The synthesis of a fluorescent chemo-sensor system based on regioselectively dansyl-tosyl-modified β- and γ-cyclodextrins

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Flexible bis-functionalized hosts, 6^A-dansyl-6^X-tosyl-modified β-cyclodextrins (X = B or G, C or F, and D or E for β-1, β-2, and β-3, respectively) and γ-cyclodextrins (X = B or H, C or G, D or F, and E for γ-1, γ-2, γ-3, and γ-4, respectively) have been synthesized to investigate their chemo-sensor potential for organic compounds such as bile acids and terpenoids. These host compounds show pure monomer fluorescence, the β-analogs showing a decrease in fluorescence intensity on accommodation of all the guests examined. On the other hand, γ-analogs exhibit a decrease in intensity on complexation of bile acids and smaller guests such as bicyclic molecules, but an increase in intensity for much smaller guests such as monocyclic and non-cyclic molecules. The extent of fluorescence variation with a guest is employed to display the sensing ability of the hosts. The sensing parameter ($\Delta I/I_0$) was used to describe the sensing ability of the hosts. Host β-analogs can detect chenodeoxycholic acid, ursodeoxycholic acid, and (−)-borneol with high sensitivity. The sequence of binding ability of these hosts is β-1 > β-2 > β-3 for bile acids, and β-2 > β-1 ≥ β-3 for terpenoids. On the other hand, γ-analogs can detect lithocholic acid, chenodeoxycholic acid, ursodeoxycholic acid, and (−)-borneol with high sensitivity. The sensing parameters of β-analogs are up to almost two times larger for ursodeoxycholic acid and three times for (−)-borneol in comparison with those of γ-analogs. The behavior of the appended moieties of the hosts during host–guest complexation is studied by induced circular dichroism (ICD), fluorescence, and ^{1}H NMR spectra. Host β- and γ-analogs show similar ICD spectral patterns. Host γ-analogs exhibit ^{1}H NMR spectral changes after addition of ursodeoxycholic acid, whereas β-analogs indicate no change. The guest-induced variations in ICD, fluorescence, and ^{1}H NMR spectra suggest that the dansyl and tosyl groups change their mutual relationship.

1. Introduction

A fluorescent sensing system of modified cyclodextrins for organic guests is a current topic in host–guest chemistry.[1] Cyclodextrins, which are torus-shaped cyclic oligomers of D-glucopyranose and are named α, β, and γ- for the hexamer, heptamer, and octamer, respectively, can include a variety of organic compounds in their cavity in an aqueous solution.[2] For at least a decade, we have studied fluorescent molecular sensing by cyclodextrins modified with chromophores, such as naphthalene,[3] anthracene,[4] fluorescein,[5] terphenyl,[6] pyrrolinone,[7] and anthranilate.[8–12] In these reports we discussed the binding abilities of these derivatives with terpenoids and bile acids as guest molecules, because they are biological substances produced by plants or animals and are utilized as crude drugs. In previous papers, we have described regioselective syntheses of bis-dansylglycine appended β- and γ-cyclodextrins to investigate their fluorescent chemo-sensor ability;[13,14] these hosts indicated a much higher sensitivity and selectivity for guests such as bile acids than those of mono-dansyl-modified β- and γ-cyclodextrins.[15,16] As a further extension of our work, we synthesized regioselectively modified dansyl-tosyl-β- and γ-cyclodextrins, which are 6^A-dansyl-6^X-tosyl-modified β-cyclodextrins (X = B or G, C or F, and D or E) and 6^A-dansyl-6^X-tosyl-modified γ-cyclodextrins (X = B or H, C or G, D or F, and E), because different sizes of appended moieties such as tosyl and dansyl groups can result in partly or totally unlike movements upon addition of a guest, which should in turn give a much better fluorescent molecular sensing system. A couple of groups have reported preparations and binding properties of bis functionalized cyclodextrins.[17–22] Unfortunately, they have not investigated the fluorescent sensing properties for guests, because the appended functional groups were fluorescence inert. Our new compounds exhibit higher molecular recognition ability for bile acids such as chenodeoxycholic acid and ursodeoxycholic acid, and terpenoids such as (−)-borneol, than those of bis-dansyl modified β- and γ-analogs.

2. Experimental

2.1 Preparations of β-1, β-2, and β-3

A mixture of 6^A,6^B-di(*p*-tosyl)-β-cyclodextrin (250 mg, 0.17 mM)[12] and sodium dansylglycinate (76 mg, 0.23 mM) in 5 mL of DMF was heated at 80 °C for 24 h. After cooling, the reaction mixture was poured into 500 mL of acetone. The resulting precipitate was filtered off and dissolved in 5 mL of DMF. The DMF soluble fraction was applied to a reversed-phase column (Lobar column LiChroprep RP18). Stepwise elution using 500 mL of 30 vol.%, 300 mL of 40 vol.%, 400 mL of 50 vol.%, and 450 mL of 55 vol.% aqueous MeOH, and 500 mL of 60 vol.% aqueous MeOH was applied to obtain β-1. Compounds β-2 and β-3 were prepared by the same procedure as for β-1.

β-1. Yield 14.5%. R_f 0.58 (butanol–ethanol–water 5:4:3 by volume, TLC; silica gel 60F254) and 0.59 (methanol–water 2:1 by volume, TLC; RP-18F254S; Merck Ltd.). ^{1}H NMR(DMSO-d_6): δ 2.83 (6H, s, N–CH_3), 3.1–3.8 (48H, m, CH_2 and C^2–C^6H of cyclodextrin), 4.0–4.6 (5H, m, O^6H of cyclodextrin), 4.7–4.9 (7H, m, C^1H of cyclodextrin), 5.5–5.9 (14H, m, O^2H and OH of cyclodextrin), 7.27 (1H, dd, J = 7.8, 7.8 Hz, aromatic H of dansyl), 7.33–7.49 (2H, m, aromatic H of tosyl), 7.59 (2H, q, J = 7.3 Hz, aromatic H of dansyl), 7.67–7.78 (2H, m, aromatic H of tosyl), 8.11 (1H, t, J = 8.3 Hz, aromatic H of dansyl), 8.28

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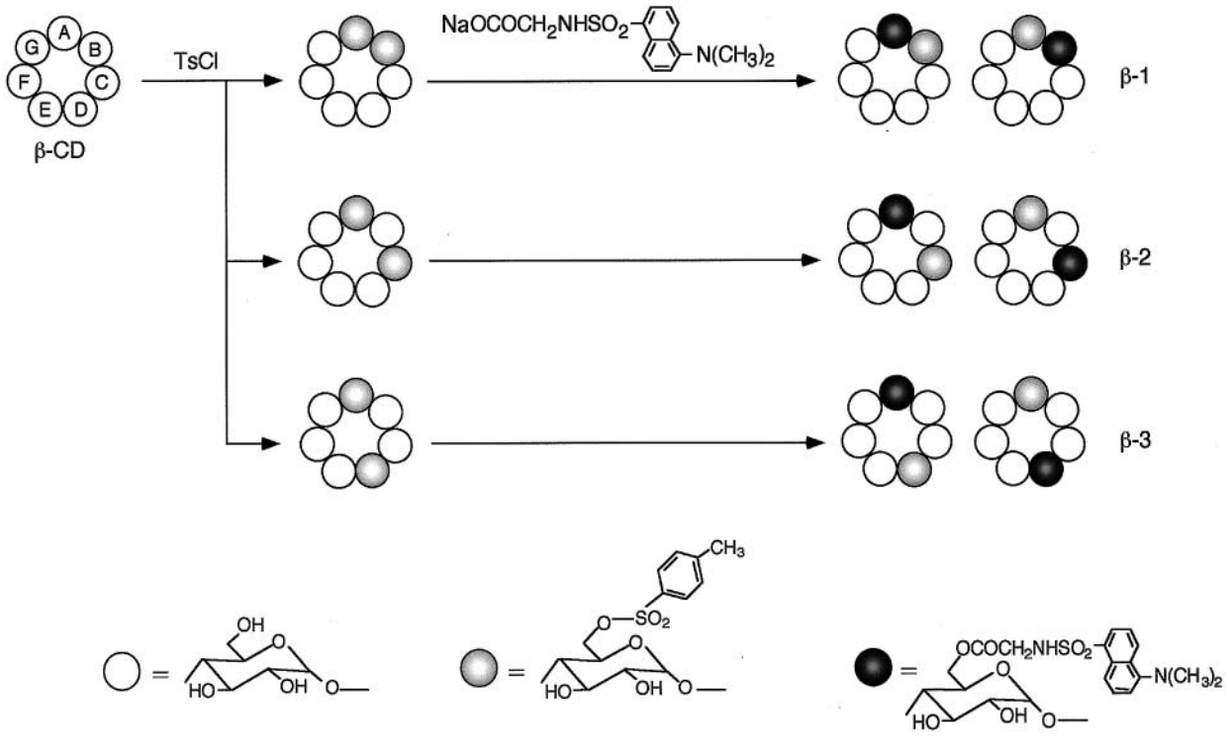


Scheme 1 Preparation of β-1, β-2, and β-3.

