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Synthesis of a Selective Fluorescent Sensing System Based on γ-Cyclodextrin Modified with Pyrene and Tosyl on the Hetero Rims

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Abstract

The hetero-functionalized host bearing pyrene and tosyl moieties substituted at the lower and upper rims of cyclodextrin, namely, mono-3^A-deoxy-3^A-pyrenebutylamido-6^X-O-mono- (*p*-toluenesulfonyl)mono-altro-γ-cyclodextrin (**mixture γ-1**; X = A, B, C, D, E, F, G, or H), has been synthesized in order to investigate its fluorescent sensing system for organic guests such as steroids and terpenoids. This host showed pure monomer fluorescence, in which the host fluorescence increased in intensity on accommodation of bile acids and terpenoids or decreased in fluorescence intensity upon addition of ketosteroids. The extent of fluorescence variation with a guest was employed to evaluate the sensing ability of the host. The sensing parameter $\Delta I/I^\circ$, where I and I° are the fluorescence intensities in the presence and absence of a guest and ΔI is $I - I^\circ$, was used to describe the sensing ability. The host detected ketosteroids with negative parameters, on the other hand, bile acids and terpenoids were recognized by the host with positive parameters. The behaviors of the appended moieties of the host during host–guest complexation were studied by induced circular dichroism (ICD) and fluorescence spectra. The host compound showed different patterns depending on the guest. The guest-induced variations in the ICD and fluorescent spectra suggest that the pyrene moiety acts to elevate the binding ability and the tosyl moiety plays a roll as a spacer to regulate the cyclodextrin cavity size.

Introduction

Cyclodextrins (CDs) have attracted interest as enzyme models or supramolecular compounds, because they can accommodate a variety of guests such as organic molecules or metals in their cavities in an aqueous solution [1–3]. CDs are torus-shaped cyclic oligomers composed of 1,4-linked D-glucopyranose and are named α-, β- and γ- for the hexamer, heptamer and octamer, respectively, bearing wide and narrow hydrophilic ends delineated by the C-2 and C-3 secondary and C-6 primary hydroxyl groups. Since CDs are spectroscopically inert, modification of the native CDs with a spectroscopically active unit can convert them into spectroscopically active hosts. For at least a decade, we have discussed fluorescent molecular sensing systems of CDs modified with chromophores such as naphthalene [4], fluorescein [5], terphenyl [6], pyrrolinone [7], anthranilate [8], and dansyl [9, 10] moieties which are substituted at the C-6 position. In these papers, we have found that these fluorescent CDs were useful as an indicator for bile acids or terpenoids and these mechanisms of the host–guest complexation were based on an induced-fit type. In contrast, there are few papers which describe the fluorescent binding property of CD derivatives substituted at the C-2 or C-3 positions [11–16]. Sulfonation of the secondary hydroxyl groups as a precursor to the modification of the CDs has been reported to be more difficult, in which the reaction conditions have required a specific sulfonation reagent [17], alkaline conditions [18, 19], strict anhydrous conditions [20, 21], and use of protected C-6 hydroxyl groups [22, 23]. Therefore, it is very interesting that a new fluorescent CD labelled at the upper and lower rims is synthesized in order to study its molecular sensory system. Teranishi and co-workers have prepared mono- and regioselectively di-sulfonyl β- and γ-CDs at the C-2 position from sulfonyl imidazole treated with molecular sieves 4A, in which these procedures are conveniently used under mild condition [24–27]. In this contribution, we describe the synthesis and fluorescent sensing ability of mono-3^A-deoxy-3^A-pyrenebutylamido-6^X-O-mono-(*p*-toluenesulfonyl)mono-altro-γ-CD (**mixture γ-1**; X = A, B, C, D, E, F, G, or H). This host exhibits a qualitative molecular recognition ability for ketosteroids, bile acids and (−)-borneol, based on an induced-fit type of complex behavior as shown in the case of other CD derivatives.

Experimental

*Preparation of mono-2-O-(p-toluenesulfonyl)γ-CD (***I***) [25]*

To a solution of 3.0 g (2.31 mmol) of γ-CD and 880 mg (3.96 mmol) of *p*-toluenesulfonyl imidazole in 50 mL of

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DMF was added 7.44 g of freshly active molecular sieve 4A (powder), and the mixture was stirred at 20 °C for 120 h. The reaction mixture was filtered to remove molecular sieve 4A, and the filtrate was poured into 300 mL of acetone. The resulting precipitate was filtered off and dissolved in 15 mL of DMF. The DMF soluble fraction was applied to a reversed-phase column (Lobar column LiChroprep RP-18, Merck Ltd. 310 × 2 mm). Stepwise elution using 300 mL of 10 vol.%, 500 mL of 20 vol.% and 40 mL of 30 vol.% aqueous MeOH, and 600 mL of 40 vol.% aqueous MeOH was applied to obtain pure compound **I** (836 mg, 24.9%). R_f 0.49 (butanol-ethanol-water 5:4:3 by volume, TLC; silica gel $60F_{254}$), and 0.61 (MeOH-water 1:1, by volume, TLC; RP-$18F_{254S}$; Merck Ltd.). ^{1}H-NMR (DMSO-d_6): δ = 2.42 (3H, s, CH_3 of *p*-tosyl), 3.3–3.8 (46H, m, C^2H—C^6H of CD), 3.87 (1H, t, J = 8.7 Hz, C^3H of glucose unit with *p*-tosyl), 4.09 (1H, d-d, J = 3.7 and 9.2 Hz, C^2H of glucose unit with *p*-tosyl), 4.34 (1H, d, J = 8.7 Hz, C^1H of glucose unit with *p*-tosyl), 4.5–4.7 (8H, m, O^6H of CD), 4.8–4.95 (7H, m, C^1H of glucose units without *p*-tosyl), 5.6–5.8 (15H, m, O^2H and O^3H of CD), 7.44 (2H, d, J = 8.4 Hz, aromatic-H of *p*-tosyl), 7.85 (2H, d, J = 8.1 Hz, aromatic-H of *p*-tosyl).

