Variations of fluorescent molecular sensing for organic guests by regioselective anthranilate modified β- and γ-cyclodextrins

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Flexible hosts, regioselectively modified, namely disodium $6^A,6^B$-, $6^A,6^C$- and $6^A,6^D$-dianthranilato-β-cyclodextrins (β-1, β-2 and β-3, respectively) and γ-cyclodextrin analogues, disodium $6^A,6^B$-, $6^A,6^C$-, $6^A,6^D$- and $6^A,6^E$-dianthranilato-γ-cyclodextrins (γ-1, γ-2, γ-3 and γ-4, respectively) have been synthesized as a sensor for organic guests including terpenoids and bile acids. These host compounds show pure monomer fluorescence, in which β-1 shows an increase in fluorescence intensity on accommodation of guest species. On the other hand, β-3 exhibits an increase in intensity on complexation of bile acids and a decrease in intensity for smaller guests such as terpenoids. Host β-2 exhibits a mixed type of β-1 and β-3. The extent of fluorescence variation with a guest is employed to display the sensing abilities of those hosts. The sensing parameter ($\Delta I/I^0$) to describe the sensing ability of the hosts was used. Host β-1 can detect both small and large guests with high sensitivity. Hosts β-2 and β-3 show a similar sensing pattern for guests, while the monoderivative (β-4) can detect small guests with higher sensitivity, but cannot detect larger guests such as bile acids. In the case of larger hosts such as γ-1, γ-2 and γ-3, they show positive parameter values for small guests such as the terpenoids examined, which means the fluorescence intensity increases on accommodation of a guest, whereas γ-4 shows negative parameter values. Host γ-3 exhibits the highest sensitivity for bile acids. The sequence of the binding ability of these hosts is γ-3 > γ-4 > γ-2 > γ-1. The behavior of the appended moieties of those hosts during a host–guest complexation are studied by induced circular dichroism (ICD) spectra and fluorescence spectra. The ICD spectral patterns of β-1, β-2 and β-3 are quite different. On the other hand, the ICD patterns of γ-cyclodextrin analogs are similar. For example, the spectrum of γ-2, alone or in the presence of a guest is very similar to that of γ-3, indicating that the movements of the appended moieties are very similar. The guest-induced variations in the fluorescence or ICD intensity suggest that the appended moieties act as a spacer or hydrophobic cap which enables the cyclodextrin to form a 1 : 1 host–guest complex.

Introduction

The detection of molecules by fluorescent sensors is of current interest.[1] One of the mechanisms for sensing is the use of host–guest complexation phenomena of cyclodextrin derivatives.[2–10] 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 undergo host–guest complexation with a variety of organic compounds in their cavities in aqueous solution.[11,12] The fluorescent active cyclodextrins have recently received increasing attention because these compounds show remarkable variations in their fluorescence spectra associated with the formation of inclusion complexes; on this basis they have been used as sensors or indicators of molecules in aqueous solution. We have reported the fluorescent sensor systems of anthranilate modified cyclodextrins, monoanthranilate modified α-, β- and γ-cyclodextrins,[13,14] or di appended β- and γ-analogs such as $6^A,6^D$-di appended β-cyclodextrin (β-3) and $6^A,6^E$-di appended γ-cyclodextrin (γ-4),[15,16] respectively, which show unique guest binding properties because of the smaller size of the sodium anthranilate moiety. We studied the binding abilities of these derivatives with terpenoids and bile acids as guest molecules, because they are biologically significant substances produced by plants or animals and are used for crude drugs. In a previous report, we described how four analogs of dinaphthalene appended γ-cyclodextrins, which are modified with naphthalene moieties at the $6^A,6^B$-, $6^A,6^C$-, $6^A,6^D$-, and $6^A,6^E$-positions of glucose units of the cyclodextrins as shown in Fig. 1, exhibit different fluorescent molecular sensing ability for guest molecules.[3] It means that the position of modification affects the sensing ability of the cyclodextrin for organic guests such as terpenoids or bile acids. As a further extension of this work, we synthesized another two β-cyclodextrin analogs and three γ-cyclodextrin ones, which are $6^A,6^B$-, and $6^A,6^C$-di appended β-cyclodextrins (β-1 and β-2, respectively) and $6^A,6^B$-, $6^A,6^C$- and $6^A,6^D$-di sodium anthranilate modified γ-cyclodextrins (γ-1, γ-2 and γ-3, respectively). In this paper, we would like to describe the fluorescent sensing abilities of these hosts compared with those of β- and γ-cyclodextrin analogues reported previously.

Experimental

Preparation of $6^A,6^B$-, $6^A,6^C$- and $6^A,6^D$-di(*p*-tosyl)β-cyclodextrins (I, II and III, respectively)[17]

β-Cyclodextrin 9.0 g (7.93 mM) was added to a solution of 9.48 g (49.8 mM) of *p*-TsCl in 180 mL of dry pyridine. The reaction mixture was stirred for 2 h at room temperature. The reaction mixture was concentrated *in vacuo* to give an oily material, which was poured into 300 mL of acetone. The resulting precipitates were filtered, and the filtrate was dissolved in 30 mL of water. The water soluble fraction was applied to a reversed-

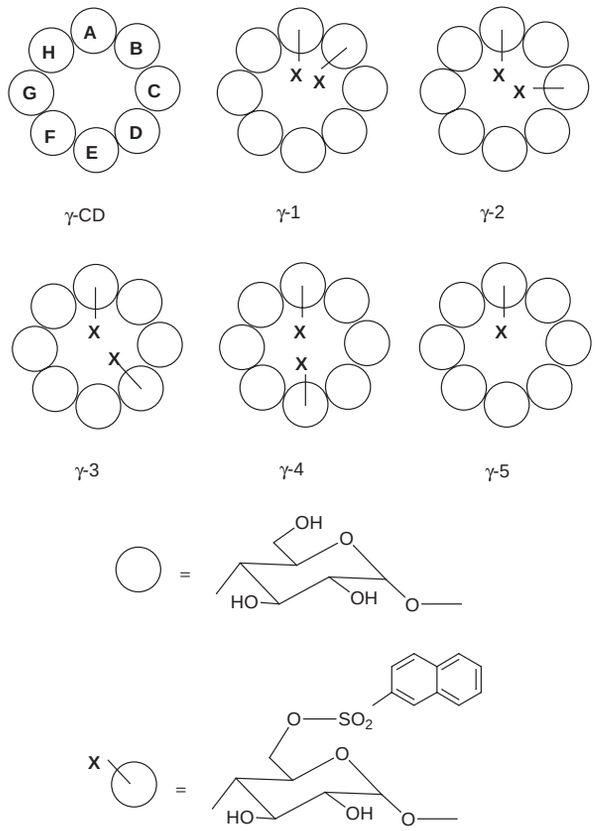


Fig. 1 Structures of dinaphthalene appended γ-cyclodextrins.

