Fluorescent Molecular Sensory System Based on Bis Pyrene-Modified γ -Cyclodextrin Dimer for Steroids and Endocrine Disruptors

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A γ -cyclodextrin dimer modified with two pyrene moieties, 6-(2-pyrenebutylate-aminoethyl)pyrenebutylate-amino-6-deoxy-bis- γ -cyclodextrin, has been synthesized in the presence of *N,N'*-dicyclohexylcarbodiimide from γ -cyclodextrin dimer linked with ethylenediamine at an upper rim. The sensing ability and binding property of the titled dimer were investigated for bile acids and endocrine disruptors. This cyclodextrin dimer showed both monomer and excimer fluorescence; the guest-induced emissions were observed as increases or decreases depending on the guest. The values $\Delta I_m/I_m^0$ and $\Delta I_{ex}/I_{ex}^0$, where I_m^0 and I_m are fluorescence intensities of monomer emission in the absence and presence of a guest and I_{ex}^0 and I_{ex} are those of excimer emission and ΔI_m and ΔI_{ex} were $I_m - I_m^0$ and $I_{ex} - I_{ex}^0$, respectively, were used as a parameter of sensitivity. This host exhibited highly sensitive molecular recognition ability for bile acids and endocrine disruptors, in which the sensing parameters obtained from monomer emission were plus or minus values, whereas the parameters obtained as excimer emission were minus ones. The behavior of the appended moieties of the host during a host-guest complexation was studied by induced circular dichroism (ICD) and fluorescence spectra. The ICD intensities of the titled dimer were decreased upon an addition of a guest. The guest-induced variations in the fluorescence and ICD intensity suggest that the appended moieties move by altering the spatial relationship in the hydrophobic space between two linked cyclodextrins.

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Introduction

Cyclodextrins (CyDs) are torus-shaped cyclic oligomers of D-glucopyranose and are named α -, β - and γ - for the hexamer, heptamer and octamer, respectively. CyDs have been studied as an enzyme model and molecular receptor, because they can include various organic compounds into their hydrophobic cavity.^{1,2} For the last decade, we have contributed to report chemo-sensory systems based on fluorescent CyDs modified with naphthalene,³ anthracene,⁴ anthranilate,⁵⁻⁹ fluorescamine,¹⁰ terphenyl,¹¹ and dansyl,¹²⁻¹⁵ in which these fluorescent CyDs detect biologically important bile acids such as lithocholic acid, chenodeoxycholic acid and ursodeoxycholic acids with high sensing ability. In the study of cyclodextrin dimers, preparations and binding properties of spectroscopically inert CyD dimers using spectroscopically active guests have been reported.¹⁶⁻¹⁹ Ueno and co-workers discussed fluorescent β -CyD dimer modified with mono-dansyl,²⁰ in which this dimer recognized bile acids and the host-guest complex formation was a 1:1. Previously, we synthesized bis dansyl-modified γ -CyD dimer which recognizes bile acids such as lithocholic acid, chenodeoxycholic acid and ursodeoxycholic acid with high sensitivity and selectivity.²¹ Mono- and bis pyrene-modified γ -CyD monomers exhibited monomer and excimer fluorescence spectra,^{22,23} in which the appended moieties of CyDs modified

with two pyrenes took face-to-face orientation derived from intramolecular excimer formation, although mono-pyrene-modified γ -CyD formed an association dimer exhibiting the excimer emission derived from intermolecular excimer formation. This fact forced us to construct a new type of chemo-sensor based on CyD dimer. In this study, we synthesized bis pyrene-modified γ -CyD dimer. Previously, we have found that our CyD derivatives can detect endocrine disruptors with high sensitivity and selectivity.^{24,25} That is why we have tried again to study the fluorescent binding ability for bile acids in addition to endocrine disruptors by the titled CyD dimer.