*Preparation of mono-3-deoxy-3-amino-mono-altro-γ-CD (***II***) [27]*

A solution of 860 mg (0.59 mmol) of compound **1** in 30 mL of 28% aqueous NH_3 was heated at 20 °C for 120 h. The reaction mixture was poured into 300 mL of acetone. The resulting precipitate was filtered off and dried to give the crude product **II** (560 mg, 72.9% crude yield). The crude compound **II** was used as the starting material for compound **III**. R_f 0.15 (methyl ethyl ketone-methanol-acetic acid 12:3:5 by volume, TLC; silica gel $60F_{254}$). ^{1}H-NMR (DMSO-d_6): δ = 3.3–3.8 (46H, m, C^2H—C^6H of CD), 3.86 (1H, m, C^3H of glucose unit with amine), 3.92 (1H, m, C^2H of glucose unit with amine), 4.5–4.8 (8H, m, O^6H of CD), 4.8–5.05 (8H, m, C^1H of CD), 5.7–5.9 (15H, m, O^2H and O^3H of CD).

*Preparation of mono-3-deoxy-3-pyrenebutylamido-mono-altro-γ-CD (***III***)*

To a cool solution (−10 °C) of 260 mg (0.90 mmol) of 1-pyrenebutylate in 4 mL of DMF was added 185 mg (0.90 mmol) of dicyclohexyl carbodiimide (DCC) and 120 mg (0.90 mmol) of 1-hydroxylbenzotriazole (1-HOBt). The reaction mixture was stirred at −10 °C for 30 min. To a stirred solution was added portionwise 583 mg (0.45 mmol) of compound **II**, and the solution was stirred at −10 °C for 30 min, and then the reaction mixture was stirred at 60 °C for 24 h. After cooling, the reaction mixture was concentrated under reduced pressure. The residue was poured into 300 mL of acetone. The resulting precipitates were filtered and dried. The DMF soluble fraction was applied on a reversed-column (Lobar column LiChroprep RP-18, Merck Ltd. 310 × 24 mm). Stepwise elution using 300 mL of 10 vol.%, 200 mL of 20 vol.% and 300 mL of 30 vol.% aqueous MeOH, and 300 mL of 40 vol.% aqueous MeOH was used to give compound **III**. The fractions were collected, and dried to afford pure compound **III** (113 mg, 15.9%, isolated yield). R_f 0.50 (butanol-ethanol-water 5:4:3 by volume, TLC; silica gel $60F_{254}$), and 0.75 (MeOH-water 2:1, by volume, TLC; RP-$18F_{254S}$; Merck Ltd.). ^{1}H-NMR (DMSO-d_6): δ = 3.2–3.8 (46H, m, C^2H—C^6H of CD), 3.85–3.9 (1H, m, C^3H of glucose unit with pyrene), 4.1–4.2 (1H, m, C^2H of glucose unit with pyrene), 4.4–4.65 (8H, m, O^6H of CD), 4.65–5.05 (8H, m, C^1H of CD), 5.6–6.0 (15H, m, O^2H and O^3H of CD), 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), 8.21–8.30 (4H, m, aromatic-H of pyrene), 8.42 (1H, d, J = 9.6 Hz, aromatic-H of pyrene).

*Preparation of mono-3^A-deoxy-3^A-pyrenebutylamido-6^X-O-mono-(p-toluene-sulfonyl)mono-altro-γ-CD (***mixture γ-1***)*

103 mg (0.065 mmol) of compound **III** was added to a solution of 96 mg (0.52 mmol) of *p*-toluenesulfonylchloride in 7 mL of pyridine, and the reaction mixture was stirred for 7 h at room temperature. The reaction mixture was concentrated in vacuo, and the product was poured into 200 mL of acetone. The resulting precipitate was filtered off and dissolved in 2 mL of DMF. The DMF soluble fraction was applied to a reversed-phase column (Lobar column LiChroprep RP-18, 240 × 10 mm). Stepwise elution using 100 mL of 10 vol.%, 100 mL of 20 vol.%, 200 mL of 30 vol.%, 250 mL of 40 vol.%, and 200 mL of 50 vol.% aqueous MeOH, and 200 mL of 60 vol.% aqueous MeOH was used to obtain **mixture γ-1** (10 mg, 8.9%, isolated yield). R_f 0.68 (butanol-ethanol-water 5:4:3 by volume, TLC; silica gel $60F_{254}$), and 0.41 (MeOH-water 2:1, by volume, TLC; RP-$18F_{254S}$; Merck Ltd.). ^{1}H-NMR (DMSO-d_6): δ = 2.42 (3H, s, CH_3 of *p*-tosyl), 3.2–3.8 (46H, m, of CD), 3.85–3.95 (1H, m, C^3H of glucose unit with pyrene), 4.1–4.2 (1H, m, C^2H of glucose unit with pyrene), 4.2–4.7 (7H, m, O^6H of CD), 4.7–5.05 (7H, m, C^1H of glucose units without pyrene), 5.4–5.5 (1H, m, C^1H of glucose unit with pyrene), 5.6–6.0 (15H, m, O^2H and O^3H of CD), 7.45 (2H, d, J = 8.1 Hz, aromatic-H of *p*-tosyl), 7.73 (2H, d, J = 7.8 Hz, aromatic-H of *p*-tosyl), 7.96 (1H, d, J = 7.5 Hz, aromatic-H of pyrene), 8.06 (1H, t, J = 7.5 Hz, aromatic-H of pyrene), 8.13 (2H, s, aromatic-H of pyrene), 8.20–8.30 (4H, m, aromatic-H of pyrene), 8.41 (1H, d, J = 9.3 Hz, aromatic-H of pyrene). Calcd. for $C_{75}H_{101}O_{42}NS{\cdot}3H_2O$: C, 50.76; H, 6.08; N, 0.79%. Found: C, 50.53; H, 6.28; N, 0.74%. FAB-MS (*m/z*): 1720, ($[M+H]^+$).

