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Original Article

Diastereomeric Difference of the Self-Inclusion Complex of *N*(*N*′-Formyl-Phenylalanyl)-Deoxyamino-*β*-Cyclodextrin Caused by the Interaction between the Arm and the Rim of the Cavity

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Abstract

A complete assignment of proton resonances for *N*(*N*′-formyl D-phenylalanyl)-deoxyamino-*β*-cyclodextrin (**1**$_D$) was performed by means of 1D and 2D NMR,^{1}H-^{1}H-COSY, ^{1}H-^{13}C-COSY, TOCSYand NOESY spectroscopy. Based on 2D-NMR ROESY and circular dichroism spectroscopy, the conformation of **1**$_D$ was determined; the phenyl group stays inside the distorted cyclodextrin (CyD) cavity forming a self-inclusion complex, which is almost the same as *N*(*N*′-formyl L-phenylalanyl)-deoxyamino-*β*-CyD (**1**$_L$). The remarkable diastereomeric difference was observed in the chemical shifts of H(5) and H(6) protons at the narrow rim of the CyD cavity and induced circular dichroism spectra. These results suggest the existence of hydrogen bonds between the hydroxyl group on CyD and the amide groups on the arms, which provides the difference in the molecular recognition properties.

Introduction

Some modified cyclodextrin (CyD)s with aromatic groups as self-guests form an intramolecular complex [1, 2]. The extent of the intramolecular complexation is affected by factors such as the nature of the link between the CyD and the guest moiety [3, 4], the chirality of the guest moiety [5], the temperature [6] and the extent of the protonation of the guest moiety. In the presence of another guest which fits the cavity, however, the conformation of the intramolecular complex changes or intermolecular complexation may take place. On the other hand, it has been known that the advantage functions of CyD could be observed even if the guest molecule stays out of the cavity. For example, enzyme-mimic hydrolysis of nitrophenyl ester [7] and exiplex emission in an aqueous solution from naphthyl moiety to amine moiety near the rim of CyD cavity [5d]. Thus, the intramolecular complexation of modified CyDs would have great potential for detecting and recognizing natural compounds as a nano-order sensing system [8] and to solve the sufficiently significant roles of 'rims' of CyD as nano interface model. In this regard, to discuss the diastereomeric differences of self-inclusion type modified CyDs in conformations at nano-level, a NMRstudy of *N*(*N*′-formyl-phenylalanyl)-deoxyamino-*β*-cyclodextrin(**1**$_D$: Figure 1) is carried out. This paper reports the strategy of proton resonances assignment and the diastereomeric difference in the modified group for conformations in aqueous solutions.

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Experimental

Synthesis

Compounds **1**$_D$ and **1**$_L$ were prepared from 6-monoamino-6-deoxy-*β*-CyD [9] and (D)- or (L)-formylphenylalanine (f-Phe; WAKO Co.) with DCC in DMF. The details have been reported previously [10].

Measurements

The circular dichroism (CD) spectra were measured on a Jasco J-820 spectropolarimeter. The NMR spectra were measured on the JEOL Lambda-500 (500 MHz) and Delta-600 (600 MHz) spectrometers using acetone as an internal reference (2.10 ppm) at 25 °C.

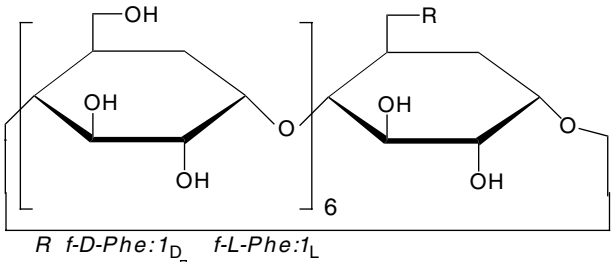

Figure 1. Structure of $\mathbf{1}_D$ and $\mathbf{1}_L$.

