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# Synthesis of Bis Dansyl-Modified β-Cyclodextrin Dimer Linked with Azobenzene and Its Fluorescent Molecular Recognition

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**Abstract**

Flexible dimeric host, 4,4′-azobenzenediamido-bis-6-(2-dansyl-aminoethyl)6-deoxy-β-cyclodextrin (**β-1**), has been synthesized in order to investigate its fluorescent molecular sensory system transforming photoisomerization. Photoisomerization of **β-1** was performed with photoirradiation by a 500W-Xe lamp using a Corning 7-37 filter. Host **β-1** showed pure monomer fluorescence, exhibiting a decrease in fluorescence intensity on accommodation of the guest, and the extent of fluorescence variation with a guest was employed to display the sensing ability of **β-1**, in which the sensing parameter $(\Delta I/I^0)$was used to describe the sensing ability. Host **β-1** could detect chenodeoxycholic acid, ursodeoxycholic acid and hyodeoxycholic acid with high selectively. The behaviors of the appended moieties of **β-1** during host-guest complexation were studied by induced circular dichroism (ICD) and fluorescence spectra and MM2 energy-minimized structure. The ICD and fluorescence intensities of **β-1** decreased on the addition of a guest. The guest-induced variations in the ICD and fluorescence spectra, and MM2 energy-minimized structure suggested that the dansyl moieties of **β-1** worked as a hydrophobic cap when a host-guest complexation occurred.

## Introduction

Recently, we have tried to investigate synthesis and fluorescent molecular sensing of multiple cyclodextrins such as linked cyclodextrin dimer and trimer [1–4]. In these systems, these cyclodextrin derivatives have fixed multi-recognition sites forming multiple complexation of guest species. Furthermore, multi-binding sites and large hydrophobic domain enclosed cyclodextrins contribute to qualitative molecular recognition of these multiple cyclodextrins. Therefore, it is very interesting that a new cyclodextin dimer, which is functionalized with both fluorecent and anchi-fluorescent molecular sensing, is synthesized. In this study, we describe the synthesis and fluorescent sensing ability of bis dansyl-appended β-cyclodextrin dimer linked with azobenzene, 4,4′-azobenzenediamido-bis-6-(2-dansyl-aminoethyl)6-deoxy-β-cyclodextrin **β-1**, forming trans- or cis-photoisomerization.

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## Experimental

### *Material*

*Preparation of 4,4′-azobenzenediamido-bis-6-(2-aminoethyl)6-deoxy-β-cyclodextrin (**I**)*

To a cool solution (−10 °C) of 4,4′-carboxylic acid (90.9 mg, 0.34 mmol) in 30 mL of DMF was added dicyclohexyl carbodiimido (DCC, 277.7 mg, 1.35 mmol) and 1-hydroxy tribenzotriazole (1-HOBt, 181.8 mg, 1.35 mmol). The reaction mixture was stirred at −10 °C for 30 min. To a stirred solution was added portionwise 6-deoxy-6-amino-β-cyclodextrin [5] (1.19 g, 1.01 mmol) heated at 80 °C for 24 h. After cooling, the reaction mixture was poured into 500 mL of acetone. The precipitate was filter off and dried. The water soluble fraction was applied on a CM-sephadex C-50 (7 × 35 cm). Stepwise elution from 2 L of water and 2 L of 1 vol.% ammonia aqueous solution were applied to give compound **I**. The fractions containing **I** were collected and evaporated *in vacuo* and then they were poured into 300 mL of acetone. The resulting precipitates were filtered and dried to afford 278 mg (31.8%, isolated yield) of pure **I**.

$R_f$: 0.15 (methyl ethyl ketone-methanol-acetic acid 12:3:5 by volume; TLC; silica gel $60F_{254}$). $^1$H-NMR ($D_2O$) $\delta$ = 2.5–2.8 (8H, m, $NCH_2$), 3.3–3.6 (28H, m, $C^2H$ and $C^4H$ of cyclodextrin), 3.6–4.0 (56H, m, $C^3H$, $C^5H$ and $C^6H$ of cyclodextrin), 4.96 (14H, s, $C^1H$ of cyclodextrin), 7.87 (4H, d, $J$ = 7.5 Hz, aromatic-H of azobenzene), 7.96 (4H, s, aromatic-H of azobenzene).

