Preparation of Polytosylated γ-Cyclodextrins

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Practical preparation of polytosylated γ-cyclodextrins, octakis(6-*O*-tosyl)-2-*O*-tosyl-γ-cyclodextrin, octakis-(6-*O*-tosyl)-γ-cyclodextrin, and heptakis(6-*O*-tosyl)-γ-cyclodextrin, has been established. The procedure involves reaction of γ-cyclodextrin with tosyl chloride in pyridine and column chromatographic separation using silica gel modified with 3-aminopropyl groups.

Like a substrate binding site of an enzyme, cyclodextrin can specifically include the guest molecule.[1] Its inclusion ability depends on the cavity. γ-Cyclodextrin, which is composed of eight glucose units, shows such a characteristic inclusion phenomenon different from α- and β-cyclodextrins as it can include two molecular species in its large cavity.[2] This property has made it possible to construct unique artificial enzymes (or receptors) using γ-cyclodextrin as a guest binding site.[3]

To construct a more sophisticated artificial enzyme (or receptor), it is essential to introduce a catalytic site into γ-cyclodextrin, where the functional groups were arranged to suit the purpose. Polysulfonylated cyclodextrins are the important key intermediates. However, there have been no reports on polysulfonylated γ-cyclodextrins in contrast to many reports of α- and β-cyclodextrin derivatives.[4] It should be due to the presence of more hydroxyl groups in γ-cyclodextrin, which on sulfonylation gives more complicated reaction mixture containing over- and undersulfonylated derivatives. Accordingly, in order to establish the procedure to prepare such novel cyclodextrin derivatives, it is crucial to secure the isolation procedure to afford the desired compound. In this report, we describe the preparation of novel polysulfonylated γ-cyclodextrins, namely, octakis-(6-*O*-tosyl)-2-*O*-tosyl-γ-cyclodextrin (**1**), octakis(6-*O*-tosyl)-γ-cyclodextrin (**2**), and heptakis(6-*O*-tosyl)-γ-cyclodextrin (**3**). The purification of compounds **1**—**3** was accomplished by liquid chromatography using silica gel modified with 3-aminopropyl groups.

Results and Discussion

Preparation of Polytosylated γ-Cyclodextrins. Polytosylated γ-cyclodextrins were prepared by the reaction of lyophilized γ-cyclodextrin with tosyl chloride in pyridine with monitoring by thin-layer chromatography (TLC). After addition of H_2O, the reaction mixture was concentrated in vacuo. To remove hydrophilic impurities, the residue was suspended in 5% aqueous CH_3CN and the insoluble material was collected by centrifugation. The precipitate contained the octatosyl derivative **2**, in which all of the primary hydroxyl groups were tosylated, accompanied with nona- and heptatosylated derivatives **1** and **3**, possessing one more and one less tosyl groups, respectively. The crude product was dissolved in 90% aqueous CH_3CN and applied on column chromatography.

Column Chromatographic Purification of Polytosylated γ-Cyclodextrins. As described previously, polysulfonylation of cyclodextrin gives complicated reaction mixture. We reported that column chromatography using reversed-phase (octadecyl- and octyl-modified silica gel) column was the useful method for the purification of polysulfonylated cyclodextrins including heptakis(6-*O*-tosyl)-β-cyclodextrin (**4**).[4e,5] However, attempted purification of polytosylated γ-cyclodextrins **1**—**3** using reversed-phase column was unsuccessful probably due to high hydrophobicity of **1**—**3**. On the basis of the separation test using high-performance liquid chromatography (HPLC), we selected silica gel modified with 3-aminopropyl groups as the stationary phase for the separation of **1**—**3**.[6] It possesses intermediate polarity between those of octadecyl-modified silica gel and unmodified silica gel. While the 3-aminopropyl-modified silica gel column (NH_2 column) is generally used for separation of oligosaccharide and nucleoside, this is the first case of success in the preparative purification of this kind of chemically modified cyclodextrin using NH_2 column. Preliminary experiment showed that capacity of NH_2 column to treat **1**—**3** is more than 10 times that of reversed-phase (octadecyl-modified silica gel) column. The purification procedure was as follows.

