Fluorescent Cyclodextrins for Molecule Sensing: Fluorescent Properties, NMR Characterization, and Inclusion Phenomena of *N*-Dansylleucine-Modified Cyclodextrins

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Abstract: Fluorophore−amino acid−cyclodextrin (CD) triad systems, *N*-dansyl-L-leucine-modified and *N*-dansyl-D-leucine-modified β-CD (**1**, **2**) and *N*-dansyl-L-leucine-modified and *N*-dansyl-D-leucine-modified γ-CD (**3**, **4**), were synthesized and characterized as fluorescent indicators of molecular recognition. Fluorescence decay analyses of these CD derivatives indicated that there exist two lifetime components, being in equilibrium with each other in aqueous solution, with the dansyl moiety included in its own cavity (self-inclusion) for the larger lifetime component while located outside the cavity for the shorter lifetime one. The structural analyses of β-CD derivatives **1** and **2** undertaken by combined use of 1D and 2D NMR spectra indicate that the dansyl moiety of **2** is more deeply included in the CD cavity than that of **1**. The leucine residue between the dansyl and the CD moieties of these hosts was more effective in enhancing the binding abilities for various guests when compared with the glycine residue of the corresponding hosts. The difference in the enantiomeric configuration of the leucine residue caused the difference in the binding constants with larger values for **1** than **2** and with the opposite trend for the γ-CD derivatives **3** and **4**. Upon guest addition, the fluorescence intensities of **1** and **2** decreased, reflecting the exclusion of the dansyl moiety from inside to outside of the β-CD cavity, while the fluorescence intensities of the γ-CD derivatives **3** and **4** depended on the guest as shown by the increase induced by cyclohexanol and the decrease by (−)-borneol and other larger guests. These guest-responsive variations of the fluorescence intensity enabled these hosts to be used as effective fluorescent indicators of molecular recognition.

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides, mostly consisting of six, seven, and eight glucose units for α-, β-, and γ-CD, respectively. They have the remarkable property of including various organic molecules in their central cavity in aqueous solution and have been used as molecular vessels for reactions, binding sites of enzyme models, solubilizers of water-insoluble substances, and molecular capsules for stabilizing unstable chemicals.[1−4] Although CDs themselves are spectroscopically inert, we have recently shown that they can be converted into spectroscopically active compounds by modification with appropriate chromophores.[5−17] On this basis, it was shown that many chromophore-modified CDs are capable of changing fluorescence and absorption intensities upon accommodation of a guest molecule, and these spectral variations were indicated to occur associated with the induced-fit conformational changes of the hosts, usually excluding the chromophore moiety from inside to outside the CD cavity upon guest binding.

So far most of these systems have been confined to CD−chromophore diad systems, and here we report on a new series of CD−amino acid−chromophore triad systems, where CD is β- or γ-CD, amino acid is D- or L-leucine, and chromophore is dansyl. The leucine residue inserted between CD and dansyl has a hydrophobic isobutyl side chain, so the moiety is expected to affect the binding and molecular recognition abilities of the hosts. It is also interesting how the stereochemical difference between D- and L-leucine units is reflected in the molecular recognition abilities in these triad systems. We also used dansylglycine-modified β- and γ-CD for comparison.

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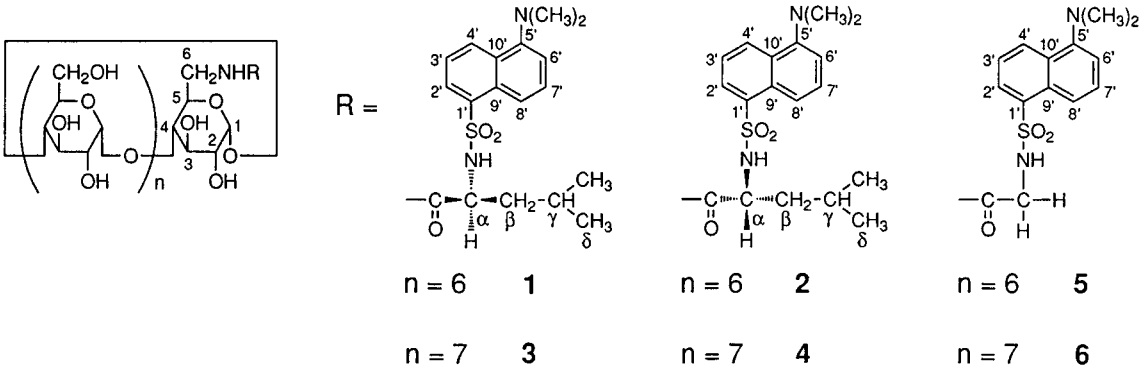
Chart 1



Experimental Section

General Methods. Thin-layer chromatography (TLC) was carried out with silica gel 60 F_{254} (Merck Co.). Absorbance and fluorescence spectra were recorded on a Shimadzu UV-3100 spectrometer and a Hitachi 850 fluorescence spectrometer, respectively. Fluorescence decay was measured by a time-correlated single-photon counting method on a Horiba NAES-550 system. A self-oscillating flash lamp filled with H_2 was used as a light source. The excitation beam was passed through the filters UV34, U340, and U350 (Hoya), and the emission beam was passed through the filters L42 (Hoya) and Y46 (Toshiba) and an aqueous solution of $NiSO_4 \cdot 6H_2O$ (500 g/L) in a 1-cm pathlength cell. The accumulated data in a memory of the system were transferred to and analyzed on a desktop computer (Hewlett-Packard HP9000 Series Model 330). Lifetime was obtained by the deconvolution with a nonlinear least-square fitting procedure. 1D and 2D NMR spectra were recorded in D_2O at 25 °C on Varian VXR-500S and UNITY plus-400 spectrometers operating at 499.843 and 399.973 MHz, respectively, for ^1H. All the NMR spectra were measured by using pulse sequences and standard procedures offered by Varian. HDO ($\delta = 4.70$) was used as an internal standard, and 3-(trimethylsilyl)propionic acid-d_4 sodium salt (TSP, $\delta = 0$) was used as an external standard. Mass spectrometry was performed on a Hitachi M-1200H mass spectrometer. Optical rotation was measured with a JASCO DIP-1000 digital polarimeter at room temperature. Elemental analyses were performed by the Analytical Division in Research Laboratory of Resources Utilization of Tokyo Institute of Technology.

Materials. β- And γ-cyclodextrin were kindly gifted from Nihon Shokuhin Kako Co., Ltd. All chemicals were reagent grade and were used without further purification unless otherwise noted. Distilled water and dimethyl sulfoxide as solvents for spectroscopy were special grade for fluorometry (Uvasol) from Kanto Chemicals. Deuterium oxide, with an isotopic purity of 99.95%, was purchased from Merck Co.