Measurements

Fluorescence and circular dichroism spectra were measured at 25 °C using 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 348 nm, and excitation and emission slits were 2.5 nm. Aqueous ethylene glycol (10 vol.%) was used as a solvent for the host

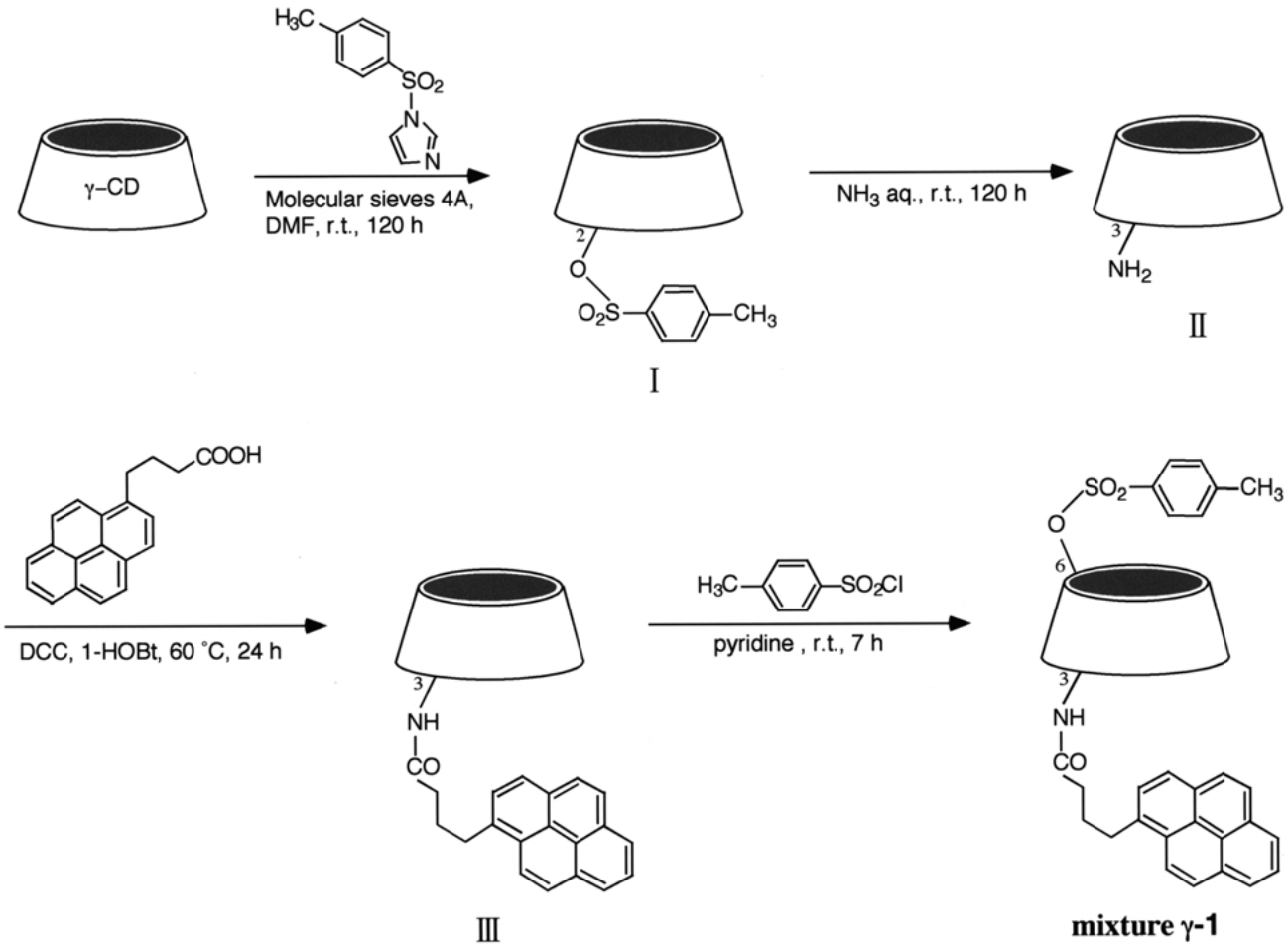


Figure 1. Preparation of **mixture γ-1**.

for spectroscopic measurements because the solubility of the host in pure water is poor. 5 μL 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 the host (2.5 mL, 1.0×10^{-6} M). This procedure gave a guest concentration of 1.0, 0.1 and 0.01 mM respectively in the sample solution.

For the circular dichroism measurements, 5 μL of guest species (0.05 M) in dimethyl sulfoxide (DMSO) were injected into a 10 vol.% ethylene glycol aqueous solution of the host (2.5 mL) to make a sample solution with the host concentration of 0.1 mM and the guest concentration of 0.1 mM.

