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Dansyl-Modified Cyclodextrins as Fluorescent Chemosensors for Molecular Recognition

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α-, β-, and γ-cyclodextrin derivatives (cyclodextrin is abbreviated as CD, hereafter) bearing an dansyl or dansylglycine moiety at the primary or secondary side of CD were synthesized as fluorescent indicators for molecular recognition. The CDs with the moiety at the secondary hydroxy side have an asymmetric cavity with a distorted CD framework because of the conversion of one glucose member to an altrose residue and the effects of this distortion of the CD ring on the sensing abilities were examined, compared with the case of symmetrical CDs bearing the dansyl moiety at the primary side. The results demonstrate that the asymmetrical CDs are unfavorable to accommodate the guest species due to the cavity narrowed by the distortion, contrasting with the observation that the symmetrical CDs exhibit remarkable sensing abilities for various guests.

INTRODUCTION

In recent years, much effort has been devoted to constructing molecular systems for sensing metal ions and molecular species.[1–6] Many of these systems exhibit changes in absorption or fluorescence intensities associated with guest binding as exemplified by fluorescent boronic acid for saccharide,[7] chelation-enhanced chemosensors for phosphate anion,[8] and bispyrene type molecular cleft for barbiturates.[9] We reported various cyclodextrin (CD)—based chemosensors, which have a dye moiety[10–12] or a fluorophore[13–27] and consequently exhibit changes in color or in fluorescence intensity associated with guest accommodation. CDs are cyclic oligosaccharides consisting of six (α), seven (β), eight (γ) and more glucose members, and form inclusion complexes with various organic guest compounds in aqueous solutions, accommodating a guest species in their central cavity. Therefore, our CD systems may be used for detecting a variety of organic substances and contrast to other systems which are useful for particular chemical species.

Among many CD systems, dansyl-modified CDs[23–28] are useful because the dansyl fluorophore is very sensitive to environmental polarity, exhibiting a strong emmision when accommodated in the hydrophobic CD cavity (self-inclusion form) whereas exhibiting a weak emmision in bulk water solution when it is ex-

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cluded from the CD cavity upon guest accommodation (Figure 1, Eq (1)). We have already examined molecule sensing abilities of dansyl- and dansylglycine-modified CDs and observed the guest-induced locational change of the dansyl moiety from inside to outside of the CD cavity. The self-inclusion of the dansyl moiety was recently substantiated by the X-ray structural analysis for the crystal of the related CD derivative.[28] In all the dansyl modified CDs so far examined, the dansyl moiety is located at the primary side of the CD with the moiety linked to C-6 without or with a spacer linkage. In this study, we have compared C-3 dansyl-modified CDs with C-6 modified ones. The β-CD derivatives with a substituent at C-3 were reported in which one glucose residue is converted into altrose when they were synthesized from C-2 tosyl β-CD via 2,3-mannoepoxide intermediate.[29–31] Molecular models indicate that the altrose-incorporated CDs have distorted CD frameworks with an asymmetrical cavity. Therefore, it is interesting to examine the molecule-sensing abilities of the C-3 CD derivatives, which should have a deformed CD cavity. We report here, for the first time, the binding and sensing abilities of fluorescent CDs with an asymmetrical cavity.

RESULTS AND DISCUSSION

Synthesis of Dansyl-modified CDs

We have prepared seven dansyl(Dns)-modified CDs, in which three CDs are C-6 modified ones abbreviated as 6-Dns-α-CD, 6-Dns-β-CD, and 6-Dns-γ-CD and four CDs are C-3 modified ones abbreviated as 3-Dns-β-CD, 3-Dns-gly-β-CD, 3-Dns-γ-CD, and Dns-gly-γ-CD where Dns-gly is dansylglycine moiety (Figure 2). The syntheses of these Dns-modified CDs were performed by reaction of dansyl chloride or dansylglycine with C-6- or C-3-amino CDs in DMF.[23,24]

The Structures of Altrose-incorpotrated CDs

Native CDs are symmetrical with a circle-like structure. However, there have been almost no