Synthesis of *N*-Dansyl-D-leucine. To a solution of D-leucine (0.75 g, 5.7 mmol) in 10 mL of 1 N NaOH aqueous solution was added a solution of dansyl chloride (2.7 g, 10.0 mmol) in 10 mL of acetone. The resulting mixture was stirred at room temperature for 4 h. After evaporation of acetone, insoluble materials were removed with filtration. The filtrate was made acidic to Congo red with 1 N HCl aqueous solution to obtain a crude product as a solid. Column chromatography on silica gel (eluted with methanol/chloroform = 1/9) afforded the desired product as a pale yellow solid (30% yield). TLC: $R_f = 0.34$ ($CHCl_3$/MeOH = 4/1). Anal. Calcd for $C_{18}H_{24}N_2O_4S_1$:C, 59.32; H, 6.64; N, 7.69, S, 8.80. Found: C, 58.57; H, 6.54; N, 7.79; S, 8.55. $[\alpha]_D$: 37.4° (*c* 0.1, methanol) ($[\alpha]_D = -37.7°$ for *N*-dansyl-L-leucine). ^1H NMR (D_2O): δ 0.01 (d, $J = 6.6$, 3H, H-δ), 0.51 (d, $J = 6.6$ Hz, 3H, H-δ'), 0.98 (m, 1H, H-γ), 1.16 (ddd, $J = 4.4$, 9.5, and 14.1 Hz, 1H, H-β), 1.28 (ddd, $J = 4.4$, 10.0 and 14.1 Hz, 1H, H-β'), 2.92 (s, 6H, N*Me*$_2$), 3.33 (dd, $J = 4.4$ and 10.0 Hz, 1H, H-α), 7.47(d, $J = 7.8$ Hz, 1H, H-6′), 7.67 (m, 2H, H-3′ and H-7′), 8.25 (dd, $J = 1.1$ and 7.4 Hz, 1H, H-2′), 8.37 (d, $J = 8.5$ Hz, 1H, H-8′), 8.43 (d, $J = 8.4$ Hz, 1H, H-4′).

Synthesis of 6-(*N*-Dansyl-L-leucylamino)-6-deoxy-β-cyclodextrin (1). To a solution of 6-amino-6-deoxy-β-cyclodextrin[11] (2.1 g, 1.9 mmol) in 10 mL of DMF was added *N*-dansyl-L-leucine (0.73 g, 2.0 mmol). After the solution was cooled below 0 °C, *N*,*N*-dicyclohexylcarbodiimide (0.45 g, 2.2 mmol) and 1-hydroxybenzotriazole (0.30 g, 2.2 mmol) were added. The resulting mixture was stirred at room temperature for 2 days. After insoluble materials were removed with filtration, the filtrate was poured into acetone, and the precipitate was collected and dried *in vacuo*. The crude product was purified by column chromatography on high porous polystyrene gel, DIAION HP-20 (eluted with water/methanol = 100/0 to 50/50). The eluent was concentrated to give the desired product as a pale yellow solid (50%

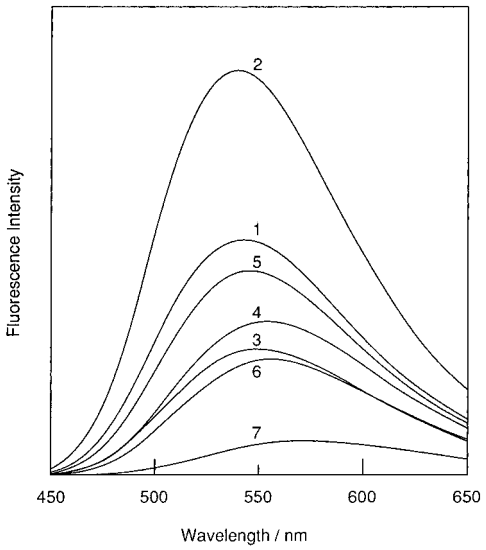


Figure 1. Fluorescence spectra of **1**−**7** (2×10^{-6} M) in aqueous solution; excitation wavelength was 370 nm.

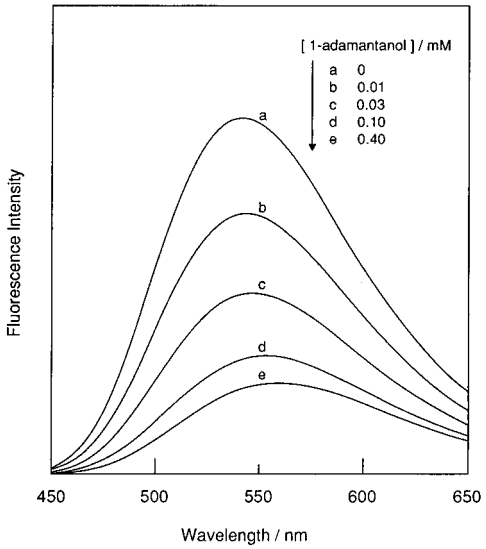
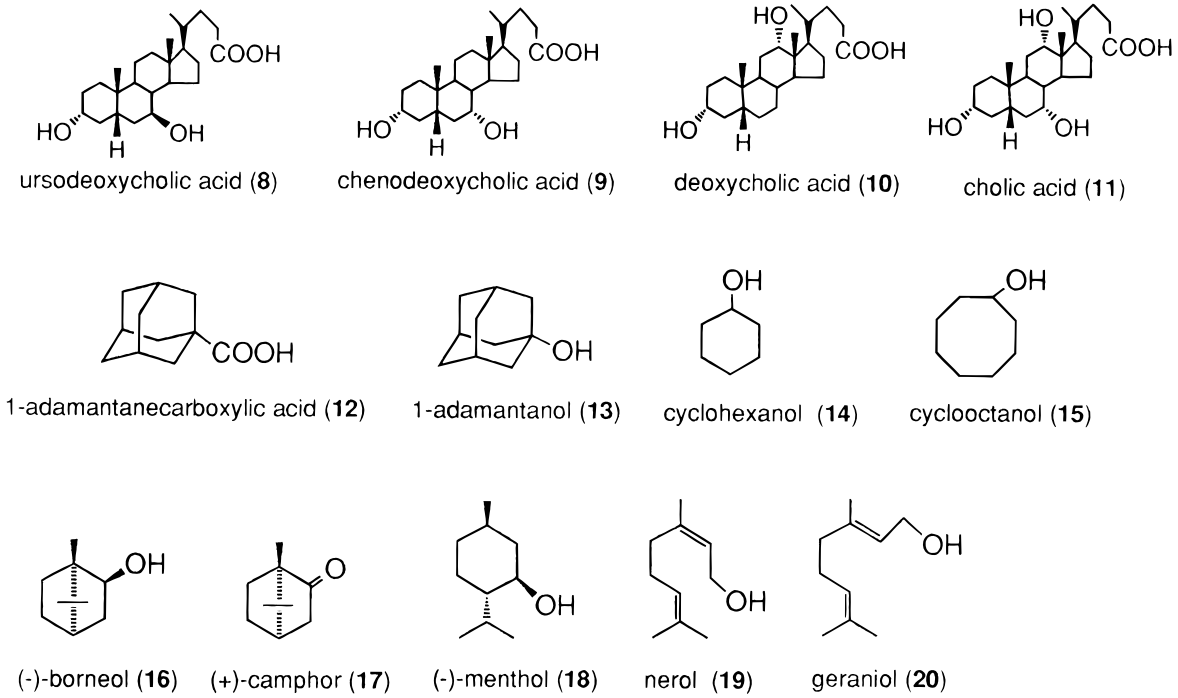


Figure 2. Fluorescence spectra of **1** (2×10^{-6} M) in aqueous solution in the presence of various concentrations of 1-adamantanol; excitation wavelength was 370 nm.

