NMR Studies of Conformations of *N*-Dansyl-L-leucine-Appended and *N*-Dansyl-D-leucine-Appended *β*-Cyclodextrin as Fluorescent Indicators for Molecular Recognition

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Received March 1, 1996 (Revised Manuscript Received January 2, 1997[⊗]*)*

The structures of *N*-dansyl-L-leucine-appended *β*-cyclodextrin (**1**) and *N*-dansyl-D-leucine-appended *β*-cyclodextrin (**2**) were estimated by the combined use of 1D and 2D NMR techniques (1D and 2D TOCSY, ROESY, PFG MQF-COSY, PFG HSQC, and NOE difference spectra). The dansyl moiety of **2** is included in its own cavity more deeply than that of **1**. The difference in the properties between **1** and **2** was interpreted by the difference in the inclusion depth of the dansyl moieties. Their conformational changes upon addition of 1-adamantanol were also studied by NMR. The chemical shifts and patterns of ^{1}H resonances for the protons of the dansyl, leucine, and cyclodextrin parts were changed upon addition of the guest. These changes indicated the exclusion of the dansyl moiety from the cyclodextrin cavity to bulk water upon addition of the guest.

Introduction

In supramolecular chemistry, there has been a great deal of interest in molecular recognition by cyclodextrins (CDs) and their derivatives.[1−9] Detailed understanding of the structure−function relationships of modified CDs will be one of the effective ways to know how to design more excellent modified CDs; however, only a few attempts have been made to determine the detailed structures of the modified CDs including the orientation of the pendant groups. The current NMR technique is a powerful tool for the structural and conformational analysis of the modified CDs.[10−17] On this basis, we have been studying the structural and conformational analyses of the modified CDs and have determined the orientation of the pendant groups of some modified CDs by the combined use of 1D and 2D NMR techniques.[14−17]

We have been studying fluorophore-appended CDs as fluorescent indicators for molecular recognition.[5,7,17] The fluorescence intensities of the fluorophore-appended CDs are decreased or increased upon addition of a guest molecule. The degree of this guest-induced variation of the fluorescence intensity depends on the polarity, shape, and size of the guest molecule, and spectroscopically inert molecules can also be detected by these indicators by changes in the fluorescence intensity. We synthesized *N*-dansyl-L-leucine-appended *β*-CD (**1**) and *N*-dansyl-D-leucine-appended *β*-CD (**2**) as new fluorescent indicators for molecular recognition (dansyl: 5-(dimethylamino)-1-naphthalenesulfonyl). Their properties were significantly different from each other, although the structural difference between them was only the enantiomeric configuration of their amino acid residues.[18] For example, the binding ability of **1** was over twice that of **2**. The degree of guest-induced variation in the fluorescence intensity of **1** was greater than that of **2**, whereas the former fluorescence intensity in the absence of the guest was smaller than the latter. These differences would be derived from the difference in the orientation of the dansyl moiety. Therefore, it is necessary to elucidate the orientation of the pendant groups in order to understand the difference in their properties. In this paper, we describe the conformational analyses of *N*-dansyl-L-leucine-appended and *N*-dansyl-D-leucine-appended *β*-CD using NMR techniques.

Experimental Section

Materials. *β*-Cyclodextrin was kindly gifted by 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 the Merck Co.

General Methods. Thin-layer chromatography (TLC) was carried out with silica gel 60 F_{254} (Merck Co.). Absorbance

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⊗ Abstract published in *Advance ACS Abstracts,* February 1, 1997.

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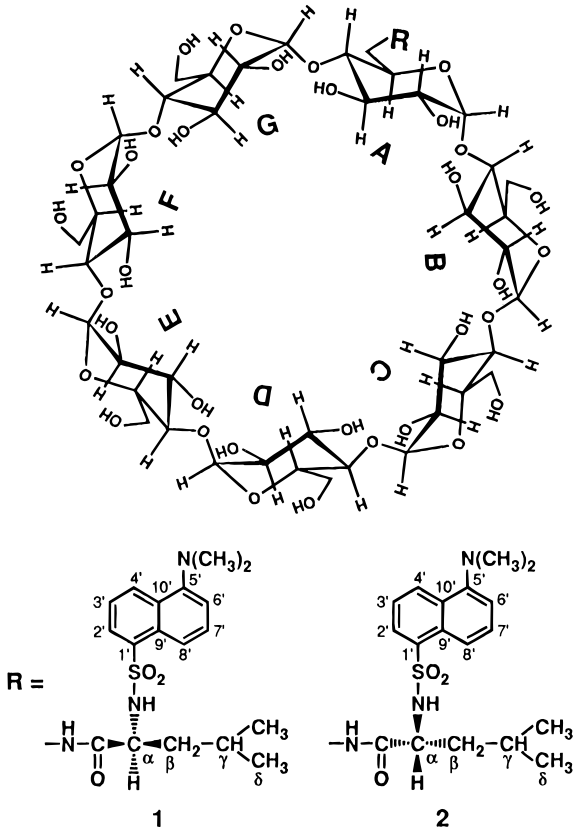
S0022-3263(96)00425-2 CCC: $14.00

Chart 1



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 the memory of the system were transferred to and analyzed on a desktop computer (Hewlett-Packard HP9000 Series Model 330). Lifetime was obtained by deconvolution with a nonlinear least-square fitting procedure. 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. Melting point was measured by a Yanaco MP-J3 micromelting point apparatus without any correction. Elemental analyses were performed by the Analytical Division in Research Laboratory of Resources Utilization of Tokyo Institute of Technology.