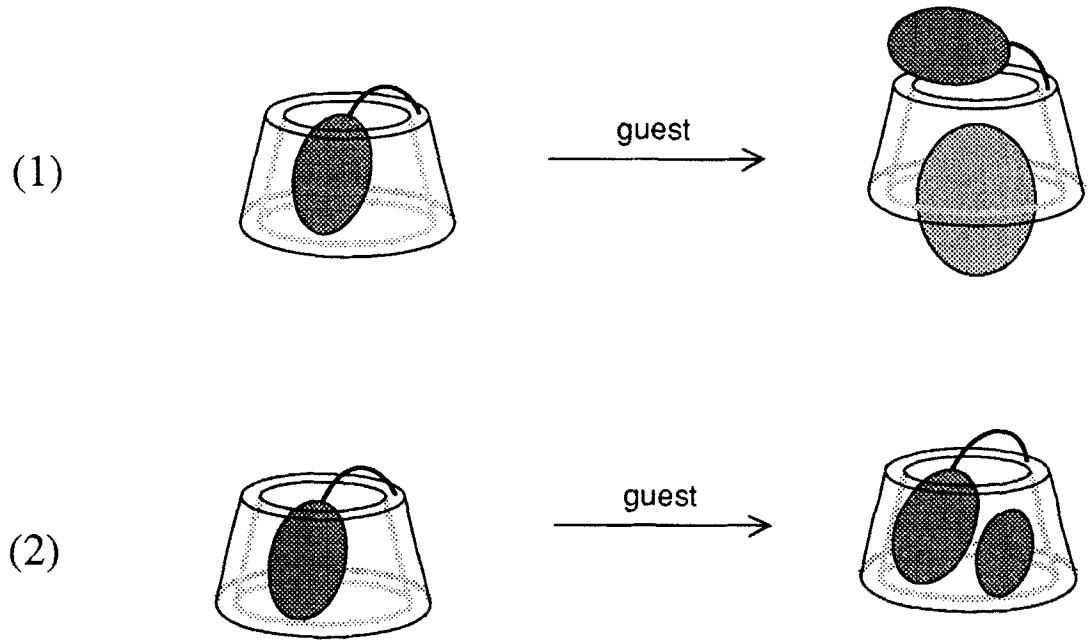


FIGURE 1 Schematic representation for induced-fit conformational changes of dansyl-modified CDs.

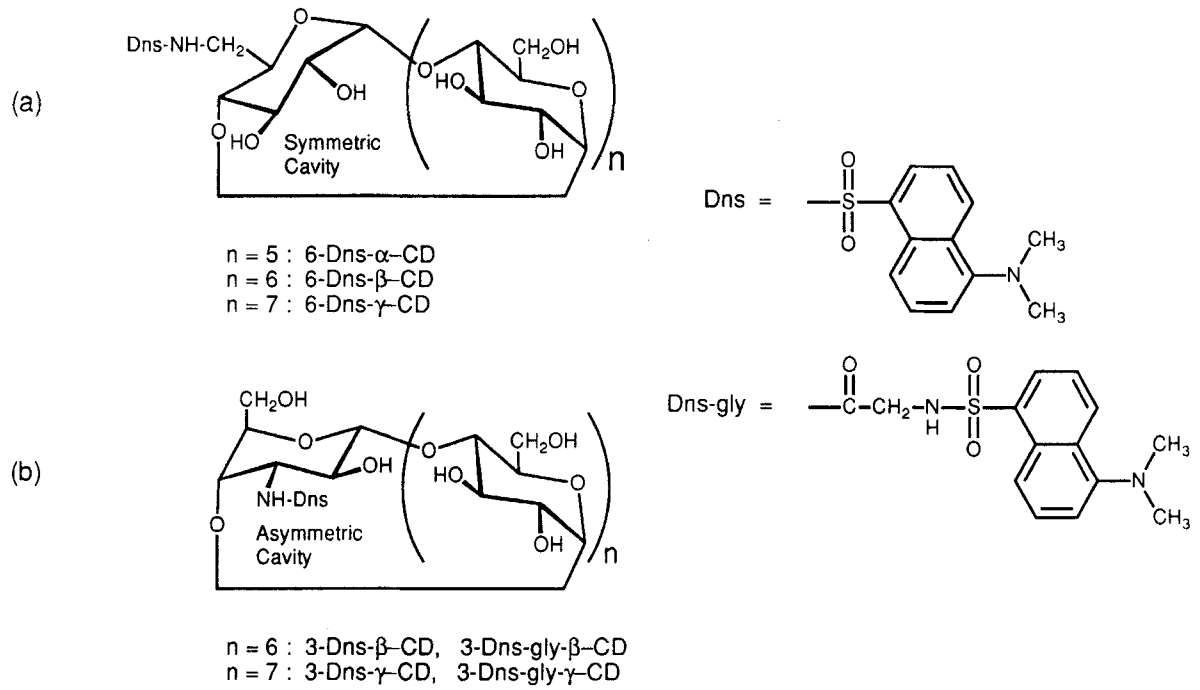


FIGURE 2 Dansyl-modified CDs bearing a dansyl moiety at the primary (a) and secondary (b) hydroxyl side.

reports on the examination of their detailed structures, although some interesting effects of the distorted altrose-incorporated CDs (asymmetrical CDs) were observed in ester hydrolysis catalized by imidazole-modified CDs. Here, we have used molecular dynamic (MD) approach to get insight into the structural features of the altrose-incorporated CDs. Figure 3 shows the structures obtained by energy minimizations. In the case of asymmetrical α-CD, MD simulation gives markedly distorted structure, in which hydrogen bonding linkages existing between secondary hydroxyls in native α-CD are broken. It seems that the cavity is too narrow to accommodate usual guests. On the other hand, altrose-incorporated β-CD and γ-CD have cavities that may include appropriate-sized guests although their cavities are narrower than native CDs.