Chart 2



yield). TLC: $R_f = 0.60$ (*n*-BuOH/EtOH/H_2O = 5/4/3). Anal. Calcd for $C_{60}H_{93}O_{37}N_3S\cdot 3H_2O$: C, 47.0; H, 6.50; N, 2.74; S, 2.09. Found: C, 47.3; H, 6.52; N, 2.76; S, 2.05. MS (ESI): *m/e* 1478 (calcd for (M − H)$^-$, 1478).

Synthesis of 6-(*N*-Dansyl-D-leucylamino)-6-deoxy-β-cyclodextrin (2). This compound was prepared by the condensation of 6-amino-6-deoxy-β-cyclodextrin and *N*-dansyl-D-leucine by the same method as for **1** (40% yield). TLC $R_f = 0.61$ (*n*-BuOH/EtOH/H_2O = 5/4/3). Anal. Calcd for $C_{60}H_{93}O_{37}N_3S\cdot 4H_2O$: C, 46.4; H, 6.56; N, 2.71; S, 2.07. Found: C, 46.5; H, 6.52; N, 2.84; S, 2.47. MS (ESI): *m/e* 1478 (calcd for (M − H)$^-$, 1478).

Synthesis of 6-(*N*-Dansyl-L-leucylamino)-6-deoxy-γ-cyclodextrin (3). This compound was prepared by the condensation of 6-amino-6-deoxy-γ-cyclodextrin[11] and *N*-dansyl-L-leucine by the same method as for **1** (45% yield). TLC: $R_f = 0.52$ (*n*-BuOH/EtOH/H_2O = 5/4/3). Anal. Calcd for $C_{66}H_{103}O_{42}N_3S\cdot 4H_2O$: C, 46.2; H, 6.52; N, 2.45; S, 1.87. Found: C, 46.2; H, 6.27; N, 2.39; S, 1.90. MS (ESI): *m/e* 1640 (calcd for (M − H)$^-$, 1640).

Synthesis of 6-(*N*-Dansyl-D-leucylamino)-6-deoxy-γ-cyclodextrin (4). This compound was prepared by the condensation of 6-amino-6-deoxy-γ-cyclodextrin and *N*-dansyl-D-leucine by the same method as for **1** (60% yield). TLC: $R_f = 0.52$ (*n*-BuOH/EtOH/H_2O = 5/4/3). Anal. Calcd for $C_{66}H_{103}O_{42}N_3S\cdot 8H_2O$: C, 44.4; H, 6.71; N, 2.35; S, 1.79. Found: C, 44.0; H, 6.24; N, 2.28; S, 1.86. MS (ESI): *m/e* 1640 (calcd for (M − H)$^-$, 1640).

Results and Discussion

Fluorescence Spectra. Figure 1 shows the fluorescence spectra of *N*-dansyl-L-leucine-modified β-CD (**1**), *N*-dansyl-D-leucine-modified β-CD (**2**), *N*-dansyl-L-leucine-modified γ-CD (**3**), *N*-dansyl-D-leucine-modified γ-CD (**4**), *N*-dansylglycine-modified β-CD (**5**),[13] *N*-dansylglycine-modified γ-CD (**6**),[14] and *N*-dansyl-L-leucine (**7**) in aqueous solution. The fluorescence intensities of the dansyl-modified CDs are much larger than that of the reference compound **7**, and among the modified CDs, **2** exhibits the largest fluorescence intensity. Since the fluorescence intensity of the dansyl moiety is sensitive to a microenvironment, being stronger in a hydrophobic environment than in a hydrophilic environment,[18−21] this result suggests that the dansyl moiety of **2** is located in the most hydrophobic environment or most deeply included in the CD cavity. On this basis, the order of the maximum intensities, **2** > **1** > **5** > **4** > **3** > **6**, may be regarded roughly as the order of the hydrophobic nature around the dansyl moiety of the fluorescent CDs, consequently suggesting that the β-CD cavity is more appropriate than the γ-CD one in including the appending dansyl moiety.

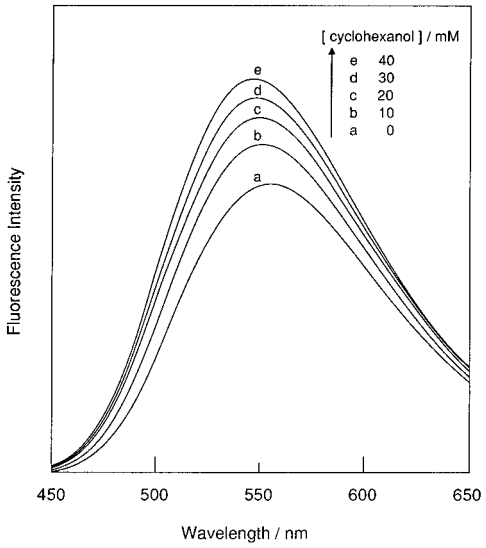


Figure 3. Fluorescence spectra of **4** (2×10^{-6} M) in aqueous solution in the presence of various concentrations of cyclohexanol; excitation wavelength was 370 nm.

The maximum wavelengths of the fluorescence spectra of **1**, **2**, **3**, **4**, **5**, **6**, and **7** are 543, 540, 550, 553, 546, 556, and 571 nm, respectively, reflecting the polarity of the environment around the dansyl moiety in each CD derivative with shorter maximum wavelength in less polar environment. This result also suggests that the dansyl moiety of **2** is located in the most hydrophobic environment.

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Table 1. Fluorescence Lifetimes of **1**−**7** Alone and in the Presence of Guest in Aqueous Solution[a]

host	guest	$\tau_{1/ns}$	A_1	τ_2/ns	A_2	χ^2
1		5.7	0.33	17.7	0.67	1.32
2		6.9	0.23	17.8	0.77	1.38
3		7.3	0.46	13.1	0.54	1.13
4		7.9	0.78	13.5	0.22	1.06
5		5.9	0.29	15.5	0.71	1.24
6		8.2	0.79	13.4	0.21	1.11
7		3.5				1.28
1	**13**	5.5	0.99	19.0	0.01	1.38
2	**13**	5.9	0.97	17.9	0.03	1.07
5	**13**	4.4	0.92	15.3	0.08	1.39
3	**16**	5.5	0.57	13.7	0.43	1.23
4	**16**	5.7	0.86	13.2	0.14	1.03
6	**16**	6.0	0.53	12.3	0.47	1.20
3	**14**	7.6	0.40	14.8	0.60	1.22
4	**14**	7.3	0.45	14.1	0.55	0.95
6	**14**	7.3	0.41	13.3	0.59	1.04

[a] [**1**−**7**] = 2×10^{-5} M, [1-adamantanol (**13**)] = 5×10^{-3} M, [(−)-borneol (**16**)] = 3×10^{-3} M, [cyclohexanol (**14**)] = 5×10^{-2} M. Decay curves were fitted to the equation: $I(t) = A_1 \exp(-t/t_1) + A_2 \exp(-t/t_2)$. χ^2 is the parameter for the goodness of the fit.