In this contribution, we would like to describe the synthesis of bis pyrene-modified γ -CyD dimer, 6-(2-pyrenebutylate-aminoethyl)pyrenebutylate-amino-6-deoxy-bis- γ -CyD (host **1**), as a new indicator with two binding and fluorescent active sites which can show monomer and excimer emissions. This fluorescent sensory system exhibited high qualitative determination for steroids and endocrine-disruptors.

Experimental

*Preparation of 6-(2-pyrenebutylate-aminoethyl)pyrenebutylate-amino-6-deoxy-bis- γ -CyD (**1**)*

To a cooled solution (-10°C) of 4-(1-pyrenyl)butanoic acid (165 mg, 0.57 mM) in 8 mL of DMF were added dicyclohexylcarbodiimide (DCC, 118 mg, 0.57 mM) and 1-hydroxybenzotriazole (HOBt, 77 mg, 0.57 mM). The reaction

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Fig. 1 Preparation of **1**. Scheme 1 Structure of bis pyrene-modified γ -CyD monomer and host **1**.

mixture was stirred at -10°C for 30 min. To this stirred solution was added portionwise 6-(2-aminoethyl)-amino-6-deoxy-bis- γ -CyD²¹ (500 mg, 0.19 mM). The solution was stirred at -10°C for another 30 min, and then the reaction mixture was stirred at 50°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 on a reversed-column (Lobar column LiChroprep RP-18, Merck Ltd., 440 mm $\times$ 37 mm). Step-wise elution from 500 mL of 10 vol.%, 1 L of 20 vol.%, 500 mL of 30 vol.% aqueous MeOH applied to give compound **1**. The fractions containing **1** were collected and evaporated *in vacuo*; and they were poured into 500 mL of acetone. The resulting precipitates were filtered and dried to afford 5 mg (0.8%, isolated yield) of pure **1**. Rf: 0.34 (butanol-ethanol-water 5:4:3 by volume; TLC; silica gel 60F₂₅₄) and 0.63 (methanol-water 2:1, by volume; TLC; RP-18F₂₅₄; Merck Ltd.). ¹H-NMR (DMSO) = 3.2–3.8 (96H, m, C²-C⁶H of CyD), 4.8–5.0 (14H, m, O⁶H of CyD), 5.7–6.0 (32H, m, O³H and O³H of CyD), 7.96 (4H, t, J = 7.2 Hz, aromatic-H of pyrene), 8.05 (2H, t, J = 7.5 Hz, aromatic-H of pyrene), 8.13 (2H, s, aromatic-H of pyrene), 8.25 (8H, quintet, J = 7.8 Hz, aromatic-H of pyrene), 8.44 (2H, t, J = 7.8 Hz, aromatic-H of pyrene). Calcd. for C₁₃₈H₁₉₂O₈₀N₂·6H₂O: C, 50.73; H, 6.29; N, 0.86%. Found: C, 50.88; H, 6.51; N, 0.82%. TOF-MS (m/z): 3159, [M]⁺.

Measurements

Fluorescence and circular dichroism spectra were measured at 25°C with 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 334 nm and excitation and emission slits were 2.5 nm wide. Ethylene glycol aqueous solution (10 vol.%) was used as a solvent for host for

Fig. 2 ICD spectra of **1** in a 10 vol.% ethylene glycol aqueous solution (5.0×10^{-5} M: —, 25°C) at various concentrations of ursodeoxycholic acid (5.0×10^{-5} M: ----, 1.0×10^{-4} M: -.-.-). Fig. 3 ICD spectra of **1** in a 10 vol.% ethylene glycol aqueous solution (5.0×10^{-5} M: —, 25°C) at various concentrations of 2,4,6-trichlorophenol (5.0×10^{-4} M: ----, 1.0×10^{-3} M: -.-.-).

the spectroscopic measurements because the solubility of the host in pure water is poor. Five microliters of guest species (0.5, 0.05 and 0.005 M) in dimethyl sulfoxide (DMSO) or MeOH were injected into 10 vol.% ethylene glycol aqueous solution of the host (2.5 mL) to make a sample solution with a host concentration of 1.0×10^{-6} M and a guest concentration of 1.0, 0.1 and 0.01 mM, respectively.

