$NaClO_4$ (μ = 0.5); the final deuterium content was >99% in each case. Values of pD were calculated as pH (measured) +0.4.[32]

Semicarbazide Trapping Experiments (Table III). Reaction mixtures contained, in a total volume of 3 mL, 0.6 mmol of NaCl or semicarbazide hydrochloride; pH was adjusted with HCl or KOH, except for the run at pH 3.61, which contained 0.3 mmol of acetate buffer. Reactions were initiated by the addition of 0.5 mL of a 0.3 mM solution of the sodium salt of **8a**. Oxindole product was determined by HPLC analysis on a Rainin C_{18} reverse-phase column (100 × 4.6 mm), with 0.02 M potassium phosphate (pH 7.0)–methanol (4:1) as eluant. Percent oxindole was determined by comparison of peak height in the control reaction (without semicarbazide) to that with semicarbazide. In another control experiment, it was shown that oxindole-3-propionic acid (**9**) does not react with semicarbazide under the experimental conditions.

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Registry No. 1, 1604-49-5; **2a**, 89311-48-8; **2b**, 89311-49-9; **3a**, 89311-50-2; **3b**, 89311-51-3; **4a**, 89311-52-4; **4b**, 89311-53-5; **5**, 32999-55-6; **6**, 5548-09-4; **7a**, 89311-54-6; **7b**, 100572-63-2; **8a**, 100572-64-3; **8b**, 100572-65-4; **9**, 2971-17-7; **10**, 100572-66-5; **11a**, 100572-67-6; **12a**, 100572-68-7; 1-methyl-DL-tryptophane, 26988-72-7; ethyl trifluoroacetate, 383-63-1; 2-bromoskatole, 1484-28-2; ethyl 2-bromoindole-3-acetate, 1912-37-4; 2-bromoindole-3-acetic acid, 1912-39-6; 2-chloroskatole, 51206-73-6.

Regiospecific Sulfonation onto C-3 Hydroxyls of β-Cyclodextrin. Preparation and Enzyme-Based Structural Assignment of 3A,3C and 3A,3D Disulfonates

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Abstract: A secondary hydroxyl (C-3–OH) of β-cyclodextrin was specifically sulfonated by β-naphthalenesulfonyl chloride in aqueous CH_3CN. This method also gave a limited mixture of the disulfonated β-cyclodextrins, which were mainly composed of 3A,3C and 3A,3D isomers. They were easily isolated by reversed-phase column chromatography and converted to the corresponding diallo epoxides. Their regiochemistries were determined by their specific amylolyses to the linear oligosaccharides.

Chemical construction of enzyme mimics by use of cyclodextrins has attracted much attention.[3] Usually, hydroxyl groups of cyclodextrins are once activated (sulfonated) for the respective functionalizations. Many studies have been focused on the sulfonations of the primary hydroxyl groups since sulfonyl chlorides react exclusively with the primary hydroxyls in pyridine and since the sulfonated cyclodextrins are stable under the sulfonation condition and also under the isolation condition.[4] Activations on secondary hydroxyl groups of cyclodextrins should also be investigated for the sake of wide development of chemical construction of enzyme mimics. Breslow reported selective preparation of 2-deoxy-2-[(*p*-tosyl)oxy]-β-cyclodextrin,[5] and Hattori described formation of 2-deoxy-2-[(*p*-tosyl)oxy]-α-cyclodextrin.[6] Recently, we showed specific preparation of 2-deoxy-2-(*m*-nitrobenzenesulfonyl)oxy]-α-cyclodextrin by the reaction of α-cyclodextrin with the corresponding sulfonyl chloride in an alkaline water.[7] In this report, we describe specific preparation of C-3 sulfonated β-cyclodextrin and its application to specific preparation of 3A,3C- and 3A,3D-disulfonated β-cyclodextrins.

Results and Discussion

β-Cyclodextrin was sulfonated with β-naphthalenesufonyl chloride in 30% aqueous CH_3CN at 40 °C to give a mixture of the C-3 sulfonate **1** and the disulfonates **2** and **3** under the reaction condition where the initial pH of the solution of β-cyclodextrin in the aqueous CH_3CN was adjusted to 12, and the pH was allowed to decrease during the reaction with β-naphthalenesulfonyl chloride. The reaction mixture was not a solution but a suspension of the sulfonyl chloride. The mixture was chromatographed by use of a reversed-phase column to afford pure sulfonates **1** (18.0%), **2** (4.4%), and **3** (4.5%). Structural assignments of these compounds are as follows. The similarity of ^{13}C NMR spectrum of **1** (Figure 1) to that of 3-deoxy-3-[(*p*-tosyl)oxy]-α-cyclodextrin[8] indicated that **1** was the C-3 sulfonate of β-cyclodextrin. Its ^1H NMR and FABMS spectra also confirmed the monosulfonation in **1**. In order to ascertain the C-3 sulfonation, **1** was converted to the allo epoxide **4**, whose structure was spectrally determined as shown below. Its FABMS spectrum showed the correct molecular ion, and its ^{13}C NMR spectrum (Figure 2) demonstrated the presence of the epoxide carbons at δ 58.9 and 56.5. A doublet absorption of one proton (J = 3.30 Hz) was observed at δ 5.05 in its ^1H NMR spectrum (270 MHz). These facts demonstrate that **4** was the allo epoxide,[8] and, therefore, its precursor **1** was the C-3 sulfonate. FABMS and ^1H NMR spectra (Figure 1) showed that **2** and **3** were disulfonates of β-cyclodextrin. Since the present sulfonation reaction gave selectively one product (**1**) as the monosulfonate, the positions of the disulfonation in **2** and **3** were expected to be the C-3 hydroxyls. This expectation was supported by their ^{13}C NMR spectra (Figure 1) where the chemical shifts of the cyclodextrin parts were very similar to that