phase column (Lobar column LiChroprep RP 18, Merck Ltd., 40–63 mm, 440 × 37 mm). Stepwise elution with 200 mL of 10 vol%, 500 mL of 20 vol%, 900 mL of 30 vol%, 800 mL of 40 vol%, and 1.4 L of 50 vol% aqueous MeOH gave 6^A,6^D-6-deoxy-6-(tolyl-*p*-sulfonyl)β-cyclodextrin **III** (315 mg, 2.75%), 6^A,6^C-6-deoxy-6-(tolyl-*p*-sulfonyl)β-cyclodextrin **II** (303 mg, 2.64%), and 6^A,6^B-6-deoxy-6-(tolyl-*p*-sulfonyl)β-cyclodextrin **I** (113 mg, 0.98%). **I**: R_f 0.55 (methanol–water 2:1 by volume; TLC; RP-18F254S; Merck Ltd.) and 0.40 (CH_3CN–H_2O 5:1 by volume; TLC; silica gel 60F254). ^{1}H-NMR (DMSO-d_6) = 3.00–3.80 (42H, m, C^2-C^6H of cyclodextrin), 4.00–4.98 (12H, m, O^6H, C^1H of cyclodextrin), 5.65–5.95 (14H, m, O^2H, O^3H of cyclodextrin), 7.44 (4H, t, J = 8.1 Hz, aromatic-H), 7.74 (4H, d-d, J = 12.6, 12.3 Hz, aromatic-H). **II**: R_f 0.59 (methanol–water 2:1 by volume; TLC; RP-18F254S; Merck Ltd.) and 0.40 (CH_3CN–H_2O 5:1 by volume; TLC; silica gel 60F254). ^{1}H-NMR (DMSO-d_6) = 3.0–3.8 (42H, m, C^2-C^6H of cyclodextrin), 4.0–4.98 (12H, m, O^6H, C^1H of cyclodextrin), 5.78–5.95 (14H, m, O_2H, O_3H of cyclodextrin), 7.40–7.46 (4H, m, aromatic-H), 7.75 (4H, d, J = 8.1 Hz, aromatic-H). **III**: R_f 0.68 (methanol–water 2:1 by volume; TLC; RP-18F254S; Merck Ltd.) and 0.40 (CH_3CN–H_2O 5:1 by volume; TLC; silica gel 60F254). ^{1}H-NMR (DMSO-d_6) = 3.20–3.80 (42H, m, C^2-C^6H of cyclodextrin), 4.0–4.98 (12H, m, O^6H, C^1H of cyclodextrin), 5.65–5.95 (14H, m, O^2H, O^6H of cyclodextrin), 7.40 (4H, d, J = 8.4 Hz, aromatic-H), 7.72 (4H, d, J = 7.5 Hz, aromatic-H).

Preparation of disodium 6^A,6β-dianthranilato-β-cyclodextrin (β-1)

A mixture of 6^A,6β-di(*p*-tosyl)β-cyclodextrin (769 mg, 0.53 mM) and sodium anthranilate (230 mg, 1.44 mM) in 40 mL of DMF was heated at 80 °C for 8 h under a nitrogen atmosphere. After cooling, the reaction mixture was poured into 500 mL of acetone. The resulting precipitates were filtered and dried. The crude product was recrystallized from water to give pure compound (339 mg, 45.1%). R_f 0.34 (CH_3CN–H_2O 5:1 by volume; TLC; silica gel 60F254). ^{1}H-NMR (DMSO-d_6) = 3.0–3.8 (42H, m, C^2-C^6H of cyclodextrin), 4.1–5.0 (12H, m, O^6H, C^1H of cyclodextrin), 5.6–6.0 (14H, m, O^2H, O^3H of cyclodextrin), 6.50 (2H, m, aromatic-H), 6.59 (2H, s, -NH), 6.73 (2H, d-d, J = 7.9, 7.9 Hz, aromatic-H), 7.21 (2H, q, J = 7.9 Hz, aromatic-H), 7.66 (2H, q, J = 7.9 Hz, aromatic-H). Calcd. for $C_{56}H_{78}O_{37}N_2Na_2 \cdot 5H_2O$: C, 44.63; H, 5.88; N, 1.86%. Found: C, 44.55; H, 5.73; N, 1.89%. MS (FAB): 1373 ($[M - 2Na + 2H]^+$).

Preparation of disodium 6^A,6^C-dianthranilato-β-cyclodextrin (β-2)

Compound β-2 was prepared by the same procedure as β-1. Yield 3.6%. R_f 0.34 (CH_3CN–H_2O 5:1 by volume; TLC; silica gel 60F254). ^{1}H-NMR (DMSO-d_6) = 3.0–3.8 (42H, m, C^2-C^6H of cyclodextrin), 4.1–5.0 (12H, m, O^6H, C^1H of cyclodextrin), 5.6–5.9 (14H, m, C^2H, C^3H of cyclodextrin), 6.4–6.7 (4H, br, aromatic-H and -NH), 6.73 (2H, d-d, J = 7.9, 7.9 Hz, aromatic-H), 6.9–7.5 (2H, m, aromatic-H), 7.72 (2H, t, J = 7.9Hz, aromatic-H). Calcd. for $C_{56}H_{78}O_{37}N_2Na_2 \cdot 6H_2O$: C, 44.10; H, 5.95; N, 1.84%. Found: C, 44.01; H, 5.92; N, 1.78%. MS (FAB): 1373 ($[M - 2Na + 2H]^+$).

Preparation of 6^A,6^B-, 6^A,6^C-, 6^A,6^D- and 6^A,6^E-di(*p*-tosyl)γ-cyclodextrins (IV, V, VI and VII, respectively) [18]

