Synthesis and fluorescent molecular sensing at exciplex emission of pyrene- and cyanobenzene-modified γ-cyclodextrins

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Received (in Cambridge, UK) 22nd August 2000, Accepted 12th January 2001
First published as an Advance Article on the web 9th February 2001

Decrease of exciplex emission of γ-2 with increase of ursodeoxycholic acid concentration (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} M).

Flexible regioselectively hetero-substituted hosts, 6^{A}-*O*-4-pyrenylbutanoyl-6^{X}-*O*-*p*-cyanobenzoyl-modified 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 in order to investigate their fluorescence sensing properties for application to organic compounds such as bile acids and cyclic alcohols. The hosts, γ-1, γ-2 and γ-3, exhibit both monomer and exciplex fluorescence, whereas γ-4 exhibits only monomer emission. In guest-induced emission of γ-1, γ-2 and γ-3, exciplex emission is suppressed while monomer emission is increased. However, γ-4 exhibits only a negligible change in monomer fluorescence in the presence of guests. The extent of exciplex fluorescence variation of γ-1, γ-2 and γ-3 with guests is recognized as the manifestation of the sensing ability of the hosts. A sensing parameter ($\Delta I_{ex}/I^{0}_{ex}$) was used to describe the sensing ability of three hosts. Host γ-analogs, γ-1, γ-2 and γ-3, are able to detect ursodeoxycholic acid, deoxycholic acid, chenodeoxycholic acid, and (−)-borneol with high sensitivity by exciplex emission. The sequence of the binding ability of these hosts is γ-2 > γ-1 > γ-3. The behaviors of the appended moieties of these hosts during the formation of host–guest complexes were studied using induced circular dichroism (ICD) and fluorescence spectra. The host γ-analogs γ-1–γ-3 exhibit different ICD patterns to γ-4 before and after addition of ursodeoxycholic acid. The guest-induced variations of ICD and fluorescence spectra changes suggest that the pyrene and cyanobenzene moieties move, altering the spatial relationship between them.

1 Introduction

Cyclodextrins (CyDs) form inclusion complexes with various organic guests in an aqueous solution.[1,2] CyDs, which are torus-shaped cyclic oligomers of D-glucopyranose, are named α-, β- and γ- for the hexamer, heptamer and octamer, respectively. In investigating the following phenomena, we determined that spectroscopically-active guests should be used because CyDs are basically inert with respect to optical spectroscopy. However, CyDs can be transformed into spectroscopically-active hosts by modification of the chromophore units. Over the past few years, chemo-sensing systems based on chromophore-modified CyDs have been reported. These host molecules and their inclusion complexes exhibit remarkable variations in their circular dichroism, absorption, and fluorescence spectra.[3–16] That is why they can be used as sensing units for organic guests or metal cations with very high sensitivity and selectivity. In particular, the fluorescence intensity changes including monomer and excimer emission of these hosts can be used as a probe to sense guest molecules because the fluorescence spectra are the most sensitive observed in spectrophotometry. Previously, we reported the fluorescence sensing system of dinaphthalene-modified γ-CyDs.[9] It was found that a combination of guest-induced intensity variations of monomer and excimer fluorescence effectively works as a sensing probe showing much more complete and accurate sensing patterns and the position of modification of the appended moieties on the rim of the CyD cavity was found to affect the sensing ability of these host compounds.

Recently, we reported a sensing system based on hetero-double-labelled cyclodextrins, which showed much higher sensing ability than that of mono-double-labelled CyDs. In a series of hetero-labelled cyclodextrin systems, we synthesized regioselectively hetero-modified γ-CyDs, which are 6^{A}-*O*-4-pyrenylbutanoyl-6^{X}-*O*-*p*-cyanobenzoyl-modified γ-CyDs (X = B or H, C or G, D or F, and E for γ-1, γ-2, γ-3, and γ-4, respectively), as a new chemo-sensing system based on the intramolecular exciplex formation of these compounds. These hosts, except γ-4, exhibit a high sensitivity for ursodeoxycholic acid and chenodeoxycholic acid by exciplex emission.

2 Experimental

2.1 Preparations of 6^{A},6^{BorH}-, 6^{A},6^{CorG}-, 6^{A},6^{DorF}-, and 6^{A},6^{E} *O*-4-pyrenylbutanoyl-*O*-*p*-tosyl-modified γ-cyclodextrins (I, II, III, and IV, respectively)

A mixture of 6^{A},6^{B}-di-*O*-(*p*-tosyl-)γ-cyclodextrins[10] (1.365 g, 0.85 mM) and sodium 4-pyrenylbutanoate (344 mg, 1.11 mM) in 10 mL of DMF was heated at 80 °C for 24 h. After cooling, the reaction mixture was poured into 300 mL of acetone. The resulting precipitates were filtered and dissolved in 10 mL of DMF. The DMF soluble fraction was separated in a reverse-phase column (Lober column Lichroprep RP-18). Stepwise elution in 1 L of aqueous MeOH at concentrations of 10, 20, 30, 40, 50, 55, 60 vol% aqueous MeOH were used to obtain **I**. Compounds **II**, **III** and **IV** were prepared by the same procedure as **I**.

I: yield 13.5%. R_f 0.52 (butanol–ethanol–water 5:4:3 by volume, TLC; silica gel $60F_{254}$) and 0.30 (methanol–water 2:1 by volume, TLC; RP-$18F_{254S}$; Merck Ltd.). ^{1}H-NMR (DMSO-d_6) 3.0–3.7 (52H, m, CH_2 and C^2–C^6H of CyD), 3.75–5.0 (6H, m, O^6H of CyD), 5.7–6.0 (16H, m, O^2H and O^3H of CyD), 7.25 (2H, d, J = 8.4 Hz, aromatic-H of tosyl), 7.66 (2H, d, J = 8.4 Hz, aromatic-H of tosyl), 7.95 (1H, d, J = 8.1 Hz, aromatic-H of pyrene), 8.06 (1H, t, J = 7.8 Hz, aromatic-H of pyrene), 8.13

 DOI: 10.1039/b006861f

(2H, s, aromatic-H of pyrene), 8.21 (2H, t, $J = 8.1$ Hz, aromatic-H of pyrene), 8.27 (4H, d, $J = 7.5$ Hz, aromatic-H of pyrene), 8.40 (1H, d, $J = 9.0$ Hz, aromatic-H of pyrene).