Results and discussion

*The preparation of mono-3^A-deoxy-3^A-pyrenebutylamido-6^X-O-mono-(p-toluene-sulfonyl)mono-altro-γ-CD (**mixture γ-1**)*

Mono-3-deoxy-pyrene-mono-altro-γ-CD (**III**) was prepared from compound **II** with excess of 1-pyrenebutylate in the presence of DCC and 1-HOBt, and 3-deoxy-pyrene-6-(*p*-toluenesulfonyl)γ-CD (**mixture γ-1**) was prepared from compound **III** with excess of *p*-toluenesulfonyl chloride in pyridine. Compounds **III** and **mixture γ-1** were separated with reverse-phase column chromatography, as shown in Figure 1. It was anticipated that the tosyl moiety of **mixture γ-1** would be substituted at the 6A to 6H-positions of γ-CD. It was reported that the composition of regioisomers of CD derivatives could be evaluated by chemical [28] and enzymatic degradation [29]. However, we were unable to synthesize **mixture γ-1** deliberately and degrade **mixture γ-1** by enzymes. Therefore, we attempted to determine the position of the tosyl moiety in γ-CD by ^{1}H-NMR analysis. Our attempt was not successful, because the ^{1}H-NMR signals at 7.45 and 7.73 ppm as doublets attributed to the aromatic protons of the tosyl group were not evidenced where the tosyl moiety is located on the upper rim of the CD. Unfortunately, Ueno and co-worker could not also identify the composition of regioisomers of CD derivatives [30]. Thus, in this paper, host **mixture γ-1** was assumed to exist as diastereomers bearing the pyrene moiety at the 3A-postion and the tosyl moiety at the 6A to 6H-positions and these diastereomers have been named **mixture γ-1**. The transformation of 2-mono-sulfonyl CDs to 3-deoxy-3-amino-(2S),(3R)-CDs has been reported [26]. Namely, compound **II** consists of seven glucose units and one altrose unit which is appended with a pyrene moiety, therefore, **mixture γ-1** is a distorted ring.

Induced circular dichroism (ICD) spectra

The ICD spectra of **mixture γ-1**, alone and with progesterone or deoxycholic acid in a 10 vol.% ethylene glycol aqueous solution were recorded to investigate the movement of the appended moieties when host–guest complexation occurred, as shown in Figures 2 and 3, respectively. The ICD spectrum of **mixture γ-1**, alone, shows positive Cotton peaks at around 268, 277, 330, and 346 nm and negative Cotton peaks at around 260 and 285–312 nm. In Figure 2, the ICD intensity of the positive bands at around 268 and 277 nm and the negative band at 285–312 nm increased upon addition of progesterone, however, the [Φ] values of the negative band at around 260 nm and the positive bands at around 330 and 346 nm decreased on addition of progesterone. On the other hand, in Figure 3, the ICD intensity of the positive bands at around 277 and 346 nm and 330 nm increased or decreased, respectively, on addition of deoxycholic acid, whereas other peaks exhibited almost no changes. These guest-induced ICD spectral changes of **mixture γ-1** were very small. It suggests that these small effects on ICD spectra will be caused by different behaviors of regioisomers of **mixture γ-1**. It is well known that the increase of the ICD intensity is ascribed to the formation of a complex between the achiral appended moiety of the CD and a chiral CD. The decrease of the ICD intensity of **mixture γ-1** upon guest addition suggests two possibilities; one is that the appended moiety moves far from the chiral environment of the CD cavity, and another is that the appended moiety is rotated to allow host–guest complexation [6, 31, 32]. The ICD patterns of **mixture γ-1** for progesterone or deoxycholic acid are different. It suggests that the hetero appended moieties of **mixture γ-1** are arranged differently when progesterone or deoxycholic acid is added, in which the pyrene moiety will be going far away from the CD cavity or rotating. On the other hand, the tosyl moiety will affect the movement of the pyrene moiety, probably, the tosyl moiety is estimated to be deeply included into the CD cavity, because of its small size. It seems that these different behaviors of the hetero appended moieties of **mixture γ-1** could bring about the variation of the fluorescent sensing ability.

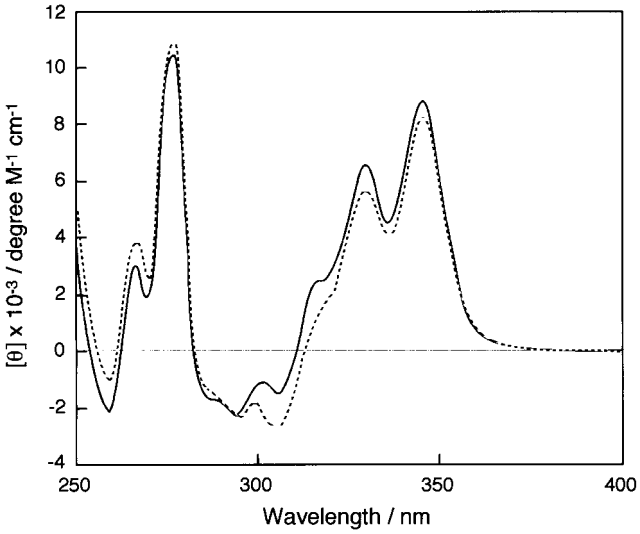


Figure 2. ICD spectra of **mixture γ-1** in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-4} M: ——, 25 °C) and containing progesterone (1.0×10^{-4} M: - - - - - -).