(1H, t, $J = 7.7$ Hz, aromatic H of dansyl), 8.43–8.47 (1H, m, aromatic H of dansyl). Calc. for $C_{63}H_{90}O_{40}N_2S_2 \cdot 6H_2O$: C, 44.83; H, 6.09; N, 1.66%. Found: C, 44.92; H, 5.98; N, 1.62%. MS(FAB): 1578 ($[M]^+$).

β-2. Yield 11.8%. R_f 0.58 (butanol–ethanol–water 5:4:3 by volume, TLC; silica gel 60F254) and 0.61 (methanol–water 2:1 by volume, TLC; RP-18F254S; Merck Ltd.). ^{1}H NMR(DMSO-d_6): δ 2.83 (6H, s, N–CH_3), 3.2–3.8 (48H, m, CH_2 and C^2–C^6H of cyclodextrin), 4.1–4.6 (5H, m, O^6H of cyclodextrin), 4.7–4.9 (7H, m, C^1H of cyclodextrin), 5.6–5.9 (14H, m, O^2H and O^3H of cyclodextrin), 7.26 (1H, d, $J = 7.8$, aromatic H of dansyl), 7.34–7.42 (2H, m, aromatic H of tosyl), 7.59 (2H, t, $J = 8.0$, aromatic H of dansyl), 7.69–7.76 (2H, m, aromatic H of tosyl), 8.10 (1H, t, $J = 7.2$, aromatic H of dansyl), 8.27 (1H, d, $J = 8.4$, aromatic H of dansyl), 8.46 (1H, d, $J = 8.1$ Hz, aromatic H of dansyl). Calc. for $C_{63}H_{90}O_{40}N_2S_2 \cdot 2H_2O$: C, 46.83; H, 5.86; N, 1.73. Found: C, 46.99; H, 6.09; N, 1.69%. MS(FAB): 1579 ($[M + H]^+$).

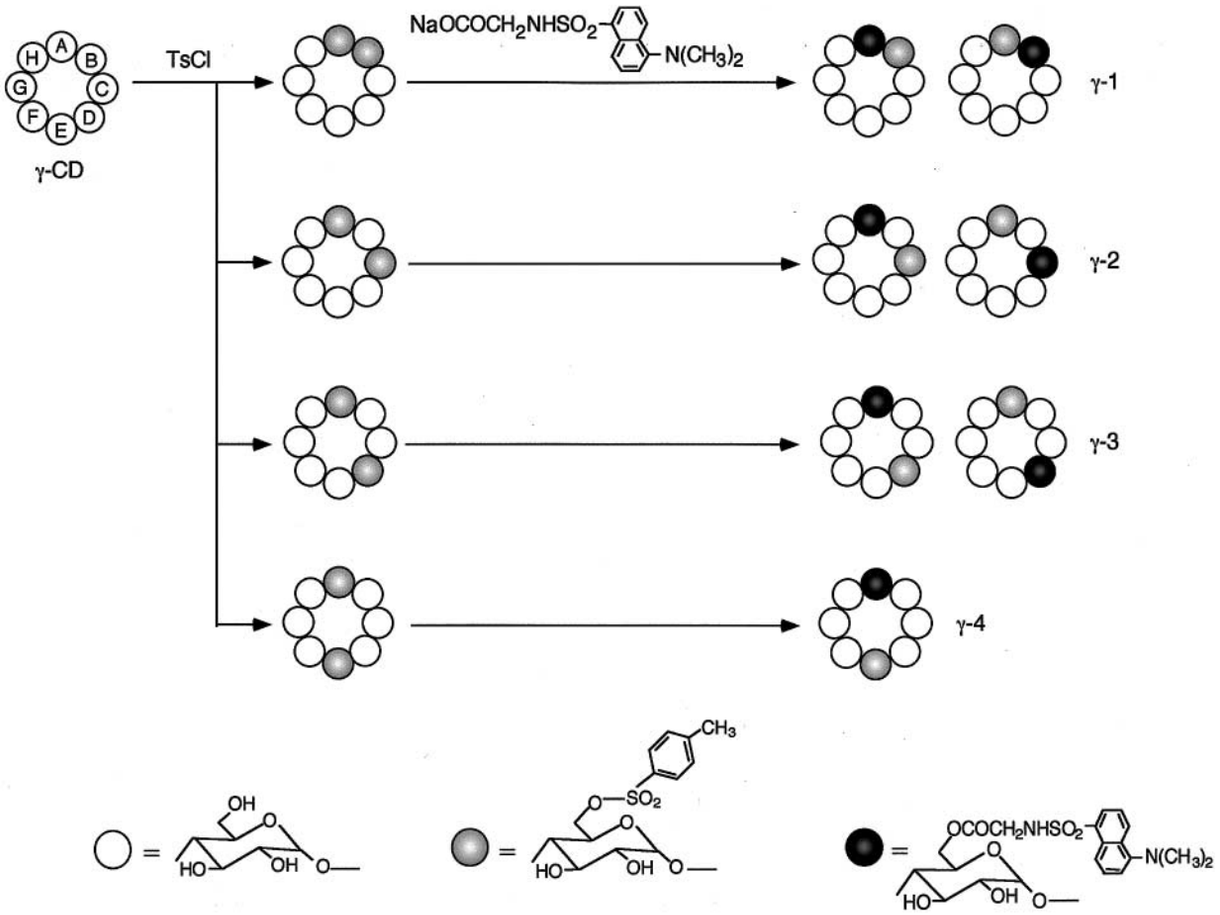
β-3. Yield 10.0%. R_f 0.56 (butanol–ethanol–water 5:4:3 by volume, TLC; silica gel 60F254) and 0.70 (methanol–water 2:1 by volume, TLC; RP-18F254S; Merck Ltd.). ^{1}H NMR(DMSO-d_6): δ 2.83 (6H, s, N–CH_3), 3.2.–3.8 (48H, m, CH_2 and C^2–C^6H of cyclodextrin), 4.0–4.6 (5H, m, O^6H of cyclodextrin), 4.75–4.95 (7H, m, C^1H of cyclodextrin), 5.6–5.9 (14H, m, O^2H and O^3H of cyclodextrin), 7.25 (1H, d, $J = 7.8$, aromatic H of dansyl), 7.41 (2H, d, $J = 6.3$, aromatic H of tosyl), 7.59 (2H, t, $J = 8.1$, aromatic H of dansyl), 7.73 (2H, dd, $J = 2.7$, 3.3, aromatic H of tosyl), 8.09 (1H, dd, $J = 3.9$, 3.9, aromatic H of dansyl), 8.26 (1H, d, $J = 9.0$, aromatic H of dansyl), 8.47 (1H, t, $J = 7.4$ Hz, aromatic H of dansyl). Calc. for $C_{63}H_{90}O_{40}N_2S_2 \cdot 4H_2O$: C, 45.82; H, 5.98; N, 1.70. Found: C, 45.92; H, 6.10; N, 1.76%. MS(FAB): 1579 ($[M + H]^+$).

2.2 Preparations of γ-1, γ-2, γ-3, and γ-4

A mixture of 6^A,6^B-di(*p*-tosyl)-γ-cyclodextrin (800 mg, 0.50 mM)[12] and sodium dansylglycinate (363 mg, 1.10 mM) in 20 mL of DMF was heated at 80 °C for 24 h. After cooling, the reaction mixture was poured into 500 mL of acetone. The resulting precipitate was filtered off and dissolved in 5 mL of DMF. The DMF soluble fraction was applied to a reversed-phase column (Lobar column Lichroprep RP18). Stepwise elution using 500 mL of 10 vol.%, 300 mL of 20 vol.%, 300 mL of 30 vol.%, 300 mL of 40 vol.%, 300 mL of 50 vol.%, and 300 mL of 55 vol.% aqueous MeOH, and 500 mL of 60 vol.% aqueous MeOH was applied to obtain γ-1. Compounds γ-2 and γ-3 were prepared by the same procedure as for γ-1 and γ-4 was prepared by the same procedure as for γ-1 but using 0.65 M of sodium dansylglycinate.