Results and discussion

Assignments of ^{1}H resonances of N(N′-formyl D-phenylalanyl)-deoxyamino-β-cyclodextrin(1_D)

The ^{1}H NMR spectrum of $\mathbf{1}_D$ are shown in Figure 2. $\mathbf{1}_D$ exhibits severely overlapped ^{1}H spectrum. Generally, in the case of sugars, the anomeric protones exhibit characteristic lowfield shifts and, therefore, the assignment can be started from the anomeric protons. Although anomeric protons of native β-CyD exhibit only one degenerated resonance, those of $\mathbf{1}_D$ exhibit seven separate resonances. By using ^{1}H-^{1}H-COSY, only H(2), H(3) and a part of H(4) resonances of $\mathbf{1}_D$ can be assigned. The assignments of H(5) and H(6) resonances are very important to determine the orientation of the formyl D-phenylalanyl group. The ^{1}H-^{13}C-COSY method is very useful because C(6) resonance is exactly assigned around 60 ppm. As shown in Figure 3, seven sets of crosspeaks between C(6)—H(6), C(1)—H(1) and C(4)-H(4) can be observed clearly. The observation of a crosspeak on ^{1}H-^{1}H-COSY with H(6) or H(4) makes it possible to assign H(5) resonances. By using TOCSY methods, all the resonances can be fully assigned.

Determination of the sequence of glucopyranose units (A–G)

In the CyDs, the distance between an H(1) of one glucopyranose unit and an H(4) of its neighbouring unit across the α-(1 → 4)glucosidic linkage is close enough to give rise to through-space NOE enhancements which allows the sequence-specific assignments of resonances of glucopyranose units along the CyD macrocycle. Figure 4 shows a part of the H(1) NOESY spectrum of $\mathbf{1}_D$, indicating the NOE connectivities between H(1) and H(4) resonances. A spin network of glucopyranose unit of which H(4) resonance has a crosspeak with H(1) resonance of the A unit, in which phenylalanyl moiety is introduced, is identified as that of the B unit, and in a similar way all spin networks of glucopyranose units up to the G unit are successively determined. The validity of sequential assignments was confirmed, as the H(1) resonance of the G unit has an NOE crosspeak with the H(4) resonance of the A unit. Proton chemical shifts of each glucopyranose group of $\mathbf{1}_D$ with respect to those of $\mathbf{1}_L$ are indicated in Figure 5.

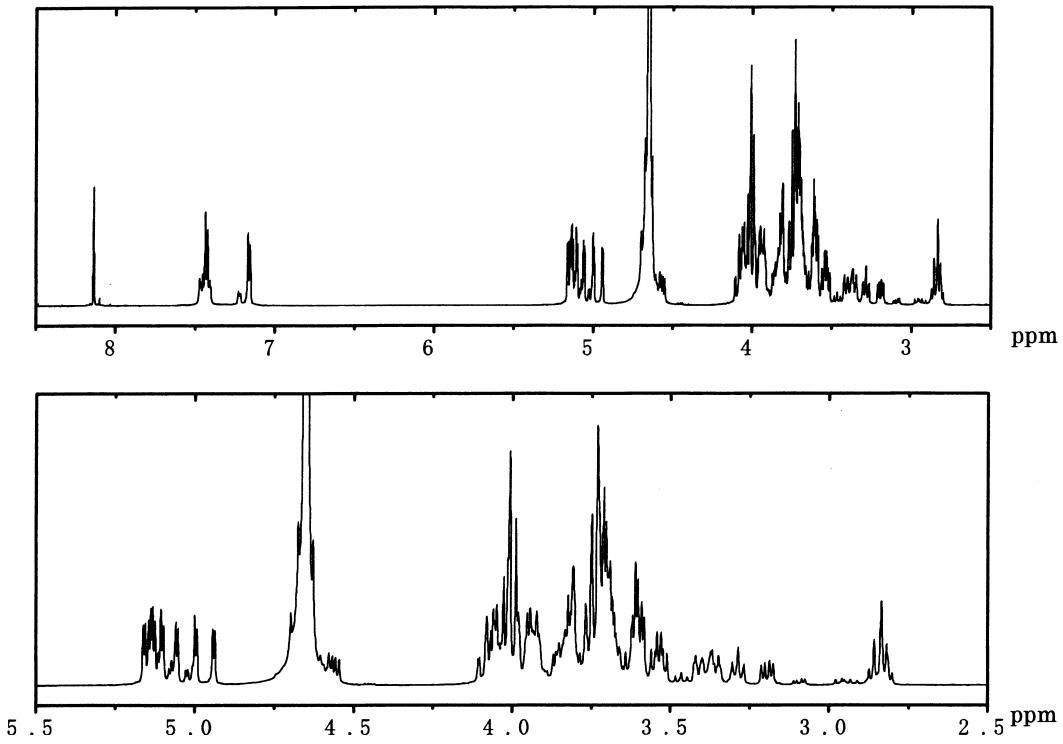

Figure 2. 500 MHz ^{1}H-NMR spectra of $\mathbf{1}_D$ covering phenyl alanyl group and CyD group (upper) and covering CyD ring (bottom) in D_2O at room temperature.

