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A High Sensitivity Fluorescent Chemo-sensory System Based on β-Cyclodextrin Dimer Modified with Dansyl Moieties

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Key words: fluorescent molecular sensing, β-cyclodextrin dimer, dansyl, bile acid, hydrophobic cap, 1 : 1 complexation

Abstract

β-Cyclodextrin dimer linked with ethylenediamine at the upper rim of the cyclodextrin has been synthesized and then modified with two dansyl moieties in the presence of N,N'-dicyclohexylcarbodiimide. The sensing ability and binding property of the title compound were investigated for steroids and terpenoids. The fluorescence intensity of this dimer was decreased when a host–guest complex was formed. The value $\Delta I/I^0$, where I^0 and I are fluorescence intensities in the absence and presence of a guest and ΔI is $I^0 - I$, was used as a parameter of sensitivity. This host exhibited a much higher sensitivity and selective molecular recognition ability for bile acids such as ursodeoxycholic acid and chenodeoxycholic acid and terpenoids such as (−)-borneol than the dansyl-modified cyclodextrins reported previously including γ-cyclodextrin dimer. The behaviors of the appended moieties of the host during the formation of host–guest complexes were studied using induced circular dichroism (ICD) and fluorescence spectra. The ICD intensity of this dimer was decreased on accommodation of a guest and this spectral pattern of the title dimer was opposite to that of bis dansyl-modified β-cyclodextrin monomer. The guest-induced variations in the fluorescence and ICD intensities suggest that this dimer formed a 1 : 1 host–guest complex and the appended moieties act as a hydrophobic cap.

Introduction

Cyclodextrins (CDs) are composed of 6, 7 or 8 α-1,4-linked D-glucopyranose units and are usually referred to as α-, β- and γ-CD, respectively. Much of the interest in natural and modified CDs is their ability to exhibit inclusion phenomena with guest molecules such as organic compounds or ions in the hydrophobic cavity of the CDs [1, 2]. Accordingly, these CDs have attracted interest as catalysts, enzyme mimics or photosynthesis models, and drug delivery systems (DDS) [3–5]. The modification of the CDs with various organic compounds such as spectroscopically, catalytic or functionalised ones gives the CDs new functions [6, 7] which are not shown by native CDs. For more than a decade, we have been studying chemo-sensory systems by CDs modified with fluorescent active units such as naphthalene [8–10], anthranilate [11–14], fluorescamine [15], terphenyl [16], and dansyl [17–21] for guest molecules such as terpenoids and steroids which are biological substances produced by plants or animals and are useful as crude drugs. In these systems, we found that these fluorescent CDs detected bile acids and terpenoids with high sensing ability and the appended moieties worked as a spacer or as a hydrophobic cap, exhibiting variations of guest-induced fluorescence and induced circular dichroism spectra. Recently, multiple CDs such as linked CD dimers have received much attention [22–40], because these dimeric CDs have two discrimination sites producing complexation with a guest compound. Unfortunately, these CD derivatives were spectroscopically inert and their molecular bindings were studied by using spectroscopically active guests. Previously, we have discussed the synthesis and chemo-sensory system of double-dansyl-labeled γ-CD dimer (γ-**1**), in which γ-**1** displayed more sensitive and selective binding ability for bile acids than mono- or double-dansyl-labeled γ-CD monomers [18, 19], and we also indicated that this fact might be due to the large hydrophobic domain of the CD dimer [41].

As an extension of this work, in this contribution, we synthesized double-dansyl-labeled β-CD dimer (β-**1**) in order to investigate its chemo-sensory system as a new indicator in comparison with γ-**1**. Because dansyl-modified β-CD monomers exhibited higher sensitivity than γ-CD analogs [17, 20], it is interesting to study the complexation properties of dimeric β-CDs modified with a sensitive fluorescent probe such as dansyl and having a large hydrophobic domain formed by the CD cavities. Our new compound β-**1** exhibits higher selectivity and sensitivity for steroidal compounds bearing hydroxyl groups on C-3 and C-7 in the steroidal framework and (−)-borneol than those of γ-**1**.

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Experimental

*Preparation of 6-(2-aminoethyl)amino-6-deoxy-bis-β-CD (**a**)*

A mixture of 6-(2-aminoethyl)amino-6-deoxy-β-CD [42] (1.163 g, 1.00 mmol) and 6-iodo-6-deoxy-β-CD [20] (1.857 g, 1.50 mmol) in 30 mL of DMF was heated at 80 °C for 24 h. After cooling, the reaction mixture was poured into 500 mL of acetone. The resulting precipitates were filtered and dried. The water soluble fraction was applied on a CM-Sephadex C-50 column (7 × 35 cm). Stepwise elution from 3 L of water and 1.5 L of 1 vol.% ammonia aqueous solution were applied to give compound **a**. The fractions containing **a** were collected and evaporated in vacuo; then they were poured into 500 mL of acetone. The resulting precipitates were filtered and dried to give 1.291 g (57.0%, isolated yield) of pure **a**. Rf: 0.17 (methyl ethyl ketone-methanol-acetic acid 12 : 3 : 5 by volume; TLC; silica gel $60F_{254}$). ^{1}H-NMR (D_2O) = 2.2 (4H, m, NCH_2), 3.4–3.6 (28H, m, C^2H and C^4H of CD), 3.65–3.85 (56H, m, C^3H, C^5H and C^6H of CD), 4.92 (14H, s, C^1H of CD).