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(2) Daiichi College.
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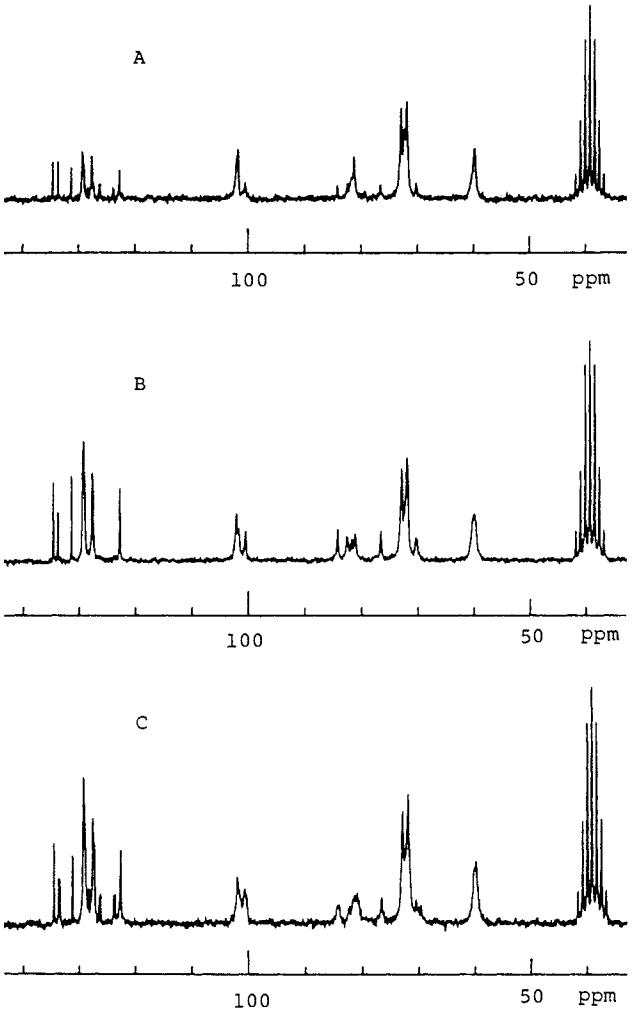


Figure 1. ^{13}C NMR spectra (25 MHz) of 3-deoxy-3-[(β-naphthylsulfonyl)oxy]-β-cyclodextrin (**1**) (1A), 3A,3D-dideoxy-3A,3D-bis[(β-naphthylsulfonyl)oxy]-β-cyclodextrin (**2**) (1B), and the 3A,3C isomer (**3**) (1C) in Me_2SO-d_6. Me_4Si was used as an internal standard.

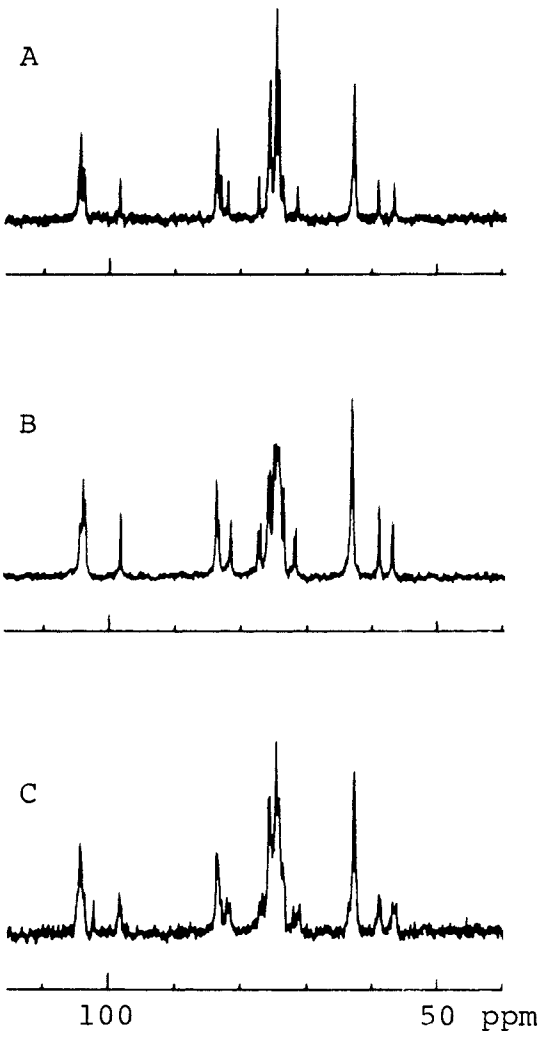
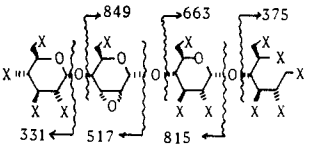