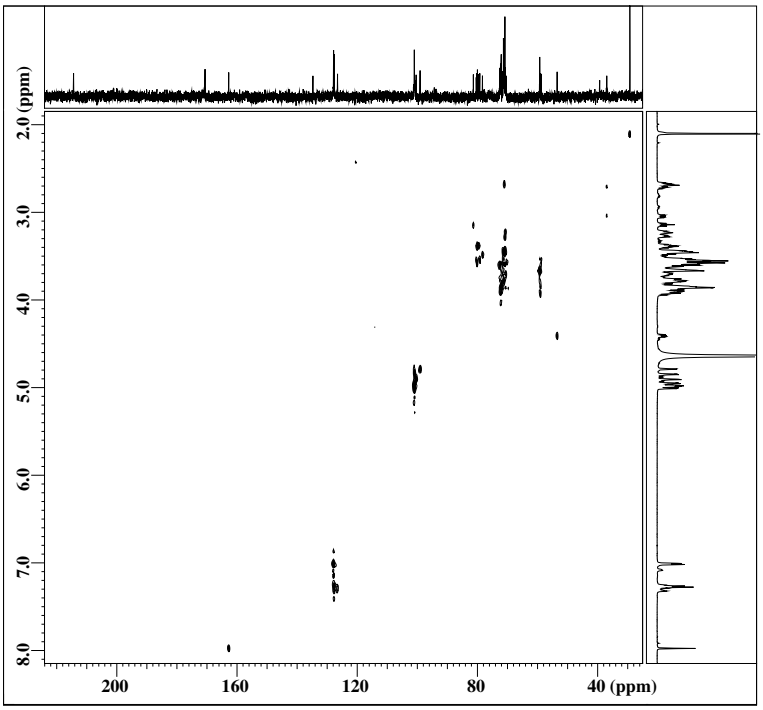

Figure 3. ^{1}H–^{13}C-COSY spectrum of $\mathbf{1}_D$.

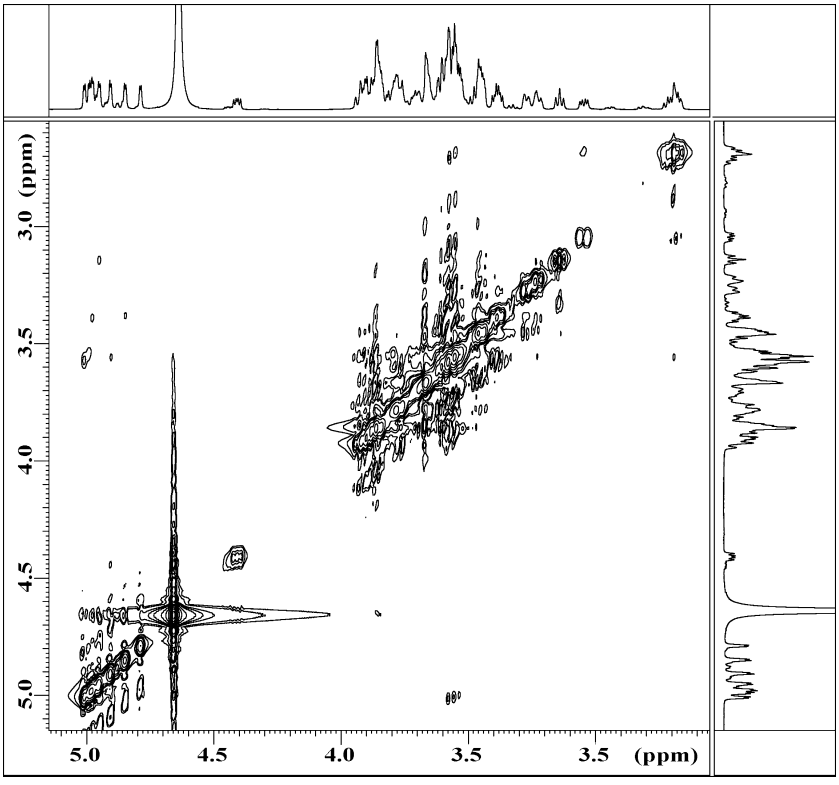

Figure 4. A part of 2D NOESY NMR spectrum of $\mathbf{1}_D$ in D_2O.

