Host−Guest Complexation Affected by pH and Length of Spacer for Hydroxyazobenzene-Modified Cyclodextrins

Tetsuo Kuwabara,*,† Kazuyo Shiba,† Hiroki Nakajima,† Mayumi Ozawa,† Naoya Miyajima,† Masakazu Hosoda,‡ Noriyuki Kuramoto,‡ and Yasutada Suzuki†

Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, 4-3-11 Takeda, Kofu 400-8511 Japan, and Graduate Program of Human Sensing and Functional Sensor Engineering, Graduate School of Engineering, Yamagata University, 4-3-16, Yonezawa 992-8510, Japan

Received: June 21, 2006; In Final Form: September 26, 2006

Three modified β-cyclodextrins appended with a hydroxyazobenzene as a dye unit, **1**, **2**, and **3**, each incorporating a different length spacer between the β-CD and the dye unit with a bis(propyl(oxyethylene)), butylene, and amide bond spacer, respectively, were synthesized in order to investigate their spectroscopic changes induced by pH and host−guest complexation as well as to investigate their conformations and guest-binding properties by means of absorption and induced circular dichroism spectroscopies in aqueous solutions. All hosts accommodated the dye unit in their own CD cavities with an orientation parallel to the CD axis, forming intramolecular complexes. When the pH of the solution changed, the structure changed in response to pH without conformational changes. Existing as the phenol form under acidic condition, they were converted to the yellow phenolate form by dissociation of a proton of the hydroxyl group in the dye unit with increasing pH (pK_{a1}; 7.62 for **1**, 7.44 for **2**, 8.00 for **3**). Further increase in pH led to the dissociation of the ammonium proton in the secondary amine group in the spacer of **1** and **2** (pK_{a2}; 8.76 for **1**, 8.67 for **2**). Upon addition of 1-adamantanol (AN) as a guest, all hosts accommodated AN in their CD cavities, forming 1:1 host−guest inclusion complexes. The complexation phenomena were accompanied with changes in the conformation of the hosts, in which the dye units of **1** and **2** are excluded to outside of the cavity, but not for **3**. The dye unit of **3** remained in the cavity, where the guest was also included partly. Therefore, the guest-binding abilities of **1** and **2** were larger than that of **3**, which has poor binding ability. The binding constants of **1**, **2**, and **3** for AN are estimated to be 7400, 1940, and 140 M^{-1} at pH 3.2, respectively. However, the guest-binding abilities of **1** and **2** were dependent on the pH of the solution. The ability of **1** under weak alkaline condition was stronger than under acidic or alkaline conditions, while that of **2** increased with increasing pH. Under the condition from neutral to weak alkaline media, **1** and **2** demonstrated color changes from colorless to yellow upon formation of inclusion complexes. When 1-adamantanecarboxylic acid (AC) was used as the charged guest, **1** and **2** bound to AC with a larger binding constant than AN. On the other hand, **1** and **2** bound to 1-adamantineamine (AA) with a smaller binding constant than AN. All these results demonstrate that the complexation phenomena depend on the pH of the solution as well as the length of the spacer of the hosts and that the electrostatic interaction between the host and the guest is also important for forming a stable complex.

Introduction

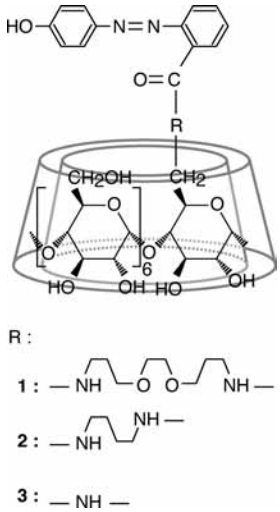
All biological structures and processes such as specific recognition of a substrate by an enzyme, folding of protein into an intricate three-dimensional form, and precise replication of DNA depend on the interplay of noncovalent interactions as well as covalent bonds. The investigation of molecular-recognizing supramolecular systems offers a powerful tool for the detailed rationalization of these processes.[1] Although small synthetic organic hosts bear little resemblance to a natural receptor, they are more advantageous not only in manageable degrees of structural complexity but also in making it possible to prepare and design the hosts so as to make them sophisticated with different functional group orientation, various degrees of flexibility, and appropriate electronic properties. Natural and modified cyclodextrins (CDs) have received much attention over recent years as aqueous-based hosts for studying the recognition of organic compounds.[2]

Our interest has been focused on molecular recognition of CD derivatives that possess a chromophore, with which guest species can be detected by spectroscopic changes.[3] The hosts usually exist as intramolecular complexes in which the appended chromophore unit is included in their own hydrophobic CD cavities. The intramolecular complexes are converted to intermolecular host−guest complexes upon the addition of guest species, changing the location of the chromophore unit from inside to outside of the cavities. Previously, we have shown that the guest-induced conformational change of the host results in remarkable changes in visible absorption, and the hosts have been used as sensory systems.[4] In particular, β-CD derivatives having a dimethylaminoazobenzene unit have been shown to be very unique as such a sensor system.[5] The positional difference in a substituent in the appending dye part in the hosts

* Corresponding author. Phone and Fax: +81-55-220-8548. E-mail: kuwabara@ab11.yamanashi.ac.jp.
† University of Yamanashi.
‡ Yamagata University.

10.1021/jp063892j CCC: $33.50
Published on Web 11/23/2006

results in the unique molecular recognition ability, although they are limited to acidic media. Furthermore, the hosts are limited in the structure with the short and rigid spacer between the CD and the chromophore units. When hydroxyazobenzene is connected to CD by a spacer with various lengths as the dye unit, the modified CD can be expected to show guest-induced color change under neutral conditions and unique molecular recognition behavior. In the present study, we have prepared three types of hydroxyazobenzene-modified β-CDs, which have a spacer with different lengths between the dye and CD units, in order to get insight into the mechanism of color change upon guest binding as well as to elucidate the role of the flexible spacer and the modified unit in guest binding. The guest-binding and inclusion phenomenon of the host will be shown to be greatly affected by the flexibility of the hosts and pH value of the solution.