γ-1. Yield 5.8%. R_f 0.55 (butanol–ethanol–water 5:4:3 by volume, TLC; silica gel 60F254) and 0.62 (methanol–water 2:1 by volume, TLC; RP-18F254S; Merck Ltd.). ^{1}H NMR(DMSO-d_6): δ 2.87 (6H, s, N–CH_3), 3.2–3.8 (52H, m, CH_2 and C^2–C^6H of cyclodextrin), 3.9–4.6 (6H, m, O^6H of cyclodextrin), 4.8–4.9 (8H, m, C^1H of cyclodextrin), 5.7–5.9 (16H, m, O^2H and O^3H of cyclodextrin), 7.26 (2H, d, $J = 7.8$, aromatic H of dansyl), 7.37 (2H, dd, $J = 8.1$, 9.0, aromatic H of tosyl), 7.58 (2H, m, aromatic H of dansyl), 7.69 (2H, d, $J = 8.3$, aromatic H of tosyl), 8.10 (1H, m, aromatic H of dansyl), 8.28 (1H, d, $J = 8.7$ Hz, aromatic H of dansyl), 8.45 (1H, m, aromatic H of dansyl). Calc. for $C_{69}H_{100}O_{45}N_2S_2 \cdot 3H_2O$: C, 46.15; H, 5.95; N, 1.56. Found: C, 46.19; H, 6.03; N, 1.62%. MS(FAB): 1741 ($[M + H]^+$).

γ-2. Yield 11.8%. R_f 0.56 (butanol–ethanol–water 5:4:3 by volume, TLC; silica gel 60F254) and 0.64 (methanol–water 2:1 by volume, TLC; RP-18F254S; Merck Ltd.). ^{1}H NMR(DMSO-d_6): δ 2.84 (6H, s, N–CH_3), 3.0–3.8 (52H, m, CH_2 and C^2–C^6H of cyclodextrin), 3.9–4.6 (6H, m, O^6H of cyclodextrin), 4.8–4.95 (8H, m, C^1H of cyclodextrin), 5.7–6.0 (16H, m, O^2H and O^3H of cyclodextrin), 7.26 (1H, d, $J = 6.9$, aromatic H of dansyl), 7.44 (2H, d, $J = 7.8$, aromatic H of tosyl), 7.58 (2H, m, aromatic H of dansyl), 7.76 (2H, d, $J = 8.3$, aromatic H of tosyl), 8.08 (1H, d, $J = 7.5$, aromatic H of dansyl), 8.27 (1H, d, $J = 8.4$, aromatic H of dansyl), 8.46 (1H, d, $J = 9.3$ Hz, aromatic H of dansyl). Calc. for $C_{69}H_{100}O_{45}N_2S_2 \cdot 8H_2O$: C, 43.95; H, 6.20; N, 1.49. Found: C, 44.05; H, 6.13; N, 1.45%. MS(FAB): 1741 ($[M + H]^+$).



Scheme 2 Preparation of γ-1, γ-2, γ-3, and γ-4.

γ-3. Yield 10.6%. R_f 0.57 (butanol–ethanol–water 5:4:3 by volume, TLC; silica gel 60F254) and 0.64 (methanol–water 2:1 by volume, TLC; RP-18F254S; Merck Ltd.). ^{1}H NMR(DMSO-d_6): δ 2.83 (6H, s, N–CH_3), 3.2–3.8 (52H, m, CH_2 and C^2–C^6H of cyclodextrin), 4.0–4.7 (6H, m, O^6H of cyclodextrin), 4.8–5.0 (8H, m, C^1H of cyclodextrin), 5.7–6.0 (16H, O^2H and O^3H of cyclodextrin), 7.25 (1H, d, J = 7.8, aromatic H of dansyl), 7.42 (2H, t, J = 8.7, aromatic H of tosyl), 7.58 (2H, m, aromatic H of dansyl), 7.75 (2H, d, J = 8.1, aromatic H of tosyl), 8.09 (1H, d, J = 6.9, aromatic H of dansyl), 8.27 (1H, d, J = 6.6 Hz, aromatic H of dansyl), 8.42–8.49 (1H, m, aromatic H of dansyl). Calc. for $C_{69}H_{100}O_{45}N_2S_2 \cdot 7H_2O$: C, 44.37; H, 6.15; N, 1.50. Found: C, 44.21; H, 5.81; N, 1.55%. MS(FAB): 1741 ($[M + H]^+$).

γ-4. Yield 10.8%. R_f 0.56 (butanol–ethanol–water 5:4:3 by volume, TLC; silica gel 60F254) and 0.81 (methanol–water 2:1 by volume, TLC; RP-18F254S; Merck Ltd.). ^{1}H NMR(DMSO-d_6): δ 2.84 (6H, s, N–CH_3), 3.2–4.0 (52H, m, CH_2 and C^2–C^6H of cyclodextrin), 3.9–4.6 (6H, m, O^6H of cyclodextrin), 4.7–5.0 (8H, m, C^1H of cyclodextrin), 5.6–6.0 (16H, O^2H and O^3H of cyclodextrin), 7.26 (1H, d, J = 7.5, aromatic H of dansyl), 7.43 (2H, d, J = 8.4, aromatic H of tosyl), 7.58 (2H, m, aromatic H of dansyl), 7.74 (2H, d, J = 8.1, aromatic H of tosyl), 8.08 (1H, d, J = 7.2, aromatic H of dansyl), 8.26 (1H, d, J = 8.4, aromatic H of dansyl), 8.46 (1H, d, J = 9.3 Hz, aromatic H of dansyl). Calc. for $C_{69}H_{100}O_{45}N_2S_2 \cdot 6H_2O$: C, 44.80; H, 6.10; N, 1.51. Found: C, 45.00; H, 6.10; N, 1.19%. MS(FAB): 1741 ($[M + H]^+$).

2.3 Measurements

Fluorescence, circular dichroism, and 2-D ^{1}H NMR spectra were measured at 25 °C, with a Perkin-Elmer LS 40B fluorescence spectrometer, a JASCO J-700 spectropolarimeter, and JEOL JNM-LA400 FT NMR system, respectively. For the fluorescence measurements, the excitation wavelength was 340 nm and emission slits were 10 nm. A 10 vol.% ethylene glycol aqueous solution was used as a solvent for hosts because their solubility in pure water is poor. Five microliters of guest species (0.5, 0.05, and 0.005 M) in dimethyl sulfoxide (DMSO) or MeOH were injected into a 10 vol.% ethylene glycol aqueous solution of host (2.5 mL) to make a sample solution with a host concentration of 1×10^{-6} M and guest concentration of 0.01, 0.1, and 1.0 mM, respectively.

3. Results and discussion

3.1 The preparation of 6^A,6^{BorG}-, 6^A,6^{CorF}-, and 6^A,6^{DorE}-dansyl-tosyl-modified β-cyclodextrins (β-1, β-2, and β-3, respectively)

Hosts β-1, β-2, and β-3 were prepared from 6^A,6^B-, 6^A,6^C-, and 6^A,6^D-di(*p*-tosyl)-β-cyclodextrins, respectively, with sodium dansylglycinate at 80 °C as shown in Scheme 1. These hosts were separated with reversed phase column chromatography (Lobar column LiChroprep RP-18, Merck Ltd., 40–63 mm, 400 × 37 mm), in yields of 14.5, 11.8, and 10.0% for β-1, β-2, and β-3, respectively. It is thought that dansyl-modified β-analogs are isolated as a mixture of diastereomers, which are 6^A,6^B- and 6^A,6^G-, 6^A,6^C- and 6^A,6^F-, and 6^A,6^D- and 6^A,6^E-dansyl-tosyl-modified β-cyclodextrins, because these diastereomers cannot be separated by reversed phase column chromatography. In this paper, if these hosts existed as diastereomers, we name them β-1, β-2, and β-3, for 6^A,6^B- and 6^A,6^G-, 6^A,6^C- and 6^A,6^F-, and 6^A,6^D- and 6^A,6^E-dansyl-tosyl-modified β-cyclodextrins, respectively. It seemed that the host–guest binding properties of these diastereomers are similar for guest molecules such as terpenes and bile acids.