γ-Cyclodextrin 9.0 g (6.94 mM) was added to a solution of 8.23 g (43.5 mM) of *p*-TsCl in 180 mL of dry pyridine, and the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was concentrated *in vacuo* to give an oily material, which was poured into 300 mL of acetone. The resulting precipitates were filtered and the filtrate was dissolved in 30 mL of water. The water soluble fraction was applied to a reversed-phase column (Lober column LiChroprep RP 18). Stepwise elution with 100 mL of 10 vol%, 200 mL of 20 vol%, 350 mL of 25 vol%, 800 mL of 35 vol% and 700 mL of 40 vol% aqueous MeOH gave 6^A,6^E-6-deoxy-6-(tolyl-*p*-sulfonyl)γ-cyclodextrin **VII** (352 mg, 3.2%) and 500 mL of 45 vol%, and 1.5 L of 50 vol% aqueous MeOH were applied to obtain 6^A,6^D-6-deoxy-6-(tolyl-*p*-sulfonyl)γ-cyclodextrin **VI** (312 mg, 2.85%), 6^A,6^C-6-deoxy-6-(tolyl-*p*-sulfonyl)γ-cyclodextrin **V** (485 mg, 4.4%), and 6^A,6^B-6-deoxy-6-(tolyl-*p*-sulfonyl)γ-cyclodextrin **IV** (334 mg, 3.0%). **IV**: R_f 0.57 (methanol–water 2:1 by volume; TLC; RP-18F254S; Merck Ltd.) and 0.40 (CH_3CN–H_2O 5:1 by volume; TLC; silica gel 60F254). ^{1}H-NMR (DMSO-d_6) = 3.2–3.8 (48H, m, C^2-C^6H of cyclodextrin), 4.1–4.95 (14H, m, O^6H, C^1H of cyclodextrin), 5.75–5.95 (16H, m, O^2H, O^3H of cyclodextrin), 7.43 (4H, d-d, J = 5.7, 5.4 Hz, aromatic-H), 7.74 (4H, d-d, J = 8.4, 8.7 Hz, aromatic-H). **V**: R_f 0.57 (methanol–water 2:1 by volume; TLC; RP-18F254S; Merck Ltd.) and 0.64 (CH_3CN–H_2O 5:1 by volume; TLC; silica gel 60F254). ^{1}H-NMR (DMSO-d_6) = 3.2–3.8 (48H, m, C^2-C^6H of cyclodextrin), 4.1–4.95 (14H, m, O^6H, C^1H of cyclodextrin), 5.70–5.95 (16H, m, O^2H, O^3H of cyclodextrin), 7.44 (4H, d-d, J = 3.0, 3.3 Hz aromatic-H), 7.77 (4H, d, J = 7.2 Hz, aromatic-H). **VI**: R_f 0.57 (methanol–water 2:1 by volume; TLC; RP-18F254S; Merck Ltd.) and 0.66 (CH_3CN–H_2O 5:1 by volume; TLC; silica gel 60F254). ^{1}H-NMR (DMSO-d_6) = 3.2–3.8 (48H, m, C^2-C^6H of cyclodextrin), 4.1–4.95 (14H, m, O^6H, C^1H of cyclodextrin), 5.6–5.95 (16H, m, O^2H, O^6H of cyclodextrin), 7.43 (4H, d, J = 7.8 Hz, aromatic-H), 7.75 (4H, d-d, J = 2.1, 1.8 Hz, aromatic-H). **VII**: R_f 0.57 (methanol–water 2:1 by volume; TLC; RP-18F254S; Merck Ltd.) and 0.81 (CH_3CN–H_2O 5:1 by volume; TLC; silica gel 60F254). ^{1}H-NMR (DMSO-d_6) = 3.2–3.9 (48H, m, C^2-C^6H of cyclodextrin), 4.1–4.95 (14H, m, O^6H, C^1H of cyclodextrin), 5.7–5.95 (16H, m, O^2H, O^6H of cyclodextrin), 7.47 (4H, d, J = 8.1 Hz, aromatic-H), 7.76 (4H, d, J = 8.1 Hz, aromatic-H).

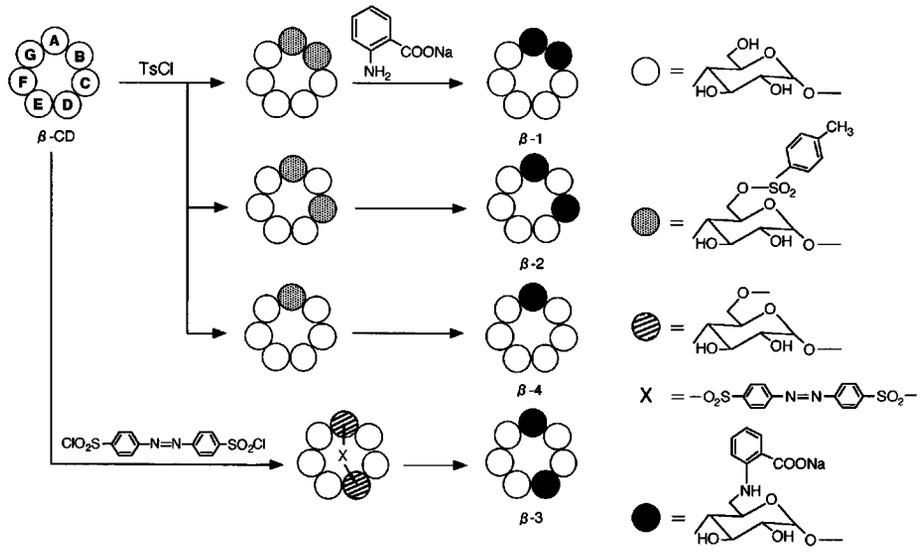


Fig. 2 Preparation of β-1, β-2, β-3 and β-4.

Preparation of disodium 6^A,6^B-dianthranilato-γ-cyclodextrin (γ-1)

A mixture of 6^A,6^B-di(*p*-tosyl)γ-cyclodextrin (638 mg, 0.40 mM) and sodium anthranilate (157 mg, 0.86 mM) in 20 mL of DMF was heated at 80 °C for 8 h under a nitrogen atmosphere. After cooling, the reaction mixture was poured into 500 mL of acetone. The resulting precipitates were filtered and dried. The crude product was recrystallized from MeOH to give a pure compound (65 mg, 10.3%). R_f 0.27 (CH_3CN–H_2O 5:1 by volume; TLC; silica gel 60F254). ^{1}H-NMR (DMSO-d_6) = 3.0–3.8 (42H, m, C^2-C^6H of cyclodextrin), 4.1–5.0 (12H, m, O^6H, C^1H of cyclodextrin), 5.6–6.0 (14H, m, O^2H, O^3H of cyclodextrin), 6.51 (2H, t, J = 7.9 Hz, aromatic-H), 6.60 (2H, s, -NH), 6.73 (2H, d, J = 7.9 Hz, aromatic-H), 7.21 (2H, q, J = 7.9 Hz, aromatic-H), 7.69 (2H, d-d J = 7.9, 7.9 Hz, aromatic-H). Calcd. for $C_{62}H_{88}O_{42}N_2Na_2 \cdot 5H_2O$: C, 44.61; H, 5.92; N, 1.68%. Found: C, 44.77; H, 5.82; N, 1.68%. MS (FAB): 1535 ($[M - 2Na + 2H]^+$).

Preparation of disodium 6^A,6^C-dianthranilato-γ-cyclodextrin (γ-2)

Compound γ-2 was prepared by the same procedure as γ-1. Yield: 42%. R_f 0.53 (CH_3CN–H_2O 5:1 by volume; TLC; silica gel 60F254). ^{1}H-NMR (DMSO-d_6) = 3.0–3.8 (42H, m, C^2-C^6H of cyclodextrin), 4.1–5.0 (12H, m, O^6H, C^1H of cyclodextrin), 5.6–5.9 (14H, m, C^2H, C^3H of cyclodextrin), 6.52 (2H, t, J = 7.9 Hz, aromatic-H), 6.62 (2H, s, -NH), 6.74 (2H, d-d, J = 7.9, 7.9 Hz, aromatic-H), 7.20 (2H, t, J = 7.9 Hz, aromatic-H), 7.72 (2H, d-d, J = 7.9, 7.9 Hz, aromatic-H). Calcd. for $C_{62}H_{88}O_{42}N_2Na_2 \cdot 5H_2O$: C, 44.61; H, 5.92; N, 1.68%. Found: C, 44.88; H, 6.27; N, 1.58%. MS (FAB): 1557 ($[M - Na + H]^+$).

