$2^{A},2^{B}$-, $2^{A},2^{C}$-, and $2^{A},2^{D}$-Bis-*O*-(*p*-tolylsulfonyl)-*β*-cyclodextrins

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A mixture of the title ditosylates was prepared by the reaction of *β*-cyclodextrin with dibutyltin oxide and tosyl chloride. Each ditosylate was isolated by reversed-phase column chromatography and the structure was assigned on the basis of conversion into a known compound or hydrolysis with Taka amylase A.

The introduction of two functional groups at the desired positions of cyclodextrins is an approach to the construction of enzyme (or receptor) mimics.[1–5] In this context the study of specific disulfonylation is important.

Tabushi et al.[6–9] reported the specific 6,6-di-*O*-sulfonylation of *β*-cyclodextrin (*β*-CD) and Boger and Knowles[10] demonstrated nonspecific 6,6,6-tri-*O*-sulfonylation of *α*-cyclodextrin (*α*-CD) but only the $6^{A},6^{C},6^{E}$ isomer was identified. Fujita et al.[11–14] prepared poly-6-*O*-sulfonylated *α*(or *β* or *γ*)-cyclodextrins by nonspecific sulfonylation.

Since Breslow et al.[15] demonstrated that *β*-CD modified with a pyridoxamine moiety on its secondary hydroxyl side[15] produced amino acids with opposite chirality to that produced from the corresponding 6-*O*-modified *β*-CD[16] in transamination reactions, disulfonylation of the secondary hydroxyl groups has been a challenging problem. However, there are few reliable methods for the specific sulfonylation of 3-OH or 2-OH.

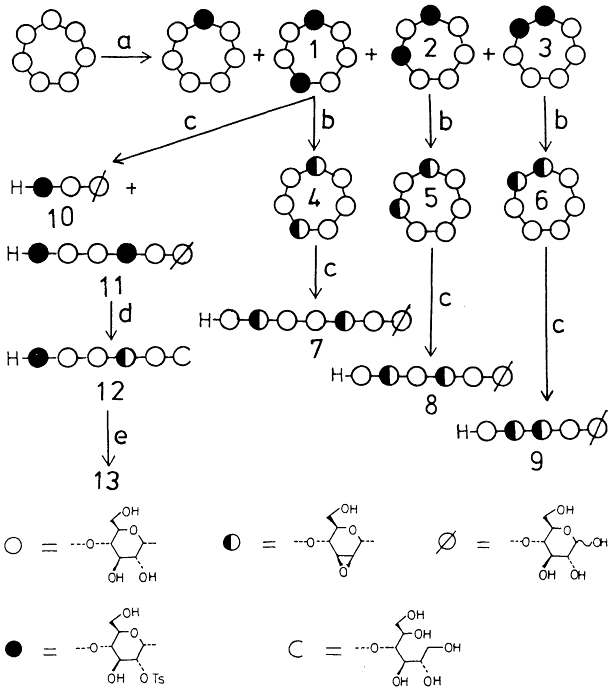
Fujita et al.[17] reported the preparation of 3-*O*-sulfonylated *α*- or *β*-CD by reaction with 2-naphthalenesulfonyl chloride in alkaline aqueous acetonitrile and its application for the preparation of $3^{A},3^{C}$- and $3^{A},3^{D}$-di-*O*-sulfonylated *β*-CDs.