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, the 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 $C_{18}H_{24}N_2O_4S$: C, 59.32; H, 6.64; N, 7.69; S, 8.78. Found: C, 59.18; H, 6.57; N, 7.64; S, 8.55. $[\alpha]_D = 37.4°$ (*c* 0.1, methanol) ($[\alpha]_D = -37.7°$ for *N*-dansyl-L-leucine). Mp 80−81 °C. ^{1}H 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 Mono-6-(*N*-dansyl-L-leucylamino)-6-deoxy-β-cyclodextrin (1). To a solution of 6-amino-6-deoxy-β-cyclodextrin[17] (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 the 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 highly 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% 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 Mono-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).

NMR Spectroscopy. 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 ^{1}H. The probe of a UNITY plus-400 spectrometer was equipped with an Actively-Shielded Pulsed Field Gradient coil. All the NMR spectra were measured 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. A 1−3 mM sample in D_2O was used for NMR spectroscopy unless otherwise noted. One-dimensional total correlation spectroscopy (1D TOCSY): 1D TOCSY spectra were obtained by the selective irradiation of the H-1 protons of the glucose unit with the shaped pulse, hermite 180,[20] followed by various mixing times (MLEV-17 spin lock) on a UNITY plus-400 spectrometer; 256 scans, 1.2 s repetition delay, 27392 data point, and 3.8 s acquisition time were used. Two-dimensional total correlation spectroscopy (2D TOCSY): 2D TOCSY spectra were obtained on a Varian VXR-500S; 100 ms MLEV-17 spin lock, 32 scans per t_1 increment, 512 × 2 t_1 increment, 2048 × 2048 data points, 0.227 s acquisition time, and 2.0 s repetition delay were used. HDO was suppressed by selective irradiation during the repetition delays. PFG multiple-quantum filtered correlated spectroscopy (PFG MQF-COSY): PFG MQF-COSY spectra were obtained on a UNITY plus-400 spectrometer; 32 scans per t_1 increment, 512 × 2 t_1 increment, 2048 × 2048 data points, 0.205 s acquisition time, and 1.5 s repetition delay were used. PFG ^{1}H−^{13}C ^{1}H detected heteronuclear single-quantum coherence spectroscopy (PFG ^{1}H−^{13}C HSQC): PFG ^{1}H−^{13}C HSQC spectra were obtained on a UNITY plus-400 spectrometer; 128 scans per t_1 increment, 128 × 2 t_1 increment, 2048 × 2048 data points, 0.285 s acquisition time, and 2.0 s repetition delay were used. Rotating frame nuclear Overhauser effect spectroscopy (ROESY): ROESY spectra were obtained on a Varian VXR-500S; 300 ms MLEV-17 spin lock, 32 scans per t_1 increment, 512 × 2 t_1 increment, 2048 × 2048 data points, 0.227 s acquisition time, and 2.0 s repetition delay were used. HDO was suppressed by selective irradiation during the repetition delays. The NOE difference spectrum of dansylamide (**4**) was obtained by subtracting the FID without NOE caused by irradiation at a no-resonance area from that with NOE caused by selective irradiation of the methyl resonance ($\delta = 2.88$ ppm) on a UNITY plus-400 spectrometer.

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Table 1. Fluorescence Lifetimes of 1−3 in Aqueous Solution[a]

	τ_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	3.5	−	−	−	1.28

[a] [**1**−**3**] = 2×10^{-5} M. Decay curves were fitted to the following equation: $I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$.

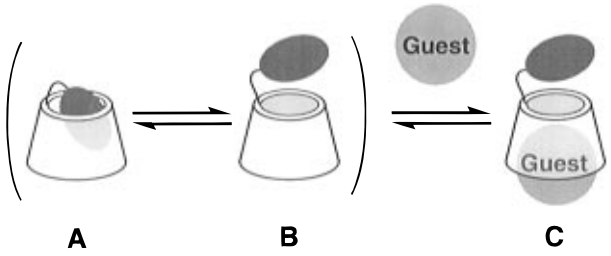

Figure 1. Conformational equilibrium in aqueous solution and guest-induced conformational change in **1** and **2**.

Results and Discussion

Fluorescence Lifetime. In aqueous solution, the pendant groups of modified CDs are flexible and not fixed in many cases.[7,17] In the case of the modified CD that has a fluorophore as a pendant, the analysis of the fluorescence decay of the fluorophore pendant provides useful information with respect to the conformational features, because the fluorescence lifetimes of usual 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 the environment and has different lifetimes when located inside the cavity and in bulk water solution.[7,19]

All the fluorescence decays of the dansyl moiety of *N*-dansyl-L-leucine-appended *β*-cyclodextrin (**1**) and *N*-dansyl-D-leucine-appended *β*-cyclodextrin (**2**) were 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 are with the dansyl moiety inside and outside the cavity, respectively.[7,19] These two species are in equilibrium as shown in parenthesis in Figure 1.

Assignments of the CD Regions of the ^{1}H Resonances of 1 and 2. The observed NMR spectra of **1** and **2** 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 indicates that there are two kinds of observable conformational isomers, which are in equilibrium, and that the dansyl moieties of **1** and **2** are located in the cavity in the major state. The concentration dependence of various spectra of **1** and **2** (10−0.1 mM) substantiates that this inclusion complex is not the intermolecular complex but the intramolecular complex (self-inclusion complex). Therefore, the NMR spectra of **1** and **2** are 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 the combined use of various of 1D and 2D NMR techniques. In addition to the method already reported,[14−17] the 1D total correlation spectroscopy (TOCSY)[21,22] and the nuclear Overhauser effect (NOE) difference spectra[27] were applied and the pulsed field gradient (PFG)[23−25] was used for the selection of a particular coherence-transfer pathway and the suppression of the HDO resonance in 2D NMR experiments. A shaped pulse was also used for increasing the selectivity of the irradiation.