Fluorescence Spectra and Molecular Recognition

Figure 4 shows fluorescence spectra of 6-Dns-β-CD, alone and in the presence of hyodeoxycholic acid as a guest. The fluorescence intensity decreases with increasing concentration of the guest species. This type of guest-induced variation in the fluorescence intensity was previously reported by other dansyl-modified β-CDs.[23,24] However, the dansyl-modified CDs so far examined have symmetrical cavities, so it is interesting to know how the dansyl-modified CDs with distorted cavities behave for guest species. The selected guest compounds used in this study are shown in Chart 1. They are (+)-borneol, (−)-borneol, (+)-menthol, (−)-menthol, 1-adamantanol, deoxycholic acid (DCA), chenodeoxy-

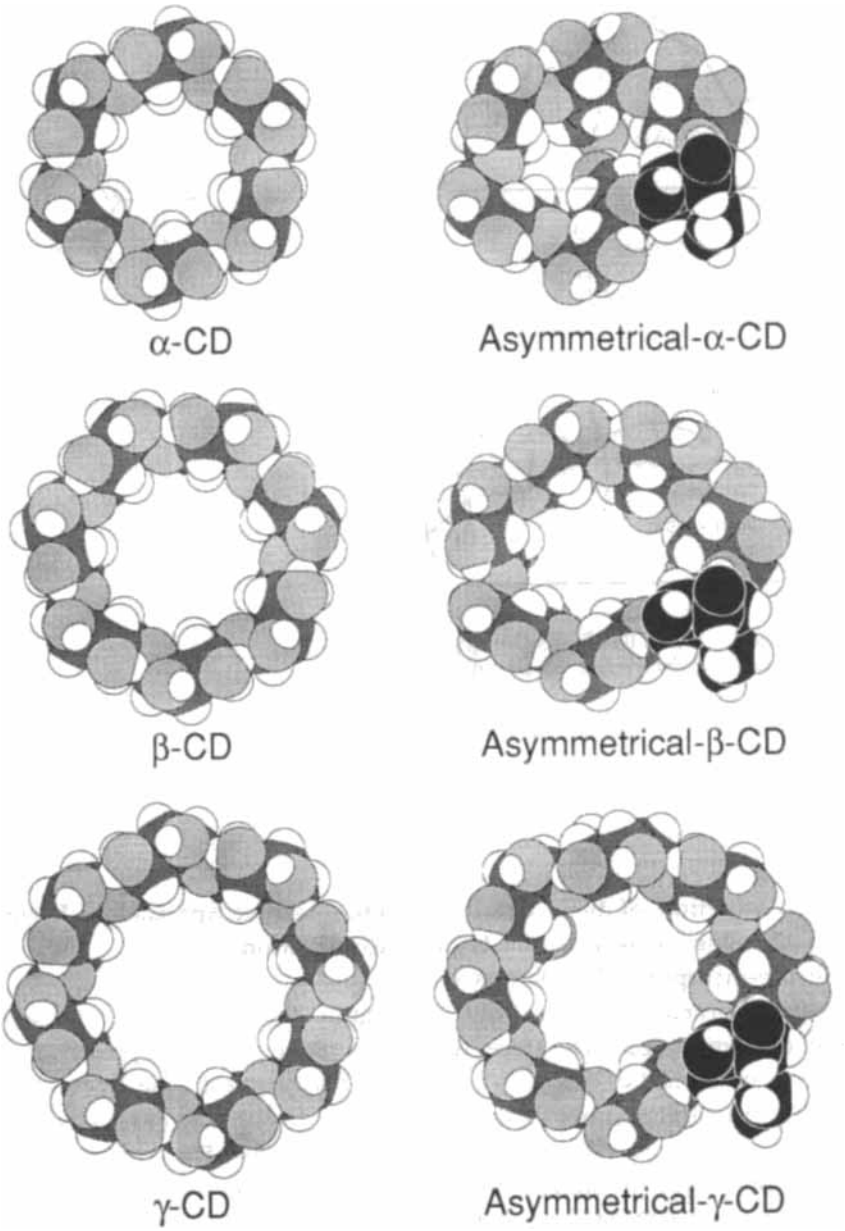


FIGURE 3 Structures of native (left) and altrose-incorporated CDs (right, the altrose residue is indicated by black) obtained by molecular dynamics.

cholic acid (CDCA), ursodeoxycholic acid, and hyodeoxycholic acid (HDCA) and only one enantiomer is shown in Chart 1 for each of borneol and menthol. It is noted that all dansyl-modified CDs used here exhibit an isosbestic point in the region of 310–360 nm (Table I) and then we adopted the isosbestic points as the excitation wavelength. Table I shows the wavelengths of emission maxima together with these excitation wavelengths. 6-Dns-α-CD exhibits no fluorescence variation for the guests examined, being consistent with the fact that the dansyl

