Crown ether-tethered cyclodextrins: superiority of the secondary-hydroxy side modification in binding tryptophan

Iwao Suzuki,*[a] Kohki Obata,[a] Jun-ichi Anzai,[a] Hiroshi Ikeda[b] and Akihiko Ueno[b]

[a] *Graduate School of Pharmaceutical Sciences, Tohoku University, Aramaki, Aoba-ku, Sendai 980-8578, Japan*
[b] *Department of Bioengineering, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midrori-ku, Yokohama, 226-8501, Japan*

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β-Cyclodextrin (β-CyD) derivatives bearing a benzo-*X*-crown-*Y* (*X* = 15, 18 and *Y* = 5, 6) moiety at the primary- or secondary-hydroxy side were synthesized for molecular recognition toward tryptophan (Trp) in zwitterionic form. ^{1}H NMR titration experiments gave binding constants for a 1 : 1 host–guest complexation process, leading to the conclusions that the benzo-18-crown-6 moiety was superior to the benzo-15-crown-5 moiety in binding Trp and that the secondary-hydroxy side modification was preferable to the primary-hydroxy side one for recognizing Trp. For the secondary-hydroxy side-modified β-CyDs, although the difference in the binding constants for D- and L-Trp were small, complexation-induced chemical shift changes and complexation-induced circular dichroism changes revealed that the hosts recognized the chirality difference of Trp. The ammonium cation part of Trp was located at the secondary-hydroxy side of the CyD cavity and is recognized by the benzo-18-crown-6 moiety attached at the secondary-hydroxy side of CyD. This interaction between the ammonium cation and the benzo-18-crown-6 was confirmed by 2D-ROESY. 2D-ROESY spectra also indicated that the benzo-18-crown-6-modified CyD accommodated the indole ring of Trp more shallowly in the CyD cavity than the benzo-15-crown-5-modified CyD.

Introduction

The major goal in molecular recognition events by a synthetic host is to achieve selective guest binding in an aqueous medium where a biological host interacts with a guest. In this regard, efforts at combining cyclodextrin (CyD), which recognizes an organic guest by the hydrophobic interaction,[1] and crown ether or cyclen, which can bind cations including an ammonium group,[2] have been conducted by several researchers.[3–8] When diaza-18-crown-6-ether was attached to β-CyD, the resulting host strongly binds *p*-nitrophenolate in the presence of alkali metal cations in organic solution.[3] The same host as well as diaza-18-crown-6-capped β-CyD was utilized for realizing energy transfer from benzene bound by the CyD cavity to transition metal cations bound by the azacrown part.[4] Cyclen-modified β-CyDs were found to be excellent artificial enzyme-mimetic systems which accelerated the hydrolysis of *p*-nitrophenyl ester in the presence of transition metal cations.[5] Recent results on CyD–crown systems in which CyD and crown ether interact with each other non-covalently revealed that these two hosts cooperatively recognized secondary amines with D/L separation in capillary electrophoresis.[6] Moreover, Teramae *et al*. found that a 1 : 2 host–guest complex between γ-CyD and a benzo-15-crown-5-ether derivative bearing a pyrene unit could be useful to detect potassium cations in an aqueous medium based on potassium cation-induced monomer–excimer changes in fluorescence.[7] However, our previous study on an azacrown-modified γ-CyD in which a fluorescent pyrene group was attached at one end of the azacrown moiety did not show cation selectivity, although its fluorescence properties were dramatically changed upon binding bile acids.[8] We considered that its poor ability in cation binding was due to the primary-hydroxy side modification as well as to the amido function through which the pyrene group was attached to the azacrown moiety.

For modified CyDs, there are two major modification sites; one is the primary hydroxy groups aligned at one end of the open torus of CyD and the other is secondary hydroxy groups aligned at the opposite side of the CyD torus.[1] Since the number of secondary hydroxy groups is double that of the primary ones, the secondary-hydroxy side of CyD should be more hydrophilic than the primary-hydroxy side. Therefore, it seems better to place an additional recognition moiety toward hydrophilic groups of a guest, such as a crown ether moiety, at the secondary-hydroxy side of CyD to construct effective host compounds capable of recognizing both hydrophobic and hydrophilic parts of a guest. It is known that some secondary-hydroxy side-modified CyDs are superior to the corresponding primary-hydroxy side-modified CyDs.[9–12] In the crown ether-tethered CyDs constructed so far, however, the position of the crown ether moiety has been limited to the primary-hydroxy side with the one exception of cyclen-modified β-CyD[4] for which the superiority of the secondary-hydroxy side modification has not been achieved through ester hydrolysis which was catalyzed by transition metal cations bound by the cyclen part.

