Fluorescent Sensors of Molecular Recognition. Modified Cyclodextrins Capable of Exhibiting Guest-Responsive Twisted Intramolecular Charge Transfer Fluorescence

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Abstract: : α-, β-, and γ-cyclodextrin derivatives bearing a *p*-(dimethylamino)benzoyl (DMAB) moiety (DMAB-αCyD, DMAB-βCyD, and DMAB-γCyD, respectively) have been synthesized as fluorescent sensors of molecular recognition. These compounds show dual fluorescence emission arising from normal planar (NP) and twisted intramolecular charge transfer (TICT) exited states, and among them strong TICT emission was observed for DMAB-βCyD. The induced circular dichroism spectra of the derivatives suggest that only DMAB-βCyD among other derivatives binds the DMAB moiety into its own cavity, forming an intramolecular inclusion complex. This conformation was confirmed by the analysis of its ^{1}H-NMR data and was related to its strong TICT emission. The intensity of the TICT emission of DMAB-βCyD decreased markedly with increasing the concentration of cyclic alchols, monoterpenes, or steroids. This observation was explained by the guest-induced location change of the DMAB moiety from inside to outside of the cavity. Since the TICT emission intensity depended on the size, shape, and polarity of the guest molecules, DMAB-βCyD was useful as a fluorescent chemosensor of molecular recognition.

Fluorescent sensors and chromogenic indicators that transform binding of molecules into spectroscopic signals are current interest.[1] However, only few sensing mechanisms have been described.[2] Cyclodextrins (CyDs) are series of cyclic oligosaccharides composed of six or more D-glucopyranose units. The six, seven, and eight D-glucopyranose members of CyDs are named as α-, β-, and γCyD, respectively, each having an approximate internal diameter of 5.7, 7.8, and 9.5 Å.[3] They have attracted great interest because of their abilities to bind various organic compounds into their cavities in aqueous solution. On this basis, modified CyDs with appropriate functional groups act as enzyme-mimic catalysts or receptors.[4] For the purpose of constructing CyD-based sensors, native CyD must be modified by chromophores, because CyDs themselves are spectroscopically inert. Ueno et al. prepared several chromophore-modified CyDs and observed that they act as sensors or indicators of molecules.[5] Pyrene-modified CyDs, for example, act as fluorescent chemosensors, changing the excimer emission intensities upon guest binding.[5a,b,g] We now report novel fluorescent sensors that detect various organic compounds by twisted intramolecular charge transfer (TICT) fluorescence.[6]

In many of the CyD-based sensors, the location change of the chromophore which is attached to CyD from inside to outside of the CyD cavities upon guest binding results in a drastic environmental change from the hydrophobic environment to the polar water one around the chromophore. From this respect, the fluorescence from the twisted intramolecular charge transfer (TICT) excited state seems interesting because the TICT emission intensity and the peak position are known to be greatly affected by solvent polarity.[7-9]

Among many compounds capable of exhibiting TICT emission, the fluorescence properties of *p*-(dimethylamino)benzonitrile and its derivatives have been extensively studied. They show dual fluorescence arising from normal planar (NP) and TICT excited states.[7] The molecules in the TICT excited state have a large dipole moment, and consequently the TICT excited state is stabilized in a polar environment. Since the intensity and the maximum wavelength of the TICT emission depend on the solvent polarity, the compounds which are capable of exhibiting TICT fluorescence may be used as probes of the environmental polarity. Koswer and Dodiuk showed that the ratio of TICT and NP emission intensities (TICT/NP) increased with increasing solvent polarity up to $E_T(30)$ of 46 (acetonitrile) and then decreased with further increase in the solvent polarity.[8] This may be related to the fact that the rate constant for the radiative decay of the TICT fluorescence is greater than that for the nonradiative one in the solvent in which polarity is lower than acetonitrile, while

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(2) For chelation-enhanced fluorescent detection, see ref 1a; for detection by cyclodextrin complexation, see refs 1b and 5. A fluorescent sensor of anthrylboronic acid for polyol detection has been reported: Yoon, J.; Czarnik, A. W. *J. Am. Chem. Soc.* **1992**, *114*, 5874.

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(5) (a) Ueno, A.; Suzuki, I.; Osa, T. *J. Am. Chem. Soc.* **1989**, *111*, 6391. (b) Ueno, A.; Suzuki, I.; Osa, T. *Anal. Chem.* **1990**, *62*, 2461. (c) Ueno, A.; Minato, S.; Suzuki, I.; Fukushima, M.; Ohkubo, M.; Osa, T.; Hamada, F.; Murai, K. *Chem. Lett.* **1990**, 605. (d) Minato, S.; Osa, T.; Ueno, A. *J. Chem. Soc., Chem. Commun.* **1991**, 107. (e) Minato, S.; Osa, T.; Morita, M.; Nakamura, A.; Ikada, H.; Toda, F.; Ueno, A. *Photochem. Photobiol.* **1991**, *54*, 593. (f) Fukushima, M.; Osa, T.; Ueno, A. *Chem. Lett.* **1991**, 709. (g) Suzuki, I.; Ohkubo, M.; Ueno, A.; Osa, T. *Chem. Lett.* **1992**, 269. (h) Wang, Y.; Ikeda, T.; Ueno, A.; Toda, F. *Chem. Lett.* **1992**, 863. (i) Ueno, A.; Kuwabara, T.; Nakamura, A.; Toda, F. *Nature* **1992**, *356*, 136. (j) Ueno, A.; Minato, S.; Osa, T. *Anal. Chem.* **1992**, *64*, 1154. (k) Ueno, A.; Chen, Q.; Suzuki, I.; Osa, T. *Anal. Chem.* **1992**, *64*, 1650.