The solution of crude product was analyzed with HPLC using NH_2 column (Fig. 1) and applied on a preparative NH_2 column for low pressure liquid chromatography. The aqueous CH_3CN gradient elution gave impure **1** containing minor by-products, and pure **2** and **3** (Fig. 2). Rechromatography with reversed-phase column gave pure **1**. The purities of **1**—**3** were

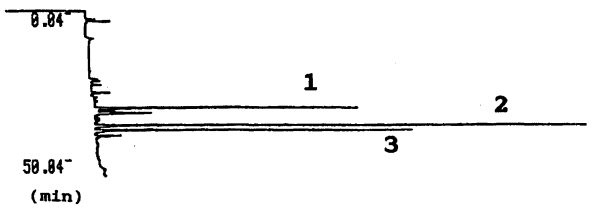

Fig. 1. HPLC of polytosylated γ-cyclodextrins **1**—**3** using NH_2 column. Eluent A: 100% CH_3CN, B: 70% aq CH_3CN, A→B linear gradient; flow rate 1.0 cm^3 min^{-1}; detection UV (220 nm).

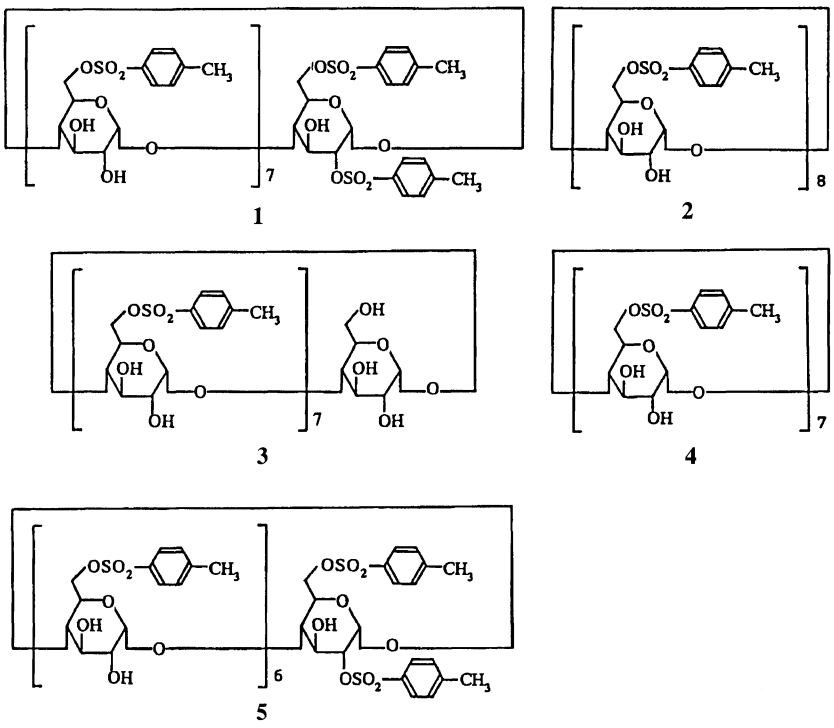

Chart 1.

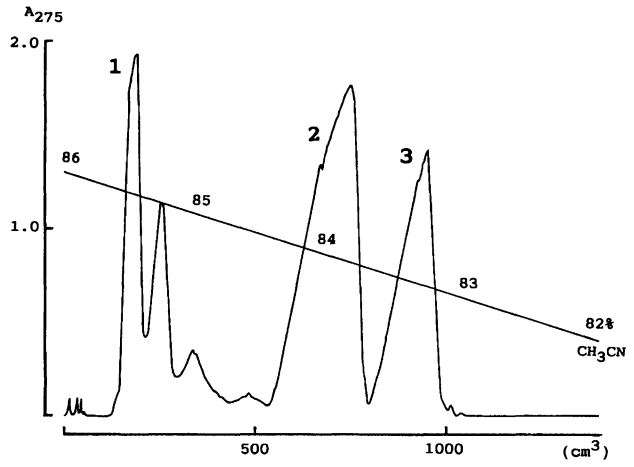

Fig. 2. NH_2 column chromatography of polytosylated γ-cyclodextrins **1**—**3**.