Determination of the orientation of the formyl D-Phe group against the macrocyclic ring by ROESY

Direct evidence for the conformation of $\mathbf{1}_D$ was obtained by another 2D NOE measurement, ROESY (Figure 6). The ROESY map indicated that *p*- and/or *m*-protons of the phenyl group have NOE crosspeaks with H(3) protons of B, C, D and G unit, with H(5) of B, C and D unit and with α protons, whereas *o*- of phenyl protons have NOE crosspeaks with H(3) protons of C and D

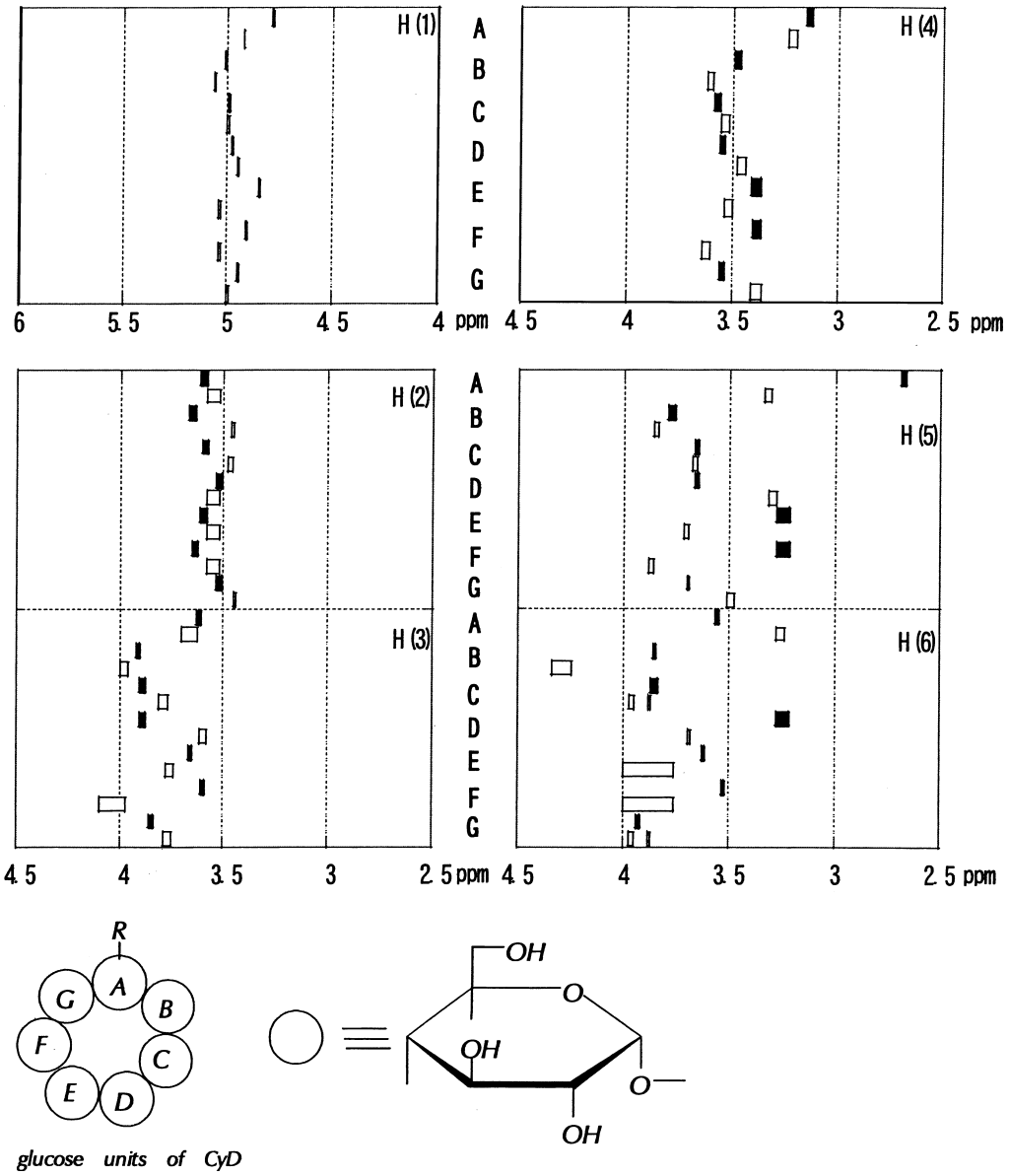

Figure 5. Proton chemical shifts of each gluco pyranose unit of A–G of $\mathbf{1}_D$(■) with respect to those of $\mathbf{1}_L$ (□). H(6) resonances of E and F unit have not be reported.

unit, with H(5) of B, C, D, E, F and G unit and with α and β protons of Phe group. Any crosspeaks cannot be observed with H(6) protons, which is different from the case of $\mathbf{1}_L$ reported previously [2(a)]. The C(1) and C(4) carbon atoms in oligosaccharides can be directly correlated with one of the torsion angles (φ°); a change in the observed ^{13}C chemical shift of ca. 2 ppm corresponds to an average change in φ angles of ca. 10° [11] (Table 1). Considering these experimental facts, we can conclude that the phenyl group stays in a distorted hydrophobic environment by penetrating into the CyD cavity from the primary-hydroxyl group side of its macrocyclic ring.