At first, the overlapped signals of the ^{1}H NMR spectra of **1** and **2** were separated into each set of resonances for the protons belonging to the same D-glucose unit using the 1D and 2D TOCSY spectra. Using the 1D and 2D TOCSY spectra and the PFG multiple-quantum filtered correlated spectroscopy (MQF-COSY) spectra,[24] all the resonances of **1** and **2** were then assigned to the protons of each glucose unit, except the sequence of the glucose units. Because the 1D TOCSY spectrum with variation of the duration of the propagation period can give not only the extracts of each set of resonances for the protons belonging to the same D-glucose unit but also the assignments of their resonances as shown in Figure 2, the combined use of 1D and 2D TOCSY and COSY spectra provides more exact assignment. It is difficult to assign the resonances of the H-6 protons of the glucose unit **A** in the ^{1}H NMR spectrum because of their dispersed and shifted resonances due to the anisotropic shielding effect of the carbonyl group of the leucine residue, but the C-6 carbon resonance of the glucose unit **A** was found at 43.5 ppm in the ^{13}C NMR spectrum. The resonances of the H-6 protons of the glucose unit **A** were assigned by the PFG ^{1}H−^{13}C ^{1}H detected heteronuclear single-quantum coherence spectroscopy (HSQC) spectrum[25] as shown in Figure 3. Finally, the sequence of the glucose units was determined by the rotating frame nuclear Overhauser effect spectroscopy (ROESY) spectrum.[26] The distance between H-1 of a glucose unit and H-4 of the adjacent glucose unit is short enough to give an NOE, which can be observed by ROESY. The glucose unit of which the H-4 resonance has a negative crosspeak with the H-1 resonance of the glucose unit **A** in the ROESY spectrum was identified as the glucose unit **B**. In a similar way, the sequences of all the glucose units were successively determined. The results of the assignments are shown in Figures 4 and 5.

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 to the upper field as shown in Figures 4 and 5. These shifts are attributed to the anisotropic ring current effects from the dansyl moieties, and this indicates that the dansyl moieties are included in the CD cavities.

Assignments of the Dansyl Regions of the ^{1}H Resonances of 1 and 2. Figure 6 shows the naphthyl region of the ^{1}H NMR spectra of **1**, **2**, and *N*-dansyl-L-leucine (**3**). The resonance patterns of **1** and **2** are

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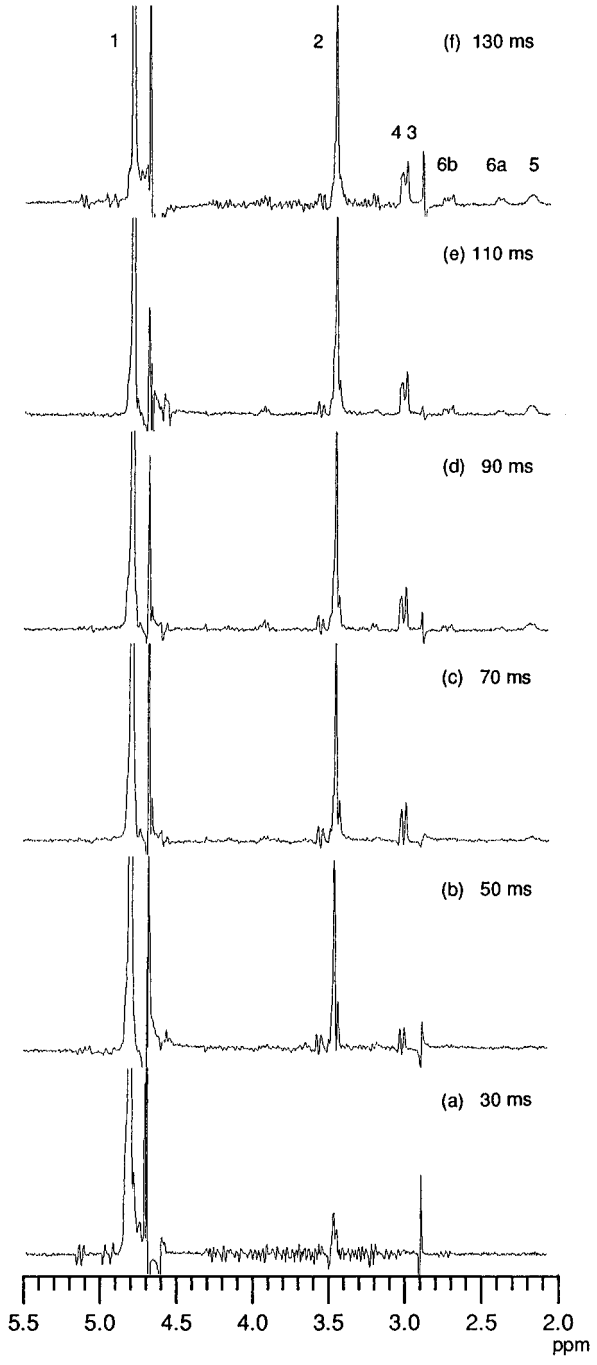

Figure 2. 400 MHz 1D-TOCSY spectra of **2**; each spectrum was obtained with a different mixing time after the selective irradiation of the anomeric proton in the glucose unit **A** with a shaped pulse, hermite 180.

different from each other and are also different from that of **3**. These spectra suggest that the dansyl moieties of **1** and **2** are included in the cavities, and the positions of their dansyl moieties in the cavities are different from each other. The ^{1}H resonances for the naphthyl region of the dansyl moiety were assigned by the use of the NOE difference spectra and the COSY spectra. Because the dimethylamino group of the dansyl moiety prefers to adopt conformation **a** rather than **b** in Figure 7,[28] the