*Preparation of 4,4′-azobenzenediamido-bis-6-(2-dansyl-aminoethyl)6-deoxy-β-cyclodextrin* ***β*-1**

To a cool solution (−10 °C) of dansylglycine (71.6 mg, 0.232 mmol) in 7 mL of DMF was added DCC (47.9 mg, 1.232 mmol) and 1-HOBt (31.4 mg, 0.232 mmol). The reaction mixture was stirred at −10 °C for 30 min. To a stirred solution was added portionwise compound **I** (150 mg, 0.058 mmol) heated at 80 °C for 24 h. After cooling, the reaction mixture was poured into 250 mL of acetone. The precipitate was filter off and dried. The DMF soluble fraction was applied on a reversed-column (Lobar column Lichroprep PR-18, Merck Ltd., 240 × 10 cm). Stepwise elution from 300 mL of 10 vol.%, 300 mL of 20 vol.% and 300 mL of 30 vol.% MeOH aqueous solution were used to give ***β*-1**. The fractions of ***β*-1** were collected and evaporated *in vacuo* and then they were poured into 200 mL of acetone. The resulting precipitates were filtered and dried to afford 78 mg (12.6%, isolated yield) of pure ***β*-1**.

$R_f$: 0.54 (butanol-ethanol-water 5:4:3 by volume; TLC; silica gel $60F_{254}$) and 0.79 (methanol-water 2:1 by volume; TLC; PR-$18F_{254}$S; Merck Ltd.). $^1$H-NMR (DMSO) $\delta$ = 2.83 (12H, s, $NCH_3$ of dansyl), 3.0–3.8 (84H, m, $C^2H$-$C^6H$ of cyclodextrin), 3.9–4.7 (12H, m, $O^6H$ of cyclodextrin), 4.75–5.95 (14H, m, $C^1H$ of cyclodextrin), 5.6–5.9 (28H, m, $O^6H$ and $O^3H$ of cyclodextrin), 7.11 (2H, d, $J$ = 8.1 Hz, aromatic-H of azobenzene), 7.23 (2H, d, $J$ = 7.8 Hz, aromatic-H of dansyl), 7.46 (2H, t, $J$ = 8.1 Hz, aromatic-H of azobenzene), 7.60 (4H, quartet, $J$ = 6.9 Hz, aromatic-H of dansyl), 7.65–7.75 (4H, m, aromatic-H of azobenzene), 8.11 (2H, d, $J$ = 6.6 Hz, aromatic-H of dansyl), 8.28 (2H, d, $J$ = 9.0 Hz, aromatic-H of dansyl), 8.46 (2H, d, $J$ = 7.6 Hz, aromatic-H of dansyl), Calcd. for $C_{130}H_{186}O_{76}N_{10}S2{\cdot}5H_2O$: C, 47.91; H, 6.06; N, 4.30%. Found: C, 47.75; H, 6.01; N, 4.35%. TOF-MS (m/z): 3168, ($[M]^+$).

*Measurements*

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

For the ICD measurements, ethylene glycol aqueous solution (10 vol.%) was used as a solvent of the host, because the solubility of the host in pure water is poor. 5 $\mu$L 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 $1.0 \times 10^{-4}$ M and guest concentrations of $1.0 \times 10^{-4}$ and $2.0 \times 10^{-4}$ M.

For the fluorescence measurements, the excitation wavelength of the fluorescence spectra was 342 nm, and the excitation and emission were 7 nm wide. 5 $\mu$L of guest species (0.05 and 0.05 M) in 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 $1.0 \times 10^{-6}$ M and guest concentrations of $1.0 \times 10^{-4}$ and $1.0 \times 10^{-5}$ M.

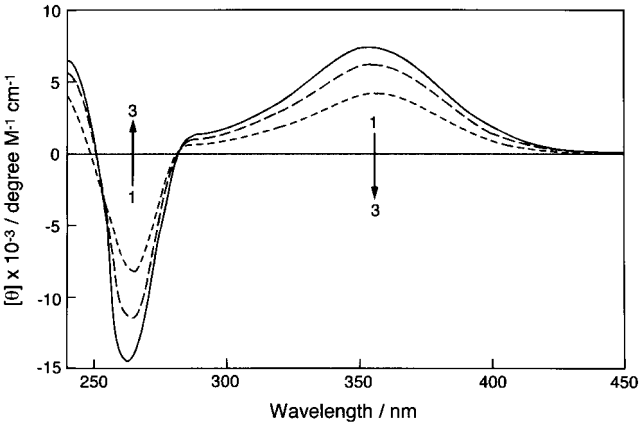



*Figure 1.* ICD spectra of ***β*-1** (trans-form) in a 10 vol.% ethylene glycol aqueous solution ($1.0 \times 10^{-4}$ M: ———, 25 °C) at various concentrations of hyodeoxycholic acid (1: 0, 2: $1.0 \times 10^{-4}$ M: — – -, 3: $2.0 \times 10^{-4}$ M: – – –).