*Preparation of 6-(2-dansyl-aminoethyl)dansyl-amino-6-deoxy-bis-β-CD (**β-1**)*

Dicyclohexyl carbodiimide (DCC, 198 mg, 0.96 mmol) and 1-hydroxytribenzotriazole (1-HOBt, 130 mg, 0.96 mmol) were added to a cooled solution (−10 °C) of dansylglycine (295 mg, 0.96 mmol) in 8 mL of DMF. The reaction mixture was stirred at −10 °C for 30 min. To a stirred solution was added portionwise compound **a** (433 mg, 0.19 mmol), the solution was stirred at −10 °C for another 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 water soluble fraction was applied to a reversed-column (Lobar column LiChroprep RP-18, Merck Ltd., 240 mm x 10 mm). Stepwise elution from 300 mL of 10 vol.% and 300 mL of 20 vol.% aqueous CH_3CN was used to give **β-1**. The fractions containing **β-1** were collected and evaporated in vacuo; then they were poured into 300 mL of acetone. The resulting precipitates were filtered and dried to give 28 mg (5.1%, isolated yield) of pure **β-1**. Rf: 0.49 (butanol-ethanol-water 5 : 4 : 3 by volume; TLC; silica gel 60F254) and 0.71 (methanol-water 2 : 1, by volume; TLC; RP-$18F_{254S}$; Merck Ltd.). ^{1}H-NMR (D_2O) = 2.86 (4H, m, NCH_2), 3.5–3.8 (56H, m, C^2H, C^3H, C^4H, and C^5H of CD), 3.8–4.1 (28H, m, C6H of CD), 4.98–5.17 (14H, m, C^1H of CD), 7.25 (1H, d, J = 7.2 Hz, aromatic-H of dansyl), 7.35 (1H, d, J = 7.2 Hz, aromatic-H of dansyl), 7.62 (4H, quartet, J = 7.5 Hz, aromatic-H of dansyl), 8.30 (2H, d, J = 8.1 Hz, aromatic-H of dansyl), 8.37 (2H, d, J = 9.6 Hz, aromatic-H of dansyl), 8.45 (2H, d, J = 7.8 Hz). Calcd. for $C_{114}H_{172}O_{74}N_6S_2 \cdot 1H_2O$: C, 47.37; H, 6.06; N, 2.91%. Found: C, 47.44; H, 6.09; N, 3.21%. TOF-MS (m/z): 2875, ($[M]^+$).

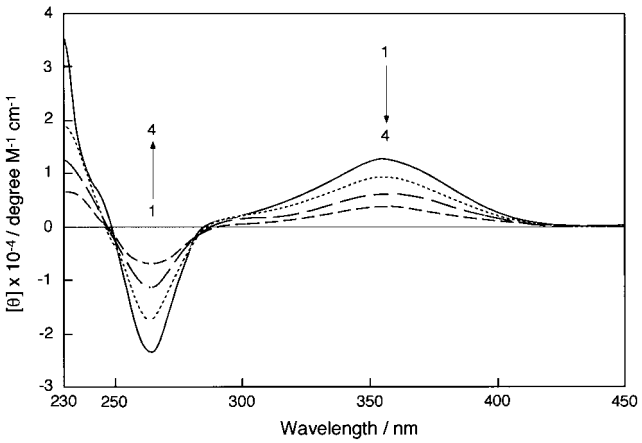


Figure 1. ICD spectra of **β-1** (5.0×10^{-5} M: ——, 25 °C) in a 10 vol.% ethylene glycol aqueous solution at various concentrations of ursodeoxycholic acid (5.0×10^{-5} M: ....., 1.0×10^{-4} M: — —, 1.5×10^{-4} M: – – –).

Measurements

Fluorescence and circular dichroism spectra were measured at 25 °C using a Perkin-Elmer LS 40B fluorescence spectrophotometer and a JASCO J-700 spectropolarimeter, respectively.

For the fluorescence measurements, the excitation wavelength of the fluorescence spectra was 340 nm and excitation and emission slits were 6 and 10 nm wide for **γ-1** and **β-1**, respectively. Ethylene glycol aqueous solution (10 vol.%) was used as a solvent for the host for the 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) to make a sample solution with a host concentration of 1.0×10^{-6} M and guest concentrations of 1.0, 0.1 and 0.01 mM, respectively.

For the circular dichroism measurements, five μL of guests 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 give a sample solution with a host concentration of 5.0×10^{-5} M and guest concentrations of 5.0×10^{-5}, 1.0×10^{-4}, and 5.0×10^{-4} M.

Energy-minimized structures

Energy-minimized structures were calculated by molecular mechanics using MM2 in CS Chem 3D. The parameters of MM2 are improved ones obtained from studies by Allinger [43] based on the TINKER system researched by Ponder [44].