Circular dichroism spectra

Whenever a chromophore is not optically active, induced circular dichroism (ICD) is observed in a chiral CyD cavity. The optically active D- and L-Phe show negative and positive ellipticities peaked around 250 nm owing to aromatic ring, respectively. Since compound **1** has two amide groups, the strongest ellipticity is observed at 220 nm in a neutral aqueous solution (Figure 7). The CD spectral patterns and maximum intensities of $\mathbf{1}_D$ and $\mathbf{1}_L$ are different from each other. When 1-adamantanol (Ad) is added to the solution of $\mathbf{1}_D$, positive ellipticity decreases and reaches strong negative ellipticity peaked at 220 nm. On the other hand, when Ad is added to the solution of $\mathbf{1}_L$, negative peaks change to positive ellipticity. It is reasonable that the Phe group is replaced by 1-adamantanol, staying outside the cavity. An approximate ICD by self-host CyD may thus be the differential spectra between the spectra with excess equivalent of guest and that without guest. ICD of $\mathbf{1}_D$ and $\mathbf{1}_L$ (dotted line) are indicated in

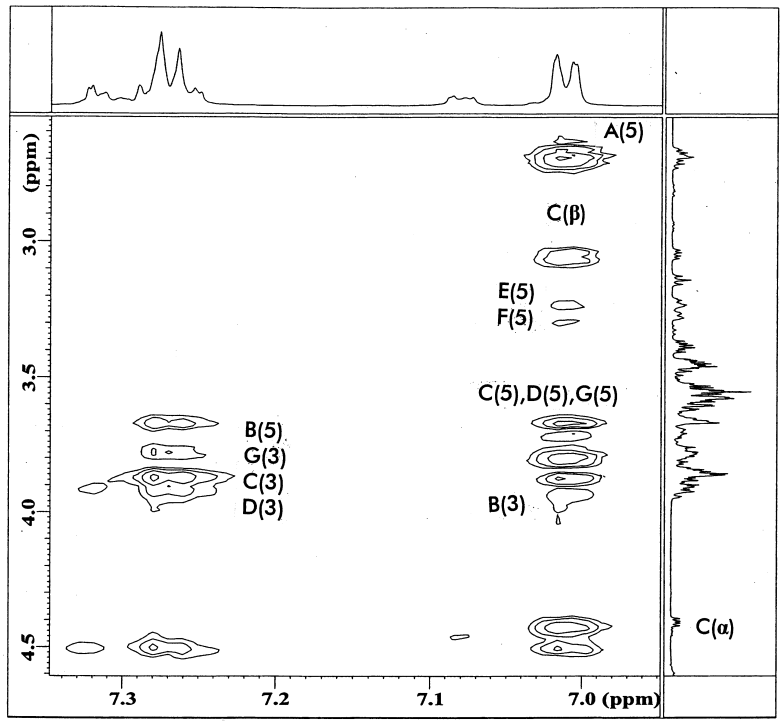

Figure 6. A part of ROESY spectrum of $\mathbf{1}_D$ covering the phenyl ring proton region in F1 axis. Assignments of crosspeaks are also shown. Resonances of H*p* and H*m* cannot be distinguished.