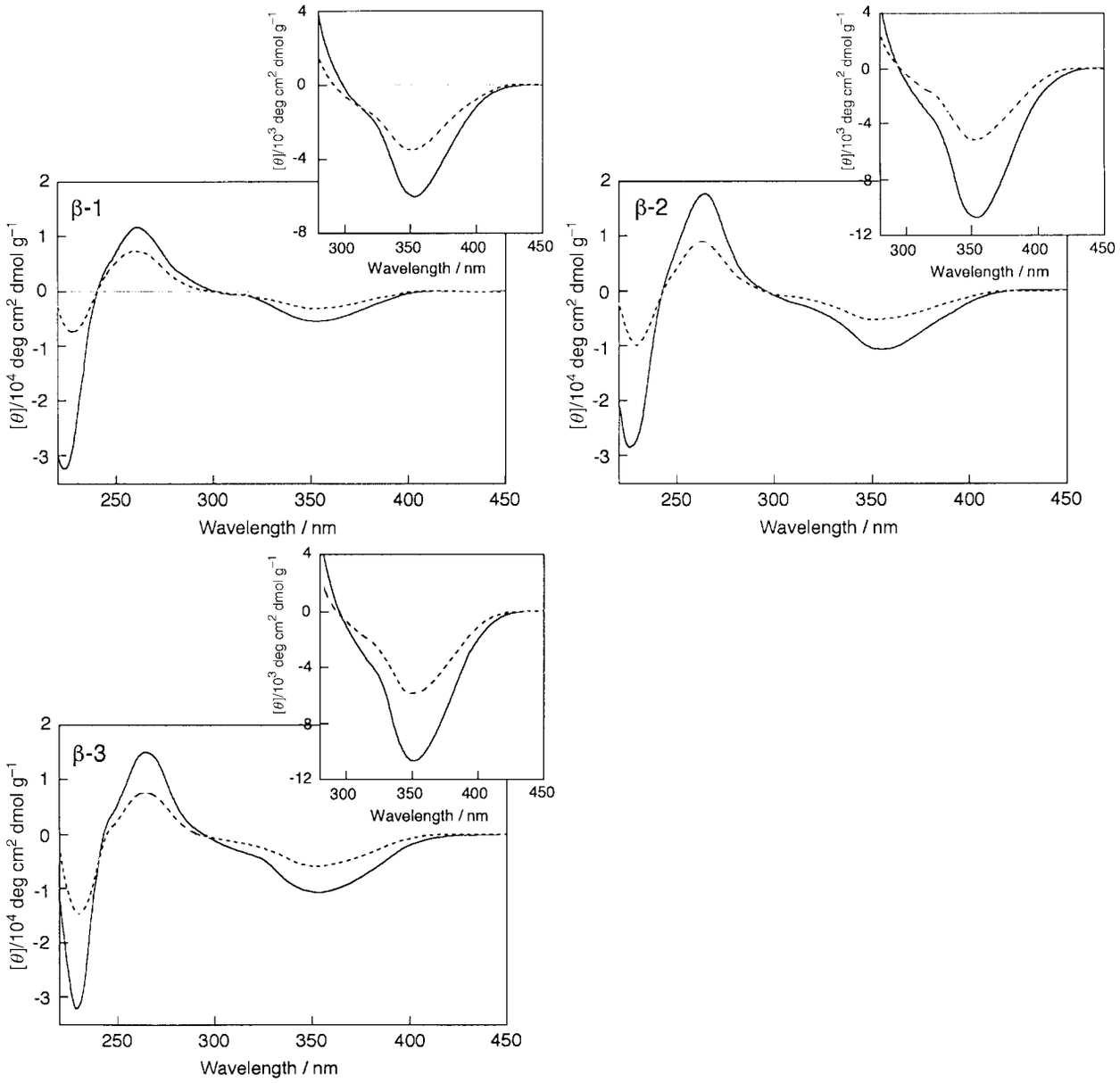


Fig. 1 Induced circular dichroism spectra of β-1, β-2, and β-3 in a 10 vol.% ethylene glycol aqueous solution (10^{-4} M: ——, 25 °C) and containing ursodeoxycholic acid (10^{4} M: - - · · - -, 25 °C).

3.2 The preparation of $6^{A},6^{BorH}$-, $6^{A},6^{CorG}$-, $6^{A},6^{DorF}$-, and $6^{A},6^{E}$-dansyl-tosyl-modified γ-cyclodextrins (γ-1, γ-2, γ-3, and γ-4, respectively)

Hosts γ-1, γ-2, γ-3, and γ-4 were prepared by the same procedure for the β-analogs, from $6^{A},6^{B}$-, $6^{A},6^{C}$-, $6^{A},6^{D}$-, and $6^{A},6^{E}$-di(*p*-tosyl)-γ-cyclodextrins, respectively, with sodium dansylglycinate, respectively. These hosts were separated with reversed phase column chromatography, in yields of 5.8, 11.8, 10.6, and 10.8% for γ-1, γ-2, γ-3, and γ-4, respectively. As with the β-analogs, it seems that dansyl-tosyl-modified γ-analogs were obtained as a mixture of diastereomers, which are $6^{A},6^{B}$- and $6^{A},6^{H}$-, $6^{A},6^{C}$- and $6^{A},6^{G}$-, and $6^{A},6^{D}$- and $6^{A},6^{F}$-dansyl-tosyl-modified γ-cyclodextrins. If these hosts exist as a diastereomer, we name them γ-1, γ-2, and, γ-3 for a mixture of $6^{A},6^{B}$- and $6^{A},6^{H}$-, $6^{A},6^{C}$- and $6^{A},6^{G}$-, and $6^{A},6^{D}$- and $6^{A},6^{F}$-dansyl-tosyl-modified γ-cyclodextrins, respectively, as illustrated in Scheme 2.

3.3 Induced circular dichroism (ICD), fluorescence, and ^{1}H NMR spectra

Fig. 1 shows the ICD spectra of the three hosts β-1, β-2, and β-3, alone, and in the presence of ursodeoxycholic acid in a 10 vol.% ethylene glycol aqueous solution. The spectra of these hosts alone exhibit a positive band around 265 nm and negative bands around 230 and 350 nm, which decrease with increasing ursodeoxycholic acid concentration. The spectral patterns of the three hosts are basically similar, but the changes of ICD intensity upon guest addition are not the same. The [θ] values of β-2 at 265 and 350 nm, are decreased to a much greater extent than those of β-1 and β-3. On the other hand, the intensity of β-1 at around 230 nm decreased more than those of β-2 and β-3. These results suggest that the movements of the appended moieties to accommodate a guest are not the same for these host molecules. These phenomena should be advantageous for molecular sensing by these hosts, because they might cause differences of sensitivity and selectivity for guest molecules. It is well known that an increase or decrease of ICD intensities is ascribed to formation of a complex between a cyclodextrin and a guest.

The ICD spectra of γ-analogs γ-1, γ-2, γ-3, and γ-4, alone and in the presence of ursodeoxycholic acid in a 10 vol.% ethylene glycol aqueous solution are shown in Fig. 2. Host γ-1, alone, shows positive and negative Cotton peaks around 325 and 375 nm, respectively. Hosts γ-2, γ-3, and γ-4 exhibit a positive Cotton peak at 325 nm and a negative peak at 360

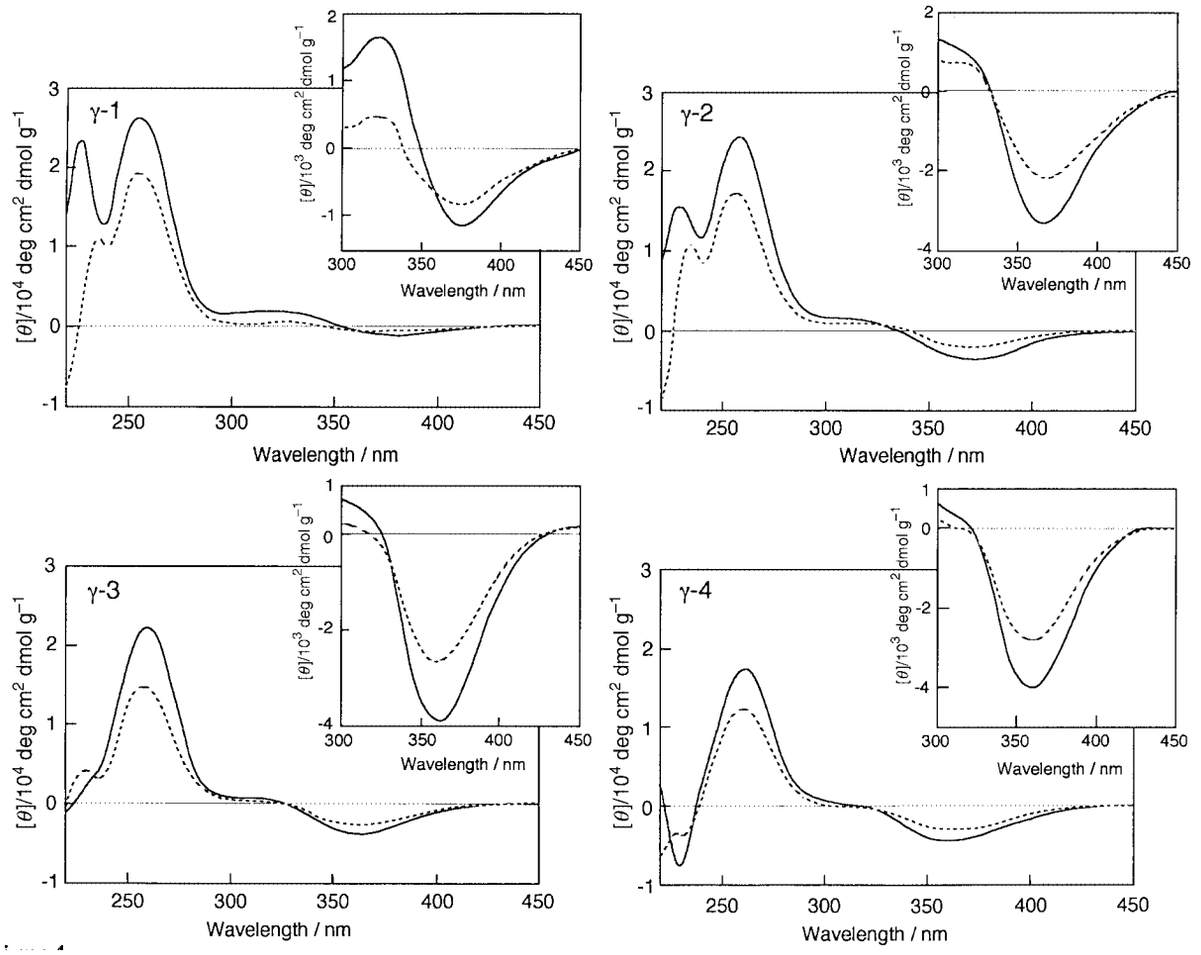
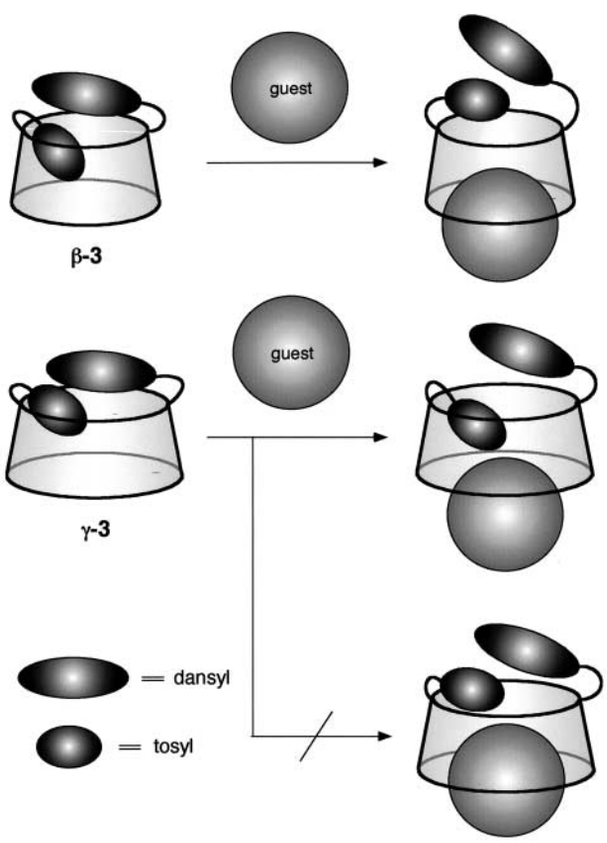