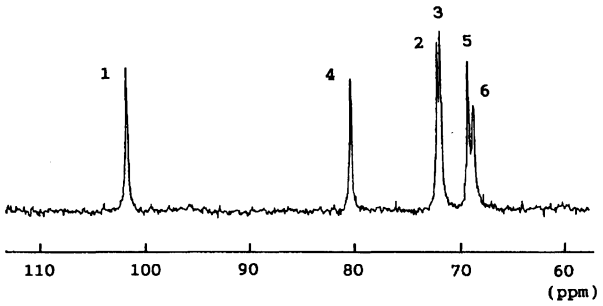

Fig. 3. The ^{13}C NMR spectrum of the cyclodextrin part of octakis(6-*O*-tosyl)-γ-cyclodextrin (**2**) in DMSO-d_6.

confirmed by HPLC analysis, since the purity of polysulfonylated cyclodextrin can be determined only with HPLC as demonstrated by Ashton et al.[4f]

Structure Determination of Polytosylated γ-Cyclodextrins. The structures of **1**—**3** were confirmed by elemental analyses and nuclear magnetic resonance (NMR) spectra (Chart 1). The ^1H and ^{13}C NMR spectra of **2** were quite simple, indicating that **2** was composed of eight identical units (Fig. 3). Their patterns were very similar to those of heptakis (6-*O*-tosyl)-β-cyclodextrin (**4**).[4e] The large downfield shift of C-6 and the small upfield shift of C-5 in its ^{13}C NMR spectrum, as compared with those of γ-cyclodextrin, indicated the sulfonylation on the 6-OH groups. Their signals were assigned by ^1H–^1H and ^1H–^{13}C correlated spectroscopy. The result of elemental analysis also supported the octakis(6-*O*-tosyl)-γ-cyclodextrin structure of **2**.

The compounds **1** and **3** were shown to be over- and undertosylated derivatives possessing nine and seven tosyl groups, respectively, by their elemental analyses and ^1H NMR spectra. The ^{13}C NMR spectrum of **1** (Fig. 4) showed the signals similar to those of **2** assignable to 6-*O*-tosyl-D-glucose moieties. In addition, there were the signals denoted as 1′, 2′, and 4′ which were assigned to those of 2,6-di-*O*-tosyl-D-glucose moiety by their chemical shifts.[7] This spectrum was similar to that of heptakis(6-*O*-tosyl)-2-*O*-tosyl-β-cyclodextrin (**5**),[4e] which also confirmed the position of overtosylation as 2-OH group. Thus, the structure of **1** was determined as octakis(6-*O*-tosyl)-2-*O*-tosyl-γ-cyclodextrin. The ^{13}C NMR spectrum of **3** (Fig. 5) also

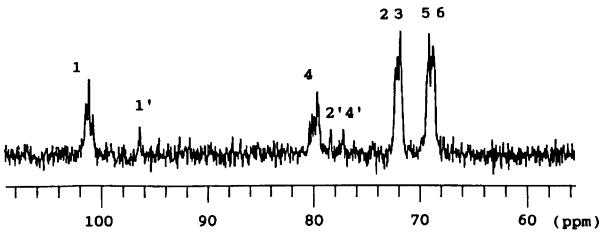

Fig. 4. The ^{13}C NMR spectrum of the cyclodextrin part of octakis(6-*O*-tosyl)-2-*O*-tosyl-γ-cyclodextrin (**1**) in DMSO-d_6.