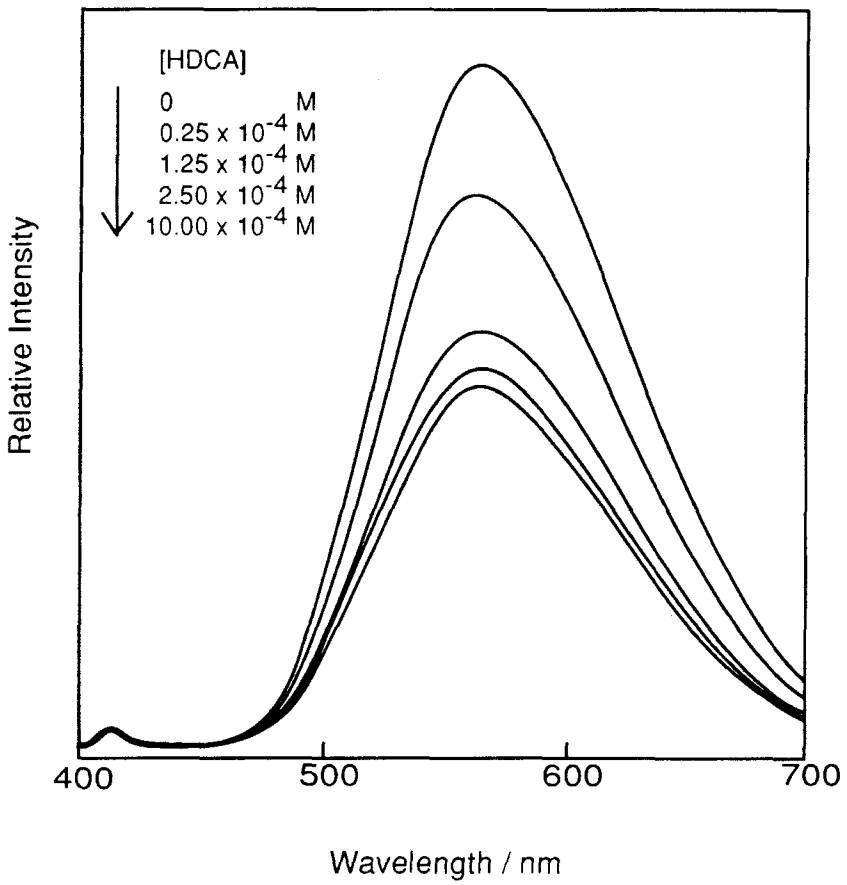
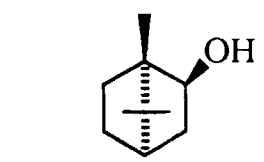
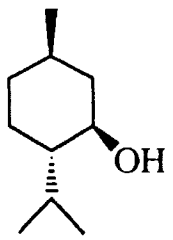


FIGURE 4 Fluorescence spectra of 6-DNS-γ-CD (2.5×10^{-5} M) at 25°C, alone and in the presence of hyodeoxycholic acid (HDCA).

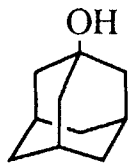
moiety is too large to be accommodated in the α-CD cavity. Tables II and III shows the sensitivity parameters ΔI/I° and binding constants (K) for various guests (0.025 mM) in pH 7.29 phosphate buffer solution, where ΔI = I–I°, I° and I are fluorescence intensities for the host alone and in the presence of guest, respectively. In the case of β-CD derivatives, 3-Dns-β-CD exhibits no variation upon guest addition, while 3-Dns-gly-β-CD exhibits almost no response for borneol, menthol, and 1-adamantanol but exhibits responses for steroid guests except for CDCA although the binding constants for them are small. Obviously this result indicates that the presence of the glycine unit is important to enable 3-Dns-gly-β-CD to bind the steroids. The glycine unit may act as a spacer to increase the flexibility of the dansyl moiety of the host, thus enabling the moiety to insert into the β-CD cavity. However, among β-CD derivatives, 6-Dns-β-CD exhibits remarkable decreases for all guests examined. The enantioselectivity of this host is remarkable for borneol with ca. 2.3 preference in binding for (−)-enatiomer although it is almost negligible for menthol. Furthermore we observed outstanding differences between the four isomeric steroids as shown by the order of binding constants HDCA (71900 M^{-1}) > UDCA (47700 M^{-1}) > CDCA (10600 M^{-1}) > DCA (345 M^{-1}). All these results demonstrate that 6-Dns-β-CD can act as a chemosensor whereas 3-Dns-β-CD and 3-Dns-gly-β-CD