Fig. 2 Induced circular dichroism spectra of γ-1, γ-2, γ-3, and γ-4 in a 10 vol.% ethylene glycol aqueous solution (10^{-4} M: ——, 25 °C) and containing ursodeoxycholic acid (10^{4} M: - - · · - -, 25 °C).

nm. A decrease of the [θ] values for these hosts upon ursodeoxycholic acid addition was observed, indicating that the appended moieties are moving far from the chiral environment of the cyclodextrin cavity as illustrated in Scheme 3. The ICD patterns in the range 240–300 nm are very similar, while in the short wavelength region 220–240 nm the spectral patterns of these hosts are different. The patterns for γ-1 and γ-2 or γ-3 and γ-4 are similar. These results suggest that the appended moieties of these hosts are arranged differently when a host–guest complexation occurs.

Fig. 3 shows fluorescence spectra of β-2 in the presence and absence of ursodeoxycholic acid in a 10 vol.% ethylene glycol aqueous solution. The fluorescence spectra of β-1, β-2, and β-3 are composed of almost pure monomer emission with a peak around 526 nm, and the intensity decreases with increasing ursodeoxycholic acid concentration. It is reported that a guest-induced fluorescence enhancement means that the appended moiety is moving into the cyclodextrin cavity deeply and a decrease means that the appended moiety is moving out of the cavity.[14] The ICD and fluorescence spectral changes of these hosts suggest that the dansyl moiety is excluded from the cyclodextrin cavity upon guest binding and acts as a hydrophobic cap.

The behavior of the tosyl moiety of the hosts during host–guest complexation was investigated by taking ^{1}H NMR spectra. Fig. 4 shows ^{1}H NMR spectra of γ-3 alone or with ursodeoxycholic acid in a 10 vol.% DMSO-d_6 D_2O solution. The peak attributed to the tosyl group of γ-3 changes from a triplet at 7.83 ppm to a doublet at 7.83 ppm upon guest addition, whereas another peak attributed to the tosyl group of γ-3 appearing at 7.49 ppm was unchanged upon addition of guest, and the signals attributed to the dansyl moiety appearing at



Scheme 3 One host–guest complexation mechanism of β-3 and γ-3.

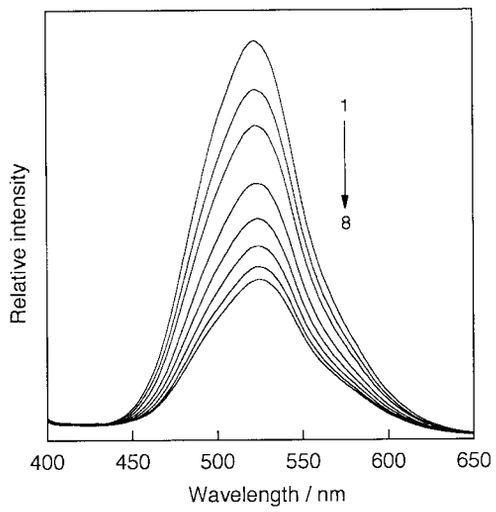


Fig. 3 Fluorescence spectra of β-2 in a 10 vol.% ethylene glycol aqueous solution (10^{-6} M, 25 °C) at various concentrations of ursodeoxycholic acid (1: 0, 2: 1.0×10^{-5}, 3: 2.0×10^{-5}, 4: 4.0×10^{-5}, 5: 6.0×10^{-5}, 6: 8.0×10^{-5}, 7: 1.0×10^{-4}, 8: 1.2×10^{-4} M).

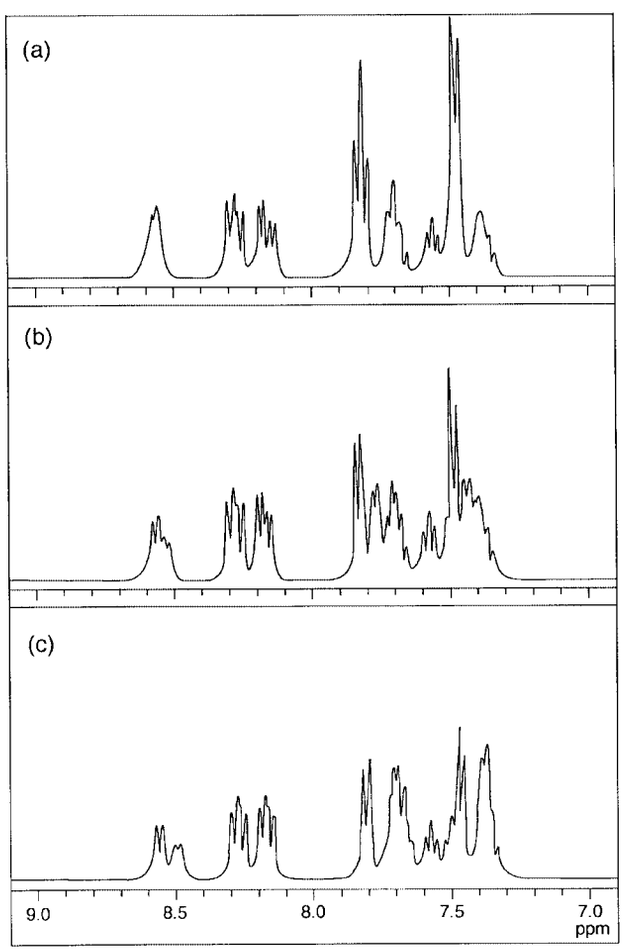


Fig. 4 ^{1}H NMR spectra of the free and complexed form of γ-3 in a 10 vol.% DMSO-d_6 D_2O solution at 25 °C: (a) R = [ursodeoxycholic acid]/[γ-3] = 0; (b) R = 0.5; (c) R = 1; where [γ-3] = 1.0×10^{-3} M in a 10 vol.% DMSO-d_6 D_2O solution. Aliquots from a 5×10^{-2} M solution of ursodeoxycholic acid in DMSO-d_6 were added directly to a 10 vol.% DMSO-d_6 D_2O solution of γ-3 in a NMR tube.