Results and Discussion

pH Dependence in the Absorption Spectra of 1, 2, and 3. Figure 1 shows the absorption and the induced circular dichroism spectra of **1** under various pH conditions together with the second-derivative curves of the absorption spectra at pH 3.67 and 11.94. Compound **1** is colorless with a strong absorption band around 350 nm at pH 4.26. With increasing pH, it exhibited a yellow color with a decrease and increase on the absorption intensities around 350 and 440 nm, respectively. This indicates the pH-induced change in the structure of the dye unit in **1** from the phenol form to a phenolate one. Compound **2**, which has a butylene spacer between the CD and the dye units, underwent similar pH-induced absorption variation to that of **1** (Figure 2a), indicating a similar change in the dye structure. The absorption variations of **1** and **2** were accompanied with three isosbestic points at about 383, 293, and 260 nm. However, we found that each isosbestic point was composed of two adjacent isosbestic points. As shown in the inset of Figure 1a, the isosbestic point at longer wavelength for **1** was observed at 381 in the acidic region and at 384 nm in the alkaline region on the boundary at pH 8.2. The isosbestic point observed at 293 nm was also composed of 292 and 294 nm below and above pH 8.2, respectively, while the two adjacent isosbestic points could not be found clearly for the isosbestic point at the shorter wavelength for **1**. In the case of **2**, two sets of two adjacent isosbestic points were also observed at 381 and 383 nm and at 291 and 295 nm on the boundary at pH 7.9. The appearance of two adjacent isosbestic points for **1** and **2** demonstrates the existence of two acid dissociation equilibria

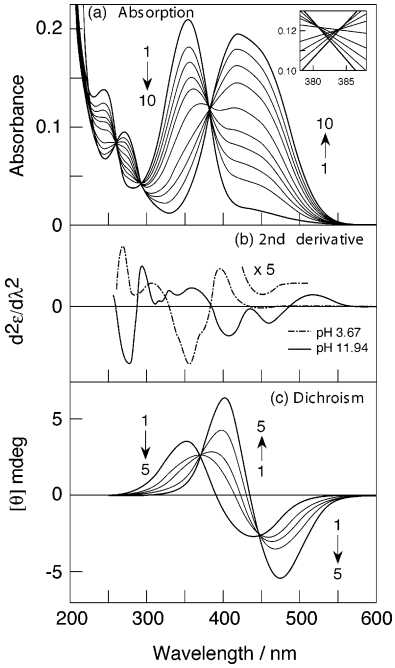


Figure 1. Absorption and the induced circular dichroism spectra of **1** at different pH values and the second-derivative curves of the absorption spectra at pH 3.67 and 11.94: (a) the pH of the solution is (1) 4.26, (2) 7.10, (3) 7.38, (4) 7.58, (5) 7.78, (6) 8.05, (7) 8.46, (8) 8.76, (9) 9.13, and (10) 11.94; (c) the pH of the solution is (1) 3.67, (2) 7.61, (3) 8.02, (4) 8.70, and (5) 11.94.

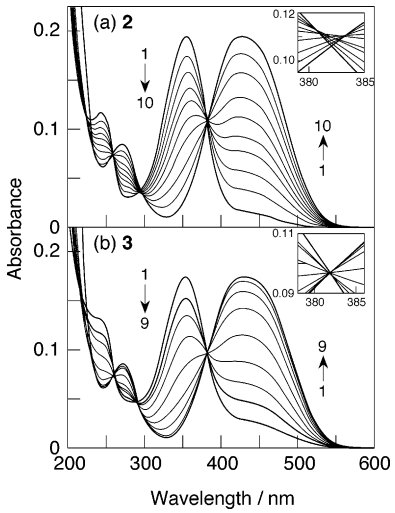


Figure 2. Absorption spectra of **2** and **3** at different pH values: (a) the pH of the solution is (1) 5.61, (2) 7.10, (3) 7.52, (4) 7.83, (5) 8.06, (6) 8.37, (7) 8.65, (8) 8.98, (9) 9.36, and (10) 11.90; (b) the pH of the solution is (1) 3.86, (2) 6.65, (3) 7.25, (4) 7.56, (5) 7.85, (6) 8.26, (7) 8.65, (8) 9.00, (9) 9.51, and (10) 12.01.

in their systems; one should be attributed to the equilibrium between the phenol form and the phenolate one in the dye unit, and the other may be attributed to the equilibrium between the amine form and the ammonium one in the secondary amine group near the CD cavity in the spacer unit.

The plots of the absorption intensities at 440 nm for **1** and **2** as a function of pH are shown in Figure 3. The curve-fitting analysis of the pH titration curves using eq 1 gave the apparent pK_a with the values of 7.62 and 8.76 for **1** and 7.44 and 8.67 for **2**, respectively (Table 1). Good fitting of the theoretical curves with the data obtained for **1** and **2** suggests the existence of two acid dissociation equilibria, which is well coincident with the observation of the two adjacent isosbestic points stated

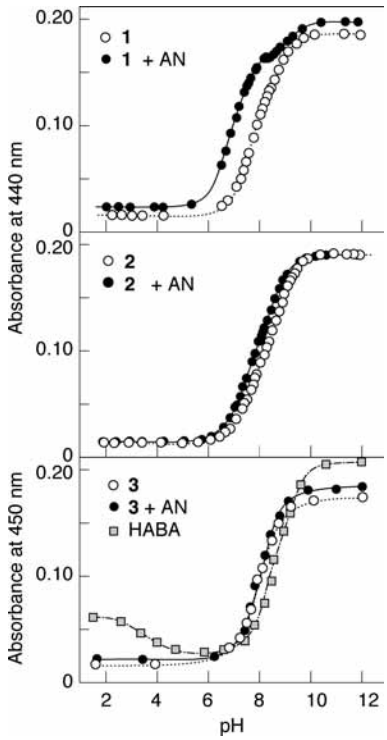


Figure 3. Plot of absorption intensity at 440 nm for **1** and **2**, and at 450 nm for **3**, as a function of pH in the absence (white circles) and presence (black circles) of 1-adamanatanol. The 1-adamantanol concentration is 5.1, 6.0, and 3 mM for **1**, **2**, and **3**.

TABLE 1: pK_a Values of the Acid Dissociation Equilibria of 1, 2, 3, and HABA

host			pK_{a1}	pK_{a2}
1	10^{-5} M		7.62	8.76
		+AN	6.90	9.10
2	10^{-5} M		7.44	8.67
		+AN	7.39	8.56
3	10^{-5} M		8.00	
		+AN	8.00	
HABA	10^{-5} M		8.64	