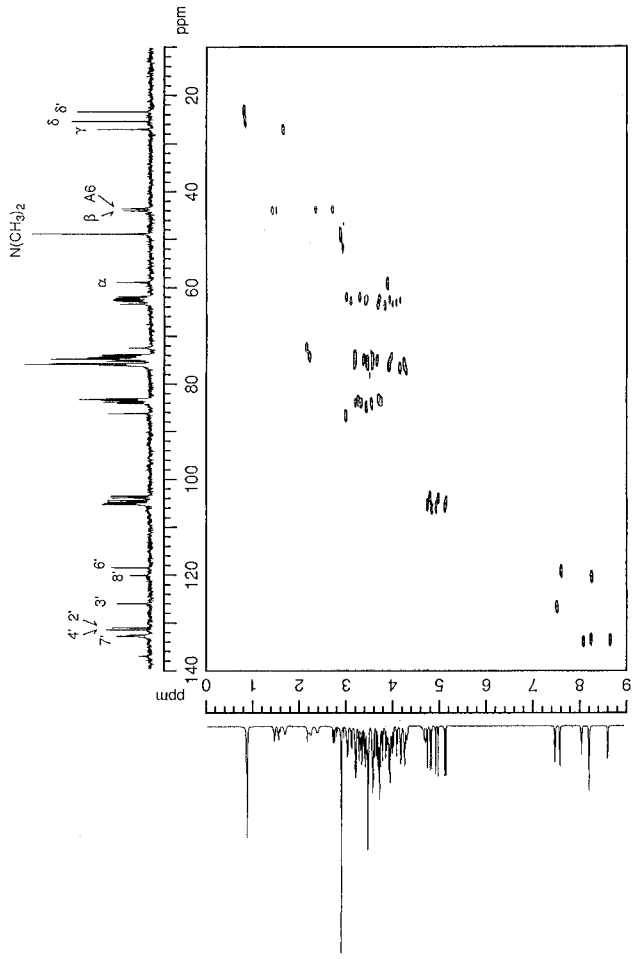

Figure 3. 400 MHz PFG ^{1}H−^{13}C HSQC spectrum of **2** in D_2O at 25 °C.

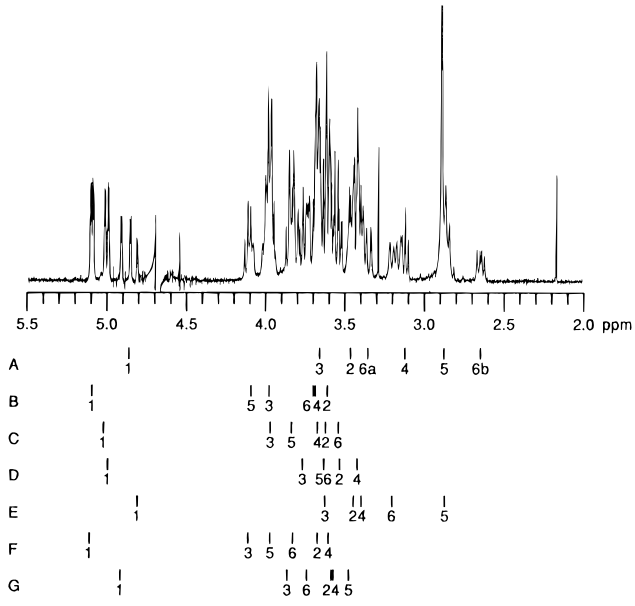

Figure 4. A part of the 500 MHz ^{1}H NMR spectrum of **1** in D_2O at 25 °C, showing only the CD region with assignments.

irradiation of the methyl resonance would produce the largest enhancement on the H-6′ proton and a smaller enhancement on the H-4′ proton. The NOE difference spectrum of dansylamide (**4**) was obtained by subtracting the FID without NOE caused by irradiation at a no-resonance area from that with NOE caused by selective

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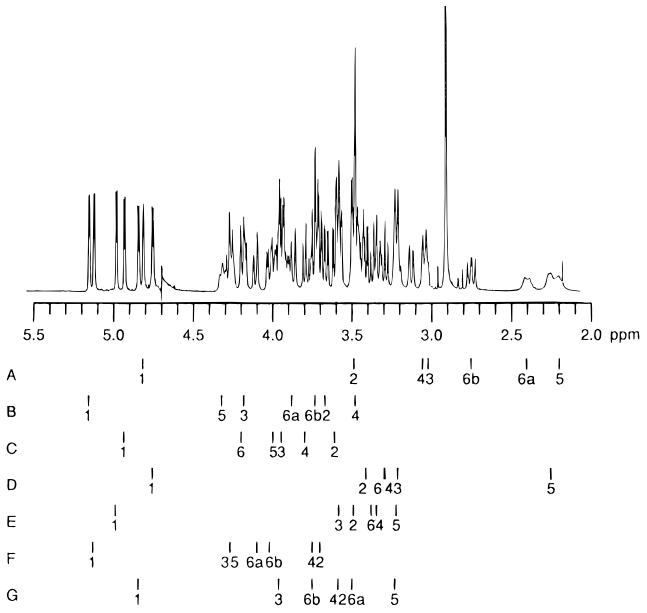

Figure 5. A part of the 500 MHz ^{1}H NMR spectrum of **2** in D_2O at 25 °C, showing only the CD region with assignments.