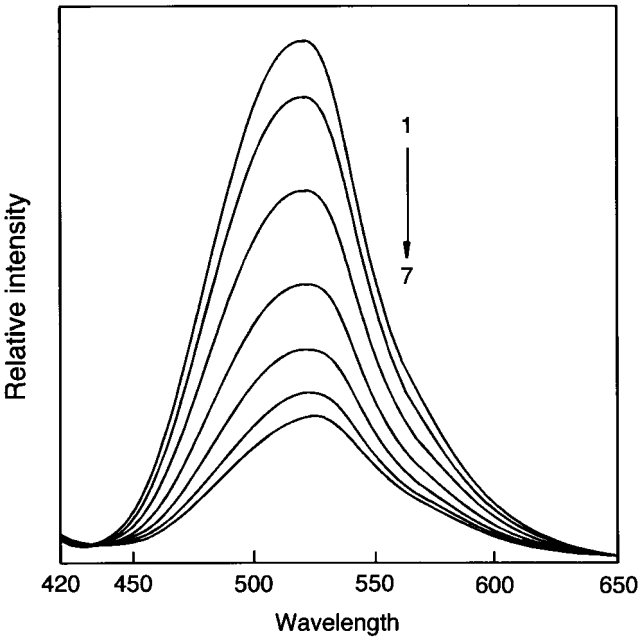



*Figure 2.* Fluorescence spectra of ***β*-1** (trans-form) in a 10 vol.% ethylene glycol aqueous solution ($1.0 \times 10^{-6}$ M 25 °C) at various concentrations of hyodeoxycholic acid (1: 0, 2: $4.0 \times 10^{-6}$, 3: $8.0 \times 10^{-6}$, 4: $1.2 \times 10^{-5}$, 6: $4.0 \times 10^{-5}$, 7: $6.1 \times 10^{-5}$ 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 [6] based on the TINKER system researched by Ponder [7].