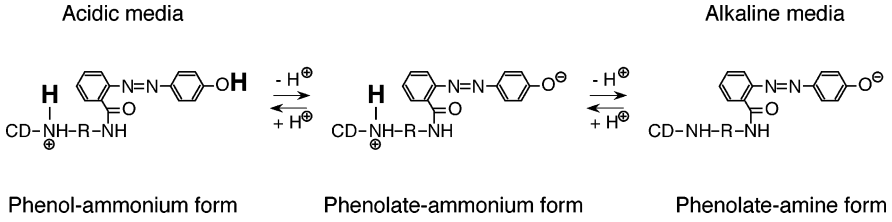
previously. Since the pK_a values of hydroxyazobenzene carboxylic acid (HABA) itself and diethylamine are 8.64 and 11.1,[6] respectively, the apparent pK_{a1} and pK_{a2} values estimated for **1** and **2** are associated with the phenol−phenolate equilibrium and the ammonium−amine one, respectively. The observation in the large absorption variations of pH titration curves in the acidic side as compared to those in the alkaline side for **1** and **2** is also one of the reasons for such an assignment (Figure 3). Therefore, **1** and **2** existing as the phenol−ammonium form under the acidic condition are converted to the phenolate−ammonium one, which is a zwitterionic structure, with increasing pH. Further increase in pH causes the dissociation of a proton on the secondary amine in the spacer, and **1** and **2** exist as the phenolate−amine form under alkaline conditions (Scheme 1). For **1** and **2**, the estimated shifts of the apparent pK_a values shifts to the acidic side as compared to those of HABA and diethylamine, especially pK_{a2}, are resulted not only from the difference in the structure but also from the hydrophobic environment around the dye unit and the secondary amine group of **1** and **2**. Therefore, it can be considered that the dye units for **1** and **2** are included in the hydrophobic CD cavities. Yoshida and co-workers also observed the equilibrium between the amine and ammonium forms of the secondary amine group connected at the C6 position of the glucose unit in the CD in the pH region similar to those of **1** and **2**, although the precise pK_a value was not obtained.[7] The difference in the apparent pK_a values as well as the titration curves between **1** and **2** is not so large but reflects the length of the spacer. Since no concentration dependency of the spectral shapes as well as their molecular extinction coefficient for **1** and **2** was observed in the range from 10^{-6} to 10^{-4} M, the inclusion of the dye unit into the CD cavity occurs not intermolecularly but intramolecularly.

Compound **3**, whose dye unit was connected to the CD unit by an amide bond, also displayed the color change from colorless to yellow when the solution became alkaline (Figure 2b). The absorption variation of **3** was basically similar to those of **1** and **2**. The isosbestic points were observed at 382, 292, and 261 nm, indicating that **3** involves only the phenol−phenolate equilibrium, which is different from that of **1** and **2**. The analysis of the pH titration curve of **3** gave the apparent pK_a with the value of 8.00 (Table 1). The shift in apparent pK_a toward the alkaline side as compared with that of **1** and **2** suggests the more hydrophilic environment around the hydroxyl group in the dye unit of **3** as compared to that of **1** and **2**. This is owing to the rigid structure of **3**. Previously, we have reported that methyl red-modified β-CD (**4**), which is different from **3** only in one substituent with a dimethylamino group in place of the hydroxyl group of **3** in the dye unit, accommodates its dye unit axially and that the dimethylamino group projecting from the secondary hydroxyl group side of its CD is exposed to bulk water.[8] So, it can be considered that **3** also includes the dye unit in its CD cavity axially, in which the hydroxyl group in the dye unit projects from the secondary hydroxyl group side of its CD. Consequently, the hydroxyl group of the dye unit in **3** is exposed to bulk water solution.

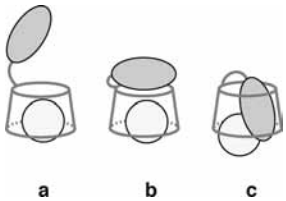
In the presence of 1-adamantanol (AN) as a guest, **1**, **2**, and **3** revealed similar pH-induced absorption variations to those in the absence of the guest. The absorption variations of **1** and **2** were accompanied with two isosbestic points at 378 and 383 nm for **1** and at 379 and 381 nm for **2**, respectively, indicating that **1** and **2** change structure from the phenol−ammonium form to the phenolate−amine one through the phenolate−ammonium one with increasing pH, which are similar changes in structure to those in the absence of AN. It was found, however, that the isosbestic points observed for **1** and **2** in the presence of AN were shifted to the blue side by 1−3 nm as compared to those in the absence of the guest. Furthermore, the pH titration curves of **1** and **2**, especially for **1**, were shifted to the acidic side by the presence of AN (Figure 3). Such guest-induced shifts in the pH titration curves are resulted from the shifts in their apparent pK_a values (Table 1). The apparent pK_{a1} values estimated for **1** and **2** were shifted to the acidic side by the presence of AN, while the apparent pK_{a2} for **1** was shifted to the alkaline side. These results indicate the guest-induced changes in the environment around the dye unit and the secondary amine group of **1** and **2**, which resulted from the guest-induced conformational change. Compounds **1** and **2** existing as the intramolecular complex forms, in which the dye units are included in their CD cavity, are converted to an intermolecular complex form upon the guest accommodation. In the intermolecular complex form, the dye units may be excluded to outside of the cavity and are exposed to the bulk water solution, as shown in Scheme 2a.

The pH-induced absorption variation of **3** in the presence of AN is also similar to that in the absence of the guest, accompanied with an isosbestic point at 382 nm. There exists the phenol−phenolate equilibrium with no shift in the apparent pK_a of **3** by the presence of the guest (Table 1). However, the

SCHEME 1: Schematic Representation for Acid−Base Dissociation Equilibria of 1, 2, and 3



SCHEME 2: Schematic Representation of Conformation for 1, 2, and 3 in the Presence of the Guest



hyperchromic effect induced by the guest was observed around 450 nm in the acidic and the alkaline regions. These suggest the guest-induced change in the conformation of **3** with no change in the environment around the hydroxyl group in the dye unit. This is explained by the fact that the hydroxyl group is exposed to the bulk water solution even in the presence of AN. The dye unit may be excluded from the cavity, or the unit penetrating the cavity is inclined by the partly accommodated guest, as shown in Scheme 2c.

Induced Circular Dichroism of 1, 2, and 3 in the Absence of the Guest. Since CD consists of chiral D-glucose units, a chromophore-modified CD exhibits induced circular dichroism in the wavelength region of the electronic transitions. The conformational features of the chromophore-modified CDs are confirmed by their induced circular dichroism spectra.[9]

Figure 1c shows the induced circular dichroism spectra of **1** at various pH values. The positive band around 350 nm and the negative one around 440 nm were observed at pH 3.67. With increasing pH, these bands were shifted to the longer wavelength side with increasing intensities, and the positive band around 400 nm and the negative one around 470 nm were observed at pH 11.94. The isoellipiticity point was observed at 375 and 445 nm. The analysis of the second derivatives of the absorption spectrum of **1** gave the electronic transitions at 355 and 450 nm at pH 3.67 and at 410 and 460 nm at pH 11.94, respectively (Figure 1b). The electronic transitions with the shorter wavelength under each condition are ascribed to the $\pi-\pi^*$ transition and those with the longer wavelength to the $n-\pi^*$ transition, respectively.[10] The transition moments of the $\pi-\pi^*$ and the $n-\pi^*$ bands under both conditions are parallel and perpendicular to the long axis of the dye unit, respectively. Therefore, the dichroism signs observed at pH 3.67 and at pH 11.94 imply that the dye unit of **1** is included in the CD cavity with an orientation parallel to the CD axis under both conditions. So the pH-induced change in the dichroism spectra of **1** is associated with change not in its conformation but in the structure of its dye unit. In any pH, **1** adopts the intramolecular complex form, in which the dye unit is accommodated in its CD cavity axially.