Three methods have been reported for the 2-*O*-sulfonylation of *β*-CD. (a) Ueno and Breslow[18] prepared 2-*O*-(*p*-tosyl)-*β*-CD by the reaction of *β*-CD with *m*-nitrophenyl *p*-tosylate in alkaline aqueous *N,N*-dimethylformamide. (b) Fujita et al. reported that the reaction of *α*- or *β*-CD with *m*-nitrobenzenesulfonyl chloride[19] or 1-naphthalenesulfonyl chloride[20] in alkaline aqueous solution gave the corresponding 2-*O*-sulfonates. The reaction with *m*-nitrobenzenesulfonyl chloride was used to synthesize $2^{A},2^{X}$-di-*O*-sulfonyl-*α*-CDs (X=B, C, and D). However, the reaction with 1-naphthalenesulfonyl chloride gave a low yield of 2-*O*-sulfonyl *β*-CD and the rate of reaction was too slow to allow further sulfonylation because of the competitive epoxidation of the monosulfonate. (c) Murakami et al.[21] reported the preparation of a 2-*O*-sulfonate by the use of dibutyltin oxide and the corresponding sulfonyl chloride in *N,N*-dimethylformamide.[22] Methods (a) and (b) did not allow accumulation of the disulfonates since the 2-*O*-sulfonylated *β*-CDs were converted into the epoxide, $(2^{A}S,3^{A}S)$-$2^{A},3^{A}$-anhydro-*β*-CD.

Method (c), on the other hand, is carried out in dry *N,N*-dimethylformamide and offers a promise for the preparation of 2,2-di-*O*-sulfonylated *β*-CDs. We now describe its use for the preparation of $2^{A},2^{B}$-, $2^{A},2^{C}$-, and $2^{A},2^{D}$-bis-*O*-(*p*-tosyl)-*β*-CDs by the reaction of *β*-CD.

Results and Discussion

Following the reaction of *β*-CD with dibutyltin oxide and *p*-toluenesulfonyl chloride in *N,N*-dimethylformamide, reversed-phase column chromatography of the products gave, in order of elution in reversed-phase HPLC, 2-*O*-*p*-tosyl (9.4%), $2^{A},2^{D}$- (**1**, 3.6%), $2^{A},2^{C}$- (**2**, 2.6%), and $2^{A},2^{B}$-bis-*O*-(*p*-tosyl) (**3**, 1.8%) derivatives (Scheme 1). That **1**—**3** were ditosylates was confirmed by their fast-atom bombardment (hereafter abbreviated to FAB) mass spectra and by their



Scheme 1. Preparation and determination of the structure of $2^{A},2^{X}$-bis-*O*-(*p*-tosyl)-*β*-cyclodextrins (**1**—**3**): (a) *p*-toluenesulfonyl chloride–Bu_2SnO–*N,N*-dimethylformamide, (b) $Ba(OH)_2$, (c) Taka amylase A, (d) $NaBH_4$, (e) Ac_2O–pyridine.

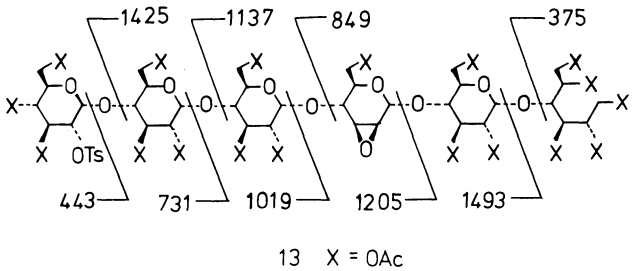
^{13}C NMR spectra which showed that two of the ^{13}C resonances were shifted up-field (see Experimental). However, the ^{13}C NMR spectra were not helpful in determining the regiochemistry of the sulfonylations.

Fujita et al.[19] reported that $2^A,2^X$-di-*O*-sulfonyl-α-CDs (X=B, C, and D) were converted easily into the corresponding ($2^AS,3^AS:2^XS,3^XS$)-$2^A,3^A:2^X,3^X$-dianhydro-α-CDs by treatment with aqueous alkali and that ($2^AS,3^AS:2^CS,3^CS$)-$2^A,3^A:2^C,3^C$-dianhydro-α-CD and ($2^AS,3^AS:2^BS,3^BS$)-$2^A,3^A:2^B,3^B$-dianhydro-α-CD were hydrolyzed by Taka amylase A to give ($2''S,3''S:2''''S,3''''S$)-$2'',3'':2'''',3''''$-dianhydromaltohexaose and ($2''S,3''S:2'''S,3'''S$)-$2'',3'':2''',3'''$-dianhydromaltopentaose, respectively, whereas ($2^AS,3^AS:2^DS,3^DS$)-$2^A,3^A:2^D,3^D$-dianhydro-α-CD was unaffected.