Upon addition of 1-adamantanol as a guest, the fluorescence intensities of **1**, **2**, and **5** decreased as shown in Figure 2. Similar phenomena were observed for all the guests used in this work (Chart 2), when the dansyl-modified β-CD derivatives were used as the hosts. This guest-induced fluorescence variation suggests that the hosts exclude the dansyl moiety from their hydrophobic cavities to the bulk water environment associated with the guest accommodation. The degrees of the fluorescence intensity variation were evaluated by a sensitivity parameter expressed as $\Delta I/I_0$, where $\Delta I = I_0 - I$ and I_0 and I are the fluorescence intensities in the absence and presence of the guest, respectively, measured at the maximum emission wavelength of the host alone. The variation of the $\Delta I/I_0$ value of **1** with increasing concentration of 1-adamantanol was fitted to a Benesi−Hildebrand type equation[22] for 1/1 host/guest complex formation similarly to the fluorophore-appended CDs already reported.[11−17]

In the cases of γ-CD derivatives **3**, **4**, and **6**, addition of a guest caused increase or decrease of the fluorescence intensity, depending on the kind of the guest. For example, their fluorescence intensities decreased upon addition of (−)-borneol, while increased upon addition of cyclohexanol (Figure 3). Both guest-induced variations were fitted to the equation for the 1/1 host/guest complex formation. These results indicate that **3**, **4**, and **6** form inclusion complexes of 1/1 stoichiometry with different structures, depending on the kind of the guest. The $\Delta I/I_0$ values for other guests are discussed in a later section.

Fluorescence Lifetimes. In aqueous solution, the pendant groups of modified CDs are flexible and not fixed in many cases, usually being located inside the cavity in one conformation and outside the cavity in another.[11,15] Although the conformational interconversion occurs too rapidly to be followed by NMR spectroscopy, the analysis of the fluorescence decay of the fluorophore pendant may provide useful information with respect to the conformational features, because of the fact that fluorescence lifetimes of many fluorophores are in the range of a measurable time scale (nanoseconds) for the conformations. This is particularly true for the fluorescent CDs with a fluorophore pendant, which is sensitive to environment and has different lifetimes when located inside the cavity and in bulk water solution.[15,32]

All the fluorescence decays of the dansyl moiety of the fluorescent CDs could be analyzed by a simple double-exponential function, and the results are summarized in Table 1. These results indicate that there are two kinds of observable conformational isomers. The longer and shorter lifetime species may be ones with the dansyl moiety inside and outside the cavity, respectively.[15,32] These two species would be in equilibrium as shown in parentheses in Figure 4. Table 1 shows fractions A_1 and A_2 of the two species and reveals that **2** has the largest fraction of the longer lifetime (A_2, 0.77). It means that the proportion of the self-inclusion form of **2** is largest among all the fluorescent hosts, being consistent with the largest fluorescence intensity of **2**. The data in Table 1 also reveal that the fractions of the longer lifetime forms of the β-CD derivatives **1**, **2**, and **5** are larger than those of the γ-CD derivatives **3**, **4**, and **6**, consistent with the previous argument that the self-inclusion forms of the β-CD derivatives are more stable than those of the γ-CD derivatives. The data indicate that the cavity of γ-CD is too large to form stable self-inclusion complexes. This is substantiated particularly by the data of **4** and **6** that the shorter lifetime forms become the major components with A_1 values of 0.78 and 0.79 for **4** and **6**, respectively. Therefore, the dansyl moiety of **4** and **6** is located predominantly in a polar environment, being in contact with water. However, it is noted that the dansyl moiety in the shorter lifetime form is still surrounded by a less polar environment than in pure bulk water, because the shorter lifetimes of **4** and **6** are still larger than the lifetime of **7** in bulk water solution.

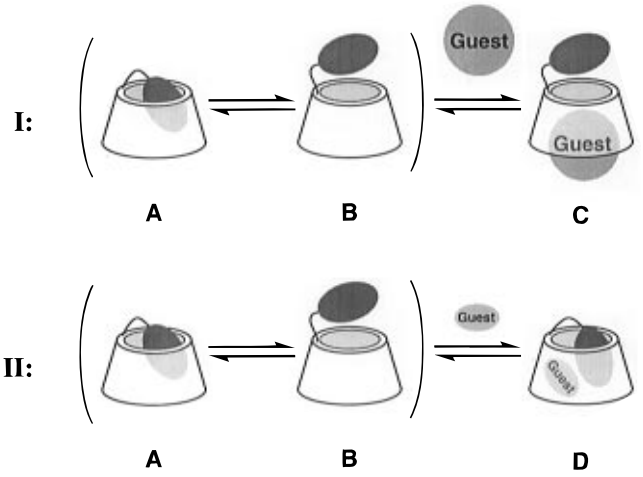

Figure 4. (I) Conformational equilibria in aqueous solution and guest-induced conformational change of β-CD derivatives (**1**, **2**, and **5**) and γ-CD derivatives (**3**, **4**, and **6**). In the case of γ-CD derivatives, this type of conformational change occurs for larger guests. (II) Conformational change of γ-CD derivatives (**3**, **4**, and **6**) occurring associated with accommodation of a smaller guest.

Upon addition of 1-adamantanol as a guest, the fractions of the shorter lifetime form of **1**, **2**, and **5** increased, while the fractions of the longer lifetime forms of the hosts decreased (Table 1). Obviously, the locational change of the dansyl moiety from inside to outside the cavity occurs in association with the guest accommodation as shown by I in Figure 4, and this conformational change of the hosts is reflected in the change in lifetime distribution. The fact that the fluorescence decay curves of the hosts are still double exponential indicates that the fluorescence lifetimes of species B and C in I of Figure 4 are similar to each other and not resolved by our lifetime measurement system.

On the other hand, the fractions of the longer lifetime component of **3** and **4** increased upon addition of cyclohexanol but decreased upon addition of (−)-borneol (Table 1). In the case of **6**, however, the addition of (−)-borneol increased the

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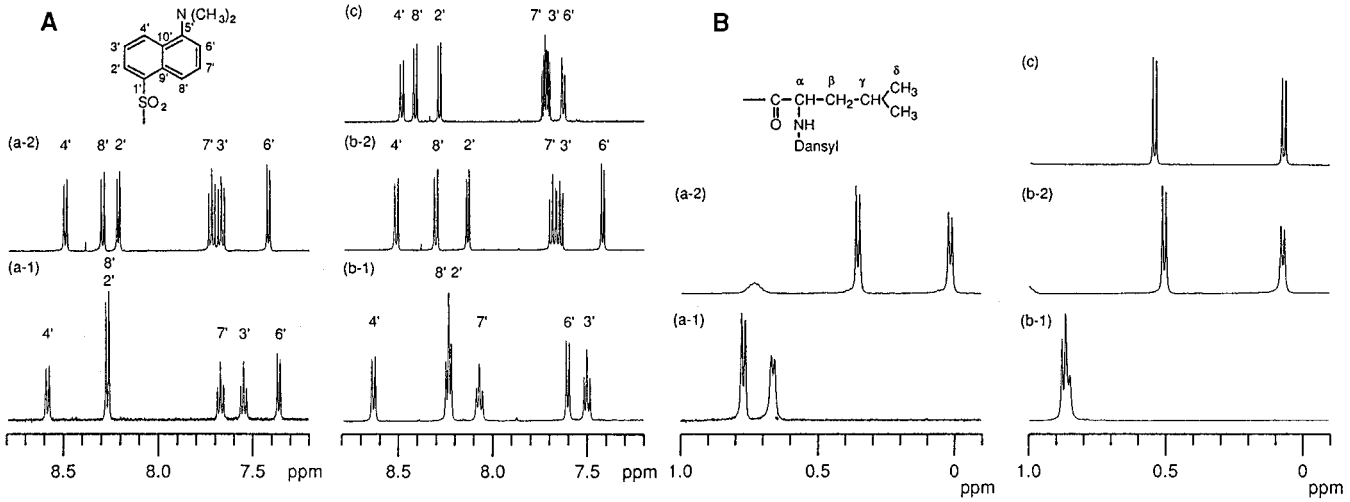

Figure 5. 500 MHz ^{1}H NMR spectra of **1** (a-1) alone, (a-2) in the presence of 1-adamantanol, **2** (b-1) alone, (b-2) in the presence of 1-adamantanol, and (c) **7** in D_2O at 25 °C in the region of (A) aromatic protons and (B) δ protons of leucine: [**1**] = [**2**] = [**7**] = [1-adamantanol] = 1×10^{-3} M.

fraction of the longer lifetime component. The results suggest that the dansyl moiety of **3** and **4** is included together with cyclohexanol in the cavity, whereas it is excluded by (−)-borneol (II in Figure 4). On the same basis, it is likely that the dansyl moiety of **6** is included together with the guest in both cases of cyclohexanol and (−)-borneol.