(6) Some of data of this paper were presented in a symposium of *Fluorescent Chemosensor of Molecular Recognition*, which was held in 204th meeting of the American Chemical Society, Washington, DC, August 26, 1992.

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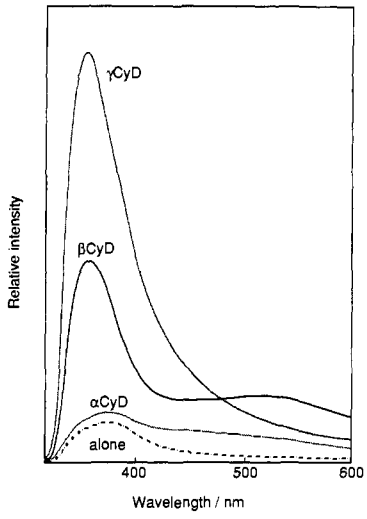

Figure 1. Fluorescence spectra of DMABE at 25 °C in a 10% acetonitrile aqueous solution (2.5×10^{-5} M), alone and in the presence of αCyD, βCyD, and γCyD. The concentration of CyDs is 10^{-4} M. The emissions around 370 and 540 nm are NP and TICT bands, respectively.

the rate constant of the nonradiative decay becomes greater than that of the radiative one in a higher solvent polarity region.[9] It is, therefore, reasonable that TICT emission is scarcely observed in an aqueous solution, which has an extremely high solvent polarity ($E_T(30)$ = 60). However, Cox et al. and Nag et al. reported a unique phenomenon that *p*-(dimethylamino)benzonitrile exhibits TICT emission even in aqueous solution when it forms inclusion complexes with CyDs.[10]

Under this circumstance, we have prepared modified CyDs bearing a *p*-(dimethylamino)benzoyl (DMAB) moiety (DMAB-CyDs) as novel fluorescent chemosensors. The abilities as fluorescent sensors as well as fluorescence and structural properties of DMAB-αCyD, DMAB-βCyD, and DMAB-γCyD are described in this paper.

Results and Discussion

Emission of *p*-(Dimethylamino)benzoic Acid Ethyl Ester. Figure 1 show the fluorescence emission spectra of *p*-(dimethylamino)benzoic acid ethyl ester (DMABE) in a 10% acetonitrile aqueous solution,[11] alone and in the presence of CyDs. DMABE hardly exhibits TICT emission in an aqueous solution, but the NP and the TICT emissions were enhanced by the presence of CyDs. In the presence of βCyD, DMABE exhibits remarkable TICT emission around 540 nm and enhanced NP emission around 370 nm. In contrast, the effects of αCyD on NP and TICT emissions are small. On the other hand, γCyD enhanced the NP emission strongly, but the effect on the TICT emission is limited. These observations may be related to the fact that the size of the βCyD cavity is suitable to include DMABE, but that of αCyD is too small to accommodate DMABE. On the other hand, γCyD can accommodate two DMABE molecules to form a 2:2 (host: guest) complex[5a] because of its wide cavity size. If such a complex is constructed, environmental polarity around the DMAB moiety might be too low to stabilize the TICT excited state or the interior of the cavity might be too rigid to twist the molecule. The induced circular dichroism (ICD) spectra of the βCyD and γCyD complexes exhibit positive bands around 370 nm (Figure 2) and

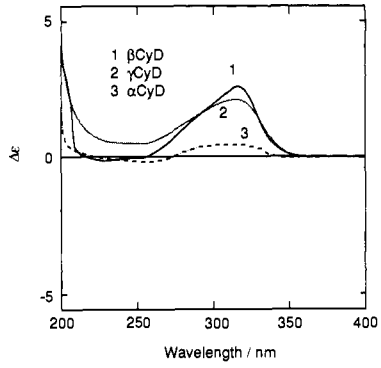

Figure 2. Induced circular dichroism spectra of DMABE at 25 °C in a 10% acetonitrile aqueous solution (2.5×10^{-5} M), in the presence of αCyD, βCyD, and γCyD. The concentration of CyDs is 10^{-4} M.

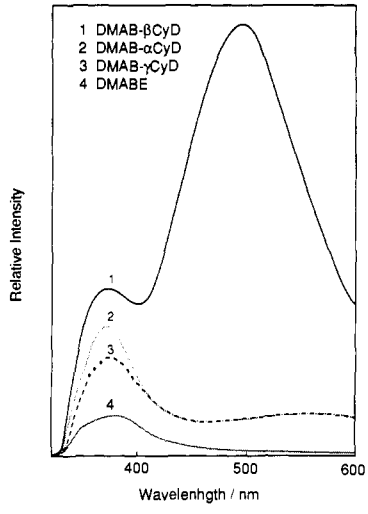

Figure 3. Fluorescence spectra of DMAB-αCyD, DMAB-βCyD, and DMAB-γCyD at 25 °C in an aqueous solution (2.5×10^{-5} M) and of DMABE in a 10% acetonitrile aqueous solution (2.5×10^{-5} M).

are consistent with the theoretical prediction[12] that the transition dipole moment of DMABE along the long axis of the molecule is parallel to the C_n axis of CyDs. In comparison with the cases of βCyD and γCyD, the magnitude of the ICD band of the αCyD–DMABE system is small, consistent with the previous argument that DMABE is difficult to include in the αCyD cavity.