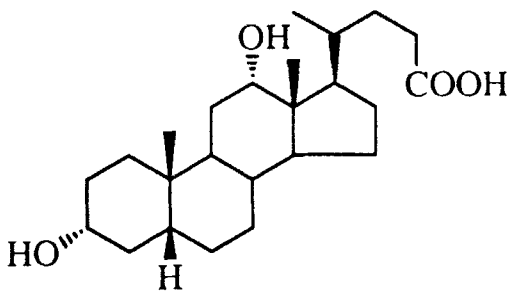
(-)-borneol



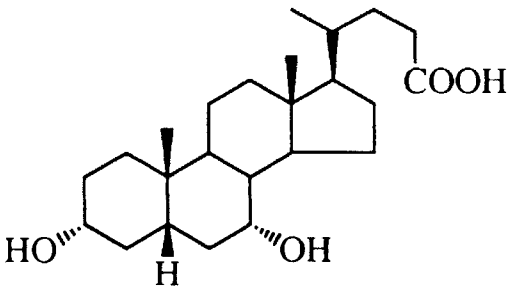
(-)-menthol



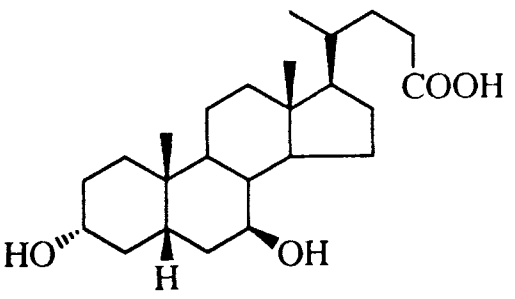
1-adamantanol



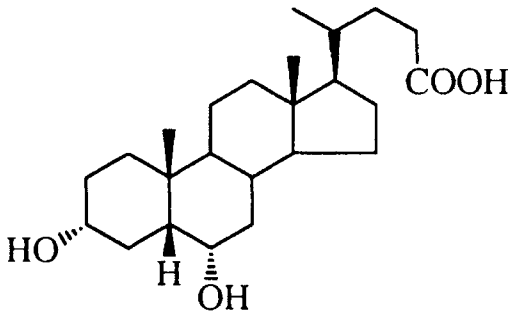
DCA



CDA



UDCA



HDCA

CHART 1

hardly act as chemosensors because of the narrowered cavity with distorted shape. In the case of γ-CD derivatives, we observed that 3-Dns-γ-CD and 3-Dns-gly-γ-CD exhibit slight enhancement in the fluorescence intensity for borneol, menthol, and 1-adamantanol although they exhibit decreases in the fluorescence intensity for much larger guests of the steroids with one exception. The guest-induced enhancement observed for 3-Dns-gly-γ-CD may be due to the co-inclusion of both the dansyl moiety and a smaller sized guest molecule in the large γ-CD cavity (Figure 1, Eq. (2)). On the other hand, 6-Dns-γ-CD exhibits decreases in the intensity

TABLE I Isosbestic points and emission maxmum of fluorescent indicators

	isosbestic point, nm	emission maxmum, nm
6-Dns-α-CD	360	560
3-Dns-β-CD	355	580
3-Dns-gly-β-CD	315	560
6-Dns-β-CD	340	560
3-Dns-γ-CD	360	578
3-Dns-gly-γ-CD	335	562
6-Dns-γ-CD	340	575

for all guest species which give values. This result suggests that a spacer unit such as the glycine residue is needed for the dansyl moiety to be co-inculded in the cavity together with a guest species, and then allows the dansyl moiety to be located snugly in the cavity, interacting with the guest molecule.

EXPERIMENTAL

Molecular Dynamics and Measurements

The models of asymmetrical CDs were constructed by using energy minimization and low temperature molecular dynamics on Insight/Discover 2.9 programs of Biosym. Model building of initial starting conformation of the asymmetrical CDs were performed by connecting one altrose ($^1C_4$ form) with other glucose residues so as to make a cyclic structure. The initial conformation of each CD was subjected to energy minimization until the maximum derivative became less than 0.01 kcal/mol. Since slow movements were required to remove too much strain in minimized structures, the temperature was reduced to 20K for 6ps MD simulation. In all calculations the CVFF force field with a harmonic potential and without cross terms and colomb atomic charges as defined by the program was applied with conjugate-gradient methods for energy minimizations. The MD simulations were carried out with the leap frog algorithm in vacuo without periodic baundary condition. An integration step of 1 femtosecond was used. There was no cut-off radius for non-bonded interaction. A distance dependent dielectric constant of 3.0r was used where r is the distance.

Thin Layer Chromatography (TLC) was performed on Kiesegel 60 F256 (Merck) silica plate, with the mixed solvent n-butanol: ethanol: water = 5:4:3 by volume. The pH value was measured by Horiba L-7LC. ^{1}H-NMR measurement were performed on Varian Gemini 200 or VXR500S.

TABLE II Guest induced emission variations and binding constants (M^{-1}) of dansyl-modified CDs for various guests[a]

Host	(+)-borneol		(−)-borneol		(+)-menthol		(−)-menthol		1-adamantanol	
	ΔI/I°	K, M^{-1}	ΔI/I°	K, M^{-1}	ΔI/I°	K, M^{-1}	ΔI/I°	K, M^{-1}	ΔI/I°	K, M^{-1}
6-Dns-α-CD	nd	(b)	nd	(b)	nd	(b)	nd	(b)	nd	(b)
3-Dns-β-CD	nd	(b)	nd	(b)	nd	(b)	nd	(b)	nd	(b)
3-Dns-gly-βCD	nd	(b)	nd	(b)	nd	(b)	nd	(b)	nd	(b)
6-Dns-β-CD	−0.07	2300	−0.09	5260	−0.07	2250	−0.08	2660	−0.07	11800
3-Dns-γ-CD	0.06	(c)	0.06	(c)	0.13	426	0.12	416	0.03	(c)
3-Dns-gly-γ-CD	0.06	264	0.06	204	0.09	294	0.10	338	0.06	265
6-Dns-γ-CD	−0.12	483	−0.12	497	nd	(b)	nd	(b)	−0.07	312

[a][host] = 2.5 × 10^{-5} M in pH = 7.25 phosphate buffer solution; ΔI = I − I° where I° and I are fluorescent intensities of host alone and in the presence of guest (1 mM); nd: no variation was detected. [b]Fluorescence changes were too small to calculate binding constants. [c]Stoichiometry is too complex to determine binding constant.