7.35–7.45, 7.55–7.8, 8.1–8.35, and 8.5–8.65 ppm, were also unchanged upon guest addition. These results suggest that tosyl and dansyl groups alter their mutual relationship or direction. On the other hand, ^{1}H NMR spectra of γ-3 in DMSO-d_6 do not change after guest addition. In DMSO, it is realized that there is little hydrophobic interaction between appended moieties such as tosyl and dansyl groups and cyclodextrin, which means that the appended moieties are on the outside of the cyclodextrin cavity. The relative position between the dansyl and tosyl moieties in the presence and absence of guest was examined by 2-D ^{1}H NMR spectra (ROESY). Fig. 5 shows ROESY ^{1}H NMR spectra of γ-3, alone or with ursodeoxycholic acid in a 10 vol.% DMSO-d_6 D_2O solution. It is observed that two cross peaks between protons of a dansyl moiety appearing at 8.57 and 8.16 ppm and protons of a tosyl moiety appearing at 7.49 ppm disappeared after guest addition. The cross peaks due to protons of the tosyl moiety and protons of guest, and protons of tosyl moiety and protons of cyclodextrin, are hardly recognized. This indicates that the interaction between dansyl and tosyl moieties is decreased when a host–guest complexation occurs. On the other hand, there is no change in the ^{1}H NMR of β-analogs after guest addition, which means the mutual relationship in space between tosyl and dansyl moieties is retained upon guest addition. The energy-minimized structure of β-3 (AD isomer) shown in Fig. 6 suggests that the tosyl moiety is included in the cyclodextrin cavity and the dansyl moiety is located outside it. The energy-minimized structure of γ-3 (AD isomer) shown in Fig. 7 suggests that the tosyl moiety is getting close to the rim of the cavity and the dansyl moiety is moving out from the cavity. The ^{1}H NMR spectra and 3-D structures suggest that the movements of the appended moieties are different between the β- and γ-analogs. It is obvious that tosyl and dansyl groups of β-3 are coming out from the cavity simultaneously upon guest addition, where the appended moieties act as a hydrophobic cap. On the other hand, it seems that the tosyl moiety of γ-3 is coming slightly into the cavity to act as a spacer to allow the guest to be included and that the dansyl moiety is going out from it as illustrated in Scheme 3. The unique behaviors of the tosyl and dansyl moieties provide a high sensitivity factor for guests.

To display the sensing ability of modified cyclodextrins, the $\Delta I/I_0$ value was used as a sensitivity parameter. Here, ΔI is $I_0 - I$, where I_0 is the fluorescence intensity for the host alone and I that for a complex. Fig. 8 shows the parameter values of β-1, β-2, and β-3 with steroids at 0.1 mM except for lithocholic acid (**7**), which was examined at 0.01 mM because 0.1 mM of lithocholic acid is not soluble in a 10 vol.% ethylene glycol aqueous solution, and terpenoids at 1.0 mM. It is evident that chenodeoxycholic acid (**8**) and ursodeoxycholic acid (**9**) were detected with remarkably high sensitivity, exhibiting values of 0.532, 0.351, and 0.246 for β-1, β-2, and β-3 and 0.575, 0.580, and 0.493 for, β-1, β-2, and β-3, respectively. Lithocholic acid (**7**) was detected with low sensitivity. Deoxycholic acid (**6**), which is different from the other steroids only in the position of one hydroxyl group, and cholic acid (**10**), which bears one more hydroxyl group than **8** and **9**, were hardly detected. The sensing factors of bile acid by β-1, β-2, and β-3 decrease in the sequence **9** < **8** < **7** < **6** = **10**, and the sensing ability of the three hosts is roughly in the order β-1 > β-2 > β-3. It is suggested that the positions of the hydroxyl groups of the guests affect the sensing ability of the host. The sensing parameter values of β-1, β-2, and β-3 for guests **8** and **9** were higher than those of mono- and bis-dansyl-modified β-analogs.[13,15] It is estimated that the dansyl moiety in dansyl-tosyl-modified β-analogs can move much more flexibly because of the smaller molecular size of the tosyl moiety than the dansyl group, in which there is less steric hindrance than that of a bis-dansyl system. All hosts showed only little sensitivity for ketosteroids. Of (−)-borneol (**11**), (+)-fenchone (**12**), and (−)-fenchone (**13**), which are bicyclic derivatives, (−)-borneol (**11**) was detected with higher sensitivity, exhibiting values of 0.446, 0.348, and 0.346 for β-2, β-3, and β-1, respectively. Cyclohexanol (**14**), cyclooctanol (**15**), and (−)-menthol (**16**), which are monocyclic derivatives, benzhydrol (**17**), which bears two aromatic rings, and nerol (**18**), which is a non-cyclic compound, were detected with high sensitivity and with positive values, except for cyclohexanol (**14**).

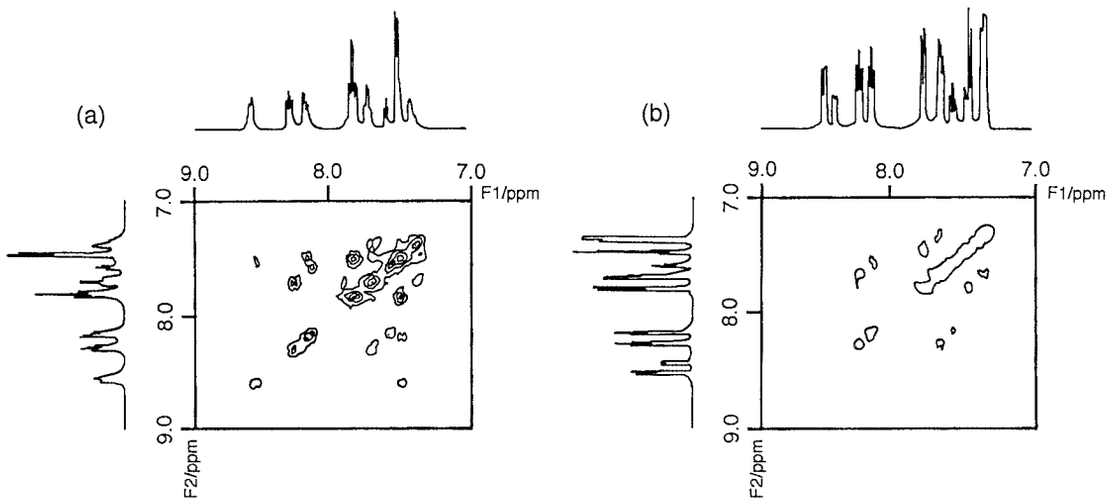
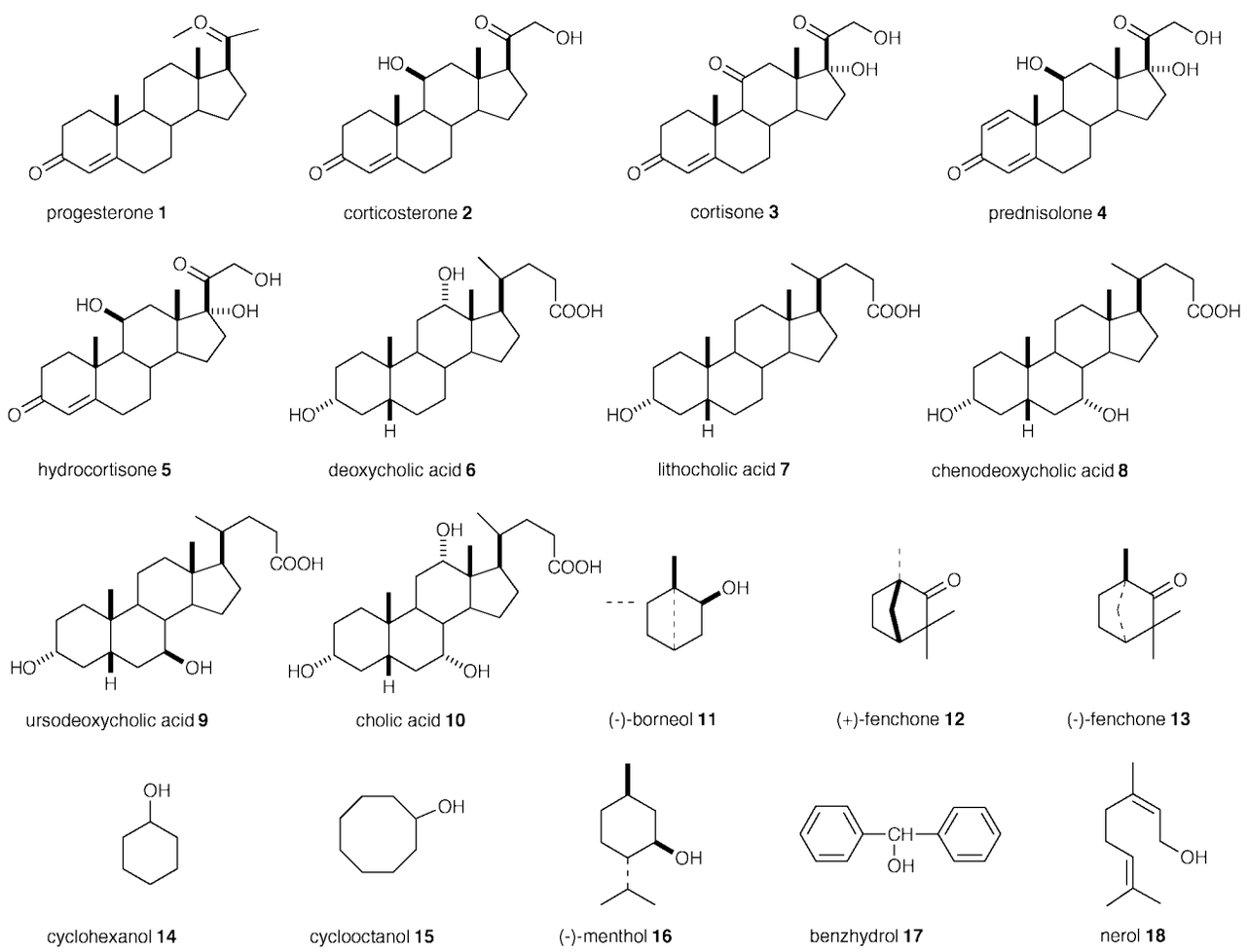


