) of the solution demonstrated that the mannoepoxide disappeared and only one product was formed. By reversed-phase column chromatography, the di-altro-*β*-CD **8**, **9**, or **10** was obtained in the high yield of 67.8%, 75.3% or 78.3%, respectively. The FAB-MS of these saccharides showed molecular ion peaks at *m/z* 1135 ($M+H^+$) and 1157 ($M+Na^+$), the same as for *β*-CD. However, the ^{1}H- and ^{13}C-NMR spectra of **8**—**10** were very different from those of *β*-CD. The NMR signals were assigned by means of H–H and C–H COSY. The ^{1}H-NMR spectra (Fig. 4) showed two upfield-shifted H-1 and two downfield-shifted H-5, which are characteristic to altroside.[6] The ^{13}C-NMR spectra (Fig. 5) exhibited downfield shifts for two C-1. From these results, we conclude that di-altro-*β*-CD's **8**—**10** are the AD, AC, and AB isomers, respectively.

Conformations of the Altrose Moieties in 8—10 It is noteworthy that the coupling constants $J_{1,2}$ of the altrose moieties in **8**—**10** are different from each other, as shown in Table 2, reflecting the conformations of these moieties. The values are between 8.4 Hz (calculated $J_{1,2}$ for the axial-axial relation) and 2.1 Hz (calculated $J_{1,2}$ for the equatorial-equatorial relation),[11] suggesting that the altrosides are in rapid equilibrium between ^{1}C$_4$ and ^{4}C$_1$ conformers and the ratios of the conformers differ. On the basis of this assumption, the percentages of the conformers were estimated (Table 2). Although methyl α-D-altroside is mainly in the ^{4}C$_1$ conformation, the ^{1}C$_4$ conformations

are preferred for the two altrosides of the AD isomer **8**. The altroside of altro-β-CD and one altroside of the AC isomer **9** show some preference for $^4C_1$ conformation. The other AC isomer **9** and both of the AB isomers **10** have the two conformations in comparable amounts. The preference of the $^1C_4$ conformer of the CD derivatives as compared with methyl α-D-altroside seems to be attributable to the hydrogen bonding between 2-OH of the altroside and the 3-OH of the adjacent glucoside. This hydrogen bonding seems to be more difficult when the macrocyclic strain caused by the existence of the altroside becomes larger. Thus, the quasi-symmetrical compound **8** has a larger $^1C_4/^4C_1$ ratio than the AC- and AB-isomers and, interestingly, than the altro-β-CD with only one altroside. The discussion described above is based on the $J_{1,2}$ values, and other J values could not be obtained. Therefore, there is another possibility with respect to the preferred conformation of the altrose parts; twist-boat conformations.

In any event, the altroside can change its conformation depending on the circumstances, that is, whether or not it is a member of the macrocyclic ring, and if it is, its position in the ring. This interesting behavior is a consequence of the small difference in conformational

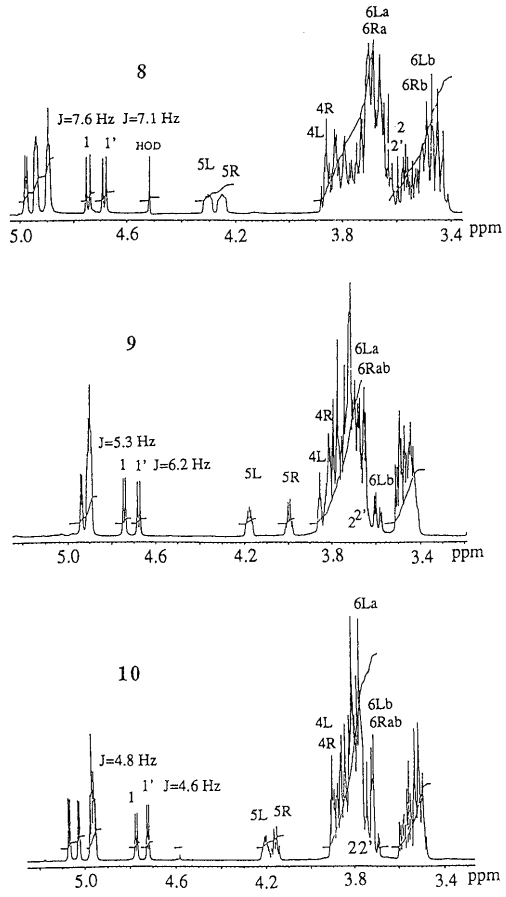


Fig. 4. ^{1}H-NMR Spectra of Di-altro-β-CDs **8**—**10** in D_2O at 35 °C (500 MHz, Internal Reference: CH_3CN)

The marked signals are those of the altrose moieties. The signals denoted as 1 or 1′ do not necessarily correspond to those denoted as L or R.