Previous reports substantiated that γ-CD, which has a larger cavity than β-CD, can include two small guest molecules.[23−26] Since the cavity of γ-CD is too large to form a stable intramolecular complex with the dansyl moiety, a small guest such as cyclohexanol may act as a spacer to form stable complexes.[5,12,14,27] Therefore, it is reasonable that both the dansyl moiety and cyclohexanol are included in the γ-CD cavity in **3**, **4**, and **6**. However, the inclusion of the dansyl moiety in the γ-CD cavity in **3** and **4** is not enhanced by (−)-borneol, which is much larger than cyclohexanol. This result suggests that there is a steric hindrance for such co-inclusion due to the presence of the isobutyl side chain of their leucine residue.

Conformational Analyses of 1 and 2 by NMR Spectroscopy. The dansyl moiety of the fluorescent CDs is flexible rather than fixed in aqueous solution, and the observed NMR spectra are the averages of the spectra of the species in various conformational states weighted by the fractions of the conformers. This situation is due to the fact that the interconversion rates are fast on the NMR time scale. The analysis of the fluorescence decay of the dansyl moiety of the CD derivatives indicates that there are two kinds of observable conformational isomers, which are in equilibrium. The dansyl moieties of **1** and **2** are located in the cavity in one of the states and are located out of the cavity in another, and the former is the major state. Therefore, the NMR spectra of **1** and **2** may be expected to provide information about their structures in the self-inclusion state.

The full assignments of the ^{1}H NMR spectra of **1** and **2** are required to elucidate their structures. Although the ^{1}H NMR spectra of **1** and **2** are complex, it is possible to assign almost all of their resonances by combined use of various 1D and 2D NMR techniques.[28−31] Their detailed NMR analyses will be reported elsewhere, and the only important results are shown in this paper.

The ^{1}H resonances for H-A5 (the proton at the C_5 position in the glucose unit A), H-E3, and H-E5 of **1** and H-A3, H-A5, H-D3, H-D5, H-E3, H-E5, and H-G5 of **2**, these protons being located at the inside of the cavities, are shifted upfield. These shifts would be attributed to the anisotropic ring current effects from the dansyl moiety, and the result indicates that the dansyl moiety is included in the CD cavities. Figure 5A shows the dansyl region of the ^{1}H NMR spectra of **1**, **2**, and **7**, and Figure 5B shows the regions of the δ protons of the leucine moiety of the ^{1}H NMR spectra of these compounds. The resonance patterns of **1** and **2** are different from each other, both being also different from the pattern of **7**. These spectra suggest that the dansyl moiety of **1** and **2** is included in the cavities, and the positions of the dansyl moiety in the cavities of **1** and **2** are different from each other.

The orientation of the dansyl moiety of **1** and **2** can be estimated from the NOE correlations between the dansyl protons and the CD protons. The NOE correlation can be observed by the ROESY spectrum, in the case when a proton of the dansyl moiety and a proton of the CD moiety are closer than 4 Å through space.[33,34] Therefore, if the dansyl moiety is included in the CD cavity, the NOE correlations between the protons of the dansyl moiety and the protons (H-3, H-5, or H-6) of the CD moiety should be observed, and consequently, on this basis, it is possible to estimate the orientation of the dansyl moiety in the CD cavity using the assigned NOE correlations.

Figures 6 and 7 show the ROESY spectra with assignments. The NOE correlations were observed between the protons of CD and the protons of the naphthyl and methyl parts of the dansyl moiety. A weak NOE correlation was observed between the protons of CD and the δ protons of the leucine moiety. The flexible movement of the δ protons would make the NOE weaker.

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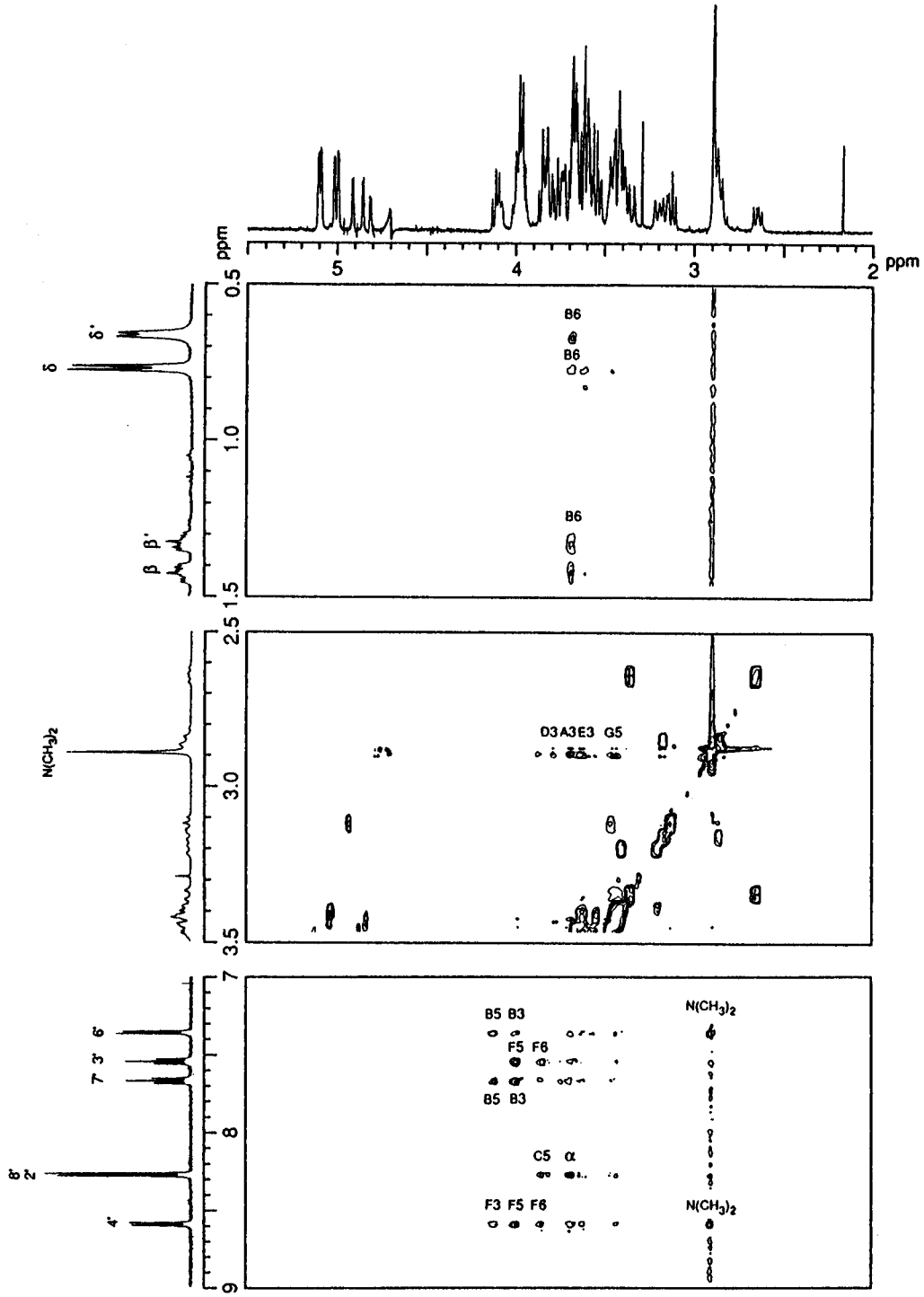