Figure 4 shows the induced circular dichroism spectra of **2** and **3** under different pH values. Compound **2** exhibited the positive dichroism band around 350 nm and the negative one around 450 nm at pH 3.63. Both bands were shifted to the longer wavelength side with increasing pH, accompanied with the isoellipiticity points at 370 and 445 nm. At pH 12.00, the

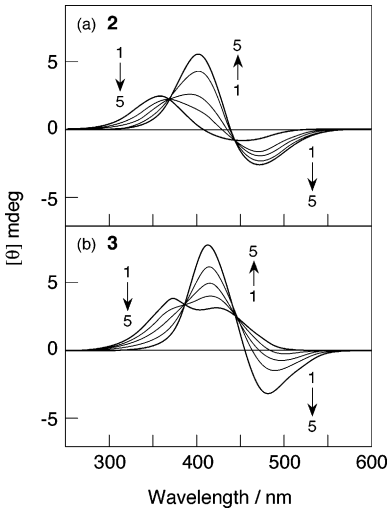


Figure 4. Induced circular dichroism spectra of **2** and **3** at different pH values: (a) the pH of the solution is (1) 3.63, (2) 7.58, (3) 8.15, (4) 8.95, and (5) 12.00; (b) the pH of the solution is (1) 3.49, (2) 7.30, (3) 7.75, (4) 8.36, and (5) 12.14.

positive and the negative bands were observed around 400 and 470 nm, respectively. This dichroism variation of **2** is similar to that of **1**, although the intensity is slightly smaller than that of **1**. The spectral analysis of **2** gave the electronic transitions at 355 and 450 nm at pH 3.63 and 410 and 460 nm at pH 12.00, which are the same wavelength to those of **1** and are ascribed to the same electronic transitions and the transition moments to those of **1** because of the same modified dye unit. Therefore, the dichroism signs of **2** demonstrate the axial inclusion of the dye unit for **2** in its CD cavity under both conditions, which is a similar conformation to that of **1**, as well as under other pH conditions.

On the other hand, the dichroism variation of **3** is somewhat different from that of **1** and **2** when the pH of the solution is varied (Figure 4b). Two positive dichroism bands observed around 370 and 430 nm at pH 3.49 changed to positive and negative dichroism bands around 410 and 490 nm at pH 12.14, respectively, accompanied with the isoellipiticity points at 383 and 450 nm. The electronic transitions were found at 355 and 450 nm at pH 3.49, and 410 and 460 nm at pH 12.14, which are the same wavelengths as those of **1** and **2**. A similar dichroism sign of **3** to those of **1** and **2** in alkaline condition indicates the similar conformation of **3** to those of **1** and **2**, although the different dichroism pattern of **3** from those of **1** and **2** in the acidic condition is associated with the different conformation of **3**. However, it is hard to consider that **3** changes its conformation in response to pH because of no occurrence of pH-induced conformation change even for **1** and **2**, which have a flexible spacer in their structures as compared to **1**. Although it is not clear that the dichroism pattern of **3** in the acidic condition is different from those of **1** and **2**, it might arise from the dye unit leaning against the CD axis in the cavity of CD due to the rigid structure of **3**. Since methyl red-modified

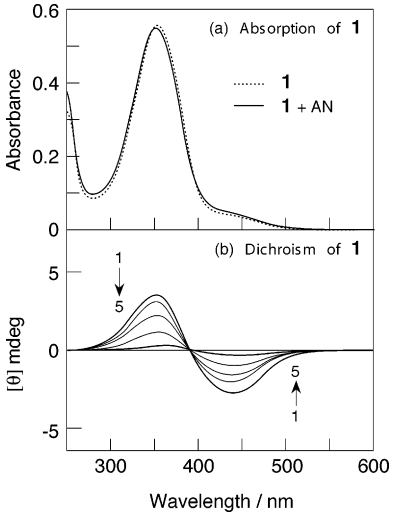


Figure 5. Absorption and the induced circular dichroism spectra of **1** at pH 3.24, alone and in the presence of 1-adamanatanol: (a) the 1-adamantanol concentration is (1) 0 and (2) 1.8 mM; (b) the 1-adamantanol concentration is (1) 0, (2) 0.04, (3) 0.16, (4) 0.46, and (5) 1.8 mM.

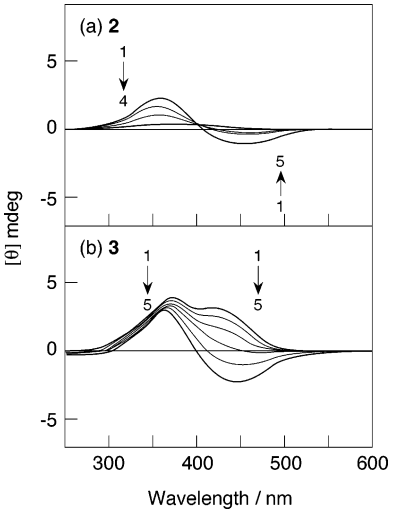


Figure 6. Induced circular dichroism spectra of **2** at pH 3.19 and **3** at pH 3.23, alone and in the presence of 1-adamanatanol: (a) the 1-adamantanol concentration is (1) 0, (2) 3.5, (3) 4.5, and (4) 5.5 mM; (b) the 1-adamantanol concentration is (1) 0, (2) 0.22, (3) 0.55, (4) 1.22, (5) 2.22, and (6) 4.23 mM.

β-CD, **4**, which has a similar structure to **3**, also adopts the axial inclusion of the dye unit in any pH,[8] **3** is likely to accommodate the dye unit axially in any pH.

In conclusion, all hosts accommodate the dye units in their own CD cavities with an orientation parallel to the CD axis in any pH, although the self-inclusion of the dye unit is accompanied with a slight inclination to the CD axis between each host. Such a difference between hosts should reflect the difference in the length of the spacer.

Guest-Induced Spectral Change of 1, 2, and 3. In order to investigate the guest-induced conformational behavior of the hosts, we measured the guest-induced spectral variations of **1**, **2**, and **3** under acidic and alkaline conditions, where **1** and **2** exist as the phenol−ammonium form and the phenolate−amine one, respectively, while **3** exists as the phenol form and the phenolate one, respectively, whether the guest is present or not in the solution.