Considering these situations, we have examined guest binding properties of four crown ether-tethered β-CyDs (**1**–**4**; Scheme 1) toward tryptophan (Trp) as a representative guest having both hydrophobic and hydrophilic parts to explore which side of the CyD cavity would be better for introducing a crown ether moiety. Although azacrown ether might better be introduced as a modifying residue because it could promise a short distance between the hydrophobic and hydrophilic recognition sites, we used a benzocrown ether unit as a modifying residue to exclude any ambiguity exerted by charged ammonium nitrogen atoms of an azacrown ether upon guest complexation.

Experimental

Materials

β-CyD was purchased from Nakarai Tesque (Kyoto, Japan). It was used without further purification for synthetic purposes

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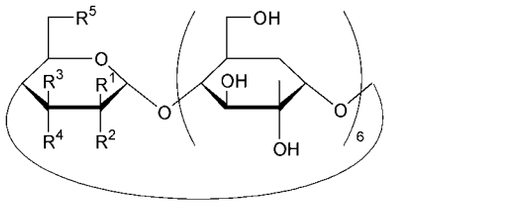
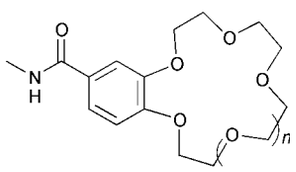


	R^1	R^2	R^3	R^4	R^5
1	H	OH	OH	H	15Cr5
2	H	OH	OH	H	18Cr6
3	OH	H	H	15Cr5	OH
4	OH	H	H	18Cr6	OH
5	OH	H	H	NHAc	OH
β-CyD	H	OH	OH	H	OH



15Cr5 n = 1
18Cr6 n = 2

Scheme 1

and was recrystallized from hot water for physicochemical measurements. 4′-Carboxybenzo-15-crown-5 and 4′-carboxybenzo-18-crown-6 were synthesized by reported procedures.[13] *N*-Acetyl-3-amino-3-deoxy-β-CyD (**5**) was previously synthesized in our laboratory.[12] D- and L-Trp were kindly given by Ajinomoto Co. Ltd. (Tokyo, Japan) or were purchased from Wako Chemical Industries Co. Ltd. (Kyoto, Japan). Deuterium oxide (99.8% D_2O) was manufactured by Isotec (OH, USA). Guaranteed reagent grade acetonitrile (Wako Chemicals) diluted with D_2O was sealed into a glass capillary and used as an external standard (1.94 ppm) for ^{1}H NMR measurements throughout this study except for 2D-ROESY measurements where a residual HOD peak was used as an internal standard.

Apparatus

^{1}H NMR titration experiments were conducted on a Varian Gemini 2000 spectrometer at 25 °C. 2D-ROESY spectra were obtained on a Varian UNITY plus-400 spectrometer. Circular dichroism spectra were recorded on a Jasco J-720 spectropolarimeter at 25 °C.

Sample preparation

Samples for ^{1}H NMR titration experiments were prepared as follows. Trp was dissolved in pH 7.4 phosphate buffer (I = 0.13 mol dm^{-3}) and lyophilized. Then, D_2O was added to the residuals and they were lyophilized again. This procedure was repeated to ensure replacement of the dissociable protons to deutrons. Finally, D_2O was added to the residuals to make the Trp concentration 2.5 mmol dm^{-3}. Separately, the CyD derivatives were dissolved in the same phosphate buffer and lyophilized. Repeated lyophilization was performed as mentioned above, and finally, the residuals were dissolved in the D_2O solution of Trp prepared as above (2.5 mmol dm^{-3}) to make the concentration of the CyD derivatives 5.0 mmol dm^{-3}.

^{1}H NMR titration and data analysis

A stock solution of Trp (2.5 mmol dm^{-3} in buffered D_2O) was used for measuring ^{1}H NMR spectra. Then portions of the buffered D_2O stock solutions of CyD derivatives (5.0 mmol dm^{-3}) were added and ^{1}H NMR spectra were collected for every addition. In this manner, the concentrations of amino acids were maintained at 2.5 mmol dm^{-3} throughout the measurements and the concentrations of CyD derivatives were varied. After the acquisition of ^{1}H NMR spectra, chemical shift values for protons originating from Trp were plotted against the concentrations of CyD derivatives, and the data were used for curve fitting analyses based on 1 : 1 host–guest complexation with the aid of nonlinear regression analysis.[14] The analysis was performed for every proton of the D- and L-isomers of Trp as the guests. The chemical shift changes induced by **1**–**5** and β-CyD were observed and the obtained binding constants were averaged.