Preparation of disodium 6^A,6^D-dianthranilato-γ-cyclodextrin (γ-3)

Compound γ-3 was prepared by the same procedure as γ-1. Yield: 16.3%. R_f 0.53 (CH_3CN–H_2O 5:1 by volume; TLC; silica gel 60F254). ^{1}H-NMR (DMSO-d_6) = 3.0–3.8 (42H, m, C^2-C^6H of cyclodextrin), 4.1–5.0 (12H, m, O^6H, C^1H of cyclodextrin), 5.6–5.9 (14H, m, C^2H, C^3H of cyclodextrin), 6.53 (2H, q, J = 7.9 Hz, aromatic-H), 6.61 (2H, s, -NH), 6.75 (2H, t, J = 7.9 Hz, aromatic-H), 7.24 (2H, q, J = 7.9 Hz, aromatic-H), 7.73 (2H, t, J = 7.9 Hz, aromatic-H). Calcd. for $C_{62}H_{88}O_{42}N_2Na_2 \cdot 3H_2O$: C, 45.59; H, 5.80; N, 1.72%. Found: C, 45.62; H, 6.66; N, 1.44%. MS (FAB): 1534 ($[M - 2Na + H]^+$).

Measurements

Ultraviolet, fluorescence, and circular dichroism spectra were measured at 25 °C, with a Perkin-Elmer Lambda 40 UV–VIS spectrophotometer, a Perkin-Elmer LS 40B fluorescence spectrometer, and a JASCO J-700 spectropolarimeter, respectively. For the fluorescence measurements, the excitation wavelength of the fluorescence spectra was 330 nm and excitation and emission slits were 5 nm. Ethylene glycol aqueous solution (10 vol%) was used as solvent for hosts for the spectroscopic measurements because the solubility of them 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 10 vol% ethylene glycol aqueous solution of hosts (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.

Determination of binding constants

The binding constants of nine hosts such as β- and γ-cyclodextrin analogs (β-1–β-4 and γ-1–γ-5, respectively) for several guests were obtained from guest-induced fluorescence variations around 424 nm by employing a Benesi–Hildbrand type equation as reported previously.[12]

Results and discussion

The preparation of 6^A,6^B-, 6^A,6^C- and 6^A,6^D-ditosylated β-cyclodextrins (I, II and III, respectively)[17]

The ditosylated β-cyclodextrins were prepared from β-cyclodextrin with excess of tosyl chloride in pyridine at room temperature. Compounds **I**, **II** and **III** were separated with reverse phase column chromatography. The di-tosylated β-cyclodextrin fractions were eluted with 50 vol% aqueous MeOH solution. The first eluted fractions gave **III**, the next fractions yielded **II** and the final ones gave **I**. Compounds β-1 and β-2 are prepared from 6^A,6^B- and 6^A,6^C-ditosyl substituted β-cyclodextrins treated with sodium anthranilate in DMF at 80 °C as shown in Fig. 2.

The preparation of 6^A,6^B-, 6^A,6^C-, 6^A,6^D- and 6^A,6^E-ditosylated γ-cyclodextrins (IV, V, VI and VII, respectively)[18]

The ditosylated γ-cyclodextrins were prepared from γ-cyclodextrin with an excess of tosyl chloride in pyridine at room temperature. Compounds **IV**, **V**, **VI** and **VII** were separated with reverse phase column chromatography. The ditosylated γ-

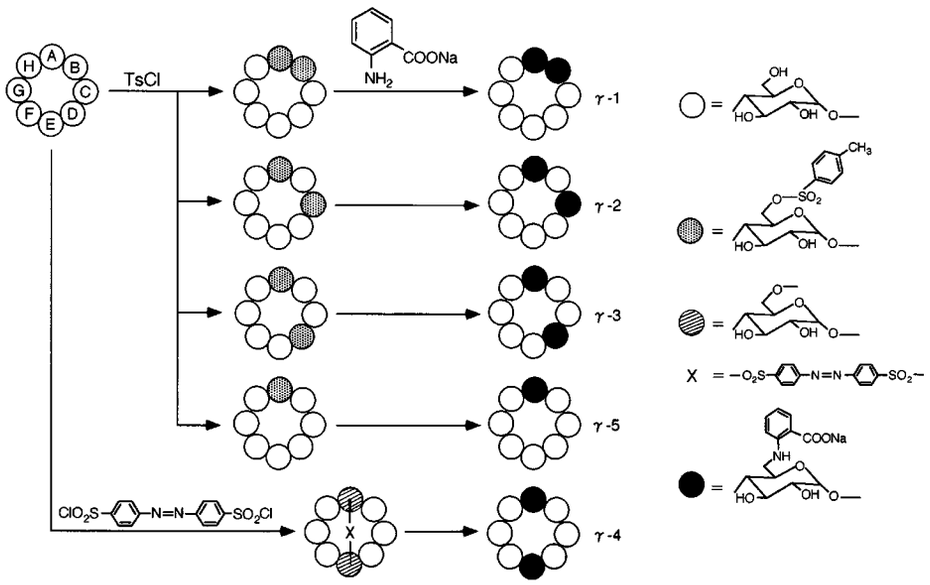


Fig. 3 Preparation of γ-1, γ-2, γ-3, γ-4 and γ-5.

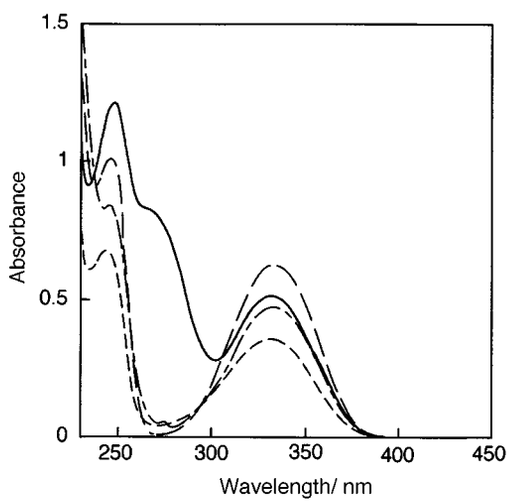


Fig. 4 Absorption spectra of β-1 (–––), β-2 (– – –), β-3 (——), and β-4 (-----) in a 10 vol% ethylene glycol aqueous solution (10^{-4} M, 25 °C).