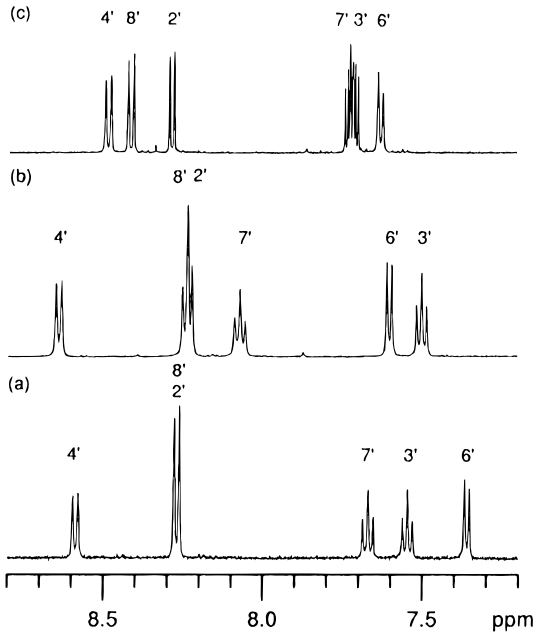

Figure 6. The naphthyl regions of the 500 MHz ^{1}H NMR spectra of (a) **1**, (b) **2**, and (c) **3** in D_2O at 25 °C.

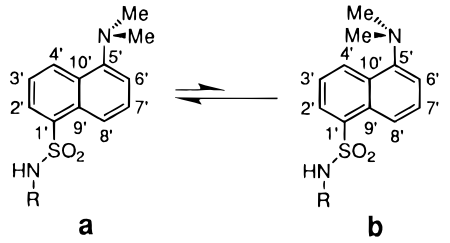

Figure 7. Conformational equilibrium of the dansyl moiety.

irradiation of the methyl resonance at 2.88 ppm (Figure 8). From this spectrum, the resonances at 7.27 and 8.53 ppm were identified as the H-6′ resonance and the H-4′ resonance, respectively. The other ^{1}H resonances of **4** were assigned by tracing the coupling relationships starting from H-6′ or H-4′ in the PFG MQF-COSY

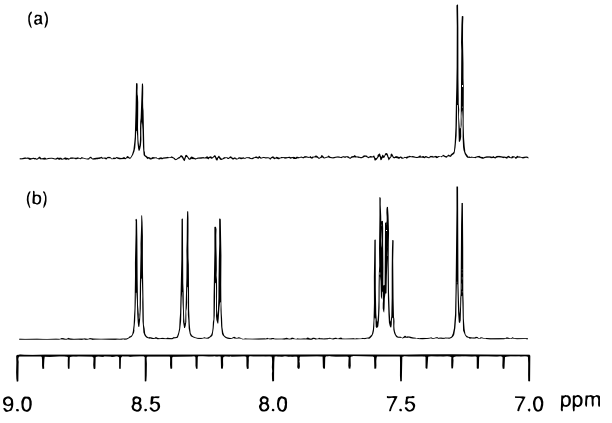

Figure 8. Aromatic regions of (a) 400 MHz NOE difference spectrum of dansyl amide (**4**) on preirradiation of $N(CH_3)_2$ signal (δ = 2.88) and (b) its normal ^{1}H NMR spectrum in methanol-d_4 at 25 °C: 1 Hz line broadening was applied in both spectra.

spectrum. The resonances of the dansyl protons of **1**, **2**, and **3** were also assigned by the same method.

Elucidation of the Orientations of the Dansylleucine Moieties. The NOE correlation between a proton of the dansyl moiety and a proton of the CD moiety will be observed by the ROESY spectrum, if they are closer than 4 Å through space.[27] 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 will be observed, and it is possible to estimate the orientation of the dansyl moiety in the CD cavity using the assigned NOE correlations. Figures 9 and 10 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 makes the NOE weaker. Although the NOE correlation between the protons of the dansyl moiety and the protons of CD moiety can be also interpreted by the head-to-head bimolecular complex in which each dansyl moiety is included in the cavity of each other (intermolecular complex), the NOE correlation between the δ protons of the leucine moiety and the CD protons cannot be interpreted by the head-to-head bimolecular complex, because some additional NOE correlation between the δ protons and the CD protons should be observed in the case of the head-to-head bimolecular complex. Therefore, the head-to-head bimolecular CD complex is completely ruled out. The head-to-tail bimolecular complex is also ruled out by the data of the NOE correlation, and the NMR data can be explained only by the self-inclusion complex (intramolecular complex). Figure 11 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 parts. The dansyl moiety of **2** is included in the cavity more deeply than that of **1**. These structures can elucidate the difference in the resonance of the δ protons of the leucine between **1** and **2**. The resonances of the δ protons of the leucine moiety of **1** are more dispersed and are shifted more upfield than those of **2**. This difference is caused by the difference in the position of the dansyl moiety, where the δ protons of **1** are more influenced by the anisotropic ring current