Following these procedures, **1—3** were treated with aqueous $Ba(OH)_2$ to give the corresponding diepoxides **4—6**. Although the ^{13}C NMR spectra of **4—6** were different from each other, they did not allow the structures to be assigned. The diepoxides **4—6** were hydrolyzed by Taka amylase A to give diepoxides (**7—9**) of maltoheptaose, maltohexaose, and maltopentaose, respectively, together with glucose (Scheme 1). The FAB mass spectra of **7—9** contained the corresponding molecular ions (see Experimental). The structures ($2''S,3''S:2''''S,3''''S$)-$2'',3'':2'''',3''''$-dianhydromaltohexaose and ($2''S,3''S:2'''S,3'''S$)-$2'',3'':2''',3'''$-dianhydromaltopentaose were assigned to **8** and **9**, respectively, by comparing the FAB mass spectra and retention times in reversed-phase HPLC with those of the authentic compounds which were prepared from ($2^AS,3^AS:2^XS,3^XS$)-$2^A,3^A:2^X,3^X$-dianhydro-α-CDs (X=B and C) as described above.[19] Therefore, **2** and **3** are the $2^A,2^C$- and $2^A,2^B$-ditosylate, respectively. Since there are only three 2,2-disulfonates, **1** must be the $2^A,2^D$ isomer and this was confirmed as follows.

Hydrolysis of 2-*O*-arylsulfonyl-β-CDs by Taka amylase A gives[20] 2″-*O*-(arylsulfonyl)maltotrioses (**10**). However, enzymic hydrolysis of **1** gave the expected known compound **10**[20] only as a minor product. The major product was **11**. Reduction of **11** with aqueous $NaBH_4$ gave **12**, the FAB mass spectrum of which contained ($M+H^+$) ion at *m/z* 1129 demonstrating that one of the two tosylated sugar units had been converted into an epoxide and that the end group was a hexitol moiety. Treatment of **12** with acetic anhydride in pyridine gave the octadecaacetate **13**, the FAB mass spectral fragmentation of which (Scheme 2) demonstrated the tosyl and the epoxy groups were located at the first and fourth glucose units, respectively. Therefore, the identity of **1** is confirmed as $2^A,2^D$-bis-*O*-(*p*-tosyl)-β-CD.

The ditosylates **1—3** may serve as starting materials for the synthesis of specifically bifunctionalized β-CDs.



13 X = OAc

Scheme. 2. FAB mass spectral fragmentation pattern of **13**.

Experimental

General. ^{13}C NMR spectra were determined with a JEOL FX 100 (25 MHz) spectrometer. FAB mass spectra were recorded with a JEOL DX-300 data system. Merck Lobar prepacked columns of LiChroprep Rp18 (A 10 mm×240 mm and B 25 mm×310 mm) and Rp8 (C 37 mm×440 mm) were used for reversed-phase column chromatography. HPLC was performed on a HITACHI L-3000 instrument with a column (4.6 mm×250 mm) of TSKgel ODS-80TM (5 μm, TOSOH).