TABLE III Guest induced emission variations and binding constants (M^{-1}) of dansyl-Modified CDs for steroid guests[a]

Indicater	deoxycholic acid		chenodeoxycholic acid		ursodeoxycholic acid		hyodeoxycholic acid	
	ΔI/I°	K, M^{-1}	ΔI/I°	K, M^{-1}	ΔI/I°	K, M^{-1}	ΔI/I°	K, M^{-1}
6-Dns-α-CD	nd	(b)	nd	(b)	nd	(b)	nd	(b)
3-Dns-β-CD	nd	(b)	nd	(b)	nd	(b)	nd	(b)
3-Dns-gly-β-CD	−0.04	116	nd	(b)	−0.13	416	−0.17	740
6-Dns-β-CD	−0.06	345	−0.16	10600	−0.26	47700	−0.29	71900
3-Dns-γ-CD	−0.06	(c)	−0.08	(c)	−0.12	(c)	−0.07	(c)
3-Dns-gly-γ-CD	0.09	265	nd	(b)	−0.13	117	−0.17	338
6-Dns-γ-CD	−0.26	3760	−0.38	6800	−0.40	17900	−0.44	37900

[a][host] = 2.5×10^{-5} M in pH = 7.25 phosphate buffer solution; $\Delta I = I - I^\circ$ where I° and I are fluorescent intensities of host alone and in the presence of guest (1 mM); nd: no variation was detected. [b]Fluorescence changes were too small to calculate binding constants. [c]Stoichiometry is too complex to determine binding constant.

Materials

Dansyl chloride and dansylglycine were purchased from Tokyo Kasei. Water used as solvent was spectroscopic grade of Cica-Merck. 3-Deoxy-3-amino-CDs (3-NH_2-CDs) and 6-deoxy-6-amino-CDs (6-NH_2-CDs) were prepared as previously reported.[19,31]

3-Deoxy-3-(N-Dansyl)Amino-β-Cyclodextrin (3-Dns-β-CD)

This compound was synthesized by the reaction of 3-NH_2-β-CD and dansyl chloride. 3-NH_2-β-CD (1.1 g, ca. 1 mmol) and dansyl chloride (0.27 g, 1 mmol) were dissolved in 25 mL of DMF and stirred at room temperature for 3 h, then the reaction mixture was poured into 300 mL of acetone. Pale green precipitates were collected on a glass-flitted funnel and dried. Then crude product (1.13 g) was obtained. This crude product was dissolved in 250 mL of water and charged into the column of high porous polystyrene resin DIAION HP-20 (4 × 15 cm). The column chromatography was performed by elution with water and then with 10, 20, and 30% methanol aqueous solution. The subsequent elution of 40% methanol aqueous solution afforded the desired product. The solution was freeze-dried, and then 0.65 g of crude product was obtained. This product (200 mg) was dissolved in water, and purified by HPLC with TSKgel ODS-120T (21.5 X 300 mm) column. An aqueous methanol solution (20 %) was used as the solvent for HPLC with the flow rate of 5 mL/min and with the absorbance at 350 nm monitored. The desired product (150 mg) was obtained as pale-green powder. The purity was checked with TLC, HPLC, ^{1}H-NMR, and elemental analysis: Anal. Calcd. for $C_{54}H_{83}O_{37}N_2S{\cdot}9H_2O$: C, 41.94; H, 6.58; N, 1.81; S, 2.07. Found: C, 41.93; H, 6.41; N, 1.85; S, 2.03; ^{1}H-NMR (D_2O, 500 MHz) δ 2.81 (s, 6H, CH_3), 3.38 (broad, 1H), 3.42 ~ 3.91 (m), 4.70 (1H, overlapped with HOD), 4.96 ~ 4.99 (m, broad, 2H), 5.02 ~ 5.04 (m, broad, 2H), 7.35 (d, 1H, aromatic), 7.60 (t, 1H, aromatic), 7.71 (t, 1H, aromatic), 8.28 (d, 1H, aromatic), 8.36 (d, 1H, aromatic), 8.48 (d, 1H, aromatic).

3-Deoxy-3-(N-Dansyl)Amino-γ-CD (3-Dns-γ-CD)

This compound was synthesized by the reaction of 3-NH_2-γ-CD and dansyl chloride. 3-NH_2-γ-CD (1.3 g, ca. 1 mmol) and dansyl chloride (0.27 g, 1 mmol) were dissolved in 25 mL of DMF and stirred at room temperature for 3 h, and then the reaction mixture was poured into 300 mL of acetone. Pale green precipitates were collected on a glass-fritted funnel and dried, giving the crude product (1.35 g). This product was dissolved in 250 mL of water and charged into the column of high porous polystyrene resin DI-

AION HP-20 (4 × 15 cm). The column chromatogrphy was performed by elution with water and then with 10, 20, 30 and 40% methanol aqueous solutions. The eluate of 40% methanol aqueous solution contained the desired product. The solution was freeze-dried, and then crude product (700 mg) was obtained. This product (200 mg) was dissolved in water, and purified by HPLC with a TSKgel ODS-120T (21.5 × 300 mm) column with a methanol aqueous solution (20 %) as an eluant under the conditions of the flow rate of 5 mL/min and the monitoring absorbance of 350 nm. Desired product (145 mg) was obtained as pale-green powder. The purity was checked with TLC, HPLC, ^{1}H-NMR, and elemental analysis: Anal. Calcd. for $C_{60}H_{93}O_{42}N_2S{\cdot}5H_2O$: C, 44.04; H, 6.34; N, 1.71; S, 1.96. Found: C, 44.02; H, 6.48; N, 2.01; S, 2.13; ^{1}H-NMR (D_2O, 500 MHz): δ 2.90 (s, 6H, CH_3), 3.01 (t, 1H), 3.20 (m, 3H), 3.29 ~ 4.10 (m), 4.18 (t, 1H), 4.22 (t, 1H), 4.42 (broad, 1H), 4.56 ~ 4.72 (broad overlapped with HOD), 4.86 (m, broad, 2H), 4.98 (d, 2H), 5.20 (broad, 2H), 7.44 (d, 1H, aromatic), 7.68 (t, 1H, aromatic), 7.83 (t, 1H, aromatic), 8.08 (d, 1H, aromatic), 8.33 (d, 1H, aromatic), 8.80 (d, 1H, aromatic).