II: yield 27.9%. R_f 0.54 (butanol–ethanol–water 5:4:3 by volume, TLC; silica gel $60F_{254}$) and 0.31 (methanol–water 2:1 by volume, TLC; RP-$18F_{254S}$; Merck Ltd.). ^{1}H-NMR (DMSO-d_6) 3.1–3.8 (52H, m, CH_2 and C^2–C^6H of CyD), 3.9–5.0 (6H, m, O^6H of CyD), 5.7–6.0 (16H, m, O^2H and O^3H of CyD), 7.31 (1H, d, $J = 8.4$ Hz, aromatic-H of tosyl), 7.38 (1H, d, $J = 8.4$ Hz, aromatic-H of tosyl), 7.67 (1H, d, $J = 7.5$ Hz, aromatic-H of tosyl), 7.74 (1H, d, $J = 7.5$ Hz, aromatic-H of tosyl), 7.92–7.96 (1H, m, aromatic-H of pyrene), 8.06 (1H, t, $J = 7.5$ Hz, aromatic-H of pyrene), 8.14 (2H, s, aromatic-H of pyrene), 8.21–8.29 (4H, m, aromatic-H of pyrene), 8.35–8.40 (1H, m, aromatic-H of pyrene).

III: yield 23.7%. R_f 0.56 (butanol–ethanol–water 5:4:3 by volume, TLC; silica gel $60F_{254}$) and 0.33 (methanol–water 2:1 by volume, TLC; RP-$18F_{254S}$; Merck Ltd.). ^{1}H-NMR (DMSO-d_6) 3.2–3.7 (52H, m, CH_2 and C^2–C^6H of CyD), 3.8–5.0 (6H, m, O^6H of CyD), 5.7–5.9 (16H, m, O^2H and O^3H of CyD), 7.39 (1H, d, $J = 7.8$ Hz, aromatic-H of tosyl), 7.41 (1H, d, $J = 8.1$ Hz, aromatic-H of tosyl), 7.73 (1H, d, $J = 8.4$ Hz, aromatic-H of tosyl), 7.75 (1H, d, $J = 8.4$ Hz, aromatic-H of tosyl), 7.94 (1H, dd, $J = 1.8$, 1.8 Hz, aromatic-H of pyrene), 8.06 (1H, t, $J = 7.8$ Hz, aromatic-H of pyrene), 8.13 (2H, s, aromatic-H of pyrene), 8.22 (2H, d, $J = 8.1$ Hz, aromatic-H of pyrene), 8.27 (2H, dd, $J = 2.1$, 1.8 Hz, aromatic-H of pyrene), 8.38 (1H, dd, $J = 2.7$, 2.7 Hz, aromatic-H of pyrene).

IV: yield 19.3%. R_f 0.62 (butanol–ethanol–water 5:4:3 by volume, TLC; silica gel $60F_{254}$) and 0.33 (methanol–water 2:1 by volume, TLC; RP-$18F_{254S}$; Merck Ltd.). ^{1}H-NMR (DMSO-d_6) 3.2–3.8 (52H, m, CH_2 and C^2–C^6H of CyD), 3.9–5.0 (6H, m, O^6H of CyD), 5.7–5.9 (16H, m, O^2H and O^3H of CyD), 7.42 (2H, d, $J = 7.8$ Hz, aromatic-H of tosyl), 7.76 (2H, d, $J = 8.1$ Hz, aromatic-H of tosyl), 7.94 (1H, d, $J = 8.4$ Hz, aromatic-H of tosyl), 8.05 (1H, t, $J = 7.8$ Hz, aromatic-H of pyrene), 8.12 (2H, s, aromatic-H of pyrene), 8.22 (2H, d, $J = 7.8$ Hz, aromatic-H of pyrene), 8.27 (2H, dd, $J = 3.3$, 2.1 Hz, aromatic-H of pyrene), 8.38 (1H, d, $J = 9.3$ Hz, aromatic-H of pyrene).

2.2 Preparation of 6^A,6^{BorH}-, 6^A,6^{CorG}-, 6^A,6^{DorF}-, and 6^A,6^E-*O*-4-pyrenylbutanoyl-*O*-*p*-cyanobenzoyl-modified γ-cyclodextrins (γ-1, γ-2, γ-3, and γ-4, respectively)

A mixture of 6^A-*O*-4-pyrenylbutanoyl-6^{BorH}-*O*-*p*-tosyl-modified γ-cyclodextrins (500 mg, 0.29 mM) and sodium *p*-cyanobenzoate in 5 mL of DMF was heated at 80 °C for 24 h. After cooling, the reaction mixture was poured into 300 mL of acetone. The resulting precipitates were filtered and dissolved in 2 mL of DMF. The DMF soluble fraction was separated in a reverse-phase column (Lober column Lichroprep RP-18). Stepwise elution in 300 mL of aqueous MeOH at concentrations of 10, 20 and 30 vol%, and then in 400 mL of aqueous MeOH at 40, 50, 55 and 60 vol% MeOH were used to obtain γ-1. Compounds γ-2, γ-3 and γ-4 were prepared by the same procedure as γ-1.