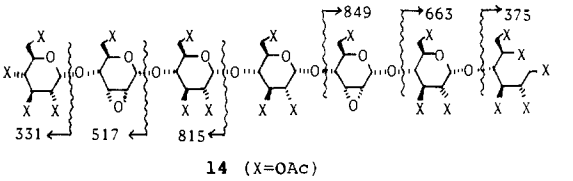
Figure 2. ^{13}C NMR spectra (25 MHz) of β-cyclodextrin allo epoxide (**4**) (A), AD-bis(allo epoxide) (**5**) (B), and AC-bis(allo epoxide) (**6**) (C) in D_2O. DSS was used as an internal standard.

of the monosulfonate **1**. The sulfonates **2** and **3** were converted to the epoxides **5** and **6**, respectively, by the treatment with aqueous K_2CO_3. [^1H NMR spectra (270 MHz) of **5** and **6** showed a doublet absorption at δ 5.19 (2 H, J = 3.18 Hz) and two doublet absorptions at δ 5.17 (1 H, J = 3.42 Hz) and at δ 5.21 (1 H, J = 3.17 Hz), respectively, demonstrating that the epoxides possessed the allo structures[8] and, therefore, their precursors, **2** and **3**, were the C-3 disulfonates.]

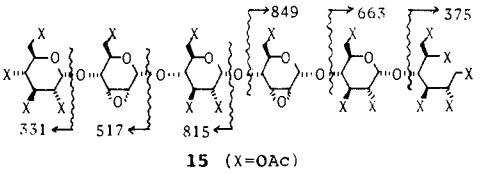
There are three possible regioisomers in the C-3 disulfonates (3A,3B, 3A,3C, and 3A,3D isomers). In order to elucidate their regiochemistry, Taka amyloylses were employed. We have found that Taka amylolysis of **4** gave a maltotetraose epoxide **7**, whose FABMS spectrum showed the correct molecular ion. Its ^{13}C NMR spectrum also supported the structural assignment of maltotetraose epoxide but did not clarify the position of the epoxy glucose unit in **7**. The epoxy maltotetraose **7** was reduced with aqueous $NaBH_4$ to give **10**, which was completely acetylated with acetic anhydride in pyridine to afford **13**. The FABMS spectrum of **10** showed the correct molecular ion and its ^{13}C NMR spectrum showed that a maltitol part and epoxy part were present in the structure of **10**, demonstrating the selective reduction on the reducing end (the hemiacetal moiety) of **7**. The complete acetylation of **10** was confirmed by the FDMS spectrum of **13**. The position of the allo epoxy group was determined by the EIMS spectral fragmentation pattern of **13** (Figure 3); the allo epoxy group was located on the second glucose unit from the nonreducing end.[9] From this observation, we expected that the diallo epoxides



13 (X=OAc)



14 (X=OAc)



15 (X=OAc)

Figure 3. EIMS spectral fragmentation pattern of **13**–**15** which showed correct molecular ions in FDMS spectra.

derived from 3A,3B, 3A,3C, and 3A,3D disulfonates would give a maltopentaose diepoxide, a maltohexaose diepoxide **9**, and a

(9) Results with respect to the Taka amylolysis of **4** and structural determination of the amylolysis product will be reported elsewhere.