2D-ROESY experiments

2D-ROESY spectra were obtained in a similar manner to that of the previous studies[15–18] and the mixing time was 350 ms. Samples for these measurements were prepared by mixing 2 mmol dm^{-3} of the host (**3** or **4**) and 6 mmol dm^{-3} of Trp in D_2O.

Obtaining circular dichroism spectra of 4–D-/L-Trp complexes

Circular dichroism spectra of **4**, D-Trp, and L-Trp were measured with 0.15 mmol dm^{-3} samples in phosphate buffer (pH 7.4). Separately, the spectra of the mixture of **4**–D-Trp and **4**–L-Trp (the concentration of each component was 0.15 mmol dm^{-3}) were measured. From the spectra of the mixture, the contributions of free **4** and D- or L-Trp, which could be calculated from the binding constant data and the circular dichroism spectra measured as above, were subtracted to afford the pure complex circular dichroism spectra.

Syntheses

***N*-(4′-Carbonylbenzo-15-crown-5)-6-amino-6-deoxy-β-CyD (1).** 4′-Carboxybenzo-15-crown-5 (1.3 mmol),[13] *N*,*N*′-dicyclohexylcarbodiimide (1.4 mmol), and 4-(dimethylamino)pyridine (0.14 mmol) were dissolved in dry DMF (10 cm^3) at 0 °C. After stirring at this temperature for 30 min, 6-amino-6-deoxy-β-CyD (0.64 mmol)[19] suspended in dry DMF (10 mL) was added to the solution. The resultant solution was stirred for 1 h at 0 °C and for 12 h at room temperature. The undissolved materials were removed by filtration and the filtrate was concentrated to *ca*. 5 cm^3 by a rotary evaporator, and the concentrated solution was dropped into acetone (250 cm^3) to form precipitates. The precipitates were collected and redissolved with a small amount of water to be subjected to successive ion-exchange column chromatography using SP-Sephadex C-25 (NH_4^+ form) and DEAE-Sephadex A-25 (HCO_3^- form). Fractions eluted with water were collected and Sephadex G-15 column chromatography was carried out. Fractions containing the product were collected and concentrated. Finally, it was subjected to preparative HPLC using an ODS column (water–MeOH linear gradient (10% MeOH–40% MeOH)) to afford pure **1** (15%). FAB MS, *m*/*z* 1427 $[M + H]^+$, 1449 $[M + Na]^+$. ^{1}H NMR ($(CD_3)_2SO$): δ 3.1–3.8 (m, others, overlapped with H_2O), 4.00–4.16 (m, 4H, Ph–O–CH_2–), 4.32–4.56 (m, 6H, C6–OH), 4.78–4.98 (m, 7H, C1–H), 5.60–5.96 (m, 14H, C2–OH and C3–OH), 6.978 (d, 1H, ^3J 8.5 Hz, phenyl H), 7.37–7.43 (m, 2H, phenyl H), 8.106 (brs, 1H, amide H). Anal. calcd. for $C_{57}H_{89}NO_{40}\cdot 5H_2O$: C, 45.09; H, 6.53; N, 0.92%; found: C, 45.08; H, 6.76; N, 0.91%.

***N*-(4′-Carbonylbenzo-18-crown-6)-6-amino-6-deoxy-β-CyD (2).** This compound was synthesized by the same procedure from 4′-carboxybenzo-18-crown-8[13] and 6-amino-6-deoxy-β-CyD. Yield was 20%. FAB MS, *m*/*z* 1472 $[M + H]^+$, 1494 $[M + Na]^+$. ^{1}H NMR ($(CD_3)_2SO$): δ 3.1–3.8 (m, others, overlapped with H_2O), 4.00–4.16 (m, 4H, Ph–O–CH_2–), 4.34–4.58 (m, 6H, C6–OH), 4.80–4.98 (m, 7H, C1–H), 5.64–5.96 (m, 14H, C2–OH and C3–OH), 6.980 (d, 1H, ^3J 8.5 Hz, phenyl H), 7.37–7.43 (m, 2H, phenyl H), 8.102 (brs, 1H, amide H). Anal calcd. for $C_{59}H_{93}NO_{41}\cdot 5H_2O$: C, 45.36; H, 6.60; N, 0.90%; found: C, 45.31; H, 6.24; N, 1.01%.