Figure 5 shows the absorption and induced circular dichroism spectra of **1**, alone and in the presence of AN at pH 3.24. Upon the addition of AN, **1** exhibited a small absorption change. The absorption maximum at 354 nm was shifted to 352 nm with a little decrease and increase in its intensity around 370 and 420 nm, respectively, accompanied with the isosbestic point at 394 nm. This result indicates no change in the structure of the dye unit but change in the conformation of **1**, associated with the formation of the 1:1 host−guest inclusion complex. In contrast with the small absorption change, a drastic dichroism change induced by the guest was observed, as shown in Figure 5b. The positive dichroism band around 350 nm and the negative one around 440 nm decreased with increasing concentration of AN, and no dichroism band was observed in the presence of 1.8 mM AN. This indicates that the dye unit changes location from inside to outside the cavity upon the guest accommodation and that the dye unit has no interaction with the CD cavity, being exposed to the bulk water solution (Scheme 2a). Compound **2** also exhibited similar guest-induced absorption and dichroism variation to those of **1** at pH 3.19, suggesting the occurrence of a similar conformational change as that of **2** (Figure 6a). The dichroism bands of **2** disappeared upon the addition of 5.5 mM AN. The larger amount of AN to cancel the dichroism signs of **2** as compared to the case of **1** suggests a stronger interaction of the dye unit with the CD cavity for **2** than in the case for **1**. The shorter spacer of **2** causes the stronger interaction of the dye unit with the CD cavity.

Such strong interaction between the dye and the CD units was observed for **3** (Figure 6b). When AN was added to the solution of **3** at pH 3.23, the positive dichroism band around 420 nm turn to the negative one, while the positive dichroism band around 360 nm decreased slightly. These indicate that the guest-induced conformation change of **3** occurs. The observation of the dichroism sign even in the presence of excess of AN (4.23 mM) indicates that the dye unit still interacts with the CD cavity and is not excluded from the CD cavity completely. From consideration with the molecular models (Corey−Pauling−Koltun model), it is possible for **3** to adopt the intermolecular inclusion complex form to accommodate the dye unit and AN simultaneously, where the dye unit is inclined by the partly accommodated AN molecule (Scheme 2c). Namely, the dye unit is not excluded at all. So, the hydroxyl group is exposed to the bulk water solution from the secondary hydroxyl group side of the CD whether the guest is present or not. This is coincident with the result obtained in the absorption measurement for **3**. Such difference in the guest-binding and conformational behavior of **3** from those of **1** and **2** is associated with the rigidity between the dye and the CD units. The conformation of the host in the complex is different, depending on the spacer length.

Under alkaline condition at pH 11.5, **1** and **2**, existing as the phenolate−amine form, displayed unique spectral behavior upon addition of AN. The addition of AN caused small absorption changes with a red shift of the absorption maximum from 420 to 430 nm for **1** (Figure 7a) and from 425 to 430 nm for **2**, respectively. The isosbestic points were observed at 432 for **1** and 398 nm for **2**, respectively. These indicate the guest-induced conformation changes of **1** and **2** and the formation of 1:1 host−guest inclusion complexes. In contrast to small absorption variations, large guest-induced dichroism changes of **1** and **2** were observed with marked difference between **1** and **2**. The positive band around 400 nm and the negative one around 470 nm for **1** decreased upon the addition of AN, accompanied with the isoellipiticity point at 400 nm at pH 11.47 (Figure 7b). This indicates that the dye unit is excluded from the inside to the outside of the cavity to accommodate the guest, resulting in the dye unit being exposed to the bulk water solution (Scheme

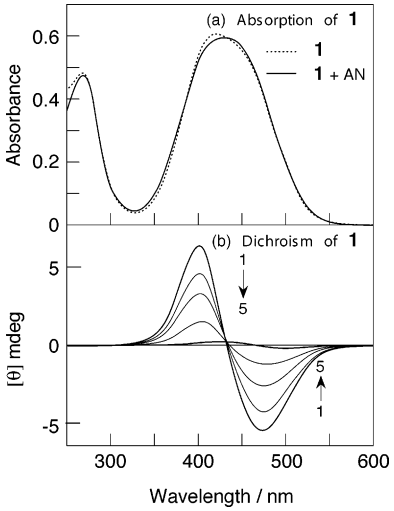


Figure 7. Absorption and the induced circular dichroism spectra of **1** at pH 11.47, alone and in the presence of 1-adamanatanol: (a, b) the 1-adamantanol concentration is (1) 0, (2) 0.04, (3) 0.16, (4) 0.46, and (5) 1.8 mM.

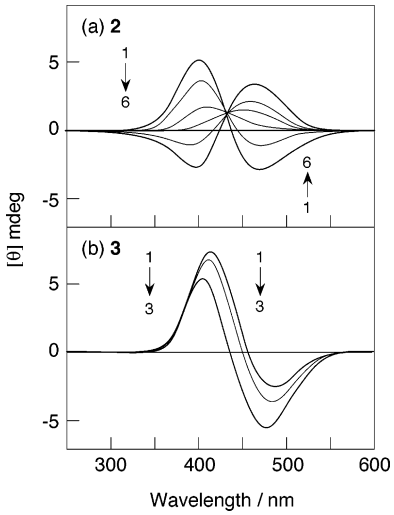


Figure 8. Induced circular dichroism spectra of **2** and **3** at pH 11.5, alone and in the presence of 1-adamanatanol: (a) the 1-adamantanol concentration is (1) 0, (2) 0.06, (3) 0.26, (4) 0.46, (5) 0.79, and (6) 2.8 mM; (b) the 1-adamantanol concentration is (1) 0, (2) 0.02, and (3) 2.8 mM.

2a). Such a conformational feature of **1** at pH 11.47 is similar to that at pH 3.24. On the other hand, the positive and the negative bands observed around 400 and 470 nm for **2** at pH 11.52, respectively, turn to the opposite upon guest addition, and the negative and positive bands were observed around 400 and 470 nm, respectively, in the presence of 2.8 mM AN (Figure 8a). The isoellipiticity point was observed at 433 nm. These suggest that the dye unit included in the CD cavity with an orientation parallel to the CD axis for **2** is excluded to the outside of the cavity, lying on the rim of the primary hydroxyl side perpendicularly (Scheme 2b). Consequently, the dye unit interacts with the CD cavity in the presence of the guest so as to cap the CD cavity. Such a guest-induced conformation change of **2** under alkaline conditions is different from that under acidic conditions as well as that of **1**. It was noted, therefore, that the conformation of the dye-modified CD in the presence of the guest is dependent not only on the length of the spacer but also on pH of the solution. Compound **3** also exhibited a small change upon the addition of AN at pH 11.47. A red shift in the absorption maximum from 430 to 440 nm was observed,

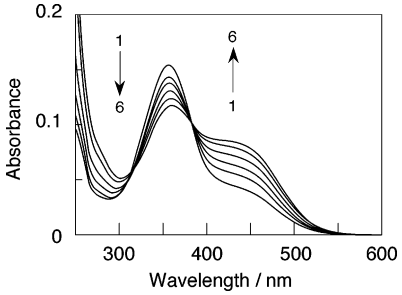


Figure 9. Absorption spectra of **1** at pH 7.20, alone and in the presence of 1-adamanatanol: the 1-adamantanol concentration is (1) 0, (2) 0.04, (3) 0.08, (4) 0.16, (5) 0.30, and (6) 0.74 mM.