Results and Discussion

Induced circular dichroism (ICD) spectra and fluorescence spectra

The ICD spectra of **β-1** alone and in the presence of ursodeoxycholic acid in a 10 vol.% ethylene glycol aqueous

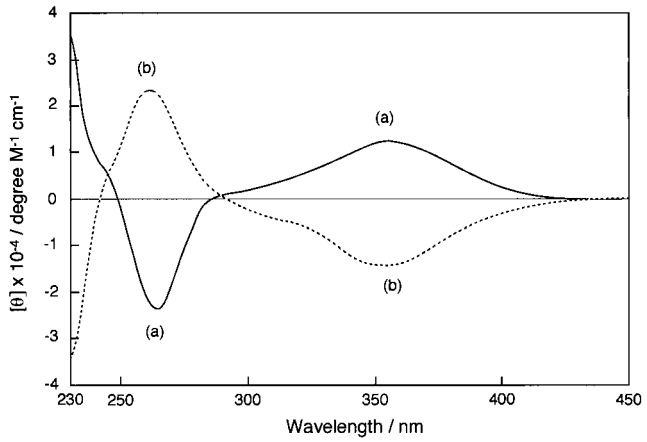


Figure 2. ICD spectra of β-**1** (5.0×10^{-5} M, a: ——, 25 °C) and $6^{A},6^{C}$-double-labelled β-cyclodextrin (1.0×10^{-4} M, b: .....) in a 10 vol.% ethylene glycol aqueous solution.

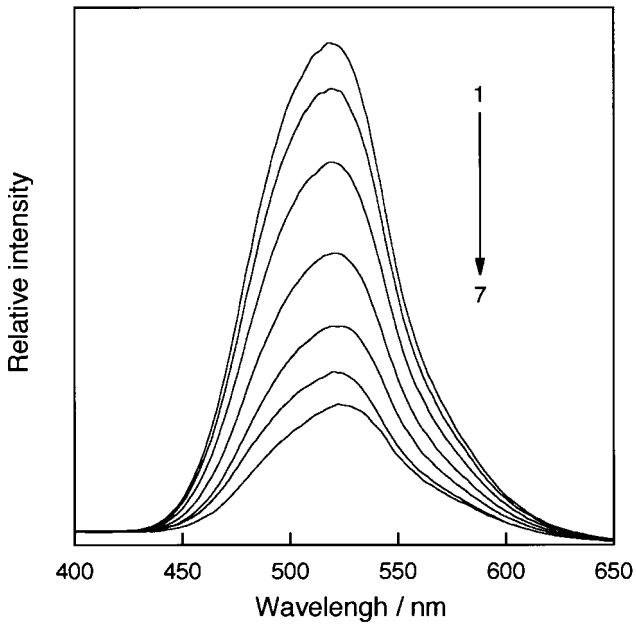


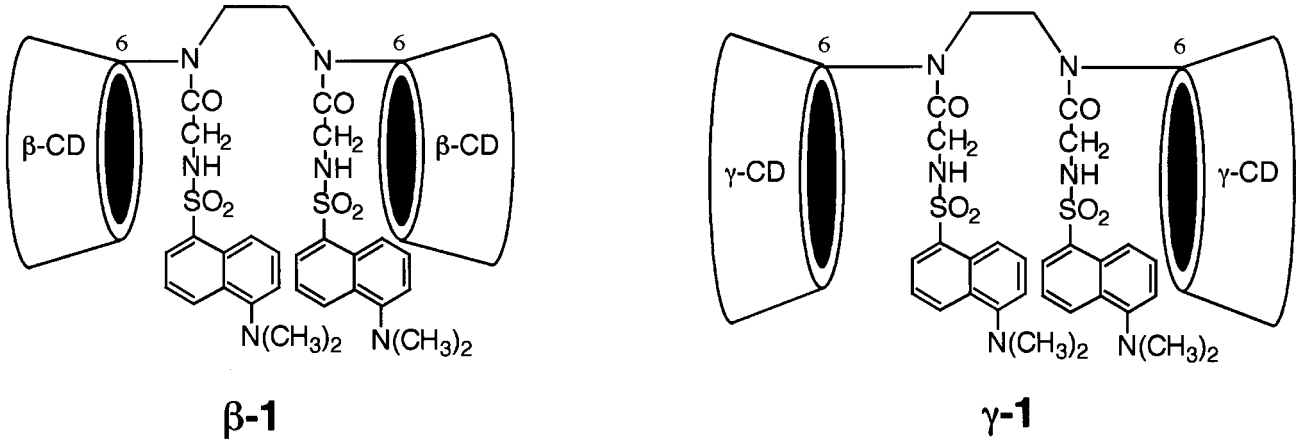
Figure 3. Fluorescence spectra of β-**1** (1.0×10^{-6} M, 25 °C) in a 10 vol.% ethylene glycol aqueous solution at various concentrations of ursodeoxycholic acid (1: 0, 2: 4.0×10^{-6}, 3: 1.2×10^{-5}, 4: 2.4×10^{-5}, 5: 4.0×10^{-5}, 6: 6.0×10^{-5}, 7: 8.3×10^{-5} M).