Fluorescence and ICD Spectra of DMAB-CyDs. Figure 3 shows fluorescence spectra of DMAB-αCyD, DMAB-βCyD, and DMAB-γCyD (2.5×10^{-5} M) in an aqueous solution together with the data of DMABE in a 10% acetonitrile aqueous solution. All samples of DMAB-CyDs show dual fluorescence emissions with peaks around 370 and 540 nm for DMAB-αCyD and DMAB-γCyD and around 370 and 495 nm for DMAB-βCyD. In each spectrum, the emissions from NP and TICT excited states appear in the shorter and longer wavelength regions, respectively.[7] DMAB-βCyD exhibits a significantly enhanced TICT emission with its peak shifted by 45 nm to a shorter wavelength compared with DMAB-αCyD and DMAB-γCyD. Hence, the environmental polarity around the DMAB moiety of DMAB-βCyD is suggested to be lower than those of DMAB-αCyD and DMAB-γCyD.

Figure 4 shows ICD spectra of these CyD derivatives in aqueous solution. DMAB-βCyD shows a trough and a peak at 290 and

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(11) Solubility of DMABE in pure water is very poor.

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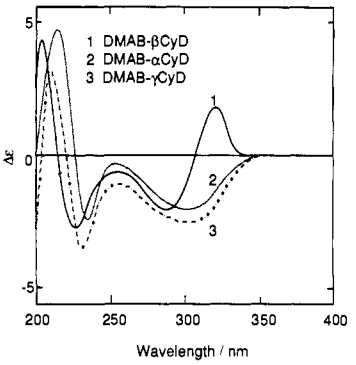

Figure 4. Circular dichroism spectra of DMAB-αCyD, DMAB-βCyD, and DMAB-γCyD at 25 °C in an aqueous solution (2.5×10^{-5} M). Temperature is 25 °C.

320 nm, respectively, whereas DMAB-αCyD and DMAB-γCyD show only a negative band in the same region. The structural features of the modified CyDs may be reflected in the ICD spectra,[13] and the conformation of DMAB-βCyD is likely to be different from those of DMAB-αCyD and DMAB-γCyD. The bands with opposite ICD signs for DMAB-βCyD suggest that there exist two species which are different in conformation. Since DMAB-αCyD as well as DMAB-γCyD exhibits a negative ICD band in the region, it is likely that the negative ICD band arises from the species in which the DMAB moiety is not included in the cavity. The results indicate that the cavity size of DMAB-γCyD is large enough to accommodate the DMAB moiety, but the moiety is not tightly included in its large cavity and does not stay in the cavity long enough to enhance the TICT emission. Consequently, the species of DMAB-βCyD, which exhibits the positive ICD band, is suggested to be one that includes its DMAB moiety in the cavity. Details on the conformations of DMAB-βCyD will be discussed in the next section.

Conformational Analysis of DMAB-βCyD by ^{1}H-NMR Spectroscopy. Modified CyDs usually give very complicated ^{1}H-NMR spectra, and several modern high-resolution 2D-NMR techniques have been used to determine their conformations.[14] Overlapped signals were separated into a set of signals of each pyranose unit by use of the TOCSY (total correlated spectroscopy)[15] spectrum, and then the signals of each pyranose unit were assigned using ^{1}H–^{1}H COSY (correlated spectroscopy)[16] and 2D ^{1}H–^{13}C HMQC (heteronuclear multiple quantum coherence)[17] spectra. The sequence of the pyranose units was determined from the ROESY spectrum, followed by determination of the location of the DMAB moiety by ROESY (rotating frame Overhauser enhancement spectroscopy).[18] Notations of the pyranose units and the protons of DMAB moiety are shown in Figure 5. The results of assignments are summarized in Figure 6. The H_3 and H_5 protons of the C, D, and F pyranose units which are located at the inner side of the cavity are shifted to the upper field by the ring-current effect of the DMAB moiety. All protons of the DMAB moiety have NOE with the protons of the inner cavity of βCyD. The ROESY spectrum of DMAB-βCyD is shown in Figure 7. The H_P protons of the DMAB moiety have NOE cross peaks with H_{5A} and H_{5E} while the H_Q protons have NOE cross peaks with H_{3E} and H_{5E}. The H_R protons of the DMAB moiety have NOE cross peaks with H_{3D} and H_{3E}. These results indicate that DMAB-βCyD includes its DMAB moiety in the cavity of itself.

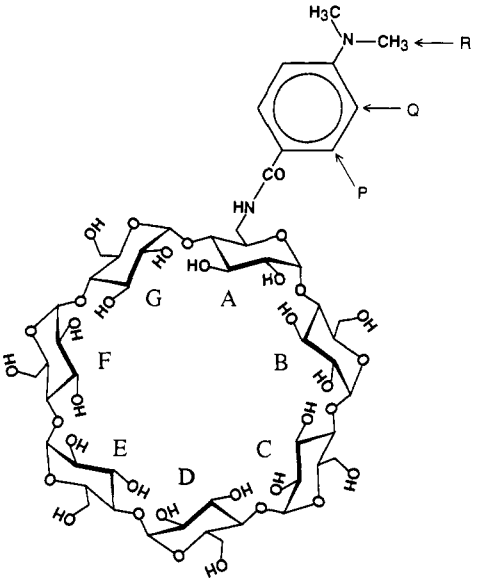

Figure 5. Notation of pyranose units and numbering of protons of the DMAB moiety of DMAB-βCyD.