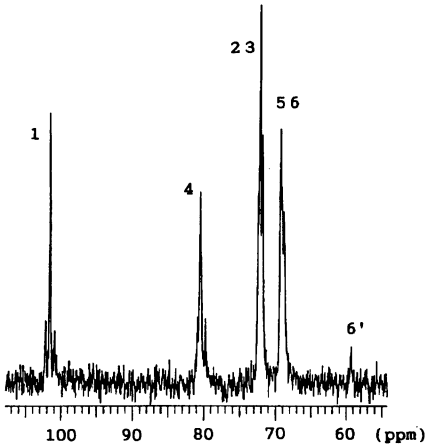

Fig. 5. The ^{13}C NMR spectrum of the cyclodextrin part of heptakis(6-*O*-tosyl)-γ-cyclodextrin (**3**) in DMSO-d_6.

showed the signals of 6-*O*-tosyl-D-glucose moieties. An additional signal at δ=59.3 due to C-6 in the unmodified glucose moiety was observed (6′ in Fig. 5), establishing the structure of **3** as heptakis(6-*O*-tosyl)-γ-cyclodextrin.

We have established here the convenient preparation of polytosylated γ-cyclodextrins, octakis(6-*O*-tosyl)-2-*O*-tosyl-γ-cyclodextrin (**1**), octakis(6-*O*-tosyl)-γ-cyclodextrin (**2**), and heptakis(6-*O*-tosyl)-γ-cyclodextrin (**3**). These compounds are considered as important intermediates for the synthesis of novel functional molecules. The studies on the construction of artificial enzyme (or receptor) using these compounds are in progress. The described separation procedure using 3-aminopropyl-modified silica gel provides an alternative method for the purification of the chemically modified cyclodextrins whose purification is difficult by the use of silica gel or reversed-phase column chromatography.

Experimental

General. ^{1}H and ^{13}C NMR spectra were determined with Varian XL-GEM200 and JEOL JNM-GSX400. TLC was run on a precoated silica-gel plate (Art 5554, Merck) with the solvent system *n*-PrOH–AcOEt–H_2O [7:7:2 (v/v/v)]. Spot detection was carried out by exposure to UV light and/or staining with 0.1% 1,3-naphthalenediol in EtOH–H_2O-H_2SO_4 [200:157:43 (v/v/v)]. Prepacked columns (Lobar column LiChroprep NH_2, 25×310 mm, and LiChroprep RP-18, 25×310 mm, Merck) were used for low-pressure liquid chromatography. Analytical HPLC was performed on a JASCO 880-PU with a LiChrospher NH_2 column (4.0×250 mm, 5 μm, Merck).

Preparation of Polytosylated γ-Cyclodextrins. Lyophilized γ-cyclodextrin (0.50 g, 3.9×10^{-4} mol) was added to a solution of tosyl chloride (1.5 g, 7.9×10^{-3} mol) in dry pyridine (10 cm^3) and stirred at 10°C for 2 h. After the addition of H_2O (5.0 cm^3), the reaction mixture was concentrated in vacuo and the residue was poured into 5% CH_3CN (200 cm^3) and neutralized by $NaHCO_3$. The precipitate was collected by centrifugation (3000 rpm, 5 min), followed by washing twice with 5% aqueous CH_3CN (200 cm^3). The crude product was dissolved in 90% aqueous CH_3CN (300 cm^3) and applied on a preparative NH_2 column. After eluting with 90% aqueous CH_3CN (1000 cm^3), a gradient elution from 86% aqueous CH_3CN (700 cm^3) to 82% aqueous CH_3CN (700 cm^3) was applied to give octakis(6-*O*-tosyl)-2-*O*-tosyl-γ-cyclodextrin (**1**) (80.5 mg) containing by-products, pure octakis(6-*O*-tosyl)-γ-cyclodextrin (**2**) (183 mg, 18.8 %), and pure heptakis(6-*O*-tosyl)-γ-cyclodextrin (**3**) (134 mg, 14.6%), which were lyophilized after evaporation of CH_3CN. The fraction containing **1** was dissolved in 80% aqueous CH_3CN (100 cm^3) and rechromatographed by a reversed-phase (RP-18) column with 80% CH_3CN (500 cm^3) and a gradient elution from 85% aqueous CH_3CN (200 cm^3) to 89% aqueous CH_3CN (200 cm^3), to give pure compound **1** (26.5 mg, 2.6%).