solution are shown in Figure 1. The ICD spectra of β-**1**, alone, show a positive band at around 355 nm and a negative band at around 265 nm. The ICD pattern of β-**1** is opposite to that of the $6^{A},6^{C}$-bis dansyl-modified β-CD monomer reported previously [20], as shown in Figure 2. It is reported that the ICD signs of the appended moiety of CD derivatives indicate the type of inclusion which can be equatorial or axial self-inclusion [45–47]. In these papers, the dansyl moiety of the β-CD derivative exhibited a positive sign at short wavelength and a negative sign at long wavelength for axial self-inclusion and the opposite signs indicated equatorial self-inclusion. Furthermore, in the case of the system where the appended moiety was capping the CD cavity, a positive ICD sign was observed at long wavelength. The energy-minimized structures obtained using molecular mechanics in CS Chem 3D (MM2) of β-**1** and $6^{A},6^{D}$-bis dansyl-modified β-CD monomer, as illustrated in Scheme 2, suggest that the appended moieties of β-**1** are close to the CD cavity, which are capping the CD cavity, are parallel to the CD equator; on the other hand, those of bis dansyl-modified β-CD monomer included into the CD cavity are parallel to the CD axis. These three-dimensional structures of β-**1** and this β-CD monomer support the differences in the ICD patterns of the CDs alone. The ICD intensities of the positive and negative Cotton peaks of β-**1** decrease upon the addition of a guest, showing larger changes of the ICD intensities than those of γ-**1**. These results suggest that the dansyl moieties of β-**1** move from the rim of the chiral environment of the CD cavity toward the outside of the CD cavity while simultaneously a guest is included into the CD cavity, in which the appended moieties of β-**1** move easier than those of γ-**1**.

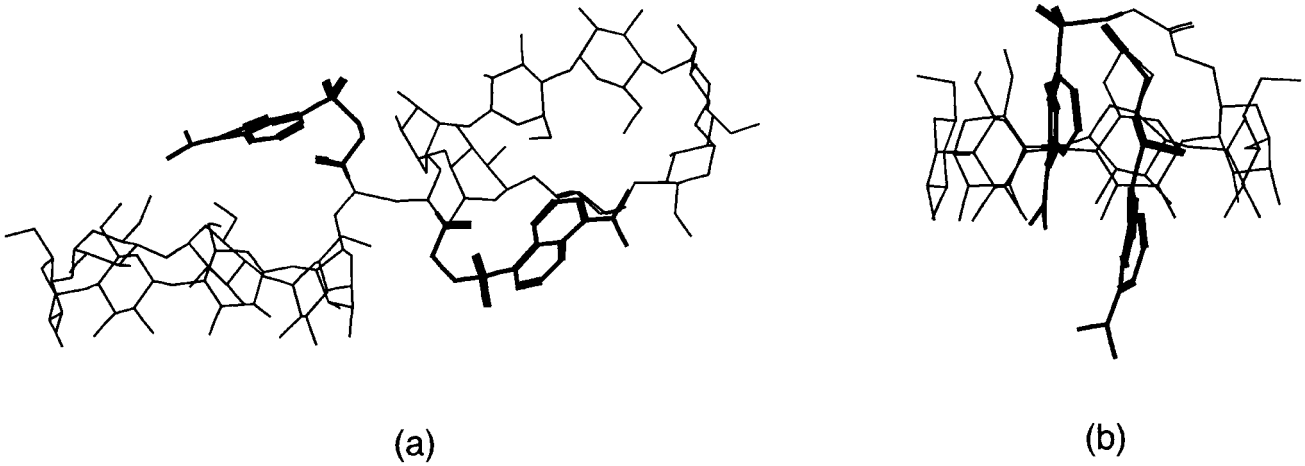
The fluorescence spectra of β-**1** in a 10 vol.% ethylene glycol aqueous solution in the absence and presence of ursodeoxycholic acid are shown in Figure 3. The fluorescence spectra of β-**1**, alone, are composed of a monomer emission with a peak at around 526 nm, and the fluorescence intensity decreases with increasing ursodeoxycholic acid concentration. It is reported that the decrease of the guest-induced fluorescence spectra indicates that the appended moiety is moving from the hydrophobic environment, which is the CD cavity, into the hydrophilic one which is bulk water [19–21]. On the other hand, enhancement means the appended moiety is moving more deeply into the hydrophobic CD cavity [14]. The decrease of the guest-responsive fluorescence intensities of β-**1** mean that the dansyl moieties move from the hydrophobic CD cavities toward the hydrophilic outside of the CD cavities, simultaneously the guest is included into its two cavities. The results obtained as ICD and fluorescence spectral changes of β-**1** suggest that the dansyl moieties move out of the CD cavity upon guest binding and act as a hydrophobic cap, as illustrated in Scheme 3, and the flexibility of the appended moieties of β-**1** is larger than that of γ-**1**. This fact will contribute to the qualitative sensing ability of β-**1**. The host–guest complexation in a 10 vol.% ethylene glycol aqueous solution are caused by the hydrophobic interaction between the CD cavity and the dansyl moiety.