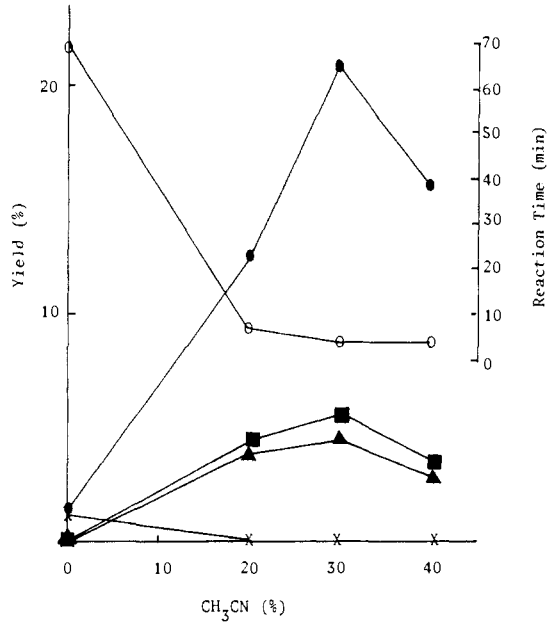


Figure 4. Effect of CH_3CN content in the solvent on the yield of **1** (●), **2** (■), **3** (▲), or 6-deoxy-6-[(β-naphthylsulfonyl)oxy]-β-cyclodextrin (×) and on the reaction time required for the pH of the reaction mixture to change from 12 to 8 (○) in the reaction of β-cyclodextrin (0.35 g) with β-naphthalenesulfonyl chloride (0.35 g) in H_2O or aqueous CH_3CN (5 mL) at 25 °C.

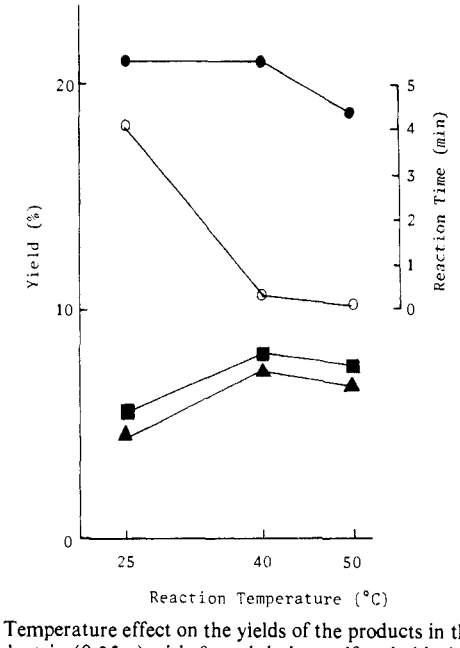


Figure 5. Temperature effect on the yields of the products in the reaction of β-cyclodextrin (0.35 g) with β-naphthalenesulfonyl chloride (0.35 g) in 30% aqueous CH_3CN solution (5 mL): ●, **1**; □, **2**; ▲, **3**; ○, reaction time required for the pH of the reaction mixture to change from 12 to 8.

maltoheptaose diepoxide **8**, respectively, by Taka amylolyses. The acetates, **14** and **15**, were obtained from **5** and **6**, respectively, by Taka amylolysis followed by $NaBH_4$ reduction and complete acetylation. The structures of the intermediate compounds **8**, **9**, **11**, and **12**, except the location of the allo epoxides, were confirmed by their FABMS and ^{13}C NMR spectra. Figure 3 shows the EIMS spectral fragmentation patterns of **14** and **15** whose FDMS spectra gave the correct molecular ions. From these EIMS spectral results, **8** was assigned to a maltoheptaose diepoxide where the epoxy groups were located on the second and the fifth glucose units from the nonreducing end, and also **9** was assigned to a maltohexaose diepoxide where the epoxy groups were located on the second and the fourth glucose units from the nonreducing unit. Therefore, their precursors, **2** and **3**, are assigned to the 3A,3D and the 3A,3C disulfonates of β-cyclodextrin, respectively.

The present reaction in aqueous CH_3CN is specific for a C-3 hydroxyl group, which was demonstrated by the predominant formation of **1** as a monosulfonate. Moreover, the formation of a limited mixture of the disulfonates (**2** and **3**) is another proof of the present C-3 specificity since formation of 30 regioisomers is possible in the case of nonspecific disulfonation. However, when the sulfonation was carried out in water instead of 30% aqueous CH_3CN, a mixture of the C-3 sulfonate (3.6%) and the C-6 sulfonate (4.2%) was obtained, and the disulfonates were also obtained as a mixture of many nonseparable components in low yield. The reaction in water was (seemingly) nonselective. The time (70 min) required for the pH of the reaction mixture to change from 12 to 8 in water was much longer than in 30% aqueous CH_3CN at 40 °C (0.3 min) where the reaction was highly selective for a C-3 hydroxyl group. Our independent kinetic studies on the epoxidation of the various cyclodextrin sulfoantes demonstrate that the half-time of the decomposition (the epoxidation) of **1** at 20 °C in a phosphate buffer (pH 12.0) was 2.5 min. This means that the long reaction time under alkaline condition decreases the yields of the C-3 mono- and disulfonates. Short reaction time is, therefore, very important for clean reaction and this was achieved by use of CH_3CN as a cosolvent. Effect of the CH_3CN concentration on the yields of the C-3 sulfonates was investigated (Figure 4). The maximum yield was obtained around the 30% CH_3CN concentration. The yields at the larger concentration than 50% CH_3CN were not obtained because of insolubility of β-cyclodextrin in the solvent. Presence of CH_3CN in the solvent increases the solubility of β-naphthalenesulfonyl chloride and, therefore, enhances the reaction rate to decrease the time for the pH change of the mixture from 12 to 8. Such short reaction time would reveal the intrinsic selectivity (C-3 sulfonation) and would not allow the accumulation of the minor product, the C-6 sulfonate which was far more stable under the alkaline condition.[8]