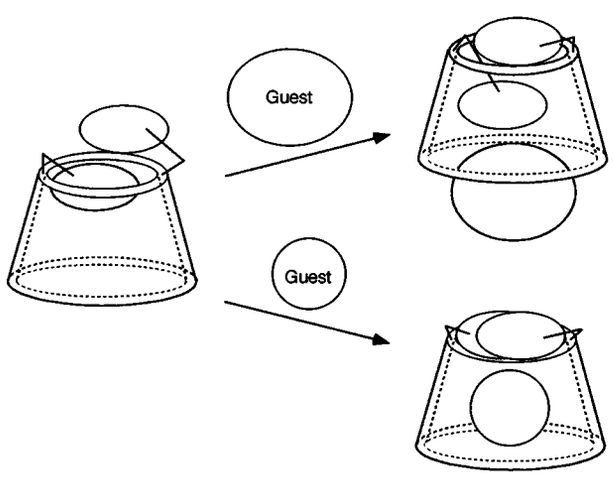


Fig. 5 Host–guest complexation mechanism of β-3 dependent on the molecular size.

cyclodextrin fractions were eluted with 40–50 vol% aqueous MeOH solution. The first eluted fractions gave **VII**, the next fractions yielded **VI** and **V**, and the final fraction gave **IV**. Compounds γ-1, γ-2 and γ-3 are prepared from 6^{A},6^{B}-, 6^{A},6^{C}- and 6^{A},6^{D}-ditosyl substituted γ-cyclodextrins treated with sodium anthranilate in DMF at 80 °C as shown in Fig. 3.

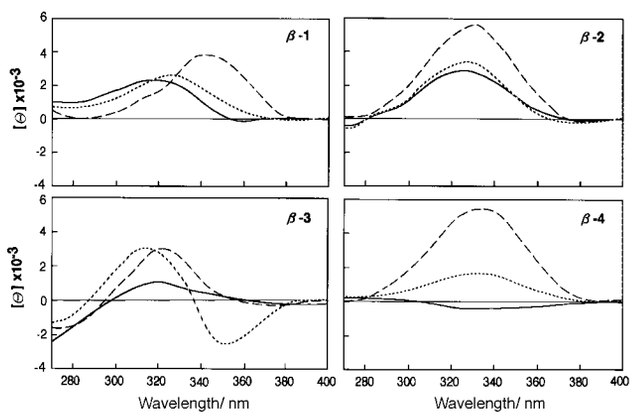


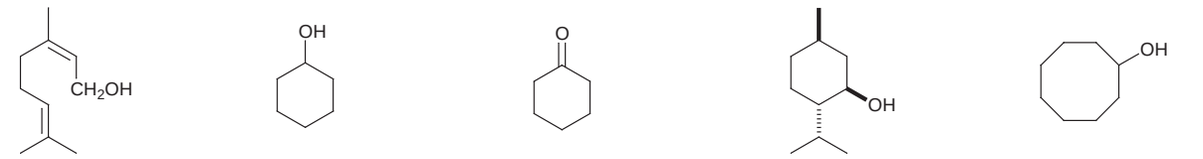
Fig. 6 Induced circular dichroism spectra of β-1, β-2, β-3 and β-4 in a 10 vol% ethylene glycol aqueous solution (10^{-4} M: ——, 25 °C) and containing (−)-menthol (10^{-4} M: ----) and ursodeoxycholic acid (10^{-4}: – –).

UV–VIS spectra

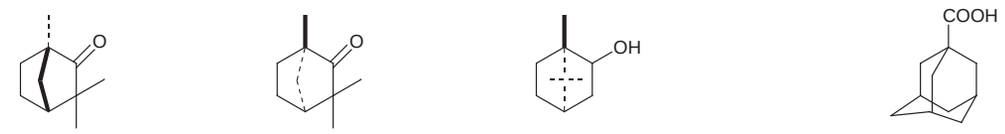
Fig. 4 shows the UV spectra of those hosts. The spectrum of β-3 exhibits peaks at 250, 270 and 340 nm, while the other hosts show two peaks at 250 and 340 nm. The spectrum for β-2 shows a small peak at 270 nm, which suggests that the environment of the appended moieties of the host sets is similar to the environment of β-3 as shown in Fig. 5, which was reported previously.[15] On the other hand, the spectra of γ-analogues are almost the same, which means that the appended moieties are located in a similar environment.

Induced circular dichroism (ICD) spectra

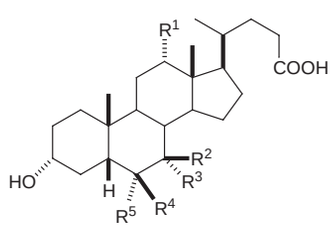
The ICD spectra of four hosts, β-1, β-2, β-3 and β-4, together with five hosts, γ-1, γ-2, γ-3, γ-4 and γ-5, alone or with (−)-menthol or ursodeoxycholic acid in a 10 vol% ethylene glycol aqueous solution were taken to investigate the movement of the appended moieties when host–guest complexation occurs. In Fig. 6, the ICD sign around 330 nm changes from a negative to a positive Cotton peak with an increase of $[\Theta]$ upon addition of a guest to β-4, indicating that the appended moiety penetrates deeply into the hydrophobic cavity of cyclodextrin,[3] as it is well known that an increase in the ICD intensity means the



nerol (**1**) cyclohexanol (**2**) cyclohexanone (**3**) (-)-menthol (**4**) cyclooctanol (**5**)



(+)-fenchone (**6**) (-)-fenchone (**7**) (-)-borneol (**8**) adamantane-1-carboxylic acid (**9**)



lithocholic acid (**10**) : R^1 , R^2 , R^3 , R^4 , R^5 = H

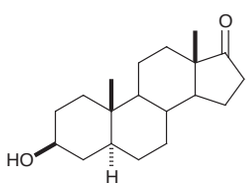
deoxycholic acid (**11**) : R^1 = OH , R^2 , R^3 , R^4 , R^5 = H

chenodeoxycholic acid (**12**) : R^1 , R^2 , R^4 , R^5 = H , R^3 = OH

ursodeoxycholic acid (**13**) : R^1 , R^3 , R^4 , R^5 = H , R^2 = OH

hyodeoxycholic acid (**14**) : R^1 , R^2 , R^3 , R^4 = H , R^5 = OH

cholic acid (**15**) : R^1 , R^3 = OH , R^2 , R^4 , R^5 = H



dehydroepiandrosterone (**16**)

Scheme 1

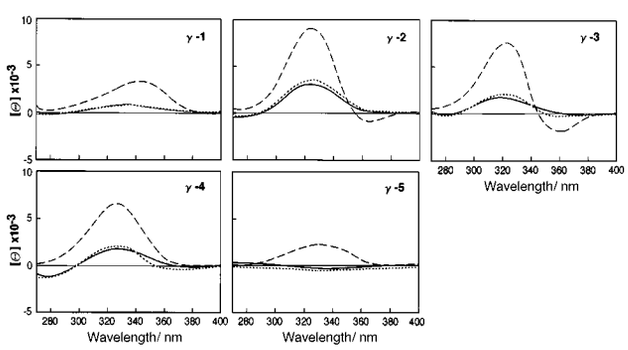


Fig. 7 Induced circular dichroism spectra of γ-1, γ-2, γ-3, γ-4 and γ-5 in a 10 vol% ethylene glycol aqueous solution (10^{-4} M: ——, 25 °C) and containing (−)-menthol (10^{-4}: -----) and ursodeoxycholic acid (10^{-4}: – –).