***N*-(4′-Carbonylbenzo-15-crown-5)-3-amino-3-deoxy-β-CyD (3).** This compound was synthesized by the same procedure from 4′-carboxybenzo-15-crown-5 and 3-amino-3-deoxy-β-CyD.[10] Yield was 57%. FAB MS, *m*/*z*: 1427 $[M + H]^+$, 1449 $[M + Na]^+$. ^{1}H NMR ($(CD_3)_2SO$): δ 3.1–3.9 (m, others, overlapped with H_2O), 4.06–4.18 (m, 4H, Ph–O–CH_2–), 4.34–4.58

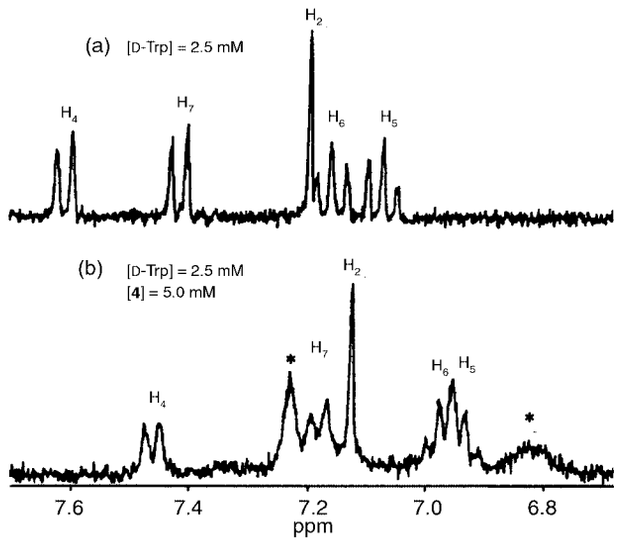


Fig. 1 Partial ^{1}H NMR spectra (aromatic region) of (a) D-Trp (2.5 mmol dm^{-3}), alone and (b) in the presence of **4** (5.0 mmol dm^{-3}) in phosphate buffer (pH 7.4).

(m, 7H, C6–OH), 4.64–4.94 (m, 7H, C1–H), 5.30–5.90 (m, 13H, C2–OH and C3–OH), 6.980 (d, 1H, ^3J 7.7 Hz, phenyl H), 7.40–7.50 (m, 2H, phenyl H), 7.955 (brs, 1H, amide H). Anal calcd. for $C_{57}H_{89}NO_{40}\cdot 4H_2O$: C, 45.63; H, 6.47; N, 0.93%; found: C, 45.50; H, 6.38; N, 0.99%.

***N*-(4′-Carbonylbenzo-18-crown-6)-3-amino-3-deoxy-β-CyD (4).** This compound was synthesized by the same procedure from 4′-carboxybenzo-18-crown-6 and 3-amino-3-deoxy-β-CyD. Yield was 23%. FAB MS, *m/z*: 1472 $[M + H]^+$, 1494 $[M + Na]^+$. ^{1}H NMR ($(CD_3)_2SO$): δ 3.2–4.0 (m, others, overlapped with H_2O), 4.10–4.18 (m, 4H, Ph–O–CH_2–), 4.30–4.58 (m, 7H, C6–OH), 4.84–4.96 (m, 7H, C1–H), 5.40–5.96 (m, 13H, C2–OH and C3–OH), 6.986 (d, 1H, ^3J 8.5 Hz, phenyl H), 7.40–7.50 (m, 2H, phenyl H), 7.946 (brs, 1H, amide H). Anal calcd. for $C_{59}H_{93}NO_{41}\cdot 4H_2O$: C, 45.87; H, 6.54; N, 0.91%; found: C, 45.77; H, 6.49; N, 0.95%.

Results and discussion

^{1}H NMR spectra

Fig. 1 shows partial ^{1}H NMR spectra (aromatic region) of D-Trp in the absence and presence of **4** in D_2O. Upon addition of **4**, signals originating from Trp were shifted towards an upfield region and broadened. This indicates that the indole part of Trp interacts with the CyD cavity of **4**. Signals assigned to the aromatic protons of **4** appeared in this region (indicated by asterisks in Fig. 1(b)), and they were also found to be broadened. This implies that the indole ring of D-Trp also interacts with the benzene ring of the benzocrown moiety of **4** and/or the mobility of the benzocrown moiety was markedly depressed upon complexation. Thus, the ^{1}H-NMR spectral change qualitatively revealed that D-Trp is positioned at the secondary-hydroxy side of **4** where the benzocrown moiety exists. A similar ^{1}H NMR spectral change was obtained upon addition of **3**, although the trends of shifts and perturbation were less pronounced. However, the magnitudes of shifts upon addition of **1**, **2**, β-CyD, and **5** were small (see Table 2), and the protons of the benzene rings of **1** and **2** were slightly perturbed. These observations suggest weak interaction between the indole ring and the CyD cavity and/or the benzocrown moiety upon complexing.