Fig. 5 ROESY ^{1}H NMR spectra of the free and complexed form of γ-3 in a 10 vol.% DMSO-d_6 D_2O solution at 25 °C: (a) R = [ursodeoxycholic acid]/[γ-3] = 0; (b) R = 1; where [γ-3] = 1.0×10^{-3} M in a 10 vol.% DMSO-d_6 D_2O solution. Aliquots from a 5×10^{-2} solution of ursodeoxycholic acid in DMSO-d_6 were added directly to a 10 vol.% DMSO-d_6 D_2O solution of γ-3 in a NMR tube.



The sensing parameters of γ-1, γ-2, γ-3, and γ-4 with steroids at 0.1 mM except for lithocholic acid (**7**), (see above), and terpenoids at 1.0 mM are shown in Fig. 9. It is obvious that lithocholic acid (**7**), even at one tenth concentration, chenodeoxycholic acid (**8**), and ursodeoxycholic acid (**9**) were detected with remarkably high sensitivity, exhibiting values of 0.286, 0.266, 0.254, and 0.249 for γ-2, γ-3, γ-1, and γ-4, 0.242, 0.241, 0.234, and 0.187 for γ-1, γ-3, γ-2, and γ-4, and 0.315, 0.301, 0.288, and 0.252 for γ-2, γ-1, γ-3, and γ-4, respectively. Deoxycholic acid (**6**) was detected with high sensitivity, exhibiting values of 0.107, 0.104, 0.096, and 0.090 for γ-1, γ-2, γ-3, and γ-4, respectively. Cholic acid (**10**) was hardly detected, due to its increased polarity. The sensing factors of bile acid by γ-1, γ-2, γ-3, and γ-4 decrease in the sequence **9** < **7** < **8** < **6** < **10**. It is suggested that the position of the hydroxyl groups of the host affects its sensing ability. The sensing parameter values of γ-1, γ-2, γ-3, and γ-4 for guests **8** and **9** were higher than those of mono- and bis-dansyl-modified γ-analogs.[14,16] It seemed that movement of the dansyl moiety of the γ-1 to γ-4 analogs is more easy than those of other bis-dansyl modified γ-analogs,

Table 1 Binding constants (K/dm^3 mol^{-1}) of β-1, β-2, and β-3 in a 10 vol.% ethylene glycol aqueous solution (10^{-6} M, 25 °C)[a]

Guest	β-1	β-2	β-3
Progesterone (**1**)	8610 ± 340[b]	2100 ± 220	3050 ± 250
Lithocholic acid (**7**)	176000 ± 8350	142000 ± 14000	263000 ± 17700
Chenodeoxycholic acid (**8**)	9260 ± 10	5820 ± 250	2260 ± 50
Ursodeoxycholic acid (**9**)	10300 ± 540	14600 ± 280	7670 ± 510
Borneol (**11**)	1970 ± 30	1700 ± 10	1440 ± 10

[a] The K values were obtained from guest-induced fluorescence variations. [b] The errors were statistically derived.

for the same reason as for the β-analogs mentioned above. All hosts show only little sensitivity for ketosteroids which have two and three hydroxyl groups. Progesterone (**1**), which bears no hydroxyl group and is more hydrophobic than the other ketosteroids, was detected with values of 0.115, 0.101, 0.094, and 0.091 for γ-1, γ-2, γ-4, and γ-3, respectively. The complexation behaviors of the four hosts are affected by the molecular structure and size, because (−)-borneol (**11**), (+)-fenchone (**12**), and (−)-fenchone (**13**), which are bicyclic derivatives, were detected with positive sensitivity factors, while monocyclic derivatives such as cyclohexanol (**14**), cyclooctanol (**15**), and (−)-menthol (**16**) were detected with negative sensitivity factors. Benzhydrol (**17**) and nerol (**18**) were detected with negative sensitivity factors.

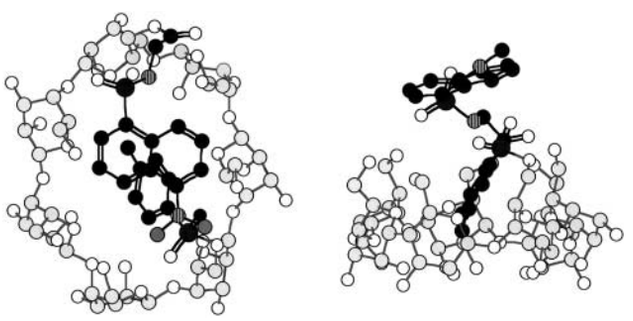


Fig. 6 Energy-minimized structure of 6^A-dansyl-6^D-tosyl-modified β-cyclodextrin obtained using molecular mechanics in CS Chem 3D.

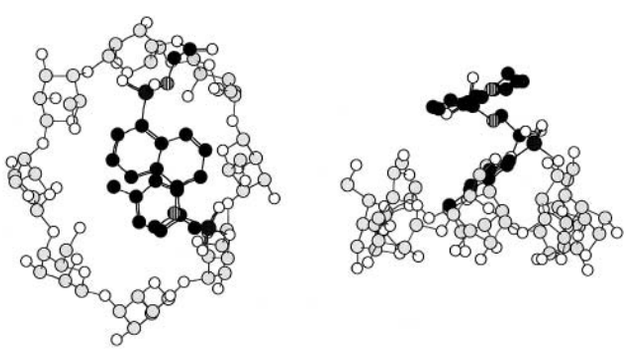


Fig. 7 Energy-minimized structure of 6^A-dansyl-6^D-tosyl-modified γ-cyclodextrin obtained using molecular mechanics in CS Chem 3D.

The guest-induced fluorescence variation at 526 nm was employed to calculate the binding constants of these hosts using eqn. (1) as reported previously.[12] Here, I is the fluorescence intensity at 468 nm (I_f for complex, I_{f0} for the host alone), [CD] the total host concentration, [G] the total guest concentration, and a and b are constants. The binding constants of seven hosts were obtained in the order to examine the correlation between the fluorescence variations and the binding abilities of the hosts. The results are shown Tables 1 and 2. The binding constants of β-1, β-2, and β-3 are in the order **7 > 9 > 8 > 1 > 11**, which are not parallel with the sensitivity factors. In the case of γ-analogs, the binding constants are in the order **7 > 1 > 6 > 8 > 9 > 11** for γ-1, **7 > 6 > 1 > 8 > 9 > 11** for γ-2, **7 > 1 > 6 > 9 > 11 > 8** for γ-3, and **7 > 6 > 8 > 9 ≥ 1 > 11** for γ-4. The order of binding constants of each host for these guests is not parallel with the order of the sensing factors. This means that the sensitivity value gives a relative but not absolute sensing ability. It is assumed that when a guest concentration is varied, the sensing ability of the hosts is also changed.

$$\frac{1}{I_f - I_{f0}} = \frac{1}{a[CD]} + \frac{1}{b[CD]K} \times \frac{1}{[G]} \quad (1)$$

3.4 Response range

Figs. 10 and 11 show response curves of β-1, β-2, β-3, γ-1, γ-2, γ-3, and γ-4 for guests lithocholic acid, ursodeoxycholic acid, and cholic acid. Those were detected by β-analogs with response ranges 10^{-6}–10^{-5}, 10^{-6}–10^{-4}, and above 10^{-4} M, respectively. In the case of γ-analogs, lithocholic acid was detected by γ-1 with range $10^{-7.5}$–$10^{-5.5}$ M, whereas other γ-analogs recognized it with response range $10^{-6.5}$–$10^{-5.0}$ M. Ursodeoxycholic acid and cholic acid were detected by γ-analogs with response ranges 10^{-5}–10^{-4} and above 10^{-4} M, respectively. Since these guests were detected with different lower detection limits by β- and γ-analogs with the order lithocholic acid ≥ ursodeoxycholic

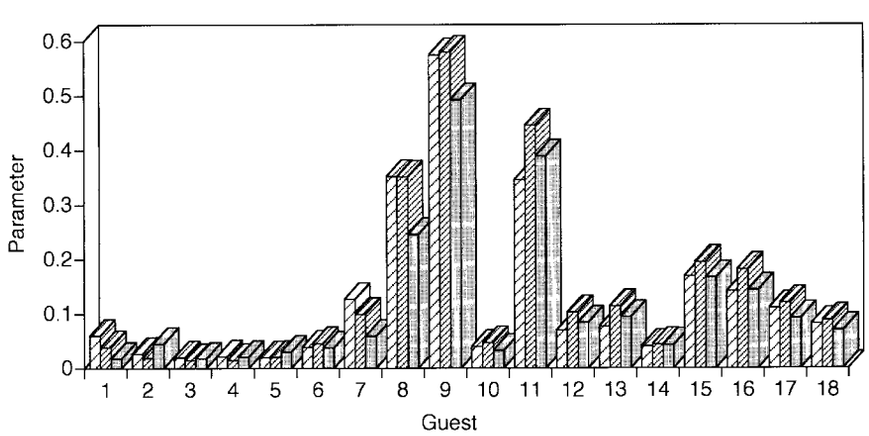


Fig. 8 Sensitivity factors of β-1 (▨), β-2 (▨), and β-3 (■) in a 10 vol.% ethylene glycol aqueous solution (10^{-6} M, 25 °C) for all guests examined.