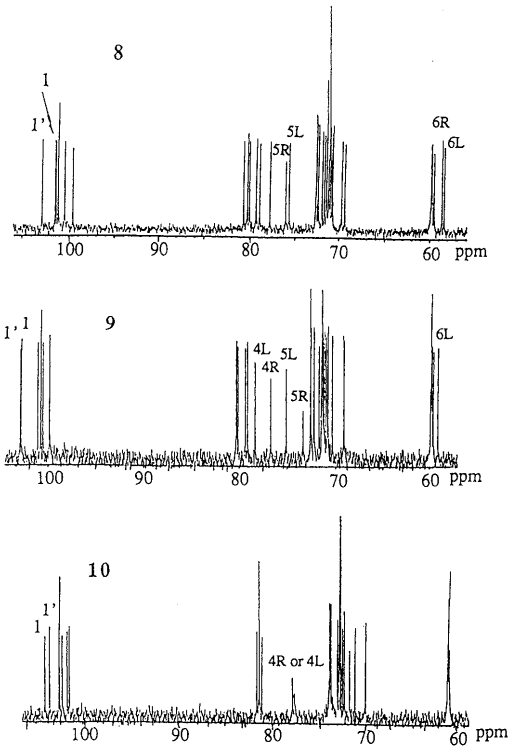
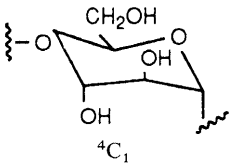
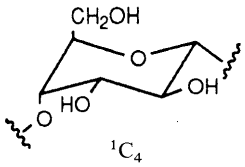


Fig. 5. ^{13}C-NMR Spectra of Di-altro-β-CDs **8**—**10** in D_2O at 35 °C (125 MHz, Internal Reference: CH_3CN)

The marked signals are those of the altrose moieties. The signals denoted as 1 or 1′ do not necessarily correspond to those denoted as L or R.

Table 2. The Value of $J_{1,2}$ and Estimation of Conformation for Altrose Moieties of Di-altro-β-cyclodextrin

Samples	$J_{1,2}$ (Hz)	Conformational percentage (%)	
		 $^4C_1$	 $^1C_4$
Methyl α-D-altroside[6]	1.9	100	0
Altro-β-CD[6]	6.6	29	71
AD-altro-β-CD **8**	7.1	21	79
	7.6	13	87
AC-altro-β-CD **9**	5.3	49	51
	6.2	35	65
AB-altro-β-CD **10**	4.6	60	40
	4.8	57	43

energy between the $^1C_4$ and $^4C_1$ conformations.

Experimental

^{1}H- and ^{13}C- NMR spectra were determined with Varian Unity plus 500 (500, 125 MHz). FAB-MS were recorded with a JEOL JMS-DX 303 spectrometer. TLC was run on precoated silica gel plates (Art. No. 5554). Spot detection was carried out by staining with 0.1% 1,3-dihydroxynaphthalene in EtOH–H_2O–H_2SO_4 (200 : 157 : 43, v/v). HPLC was performed using Tosoh CCPD with Cosmosil 10C18 (Nacalai Tesque, 20 × 150 mm) or Tosoh TSK ODS 80Tm (4.6 × 150 mm) equipped with a UV spectrophotometer or a differential refractometer as the detector. Lyophilization was carried out with an Eyela Freeze FD-5N. Optical rotation was measured with a JASCO DIP-370 digital polarimeter. Each sample was dried at 105 °C in a vacuum for 4 h before measurement of optical rotation. Desalting was undertaken with a Tosoh Fresaltor IE-Labo.

Syntheses of 2^A,2^D-, 2^A,2^C-, and 2^A,2^B-Di-(*O*-mesitylenesulfonyl)-β-CDs (2—4) A solution of β-CD (102 mg, 0.090 mmol) in dry DMF (4 ml) was purged with nitrogen gas, then NaH (7 mg, 0.29 mmol) was added followed by replacement of the nitrogen atmosphere. The mixture was stirred for 48 h under nitrogen at room temperature. Then a solution of mesitylenesulfonyl chloride (50 mg, 0.24 mmol) in dry DMF (0.5 ml) was added *via* a syringe and stirring was continued for 2 h. The reaction was terminated by addition of ice water, followed by evaporation of the mixture in a vacuum. The residue was dissolved into 40% aqueous MeOH (20 ml), filtered, and chromatographed on a Merck Lobar column Rp 18 B-type with a gradient from 40% aqueous MeOH (900 ml) to 70% aqueous MeOH (900 ml) to give **2** (7.8 mg, 5.8%), **3** (9.9 mg, 7.4%), and **4** (15.9 mg, 11.8%), along with 2-*O*-mesitylenesulfonyl-β-CD **1** (33.5 mg, 28.3%).