Preparation and Isolation of $2^A,2^X$-Bis-*O*-(*p*-tosyl)-β-CDs (1—3, X=B—D). A mixture of β-cyclodextrin (5.25 g, 4.63 mmol) and dibutyltin oxide (11.52 g, 46.3 mmol) in anhydrous *N,N*-dimethylformamide (35 mL) was stirred at 95—100 °C for 2 h under nitrogen. The mixture was cooled to 0 °C, triethylamine (5.61 g, 55.5 mmol) was added, followed dropwise by a solution of *p*-toluenesulfonyl chloride (8.84 g, 46.3 mmol) in anhydrous *N,N*-dimethylformamide (10 mL). The mixture was stirred for 10 h at room temp and then concentrated in vacuo to 20 mL. The residual yellow syrup was stirred vigorously with acetone (500 mL) for 20 min, the solid was collected, washed with acetone, and dried in vacuo. A solution in water (250 mL) was filtered and chromatographed on column C. After elution with aqueous 20% methanol (1 L), gradient elution from aqueous 30% methanol (1 L) to aqueous 60% methanol (1 L) was applied to give 2-*O*-(*p*-tosyl)-β-CD (561 mg, 9.4%) and a mixture of $2^A,2^X$-bis-*O*-(*p*-tosyl)-β-CDs (X=B—D). The mixture was concentrated in vacuo and chromatographed on column B with gradient elution from aqueous 40% methanol (1 L) to aqueous 60% methanol (1 L) to give $2^A,2^D$-(**1**, 240 mg, 3.6%), $2^A,2^C$-(**2**, 174 mg, 2.6%), and $2^A,2^B$-bis-*O*-(*p*-tosyl)-β-CD (**3**, 122 mg, 1.8%). ^{13}C NMR (Me_2SO-d_6, internal Me_4Si, characteristic nonaromatic carbons), **1** δ=59.8, 69.1, 71.9, 72.2, 72.9, 79.5, 81.4, 98.1 (two shifted C-1 resonances), 101.8, **2** δ=59.7, 69.2, 70.2, 72.0, 72.8, 79.6, 80.9, 81.4, 98.1 (two shifted C-1 resonances), 101.8, **3** δ=59.8, 68.8, 69.1, 71.9, 72.9, 79.2, 80.2, 81.1, 81.5, 97.1 (a shifted C-1 resonance), 97.7 (a shifted C-1 resonance), 101.8; FABMS *m/z* **1** 1443 ($M+H^+$), **2** 1443 ($M+H^+$), **3** 1443 ($M+H^+$).

Epoxidation of $2^A,2^X$-Bis-*O*-(*p*-tosyl)-β-CDs (1—3, X=B—D). A solution of **1** (30.8 mg) in 0.05 M $Ba(OH)_2$ (3 mL; 1 M=1 mol dm^{-3}) was stirred at room temperature for 1.5 h, then neutralized with 0.5 M H_2SO_4, filtered, applied to a column (2.6 cm×90 cm) of Bio-Gel P-2 (200—400 mesh, Bio-Rad Laboratories), and eluted with water to give ($2^AS,3^AS:2^DS,3^DS$)-$2^A,3^A:2^D,3^D$-dianhydro-β-CD **4** (23.1 mg, 74%).

Similarly, **2** (15.0 mg) and **3** (25.2 mg) gave ($2^AS,3^AS:2^CS,3^CS$)-$2^A,3^A:2^C,3^C$-dianhydro-β-CD (**5**, 11.3 mg, 84.8%) and ($2^AS,3^AS:2^BS,3^BS$)-$2^A,3^A:2^B,3^B$-dianhydro-β-CD (**6**, 17.8 mg, 97.8%),

respectively. ^{13}C NMR [D_2O, internal $Me_3Si(CH_2)_3SO_3Na$, characteristic absorptions] **4** δ=51.7, 56.5, 62.7, 63.5, 74.3, 75.0, 82.2, 82.6, 83.1, 100.0, 100.2, 103.9, **5** δ=51.9, 56.6, 62.7, 63.4, 74.3, 74.6, 82.3, 82.7, 83.2, 100.1, 103.6, 104.1, **6** δ=51.7, 52.2, 56.7, 62.8, 63.4, 74.4, 75.6, 82.8, 83.3, 99.0, 100.2, 103.0, 104.1; FABMS *m/z* **4** 1121 ($M+Na^+$), 1137 ($M+K^+$), **5** 1121 ($M+Na^+$), **6** 1099 ($M+H^+$), 1137 ($M+K^+$).