1: ^{1}H NMR (200 MHz, DMSO-d_6–D_2O) δ=2.36 (27H, s, CH_3), 3.06—3.33, 3.35—3.97, 4.06—4.40, 4.55—4.70, 4.77, and 4.95 (H-1, 2, 3, 5, 6 of 6-*O*-tosyl-D-glucose moieties and 2,6-di-*O*-tosyl-D-glucose moiety), 7.28—7.51 and 7.56—7.89 (36H, aromatic protons); ^{13}C NMR (80 MHz, DMSO-d_6) δ=21.1 (CH_3), 68.6, 68.7, 68.9, and 69.1 (C-5 and C-6), 71.8, 72.1, and 72.3 (C-2 and C-3), 77.2 and 78.4(C-2′ and C-4′ of 2,6-di-*O*-tosyl-D-glucose), 79.4, 79.5, 79.9, 80.1, 80.4 (C-4), 96.4 (C-1′), 100.7, 100.8, 101.1, and 101.4 (C-1), 125.5, 127.5, 127.8, 127.9, 129.9, 132.3, 132.5, 133.0, and 144.8 (aromatic carbons). Anal. Found: C, 48.26; H, 4.89; S, 10.50%. Calcd for $C_{111}H_{134}O_{58}S_9\cdot4H_2O$: C, 48.36; H, 5.19; S, 10.47%.

2: ^{1}H NMR (400 MHz, DMSO-d_6–D_2O) δ=2.38 (24H, s, CH_3), 3.17—3.26 (16H, m, H-2 and H-4), 3.51 (8H, t, $J_{3,2}$=$J_{3,4}$=9.5 Hz, H-3), 3.70 (8H, m, $J_{5,4}$=9 Hz, $J_{5,6}$<1 Hz, $J_{5,6'}$< 1 Hz, H-5), 4.15 (8H, br d, $J_{6,5}$<1 Hz, $J_{6,6'}$=10 Hz, H-6), 4.30 (8H, br d, $J_{6',5}$<1 Hz, $J_{6',6}$=10 Hz, H-6′), 4.65 (8H, d, $J_{1,2}$=3.5 Hz, H-1), 7.38 (16H, d, J=8 Hz, aromatic protons), 7.71 (16H, d, J=8 Hz, aromatic protons); ^{13}C NMR (100 MHz, DMSO-d_6) δ=21.0 (CH_3), 68.8 (C-6), 69.3 (C-5), 71.9 (C-2), 72.2 (C-3), 80.4 (C-4), 101.6 (C-1), 127.4, 129.7, 132.5, and 144.6 (aromatic carbons). Anal. Found: C, 47.22; H, 5.10; S, 9.61%. Calcd for $(C_{13}H_{16}O_7S)_8\cdot6H_2O$: C, 47.34; H, 5.35; S, 9.72%.