Figure 6. 500 MHz ROESY spectrum of **1** in D_2O at 25 °C with mixing time of 300 ms, indicating the cross peaks between the protons of the dansyl-L-leucine moiety and the protons of the β-CD moiety.

Figure 8 shows the structures of **1** and **2** estimated from the NOE data and the degree of the anisotropic ring current effect from the dansyl moiety in the ^{1}H NMR spectra of the CD protons. The dansyl moiety of **2** is included in the cavity more deeply than that of **1**. These structures can elucidate the difference of the resonance of the δ protons of the leucine between those of **1** and **2**. The resonances of the δ protons of the leucine moiety of **1** are more dispersed and are shifted more upfield than that of **2**. This difference is caused by the difference in the position of the dansyl moiety and indicates that the δ protons of **1** are more influenced by the anisotropic ring current effects of the dansyl moiety than those of **2**. The deeper inclusion of the dansyl moiety of **2** into its own cavity would make the more stable self-inclusion complex, and the difference in the stability of the self-inclusion complexes between **1** and **2** would be reflected in their binding abilities.

Upon addition of 1-adamantanol, ^{1}H NMR spectra of **1** and **2** were changed as shown in Figure 5. The ^{1}H resonances of the dansyl moiety in the presence of the guest are different from those of the hosts alone and are similar to that of **7**. The resonances of the δ protons of the leucine moiety of **1** and **2** were also changed in a similar way upon addition of the guest. These changes suggest that the dansyl moiety is excluded from the cavity upon addition of the guest. In the formed inclusion complexes of **1** and **2**, the dansyl moiety causes a larger anisotropic ring current effect on the δ protons of the leucine moiety, and the resonances of the δ protons of the leucine moiety are more dispersed.

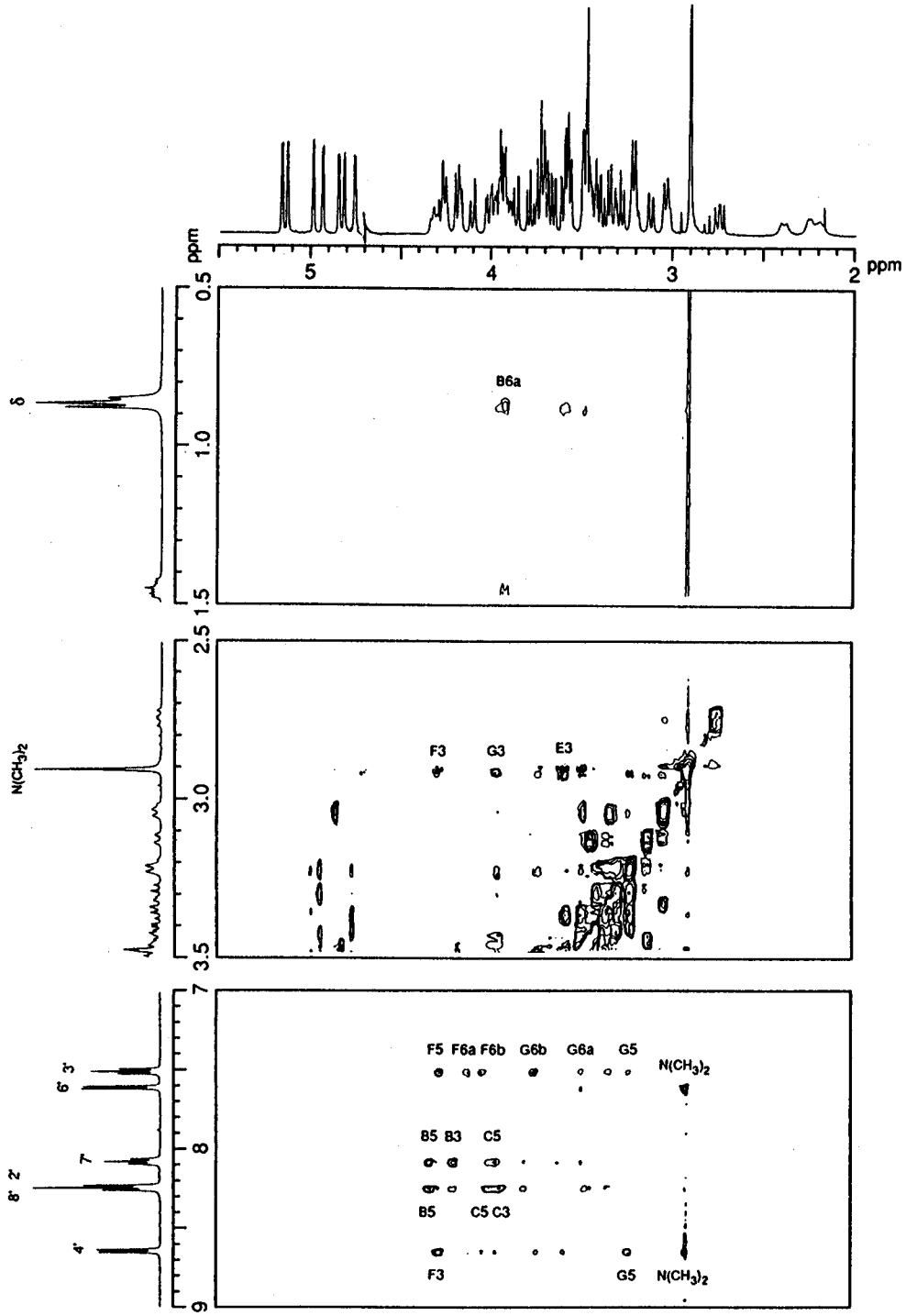
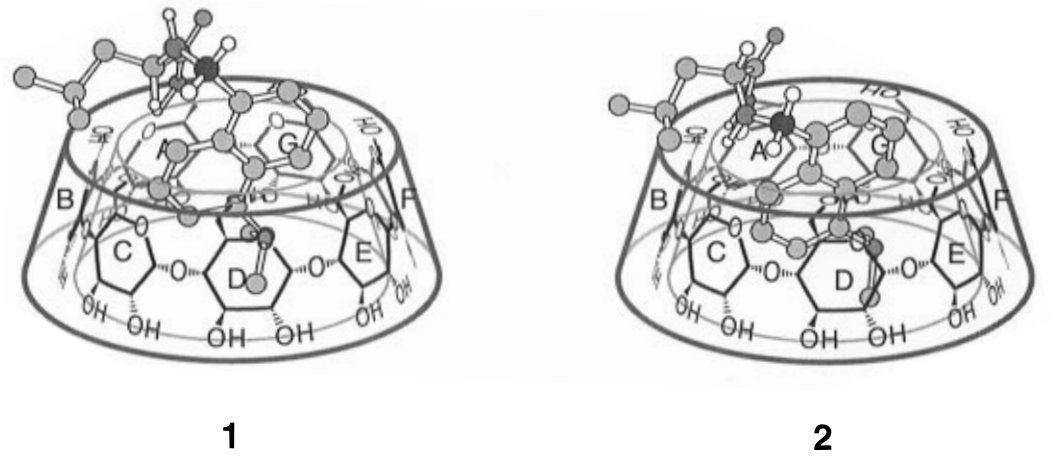