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 N. L. Allinger²⁷ based on TIKER system researched by J. W. Ponder.²⁸

Results and Discussion

The preparation of 6-(2-pyrenebutylate-aminoethyl)pyrene-butylate-amino-6-deoxy-bis- γ -CyD (**1**)

Bis pyrene-modified γ -CyD dimer (**1**) was prepared from γ -CyD dimer²¹ (6-(2-aminoethyl)-amino-6-deoxy-bis- γ -CyD) with an excess of 4-(1-pyrenyl)butanoic acid in the presence of DCC and HOBT, and separated with reverse phase column chromatography, as shown in Fig. 1.

Scheme 2 Energy-minimized structure of host **1** using molecular mechanics in MM2 (steric energy is 273.46 kcal mol⁻¹).

Induced circular dichroism (ICD) spectra

The ICD spectra of host **1** alone and in the presence of ursodeoxycholic acid or 2,4,6-trichlorophenol in a 10 vol.% ethylene glycol aqueous solution are shown in Figs. 2 and 3, respectively. The ICD spectra of host **1** alone shows positive bands at around 240, 250, 290, 340, and 355 nm and a negative band at around 405 nm. It was reported that the pyrene moiety of mono-modified CyD monomer was included into the CyD cavity parallel to the CyD axis.²² The ICD pattern of host **1** is opposite to that of mono-pyrene-modified CyD monomer. It is substantiated that pyrene moieties of host **1** close to the CyD cavity are parallel to the CyD equator. This is supported by the energy-minimized structure of host **1** using molecular mechanics in MM2 (steric energy is 273.46 kcal mol⁻¹), as illustrated in Scheme 2. The ICD intensities of positive and negative Cotton peaks of host **1** are decreased with increasing guest concentration. These results suggest that the two pyrene moieties of this host go away from the rim of the hydrophobic CyD cavity, while simultaneously a guest is included into the CyD cavity.

Fluorescence spectra

The fluorescence spectra of host **1** in a 10 vol.% ethylene glycol aqueous solution in the presence and absence of ursodeoxycholic acid are shown in Fig. 4. The fluorescence spectra of host **1** are composed of monomer and excimer emissions with the peaks at around 377 and 397 nm, and 475 nm, respectively. The excimer emission observed in host **1** indicates that the two pyrene moieties are located in face-to-face orientation, which is needed for excimer formation.²³ The fluorescence intensities of the monomer and excimer emissions increase and decrease, respectively, with increasing bile acid concentration. However, the monomer and excimer emissions of host **1** in a 10 vol.% ethylene glycol aqueous solution decrease with increasing 2,4,6-trichlorophenol concentration, as shown in Fig. 5. The decrement of the guest-induced excimer emission means that the two appended moieties of the host are far away from each other, quenching excimer emission of the host,³ and the reduction of the monomer emission upon addition with the guest indicates that the appended moiety is moving out of the CyD cavity.^{14,15,26} The results obtained as ICD and fluorescence spectral changes of the host **1** with the guest suggest that the two pyrene moieties are far away from the CyD cavity simultaneously when guests are included into the CyD cavities, being off to the side, as illustrated in the energy-minimized structure of host **1** with a guest such as ursodeoxycholic acid (steric energy is 365.25 kcal mol⁻¹) in Scheme 3. Furthermore, when a guest such as 2,4,6-trichlorophenol is added, it seems that two pyrene moieties of host **1** come out of the hydrophobic domain enclosed with two

Fig. 4 Fluorescence spectra of **1** in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-6} M, 25°C) at various concentrations of ursodeoxycholic acid (1: 0, 2: 6.0×10^{-5} , 3: 2.0×10^{-4} , 4: 4.2×10^{-4} , 5: 7.1×10^{-4} , 6: 1.1×10^{-3} M).