γ-1: yield 4.5%. R_f 0.49 (butanol–ethanol–water 5:4:3 by volume, TLC; silica gel $60F_{254}$) and 0.57 (methanol–water 2:1 by volume, TLC; RP-$18F_{254S}$; Merck Ltd.). ^{1}H-NMR (DMSO-d_6) 3.1–3.7 (52H, m, CH_2 and C^2–C^6H of CyD), 3.8–5.0 (6H, m, O^6H of CyD), 5.8–6.2 (16H, m, O^2H and O^3H of CyD), 7.95 (1H, t, $J = 8.1$ Hz, aromatic-H of pyrene), 8.06 (1H, t, $J = 8.1$ Hz, aromatic-H of pyrene), 8.14 (2H, s, aromatic-H of pyrene), 8.22–8.29 (8H, m, aromatic-H of pyrene and cyanobenzene), 8.40 (1H, d, $J = 9.6$ Hz, aromatic-H of pyrene). Calcd. for $C_{76}H_{97}O_{42}N{\cdot}5H_2O$: C, 51.12; H, 6.04%. Found: C, 51.22; H, 6.15%. MS (FAB): 1695 ($[M - H]^+$).

γ-2: yield 4.6%. R_f 0.52 (butanol–ethanol–water 5:4:3 by volume, TLC; silica gel $60F_{254}$) and 0.49 (methanol–water 2:1 by volume, TLC; RP-$18F_{254S}$; Merck Ltd.). ^{1}H-NMR (DMSO-d_6) 3.2–3.7 (52H, m, CH_2 and C^2–C^6H of CyD), 3.9–5.0 (6H, m, O^6H of CyD), 5.7–5.9 (16H, m, O^2H and O^3H of CyD), 7.96 (1H, d, $J = 7.8$ Hz, aromatic-H of pyrene), 8.06 (1H, t, $J = 7.7$ Hz, aromatic-H of pyrene), 8.13 (2H, s, aromatic-H of pyrene), 8.22–8.29 (8H, m, aromatic-H of pyrene and cyanobenzene), 8.39 (1H, d, $J = 9.0$ Hz, aromatic-H of pyrene). Calcd. for $C_{76}H_{97}O_{42}N{\cdot}8H_2O$: C, 49.62; H, 6.19%. Found: C, 49.53; H, 6.24%. MS (FAB): 1694 ($[M - 2H]^+$).

γ-3: yield 12.0%. R_f 0.58 (butanol–ethanol–water 5:4:3 by volume, TLC; silica gel $60F_{254}$) and 0.56 (methanol–water 2:1 by volume, TLC; RP-$18F_{254S}$; Merck Ltd.). ^{1}H-NMR (DMSO-d_6) 3.2–3.7 (52H, m, CH_2 and C^2–C^6H of CyD), 3.8–5.0 (6H, m, O^6H of CyD), 5.7–5.9 (16H, m, O^2H and O^3H of CyD), 7.96 (1H, d, $J = 7.8$ Hz, aromatic-H of pyrene), 8.06 (1H, t, $J = 7.8$ Hz, aromatic-H of pyrene), 8.13 (2H, s, aromatic-H of pyrene), 8.22–8.29 (8H, m, aromatic-H of pyrene and cyanobenzene), 8.39 (1H, d, $J = 9.6$ Hz, aromatic-H of pyrene). Calcd. for $C_{76}H_{97}O_{42}N{\cdot}8H_2O$: C, 49.62; H, 6.19%. Found: C, 49.57; H, 6.30%. MS (FAB): 1697 ($[M + H]^+$).

γ-4: yield 28.4%. R_f 0.48 (butanol–ethanol–water 5:4:3 by volume, TLC; silica gel $60F_{254}$) and 0.62 (methanol–water 2:1 by volume, TLC; RP-$18F_{254S}$; Merck Ltd.). ^{1}H-NMR (DMSO-d_6) 3.2–3.7 (52H, m, CH_2 and C^2–C^6H of CyD), 3.8–5.0 (6H, m, O^6H of CyD), 5.7–6.0 (16H, m, O^2H and O^3H of CyD), 7.96 (1H, d, $J = 8.1$ Hz, aromatic-H of pyrene), 8.06 (1H, t, $J = 7.2$ Hz, aromatic-H of pyrene), 8.13 (2H, s, aromatic-H of pyrene), 8.22–8.29 (8H, m, aromatic-H of pyrene and cyanobenzene), 8.40 (1H, d, $J = 9.0$ Hz, aromatic-H of pyrene). Calcd. for $C_{76}H_{97}O_{42}N{\cdot}5H_2O$: C, 51.11; H, 6.04%. Found: C, 51.12; H, 5.83%. MS (FAB): 1696 (M^+).

Hosts γ-1, γ-2, γ-3, and γ-4 were prepared from compounds, **I**, **II**, **II**, and **IV**, respectively, using sodium *p*-cyanobenzoate at 80 °C, as shown in Fig. 1. These hosts were separated by reverse-phase column chromatography (Lobar column LiChroprep RP-18, Merck Ltd. 40–63 mm, 400 × 37 mm), for which the yields were 4.5, 4.6, 12.0, and 28.4%, for γ-1, γ-2, γ-3, and γ-4, respectively. It is suspected that γ-1, γ-2, and γ-3 are isolated as a mixture of diastereomers, including 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- pyrene–cyanobenzene-modified γ-CyDs, respectively. This is because these diastereomers cannot be separated by reverse-phase column chromatography and the existing ratio of these diastereomers was unable to be determined by ^{1}H-NMR analysis. In this paper, the hosts were assumed to exist as diastereomers and have been named γ-1 for 6^A,6^B- and 6^A,6^H-, γ-2 for 6^A,6^C- and 6^A,6^G-, and γ-3 for 6^A,6^D- and 6^A,6^F- pyrene–cyanobenzene-modified γ-CyDs.