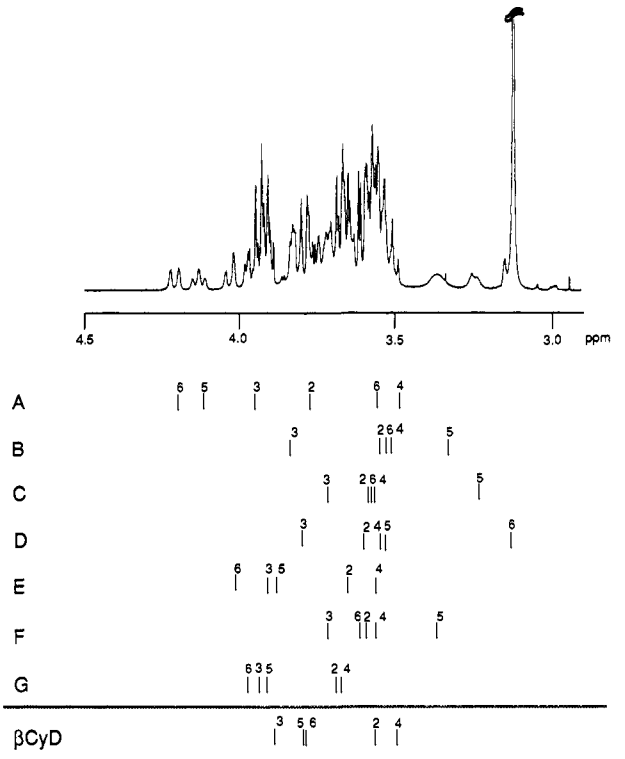

Figure 6. ^{1}H-NMR spectrum of DMAB-βCyD in D_2O at 25 °C, in the region of cyclodextrin protons, except for C1 protons, and assignments of the protons.

But signal broadening was observed at the H_{3C} (3.22 ppm), H_{5B} (3.30 ppm), and H_{5F} (3.34 ppm) protons. These three protons are located in the cavity and face the DMAB moiety, and then they are shifted to a higher field than that of native βCyD by the ring-current effect of the DMAB moiety. These facts suggest an equilibrium of the conformational isomers of DMAB-βCyD, i.e., the DMAB moiety is not fixed in the cavity but moves slowly compared with the NMR time scale. This observation may be related to the fact that the amide bond, which links the DMAB moiety to βCyD, is not flexible and the DMAB moiety is bulky enough to limit the movement in the cavity of βCyD. These results are consistent with the structural features of DMAB-βCyD suggested from the fluorescence and ICD spectra.

Guest-Induced Conformational Change of DMAB-βCyD. Figure 8 shows fluorescence spectra of DMAB-βCyD alone and in

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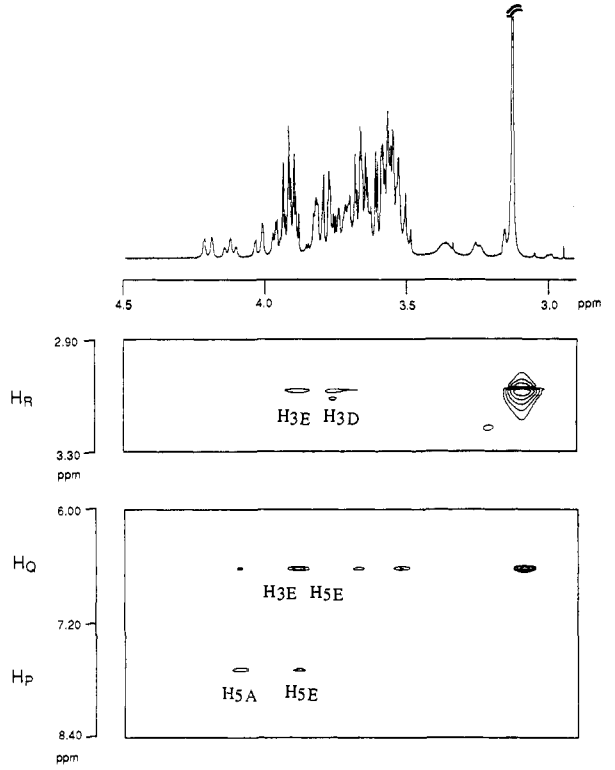

Figure 7. Parts of ROESY spectrum of DMAB-βCyD in D_2O at 25 °C with mixing time of 300 ms, covering the protons of *N,N*-dimethyl (H_R) and aromatic ring (H_P and H_Q). Assignments of cross peaks are also shown.

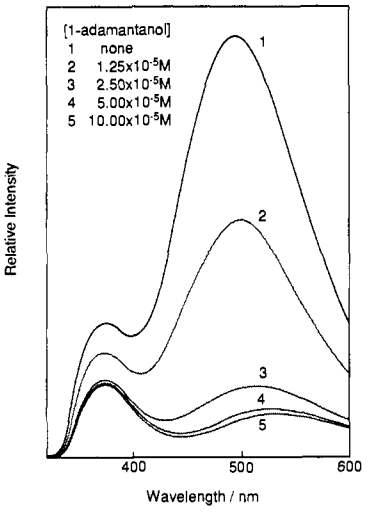

Figure 8. Fluorescence spectra of DMAB-βCyD (2.5×10^{-5} M) at 25 °C alone and in the presence of 1-adamantanol (1.25×10^{-5}–1.0×10^{-4} M).

the presence of 1-adamantanol. The TICT fluorescence intensity of DMAB-βCyD decreased markedly, while its NP emission intensity decreased moderately with increasing concentration of 1-adamantanol. It is noted that the position of the TICT emission maximum was shifted to a longer wavelength by the addition of 1-adamantanol.[19] Since the TICT emission intensity and its peaks position are very sensitive to the environmental polarity, the guest responsive variation in the TICT emission suggests that the location of the DMAB moiety was changed from the hydrophobic environment of the inner cavity of DMAB-βCyD to the polar environment of the bulk water by forming an intermolecular inclusion complex with the guest molecule.