appended moiety is located in the chiral environment of the cyclodextrin cavity. On the other hand, the ICD spectral patterns of β-1, β-2 and β-3 are different. This difference in the ICD spectra suggests that the movements of the appended moieties of these hosts are quite different. It is suggested that one appended moiety is included in the cavity and another one is located on the rim or outside of the cavity because the Corey–Pauling–Koltun (CPK) model indicates that the cavity space is not large enough to include both of the appended moieties. In the case of γ-analogs, the ICD spectra of γ-1, γ-2, γ-3, γ-4 and γ-5 alone or in the presence of (−)-menthol or ursodeoxycholic acid in a 10 vol% ethylene glycol aqueous solution are shown in Fig. 7. Each host shows a positive peak around 325 nm, except in the case of γ-5, which exhibits a weak negative band. The ICD patterns of γ-1 and γ-5 with ursodeoxycholic acid are very similar. On the other hand, the patterns alone or with (−)-menthol are quite different. It is probable that the location or orientation of each of the appended moieties in the cyclodextrin cavity are similar. It means that two appended moieties of γ-1 show similar behavior to those of γ-5. Hosts γ-2 and γ-3 exhibit similar ICD patterns with ursodeoxycholic acid, too. On the other hand, γ-4 shows a different pattern. These results suggest that the movement of the appended moieties in these hosts is different, affected by the modification positions on the rim of the cyclodextrins.

Fluorescence spectra

Fig. 8 shows the fluorescence spectra of β-2 in a 10 vol% ethylene glycol aqueous solution. The fluorescence spectra of these hosts are composed of almost pure monomer emission with a peak around 424 nm, which are similar to γ-cyclodextrin analogs. The fluorescence intensity of β-1 increases upon addition of all guests examined, indicating that each of the appended moieties is included in the cyclodextrin cavity associated with a host–guest complexation.[9] On the other hand, when a small guest such as (−)-menthol was added to β-3 solution, the fluorescence intensity decreased. This indicates that the movement of the appended moieties for β-1 is different from that of β-3. The fluorescence intensities of γ-1, γ-2 and γ-3 also increase with almost all guests (except (−)-menthol) examined, suggesting that each of the appended moieties is included in the cyclodextrin cavity associated with a host–guest complexation. It is demonstrated that two anthranilate moieties

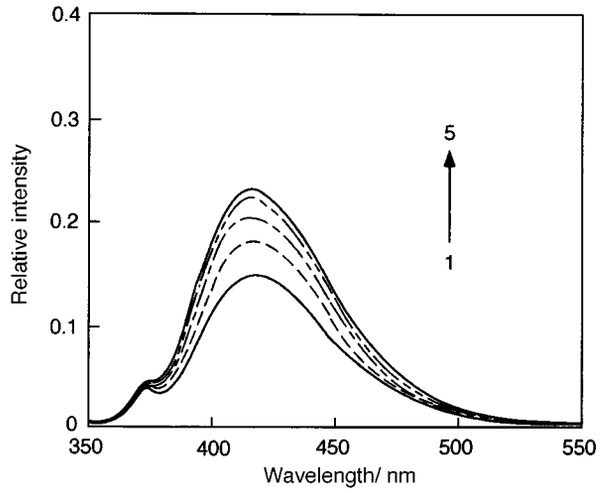


Fig. 8 Fluorescence spectra of β-2 (10^{-6} M, 25 °C) in a 10 vol% ethylene glycol aqueous solution at various concentrations of ursodeoxycholic acid (1: 0, 2: 2.0×10^{-6}, 3: 6.0×10^{-6}, 4: 2.2×10^{-5}, 5: 4.0×10^{-5} M).

are included in the cyclodextrin cavity because the cavity size is larger than that of β-cyclodextrin. On the other hand, γ-4 exhibits a decrease in fluorescence intensity upon inclusion of small guests such as terpenoids, and an increase of the fluorescence intensity with large guests such as bile acids. As reported previously, the extent of the variation of the fluorescence intensity of these hosts depended on the nature of a guest, even at low concentrations; therefore, those hosts can be used as molecular sensors, as seen in the cases of sodium anthranilate modified cyclodextrin analogues reported previously. To show the sensing ability of modified cyclodextrins, the $\Delta I/I^0$ value was used as a sensitivity parameter. Here ΔI is $I - I^0$, where I^0 is the fluorescence intensity for the host alone, and I is the fluorescence intensity for a complex. The parameters for steroids, biologically important substances, are obtained. Because of the solubility of these guests, all steroids were examined at 0.01 mM. Compounds β-1 exhibit positive parameter values for guests examined, indicating an increase in the fluorescence intensity upon guest addition. On the other hand, β-3 shows negative parameters for a smaller guest, but positive ones for larger guests such as bile acids. Compound β-3 detects bile acids with the highest sensitivity and β-1 and β-2 show almost the same sensitivity. Lithocholic acid was detected by these hosts as in sequence β-3 > β-2 > β-1 > β-4, which means that a change in position of the appended moieties affects the sensing ability for bile acids. The sensitivity of β-3 is probably due to the fact that the sodium anthranilate moieties of β-3 can move easily because the distance between the caps is increased in these hosts when a guest is included in the cyclodextrin cavity. Hosts β-1, β-2 and β-3 barely recognize cholic acid. The weak sensitivity of these hosts for cholic acid seems to be caused by its higher polarity in comparison with the other guests because cholic acid has three hydroxy groups in its structure. On the other hand, dehydroepiandrosterone, which has only one hydroxy group in its structure, was hardly detected by these hosts. Although the reason for the differences observed among the steroidal compounds is not clear, it would appear to be linked to the number of hydroxyl groups of the cyclodextrin and the steroids. Fig. 9 shows the parameter values of β-1, β-2, β-3 and β-4 obtained with guests at 1.0 mM, except for adamantane-1-carboxylic acid, which was examined at 0.1 mM because 1.0 mM adamantanecarboxylic acid is not soluble in the host solution. Among these hosts, β-1 shows the highest sensing ability for small guests (**1**–**9**), including terpenoids and adamantane-1-carboxylic acid. Hosts β-1 and β-4 display similar sensing patterns for small guests, but quite different patterns for bigger guests such as bile acids. On the other

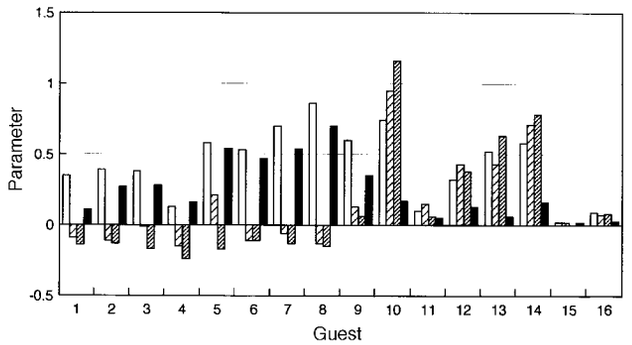


Fig. 9 Guest-induced variations of the monomer emission intensities of β-1 (□), β-2 (▨), β-3 (▨), and β-4 (■) in a 10 vol% ethylene glycol aqueous solution (10^{-6} M, 25 °C) for all guests examined.