Fig. 5 Fluorescence spectra of **1** in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-6} M, 25°C) at various concentrations of 2,4,6-trichlorophenol (1: 0, 2: 4.0×10^{-5} , 3: 1.2×10^{-4} , 4: 2.4×10^{-4} , 5: 4.0×10^{-4} , 6: $\times 10^{-4}$, 7: 8.3×10^{-4} , 8: 1.1×10^{-3} M).

linked CyDs, as illustrated for the energy-minimized structure of host **1** with 2,4,6-trichlorophenol (steric energy is 167.34 kcal mol⁻¹) in Scheme 4.

In order to display the sensing ability of host **1**, the $\Delta I_{m1}/I_{m1}^0$, $\Delta I_{m2}/I_{m2}^0$ and $\Delta I_{ex}/I_{ex}^0$ were used as a sensitivity parameters. Here, ΔI_{m1} , ΔI_{m2} and ΔI_{ex} are $I_{m1} - I_{m1}^0$, $I_{m2} - I_{m2}^0$ and $I_{ex} - I_{ex}^0$, respectively, where I_{m1}^0 and I_{m2}^0 are the intensities in monomer emissions at 377 and 397 nm, respectively, and I_{ex}^0 is the intensity in excimer emission at 475 nm for the host alone, and I_{m1} , I_{m2} and I_{ex} are those for a complex. Figure 6 shows the parameter values of host **1** with steroids at 0.1 mM except for lithocholic acid (**2**), which was examined at 0.01 mM because 0.1 mM of guest **2** is not soluble in a 10 vol.% ethylene glycol aqueous solution. Among the steroidal guests, host **1** detects ursodeoxycholic acid (**4**), which bears two hydroxyl groups on C-3 and C-7 of the steroidal framework, with the greatest sensitivity, exhibiting $\Delta I_{m1}/I_{m1}^0$, $\Delta I_{m2}/I_{m2}^0$ and $\Delta I_{ex}/I_{ex}^0$ values of 0.597, 0.568 and -0.323 , respectively. Chenodeoxycholic acid (**3**), which is the diastereoisomer of guest **4**, is detected by host **1** with the next highest sensitivity, exhibiting $\Delta I_{m1}/I_{m1}^0$, $\Delta I_{m2}/I_{m2}^0$ and $\Delta I_{ex}/I_{ex}^0$ values of 0.519, 0.515 and -0.253 , respectively. Lithocholic acid (**2**), which bears one hydroxyl group on C-3 of the steroidal framework, is detected by host **1** with high sensitivity, exhibiting $\Delta I_{m1}/I_{m1}^0$, $\Delta I_{m2}/I_{m2}^0$ and $\Delta I_{ex}/I_{ex}^0$ values of

Scheme 3 Host-guest complexation mechanism of host **1** with ursodeoxycholic acid. Scheme 4 Host-guest complexation mechanism of host **1** with 2,4,6-trichlorophenol.

Scheme 5 Guest molecules-1.

0.505, 0.507 and -0.233 , respectively. Deoxycholic acid (**5**), which bears two hydroxyl groups on C-3 and C-12 of the steroidal framework, is detected by host **1** with high sensitivity, exhibiting $\Delta I_{m1}/I_{m1}^0$, $\Delta I_{m2}/I_{m2}^0$ and $\Delta I_{ex}/I_{ex}^0$ values of 0.422, 0.409 and -0.209 , respectively. Cholic acid (**6**), which bears three hydroxyl groups on C-3, C-7 and C-12 of the steroidal framework, is detected by host **1** with high sensitivity, exhibiting $\Delta I_{m1}/I_{m1}^0$, $\Delta I_{m2}/I_{m2}^0$ and $\Delta I_{ex}/I_{ex}^0$ values of 0.218, 0.239