Heating the reaction mixture is expected to increase the solubility of β-naphthalenesulfonyl chloride and the reaction rates of the sulfonation and the epoxidation. Since the former two and the last one have an opposite effect on the yields of the desirable products, **1–3**, an optimum temperature for the production of **1–3** is hopefully expected to be present. The sulfonation reaction was carried out in the thermostated bath, and the products were analyzed by reversed-phase HPLC to show that the favorable temperature was 40 °C (Figure 5). Therefore, the sulfonation condition mentioned above (in 30% aqueous CH_3CN at 40 °C) was employed for specific preparation of **1–3**.

Ueno and Breslow reported tosylation on a C-2 hydroxyl group of β-cyclodextrin by the reaction with *m*-nitrophenyl *p*-tosylate in aqueous DMF (pH 9.9).[5a] The production of β-cyclodextrin manno epoxide by treatment of the C-2 tosylate with aqueous K_2CO_3 was also described by Breslow.[5b] Our result is quite different from their result. Our method can give not only the C-3 sulfonate (and the allo epoxide) but also the C-3 disulfonates (and the diallo epoxides) in high degree of purity. This difference may be due to the difference in the sulfonating reagents, since our preliminary study showed that the reaction of β-cyclodextrin with α-naphthalenesulfonyl chloride gave mainly the C-2 sulfonate. Hattori and co-workers described the formation of the C-6 tosylate by the reaction of β-cyclodextrin with *p*-tosyl chloride in water under the alkaline condition where the pH of the reaction mixture was maintained at 12 during the reaction.[6] Under such a condition, the C-2 and C-3 sulfonates must be decomposed to form the corresponding epoxides even if they were main products of the sulfonation reaction. Therefore, under their reaction condition, the C-6 tosylate might become only one product that could be isolated.

Discrimination between the 3A,3C (**3**) and 3A,3D (**2**) isomers was practically impossible by use of IR and ^1H NMR (100 MHz) spectra. The ^{13}C NMR and circular dichroism spectra could achieve clear discrimination between them. Particularly, the latter spectra of **2** and **3** (Figure 6) were quite different from one another and from that of **1**. These observations show the interaction of

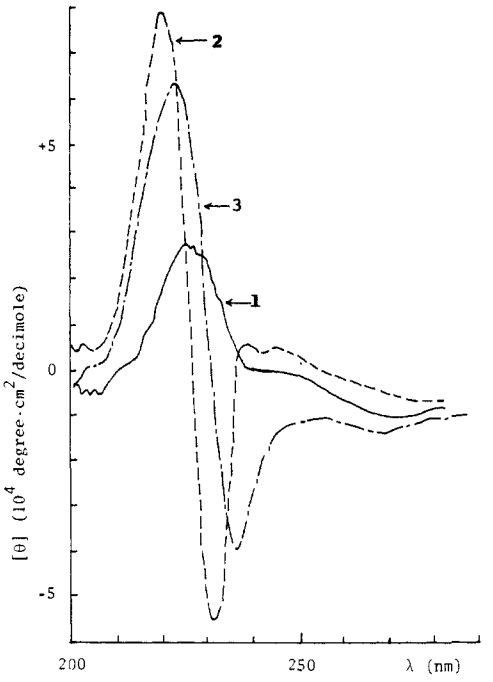


Figure 6. Circular dichroism spectra of **1–3** in phosphate buffer (pH 6.86).

the two chromophores (exciton coupling of ^1B_b absorptions)[10] in the dinaphthyl sulfonates and its dependency on the regiochemistry of the two chromophores.

The present specific reaction gave the 3A,3B disulfonate, if any, in the yield less than 0.2%. This maximum value was estimated from the HPLC. Practically negligible formation of the 3A,3B isomer may be due to steric repulsion between two naphthylsulfonyl moieties on the neighboring two glucose units.[11]

In ordinary chemical reactions, there is no question that reaction at the primary hydroxyl will predominate over reaction at the secondary. However, the present result is not this case. Possibly the present reaction proceeds through formation of inclusion complex of β-naphthalenesulfonyl chloride by β-cyclodextrin where the chlorosulfonyl group is expected to be located at the secondary hydroxyl side.