2.3 Measurements

Fluorescence and circular dichroism spectra were measured at 25 °C using a Perkin-Elmer LS 40B fluorescence spectrometer and a JASCO J-720 spectropolarimeter, respectively. For fluorescence measurements, the excitation wavelength of the fluorescence spectra was 355 nm and the excitation and emission slits were 10 nm. Aqueous ethylene glycol (10 vol%) was used as the solvent for the hosts for spectroscopic measurements because the solubility of these hosts in pure water is poor. Five μL of guest species (0.5 and 0.05 M), in dimethyl sulfoxide (DMSO) or MeOH, were injected into a 10 vol% ethylene glycol aqueous solution of the hosts (2.5 mL) to make a sample solution with a host concentration of 2.0×10^{-6} M and a guest concentration of 1.0 and 0.1 mM, respectively.

2.4 Determination of binding constants

The binding constants of three hosts, γ-1, γ-2 and γ-3, for several guests were obtained from guest-induced exciplex fluorescence variations around 478 nm by employing a Benesi–Hildebrand-type equation, as reported previously.[10]

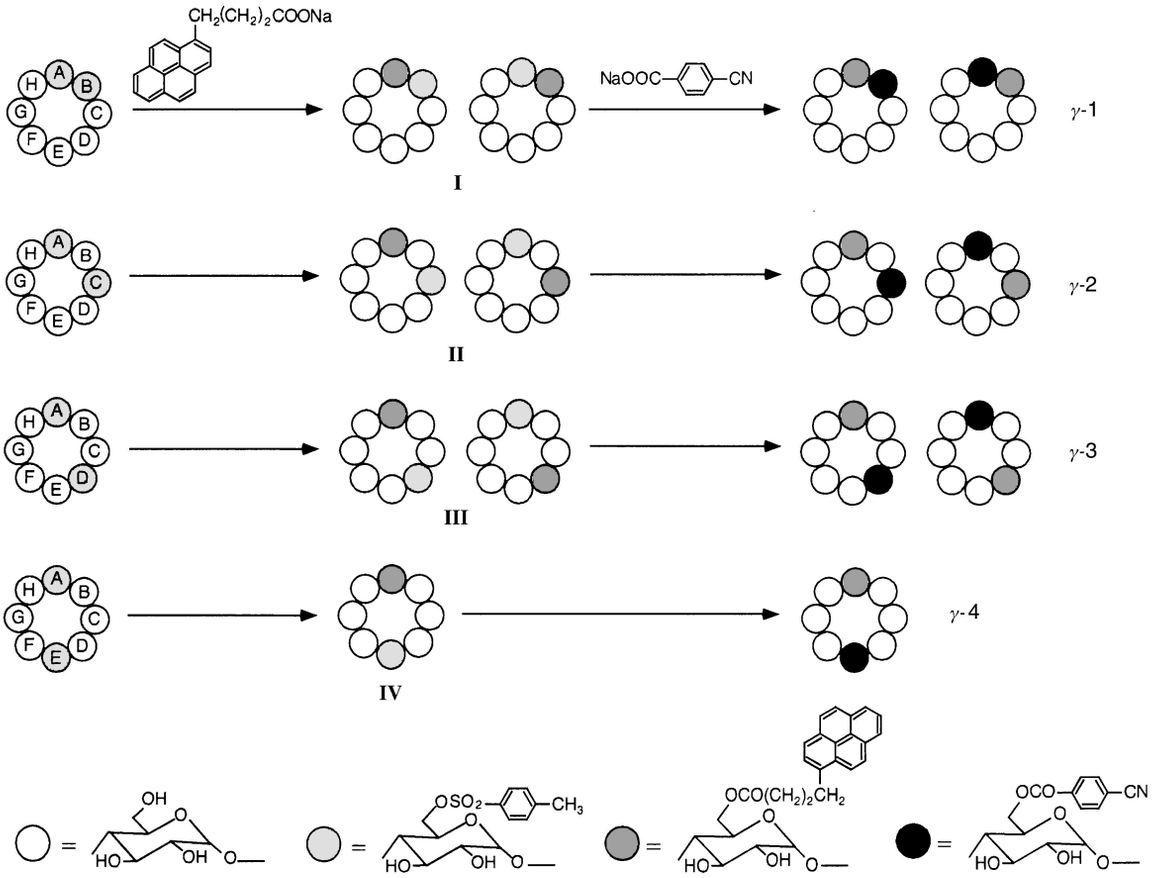


Fig. 1 Preparation of γ-1, γ-2, γ-3 and γ-4.