and -0.127 , respectively, although, these values are half as much as sensing values for guest **5**. The sensing parameter values, $\Delta I_{m1}/I_{m1}^0$, $\Delta I_{m2}/I_{m2}^0$ and $\Delta I_{ex}/I_{ex}^0$, of host **1** for these steroidal compounds decrease in the sequence; **4** > **3** > **2** > **5** > **6**. It seems that these distinctions depend on the position of the hydroxyl groups of the guest steroidal framework in the order; C-3 and C-7 > C-3 > C-3 and C-12 > C-3, C-7 and C-12. Such an order indicates that the position of the hydroxyl groups of the

Scheme 6 Guest molecules-2.

Fig. 6 Sensitivity factors of **1** in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-6} M, 25°C) for guests **2**–**6** at 377 (□), 397 (▨), and 475 (■) nm (guest concentrations: guest **2**, 0.01 mM; guests **3**–**6**, 0.1 mM).

guest affects the sensing ability of host **1**. The sensing ability of host **1** for the guests is higher than that of bis pyrene-modified γ -CyDs, reported previously.²³ Such high ability is probably caused by the fact that the appended moieties of the CyD dimer move more easily than those of the CyD monomer, due to the large hydrophobic domain of the CyD dimer, when a guest is simultaneously included into the CyD cavity. This suggests that the CyD dimer can give higher sensing ability for the big guest such as bile acid than the CyD monomer.

Figure 7 shows the parameter values of host **1** with 1,2-dichlorobenzene (**7**), 3,4-dichlorophenol (**8**), 2,4,6-trichlorophenol (**9**), *p*-nonylphenol (**13**), bisphenol A (**15**), and diethylphthalate (**16**) at 1.0 mM, 3,4-dichlorobenzoic acid (**10**) and 2,4-dichlorophenoxyacetic acid (**14**) at 0.1 mM, and 4-chlorodiphenyl (**11**) and dibenzofuran (**12**) at 0.01 mM, because 0.5 M of guests **10** and **14**, and 0.05 M of guests **11** and **12** are not soluble in a solvent such as MeOH or DMSO. Guest **9**, which is phenol bearing three chlorines, is detected with the highest sensitivity by host **1**, exhibiting the values $\Delta I_{m1}/I_{m1}^0$, $\Delta I_{m2}/I_{m2}^0$ and $\Delta I_{ex}/I_{ex}^0$ of -0.193 , -0.137 and -0.499 , respectively. The sensing parameters obtained through exciplex

Fig. 7 Sensitivity factors of **1** in a 10 vol.% ethylene glycol aqueous solution (1.0×10^{-6} M, 25°C) for guests **7**–**16** at 377 (□), 397 (▨), and 475 (■) nm (guest concentrations: guest **7**, **8**, **9**, **13**, **15**, and **16**, 0.1 mM; guests **10** and **14**, 0.1 mM; guests **11** and **12**, 0.01 mM).