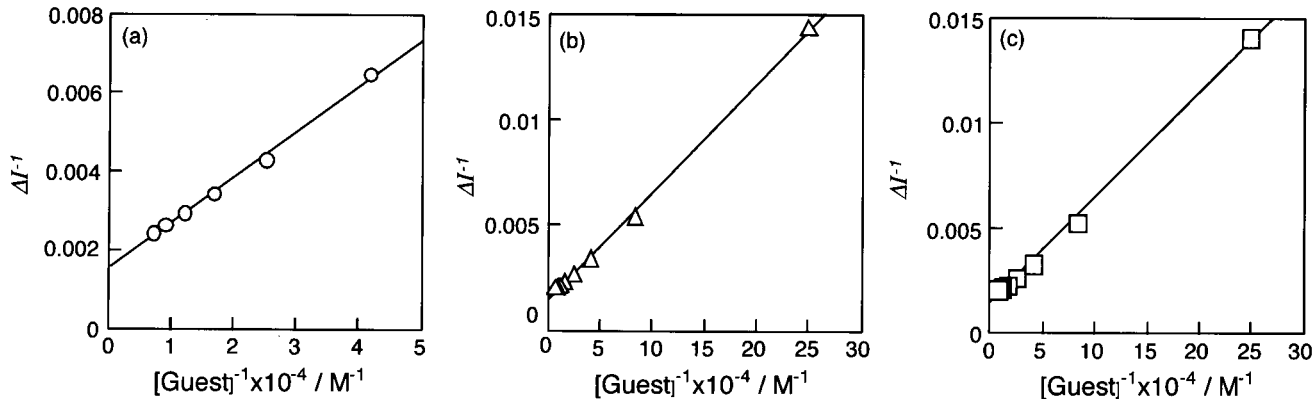
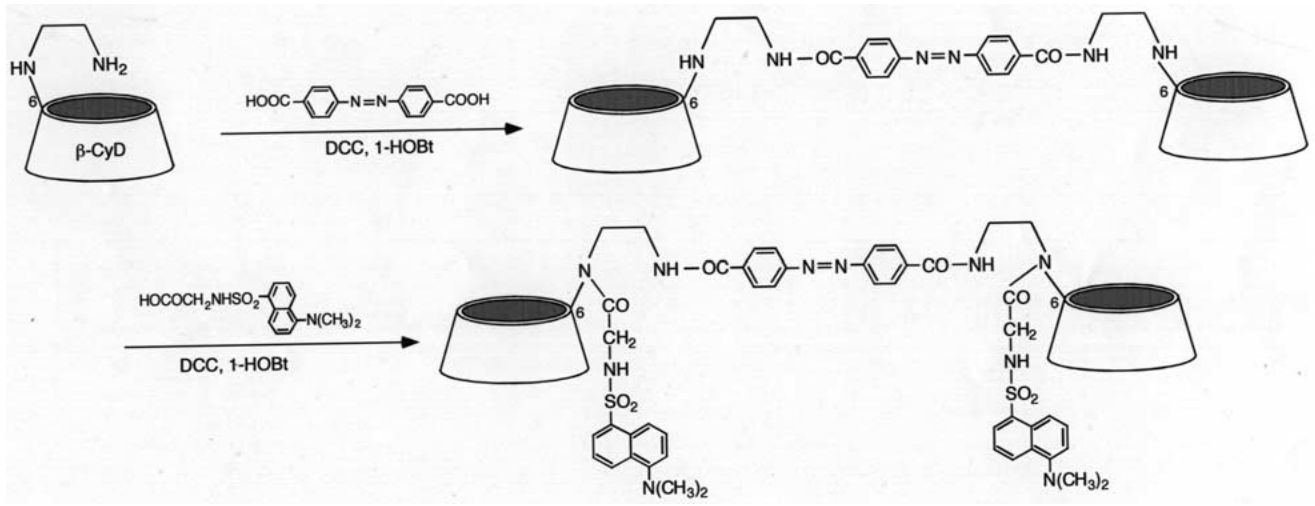
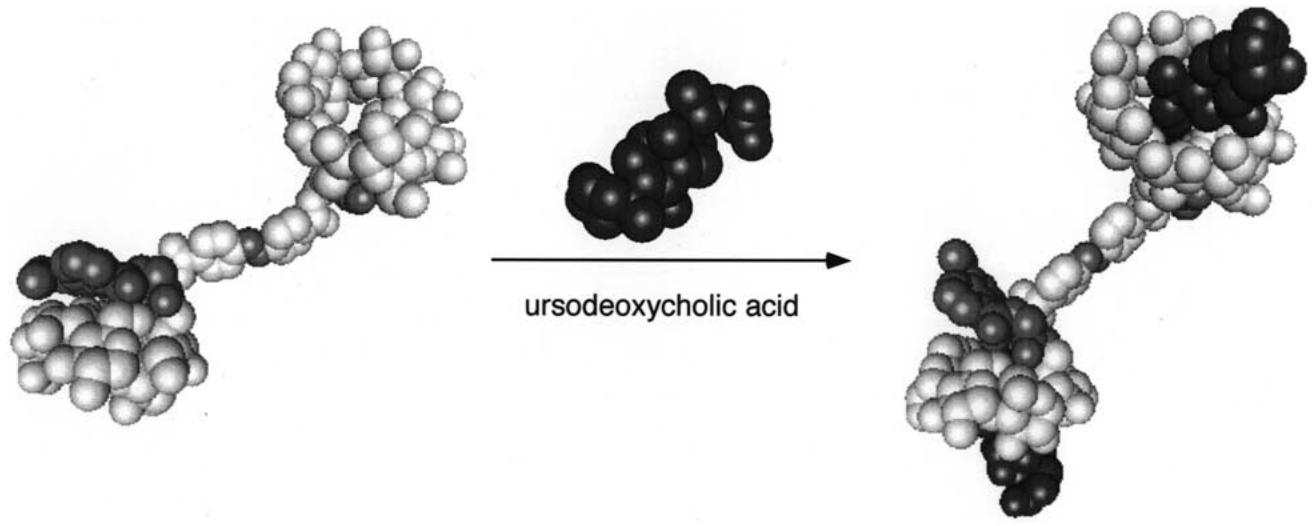