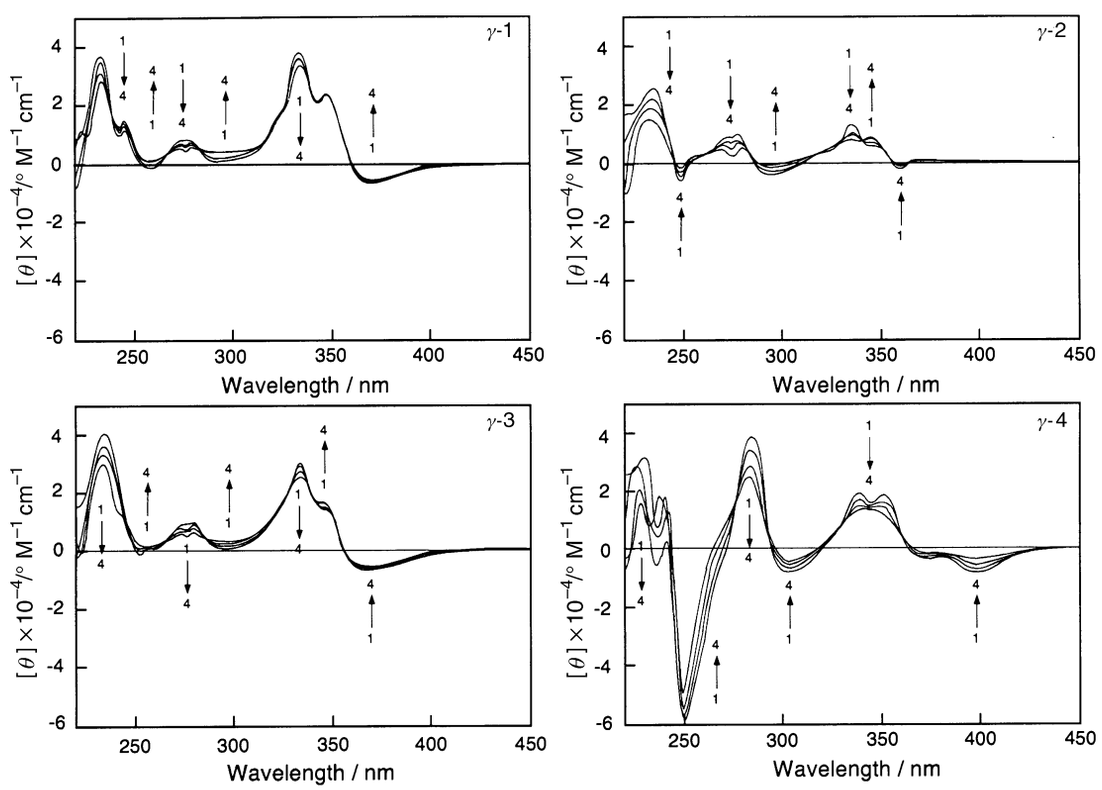


Fig. 2 ICD spectra of γ-1, γ-2, γ-3, and γ-4 in a vol% ethylene glycol aqueous solution (1.0×10^{-4} M, 25 °C) at various concentrations of ursodeoxycholic acid (1: 0, 2: 6.0×10^{-5}, 3: 1.0×10^{-4}, 4: 1.4×10^{-4} M).

3 Results and discussion

3.1 Induced circular dichroism (ICD) spectra

The ICD spectra of four hosts, 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. The ICD spectra of γ-1, γ-2 and γ-3, alone, show positive bands at around 235, 275, 280, 335, and 350 nm, and the [θ] values of γ-4, alone, are positive at around 230, 240, 285, 335, and 350 nm. The spectra of γ-1 and γ-3, alone, exhibit a negative band at around 370 nm

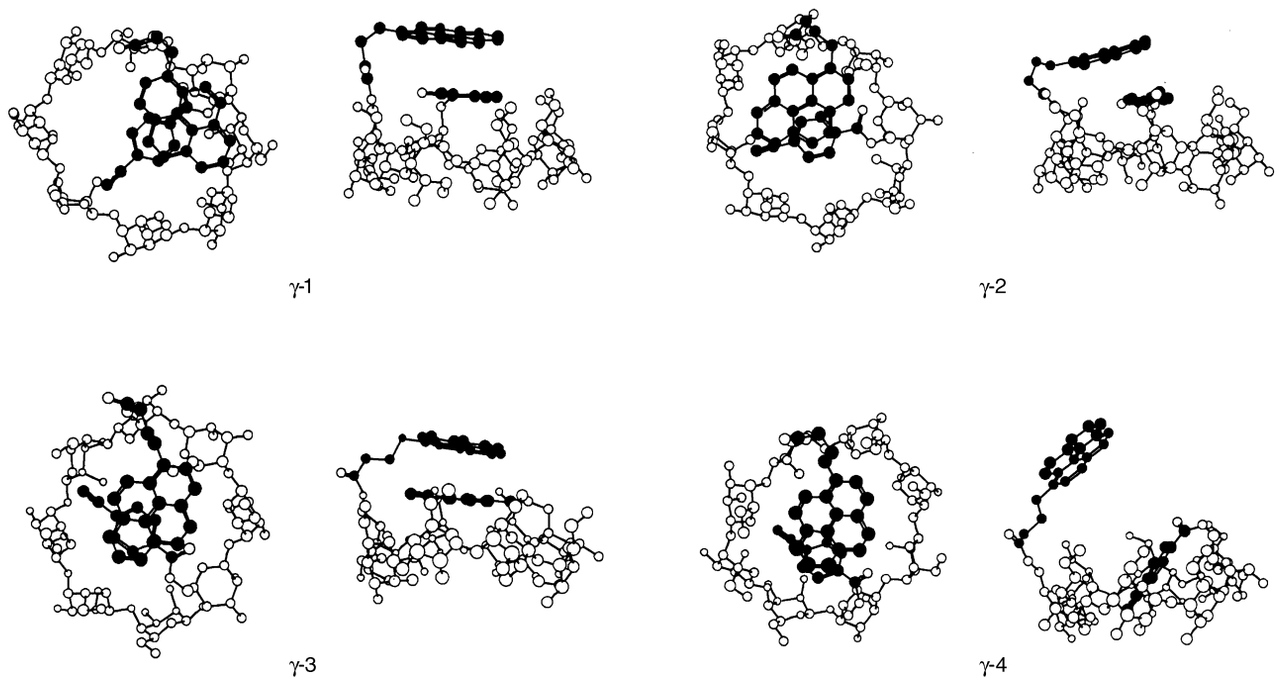


Fig. 3 Energy-minimized structures of γ-1, γ-2, γ-3, and γ-4 obtained using molecular mechanics in CS Chem 3D.

and that of γ-2 exhibits negative bands at around 250, 295 and 360 nm. By contrast, the spectra of γ-4, alone, exhibit an intense negative band at around 250 nm, and other smaller bands at 305, 375, and 400 nm. Each of the hosts exhibits different ICD patterns. This suggests that the appended moieties of each host adopt different positions. The energy-minimized structures of the four hosts obtained using molecular mechanics in CS Chem 3D (MM2), as shown in Fig. 3, suggest that the hetero-appended moieties of γ-1 and γ-3 are located parallel to the CyD equator, whereas those of γ-2 are inclined to the CyD equator. Although, it is recognized that the hetero-appended moieties of γ-4 are not parallel; the pyrene moiety is located outside the CyD cavity and the cyanobenzene moiety is included inside the CyD cavity. These three-dimensional structures of the four hosts support the differences in the ICD patterns of the hosts alone. The ICD intensity and positive and negative Cotton peaks of the hosts decrease on the addition of a guest. Furthermore, the decrease in [θ] values of γ-4 at each Cotton peak is greater than those of the other hosts. It seems that the cyanobenzene moiety of γ-4 moves away from the chiral environment of the CyD cavity upon addition of a guest.