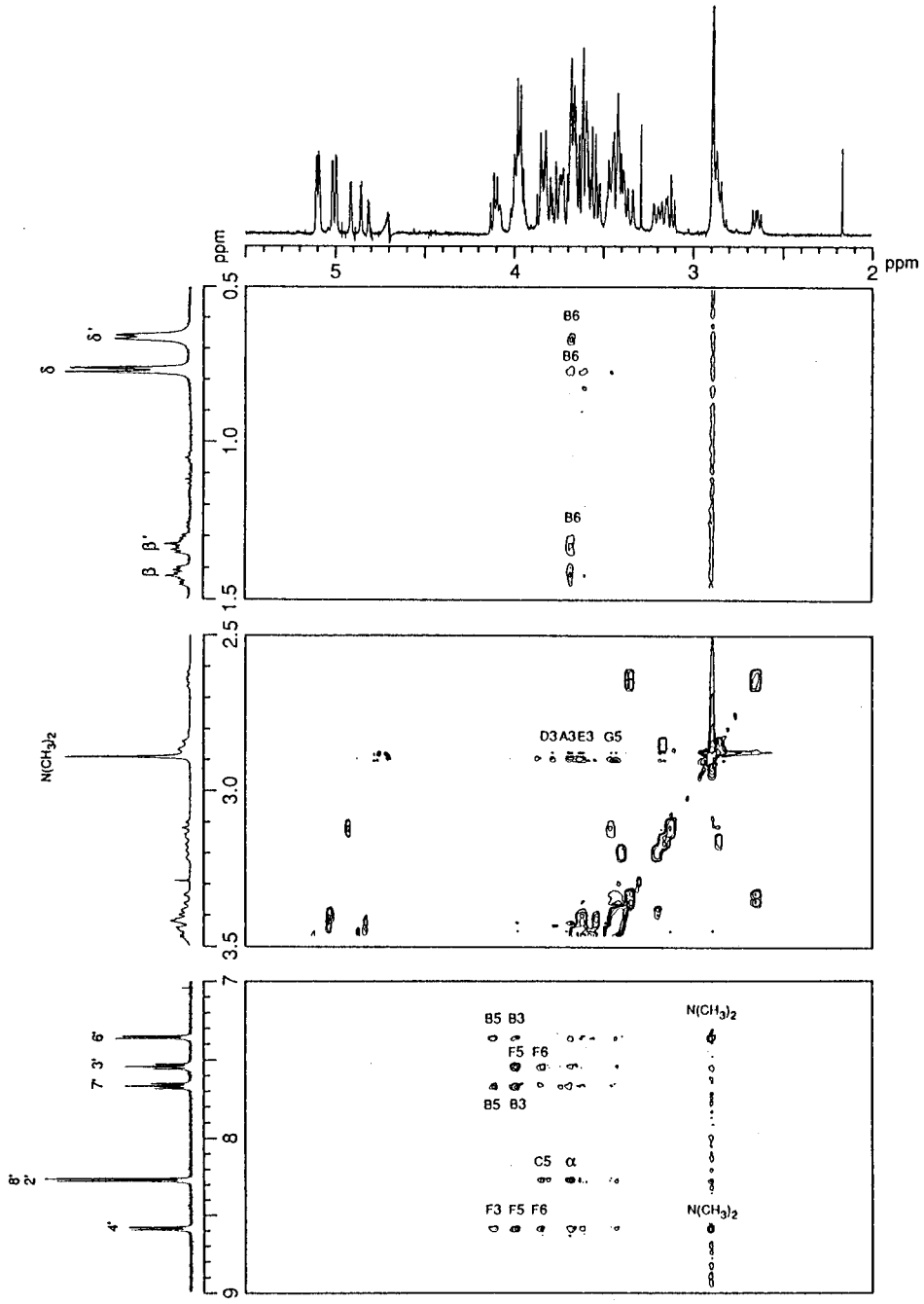

Figure 9. Portions of the 500 MHz ROESY spectrum of **1** in D_2O at 25 °C with mixing time of 300 ms, indicating the cross peaks between the protons of the dansyl-L-leucine moiety and the protons of the β-CD moiety.

effects of the dansyl moiety than those of **2**. The difference in the fluorescence intensity of **1** and **2** is also interpreted by the difference in the inclusion depth of the dansyl moiety, because the fluorescent intensity is sensitive to the microenvironment, and the fluorescence intensity is stronger in a hydrophobic environment than in a hydrophilic environment.[29−32]

The degree of the stability in the self-inclusion state can be compared between **1** and **2** by the use of the fluorescence lifetime data, because the equilibrium constant between the species **A** and the species **B** in Figure 1 can be roughly estimated from the A_2/A_1 ratio. The equilibrium constants for **1** and **2** were calculated to be 2.0 and 3.4, respectively. Therefore, the self-inclusion state of **2** is 1.7-fold more stable than that of **1**. The difference in the stability in the self-inclusion state produces the difference in the binding ability, because the guest binding is the competitive action with the dansyl moiety binding. In fact, the difference in the stability in the self-inclusion complex between **1** and **2** (i.e. 1.7-fold) is almost consistent with the difference in the binding ability (i.e. about 2-fold or more).

The difference in the self-inclusion depth is attributed only to the difference in the enantiomeric configuration of the leucine residue. The CPK model experiment suggested that the self-inclusion complex formation of **1** required larger angle bending in the spacer part than