SCHEME 3: Schematic Representation of Guest-Induced Change in Color and Conformation of 1 and 2

although the isosbestic point was not observed clearly. In the dichroism spectra, the positive band around 410 nm decreased and the negative one around 480 nm increased upon AN addition, which is a different dichroism variation from that of **1** and **2** (Figure 8b). The dye unit of **3** has an interaction with the CD cavity even in the presence of 2.8 mM AN. The guest-induced conformation change of **3** at pH 11.47 may seem to be not so large as compared to those of **1** and **2**. This may be due to the rigid structure of **3**.

When the guest was added near the neutral condition, **1** and **2** displayed a color change. Figure 9 shows the absorption spectra of **1**, alone and in presence of AN. The colorless solution containing **1** changed to yellow upon addition of AN at pH 7.20. The absorbance around 350 nm for **1** decreased upon addition of AN, while that around 450 nm increased. This suggests the structural conversion of the dye unit from the phenol form to the phenolate one by dissociation of the proton of the hydroxyl group in the dye unit in **1**, associated with the exclusion of the dye unit from the inside to outside of the cavity upon accommodation of AN, as shown in Scheme 3. The appearance of the isosbestic point at 383 nm indicates that **1** forms a 1:1 inclusion complex with AN. Compound **2** also showed the color change from colorless to yellow upon guest addition at pH 8.40 (Figure 10). This reflected the similar structural and conformational changes of **2** to those of **1**. The isosbestic point observed at 376 nm indicates that **2** also forms a 1:1 inclusion complex with the guest. On the other hand, **3** showed no change in color upon addition of the guest under the neutral conditions.

Guest-Binding Properties of 1, 2, and 3. The spectral variations of **1**, **2**, and **3** induced by formation of the inclusion complex are used for determining the binding constants. The binding constants of the hosts could be evaluated by a nonlinear least-square curve-fitting analysis of the guest-induced absorption variations of the host. The results obtained are summarized in Table 2. The binding constant of **1** for AN was estimated to be 7400 M^{-1} at pH 3.2, while that of **2** is 1940 M^{-1}, which is about a quarter of the value for **1**. Compound **3** bound to AN with a binding constant of 130 M^{-1}. The fact that the binding ability of the native β-CD with AN (5900 M^{-1} at pH 4.0) is similar to that of **1** rather than **2** and **3** is coincident with the result obtained in the dichroism spectra of **1** at pH 3.24, in which the dye unit of **1** has no interaction with the CD cavity in the presence of AN. On the contrary, the dye unit of **3** interacting

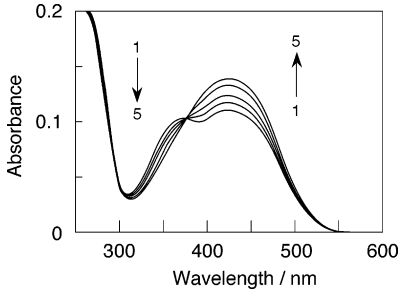


Figure 10. Absorption spectra of **2** at pH 8.40, alone and in the presence of 1-adamanatanol: the 1-adamantanol concentration is (1) 0, (2) 0.07, (3) 0.13, (4) 0.5, and (5) 3 mM.

TABLE 2: Host−Guest Binding Constants (M^{-1}) of 1, 2, 3, and β-CD for AN

	pH			
host	3.2	7.20	8.40	11.5
1	7400	8180	16800	4000
2	1940	1810	4510	6200
3	130	150		370
β-CD	5400[a]		6900[b]	

[a] pH = 4.0. [b] pH = 9.3.

with its CD cavity strongly results in the small binding constant. The tight inclusion of the dye unit in its CD cavity for **3** prevents guest binding. Therefore, it can be concluded that the dye unit acts as an inhibitor against guest binding with shortening the length of the spacer. When the pH of the solution was set at 7.2, the binding constant for AN is estimated to be 8180, 1810, and 150 M^{-1} for **1**, **2**, and **3**, respectively. These values are almost the same as those obtained at pH 3.2. Under the condition below pH 7.2, **1**, **2**, and **3** would exist as the protonated form: the phenol−ammonium form for **1** and **2**, and the phenol form for **3**. Therefore, it can be considered that the binding abilities of the hosts for AN are independent of pH below pH 7.2 and are governed only by the length of the spacer. At pH 8.40, however, it was observed that **1** and **2** revealed ca. 2 times larger binding constants for AN as compared to those at pH 7.20, while negligible pH dependency was reported in the binding constant of β-CD for AN.[11] This demonstrates that the dye unit of **1** acts as a promoter for guest binding and that of **2** loses the inhibitory character. Above pH 8.4, however, **1** lost the guest-binding ability, as shown by the fact that the binding constants of **1** are 5910 and 4000 M^{-1} at pH 8.8 and 11.5, respectively. The guest-binding ability of **1** reverts to that of the native β-CD with further increase of pH. The fact that **1** existing as the phenolate−amine form has similar guest-binding ability to the native β-CD is also in good agreement with the result obtained in the dichroism spectra, which shows no interaction of the dye unit with the CD cavity for **1** at pH 11.5 in the presence of the guest. On the other hand, **2** increased the guest-binding ability with increasing pH. The binding constant of **2** is estimated to be 6200 M^{-1} at pH 11.50. Since the interaction of the dye unit with the CD cavity for **2** in the presence of AN under the alkaline condition was observed in the dichroism measurement, the dye unit of **2** acts as a hydrophobic cap for guest binding rather than an inhibitory character. On the other hand, **3** revealed very poor guest-binding ability as compared to those of **1** and **2** even in the higher pH. All these results suggest that the guest-binding ability of the host is regulated not only by the length of the spacer but also by pH of the solution because the conformations of the hosts in the complexes are influenced by these factors when the guest is the neutral form.

TABLE 3: Host−Guest Binding Constants (M^{-1}) of 1 and 2 for Adamantane Derivatives

	AC		AA	
host	7.20	8.40	7.20	8.40
1	25900	16400	1020	1310
2	4610	6380	110	620

When 1-adamantanaecarboxylic acid (AC), which has a carboxyl group in place of the hydroxyl group of AN, was used as the guest, the binding constant of **1** for AC is 3 times larger than AN at pH 7.2, while it is the same at pH 8.4 (Table 3). Similar trends were observed for **2**. The binding constant of **2** for AC is 2.5 and 1.4 times larger than those for AN at pH 7.2 and 8.4, respectively. Because of the negative charge of AC under these conditions, the binding of AC with the hydrophobic CD cavity is unfavorable.[12] The reason for the larger binding constant for **1** at pH 7.2 may be due to the interaction between the positive charge of the ammonium unit in the spacer in **1** and the negative charge of AC. This electrostatic interaction causes the formation of a stable complex and disappears when the ammonium form in the spacer unit of **1** is converted to the neutral amine with increasing pH. On the other hand, smaller guest-binding ability of **1** and **2** was observed for 1-adamantineamine (AA), which has the amino group in the adamantine framework. The binding constants for AA at pH 7.2 and 8.4 are estimated to be 1030 and 1310 M^{-1} for **1** and 110 and 620 M^{-1} for **2**, respectively. Since AA exists as the ammonium form under these conditions, the positive charges of the host and the guest cause repulsion. All these results demonstrate that the charge effect of the guest is also important for guest binding.