Experimental Section

General Methods. ^{1}H NMR spectra were determined with a JEOL FX-90Q (90-MHz), a JEOL FX-100 (100-MHz), or a JEOL JNM GX-270 (270-MHz) spectrometer. ^{13}C NMR spectra were obtained with a JEOL FX-90Q (22.5-MHz) or a JEOL FX-100 (25-MHz) spectrometer. Fast atom bombardment mass (FABMS), field desorption mass (FDMS), and electron impact mass (EIMS) spectra were recorded with a JEOL JMS DX-300, DX-303 or D-300/JMA 3500 data system. Circular dichroism spectra were determined with a Jasco 20C spectrometer. Thin-layer chromatography (TLC) was run with precoated silica gel plates (Merck, Art 5554). Spot detection was carried out by UV light and/or staining with 0.1% 1,3-dihydroxynaphthalene in EtOH–H_2O–H_2SO_4 (200:157:43 v/v/v). A solvent of TLC development was *n*-C_3H_7OH–AcOEt–H_2O (7:7:5 v/v/v) where the R_f value of β-cyclodextrin was 0.09. Merck Lobar prepacked column (LiChroprep RP18 column, 25 × 310 nm) was used for reversed-phase column chromatography. High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC3A with a Zorbax ODS column (4.6 × 250 mm, 5μm, DuPont) or with a Chemcosorb 5-ODS-H column (4.6 × 100 mm, 5μm, Chemco).

Sulfonates of β-Cyclodextrin (1–3). Powdered β-naphthalenesulfonyl chloride (3.5 g) was added in one portion to 50 mL of 30% aqueous CH_3CN solution (pH 12.0, adjusted by addition of aqueous NaOH) of β-cyclodextrin (3.5 g), which was thermostated at 40 °C. The suspension was vigorously stirred, and the pH of the suspension was allowed to decrease rapidly. During this reaction, the reaction vessel was kept in the bath thermostated at 40 °C. After the mixture became neutral, it was filtered and applied on a reversed-phase column. After an elution with 10% aqueous CH_3CN (126 mL), a gradient elution from 10% aqueous CH_3CN (1.5 L) to 50% aqueous CH_3CN (1.5 L) was applied to give pure **1–3**, which were lyophilized: **1**, 736 mg (18.0%); **2**, 207 mg (4.4%); **3**, 210 mg (4.5%). Physical data for these compounds are as follows.

1: ^{1}H NMR (90 MHZ, Me_2SO-d_6) δ 3.0–4.0 (42 H), 4.3–4.6 and 5.5–6.1 (OH), 4.65–5.1 (C_1–H, 7 H), 7.65–8.3 (6 H), 8.65 (1 H); ^{13}C NMR (25 MHz, Me_2SO-d_6) (Figure 1); FABMS *m/z* 1325 (M + H^+); R_f value on TLC 0.38.

2: ^{1}H NMR (90 MHz, Me_2SO-d_6) δ 2.8–4.3 (42 H), 4.3–4.7 and 5.5–6.2 (OH), 4.68–5.1 (C_1–H, 7 H), 7.5–8.3 (12 H), 8.66 (2 H); ^{13}C NMR (25 MHz, Me_2SO-d_6) (Figure 1); FABMS *m/z* 1515 (M + H^+); R_f value on TLC 0.51.

3: ^{1}H NMR (90 MHz, Me_2SO-d_6) δ 2.6–4.2 (42 H), 4.2–4.7 and 5.3–6.06 (OH), 4.75–5.1 (C_1–H, 7 H), 7.36–8.38 (12 H), 8.53–8.7 (2 H); ^{13}C NMR (25 MHz, Me_2SO-d_6) (Figure 1); FABMS *m/z* 1515 (M + H^+); R_f value on TLC 0.52.

A similar reaction was carried out in water (pH 12.0) at 29 °C. After filtration, the filtrate was applied on a reversed-phase column. The products, the C-3 sulfonate **1** and the C-6 sulfonate, were isolated in 3.6% and 4.2% yield, respectively. The structural assignment of the latter sulfonate was made by comparing its HPLC and TLC retention times and ^{13}C NMR and ^{1}H NMR spectra with those of the authenic specimen which was easily prepared by the reaction of β-cyclodextrin with β-naphthalenesulfonyl chloride in pyridine.

Effect of Acetonitrile on the Yields of the Sulfonates. Powdered β-naphthalenesulfonyl chloride (350 mg) was added to a solution of β-cyclodextrin (350 mg) in 5 mL of water or aqueous CH_3CN (pH 12.0) and stirred until the pH of the mixture became neutral. The mixture was filtered and analyzed by reversed-phase HPLC. The yield of the product was obtained by HPLC with *p*-methoxyphenol as an internal standard. The result is shown in Figure 4.

Effect of the Reaction Temperature on the Yields of the Sulfonates. The reaction was carried out in a bath thermostated at given temperature similarly to that described in the experiment on the effect of acetonitrile. The result is shown in Figure 5.