Binding constants for Trp

Chemical shift changes of guest protons associated with varying the concentrations of CyD derivatives allowed us to obtain binding constants for the 1 : 1 host–guest complexation process, with the aid of non-linear least squares regression analysis. Typical examples of the analyses are depicted in Fig. 2, which was obtained for D-Trp and **4**. A good correlation between the observed chemical shifts and the theoretically generated binding isotherms strongly indicates that the complexation process is actually 1 : 1. The binding constants obtained for D- and L-Trp are compiled in Table 1.

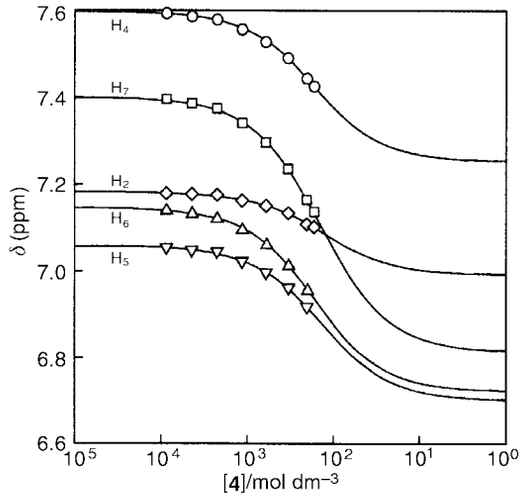


Fig. 2 Plots of chemical shifts of D-Trp protons as a function of **4** concentration. Solid lines are theoretically generated best-fitted binding isotherms based on 1 : 1 host–guest complexation.

Table 1 1 : 1 Host–guest binding constants (K) of β-CyD derivatives for Trp in aqueous solutions determined by chemical shift changes induced by the hosts[a]

	D-Trp		L-Trp	
Host	K/dm^3 mol^{-1}	$-\Delta G°$/ kJ mol^{-1}	K/dm^3 mol^{-1}	$-\Delta G°$/ kJ mol^{-1}
1	48.6 ± 4.1	9.61 ± 0.21	53.2 ± 3.5	9.84 ± 0.16
2	65.0 ± 7.1	10.3 ± 0.3	64.6 ± 4.9	10.3 ± 0.2
3	115 ± 2	11.8 ± 0.1	108 ± 3	11.6 ± 0.1
4	188 ± 10	13.0 ± 0.1	171 ± 5	12.7 ± 0.1
5	33.5 ± 3.2	8.68 ± 0.22	31.2 ± 4.5	8.47 ± 0.29
β-CyD	31.4 ± 1.9	8.53 ± 0.15	33.4 ± 1.8	8.69 ± 0.13

[a] See Experimental section for detailed experimental conditions.

β-CyD weakly binds D-Trp and L-Trp with binding constants of around 32 dm^3 mol^{-1} which is fairly consistent with a previously reported value (22.2 dm^3 mol^{-1}, at pH 9.88).[20] The indole ring seems to be bound by the β-CyD cavity when Trp and β-CyD form a host–guest complex, because the CyD cavity is hydrophobic and rejects accommodation of the other part of Trp (zwitterionic α-amino acid). Since indole itself was reportedly bound by β-CyD with a binding constant of 184 dm^3 mol^{-1},[21] the presence of the amino acid part decreased the stability of the interaction between the indole ring and the β-CyD cavity. It is noted that the indole ring is sufficiently hydrophilic to become a head group when *N*-alkylindole derivatives form a liposome.[22]

The weak binding for Trp was not improved by introduction of an acetyl group at the secondary-hydroxy side of β-CyD. A slight improvement in the stability of Trp complexes was achieved by **1** and **2**, which had a benzocrown moiety at the primary-hydroxy side of β-CyD. There are two possibilities to account for this improvement; one is a cooperative effect of the hydrophobic interaction between the β-CyD cavity and the indole ring of Trp with a cation–dipole interaction operating between the ammonium group of Trp and the benzocrown