Conclusion

The dye-modified CDs, **1**, **2**, and **3**, all appending the 4-hydroxyazobenzene residue, have a different spacer between the dye and CD units. The dye parts are accommodated in the CD cavity axially regardless of the length of the spacer. The change in pH of the solution gives rise to their structures without the conformational changes. However, the guest-binding abilities and the guest-induced conformational features of the hosts were markedly different between hosts. These results demonstrate that the guest-binding and the spectroscopic properties of dye-modified CDs can be remarkably modulated by varying the spacer lengths as well as the dye structures. These findings may be useful for constructing supramolecular systems, the recognition, reaction, or catalytic action of which can be regulated by pH and the flexibility of the hosts.

Experimental Section

Measurements. ^{1}H NMR spectra were recorded on Bruker AVANCE 400 spectrometer. Absorption and circular dichroism spectra were recorded on Shimadzu UV-2500 and UV-3100 spectrophotometers and a JASCO J-820, respectively. All of the measurements were performed at 25 °C in an aqueous solution. The concentration of **1**, **2**, and **3** was 0.01 mM for the measurement of absorption spectra and 0.03 mM for that of the dichroism one. The pH of the solutions was measured on a Beckman 720 pH meter, which was calibrated at 25 °C with pH standard solutions of pH 4.01, 6.86, and 9.22. Hydrochloric acid and sodium hydroxide were used to set pH for pH titration experiments. Phosphate buffer was used in the measurement of the binding constants of **1** and **2** at pH 7.2, while borate buffer was used at pH 8.4 and 8.8. The acidic and alkaline solutions at pH 3.2 and 11.5 were made by hydrochloric acid and sodium hydroxide, respectively.

In the acid dissociation equilibria for **1** and **2**, there are two equilibria involving three chemical species with the phenol−ammonium, the phenolate−ammonium and the phenolate−amine forms. So, the pK_a values of **1** and **2** were estimated by curve-fitting analysis with the following equation:[13]

$$I_{obs} = \frac{I^- + (K_{a1}/[H^+])I^0 + (K_{a1}K_{a2}/[H^+]^2)I^+}{1 + (K_{a1}/[H^+]) + (K_{a1}K_{a2}/[H^+]^2)} \quad (1)$$

where I_{obs} is the absorbance at 440 nm for **1** and **2**. K_{a1} and K_{a2} are the values of the acid dissociation constants between the phenol and phenolate form and between the ammonium and amine form. Here, at a certain pH, **1** and **2** are assumed to exist as the phenol−ammonium, the phenolate−ammonium, or the phenolate−amine form, and their absorbance can be expressed as I^+, I^0, and I^-, respectively. [H^+] is the concentration of proton, which can be evaluated from pH of the solutions. On the other hand, **3** involves an acid dissociation equilibrium between the phenol and phenolate form. So, the pK_a values of **3** were estimated by the following equation, in which K_{a2} equals 0 in eq 1. Therefore, the following equation was used for estimation of pK_a for **3**. In the case of **3**, I_{obs} expresses the absorption intensity at 450 nm.[8]

$$I_{obs} = \frac{I^- + (K_a/[H^+])I^0}{1 + (K_a/[H^+])} \quad (2)$$

Determination of Association Constants. The following equation describes the complexation equilibrium

$$H + G \rightleftarrows H-G$$

where H, G, and H−G represent free host, free guest, and host−guest complex, respectively. This means the host forms only a 1:1 complex with the guest. The binding constant of the host for the guest, *K*, is estimated by fitting the following equation to the data obtained.[8]

$$\Delta I = \frac{\Delta I_{max}[(G_0 + H_0 + 1/K) - \{(G_0 + H_0 + 1/K)^2 - 4G_0H_0\}^{1/2}]}{2H_0} \quad (3)$$

where H_0 and G_0 represent the initial concentration of host and guest, ΔI represents the difference of absorption intensity at 440 nm for the host alone and in the presence of the guest, and ΔI_{max} is its value when all hosts exist as the inclusion complex. The value of ΔI was used at 370 nm for estimating the binding constant, *K*, at pH 3.2 for all hosts, while the value of ΔI at 440 nm were used for estimating *K* from pH 7. 2 to pH 8.4 for **1** and **2**, respectively. At pH 11.5, the value of ΔI at 420 nm was used for **1** and **2**, and at 450 nm for **3**.

Materials. The β-CD was a kind gift from Nihon Shokuhin Kako Co. Ltd. All chemicals were reagent grade and were used without further purification unless otherwise noted.

Synthesis. *6-Deoxy-6-amino-{10-[2-(4-hydroxyphenylazo)-benzoyl]amino-4,7-dioxadecyl}-β-cyclodextrin (**1**).* A dimethylacetamide (DMAc) solution of HABA (1.12 g, 4.64 mmol), dicyclohexylcarbodiimide (DCC)(1.07 g, 5.22 mmol), and 1-hydroxybenzotriazole (HOBt) (0.70 g, 5.22 mmol) was stirred at 0 °C for 20 min. To the mixture was added 6-deoxy-6-amino-(10-amino-4,7-dioxadecyl)-β-CD (1.50 g, 1.16 mmol), and the resulting solution was stirred at 0 °C for 30 min and subsequently at room temperature for 7 days. After the insoluble material was removed by filtration, the filtrate was concentrated by a rotary evaporator and was poured into acetone (1 L) to form precipitates, followed by washing with acetone (300 mL) several times. The crude product was charged on a column of QAE Sephadex and eluted with an aqueous solution of ammonium hydrogen carbonate (0−3 mM). After the fraction containing **1** was concentrated by a rotary evaporator to 100 mL, a small amount of diluted hydrochloric acid was added to set the pH at ca. 4. The solution was poured into acetone (1 L) to obtain the desired product as a yellow powder (0.40 g 22.7%). The purity of the product was checked with TLC, ^{1}H NMR, and elemental analysis: R_f 0.09 (*n*-butanol−ethanol−water 5:4:3); ^{1}H NMR (D_2O; 400 MHz) 1.80−1.60 (4H bs), 2.91 (4H m), 3.04−3.15 (4H m), 3.20−4.0 (m), 4.83−4.95 (7H, m), 6.97 (2H, d), δ 7.50−7.40 (6H, m), 7.65 (2H, d). Anal. Calcd for $C_{63}H_{96}N_4O_{38}\cdot 9H_2O$: C, 45.05; H, 6.84; N, 3.34%. Found: C, 44.95; H, 6.10; N, 3.23%.