Detection of organic guests by β-**1** *and* γ-**1**

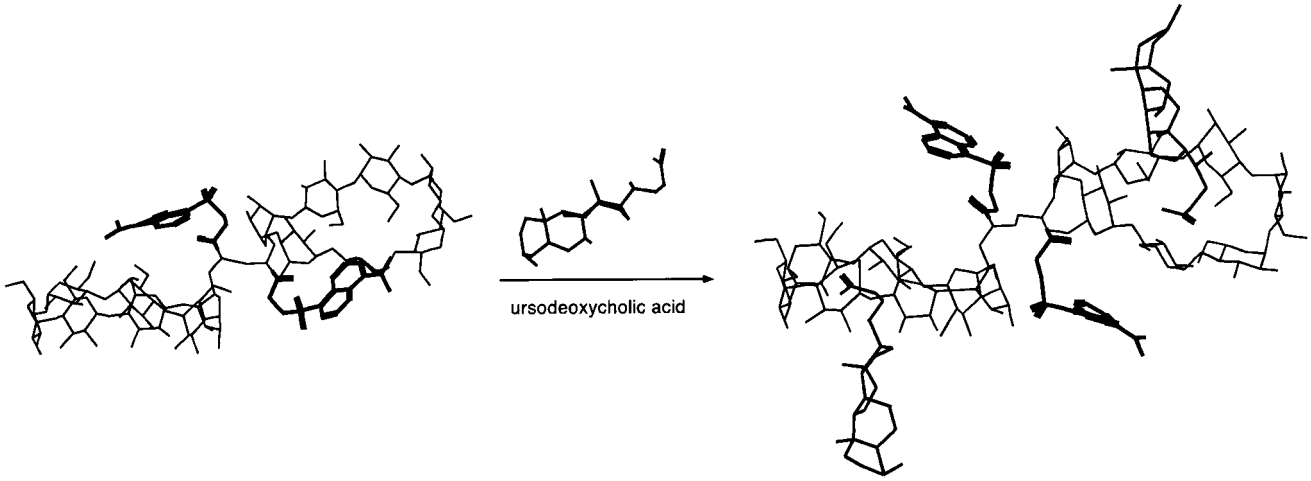
As reported previously [8], the variation in the fluorescence intensity of modified CDs is affected by the presence of guest molecules, even at low concentrations, therefore, these hosts can be used as fluorescent molecular sensors. In order to display the sensing ability of the CD dimers such as β-**1** and γ-**1**, $\Delta I/I^0$ was used as a sensitivity parameter. Here, ΔI is $I^0 - I$, where I^0 is the fluorescence intensity for the host alone and I is that for a complex. Figure 4 shows the parameter values of β-**1** and γ-**1** with steroids at 0.1 mM except for lithocholic acid, 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. Among the steroidal guests, ursodeoxycholic acid (**9**), which bears two hydroxyl groups on C-3 and C-



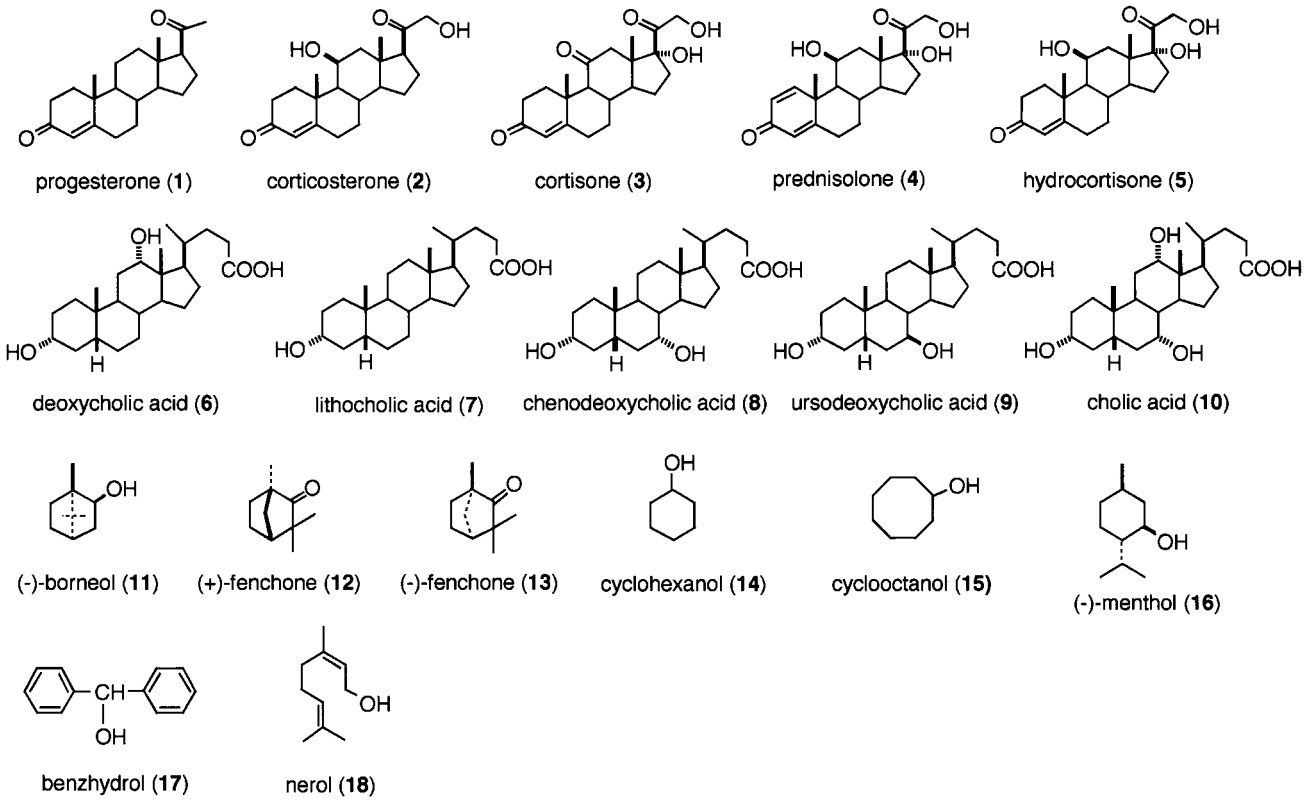
Scheme 1. Structures of β-**1** and γ-**1**.