emission of host **1** for guest **9** were three times as high as those obtained through monomer emission of host **1** for guest **9**. Guest **16**, which is phthalate diester, is detected by host **1**, exhibiting the values $\Delta I_{m1}/I_{m1}^0$, $\Delta I_{m2}/I_{m2}^0$ and $\Delta I_{ex}/I_{ex}^0$ of -0.124 , -0.135 and -0.030 , respectively. These sensing parameters obtained through monomer emission of host **1** for guest **16** were minus values and the one obtained through exciplex emission of host **1** for guest **16** was a smaller minus value, which can be neglected. Guest **13**, which is phenol having a long alkyl chain, is detected by host **1**, exhibiting the values $\Delta I_{m1}/I_{m1}^0$, $\Delta I_{m2}/I_{m2}^0$ and $\Delta I_{ex}/I_{ex}^0$ of 0.122, 0.098 and 0.069, respectively. Guest **7**, which bears two chlorines, is detected by host **1**, exhibiting the values $\Delta I_{m1}/I_{m1}^0$, $\Delta I_{m2}/I_{m2}^0$ and $\Delta I_{ex}/I_{ex}^0$ of 0.091, 0.099 and -0.052 , respectively. Guest **8**, which has one more hydroxyl group than guest **7**, and guest **15**, which bears two benzene rings and hydroxyl groups, are detected by host **1**, exhibiting the value $\Delta I_{ex}/I_{ex}^0$ of -0.105 and -0.103 , respectively, whereas the values $\Delta I_{m1}/I_{m1}^0$ and $\Delta I_{m2}/I_{m2}^0$ are negligible. Guests **10**, **11** and **12**, which are dioxin analogs, and guest **14**, which has two chlorines, are detected with low sensitivity and the values $\Delta I_{m1}/I_{m1}^0$, $\Delta I_{m2}/I_{m2}^0$ and $\Delta I_{ex}/I_{ex}^0$ are negligible. The results obtained from the sensing parameters of host **1** were explained by assuming that the host detected endocrine disruptor such as 2,4,6-trichlorophenol with more sensitive and selective excimer and monomer fluorescence spectral variations, indicating a large spectral change of excimer emission. It is recognized this dimer can be a new indicator as a selective sensor of these chemicals.

Binding constants

The guest-induced monomer and excimer fluorescence variations at 377 and 397 nm, and 475 nm, respectively, were employed to calculate the binding constants of the host **1**. The binding constants are calculated by using Benesi-Hildebrand equation (1), as reported previously.²⁶

$$\frac{1}{I_A - I_A^0} = \frac{1}{a[CD]} + \frac{1}{a[CD]K} \times \frac{1}{[G]} \quad A = m1, m2 \text{ and } ex \quad (1)$$

Here I is the fluorescence intensity at 377, 397 and 475 nm (I_{m1} , I_{m2} , and I_{ex} for complex, I_{m1} , I_{m2} , and I_{ex} for the host alone, respectively), $[CD]$ is the total host concentration, $[G]$ is the total guest concentration, a is a constant. A computer simulation using fluorescent intensity at three fixed wavelength

Fig. 8 Binding curves of **1** in 10 vol.% ethylene glycol aqueous solution (1.0×10^{-6} M, 25°C) for ursodeoxycholic acid at 377 (a), 397 (b) and 475 nm (c).

Fig. 9 Binding curves of **1** in 10 vol.% ethylene glycol aqueous solution (1.0×10^{-6} M, 25°C) for 2,4,6-trichlorophenol at 377 (a), 397 (b) and 475 nm (c).

of 377, 397 and 475 nm as a function of guest concentration proved that experimental data could fit to linear equations, indicating a Benesi-Hildebrand type equation for a 1:1 complex formation, as shown in Figs. 8 and 9. The host-guest complex formation seems to be a 1:2, although it is obvious that the formation is a 1:1 type, because host **1** has two cavities which can include a guest into each cavity. The linear equations are $y = 1.86 \times 10^{-3}x + 2.83 \times 10^{-3}$, $y = 3.23 \times 10^{-3}x + 4.61 \times 10^{-3}$ and $y = -8.88 \times 10^{-3}x - 6.92 \times 10^{-3}$ for chenodeoxycholic acid at 377, 397 and 475 nm, respectively; $y = 2.44 \times 10^{-3}x + 2.36 \times 10^{-3}$, $y = 3.98 \times 10^{-3}x + 3.59 \times 10^{-3}$ and $y = -1.07 \times 10^{-2}x - 6.26 \times 10^{-3}$ for ursodeoxycholic acid at 377, 397 and 475 nm, respectively; and $y = -1.91 \times 10^{-3}x - 2.80 \times 10^{-3}$, $y = -3.28 \times 10^{-3}x - 8.56 \times 10^{-3}$ and $y = -1.38 \times 10^{-2}x - 1.10 \times 10^{-3}$ for 2,4,6-trichlorophenol at 377, 397 and 475 nm, respectively. Such results are evidences for the formation of a 1:1 complex.^{20,21} The binding constants of host **1** 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 host **1** obtained from the fixed fluorescence wavelength are decreased in the sequence 377 nm (monomer) > 397 nm (monomer) > 475 nm (excimer) for guests **3** and **4** and 397 nm (monomer) > 377 nm (monomer) > 475 nm (excimer) for guest **9**. The sequence of binding constants of the host for the guests is not parallel with the sequence of the sensing factors.