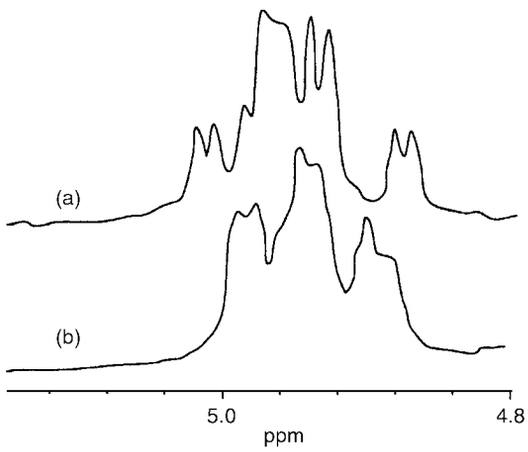


Fig. 3 Partial ^{1}H NMR spectra (anomeric proton region) of **2** (a) and **4** (b) in phosphate buffer (pH 7.4).

moiety of **1** or **2**, and the other is a hydrophobic cap effect[23] exerted by the benzocrown moiety. The latter was considered as the main factor in the improvement of the stability of Trp complexes of **1** and **2**, because of the slight perturbation in the chemical shifts of the benzocrown protons upon complexing.

On the other hand, the secondary-hydroxy side-modified β-CyDs, **3** and **4**, bind Trp strongly. It is known that the secondary-hydroxy side-modified CyDs prepared through 2-*O*-tosylated-CyD, 2,3-mannoepoxide-CyD, and 3-amino-3-deoxy-CyD have a distorted cavity resulting from a conformational inversion of the modified glucopyranose unit from $^4C_1$ to $^1C_4$ or skewed boat conformation.[9–12,24] This distortion results in different guest binding capability as found for simple *N*-acetylamino-3-deoxy-3-amino-β- and γ-CyDs.[12] The distorted cavity breaks the symmetry of the CyD molecule, and thus, correlates with widespread and well-separated anomeric (C1–H) protons of CyD in ^{1}H NMR. This ring distortion effect would not participate strongly in the guest binding behavior of **3** and **4**. Indeed, as seen in Fig. 3, the anomeric proton signals of **4** were observed in the range 4.8–5.0 ppm with modest separation. A similar spectrum was obtained for **3**. However, the anomeric proton signals of **2** for which intrinsic ring distortion should not occur through the synthetic route also exhibited modestly separated signals (Fig. 3a). This separation may be due to the presence of the large modified residue of the benzocrown moiety. Thus, we considered that the β-CyD cavity of **3** and **4** was not greatly distorted, and concluded that the larger binding constants of **3** and **4** for Trp did not originate from the distortion of the cavity. Even if the cavity is distorted, binding capability for Trp would not be perturbed by the cavity distortion, because **5**, the cavity of which is distorted, showed binding capability comparable to β-CyD itself.

Comparison of the binding constants of **3** and **4** for Trp reveals **4** is a better host in binding Trp. This is attributable to a cation–dipole interaction between the ammonium moiety of Trp and the benzo-18-crown-6 moiety of **4**. Since benzo-18-crown-6 binds the ammonium group even in an aqueous medium, though the binding constant was remarkably small,[25] **4** can recognize the ammonium moiety of Trp, and thus, the interaction between the β-CyD cavity of **4** and the indole ring of Trp is stabilized by the additional polar interaction. Benzo-15-crown-5 is less effective in recognizing the ammonium cation of Trp than benzo-18-crown-6. This difference in the affinity to the ammonium cation causes the difference of the binding abilities of **3** and **4** to Trp.

Since the amino acid moiety of Trp is strongly hydrophilic, it would exist at the more hydrophilic secondary-hydroxy side of β-CyD when Trp forms a complex with β-CyD. The difference in binding capability between **1** and **3** or **2** and **4** is probably due to this difference in disposition of the amino acid moiety of Trp. The benzocrown moiety of **3** and **4** is attached at the more hydrophilic secondary-hydroxy side and they are effective in capturing the ammonium group when complexing with Trp as compared to the same benzocrown moiety of **1** and **2**, which is attached at the less hydrophilic primary-hydroxy side.

Table 2 CIS values of the Trp protons in the host–guest complexes with the crown ether-tethered CyDs

		CIS (ppm)[a]				
Host	D/L[b]	H2	H4	H5	H6	H7
1	D	(+)	(−)	−0.11	−0.10	(−)
	L	(+)	(−)	−0.12	−0.12	(−)
2	D	(+)	(−)	−0.11	−0.15	(−)
	L	(+)	(−)	−0.12	−0.15	(−)
3	D	(−)	−0.31	−0.48	−0.80	−0.53
	L	(−)	−0.30	−0.47	−0.77	−0.39
4	D	−0.19	−0.35	−0.36	−0.42	−0.59
	L	(−)	−0.37	−0.32	−0.38	−0.38
5	D	+0.03	(−)	−0.06	−0.07	(−)
	L	+0.05	(−)	−0.06	−0.07	(−)
β-CyD	D	+0.08	(−)	−0.06	−0.07	(−)
	L	+0.09	(−)	−0.06	−0.07	(−)

[a] Negative and positive values indicate upfield and downfield shifts, respectively, and (−) and (+) signs indicate small upfield and downfield shifts, respectively. [b] Configuration of Trp.