Epoxides of β-Cyclodextrin (4–6). A solution of **1**, **2**, or **3** (300 mg) in 0.25% aqueous K_2CO_3 (30 mL) was stirred for 5 h at room temperature, neutralized by addition of dilute HCl, and chromatographed by a reversed-phase column with an elution of water to give pure **4**, **5**, or **6**, respectively. They were lyophilized: **4**, 242 mg (93%), **5**, 200 mg (92%), **6**, 155 mg (71%). Physical data for these compounds are as follows.

4: ^{1}H NMR (270 MHz, D_2O) 3.3–4.0 (42 H), 5.05 (C_1–H, 1 H, d, J = 3.30 Hz), 4.93–4.81 (C_1–H, 6 H); ^{13}C NMR (25 MHz, D_2O) (Figure 2); FABMS *m/z* 1139 (M + Na^+); R_f value on TLC 0.10.

5: ^{1}H NMR (270 MHz, D_2O) δ 3.3–4.1 (42 H), 4.85–5.0 (C_1–H, 5 H), 5.19 (C_1–H, 2 H, d, J = 3.18 Hz); ^{13}C NMR (25 MHz, D_2O) (Figure 2); FABMS *m/z* 1099 (M + H^+); R_f value on TLC 0.10.

6: ^{1}H NMR (270 MHz, D_2O) δ 3.3–4.1 (42 H), 4.85–5.0 (C_1–H, 5 H), 5.17 (C_1–H, 1 H, d, J = 3.42 Hz), 5.21 (C_1–H, 1 H, d, J = 3.17 Hz); ^{13}C NMR (25 MHz, D_2O) (Figure 2); FABMS *m/z* 1099 (M + H^+); R_f value on TLC 0.10.

Taka Amylolyses of Epoxides (5 and 6) of β-Cyclodextrin. A solution of the epoxide (**5** or **6**, 100 mg) and Taka amylase (Sigma X-A, 100 mg) in 0.2 M acetate buffer (10 mL) containing 0.01 M of $CaCl_2$ was kept at 40 °C for 1 h. After 1% aqueous NaOH was added to make the solution alkaline (pH 10) for denaturation of the enzyme, the supernatant obtained by centrifugation of the mixture was evaporated to almost dryness in vacuo. The residue was dissolved in a small amount of water, filtered, and chromatographed by a reversed-phase column with an elution of water to give pure **8** (90 mg, 87%) or **9** (72 mg, 83%). Physical data of these compounds are as follows.

8: ^{13}C NMR (22.5 MHz, D_2O, characteristic absorptions) δ 54.1, 57.9, 63.1, 94.5, 96.9, 98.4, 98.5, 102.2; FABMS *m/z* 1117 (M + H^+); R_f value on TLC 0.05.

9: ^{13}C NMR (22.5 MHz, D_2O, characteristic absorptions) δ 54.1, 58.0, 63.1, 63.2, 94.6, 97.0, 98.5, 98.6, 102.2; FABMS *m/z* 977 (M + Na^+), 993 (M + K^+); R_f value on TLC 0.06.

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(11) In our preliminary study, we isolated a trisubstituted β-cyclodextrin **16** other than **1–3**. Its ^{13}C NMR, ^{1}H NMR, and FABMS spectra showed the sulfonation on three C-3 hydroxyls and the spectra of the epoxide **17** derived from **16** also confirmed the positions of the sulfonation. From the resistance of **17** to the Taka amyloylsis, the regiochemistry of **16** was tentatively assigned to 3A,3C,3E. Although five regioisomers are likely formed in the trisulfonation of C-3 hydroxyls, one isomer **16** was mainly produced. Moreover, the additional monosulfonation of the 3A,3D-(**2**) and the 3A,3C-(**3**) isomers gave only one trisulfonate, **16**. These observations are also explicable by the inhibition of 3A,3B-disulfonation as mentioned in the text.

Reduction of Epoxides (8 and 9). A solution of **8** or **9** (50 mg) in 1% aqueous $NaBH_4$ (5 mL) was kept at room temperature for 1 day. After neutralization of the solution by addition of dilute HCl, the mixture was chromatographed by a reversed-phase column with an elution of water to give pure **11** (42 mg, 83%) or **12** (47 mg, 93%). Physical data of these compounds are as follows.

11: ^{13}C NMR (22.5 MHz, D_2O, characteristic absorptions) δ 54.1, 57.9, 63.2 65.0, 65.5, 96.9, 98.4, 98.6, 102.3, 102.9; FABMS m/z 1119 ($M + H^+$); R_f value on TLC 0.05.

12: ^{13}C NMR (22.5 MHz, D_2O, characteristic absorptions) δ 54.0, 57.9, 62.9, 63.2, 65.0, 65.5, 96.9, 98.4, 98.6, 102.9; FABMS m/z 957 ($M + H^+$); R_f value on TLC 0.05.