Response ranges

It is very important to investigate the lower detection limits for endocrine disruptors, because endocrine disruptors exist in rivers and lakes at low concentrations of 10^{-10} – 10^{-12} g/L, at which these chemicals must be directly and conveniently detected. Figure 10 shows response curves of host **1** for 2,4,6-trichlorophenol. 2,4,6-Trichlorophenol was detected by host **1** at

Fig. 10 Fluorescence variations of **1** in 10 vol.% ethylene glycol aqueous solution (1.0×10^{-6} M, 25°C) for 2,4,6-trichlorophenol as a function of the guest concentration at 377 (○), 397 (△) and 475 (□) nm.

excimer emission with response range of $10^{-5.5}$ – 10^{-3} M and at monomer emissions with response ranges of above 10^{-4} M. The response range of host **1** for 2,4,6-trichlorophenol at excimer emission started at a lower concentration than those of host **1** for this guest at monomer emissions. Additionally, the lower detection limit of host **1** at excimer emission for this guest is lower than those of fluorescent homo- or hetero-modified CyDs reported previously^{24,25} at monomer emission. It is recognized that this fluorescent dimer detects 2,4,6-trichlorophenol at excimer emission with much more remarkable selectivity.

Table 1 Binding constants ($K \text{ mol}^{-1} \text{ dm}^3$) of host **1** in a 10 vol.% ethylene glycol aqueous solution ($1.0 \times 10^{-6} \text{ M}$, 25°C)

Guest	Binding constant, K^a (a/constant)		
	377 nm	397 nm	475 nm
Chenodeoxycholic acid (3)	12300 ± 250^b (3.53×10^8)	11000 ± 900 (2.17×10^8)	8200 ± 490 (-1.45×10^8)
Ursodeoxycholic acid (4)	11600 ± 740 (4.24×10^8)	6780 ± 360 (2.78×10^8)	3040 ± 170 (-1.60×10^8)
2,4,6-Trichlorophenol (9)	1800 ± 60 (-3.58×10^8)	2790 ± 90 (-1.17×10^8)	1020 ± 35 (-9.09×10^8)

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

Conclusion

Bis pyrene-modified γ -CyD dimer has been prepared to investigate the sensing ability for organic guests such as bile acids and endocrine disruptors. This dimer shows both monomer and excimer fluorescence, the variations of which were used as a parameter to describe the sensing ability. This host compound can detect the guests at monomer and excimer fluorescence with high sensitivity and selectivity, in which the host can distinguish bile acids by the position of the hydroxyl groups and can detect 2,4,6-trichlorophenol, which is a dioxin analog with high selectivity. The appended moieties move more easily in the large hydrophobic domain of the CyD dimer, when a guest is included into the CyD cavity to work as a hydrophobic cap which is covering the hydrophobic domain of the CyD dimer. In this system, the appended moieties exhibit large fluorescence spectral changes which appear as a decrement of fluorescence spectra; therefore, this suggests that the appended moieties act to elevate the binding ability. The fluorescent chemo-sensory system based on bis pyrene-modified CyD dimer can show a higher sensing for 2,4,6-trichlorophenol than the CyD analogs reported by our group.

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