Potassium effect on binding Trp

As mentioned above, we consider the ammonium group of Trp would be recognized by the benzo-18-crown-6 moiety of **4**, though there is another possibility that carboxylate anion is recognized by K^+ bound by the benzo-18-crown-6 moiety, because our conditions for measuring ^{1}H NMR included a considerable amount of K^+ (0.0187 mol dm^{-3}) as counter cations of the phosphate buffer, and because it is known that benzo-18-crown-6 can bind K^+ even in aqueous solution.[25] To check this point, we obtained binding constants of **4** for Trp under the conditions of increased KCl concentration of 0.1 mol dm^3 of KCl. If a binding mode in which carboxylate anion is recognized by a K^+–benzo-18-crown-6 complex is operative, binding constants of **4** for Trp should be increased by addition of K^+. On the other hand, if an ammonium cation is directly recognized by the benzo-18-crown-6 moiety of **4**, competitive binding between potassium and ammonium cations should lead to smaller binding constants for Trp in the presence of an excess amount of K^+. Binding constants of **4** in the presence of 0.1 mol dm^{-3} K^+ were 158 ± 4 and 116 ± 4 dm^3 mol^{-1} for D- and L-Trp, respectively, being decreased from 188 ± 10 and 171 ± 5 dm^3 mol^{-1} for D- and L-Trp, respectively. This indicates that the latter hypothesis that the ammonium cation of Trp is captured by the benzo-18-crown-6 moiety is valid. Experimentally no effect upon the binding capability of **4** was observed when NaCl (0.1 mol dm^{-3}) was added (binding constants for D- and L-Trp were 180 ± 12 and 166 ± 11 dm^3 mol^{-1}). It is noteworthy that in the presence of an excess amount of K^+, the enantioselectivity of **4** was improved, though the stabilities of the **4**–Trp complexes were reduced.

Enantioselectivity upon binding Trp

From the data in Table 1, none of the host compounds examined here showed significant enantioselectivity in a thermodynamic manner. However, the enantioselectivity which emerged in the presence of K^+ implies that **4** (and other hosts) binds optical isomers of Trp with different conformations. Indeed, complexation-induced shifts (CIS; defined as $\delta_{\text{complexed guest}} - \delta_{\text{free guest}}$) of Trp protons, especially of H2 and H7 protons on the indole ring, were different between D- and L-Trp upon complexing with **4**, as seen in Table 2. Thus, it would be considered that conformations of Trp in the β-CyD cavity of **4** were different

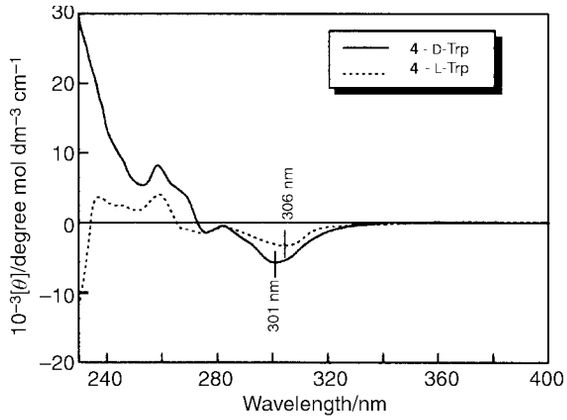


Fig. 4 Circular dichroism spectra of **4**–D-Trp (solid line) and **4**–L-Trp (dashed line) complexes in phosphate buffer (pH 7.4).

between D- and L-isomers. To verify this point, we measured circular dichroism (CD) spectra for **4**–D-Trp and **4**–L-Trp systems. Since both host and guest are optically active and have chromophores, they are CD active. This means that we had to estimate CD spectra derived from the pure complexes of **4**–Trp on the basis of the obtained binding constants. The subtracted spectra which would be for pure host–guest complexes of **4**–Trp systems are shown in Fig. 4. Although the subtracted spectra are weak due to the weak intrinsic CD bands of both host and guests, the differences between **4**–D-Trp and **4**–L-Trp were distinct; *i.e.* below 250 nm, the **4**–D-Trp complex showed positive CD while the **4**–L-Trp complex had nearly no CD signal, and the signal intensity of the CD spectrum around 300 nm was stronger for the **4**–D-Trp complex than for the **4**–L-Trp complex. In addition, the trough-bottom positions are different; the bottom-minimum for the **4**–L-Trp complex is found at 306 nm. This value is shifted by 5 nm as compared to that for the **4**–D-Trp complex (301 nm). Since a CD spectrum of a CyD complex is markedly sensitive to the location of the guest species in and near the CyD cavity,[26] those differences of the CD spectra of the complexes, together with the CIS differences, reveal that **4** can recognize the chirality of Trp upon binding.