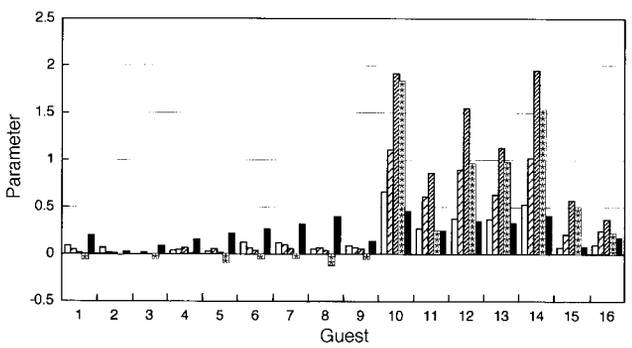


Fig. 10 Guest-induced variations of the monomer emission intensities of γ-1 (□), γ-2 (▨), γ-3 (▨), γ-4 (▣), and γ-5 (■) in a 10 vol% ethylene glycol aqueous solution (10^{-6} M, 25 °C) for all guests examined.

hand, β-2 and β-3 show similar sensing behavior for all guests examined. Fig. 10 shows the parameter values of γ-1, γ-2, γ-3, γ-4 together with the mono-substituted γ-analog (γ-5). Among these hosts, γ-5 shows the highest sensing ability for small guests such as terpenes. Hosts γ-1 and γ-5 display similar sensing patterns for larger guests. The sensing abilities of these hosts for bile acids are roughly in the sequence; γ-3 > γ-4 > γ-2 > γ-1 > γ-5, which shows the effect of the position of modification on the sensing ability for bile acids. Among the steroidal guests, these hosts detect lithocholic acid with the greatest sensitivity, exhibiting values of 1.92, 1.84, 1.11 and 0.66 for γ-3, γ-4, γ-2 and γ-1, respectively. Hyodeoxycholic acid and chenodeoxycholic acid, which bear an extra hydroxy group compared with lithocholic acid, were detected with the next highest sensitivity. Deoxycholic acid, which differs from the other steroids only in the position of one hydroxy group, was detected with lower sensitivity. Cholic acid, which bears one more hydroxy group than chenodeoxycholic acid and ursodeoxycholic acid was hardly detected, probably due to its increased polarity. On the other hand, dehydroepiandrosterone, which has only one hydroxy group in its structure, was hardly detected by these hosts. The guest-induced fluorescence variation at 424 nm was employed to calculate the binding constants of these hosts using eqn. (1) as reported previously.[14]

$$\frac{1}{I_f - I_{f0}} = \frac{1}{a[CD]_0} + \frac{1}{a[CD]_0 K} \times \frac{1}{[G]_0} \qquad (1)$$

The binding constants of four hosts including their monoderivatives for several guests were obtained in order to examine the correlation between the fluorescence variations and the binding abilities of the hosts. The results are shown in Table 1. The binding constants are in the orders **10** > **14** > **13** > **12** for β-1 and β-2, and **14** > **10** > **13** > **12** for β-3, and **10** > **13** > **14** > **12** for β-4, which are roughly parallel with the sensitivity factors

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

Guest	β-1	β-2	β-3	β-4
(−)-Menthol (**4**)	8400 ± 1200[b]	47100 ± 1500	30000 ± 1200	16400 ± 1000
(−)-Borneol (**8**)	355000 ± 28700	115000 ± 6300	65000 ± 1500	28200 ± 1200
Adamantane-1-carboxylic acid (**9**)	405000 ± 6400	91800 ± 5100	528000 ± 45000	337000 ± 18500
Lithocholic acid (**10**)	962000 ± 29600	1570000 ± 37800	180000 ± 22000	413000 ± 11200
Chenodeoxycholic acid (**12**)	46900 ± 3000	45600 ± 4000	61000 ± 1300	32200 ± 2700
Ursodeoxycholic acid (**13**)	231000 ± 6100	303000 ± 8100	170000 ± 8500	290000 ± 19200
Hyodeoxycholic acid (**14**)	266000 ± 10300	561000 ± 22900	350000 ± 9800	226000 ± 16900

[a] The K values were obtained from guest-induced fluorescence variations. [b] The errors were assessed by statistical tests.

Table 2 Binding constants (K/mol^{-1} dm^3) of γ-1, γ-2, γ-3, γ-4 and γ-5 in a 10 vol% ethylene glycol aqueous solution (10^{-6} M, 25 °C)[a]

Guest	γ-1	γ-2	γ-3	γ-4	γ-5
(−)-Menthol (**4**)	1050 ± 80[b]	780 ± 30	2050 ± 150	240 ± 20	750 ± 40
Lithocholic acid (**10**)	1500000 ± 31700	1160000 ± 113000	1090000 ± 64600	1400000 ± 90000	600000 ± 22000
Deoxycholic acid (**11**)	64000 ± 5200	149000 ± 6600	106000 ± 600	76000 ± 14000	79000 ± 4100
Chenodeoxycholic acid (**12**)	43400 ± 1100	339000 ± 12300	211000 ± 9800	78000 ± 2100	120000 ± 3000
Ursodeoxycholic acid (**13**)	134000 ± 11700	171000 ± 11600	175000 ± 9500	95000 ± 2200	270000 ± 9900
Hyodeoxycholic acid (**14**)	158000 ± 10800	661000 ± 19500	457000 ± 17500	190000 ± 5200	610000 ± 3300
Cholic acid (**15**)	58100 ± 5100	32000 ± 800	15900 ± 1800	15000 ± 1500	13000 ± 1300
Dehydroepiandrosterone (**16**)	27800 ± 2900	38600 ± 2400	60800 ± 2500	26000 ± 600	120000 ± 9600

[a] The K values were obtained from guest-induced fluorescence variations. [b] The errors were assessed by statistical tests.

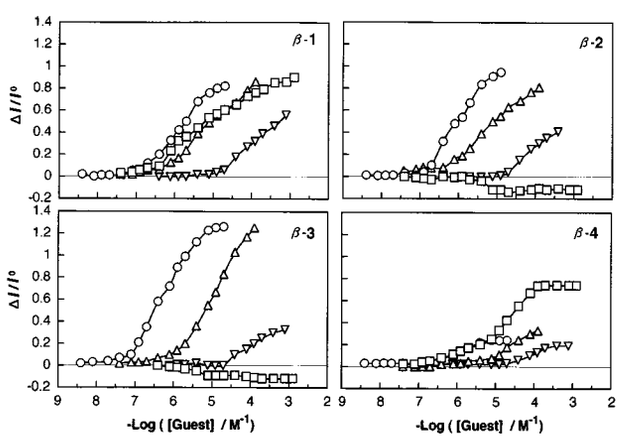


Fig. 11 Fluorescence variations of β-1, β-2, β-3 and β-4 in a 10 vol% ethylene glycol aqueous solution (10^{-6} M, 25 °C) for (−)-borneol (□), lithocholic acid (○), ursodeoxycholic acid (△), and cholic acid (▽) as a function of guest concentration.