Figure 7. 500 MHz ROESY spectrum of **2** in D_2O at 25 °C with mixing time of 300 ms, indicating the cross peaks between the protons of the dansyl-D-leucine moiety and the protons of the β-CD moiety.

The resonances of CD protons were also changed upon addition of 1-adamantanol. The upfield shifts of the protons (H-3, H-5 or H-6) of CD, arising from the ring current effects of the dansyl moiety, were not observed in the presence of the guest. The resonances for the anomeric protons of **1** and **2** in the absence of the guest were dispersed in the range from 4.7 to 5.2 ppm, showing distinctly separated peaks for all glucose units. Upon addition of the globular guest, 1-adamantanol, these spectral dispersions were collapsed and the resonances were almost overlapped around 5.0 ppm, This spectral change also indicates the exclusion of the dansyl moiety from the cavities of **1** and **2**.

Binding Constants of the Fluorescent CDs (1−6) for Various Guests. The binding constants of the fluorescent CDs (**1**−**6**) for various guests were obtained from their guest-induced variations of the sensitivity parameter expressed as $\Delta I/I_0$ (Table 2). All the variations were fitted with the Benesi−Hildebrand type equation for the 1/1 host/guest complex formation as described in the previous section. Binding constants of **3**, **4**, and **6** for some guests were too small to be determined.

The order of the binding abilities of the β-CD derivatives for all the guests is **5** < **2** < **1**. This result indicates that the isobutyl side chain of the leucine moiety increases the hydrophobic nature of the environment around the guest, thus resulting in the larger binding abilities. It is interesting that the binding constants of **1** are over two times larger than those of **2** for most of the guests. One extreme result is that the binding constant of **1** for cholic acid (**11**) is 27.3-fold larger than that



1 **2**

Figure 8. Structures of **1** and **2** estimated from the NMR data. The energy was not minimized.

Table 2. Binding Constants of **1**−**6** for the Guests Shown in Chart 2[a]

	K/M^{-1}					
guest	**1**	**2**	**3**	**4**	**5**	**6**
8	77100	41000	6250	24600	37600	4110
9	21900	11800	5620	16100	10200	2660
10	2660	1520	5600	9420	1030	658
11	1650	588	1320	1960	60.4	*b*
12	59300	20400	*b*	*b*	9630	*b*
13	54200	19900	116	370	8910	*b*
14	1740	860	27.1	43.5	540	29.3
15	6110	2450	*b*	*b*	1700	76.2
16	26000	9230	358	1430	4020	*b*
17	8470	2160	138	852	1230	*b*
18	3690	1830	436	352	1010	116
19	2860	1470	*b*	*b*	83.3	185
20	1650	1230	*b*	*b*	68.4	26.5

[a] The binding constants for guests **8**−**12** were measured in phosphate buffer ($I = 0.01$, pH 7.0) at 25 °C, and the binding constants for the other guests were measured in aqueous solution at 25 °C. [b] The values are too small to be accurately determined.

of **5**, but that of **2** is only 9.7-fold larger than that of **5**. It is worth noting that the large difference in the binding ability is caused only by the difference in the enantiomeric configuration of the amino acid residue. This fact substantiates the unique feature of this system that not only the hydrophobicity of the isobutyl side chain but also the enantiomeric configuration of the amino acid residue is an important factor to determine the binding abilities of the hosts.

The order of the binding abilities of the γ-CD derivatives is **6** < **3** < **4** for most of the guests. It implies that the binding constants of the D-leucine-modified γ-CD (**4**) are larger than those of the L-leucine-modified γ-CD (**3**) with one exception for (−)-menthol (**18**). It is rather surprising that the effect of the leucine chirality of the γ-CD derivatives is opposite to that of the β-CD derivatives in guest binding. The enantiomeric configuration of the amino acid residue is a dominant factor for determining the positions of the isobutyl side chain and the dansyl moiety in the complexes, and the above result indicates that the L- and D-configurations are more favorable for forming stable inclusion complexes with guests in the case of the β- and γ-CD derivatives, respectively. The binding constants of the γ-CD derivatives are smaller than those of the β-CD derivatives in most cases, but one can see an exception for deoxycholic acid (**10**) as shown by the fact that the binding constants of the γ-CD derivatives **3** and **4** are 2.1- and 6.2-fold larger for **10**, respectively, than those of the corresponding β-CD derivatives **1** and **2**. Another exception is seen in the binding constant of **4** for **9** and **11**, the value being 1.4- and 3.3-fold larger than that of **2**, respectively. The difference in guest binding between β- and γ-CD derivatives indicates that the fitness of the cavity size for the guest is another significant factor in the stabilization of the inclusion complexes.

Sensitivities and Selectivities of Fluorescent CDs in Molecule Sensing. Compounds **1**−**4** can be used as fluorescent indicators of molecular recognition, since their guest-induced variations in the fluorescence intensities depend on the kind of the guest as described above. From this viewpoint, we examined the sensitivities and molecular recognition abilities of the fluorescent CDs for the guests shown in Chart 2 using the $\Delta I/I_0$ value as a sensing parameter and the results are shown in Figures 9 and 10. Here, since ΔI is equated as $I_0 - I$, the $\Delta I/I_0$ value becomes positive when the fluorescence intensity decreases upon guest addition.

Figure 9 shows that the fluorescence intensities of the β-CD derivatives **1** and **2** (2 μM) always decreased upon addition of the guests (A, 10 μM; B, 100 μM) and **1** and **2** exhibit similar trends in the guest dependency of the $\Delta I/I_0$ value, although the $\Delta I/I_0$ value of **1** is larger than that of **2** in any case. Four steroidal compounds such as ursodeoxycholic acid (**8**), chenodeoxycholic acid (**9**), deoxycholic acid (**10**), and cholic acid (**11**) were detected by **1** and **2** with the sensitivity order **11** < **10** < **9** < **8**, and this order is the same as the order of their binding constants. These compounds have the same steroidal framework, and **8** and **9** are isomers with the difference of stereochemistry of the hydroxyl group at C-7. Compound **10** is the regioisomer of **8** and **9** with one hydroxyl group at C-12 in place of the C-7 of **8** and **9**. Compound **11** has one more hydroxyl group than **8**, **9**, and **10**. These differences were reflected in the different sensitivity values of these steroids and accordingly in the sensitivity order as indicated above. 1-Adamantanecarboxylic acid (**12**) and 1-adamantanol (**13**) are known as good guests for β-CD, and here, they give large $\Delta I/I_0$ values comparable with those of steroidal compounds. The $\Delta I/I_0$ values for smaller guests, cyclohexanol (**14**) and cyclooctanol (**15**) were not so large, but the difference of the ring size is reflected to some extent in the larger values for **15**. (−)-Borneol (**16**) and (+)-camphor (**17**) have the same framework although with different functional groups, a hydroxyl group for **16** and a ketone group for **17** at the same position. This difference was detected by **1** and **2** with higher sensitivity values for **16** than those for **17**. Nerol (**19**) and geraniol (**20**) are geometrical isomers and were detected with similar low sensitivities. A cyclic monoterpene, (−)-menthol (**18**), was detected with a larger sensitivity than the open chain monoterpenes **19** and **20**. The results of a series of monoterpenes **16**−**20** clearly indicate that the order