(19) In the presence of 10^{-4} M of 1-adamantanol, the peak position of TICT emission was 540 nm.

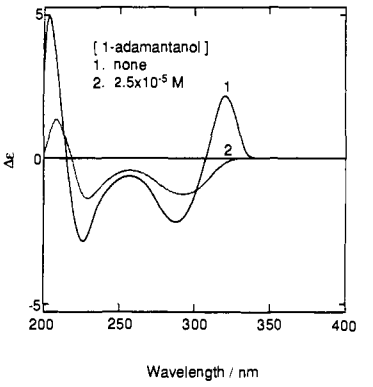

Figure 9. Circular dichroism spectra of DMAB-βCyD (2.5×10^{-5} M) at 25 °C, alone and in the presence of 1-adamantanol (2.5×10^{-5} M).

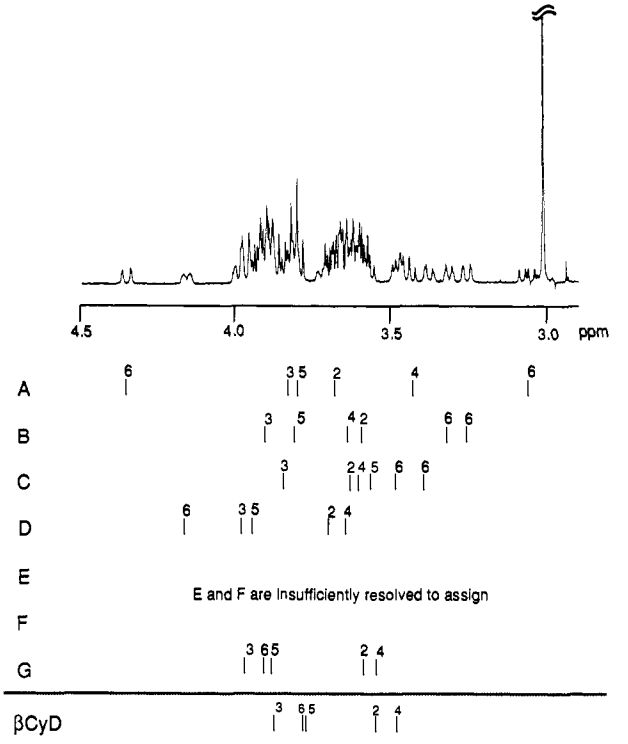

Figure 10. ^{1}H-NMR spectrum of DMAB-βCyD in the presence of 1-adamantanol in D_2O at 25 °C, in the region of cyclodextrin protons, except for C1 protons, and assignments of the protons.

Figure 9 shows ICD spectra of DMAB-βCyD alone and in the presence of 1-adamantanol. A pair of positive and negative bands observed in the ICD pattern for DMAB-βCyD in the 250–350-nm region changed to a simple negative band upon addition of 1-adamantanol. This result suggests that the conformation of DMAB-βCyD, after it accommodates 1-adamantanol, is very similar to that of DMAB-αCyD and DMAB-γCyD. Figure 10 shows the ^{1}H-NMR spectrum of DMAB-βCyD in the presence of 1-adamantanol for the protons of CyD except for those of C1 with assignment. This spectrum is very different from that shown in Figure 6 and indicates that the conformation of 1-adamantanol-included DMAB-βCyD is different from that of the host alone. The signals of H_{6B} and H_{6C} protons are shifted upfield. This would be explained by the ring-current effect of the DMAB moiety. The protons of the DMAB moiety have no NOE cross peak with any of the protons of CyD. This result is consistent with the conclusion from the 1-adamantanol-induced variations in fluorescence and ICD spectra; that is, the DMAB moiety is not included in the cavity in the complex. All spectral data suggest that there exist three conformational isomers for DMAB-βCyD in aqueous solution as shown in Figure 11. DMAB-βCyD is in an equilibrium between I and II, and I is the intramolecular

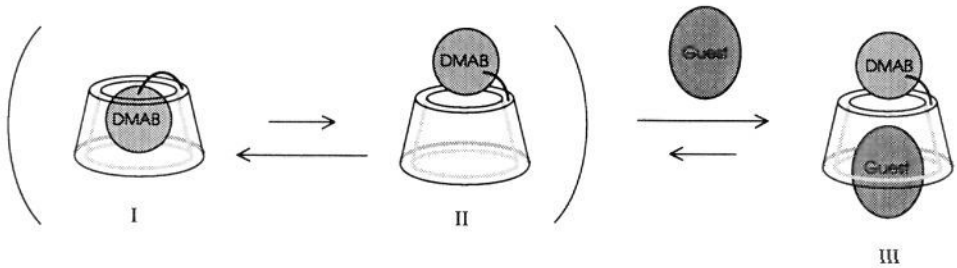

Figure 11. Schematic illustration of the equilibrium between intramolecular and intermolecular inclusion complexes.