Scheme 2. Energy-minimized structures of β-**1** (a) and $6^{A},6^{C}$-double-dansyl-labelled β-cyclodextrin (b) obtained using molecular mechanics in CS Chem 3D (MM2).



Scheme 3. One possible host–guest complexation mechanism of β-**1**.



Scheme 4. Guest molecules.

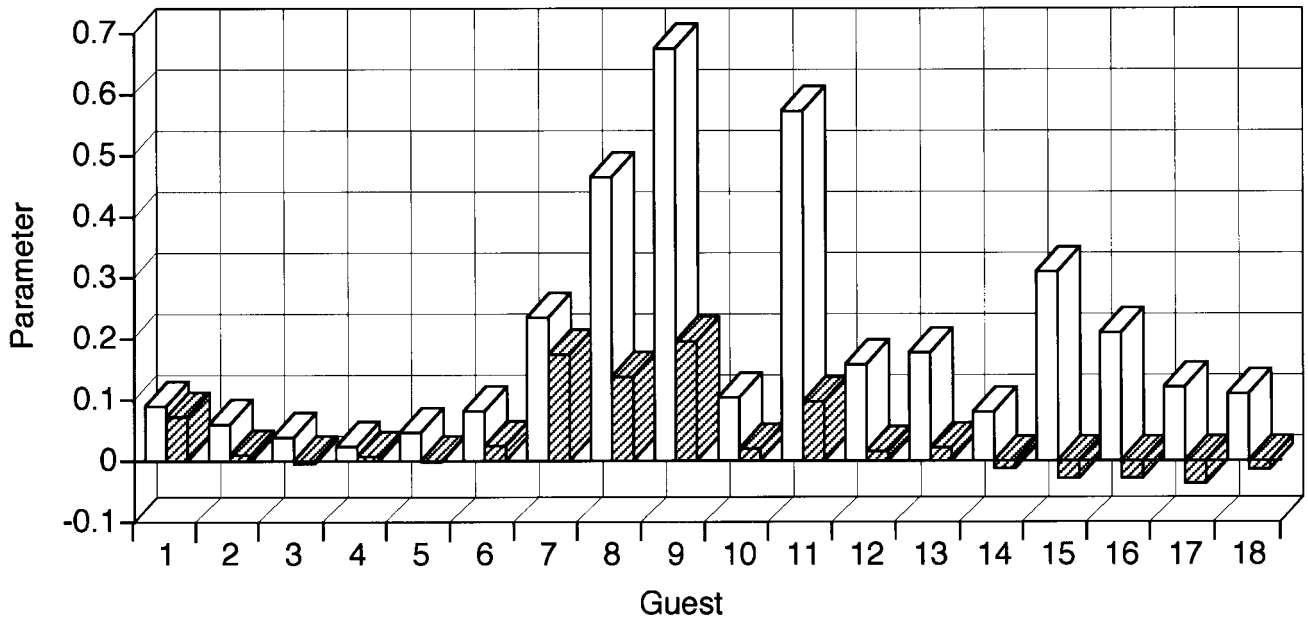


Figure 4. Sensitivity factors of β-**1** (□: 1.0 × 10^{-6} M, 25 °C) and γ-**1** (▨: 1.0 × 10^{-6} M, 25 °C) for all guests examined.