Hydrolysis of (2^A*S*,3^A*S*:2^X*S*,3^X*S*)-2^A,3^A:2^X,3^X-dianhydro-β-CD (4—6, X=B—D) with Taka Amylase A. A solution of (36 mg) and Taka amylase A [α-amylase Type X-A (Sigma), 36 mg] in 0.2 M acetate buffer (3 mL, pH 5.5) containing 0.01 M $CaCl_2$ was kept at 40 °C for 2 days, then heated at 100 °C for 10 min, filtered, applied to a column (2.6 cm×90 cm) of Bio-Gel P-2 and eluted with water to give (2″*S*,3″*S*:2‴″*S*,3‴″*S*)-2″,3″:2‴″,3‴″-dianhydromaltoheptaose (**7**, 17.6 mg, 48.2%).

Similarly, **5** (26 mg) and **6** (59 mg) gave (2″*S*,3″*S*:2‴″*S*,3‴″*S*)-2″,3″:2‴″,3‴″-dianhydromaltohexaose (**8**, 6.0 mg, 26.5%) and (2″*S*,3″*S*:2‴*S*,3‴*S*)-2″,3″:2‴,3‴-dianhydromaltopentaose (**9**, 32.0 mg, 88.8%), respectively. FABMS *m/z* **7** 1117 ($M+H^+$), 1139 ($M+Na^+$), **8** 955 ($M+H^+$), 977 ($M+Na^+$), 993 ($M+K^+$), **9** 793 ($M+H^+$), 815 ($M+Na^+$).

Hydrolysis of 2^A,2^D-Bis-*O*-(*p*-tosyl)-β-CD 1 with Taka Amylase A. A solution of **1** (60 mg) and Taka amylase A (180 mg) in 0.2 M acetate buffer (8 mL, pH 5.5) containing 0.01 M $CaCl_2$ was kept at 40 °C for 2 days, then worked-up as described above. The filtrate was chromatographed on column A. After elution of aqueous 5% methanol (50 mL), gradient elution from aqueous 10% methanol (500 mL) to aqueous 50% methanol (500 mL) was applied to give 2″-*O*-(*p*-tosyl)maltotriose (**10**, 9 mg, 32.9%) and 2″,2‴″-bis-*O*-(*p*-tosyl)maltohexaose (**11**, 36.0 mg, 66.7%). FABMS *m/z* **10** 659 ($M+H^+$), 681 ($M+Na^+$), **11** 1299 ($M+H^+$), 1321 ($M+Na^+$).

Reduction of 2″,2‴″-Bis-*O*-(*p*-tosyl)maltohexaose 11 with $NaBH_4$. A solution of **11** (15.5 mg) in aqueous 1% $NaBH_4$ (5 mL) was kept at room temp for 16 h, then neutralized with aqueous 5% HCl, filtered, and chromatographed on column A with gradient elution from aqueous 20% methanol (500 mL) to aqueous 50% methanol (500 mL) to give *O*-2-*O*-(*p*-tosyl)-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-[(2*S*,3*S*)-2,3-anhydro-α-D-glucopyranosyl]-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucitol (**12**, 7.5 mg, 55.9%). FABMS *m/z* 1129 ($M+H^+$).

A solution of **12** (3 mg) was treated conventionally with acetic anhydride (1.5 mL) in pyridine (1.5 mL) at room temp for 2 days. The crude product was purified by reversed-phase HPLC with gradient elution from aqueous 50% CH_3CN to aqueous 80% CH_3CN to give *O*-2-*O*-(*p*-tosyl)-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-*O*-[(2*S*,3*S*)-2,3-anhydro-α-D-glucopyranosyl]-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucitol octadecaacetate (**13**). FABMS *m/z* 1885 ($M+H^+$) (see Scheme 2).

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