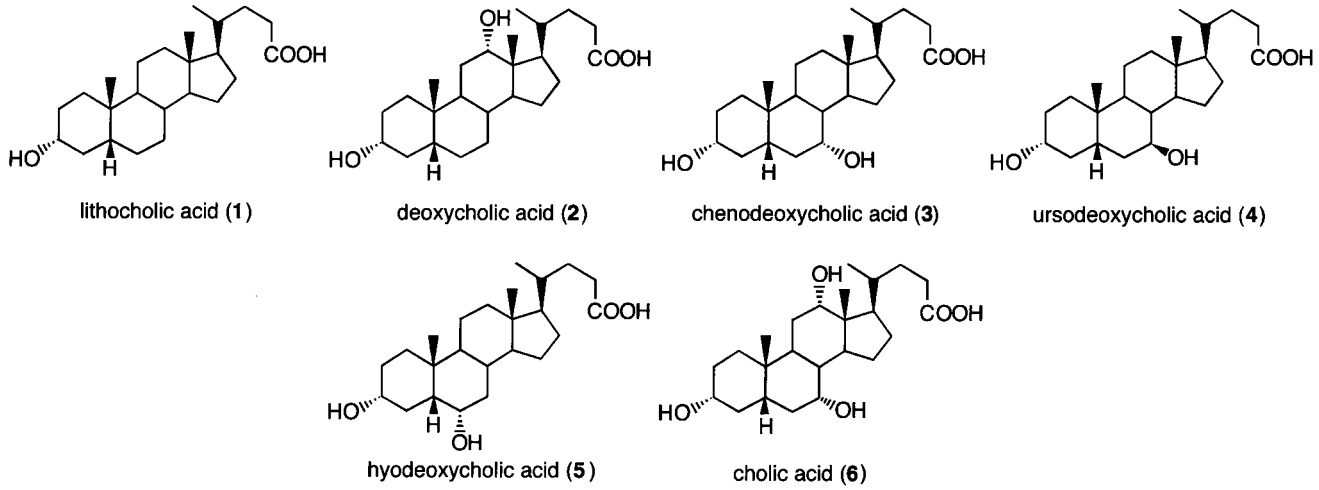

*Figure 3.* Binding curves of **β-1** (trans-form) in a 10 vol.% ethylene glycol aqueous solution ($1.0 \times 10^{-6}$ M, 25 °C) for chenodeoxycholic acid (a), ursodeoxycholic acid (b) and hyodeoxycholic acid (c).




*Scheme 1.* Preparation of **β-1**.




*Scheme 2.* Estimated complexation of **β-1** (trans-form) with a guest obtained as MM2-minimized space-filling structure.




*Scheme 3.* Guest molecules.

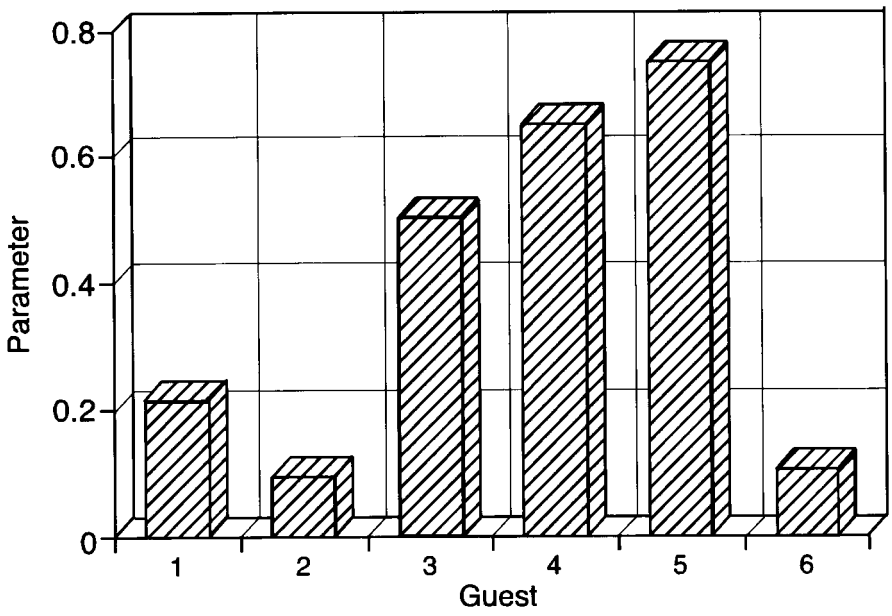



*Figure 4.* Sensing factors of **β-1** (trans-form) in a 10 vol.% ethylene glycol aqueous solution ($1.0 \times 10^{-6}$ M, 25 °C) for all guests examined (guest concentrations; **1** and **3–6**: 0.1 mM, **2**, 0.01 mM).