3: ^{1}H NMR (200 MHz, DMSO-d_6–D_2O) δ=2.38 (21H, s, CH_3), 3.15—3.40, 3.45—3.62, and 3.65—3.82 (H-2, 3, 4, 5 of 6-*O*-tosyl-D-glucose moieties and H-2, 3, 4, 5, 6 of unsubstituted glucose moiety), 4.11—4.24 and 4.26—4.40 (14H, H-6), 4.66—4.75 and 4.81—4.90 (8H, H-1), 7.42 (14H, br d, aromatic protons), 7.75 (14H, m, aromatic protons); ^{13}C NMR (80 MHz, DMSO-d_6) δ=21.2 (CH_3), 59.3 (C-6′ of unsubsitiuted glucose moiety), 68.5, 68.9, 69.2, and 69.4 (C-5, 6), 71.4, 71.7, 72.0, 72.3, and 72.5 (C-2, 3), 79.8, 80.1, 80.6, and 81.0 (C-4), 101.0, 101.1, 101.5, 101.7, 101.9, 102.2, and

102.3 (C-1), 127.6, 130.0, 132.6, 132.7, and 144.9 (aromatic carbons). Anal. Found: C, 47.42; H, 4.93; S, 9.12%. Calcd for $C_{97}H_{122}O_{54}S_7 \cdot 4H_2O$: C, 47.58; H, 5.35; S, 9.17%.

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References

1) M. L. Bender and M. Komiyama, "Cyclodextrin Chemistry," Springer-Verlag, Berlin (1978); I. Tabushi, *Acc. Chem. Res.*, **15**, 66 (1982); J. Stzejtli, "Cyclodextrin Technology," Kluwer Academic Publishers, Dordrecht (1988).

2) A. Ueno, Y. Tomita, and T. Osa, *J. Chem. Soc., Chem. Commun.*, **1983**, 976; T. Tamaki, *Chem. Lett.*, **1984**, 53; T. Tamaki, T. Kokubu, and K. Ichimura, *Tetrahedron*, **43**, 1485 (1987).

3) A. Ueno, F. Moriwaki, T. Osa, F. Hamada, and K. Murai, *J. Am. Chem. Soc.*, **110**, 4323 (1988); W. Tagaki, K. Yano, K. Yamanaka, H. Yamamoto, and T. Miyasaka, *Tetrahedron Lett.*, **31**, 3897 (1990); A. Ueno, *J. Am. Chem. Soc.*, **113**, 7034 (1991).

4) a) V. W. Lautsch, R. Wiechert, and H. Lehmann, *Kolloid-Z.*, **135**, 134 (1954); b) S. Umezawa and K. Tatsuta, *Bull. Chem. Soc. Jpn.*, **41**, 464 (1968); c) F. Cramer, G. Mackensen, and K. Sensse, *Chem. Ber.*, **102**, 494 (1969); d) R. Breslow, M. F. Czarniecki, J. Emert, and H. Hamaguchi, *J. Am. Chem. Soc.*, **102**, 762 (1980); e) H. Yamamura and K. Fujita, *Chem. Pharm. Bull.*, **39**, 2505 (1991); f) P. R. Ashton, P. Ellwood, I. Staton, and J. F. Stoddart, *J. Org. Chem.*, **56**, 7274 (1991).

5) K. Fujita, H. Yamamura, A. Matsunaga, T. Imoto, K. Mihashi, and T. Fujioka, *J. Am. Chem. Soc.*, **108**, 4509 (1986); K. Fujita, H. Yamamura, T. Imoto, T. Fujioka, and K. Mihashi, *J. Org. Chem.*, **53**, 1943 (1988); K. Fujita, H. Yamamura, and T. Imoto, *Tetrahedron Lett.*, **32**, 6737 (1991).

6) For isolation of polytosylated γ-cyclodextrins, we tested reversed-phase (RP-8 and RP-18) columns, a 3-cyanopropyl-modified silica-gel (CN) column, a 1,2-dihydroxy-3-propoxypropyl-modified silica gel (DIOL) column, and an unmodified silica-gel column besides NH_2 column.

7) Ueno and Breslow demonstrated that tosylation of 2-OH of cyclodextrin caused the downfield shift of C-2 (α carbon), the upfield shifts of C-1 and C-3 (β carbons), and the smaller upfield shift of C-4 (γ carbon): A. Ueno and R. Breslow, *Tetrahedron Lett.*, **23**, 3451 (1982).