3.2 Fluorescence spectra

The fluorescence spectra of γ-1, γ-2, γ-3, and γ-4 in a 10 vol% aqueous ethylene glycol solution in the presence and absence of ursodeoxycholic acid are shown in Fig. 4. The spectra of γ-1, γ-2 and γ-3, alone, exhibit exciplex emission with a peak at around 478 nm, and also monomer emission, whereas the spectrum of γ-4 is composed solely of monomer emission with much weaker intensity than those of other three hosts. It has been reported that the exciplex intensity of the β-(1-pyrenyl) ethyl-*p*-cyanobenzoate system has been observed in less polar media such as the CyD cavity.[24] The exciplex emission observed in these hosts, γ-1, γ-2 and γ-3, indicates that hetero-moieties, pyrene and cyanobenzene, adopt a face-to-face orientation,[9,17–23] which is needed for exciplex formation. On the other hand, the pyrene moiety of γ-4 appears to exist outside of the CyD cavity, because there are small monomer and no exciplex emissions. The exciplex emission of γ-1, γ-2 and γ-3 decreases with increasing ursodeoxycholic acid concentration. However, the fluorescence spectra of γ-4 change only negligibly upon addition of guests. The results obtained in the ICD and fluorescence spectra suggest that pyrene moieties of γ-1, γ-2 and γ-3 move far away from the chiral environment of the CyD cavity at the time when a guest is included in the CyD cavity, and acts as a hydrophobic cap. On the other hand, it appears that the pyrene moiety of γ-4 exists outside of the CyD cavity before and after addition of guests, therefore γ-4 does not exhibit exciplex emission and its monomer emission is small because the appended moieties exist in a less hydrophobic environment outside of CyD cavity. Furthermore, as illustrated in Scheme 1, it is envisaged that the cyanobenzene moieties of γ-1, γ-2 and γ-3 are excluded from the CyD cavity upon the addition of guests, thus quenching exciplex emission between the pyrene and cyanobenzene moieties, and the cyanobenzene of γ-4 moves from inside the CyD cavity to the CyD rim on the addition of guests. As reported previously,[10] the degree of variation in the fluorescence intensity of modified CyDs is affected by the presence of guest molecules, even at low concentrations, therefore, these hosts can be used as fluorescent molecule sensors. In order to evaluate the sensing ability of these hosts, the $\Delta I_{ex}/I^0_{ex}$ value was used as a sensitivity parameter. Here, ΔI_{ex} is $\Delta I^0_{ex} - I_{ex}$, where I^0_{ex} and I_{ex} are the intensities of exciplex emission at around 478 nm for each host, alone and in the presence of a guest, respectively. The parameter values of γ-1, γ-2, and γ-3 obtained using steroids at 0.1 mM and alcohols such as (−)-borneol and cyclooctanol at 1.0 mM are shown in Fig. 5. Ursodeoxycholic acid (**1**) and chenodeoxycholic acid (**3**), which are diastereoisomers, bearing two hydroxy groups on C-3 and C-7 in a steroidal framework, are detected with remarkably high sensitivity, exhibiting values of 0.834, 0.764 and 0.398 for γ-2, γ-1 and γ-3, and 0.814, 0.731 and 0.455 for γ-2, γ-1 and γ-3, respectively. Deoxycholic acid (**2**), which is a constitutional isomer of guests **1** and **3**, bearing two hydroxy groups on C-3 and C-12 in a steroidal framework, and (−)-borneol (**4**) are detected with high sensitivity, exhibiting values of 0.609, 0.578 and 0.455 for γ-2, γ-1 and γ-3 and 0.684 and 0.622 for γ-2 and γ-1, respectively. However, host γ-3 is relatively insensitive to guest **4**, exhibiting a value of 0.124. The sensing ability of γ-1 and γ-2 for cyclooctanol (**5**) is lower than for other guests, exhibiting values of 0.212 and 0.208 for γ-1 and γ-2, respectively. However, the sensing ability of γ-3 for guest **5** is higher than that of γ-3 for guest **4**, with a value of 0.165. The sensing factors for bile acids by γ-1, γ-2 and γ-3