3-Deoxy-3-(N-Dansylglycyl)Amino-β-CD (3-Dns-Gly-β-CD)

This compound was synthesized by the condensation reaction of 3-NH_2-β-CD and dansylglycine. Dansylglycine (0.46 g, ca. 0.15 mmol) and DCC (0.30 g, 0.15 mmol) were dissolved in 10 mL of DMF below 5 °C and stirred for 2 hours, then 3-NH_2-β-CD (2.5 g, ca. 2 mmol) was added to above solution and stirred at room temperature for 36 h, and then the reaction mixture was poured into 300 mL of acetone. Pale green precipitates were collected on a glass-fritted funnel and dried. The crude product (2 g) was dissolved in 300 mL of water and column chromatography was performed on ion exchange resin CM-Sephadex C-25 (4 × 10 cm) with water as an eluant. The product fraction was freeze-dried to give 0.54 g of crude product as pale-yellow powder. This product was dissolved with water and purified by HPLC on TSKgel ODS-120T column (21.5 × 300 mm) with a methanol aqueous solution (20 %) as an eluant under the conditions of 5 mL/min and the monitoring absorbance of 350 nm to give desired product (140 mg) as pale-green powder. The purity was checked with TLC, HPLC, ^{1}H-NMR, and elemental analysis: Anal. Calcd. for $C_{56}H_{85}O_{37}N_3S{\cdot}8H_2O$: C, 42.88; H, 6.49; N, 2.68; S, 2.04. Found: C, 43.20; H, 6.37; N, 2.56; S, 1.92; ^{1}H-NMR (D_2O, 500 MHz) δ 2.38 (broad, 1H), 2.61 (broad, 1H), 2.87 (s, 6H, CH_3), 3.09 (t, 1H), 3.21 ~ 3.29 (m, 4H), 3.42 ~ 4.10 (m), 4.18 (t, 1H), 4.30 (t, 1H), 4.50 (broad, 1H), 4.61 (broad, 1H), 4.92 (t, 1H), 4.95 (broad, 1H), 4.95 (broad, 1H), 5.02 (d, 1H), 5.08 (broad, 1H), 7.49 (d, 1H, aromatic), 7.73 (t, 1H, aromatic), 7.90 (t, 1H, aromatic), 8.13 (d, 1H, aromatic), 8.39 (d, 1H, aromatic), 8.63 (d, 1H, aromatic).

3-Deoxy-3-(N-Dansylglycyl)Amino-γ-CD (3-Dns-Gly-γ-CD)

This compound was synthesized by the condensation reaction of 3-NH_2-γ-CD and dansylglycine. Dansylglycine (0.46 g, ca. 0.15 mmol) and DCC (0.30 g, 0.15 mmol) were dissolved in 10 mL of DMF below 5 °C and stirred for 2 h, and then 3-NH_2-γ-CD (2.5 g, ca. 2 mmol) was added to above solution and stirred at room temperature for 36 h. The reaction mixture was poured into 300 mL of acetone. Pale green precipitates were collected on a glass-flitted funnel and dried. The crude product (2 g) was dissolved in 300 mL of water and column chromatography on ion exchange resin CM-Sephadex C-25 (4 × 10 cm) was performed with water as a eluant. The product fraction was freeze-dried to give crude product (0.54 g) as pale-yellow powder. This product was dissolved with water and purified by HPLC on TSKgel ODS-120T column (21.5 × 300 mm) with a methanol aqueous solu-

tion (20 %) as an eluant under the conditions of the flow rate of 5 mL/min and the monitoring absorbance of 350 nm. The desired product (140 mg) was obtained as pale-green powder. The purity was checked with TLC, HPLC, ^{1}H-NMR, and elemental analysis: Anal. Calcd. for $C_{62}H_{95}O_{42}N_3S{\cdot}7H_2O$: C, 43.48; H, 6.44; N, 2.45; S, 1.87. Found: C, 42.93; H, 6.08; N, 2.44; S, 1.82; ^{1}H-NMR (D_2O, 500 MHz) δ 2.89 (s, 6H, CH_3), 2.95 ~ 4.24 (m), 4.47 (broad, 1H), 4.76 (d, 1H), 4.93 ~ 4.95 (m, 2H), 4.98 (d, 1H), 5.04 (d, 1H), 5.07 (d, 1H), 5.08 (d, 1H), 7.35 (d, 1H, aromatic), 7.56 (t, 1H, aromatic), 7.76 (t, 1H, aromatic), 8.06 (d, 1H, aromatic), 8.11 (d, 1H, aromatic), 8.62 (d, 1H, aromatic).