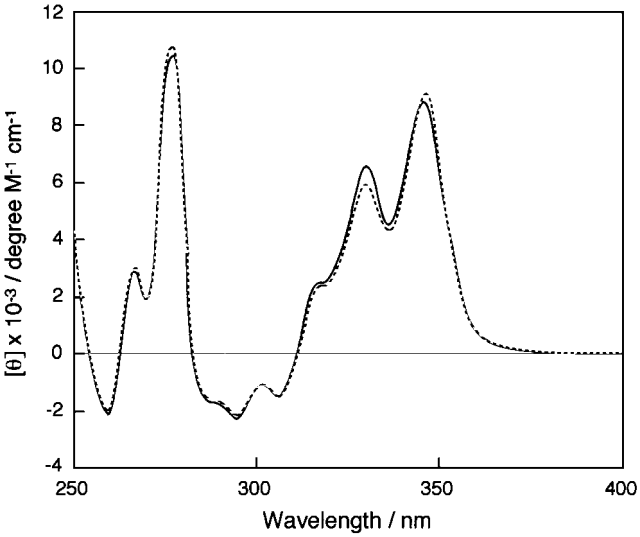
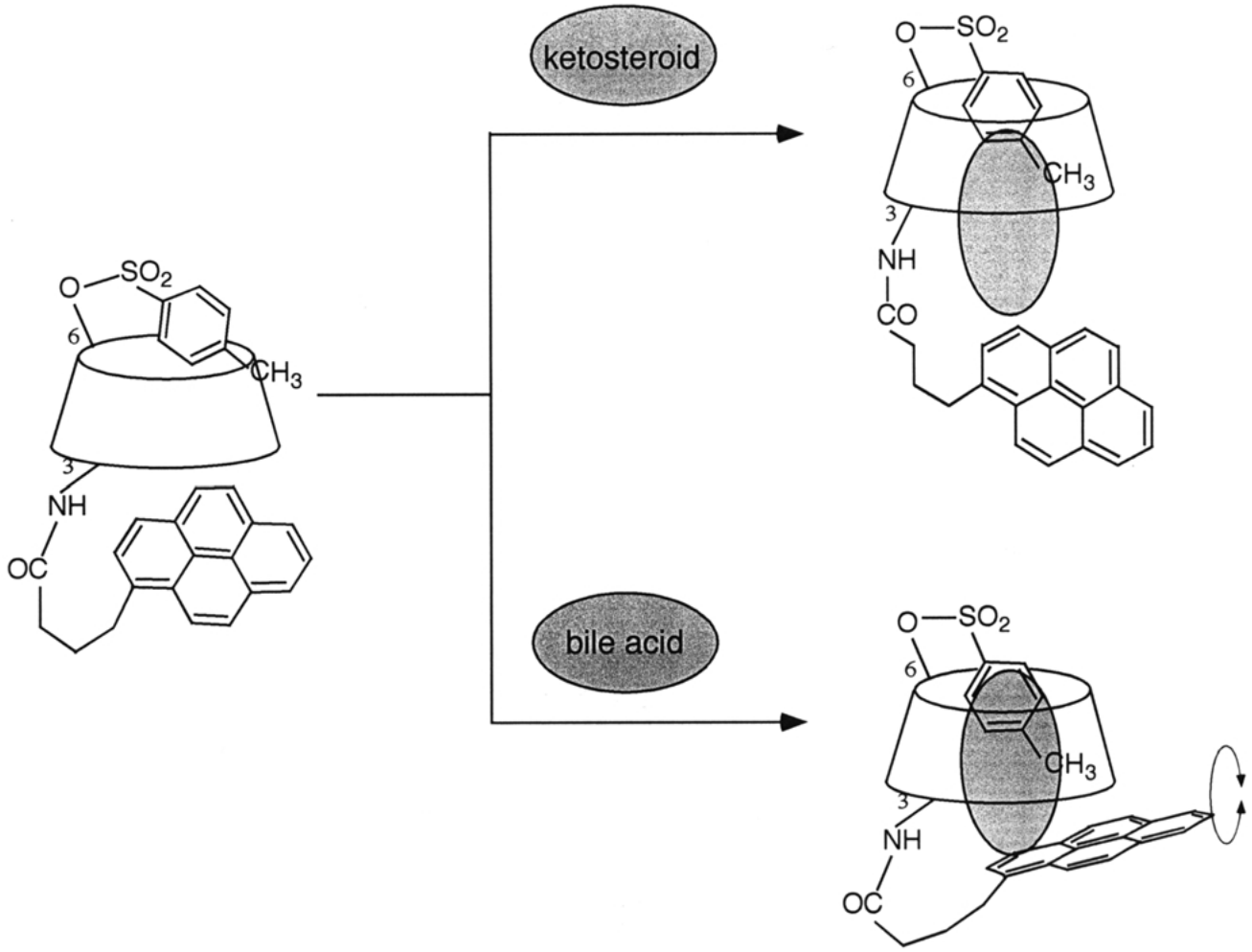


Figure 3. ICD spectra of **mixture γ-1** in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-4} M: ——, 25 °C) and containing deoxycholic acid (1.0×10^{-4} M: - - - - - -).

Fluorescence spectra

Figure 4 shows the fluorescence spectra of **mixture γ-1** with and without progesterone in a 10 vol.% ethylene glycol aqueous solution. The fluorescence spectra of **mixture γ-1** are composed of pure monomer emission with peaks around 377, 397 and 417 nm, and the intensity decreases with increasing progesterone concentration. In contrast, the fluorescence spectra of **mixture γ-1** before and after addition of lithocholic acid in a 10 vol% ethylene glycol aqueous solution as shown in Figure 5 exhibit an increase of its intensity as the concentration of lithocholic acid is increased. These guest-induced fluorescent spectral changes were also small similar to the case of those of ICD spectral changes. It will be caused by different behaviors of regioisomers of **mixture γ-1** as mentioned above in the ICD section. It is reported that a guest-induced fluorescence enhancement means that the appended moiety is coming into the hydrophobic environment of the CD cavity and a decrease indicates that the appended moiety is moving to the bulk water from the hydrophobic CD cavity. The results obtained as ICD and fluorescence spectral changes of **mixture γ-1** suggest two mechanisms of host–guest complexation; one is that the pyrene moiety is enclosed in the CD cavity and is displaced when ketosteroid is added, and another is that the pyrene moiety is displaced from the CD cavity when bile acid is added, at the same time the tosyl moiety is included into the CD cavity in these two patterns, as illustrated in Scheme 1.