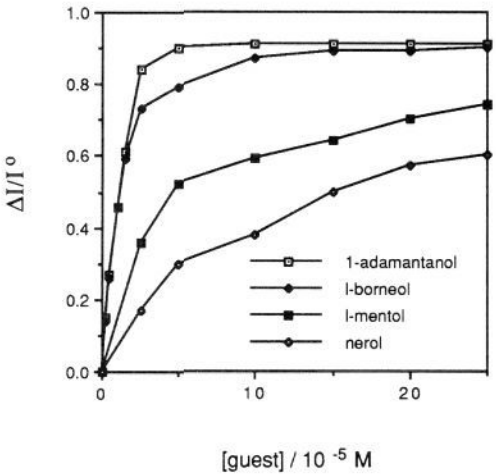

Figure 12. Fluorescence titration of DMAB-βCyD (2.5×10^{-5} M) at 25 °C, as a function of guest concentration ($(0.25–25) \times 10^{-5}$ M).

inclusion complex that exhibits the strong TICT emission. However, it changes its conformation upon addition of guest toward III. As a result, the TICT emission, which arises from I, decreases upon guest binding.

Fluorescent Sensor Using TICT Emission Intensity. As described above, DMAB-βCyD shows remarkably stronger TICT emission than others and decreases its TICT emission intensity associated with the formation of intermolecular inclusion complexes with added guest compounds. Hence, we selected DMAB-βCyD as a fluorescent sensor for detecting various molecular species.

The value of $\Delta I/I^\circ$ was used as the measure of the sensitivity of DMAB-βCyD, where $\Delta I = I^\circ - I$, and I° and I are the TICT emission intensities in the absence and presence of guest, respectively, measured at 495 nm. The binding constants of DMAB-βCyD for guest compounds were obtained from the guest-induced variations in the TICT emission intensity of DMAB-βCyD. Some examples of fluorescence titration are shown in Figure 12. The sensitivities and binding constants are summarized in Table I. The sensitivities of DMAB-βCyD for cyclohexanol (**1**), cyclooctanol (**2**), and cyclododecanol (**3**) are in the order of **1** < **3** < **2**, indicating that the size of **2** fits that of the cavity of DMAB-βCyD while **1** and **3** are too small or too large to be accommodated in the cavity. Adamantane derivatives are known to be good guests to βCyD, and here 1-adamantanol (**4**) and 1-adamantanecarboxylic acid (**5**) caused greater decreases in the TICT emission intensity than **1**, **2**, and **3**. Nerol (**6**) and geraniol (**7**), which are acyclic monoterpenes and exist as geometrical isomers with cis and trans forms, respectively, were detected with low degrees of sensitivity with a 1.2-fold preference for cis. Menthols ((*d*), **9**; (*l*), **10**) are chiral cyclic monoterpenes and were detected more easily than the acyclic monoterpenes with a 1.4-fold preference for the (*l*)-isomer. *l*-Borneol (**8**), which is a bicyclic monoterpene, was detected with the highest sensitivity among all monoterpenes examined here. These results indicate that DMAB-βCyD is capable of discriminating the structures of the guest with limited extents of enantiomer and geometric differentiation. When five biologically important steroidal compounds, choric acid (**11**), deoxycholic acid (**12**), chenodeoxycholic acid (**13**), ursodeoxycholic acid (**14**), and lithocholic acid (**15**), were used as guests, marked differences in the sensitivity among them were observed in spite of the fact that all of them have the same steroid framework as a major part of their structures. Compounds **13** and **14** are geometrical isomers, with the difference in the stereochemistry of one hydroxyl group at C-7 of the steroidal framework, and were detected with similar high sensitivities. However, **12**, which is another isomer with a hydroxyl group at C-12 in place of that at C-7, was detected with an extremely lower sensitivity than **13** and **14**. Compounds **11** and **15** have one less and one more hydroxyl group, respectively, than **12**, **13**, and **14**, and **11** was detected with the smallest sensitivity, while **15** was detected with larger sensitivity than **12** and **13**. This observation suggests that the polarity of the guest molecules, in addition to size, is an important factor that governs the sensitivities of DMAB-βCyD.

Table I. Guest-Induced Variations of TICT Emission Intensities of DMAB-βCyD in an Aqueous Solution[a]

guests	$\Delta I/I^\circ$	K (M^{-1})
cyclohexanol (**1**)	0.12	2000
cyclooctanol (**2**)	0.33	50000
cyclododecanol (**3**)	0.26	28000
1-adamantanol (**4**)	0.84	128000
1-adamantanecarboxylic acid (**5**)	0.93	220000
nerol (**6**)	0.17	4000
geraniol (**7**)	0.14	3000
l-borneol (**8**)	0.65	59000
d-menthol (**9**)	0.25	10000
l-menthol (**10**)	0.36	18000
choric acid (**11**)	0.27	27000
deoxychoric acid (**12**)	0.32	47000
chenodeoxychoric acid (**13**)	0.82	51000
ursodeoxychoric acid (**14**)	0.93	178000
lithchoric acid (**15**)	0.83	158000

[a] Measured at 25 °C. Excitation wavelength was 310 nm. $\Delta I = I^\circ - I$ where I° and I are the TICT emission intensities in the absence and in the presence of guest molecule, respectively.