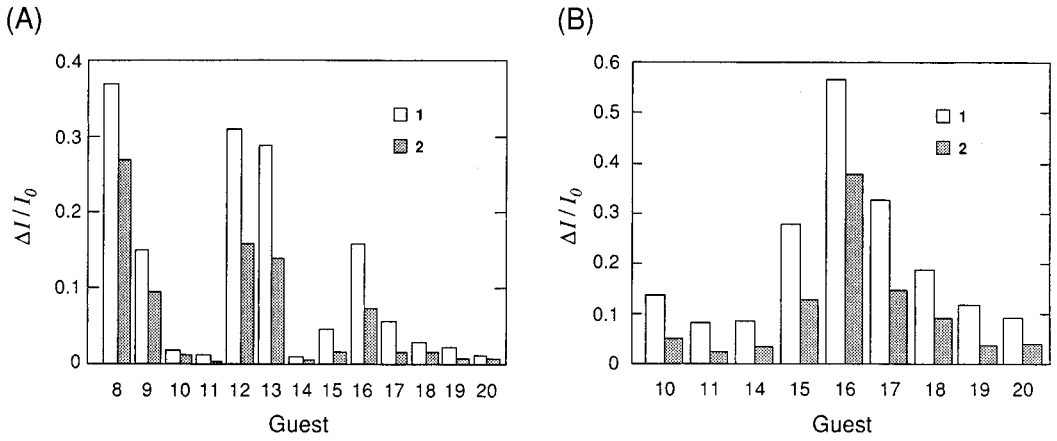


Figure 9. Sensitivity parameters ($\Delta I/I_0$) of **1** and **2** for various guests: [**1**−**2**] = 2×10^{-6} M, [guest] = (A) 10^{-5} M, (B) 10^{-4} M. λ_{ex} = 370 nm. λ_{em} = 543 nm for **1** and 540 nm for **2**.

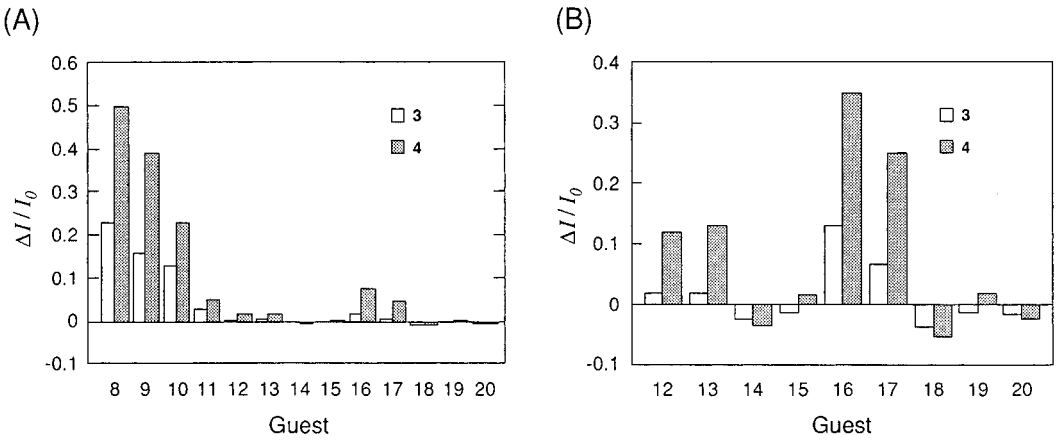


Figure 10. Sensitivity parameters ($\Delta I/I_0$) of **3** and **4** for various guests: [**3**−**4**] = 2×10^{-6} M, [guest] = (A) 10^{-4} M, (B) 10^{-3} M. λ_{ex} = 370 nm. λ_{em} = 550 nm for **3** and 553 nm for **4**.

of the sensitivity for the terpenes is acyclic < monocyclic < bicyclic terpenes.

Figure 10 shows the variations of the $\Delta I/I_0$ value of the γ-CD derivatives **3** and **4** (2 μM) induced by guest addition (A, 0.1 mM; B, 1 mM). The guest dependency is similar for **3** and **4** with exceptions for **15** and **19**, although the $\Delta I/I_0$ value of **4** is larger than that of **3**, indicating that the D-leucine-modified γ-CD **4** is more sensitive to the guest than the L-leucine-modified γ-CD **3**. It is noted that this chirality dependency is opposite to that of the corresponding β-CD derivatives **1** and **2**, and this result agrees with the opposite chirality dependency described for the binding data. Detailed examination of Figure 10 reveals that the steroidal compounds were selectively detected with the same sensitivity order **11** < **10** < **9** < **8** as that detected by the fluorescent β-CDs **1** and **2**, and the $\Delta I/I_0$ values for 1-adamantanecarboxylic acid (**12**), 1-adamantanol (**13**), and (−)-borneol (**16**) are rather depressed. Another remarkable result is that the negative $\Delta I/I_0$ values are seen for cyclohexanol (**14**), cyclooctanol (**15**), (−)-menthol (**18**), nerol (**19**), and geraniol (**20**). Since these guest compounds belong to the category of smaller guests among the guests examined here, it is reasonable that the complexation proceeds according to the case II in Figure 4.

Molecule sensing abilities of the fluorescent CDs were examined for some enantiomeric isomers such as camphor or menthol. However, their molecular recognition abilities for enantiomeric isomers were not so large, probably due to the symmetric shape of the cavity and the flexibility of the dansyl moiety. If the fluorescent CDs have a coordination group for specific interaction with the guests, the sensing abilities for enantiomeric isomers might be improved. The construction of more effective molecule sensing systems are now underway.

Conclusion

A new series of CD−amino acid−chromophore triad systems, where CD was β- or γ-CD, amino acid was D- or L-leucine, and chromophore was dansyl, were prepared for molecule sensing, and their abilities as fluorescent indicators of molecular recognition were studied. The insertion of the leucine residue between CD and the dansyl residue improved the binding ability of the hosts when compared with the glycine residue of the corresponding hosts, and the difference in the enantiomeric configuration of leucine caused the difference in the sensitivity of molecular recognition. The fluorescence intensities of β-CD derivatives always decreased upon guest addition, but the guest-induced variation of the fluorescence intensities of the γ-CD derivatives depended on the guest as shown by the increase by cyclohexanol and the decrease by (−)-borneol and other larger guests. The results demonstrate that the presence of a hydrophobic side chain of amino acid in the fluorescent CDs improves their molecule sensing abilities and the guest binding, and molecule recognition behavior of these sensors are delicately affected by the chirality of the amino acid.

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