Acetylation of 11 and 12. A solution of **11** or **12** (5 mg) and acetic anhydride (0.25 mL) in pyridine (0.25 mL) was allowed to stand at room temperature for 4 h and concentrated by evaporation of volatile materials together with a stream of nitrogen. After dry chloroform (0.5 mL) was added to the residue, the evaporation was repeated. This procedure was carried out 2 more times. The crude products were purified by reversed-phase HPLC with a gradient elution from 50% aqueous CH_3CN to 80% aqueous CH_3CN to give pure **14** (4.2 mg, 37%) and **15** (5.3 mg, 49%). They were analyzed by FDMS and EIMS spectra. The EIMS spectral fragmentations observed are shown in Figure 3. FDMS m/z **14**: 1981 ($M + Na^+$); **15**: 1693 ($M + Na^+$).

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Asymmetric Synthesis. Metal Complex Mediated Synthesis of Chiral Glycine by Enantioselective Proton Exchange

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Abstract: The complex $[Co((S,S)\text{-proam})(picgly)]^+$, a species containing a chiral tridentate triamine ligand, (*S,S*)-proam, and a tridentate ligand incorporating a glycine residue, picgly, has been prepared. The α-protons of the coordinated glycine residue exchange at different rates in basic D_2O solutions. The difference in rate was found to be 7.8:1 in favor of the *pro-S* proton at pD 11.2 at 25 °C with a $NaHCO_3/Na_2CO_3$ buffer. It is proposed that the origins of this enantiosection arise from both steric and hydrogen-bonding effects as inferred from the determined crystal structure of the complex. A kinetic analysis of the exchange process shows that the system is essentially that of an asymmetric synthesis ($CH_2 \rightarrow CHD$) followed by a reinforced kinetic resolution ($CHD \rightarrow CD_2$). As such, the optical purity of the chiral glycine (NH_2CHDCO_2H) continuously increases with the extent of reaction. This was confirmed. It is suggested that the present kinetic relationships are representative of the majority of asymmetric syntheses involving enantiotopic atoms or groups, and it follows that, for such systems, quoting an enantiomeric excess has meaning only when the extent of reaction is specified.

The remarkable ability of enzymes to react selectively with the enantiotopic hydrogen atoms of a methylene group[1] has so far proved difficult to reproduce synthetically. Thus, in relation to the present work, we note that the *pro-S* hydrogen atom of glycine is preferentially exchanged by serine hydroxymethyltransferase in the presence of pyridoxyl phosphate and tetrahydrofolic acid.[2] In deuterated water chiral glycine (NH_2CHDCO_2H) is obtained in the absence of formaldehyde by this enzymatic reaction. Since the discovery that the methylene protons of an amino acid coordinated to a transition-metal complex were exchanged under basic aqueous conditions, there have been a number of attempts to reproduce the selectivities of the enzymatic reactions.[3] The more notable of these attempts were described by Golding and Sargeson,[4] who studied the relative exchange rates of the *pro-S* and *pro-R* glycine protons of the chiral complex bis(ethylenediamine)(*N*-benzylglycinato)cobalt(III), $[Co(en)_2(N\text{-bzgly})]^{2+}$. They observed a selectivity of about 4:1 in a Na_2PO_4/D_2O buffer at pD 10.5, and they attributed this selectivity to the preferred chirality of the nitrogen atom substituents of the coordinated *N*-bzgly ligand induced by the chiral disposition of the two en chelate rings. Curiously, subsequent work showed that the replacement of the benzyl group in $[Co(en)_2(N\text{-bzgly})]^{2+}$ by either 2-methylbenzyl or 1-methylenenaphthyl gave systems which showed no selectivity in the exchange of the methylene glycine protons.[5] A more recent report by Belokon[6] using the bis(3-methylsalicyaldimo)(glycinato)cobalt(III) complex described the preferential exchange of the methylene protons of the coordinated glycine group.

This paper describes a different approach to the selective exchange of the methylene protons of a coordinated glycine ligand. We also demonstrate how the kinetics of exchange can be employed to produce chiral glycine to any degree of chiral purity. The system chosen is $[Co((S,S)\text{-proam})(picgly)]^+$, a cobalt(III) complex consisting of two tridentate ligands, one of which contains the glycine residue.

Strategy and Synthesis. A coordinated glycinate molecule is devoid of puckering, and hence its enantiotopic methylene protons lie above and below the mean molecular plane. Exchange of these protons by hydroxide ions presumably involves a transition-state geometry consisting of a linear carbon–hydrogen–oxygen aggregate.

In order to distinguish the two methylene protons, a chiral auxiliary is required which protects one or other of the coordinated glycinate methylene protons from attack by hydroxide ions or which, in some way, directs hydroxide ion attack preferentially to one or other of these protons. The chiral auxiliary preferably should possess a C_2 axis in order to avoid isomeric ambiguity in an octahedral complex.

With these considerations in mind, we prepared the chiral auxiliary, (*S,S*)-proam, a tridentate ligand derived from (*S*)-

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