7 in a steroidal framework, was detected with the greatest sensitivity, exhibiting values of 0.675 and 0.196 for β-**1** and γ-**1**, respectively. Host β-**1** detected chenodeoxycholic acid (**8**), which is the diastereoisomer of **9**, with the next highest sensitivity, exhibiting a value of 0.465, whereas the sensing parameter value of β-**1** for **8** was 0.138. These sensing values of β-**1** for **8** and **9** are almost three times as large as those of γ-**1** and are the highest observed in the dansyl-modified CD systems reported so far [17–21, 41]. It means that the appended moieties of β-**1** move easier from the hydrophobic environment to the hydrophilic one than γ-**1** and dansyl-modified CD monomers when the guest is included into the CD cavity. This is probably caused by the fact that steric hindrance in the appended moieties of β-**1** is less than that of γ-**1** and those of the β- and γ-CD monomers, because the two appended moieties of β-**1** are not closer than those of the monomers and not included deeper into the CD cavity than those of γ-**1**. Lithocholic acid (7), which bears only one hy-

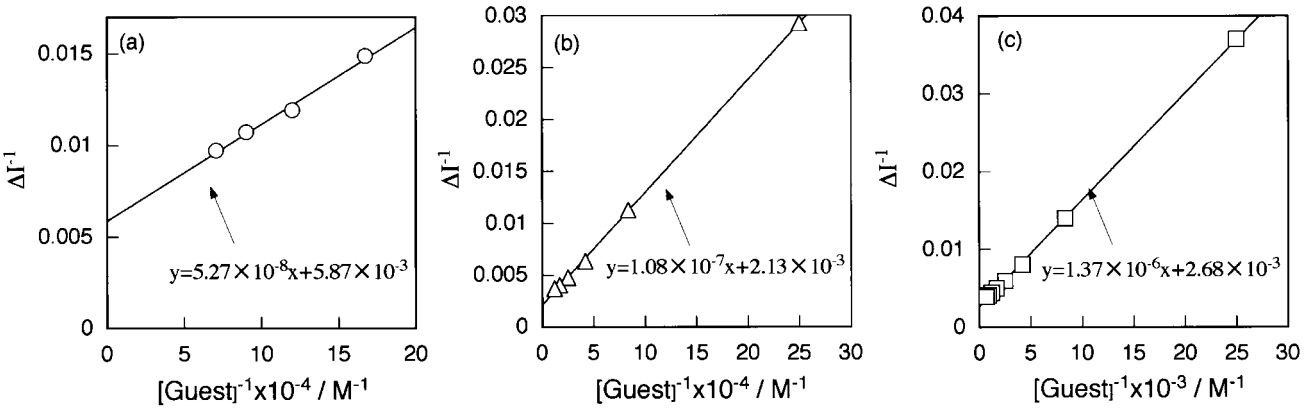


Figure 5. Binding curves of β-**1** (1.0×10^{-6} M, 25 °C) in a 10 vol.% ethylene glycol aqueous solution for lithocholic acid (a), ursodeoxycholic acid (b) and (−)-borneol (c).

droxyl group on C-3 in a steroidal framework, was detected with high sensitivity, exhibiting values of 0.235 and 0.174 for β-**1** and γ-**1**, respectively. Deoxycholic acid (**6**), which has two hydroxyl groups on C-3 and C-12 in a steroidal framework, was detected with low sensitivity, exhibiting values of 0.082 and 0.025 for β-**1** and γ-**1**, respectively. These sensing parameters of β-**1** and γ-**1** for guests **7** and **8** are smaller than those reported for the dansyl-modified CD monomers [17–21]. Cholic acid (**10**), which bears one more hydroxyl group on C-12 in a steroidal framework than **8** and **9**, was hardly detected. These results indicate that hosts β-**1** and γ-**1** detect the guests which have hydroxyl groups on C-3 and C-7 in the steroidal framework and hardly recognize the guests which have only one hydroxyl group on C-3 or a hydroxyl group on C-12 in the steroidal framework, suggesting that these hosts exhibit selective sensing ability for bile acids by distinguishing the position of the hydroxyl group of the guests. Hosts β-**1** and γ-**1** showed only low sensitivity for ketosteroids which have two or three hydroxyl groups. Progesterone (**1**), which bears no hydroxyl group and is more hydrophobic than the other ketosteroids, was detected with values of 0.090 and 0.072 for β-**1** and γ-**1**, respectively, which is higher than those of the other ketosteroids. These results obtained as sensing parameters of β-**1** for bile acids and ketosteroids indicate that γ-**1** recognizes the shapes of steroids in addition to the positions of the hydroxyl groups of bile acids as mentioned above, in which β-**1** can detect bile acids forming a *cis* AB fusion in the steriodal framework and hardly detects the ketosteroids forming a *trans* AB fusion in the steroidal framework. Host β-**1** detected (−)-borneol (**11**), (+)-fenchone (**12**) and (−)-fenchone (**13**), which are bicyclic derivatives, with high sensitivity, showing values of 0.573, 0.158 and 0.177, respectively, whereas host γ-**1** detected these guests with low sensing values, where the sensing parameters of γ-**1** for guests **12** and **13** are negligible. Among these guests, it seems that guest **11** just fits the smaller β-CD cavity. Cyclooctanol (**15**) and (−)-menthol (**16**), which are monocyclic derivatives, benzhydrol (**17**), which bears two aromatic rings, and nerol (**18**), which is a noncyclic compound, were detected by β-**1** with high sensitivities, with sensing values of 0.310, 0.210, 0.121, and 0.110, respectively, whereas cyclohexanol (**14**) was detected by β-**1** with low sensitivity. On the other hand, γ-**1** showed two different recognition patterns, viz larger parameters for larger guests such as bile acids and smaller ones for smaller guests such as terpenoids. These results suggest that a much more advanced sensing system has been constructed here because the combination of β-**1** and γ-**1** can show a much higher selective recognition pattern for guest molecules.