The binding constants of DMAB-βCyD for **1**-**15** ranged from 2000 M^{-1} for **1** to 220 000 M^{-1} for **5**. Although the binding constants of DMAB-βCyD for the guest molecules are not totally parallel to the sensitivities, there exists a good correlation between them when the guests are divided into three groups, as shown by the orders **1** < **3** < **2** < **4** < **5** for cyclic alcohols and adamantane derivatives, **7** < **6** < **9** < **10** < **8** for monoterpenes, and **11** < **12** < **13** < **15** < **14** for steroidal compounds. All these results demonstrate that DMAB-βCyD acts as an effective chemosensor of molecular recognition for detecting a variety of organic compounds by TICT fluorescence.

Experimental Section

Materials. α-, β-, and γ-cyclodextrin were kindly donated by Nihon Shokuhin Kako Co., Ltd. *p*-(Dimethylamino)benzoic acid and all guest reagents were purchased from chemical suppliers and used without further

purification. The water used for photometry and the deuterium oxide used for NMR study were obtained from Cica-Merck and Merck, respectively.

Measurements. Absobance, fluorescence, and circular dichroism spectra were measured by a Shimadzu UV3100 spectrophotometer, a Hitach 850 fluorescence spectrophotometer, and a JASCO J-600 spectropolarimeter, respectively. The excitation wavelength of the fluorescence spectra was always 310 nm. ^{1}H-NMR spectra were measured by a Varian VXR 500S spectrometer (500 MHz). TOCSY and ROESY spectra were measured with mixing times of 100 and 300 ms, respectively.

Synthesis. The synthesis of some of monosubstituted CyDs (TsO-αCyD, NH_2-αCyD) was previously reported,[20] but we prepared these compounds by modified or different procedures as described below.

Mono-6-*O*-(*p*-tolylsulfonyl)-α-cyclodextrin (TsO-αCyD). αCyD (10 g) was dissolved in pyridine (750 mL) with stirring. *p*-Toluenesulfonyl chloride (10 g) was added in small portions to the αCyD solution cooled below 5 °C, and the resulting solution was stirred at room temperature for about 5 h. The progress of the reaction was monitored by TLC (SiO_2; butanol/ethanol/water = 5:4:3 by volume). The solution was poured into acetone (2000 mL) with stirring, and the fine white precipitates that formed were allowed to settle over 1 h. After filtration, the residue was washed with acetone and dried *in vacuo* overnight to give 12 g of the crude product, which shows *p*-anisaldehyde-positive TLC spots with R_f = 0.29, 0.47, and 0.51 for αCyD, TsO-αCyD, and $(TsO)_2$-αCyD, respectively. The crude product (10 g) was dissolved in water (1000 mL) with stirring, and the solution was charged into an activated charcoal column (100-g dry weight, 5 × 25 cm) and eluted with MeOH/H_2O = 20/80 to 50/50 and then *i*-PrOH/H_2O = 30/70 to 50/50. The eluent of MeOH/H_2O = 20/80 to 50/50 contained native αCyD (5.7 g). The eluent of PrOH/H_2O = 30/70 to 40/60 contained the desired product TsO-αCyD (2.1 g). The eluent of PrOH/H_2O = 50/50 contained $(TsO)_2$-αCyD. ^{1}H NMR (D_2O, 500 MHz): δ 2.42 (s, 3H), 3.50 (1H), 3.55–3.65 (m), 3.75–4.0 (m), 4.44 (m, 2H), 4.95 (m, 2H), 5.03–5.06 (m, 4H), 7.54 (d, J = 8.2 Hz, 2H, aromatic), 7.85 (d, J = 8.2 Hz, 2H, aromatic)

Mono-6-deoxy-6-amino-α-cyclodextrin (NH_2-αCyD). This compound was synthesized by a modified method of Borger et al.[21] TsO-αCyD (4.67 g) was suspended in water (50 mL) at 80 °C, and sodium azide (3 g) was added. The reaction was carried out with stirring at 80 °C, for 5 h. After being cooled to room temperature, the solution was poured into acetone (300 mL). The resulting precipitate was dried *in vacuo* to give the azide product as a white powder (3.08 g). TLC: R_f = 0.45 (*n*-BuOH/EtOH/H_2O = 5:4:3 by volume). The product (3.08 g) and triphenylphosphine (1.5 g) were dissolved in *N*,*N*-dimethylformamide (DMF, 50 mL), and to the solution was added 10 mL of concentrated NH_3(aq). The mixture was stirred at room temperature for about 4 h, and then the solution was poured into acetone (300 mL), giving a crude product as a white precipitate (3.01 g). This product was purified by ion-exchange column chromatography on a Sephadex CM-25 column, eluted with 1 N NH_3(aq) (500 mL) as solvent. The eluent after evaporation gave 2.50 g of the desired product. ^{1}H NMR (D_2O, 500 MHz): δ 2.86 (dd, $J_{5,6a}$ = 7.2 Hz, $J_{6a,6b}$ = 13.9 Hz, 1H), 3.16 (dd, $J_{5,6b}$ = 3.4 Hz, $J_{6a,6b}$ = 13.9 Hz, 1H), 3.47 (1H), 3.55–3.67 (m), 3.80–4.00 (m), 5.05 (m, 6H).