As reported previously, the extent of the variation of the fluorescence intensity of the host depended on the nature of a guest, even at low concentrations; therefore, the host can be used as an indicator of molecular recognition. In order to evaluate the sensing ability of **mixture γ-1** initially, the $\Delta I_{m1}/I^{\circ}_{m1}$, $\Delta I_{m2}/I^{\circ}_{m2}$ and $\Delta I_{m3}/I^{\circ}_{m3}$ values were used as



Scheme 1. One of the possible host–guest complexation mechanisms of **mixture γ-1**.

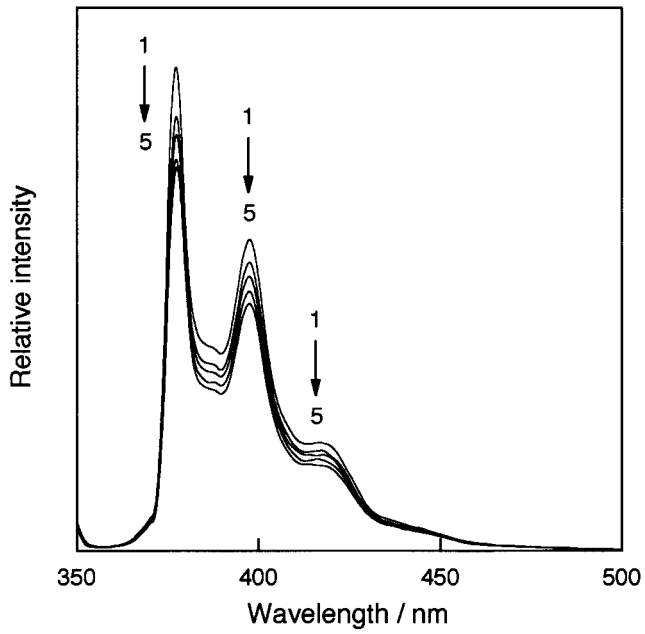


Figure 4. Florescence spectra of **mixture γ-1** in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-6} M: ——, 25 °C) at various concentration of progesterone (1: 0, 2: 4.0×10^{-6}, 3: 2.4×10^{-5}, 4: 8.3×10^{-5}, 5: 1.4×10^{-4} M).

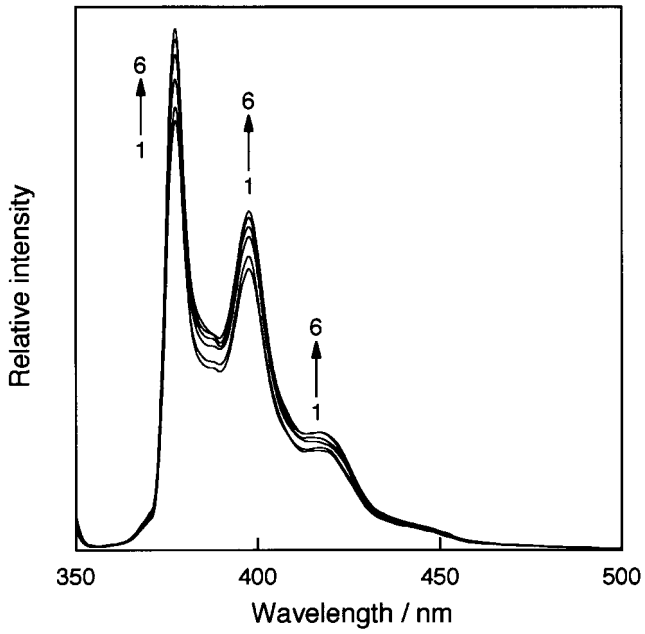
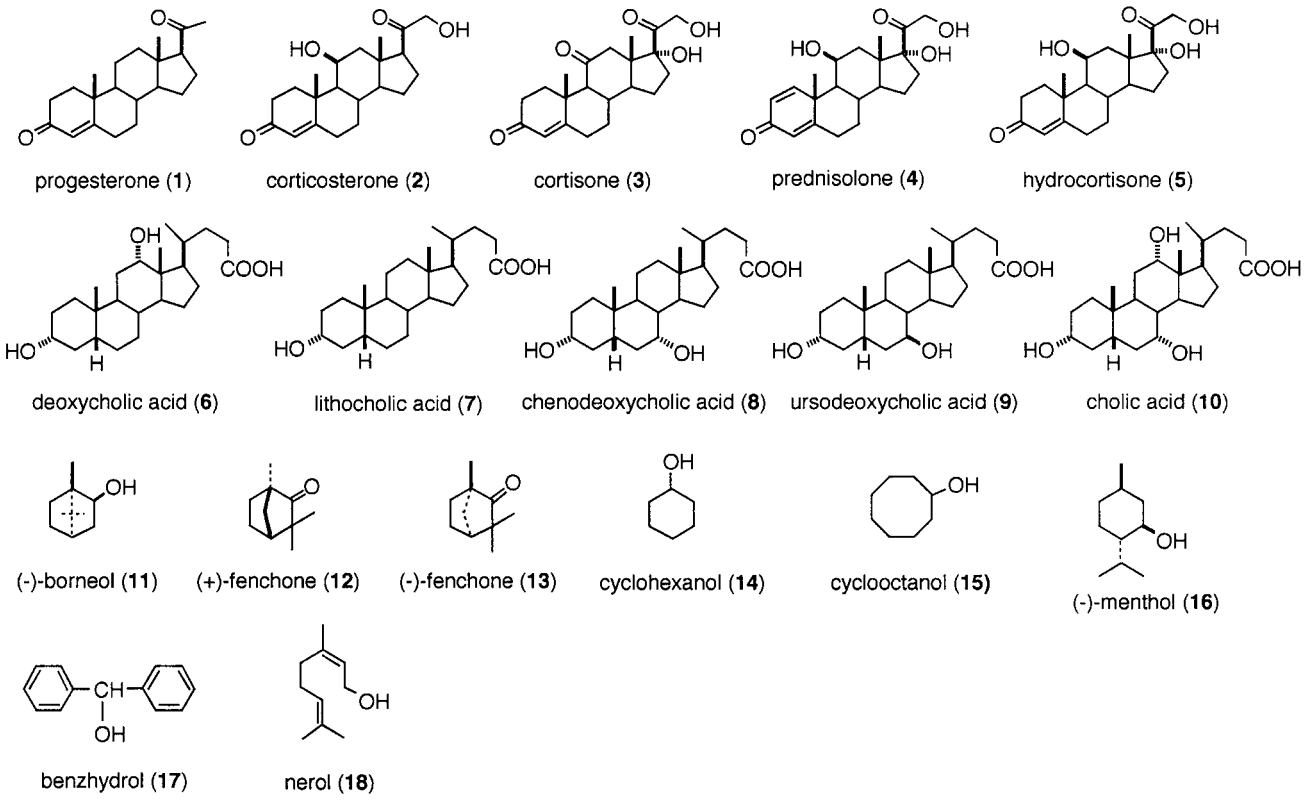