Table 2 Binding constants (K/dm^3 mol^{-1}) of γ-1, γ-2, γ-3, and γ-4 in a 10 vol.% ethylene glycol aqueous solution (10^{-6} M, 25 °C)[a]

Guest	γ-1	γ-2	γ-3	γ-4
Progesterone (**1**)	16100 ± 1430[b]	9610 ± 920	21700 ± 1120	1570 ± 120
Deoxycholic acid (**6**)	13600 ± 1030	23300 ± 1640	11700 ± 1390	9640 ± 340
Lithocholic acid (**7**)	136000 ± 9280	163000 ± 5100	124000 ± 10200	199000 ± 14700
Chenodeoxycholic acid (**8**)	3560 ± 480	3870 ± 320	810 ± 10	3110 ± 570
Ursodeoxycholic acid (**9**)	3220 ± 220	3240 ± 250	7570 ± 550	1580 ± 180
Borneol (**11**)	640 ± 70	300 ± 50	930 ± 50	590 ± 40

[a] The K values were obtained from guest-induced fluorescence variations. [b] The errors were statistically derived.

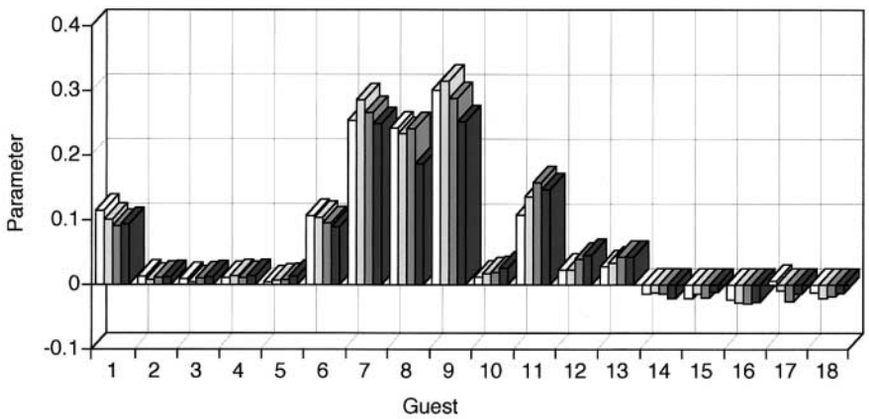


Fig. 9 Sensitivity factors of γ-1 (□), γ-2 (□), γ-3 (▒), and γ-4 (■) in a 10 vol.% ethylene glycol aqueous solution (10^{-6} M, 25 °C) for all guests examined.

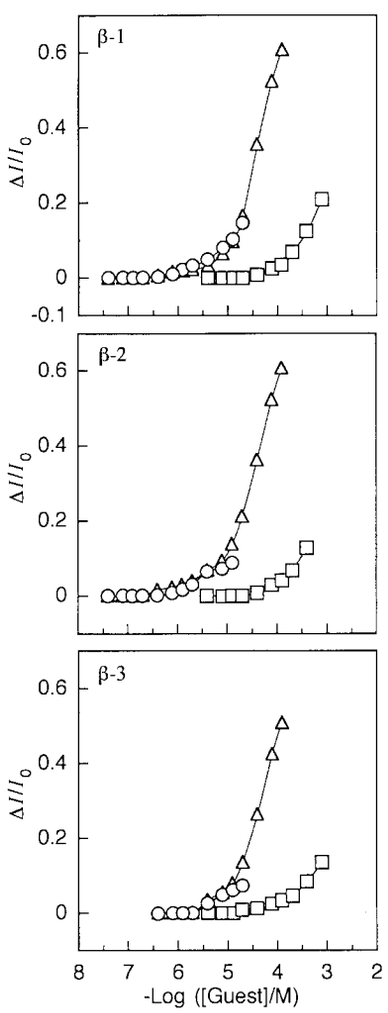


Fig. 10 Fluorescence variations of β-1, β-2, and β-3 in a 10 vol.% ethylene glycol aqueous solution (10^{-6} M, 25 °C) for lithocholic acid (○), ursodeoxycholic acid (□), and cholic acid (△) as a function of guest concentration.

acid > cholic acid and lithocholic acid > ursodeoxycholic acid > cholic acid, respectively, they are expected to have different response ranges when their concentrations are varied. This suggests that all hosts give a clear concentration dependency for the guests, reflecting the sensitivities of the system for the guests.

4. Conclusion

Seven bis-functionalized analogs of dansyl-tosyl-modified β- and γ-cyclodextrins have been investigated for their sensing ability toward organic guests including bile acids and terpenoids, which are biologically significant substances. These hosts show pure monomer fluorescence, the variation of which was used as a parameter to describe the sensing ability. Introduction of two different kinds of functional groups such as dansyl and tosyl groups, which are in different positions such as 6^A and 6^X on the cyclodextrin cavity, alters and improves the sensing ability of these hosts, those for bile acids such as chenodeoxycholic acid and ursodeoxycholic acid, and terpenoids such as (−)-borneol are higher than with bis-dansyl modified β- and γ-analogs. It is recognized that cooperation of dansyl and tosyl moieties of the hosts works to elevate the binding ability compared with those of mono- and bis-dansyl modified β- and γ-cyclodextrins reported previously.[13–16] Fluorescence chemo-sensor systems using such modified cyclodextrins are very convenient and useful, because the chemical modification of a guest, even when spectroscopically inert, is not necessary; a guest can be examined directly in this system. It seems quite possible to detect endocrine-disrupting chemicals exited in lakes or rivers directly with sensitivity and selectivity. We are attempting to apply fluorescent cyclodextrins to the direct detection of environmental hormones.[23]

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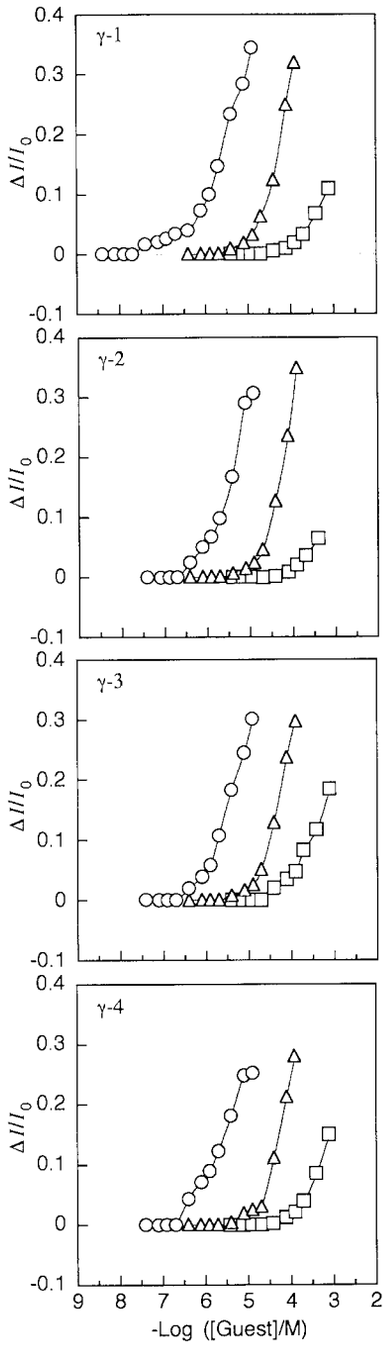

Fig. 11 Fluorescence variations of γ-1, γ-2, γ-3, and γ-4 in a 10 vol.% ethylene glycol aqueous solution (10^{-6} M, 25 °C) for lithocholic acid (○), ursodeoxycholic acid (□), and cholic acid (△) as a function of guest concentration.

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