Binding constants

The guest-induced fluorescence variation at 526 nm was employed to calculate the binding constants of the hosts. A computer simulation using fluorescent intensity at a fixed wavelength as a function of guest concentration proved that experimental data could be fitted to linear equations very well, indicating a Benesi–Hildebrand type equation for 1 : 1 complex formation, as shown in Figure 5. If the complex has 2 : 2 stoichiometry, the experimental data cannot be linear in a Benesi–Hildebrand plot. This fact means that the complex formation is 1 : 1 not 2 : 2. The host–guest complex formation might be expected to be 1 : 2 which means one host can include two guests into its cavity, although it is obvious that the formation is 1 : 1, because the host has two cavities which can include a guest into each cavity. The linear equations are evidence for the formation of a 1 : 1 complex [38, 41, 48, 49]. The binding constants are calculated using Equation (1), as reported previously [12].

$$\frac{1}{I_f - I_f^0} = \frac{1}{a[\mathrm{CD}]} + \frac{1}{a[\mathrm{CD}]K} \times \frac{1}{[\mathrm{G}]}. \qquad (1)$$

Here, I is the fluorescence intensity at 526 nm (I_f for complex, I_f^0 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 β-**1** for bile acids are almost three times as large as those of γ-**1** and the sequence of these constants of β-**1** and γ-**1** for bile acids is **7** > **9** > **8**. The binding constant of β-**1** for guest **7** is the highest for all the guests examined, whereas the sensing parameter of β-**1**

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

Guest	Binding constant[a]	
	β-**1**	γ-**1**
Lithocholic acid (**7**)	111000 ± 9880[b]	43500 ± 4240
Chinodeoxycholic acid (**8**)	11100 ± 770	3720 ± 320
Ursodeoxycholic acid (**9**)	19600 ± 840	6850 ± 620
(−)-Borneol (**11**)	1950 ± 50	–
Cyclooctanol (**15**)	590 ± 50	–

[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.

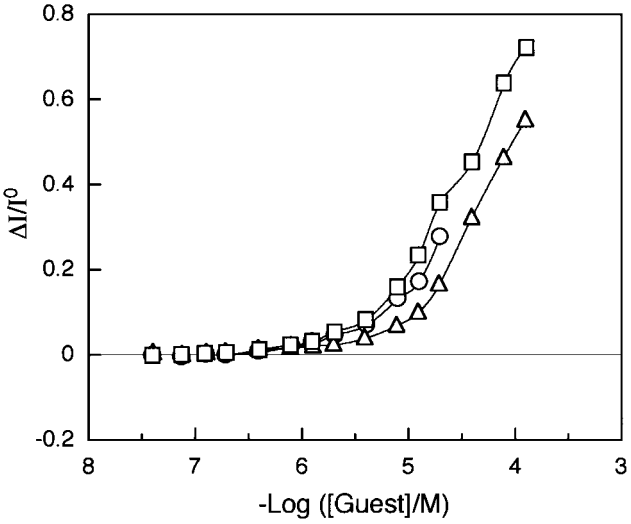


Figure 6. Fluorescence variations of β-**1** (1.0×10^{-6} M, 25 °C) in a 10 vol.% ethylene glycol aqueous solution for lithocholic acid (○), chenodeoxycholic acid (△) and ursodeoxychlic acid (□) as a function of guest concentration.

for guest **7** is not higher than for other guests. The saturation phenomenon of β-**1** with guest **7** was observed around $10^{-4.5}$ M of guest **7**, as shown in Figure 6, whereas the response range of β-**1** for guests **8** and **9** was $10^{-5.5}$–10^{-4} M. It suggests that the formation of a complex between β-**1** and guest **7** is very strong and seems to be the reason why guest **7** has the highest binding constant. These constants of β-**1** for guests **8**, **9** and **11** are almost higher than those of dansyl-modified CD monomers [19–21]. It suggests that β-**1** will exhibit a more sensitive and selective binding ability than the γ-analog and the β- and γ-CD monomers, even if a guest concentration is varied.

Conclusions

Bis dansyl-modified β-CD dimer has been prepared to investigate the sensing ability for organic guests such as bile acids and terpenes. This dimer shows pure monomer fluorescence, the variation of which was used as a parameter to describe the sensing ability. This host can detect guests such as chenodeoxycholic acid and ursodeoxycholic acid, which bear hydroxyl groups on C-3 and C-7 of the steroidal framework, and (−)-borneol with higher sensitivity and selectivity than CD monomers. It means that this host can recognize the shape and size of the guest compounds, in which the dansyl moieties work as a hydrophobic cap to elevate the binding ability. In this system, two binding sites and a large hydrophobic domain made up by two CDs contributes to the qualitative molecular recognition of this host. Now we are investigating fixed multi-recognition sites such as tri and tetra fluorescent active CD analogs.

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