Figure 5. Florescence spectra of **mixture γ-1** in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-6} M: ——, 25 °C) at various concentration of lithocholic acid (1: 0, 2: 1.2×10^{-6}, 3: 4.0×10^{-6}, 4: 8.3×10^{-6}, 5: 1.1×10^{-5}, 6: 1.4×10^{-5} M).



Scheme 2. Guest molecules

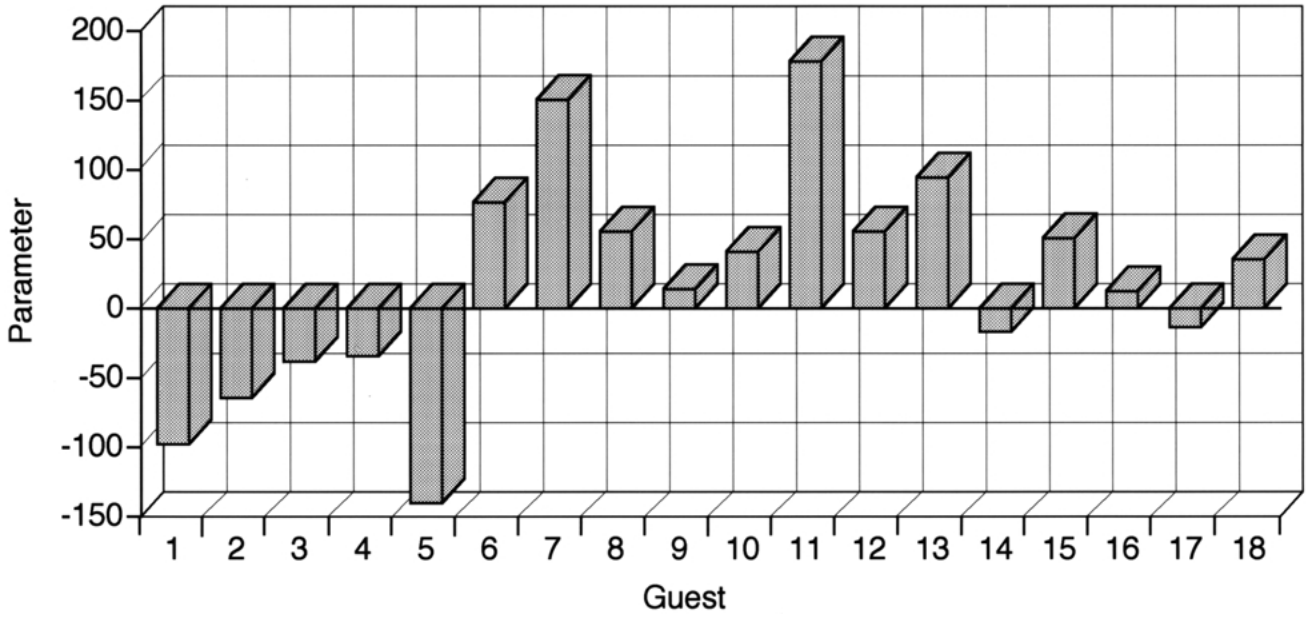


Figure 6. Sensitivity factors of **mixture γ-1** in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-6} M, 25 °C) for steroids and small guests examined at 377 nm.

Table 1. Binding constants (K/mol^{-1} dm^{-3}) of **mixture γ-1** in a 10 vol% ethylene glycol aqueous solution (1.0×10^{-6} M, 25 °C)

Guest	Binding constant/K[a]		
	377 nm	397 nm	475 nm
Progesterone (**1**)	20,100 ± 2,170[b]	15,100 ± 1,230	12,400 ± 1,000
Hydrocortisone (**5**)	108,000 ± 3,900	113,000 ± 5,020	221,000 ± 13,600
Lithocholic acid (**7**)	67,600 ± 4,400	150,000 ± 8,430	39,800 ± 860
Borneol (**11**)	320 ± 30	890 ± 70	1,200 ± 20

[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.