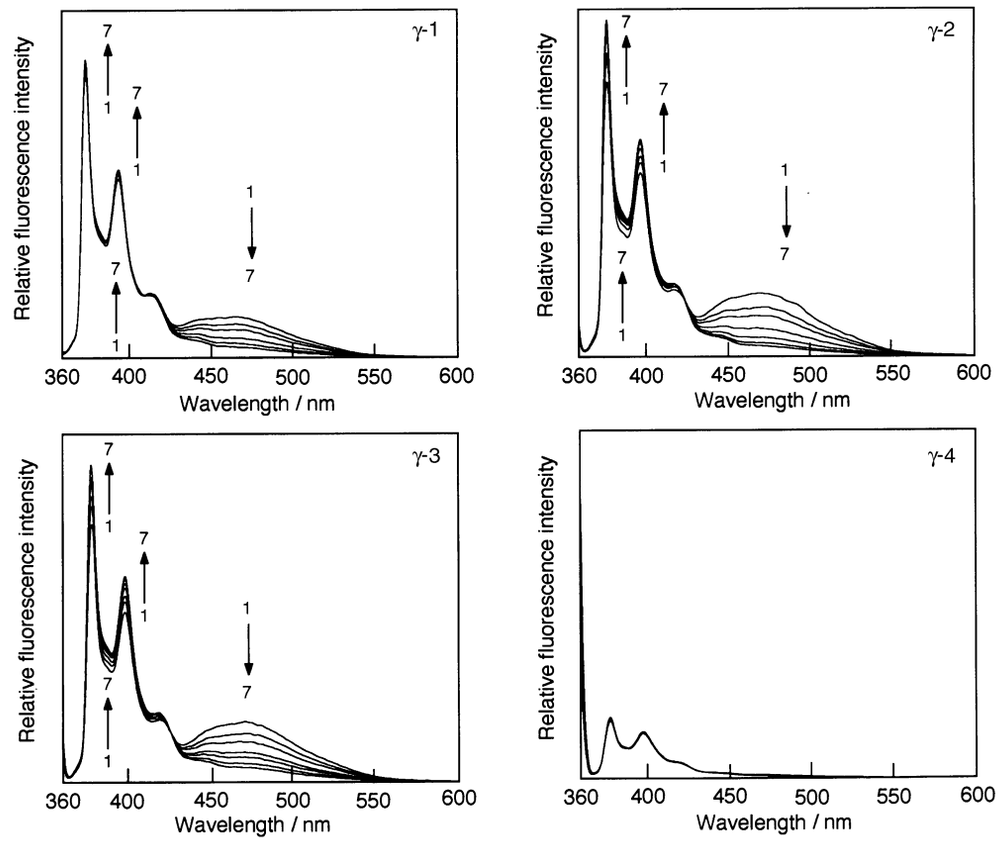
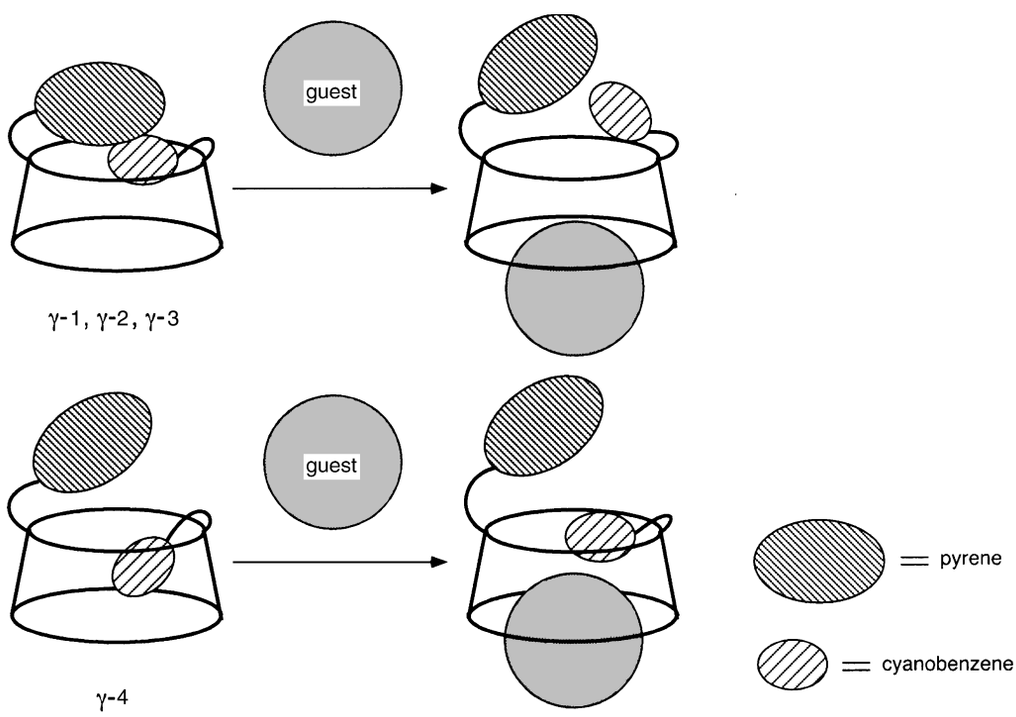


Fig. 4 Fluorescence spectra of γ-1, γ-2, γ-3, and γ-4 in a 10 vol% ethylene glycol aqueous solution (2.0×10^{-6} M, 25 °C) at various concentrations of ursodeoxycholic acid (1: 0, 2: 1.0×10^{-5}, 3: 2.0×10^{-5}, 4: 4.0×10^{-5}, 5: 6.0×10^{-5}, 6: 8.0×10^{-5}, 7: 1.0×10^{-4} M).



Scheme 1 Proposals for the host–guest complexation mechanisms of γ-1, γ-2, γ-3 and γ-4.

decrease in the sequence, **1** > **3** > **2**. This indicates that the position of hydroxy groups in the guests affects the sensitivity of the hosts. What is probably happening is that guests **1** and **3** enter the CyD cavity from the site of carboxylic acid and not the hydroxy group site C-3 in the steroidal group. This would occur by hydrophobic interaction and hydrogen bonding between the carboxylic acid and the hydrophobic group of the CyD cavity. It is also probable that guest **2** enters into the CyD cavity from the hydroxy group site C-3 in the steroidal framework because the area around the carboxylic acid is crowded with hydroxy groups at the 12-position in the steroidal framework. The sensitivity of the hosts for all the guests is roughly in the order γ-2 > γ-1 > γ-3. This suggests that the position of hetero-appended moieties, pyrene and cyanobenzene, in the CyD rim of the hosts affects their ability to recognize guests. The $6^{A},6^{CorG}$ modification indicates the highest sensitivity,