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Mono-6-deoxy-6-((*p*-(dimethyamino)benzoyl)amino)-α-cyclodextrin (DMAB-αCyD). NH_2-αCyD (1.0 g) was suspended in DMF (25 mL) and cooled below 0 °C. *p*-(Dimethylamino)benzoic acid (2.0 g), *N*,*N*-dicyclohexylcarbodiimide (DCC, 2 g), and 4-(dimethylamino)pyridine (0.1 g) were dissolved in DMF (15 mL) and cooled to 0 °C. The suspension of NH_2-αCyD (1.0 g) in DMF (25 mL) was added to the above solution with stirring and reacted for 72 h at 0 °C. Then, the solution was poured into acetone (200 mL), and the formed precipitates were dried *in vacuo* overnight, giving 0.92 g of the crude product. This crude product was purified by column chromatography with Sephadex CM-25 and Sephadex G-10, and the product was obtained as white powder (0.25 g). Anal. Calcd for $C_{45}H_{70}O_{30}N_2 \cdot 9H_2O$: C, 42.19; H, 6.92; N, 2.19. Found: C, 42.18; H, 6.49; N, 2.13. ^{1}H NMR (D_2O, 500 MHz): δ 2.95 (s, 6H, CH_3), 3.15 (1H), 3.26 (1H), 3.40 (1H), 3.78 (1H), 3.85–4.00 (m), 4.15–4.25 (m, 2H), 4.92 (d, J = 3.0 Hz, 2H), 4.97 (d, J = 3.5 Hz, 2H), 4.99 (d, J = 3.5 Hz, 2H), 5.03 (d, J = 3.5 Hz, 4H), 5.07 (d, J = 3.5 Hz, 2H), 6.88 (d, J = 9.0 Hz, 2H aromatic), 7.71 (d, J = 9.0 Hz, 2H aromatic).

Mono-6-deoxy-6-amino-β-cyclodextrin (NH_2-βCyD). This compound was synthesized according to the same procedure as NH_2-αCyD. ^{1}H NMR (D_2O, 500 MHz): δ 2.86 (dd, $J_{5,6a}$ = 7.3 Hz, $J_{6a,6b}$ = 14.1 Hz, 1H), 3.10 (dd, $J_{5,6b}$ = 3.4 Hz, $J_{6a,6b}$ = 14.1 Hz, 1H), 3.47 (1H), 3.56–3.66 (m), 3.81–4.98 (m), 5.07 (m, 7H).

Mono-6-deoxy-6-((*p*-(dimethylamino)benzoyl)amino)-β-cyclodextrin (DMAB-βCyD). This compound was synthesized in the same manner as DMAB-αCyD and isolated by HPLC with an ODS column. Fractions containing the product were evaporated, and the desired product was obtained as white powder. Anal. Calcd for $C_{51}H_{80}O_{35}N_2 \cdot 6H_2O$: C, 44.67; H, 6.61; N, 2.04. Found: C, 44.69; H, 6.67; N, 2.02. ^{1}H NMR (D_2O, 500 MHz): δ 2.86 (s, 6H, CH_3), 3.18 (1H, overlapped with methyl proton signals of the DMAB moiety), 3.22 (1H, broad), 3.30 (H, broad), 3.34 (1H, broad), 3.34–3.95 (m, majority), 4.09 (1H), 4.10 (1H, broad), 4.92 (d, J = 3.0 Hz, 2H), 4.93 (d, J = 3.5 Hz, 2H), 4.95 (d, J = 4.0 Hz, 2H), 5.01 (d, J = 3.5 Hz, 4H), 5.03–5.04 (m, 2H), 5.13 (d, J = 3.5 Hz, 2H), 6.68 (d, J = 8.9 Hz, 2H aromatic), 7.80 (d, J = 8.9 Hz, 2H aromatic).

Mono-6-deoxy-6-amino-γ-cyclodextrin (NH_2-γCyD). This compound was synthesized and isolated in the same manner as NH_2-αCyD and NH_2-βCyD, except for the use of mono-6-(2-naphthylsulfonyl)-γ-cyclodextrin[22] in place of tosyl derivatives. ^{1}H NMR (D_2O, 500 MHz): δ 2.86 (dd, $J_{5,6a}$ = 7.5 Hz, $J_{6a,6b}$ = 13.9 Hz, 1H), 3.08 (dd, $J_{5,6b}$ = 3.6 Hz, $J_{6a,6b}$ = 13.9 Hz, 1H), 3.48 (1H), 3.57–3.67 (m), 3.79–4.95 (m), 5.11 (m, 8H).

Mono-6-deoxy-6-((*p*-(dimethylamino)benzoyl)amino)-γ-cyclodextrin (DMAB-γCyD). This compound was synthesized in the same manner as DMAB-αCyD and DMAB-βCyD and isolated by HPLC with an ODS column. Anal. Calcd for $C_{57}H_{90}O_{40}N_2 \cdot 5H_2O$: C, 44.64; H, 6.57; N, 1.83. Found: C, 44.50; H, 6.57; N, 1.81 ^{1}H NMR (D_2O, 500 MHz): δ 2.95 (s, 6H, CH_3), 3.25 (m, 2H), 3.45–3.70 (m), 3.80–3.95 (m), 4.05 (1H), 4.98 (d, J = 4.0 Hz, 2H), 5.01 (d, J = 3.5 Hz, 2H), 5.03 (d, J = 4.0 Hz, 2H), 5.05–5.08 (m, 4H), 5.15 (d, J = 4.0 Hz, 2H), 6.88 (d, J = 8.8 Hz, 2H aromatic), 7.72 (d, J = 8.8 Hz, 2H aromatic).

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