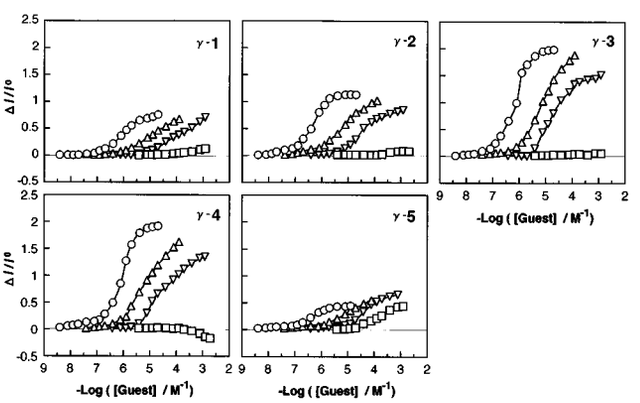


Fig. 12 Fluorescence variations of γ-1, γ-2, γ-3, γ-4 and γ-5 in a 10 vol% ethylene glycol aqueous solution (10^{-6} M, 25 °C) for (−)-borneol (□), lithocholic acid (○), ursodeoxycholic acid (△), and cholic acid (▽) as a function of guest concentration.

except in the case of β-4. In contrast, the order of the binding constants of each host for small guests is not parallel with the order of the sensitivity factor. This means that the sensitivity value gives a relative, but not an absolute, measure of the sensing ability. The binding constants of γ-1, γ-2, γ-3, γ-4 and γ-5[6] for several guests were also obtained in order to examine the correlation between the fluorescence variations and the binding abilities of the hosts. The binding constants obtained are shown in Table 2. The binding constants are in the order **10** > **14** > **13** > **11** > **15** > **12** > **16** > **4** for γ-1 and **10** > **14** > **12** > **13** > **11** > **16** > **15** > **4** for γ-2, γ-3 and **10** > **14** > **12** > **13** > **11** > **16** > **15** > **4** for γ-4, roughly parallel with the sensitivity factors.

Response ranges

Figs. 11 and 12 show response curves of β-1, β-2, β-3 and β-4 and γ-1, γ-2, γ-3, γ-4 and γ-5 for a couple of guests such as (−)-borneol (**8**), lithocholic acid (**10**), ursodeoxycholic acid (**13**), and cholic acid (**15**), respectively. Since these guests were detected with remarkably different responses by the hosts, they are expected to have different response ranges when the guest concentrations are varied. All hosts give clear concentration dependency for the guests, reflecting the sensitivities of the system for the guests. In the case of (−)-borneol (**8**), the response curves of β-analogs of the hosts are different. Hosts β-1 and β-4 show positive parameter values with response ranges of 10^{-7}–10^{-3} M, and $10^{-6.5}$–10^{-4} M, respectively. On the other hand, β-2 and β-3 show negative parameter values, in which there is no clear concentration dependency. Hosts γ-1, γ-2, γ-3 and γ-4 give very clear concentration dependency for bile acids. On the other hand, γ-5 shows a clear concentration dependency for (−)-borneol (**8**) with a response range of 10^{-5} to 10^{-3} M.

Pattern recognition of organic compounds

It is interesting to examine the responses of the nine hosts for each guest compound. Fig. 13 shows the sensing parameters ($\Delta I/I^0$) of nine hosts for each guest at the guest concentration of 1.0 mM, 0.1 mM or 0.01 mM. As can be seen in Fig. 13, (−)-menthol is hardly detected by the nine hosts, exhibiting the parameter value range from 0.18 to 0.02. On the other hand,

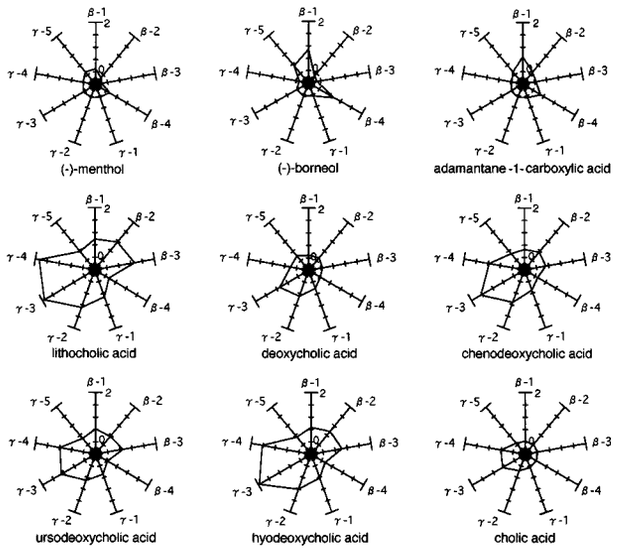


Fig. 13 Variations of sensitivity factors of the nine hosts (10^{-6} M) induced by various organic guests. The guest concentration; (−)-menthol and (−)-borneol: 1 mM, adamantane-1-carboxylic acid: 0.1 mM, bile acid: 0.01 mM.

other guests such as lithocholic acid, chenodeoxycholic acid, ursodeoxycholic acid, and hyodeoxycholic acid give expanded lines with shapes resembling a distorted nonagon. Hosts β-1 and β-4 detect (−)-borneol and adamantane-1-carboxylic acid with high sensitivity, but barely detect larger guests such as bile acids. Host γ-3 recognizes bile acids, except deoxycholic acid and cholic acid, with great sensitivity and γ-4 shows the next highest sensitivity. These results demonstrate that each guest has its own shape and that shape representation is an indication of molecular recognition of the hosts.

Conclusion

Seven analogues of dianthranilate modified β- and γ-cyclodextrins along with their monoanthranilate derivatives have been investigated for their sensing ability towards organic guests including terpenoids and bile acids, 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. The position of modification affects the sensing ability of these hosts, in which the $6^{A},6^{D}$-modification causes the highest sensitivity, probably due to the ease of movement of the appended moieties in the $6^{A},6^{D}$-position upon addition of a guest. It is recognized that the appended moieties of these hosts act as a spacer or hydrophobic cap to elevate the binding ability. It is obvious that the fluorescent-sensory system using such modified cyclodextrins is a very convenient and useful method, because the chemical modification of a guest, even if spectroscopically inert, is not necessary; a guest can be examined directly in the system. The set of nine sensors including three β-cyclodextrins of this series forms different shaped heptagons for different guests from fluorescence responses. Molecular recognition as indicated by such shape representation for the responses of plural sensors might become an important approach to sensing molecules.

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