2: FAB-MS *m/z*: 1499 ($M+H^+$). ^{13}C- and ^{1}H-NMR see Figs. 2 and 3. $[\alpha]_D$ 130.7 ($t=22$ °C, $c=0.4$, H_2O). *Rf*, 0.53 (*n*-PrOH : AcOEt : H_2O = 7 : 7 : 5, v/v). *Anal.* Calcd for $C_{60}H_{90}O_{39}S_2 \cdot 5H_2O$: C, 45.34: H, 6.34: S, 4.03. Found: C, 45.13: H, 6.09: S, 4.12. **3**: FAB-MS *m/z*: 1499 ($M+H^+$). ^{13}C- and ^{1}H-NMR see Figs. 2 and 3. $[\alpha]_D$ 112.6 ($t=22$ °C, $c=0.4$, H_2O). *Rf*, 0.50 (*n*-PrOH : AcOEt : H_2O = 7 : 7 : 5, v/v). *Anal.* Calcd for $C_{60}H_{90}O_{39}S_2 \cdot 7H_2O$: C, 44.33: H, 6.45: S, 3.95. Found: C, 44.03: H, 6.10: S, 3.85. **4**: FAB-MS *m/z*: 1499 ($M+H^+$). ^{13}C- and ^{1}H-NMR see Figs. 2 and 3. $[\alpha]_D$ 127.0 ($t=23$ °C, $c=0.6$, H_2O). *Rf*, 0.44 (*n*-PrOH : AcOEt : H_2O = 7 : 7 : 5, v/v). *Anal.* Calcd for $C_{60}H_{90}O_{39}S_2 \cdot 7H_2O$: C, 44.33: H, 6.45: S, 3.95. Found: C, 44.46: H, 6.02: S, 3.78.

Preparation of 2^A,3^A : 2^D,3^D-, 2^A,3^A : 2^C,3^C-, or 2^A,3^A : 2^B,3^B-Di-mannoepoxy-β-CDs (5—7) Di-mannoepoxy-β-CD **5**, **6**, or **7** was prepared from **2**, **3**, or **4**, respectively, by means of a modification of the reported method.[7]

A solution of **2** (823 mg, 0.57 mmol) in 1 M NaOH (5 ml) was stirred overnight at 4 °C, neutralized with 1 M HCl, desalted, and filtered. The filtrate was subjected to column chromatography on a Merck Lobar column Rp 18 C-type with a gradient from water (1 l) to 50% aqueous MeOH (1 l) to give **5** (442 mg, 70.8%). Similarly, **3** or **4** afforded **6** or **7** in 63.4% or 68.0% yield, respectively. Their TLC and HPLC behavior, FAB-MS and ^{1}H- and ^{13}C-NMR spectra were compared with those of authentic samples.[7]

Preparation of AD-, AC-, or AB-Di-altro-β-CDs (8—10) A solution of **5** (174 mg, 0.158 mmol) in water (34 ml) was refluxed for 6 d. The solution was filtered and the product was separated by reversed-phase HPLC (column, Cosmosil 10C18; eluent, 2% aqueous CH_3CN), followed by lyophilization to give AD-di-altro-β-CD **8** (122 mg, 67.9%). Similarly, **9** or **10** was obtained in 75.3% or 78.3% yield from **6** or **7**, respectively. **8**: FAB-MS *m/z*: 1135 ($M+H^+$), 1157 ($M+Na^+$). ^{13}C- and ^{1}H-NMR see Figs. 5 and 4. $[\alpha]_D$ 108.3 ($t=13$ °C, $c=0.4$, H_2O). *Rf*, 0.38 (*n*-PrOH : AcOEt : H_2O = 8 : 7 : 6, v/v, double development). *Anal.* Calcd for $C_{42}H_{70}O_{35} \cdot 3H_2O$: C, 42.43: H, 6.44. Found: C, 42.47: H, 6.11. **9**: FAB-MS *m/z*: 1135 ($M+H^+$), 1157 ($M+Na^+$). ^{13}C- and ^{1}H-NMR see Figs. 5 and 4. $[\alpha]_D$ 108.7 ($t=13$ °C, $c=0.4$, H_2O). *Rf*, 0.42 (*n*-PrOH : AcOEt : H_2O = 8 : 7 : 6, v/v, double development). *Anal.* Calcd for $C_{42}H_{70}O_{35} \cdot 4H_2O$: C, 41.79: H, 6.51. Found: C, 41.86: H, 6.17. **10**: FAB-MS *m/z*: 1135 ($M+H^+$), 1157 ($M+Na^+$). ^{13}C- and ^{1}H-NMR see Figs. 5 and 4. $[\alpha]_D$ 118.5 ($t=13$ °C, $c=0.4$, H_2O). *Rf*, 0.46 (*n*-PrOH : AcOEt : H_2O = 8 : 7 : 6, v/v, double development). *Anal.* Calcd for $C_{42}H_{70}O_{35} \cdot 4H_2O$: C, 41.79: H, 6.51. Found: C, 41.54: H, 6.16.

Acknowledgment The authors are grateful to Japan Maize Products Co. Ltd. for providing β-cyclodextrin.

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