Table 1. Association constants of self-inclusion type modified CyD

Guest	Host (K/M^{-1})		
	$\mathbf{1}_D$	$\mathbf{1}_L$	β-CyD
ANS[a]	78 ± 11	68 ± 19	78 ± 8
TNS[a]	167 ± 27	207 ± 29	2:1 complex
1-Naphthol[a]	133000	171000	1:2 complex
Dansyl-D-Phe[b]	160 ± 36	139 ± 24	197 ± 20
Dansyl-L-Phe[b]	83 ± 28	231 ± 45	153 ± 14
Dansyl-D-Ala[b]	42 ± 13	113 ± 18	179 ± 13
Dansyl-L-Ala[b]	54 ± 10	95 ± 17	114 ± 13
f-D-Phe[b]	–[c]	–[c]	37 ± 1
f-L-Phe[b]	–[c]	–[c]	35 ± 1

[a] Taken from Ref. [4b]. Determined with fluorescence intensity at 504 nm excited around 350 nm, pH 7.0 solution (1/15 M phosphate buffer), 25 °C with 5.0×10^{-5} M of guest molecule. [ANS] = 2.0×10^{-5} M, [CyD] = 1.0×10^{-3} M, [guest] = $0–10^{-2}$ M.
[b] Taken from Ref. [5c].
[c] The value was too small to be determined.

Figure 8. ICD owing to phenyl ring indicated the dependency on the concentration of 1-adamantanol, which suggested that phenyl groups of $\mathbf{1}_D$ and $\mathbf{1}_L$ are included in CyD cavity. The CD spectra were too complicated to assign the structure. Applying the general rule [12], the amide group of $\mathbf{1}_D$ is located outside the CyD cavity staying parallel to the rim of the CyD cavity, while the amide group of $\mathbf{1}_L$ is included in the CyD cavity.

Differences on conformation between $\mathbf{1}_D$ and $\mathbf{1}_L$ in aqueous solution

Based on the results of the NMR and CD spectra, the conformational difference between $\mathbf{1}_D$ and $\mathbf{1}_L$ are suggested as below: the phenyl groups of $\mathbf{1}_D$ and $\mathbf{1}_L$ are included inside the distorted CyD cavity; the location of the phenyl ring of $\mathbf{1}_D$ is almost the same as that of $\mathbf{1}_L$. The remarkable diastereomeric difference was observed with the chemical shifts of H(5) and H(6) protons on the narrow rim of the CyD cavity and ICD spectra. This result suggests the possibility of hydrogen bonds between the hydroxyl group on CyD and the amide group on the arm. Moreover, NOESY and ROESY spectra show cross peaks due not only to cross relaxation but also to spin exchange, and can therefore be used also to extract dynamics of intramolecular exchange [13]. The two contributions can be distinguished in principle by rotating frame experiments, using NOESY with variable mixing times. The diastereomeric difference in the intramolecular complexes does not exist in the conformations of the Phe groups against the CyD cavity, but in dynamics.

We have already reported that the intramolecular complex of 'cap type mononaphthylethyl-amino-β-CyD' indicates enebtioselective exciplex formation even in an aqueous solution.2 In such a system, one naphthyl group of *R*-form is deeply and rigidly included in the distorted CyD cavity, while a naphthyl group of