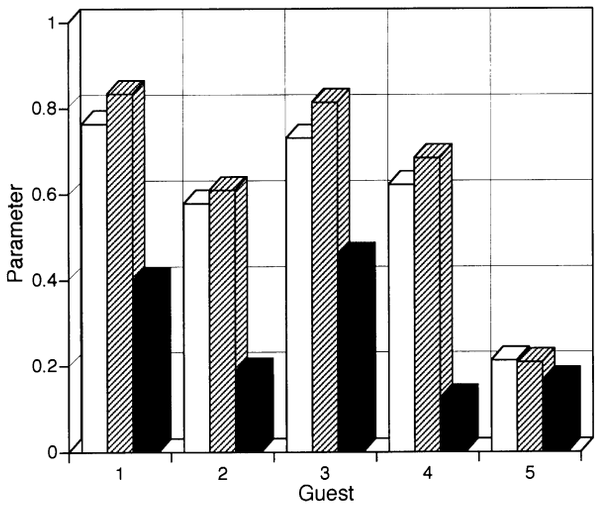
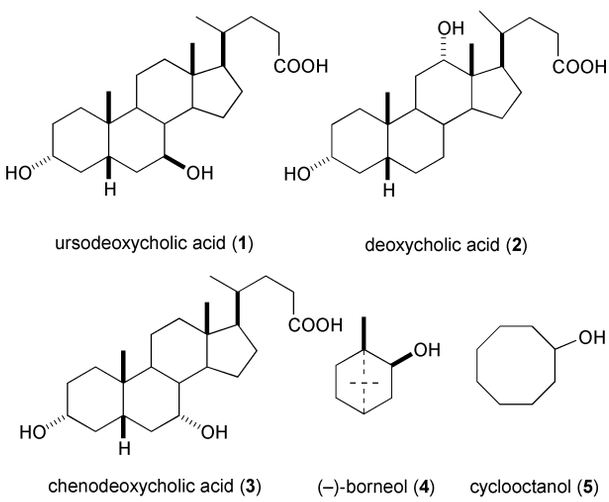


Fig. 5 Guest-induced intensity variations of exciplex emission ($\Delta I_{ex}/I^0_{ex}$) at 478 nm of γ-1 (□), γ-2 (▨), and γ-3 (■) in a 10 vol% ethylene glycol aqueous solution (2.0×10^{-6} M, 25 °C) for the guests examined.



probably due to the ease of movement of appended moieties in the 6^A,6^{CorG}-position upon addition of guests. These sensing parameters by exciplex emissions are higher than those given from excimer emissions of dipyrene or dinaphthalene-modified γ-CyDs reported previously.[9,23] It is the first example, to the best of our knowledge, of the detection of organic guests based on exciplex emission with higher sensitivity in the CyD systems than those of excimer emission, which should be a favorable and useful new CyD chemo-sensor system.

3.3 Binding constants

The guest-induced fluorescence of exciplex emission variation at 478 nm was used in eqn. (1) to calculate the binding constants, *K*, of the hosts.

$$\frac{1}{I_{ex} - I^0_{ex}} = \frac{1}{a[CD]} + \frac{1}{b[CD]K} \times \frac{1}{[G]} \qquad (1)$$

Here, *I* is the fluorescence intensity of exciplex emission at 478 nm (I_{ex} for complex, I^0_{ex} for the host alone), [CD] is the total host concentration, [G] is the total guest concentration, *a* and *b* are constants. The binding constants of three hosts, γ-1, γ-2 and γ-3, were obtained in order to examine the correlation between the variations of exciplex fluorescence and binding of the hosts. The results are listed in Table 1. The binding constants are in the order **3** > **2** > **1** > **4** > **5** for γ-1 and γ-2, and **3** > **1** > **2** > **4** > **5** for γ-3. The order of binding constants of the three hosts does not parallel the order of sensitivity, which indicates that the sensitivity values obtained are relative and not absolute. It is assumed that when a guest concentration range is varied, the sensing ability of the hosts changes also.

Table 1 Binding constants (K/dm^3 mol^{-1}) of γ-1, γ-2 and γ-3 in a 10 vol% ethylene glycol aqueous solution (2.0×10^{-6} M, 25 °C)[a]

Guest	K/mol^{-1} dm^3		
	γ-1	γ-2	γ-3
Ursodeoxycholic acid (**1**)	14 300 ± 620[b]	23 400 ± 1180	22 500 ± 860
Deoxycholic acid (**2**)	20 600 ± 1160	25 400 ± 2000	9300 ± 330
Chenodeoxycholic acid (**3**)	36 300 ± 2420	31 200 ± 1350	28 300 ± 770
Borneol (**4**)	2500 ± 50	3450 ± 230	2780 ± 140
Cyclooctanol (**5**)	1460 ± 80	930 ± 90	890 ± 60

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

4 Conclusion

Four hetero-, pyrene- and cyanobenzene-modified γ-cyclodextrins were investigated as new chemo-sensors for organic guests such as bile acids and terpenoids, which are biologically significant substances. Some of these hosts, γ-1, γ-2 and γ-3, exhibit monomer and exciplex fluorescence. To the best of our knowledge, this is the first time that this phenomenon has been observed in the area of CyD chemistry. The variation of host exciplex emissions was used as a parameter for describing their sensing ability. The introduction of hetero functional groups such as pyrene and cyanobenzene, which are in different positions such as 6^A and 6^X in the CyD cavity, gives new sensing factors that impart high sensitivity and selectivity to these hosts. A fluorescent molecular sensory system using such hetero-modified CyDs is a very convenient and useful method because guests can be detected directly in this system, even if the guest is spectroscopically inert. In the present study it has been established that the modification of host molecules with hetero-groups is an attractive method for improving or altering host functionality, and will result in the emergence of a next generation of host–guest chemistry.

Acknowledgements

This study was supported by a Grant-in-Aid for Specially Promoted Research (No. 404: Molecular Synchronization for Design of New Materials System) from the Ministry of Education, Science, Sports and Culture of Japan.

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