6-Deoxy-6-(N-Dansyl)Amino-α-CD (6-Dns-α-CD)

This compound was synthesized by the reaction of 6-NH_2-α-CD and dansyl chloride. 6-NH_2-α-CD (0.70 g, ca. 0.72 mmol) and dansyl chloride (0.27 g, 1 mmol) were dissolved in 30 mL of DMF and stirred at room temperature for 3 h, and then the reaction mixture was poured into 300 mL of acetone. Pale green precipitates were collected on a glass-fritted funnel and dried. This crude product (0.88 g) was dissolved in 300 mL of water and column chromatography was performed on ion exchange resin CM-Sephadex C-25 (4 × 10 cm) with water as an eluant. The product fraction was freeze-dried, and then desired product (0.6 g) was obtained as pale-yellow powder. The purity was checked with TLC, HPLC, ^{1}H-NMR, and elemental analysis: Anal. Calcd. for $C_{48}H_{72}O_{31}N_2S{\cdot}9H_2O$: C, 41.17; H, 6.02; N, 2.32; S, 2.66. Found: C, 42.18; H, 6.49; N, 2.13; S, 2.30; ^{1}H-NMR (D_2O, 500 MHz) δ 2.69 (d, 1H), 2.90 (s, 6H, CH_3), 3.23 ~ 3.64 (m, 7H), 3.65 ~ 4.09 (m), 4.75 (d, 1H), 4.83 (d, 1H), 4.87 (d, 1H), 5.02 (d, 1H), 5.07 (d, 1H), 7.46 (d, 1H, aromatic), 7.72 (m, 2H, aromatic), 8.11 (d, 1H, aromatic), 8.24 (d, 1H, aromatic), 8.51 (d, 1H, aromatic).

6-Deoxy-(6-Dansyl)Amino-β-CD (6-Dns-β-CD)

This compound was synthesized by the reaction of 6-NH_2-β-CD and dansyl chloride. 6-NH_2-β-CD (2.0 g, 1.8 mmol) and dansyl chloride (0.48 g, 1.8 mmol) were dissolved in 65 mL of DMF and stirred at room temperature for 3 h, and then the reaction mixture was poured into 500 mL of acetone. Pale green precipitates were collected on a glass-fritted funnel and dried. This crude product (2.56 g) was dissolved in 75 mL of hot water (100 °C) and recrystallized in room temperature to give desired product (1.4 g). The purity was checked with TLC, HPLC, ^{1}H-NMR, and elemental analysis: Anal. Calcd. for $C_{54}H_{83}O_{37}N_2S{\cdot}7H_2O$: C, 42.94; H, 6.04; N, 2.02; S, 2.32. Found: C, 42.95; H, 6.34; N, 2.10; S, 2.43; ^{1}H-NMR (D_2O, 500 MHz): δ 2.40 (broad, 1H), 2.95 (s, 6H, 3), 3.14 ~ 4.14 (m), 4.78 (1H, overlapped with HOD), 4.85 (d, 1H), 4.96 (d, 1H), 5.02 (d, 1H), 5.098 (d, 1H), 7.58 (m, 2H, aromatic), 7.92 (t, 1H, aromatic), 8.28 (d, 1H, aromatic), 8.44 (d, 1H, aromatic), 8.50 (d, 1H, aromatic).

6-Deoxy-6-(N-Dansyl)Amino-γ-CD (6-Dns-γ-CD)

This compound was synthesized by the reaction of 6-NH_2-γ-CD and dansyl chloride. 6-NH_2-γ-CD (1.0 g, 0.77 mmol) and dansyl chloride (0.20 g, 0.75 mmol) were dissolved in 25 mL of DMF and stirred at room temperature for 3 h, and then the reaction mixture was poured into 300 mL of acetone. Pale green precipitates were collected on a glass-fritted funnel and dried. This crude product (2 g) was dissolved in 250 mL of water and column chromatography on ion exchange resin CM-Sephadex C-25 (4 × 10 cm) was performed with water as an eluant. The product fraction was freeze-dried to give crude product (2 g). Column chromatography on Sephadex LH-20 with a DMF aqueous solution (50 %) as an eluant gave pure product (455 mg) as pale-green powder. The purity was checked with TLC, HPLC, ^{1}H-NMR, and elemental analysis: Anal. Calcd. for $C_{54}H_{83}O_{37}N_2S{\cdot}6H_2O$: C, 43.56; H, 6.40; N, 1.69; S, 1.94. Found: C, 43.42; H, 6.23; N, 1.63; S, 2.03; ^{1}H-NMR (D_2O, 500

MHz) δ 2.66 (d, 1H), 2.80 (t, 1H), 3.00 (s, 6H, CH_3), 3.15 ~ 4.39 (m), 4.90 (d, 1H), 5.04 (d, 1H), 5.06 (d, 1H), 5.08 (d, 1H), 5.15 (d, 1H), 5.20 (d, 1H), 7.40 (d, 1H, aromatic), 7.64 (t, 1H, aromatic), 7.73 (t, 1H, aromatic), 8.21 (d, 1H, aromatic), 8.30 (d, 1H, aromatic), 8.65 (d, 1H, aromatic).

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