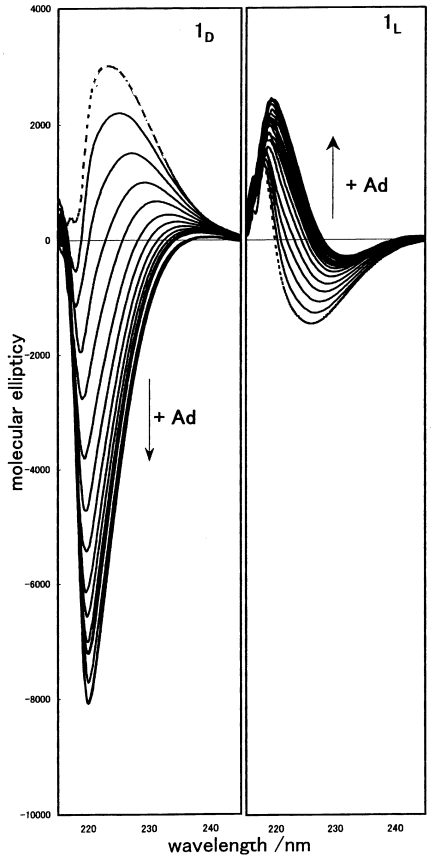

Figure 7. Circular dichroism spectra of of $\mathbf{1}_D$ (left) and $\mathbf{1}_L$ (light) in neutral aqueous solution with and without 1-adamantanol, covering the amide bond.

Table 2. Chemical shifts of anomeric and agriconic carbons

Assignment	δ/ppm			
	β-CyD	6-Amino-β-CyD	$\mathbf{1}_D$	$\mathbf{1}_L$
C1(Δδ)[a]	103.71	102.87(0.19)	99.10(1.90)	102.80(0.99)
		103.06	100.40	103.02
			100.80	103.62
			100.90	103.79
			101.00	
C4(Δδ)[a]	82.74	82.04(1.96)	78.30(3.10)	82.04(4.01)
		82.26(0.22)[b]	79.20(1.80)[b]	82.26(1.19)[b]
		84.00[c]	79.60	82.47
			80.00	82.72
			80.10	83.23
			81.40[c]	86.05[c]

[a] The variation of δ.
[b] The variation of δ except the δ of C(4) carbon in substituted glucopyranose unit.
[c] The δ of C(4) carbon in substituted glucopyranose unit.

S-form is located outside the CyD cavity staying parallel to the rim of the CyD cavity. The latter naphthyl group enhanced the hydrophobicity around the CyD rim. This system is the example that only one chirality of

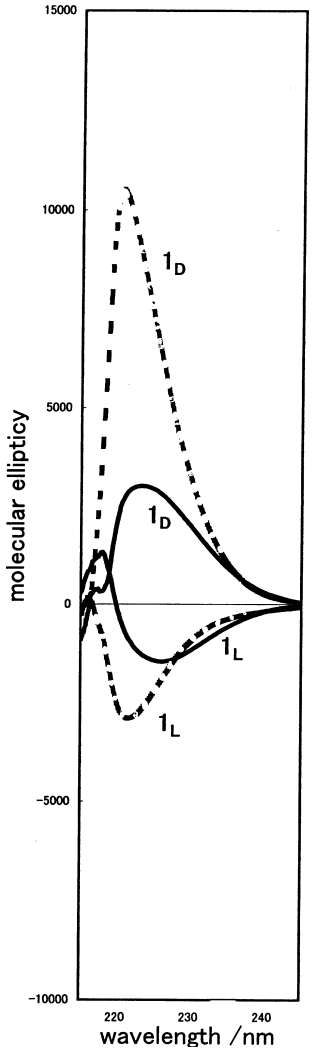

Figure 8. Calculated induced circular dichroism spectra of $\mathbf{1}_D$ and $\mathbf{1}_L$.

the modified group changes the total macromolecular conformation, being reflected in the output fluorescence signal difference. In the present system, one chirality of the modified group changes the interaction at nano interface and dynamics and which provides the difference in the molecular recognition abilities (Table 2).

Conclusion

Complete assignment of both proton and carbon resonances for $\mathbf{1}_D$ with NMR has been successful. The phenyl group of $\mathbf{1}_D$ is also included in self-cavity from the narrow site staying in the CyD cavity. The structure of $\mathbf{1}_L$ is almost the same as that of $\mathbf{1}_D$. The conformational difference exists in the site of the 'arm'. The location site of two amide groups of $\mathbf{1}_D$ is different from that of $\mathbf{1}_L$. This phenomenon can be explained by hydrogen bonding between hydroxyl group on CyD and the formyl group or the amide group on the arm.

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