*6-Deoxy-6-amino-{4-(2-[4-hydroxyphenylazo]benzoyl)aminobutyl}-β-cyclodextrin (**2**).* A DMAc solution of HABA (1.12 g, 4.64 mmol), DCC (1.07 g, 5.22 mmol), and HOBt (0.70 g, 5.22 mmol) was stirred at 0 °C for 20 min. To the mixture was added 6-deoxy-6-amino-(4-aminobutyl)-β-CD (1.40 g, 1.16 mmol), and the resulting solution was stirred at 0 °C for 30 min and subsequently at room temperature for 10 days. The product was purified in the same manner as in the case of **1** except for the concentration of ammonium hydrogen carbonate, which was 0−2.5 mM. The product was obtained as a yellow powder (0.25 g 15.0%). The purity of the product was checked with TLC, ^{1}H NMR, and elemental analysis: R_f 0.07 (*n*-butanol−ethanol−water 5:4:3); ^{1}H NMR (D_2O; 400 MHz) 1.58 (4H, bs), 2.70−2.90 (3H, bd), 3.05 (2H, bt), 3.28 (2H, bd), 3.35−4.0 (m), 4.88−5.0 (7H, m), 6.95 (2H, d), 7.45−7.62 (4H, m), 7.63 (2H, d). Anal. Calcd for $C_{59}H_{88}N_4O_{36}\cdot HCl\cdot 4H_2O$: C, 46.08; H, 6.36; N, 3.64; Cl, 2.42%. Found: C, 46.12; H, 6.24; N, 3.21; Cl, 2.05%.

*6-Deoxy-6-amino-{2-[4-hydroxyphenylazo]benzoyl}-β-cyclodextrin (**3**).* A DMAc solution of HABA (1.74 g, 7.0 mmol), DCC (1.63 g, 7.7 mmol), and HOBt (1.07 g, 7.9 mmol) was stirred at 0 °C for 10 min. To the mixture was added 6-deoxy-6-amino-β-CD (4.0 g, 3.5 mmol), and the resulting solution was stirred at 0 °C for 1 h and then at room temperature for 2 days, The product was purified in the same manner as in the case of **1** and **2**. The product was obtained as a yellow powder (0.062 g 1.3%). The purity of the product was checked with TLC, ^{1}H NMR, and elemental analysis: R_f 0.41 (*n*-butanol−ethanol−water 5:4:3); ^{1}H NMR (D_2O; 400 MHz) 3.10 (1H, d), 3.20 (1H, d), 3.25−3.96 (m), 4.75−5.06 (7H, m), 6.93 (2H, d), 7.47 (2H, m), 7.58 (2H, m) 7.67 (2H, d). Anal. Calcd for $C_{55}H_{79}N_3O_{36}\cdot 5H_2O$: C, 45.61; H, 6.19; N, 2.90. Found: C, 45.55; H, 5.61; N, 2.80.

Acknowledgment. We are grateful to Nihon Shokuhin Kako Co. Ltd. for a generous gift of β-CD. This work was partly supported by a Grant from The Asahi Glass Foundation (No. 02A-C01-P054) and by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan. The authors thank Professor Masato Nanasawa (Yamanashi University) for helpful discussion. This paper is dedicated to the late Professor Akihiko Ueno of the Tokyo Institute of Technology.

References and Notes

(1) Lehn, J.-M. *Supramolecular Chemistry*; VCH: Weinheim, Germany, 1995.

(2) (a) Szeijtli, J., Osa, T., Eds.; *Cyclodextrins Comprehensive Supramolecular Chemistry*; Pergamon: Oxford, 1996; Vol. 3. (b) Szeijtli, J.

Cyclodextrin and Inclusion Compounds; Academic: Kiado, Budapest, 1982. (c) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer-Verlag: New York, 1978. (d) Liu, Y.; Li, X.-Q.; Chen, Y.; Guan, X.-D. *J. Phys. Chem. B* **2004**, *108*, 19541−19549.

(3) (a) Ueno, A. *Adv. Mater*. **1993**, *5*, 132−134. (b) Kuwabara, T.; Shiba, K.; Ozawa, M.; Miyajima, N.; Suzuki, Y. *Tetrahedron Lett*. **2006**, *47*, 4433−4436.

(4) (a) Kuwabara, T.; Takamura, M.; Nakamura, A.; Ueno, A.; Toda, F. *J. Org. Chem.* **1998**, *63*, 8729−8735. (b) Matsushita, A.; Kuwabara, T.; Nakamura, A.; Ikeda, H.; Ueno, A. *J. Chem. Soc., Perkin Trans. 2* **1997**, 1705−1710. (c) Kuwabara, T.; Takamura, M.; Matsushita, A.; Nakamura, A.; Ueno, A.; Toda, F. *Supramol. Chem.* **1996**, *8*, 13−15. (d) Kuwabara, T.; Matsushita, A.; Nakamura, A.; Ueno, A.; Toda, F. *Chem. Lett.* **1993**, 2081−2084.

(5) (a) Kuwabara, T.; Nakajima, H.; Nanasawa, M.; Ueno, A. *Anal. Chem.* **1999**, *71*, 2844−2849. (b) Ueno, A.; Kuwabara, T.; Nakamura, A.; Toda, F. *Nature* **1992**, *356*, 136−137.

(6) *Dictionary of Organic Compounds*; Chapman & Hall, Electronic Publishing Division.

(7) (a) Nakashima, H.; Takenaka, Y.; Higashi, M.; Yoshida, N. *J. Chem. Soc., Perkin Trans. 2* **2001**, 2096−2103. (b) Takenaka, Y.; Higashi, M.; Yoshida, N. *J. Chem. Soc., Perkin Trans. 2* **2002**, 615−620.

(8) Kuwabara, T.; Nakamura, M. A.; Ueno, A.; Toda, F. *J. Phys. Chem.* **1994**, *98*, 6297−6303.

(9) Harada, K.; Uedaira, H. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 375−379.

(10) Kronor, J.; Bock, H. *Chem. Ber*. **1968**, *101*, 1922.

(11) Wang, Y.; Ikeda, T.; Ueno, A.; Toda, F. *Tetrahedoron Lett. 34*, 4971−4774.

(12) Eftink, M. R.; Andy, M. L.; Bystrom, K.; Perlmutter, H. D.; Kristol, D. S. *J. Am. Chem. Soc*. **1989**, *111*, 6765−6772.

(13) Aoyagi, T.; Nakamura, A.; Ikeda, H.; Ikeda, T.; Mihara, H.; Ueno, A. *Anal. Chem.* **1997**, *69*, 659−663.