## Results and discussion

### *Induced circular dichroism (ICD) spectra*

Figure 1 shows the ICD spectra of **β-1** (trans-form), alone, in the presence of hyodeoxycholic acid in a 10 vol.% ethylene glycol aqueous solution. The spectra of **β-1** (trans-form) alone exhibit a positive band around 355 nm and negative band around 262 nm, which decrease with increasing hyodeoxycholic acid concentration. As 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 cyclodextrin and chiral cyclodextrin. On the other hand, it was reported that decrease of the ICD intensity upon guest addition suggest two possibilities; one is that the appended moiety move far from the chiral environment of the cyclodextrin cavity, and another is that the appended moiety is rotated to allow host-guest complexation [1–4, 8, 9]. Such a result suggests that dansyl moieties of **β-1** (trans-form) moves from the rim of the chiral cyclodextrin toward the outside bulk water environment while simultaneously a guest is included in the cyclodextrin cavity.

### *Fluorescent spectra*

Figure 2 shows the fluorescence spectra of **β-1** (trans-form) in a 10 vol.% ethylene glycol aqueous solution in the absence and presence of hyodeoxycholic acid. The fluorescence spectra of **β-1** (trans-form) are composed of monomer emission with a peak at around 520 nm, and the fluorescence intensity decreases with increasing hyodeoxycholic acid concentration. It is reported that the guest-induced fluorescence decrease means that the appended is moving out of the cyclodextrin cavity, whereas an enhancement means the appended moiety is moving more deeply in to the cavity [10–12]. The results obtained as ICD and fluorescencespectral changes **β-1** (trans-form), and its MM2-minimized structure suggest that the dansyl moieties move out from the

cyclodextrin cavity upon guest binding and play a roll as a hydrophobic cap. Additionally, a computer simulation using fluorescence intensity at 520 nm as a function of the guest concentration proved that experimental data fit to a Benesi-Hildebrand type equation for 1:1 complex formation, as shown in Figure 3. This is evidence for the formation of a 1:1 complex not 2:2 complex [13], as illustrated in Scheme 2, because the host has two cavities which can include a guest into each cavity [1–3].

To evaluate the sensing ability of **β-1** (trans-form), the $\Delta I/I^0$ was used as sensitivity parameter. Here, $\Delta I$ is $I^0 - I$, where $I^0$ is the fluorescence intensity for **β-1** (trans-form), and $I$ is that for complex. Figure 4 shows the parameter values of **β-1** (trans-form) with steroids at 0.1 mM except for lithocholic acid (**1**), which was examined at 0.01 mM because 0.1 mM of guest **1** is not soluble in a 10 vol.% ethylene glycol aqueous solution. Hyodeoxycholic acid (**5**), which bears two-hydroxyl groups on C-3 and C-6 of the steroidal framework, is detected by **β-1** with the greatest sensitivity, exhibiting a value of 0.749. Ursodeoxycholic acid (**4**), which bears two hydroxyl groups on C-3 and C-7 of the steroidal framework, is detected by **β-1** with the next highest sensitivity, exhibiting a value of 0.651. Chenodeoxycholic acid (**3**), which is the diastereoisomer of guest **4**, is detected by **β-1** with high sensitivity, exhibiting a value of 0.504. Lithocholic acid (**1**), deoxycholic acid (**2**) and cholic acid (**6**), which bear only one hydroxyl group on C-3, two hydroxyl groups on C-3 and C-12, and three hydroxyl groups on C-3, C-7 and C-12 of the steroidal framework, respectively, are detected by **β-1** with low sensitivity, exhibiting values of 0.212, 0.094 and 0.104, respectively. These results mean that host **β-1** exhibits high sensitivity for steroids which have two hydroxyl groups at C-3 and C-6 or C-7 in the steroidal framework. It suggests that host **β-1** shows selective molecular recognition for steroidal compounds. Now we are investigating the host-guest complexation system base on the cis-form of this host.

## Acknowledgments

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