a sensitivity parameter. Here, ΔI_{m1} is $\Delta I_{m1} - I^{\circ}_{m1}$, ΔI_{m2} is $\Delta I_{m2} - I^{\circ}_{m2}$ and ΔI_{m3} is $\Delta I_{m3} - I^{\circ}_{m3}$, where I^{0}_{m1} and I_{m1}, I°_{m2} and I_{m2}, and I°_{m3} and I_{m3} , are the intensities of the fluorescence spectra at 377, 397 and 417 nm for the host alone and in the presence of a guest, respectively. These parameters at each fixed wavelength for each guest obtained exhibited almost the same values. Therefore, in this present study, the ΔI_{m1} value, exhibiting the value of the guest-induced spectral changes, was used as a sensitivity parameter, because the guest-responsive spectral alternations at 377 nm were larger than those at 397 and 417 nm. The parameter values of **mixture γ-1** were obtained using 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 are shown in Figure 6. Among ketosteroids formed with a *trans* AB fusion, hydrocortisone (**5**), which has two hydroxyl groups at C-11 and C-17 in the steroidal framework, is detected by **mixture γ-1** with the highest values of ΔI_{m1} of 140.1. Progesterone (**1**), which bears no hydroxyl group and is more hydrophobic than the other ketosteroids, is detected by **mixture γ-1** with the next highest values of ΔI_{m1} of 97.8. Corticosterone (**2**), which bears one hydroxyl group at C-11 in the steroidal framework, cortisone (**3**), which bears one hydroxyl group and one more ketone at C-17 and C-11, respectively, and prednisolone (**4**), which bears two hydroxyl groups at C-11 and C-17 in the steroidal framework and one more double bond between C-1 and C-2 than the other ketosteroids, are detected by **mixture γ-1** with the values of ΔI_{m1} of −64.4, −38.1, and −34.3, respectively. These results suggest that host **mixture γ-1** selectively recognizes guests such as **1** and **5**. Guest **1** probably enters the CD cavity from the side of the carboxylic acid and not the ketone group side at C-3 in the steroidal framework. It is also probable that guest **5** enters into the CD cavity from the ketone group side at C-3 in the steroidal framework, because the carboxylic acid is located next to the hydroxyl group at C-17 in the steroidal framework. Among bile acids formed with a *cis* AB fusion, lithocholic acid (**7**), which bears only one hydroxyl group at C-3 in the steroidal framework, is detected with the highest values of ΔI_{m1} of 150.6. Deoxycholic acid (**6**), which bears two hydroxyl groups at C-3 and C-12 in the steroidal framework, and chenodeoxycholic acid (**8**), which bears two hydroxyl groups in the steroidal framework, are detected with the next highest values of ΔI_{m1} of 76.7 and 55.5, respectively. On the other hand, cholic acid (**10**), which bears three hydroxyl groups at C-3, C-7 and C-12 and is more hydrophilic than the other bile acids, and ursodeoxycholic acid (**9**), which is a diastereomer of guest **8**, are detected with low values of ΔI_{m1} of 41.0 and 14.1, respectively. These results indicated that **mixture γ-1** recognizes bile acids having a hydroxyl group at C-3 in the steroidal framework. Furthermore, **mixture γ-1** also detects bile acids bearing hydroxyl groups at C-3 and C-7 with a *cis* formation in the steroidal framework such as chenodeoxycholic acid, whereas bile acids bearing hydroxyl groups at C-3 and C-7 with a *trans* formation in the steroidal framework such as ursodeoxycholic acid are hardly sensed. The sensing parameters of **mixture γ-1** have negative or positive values for ketosteroids and bile acids, respectively. This indicates that **mixture γ-1** is able to distinguish the steroids with *cis* AB fusion and *trans* AB fusion, whilst fluorescent CD derivatives reported previously [9, 10, 33] have hardly sensed these ketosteroids except for progesterone. This fact will be caused by the cooperation of the hetero moieties at the hetero rims alter the hydrophobic environment of the CD cavity. For that reason, **mixture γ-1** can recognize ketosteroids, whereas other fluorescent CD derivatives labelled at only the upper rim can hardly recognize them. It is clear that the modifications on the hetero rims of the CD is effective for sensing ketosteroids and distinguishing steroidal compounds. Among the small guests, (−)-borneol (**11**), (+)- and (−)-fenchone (**12**, **13**, respectively), which are bicyclic compounds, are detected by **mixture γ-1** with the highest or the next highest sensing values of ΔI_{m1} of 178.0, 55.58 and 94.5, respectively. However the sensing values of other small guests are low. It seems that bicyclic compounds such as guests **11**, **12** and **13** just fit in the γ-CD cavity.

The host–guest fluorescence variation at 377, 397 and 417 nm were employed to calculate the binding constants of **mixture γ-1** using Equation (1), as reported previously [33].

$$\frac{1}{I_A - A^{\circ}_A} = \frac{1}{a[\mathrm{CD}]} + \frac{1}{a[\mathrm{CD}]K} \times \frac{1}{[G]} \quad A = m1, m2 \text{and} m3. \tag{1}$$

Here, I is the fluorescence intensity at 377, 397, 417 nm (I_A for complex, I°_A for the host alone), [CD] is the total host concentration, $[G]$ is the total guest concentration, a is a constant. The binding constants of the host were obtained in order to examine the correlation between the fluorescence variations and the binding of the host. The results are listed in Table 1. The binding constants of **mixture γ-1** for bile acids are in the order **5** > **7** > **1**, which are roughly parallel with the order of the sensitivity factors.

Conclusion

γ-CD modified with pyrene and tosyl at the hetero rims has been synthesized to investigate its sensing ability for steroids and terpenes. The variation of its fluorescence intensity was used as a parameter to describe the sensing ability. Introduction of two different kinds of functional groups such as pyrene and tosyl, which are in different positions such as C-3 and C-6 on the CD hetero rims, produces highly selective molecular recognition which exhibits distinctive sensing ability for steroidal compounds. It is clear that the cooperation of the pyrene and tosyl moieties of the host fulfills its new function which can increase the sensing ability.

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