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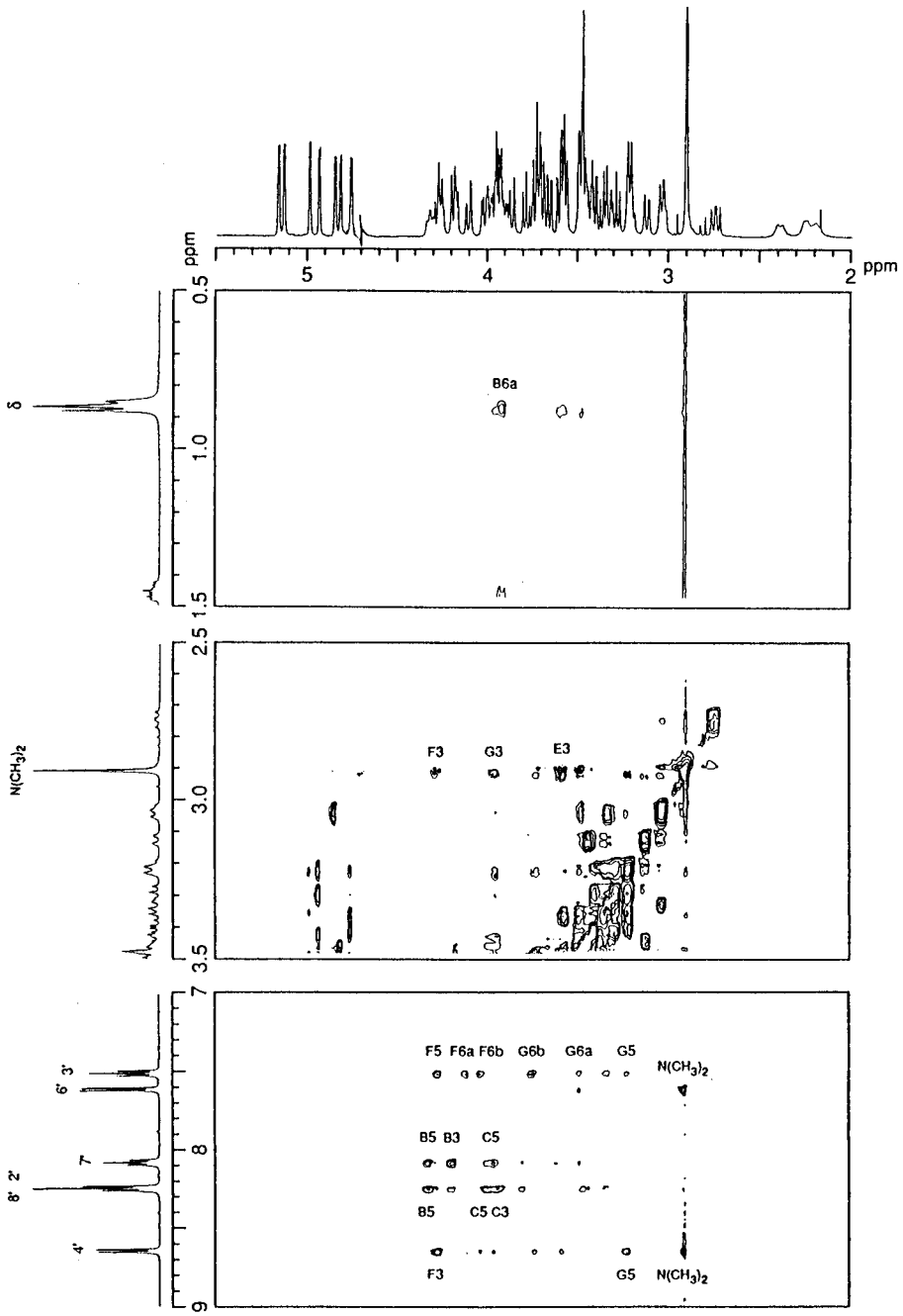

Figure 10. Portions of the 500 MHz ROESY spectrum of **2** in D_2O at 25 °C with mixing time of 300 ms, indicating the cross peaks between the protons of the dansyl-D-leucine moiety and the protons of the *β*-CD moiety.

that of **2**. This energy defect for the angle bending is an important factor for the determination of the self-inclusion depth, and the deeper inclusion of the dansyl moiety into its own cavity results in the stronger van der Waals interaction and the more stable self-inclusion complex. Hydroxyl groups of CD moiety would form hydrogen bonding with the dimethylamino group or the sulfonamide group of the dansyl moiety, and this hydrogen bonding is another important factor for the stabilization of the self-inclusion complex. More detailed structural studies are needed for discussion about the stabilization factors and are now under way.

Conformational Change upon Addition of 1-Adamantanol. Upon addition of 1-adamantanol, the fluorescence intensities of **1** and **2** were dramatically decreased. 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 (Figure 1). These conformational changes are also supported from the changes in their ^1H NMR spectra as shown in Figures 12, 13, and 14. The ^1H resonances of the dansyl moieties of **1** and **2** in the presence of 1-adamantanol are different from those of the hosts alone, but are similar to those of **3** or **4**. The resonances of the *δ* protons of the leucine moieties of **1** and **2** were also changed in a similar way. These changes suggest that the dansyl moiety is excluded from the cavity upon addition of the guest molecule. The dansyl moiety excluded from the cavity 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 13).

The resonances of the CD protons were also changed upon addition of 1-adamantanol. The upfield shifted

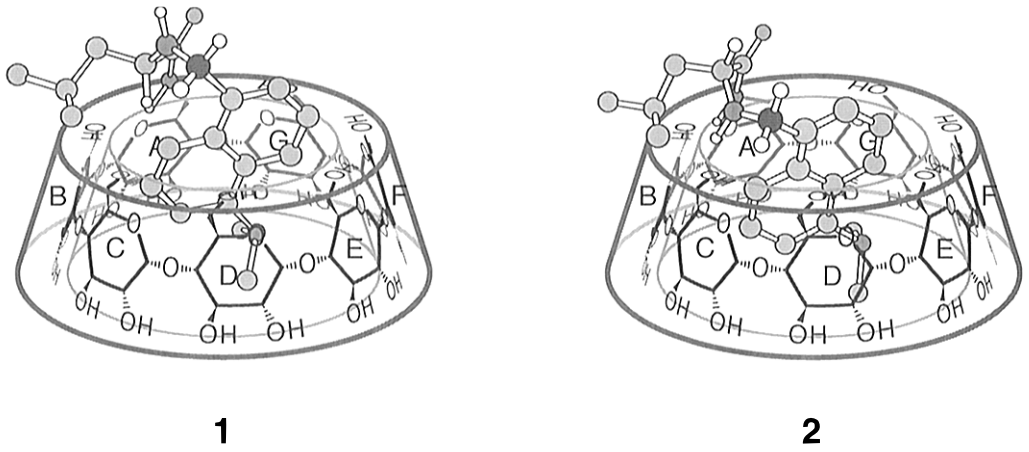

Figure 11. The estimated structures of **1** and **2**. The energy was not minimized.

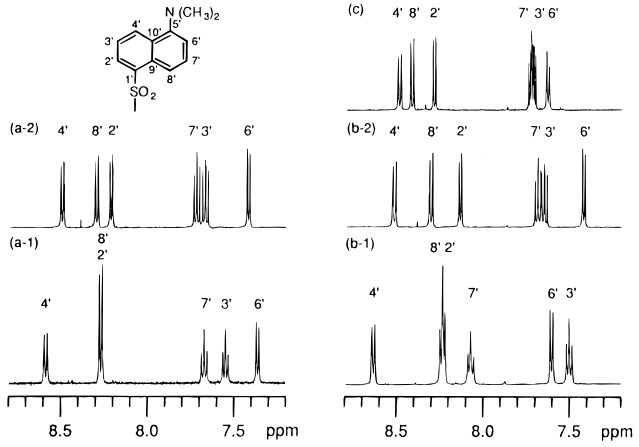

Figure 12. The aromatic regions of the 500 MHz ^{1}H NMR spectra of **1** (a-1) alone, (a-2) in the presence of 1-adamantanol, **2** (b-1) alone, (b-2) in the presence of 1-adamantanol, and (c) **3** in D_2O at 25 °C.

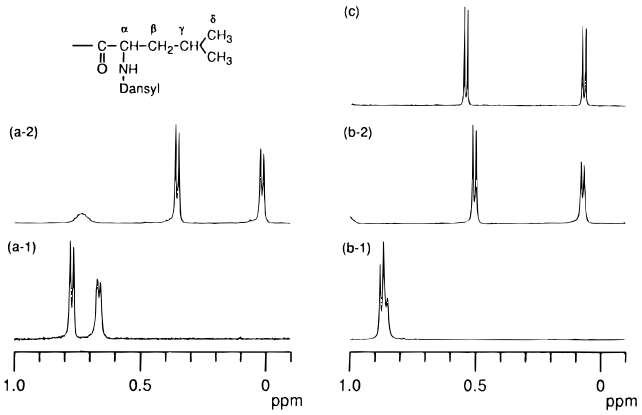

Figure 13. Portions of the 500 MHz ^{1}H NMR spectra of **1** (a-1) alone, (a-2) in the presence of 1-adamantanol, **2** (b-1) alone, (b-2) in the presence of 1-adamantanol, and (c) **3** in D_2O at 25 °C, showing the region of δ protons of leucine.

peaks due to the ring current effect of the dansyl moiety were not observed in the spectra. The resonances for the anomeric protons of **1** and **2** were also changed upon addition of 1-adamantanol. The resonances for the anomeric protons of **1** and **2** in the absence of the guest are dispersed in the range from 4.7 to 5.2 ppm, and all of them are completely separated as shown in Figure 14. Upon addition of the globular guest, 1-adamantanol, these spectral dispersions were collapsed, and the resonances were almost overlapped around 5.0 ppm (Figure 14). This change also indicates the exclusion of the dansyl moiety. In general, the resonances of the anomeric protons are dispersed, if self-inclusion of a pendant group occurs.[15−17] Even if the pendant group is not included in its own cavity but is fixed at a position near the entrance of the cavity, the resonances are dispersed to some extent. These spectral dispersions of the anomeric protons represent the reduction of the sevenfold symmetry of the modified β-CD. However, if the globular guest is included and the pendant group is moved away from the cavity, this spectral dispersion is lost.[15−17] The spectral changes in the CD protons of **1** and **2** also suggest the exclusion of the dansyl moieties from the cavities and the reduction of the distortion of the CD macrocyclic rings.

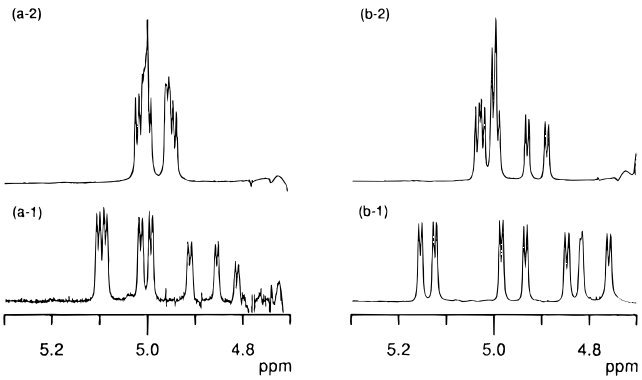

Figure 14. Portions of the 500 MHz ^{1}H NMR spectra of **1** (a-1) alone, (a-2) in the presence of 1-adamantanol, **2** (b-1) alone, and (b-2) in the presence of 1-adamantanol in D_2O at 25 °C, showing the region of the anomeric protons of CD.

In conclusion, the structures of *N*-dansyl-L-leucine-appended β-CD (**1**) and *N*-dansyl-D-leucine-appended β-CD (**2**) were estimated by the combined use of 1D and 2D NMR techniques. The dansyl moiety of **2** was included in its own cavity more deeply than that of **1**. Only the difference in the enantiomeric configuration in the spacer unit caused the difference in the inclusion depth, and it is due to the difference in the angle bending to make the self-inclusion complex. The difference in their properties was interpreted by the difference in the inclusion depth of the dansyl moieties. The exclusion of the dansyl moiety upon addition of the guest was also monitored by NMR.

JO960425X