CIS values for the other host–guest pairs did not change with respect to stereoisomers. This strongly indicates that no chiral recognition occurred for the hosts **1**–**3** and **5**. The reason why chiral recognition was observed only for **4**–Trp complexes probably relates to the simultaneous binding, that is the CyD cavity binds the indole ring and the benzo-18-crown-6 moiety binds the ammonium group of Trp. This cooperative binding is likely to be essential for chiral recognition.

Considering the small difference in the stability of the **4**–D-Trp and **4**–L-Trp complexes, we conclude that although **4** actually recognized the chirality of Trp upon complexing, as suggested by the CIS data and CD spectra, those conformational differences were too small to give apparent differences in the stability of the complexes. This may be attributable to the intrinsically weak interactions operating between the indole ring of Trp and β-CyD as well as between benzo-18-crown-6 and an ammonium cation in water.

Binding conformations for 3 and 4 with Trp

In order to get an insight into the structures of the complexes of the secondary-hydroxy side-modified CyDs **3** and **4** with Trp, we measured 2D-ROESY[27,28] spectra, which are a powerful tool to explore conformations of host–guest complexes of CyDs.[15–18,28,29] It is noted that in order to extract the information of the whole structure of the host–guest complex from ^{1}H NMR spectral data, it is essential to achieve unambiguous assignment of all the protons of a host and guest. However, the ^{1}H NMR spectra of **3** and **4**, in the absence and presence of Trp, were too complicated to assign them fully. Therefore, we only

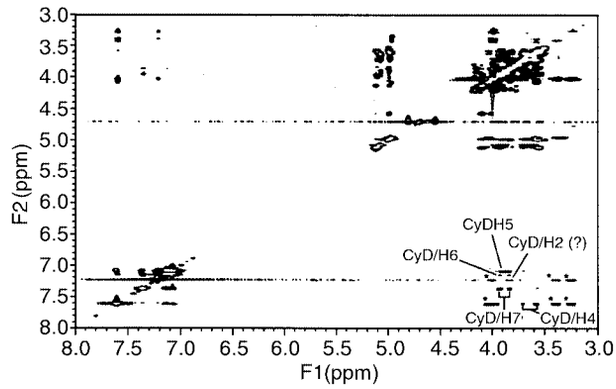


Fig. 5 2D-ROESY spectrum of a mixture of **4** and D-Trp.

Table 3 Relative NOE magnitudes observed for **3** or **4** complexes with Trp

		NOE magnitude[a]				
Host	D/L[b]	H2	H4	H5	H6	H7
3	D	0	1 (3.5–3.8)	1 (3.8)	0	2 (3.5–4.0)
	L	1 (3.9)	1 (3.9)	0	0	1 (3.6–3.8)
4	D	1 (3.7–3.8)	2 (3.5–3.8)	3 (3.8–4.0)	1 (3.8–4.0)	3 (3.8–4.0)
	L	2 (3.7–3.8)	2 (3.5–3.8)	3 (3.8–4.0)	1 (3.8–4.0)	3 (3.8–4.0)

[a] Relative magnitudes of NOE signals are indicated by 0 (not observed), 1 (weak), 2 (medium), and 3 (strong). The values in parentheses indicate chemical shift values of CyD protons to which NOEs of the Trp protons were observed. [b] Configuration of Trp.

focused on how Trp is accommodated in the CyD cavities of **3** and **4**. For this purpose, it is necessary only to assign all the protons of Trp. Fig. 5 shows the typical 2D-ROESY spectrum for the **4**–D-Trp complex. In this spectrum several intermolecular NOEs were found as well as intramolecular NOEs, indicating that, for instance, the H7 proton of Trp would be located close to the CyD protons. Table 3 shows the observed NOEs between CyD protons and D- or L-Trp protons, in which the numbers indicate the relative magnitudes of NOEs. It is noted that although the circular dichroism measurements indicated the conformational difference between D-Trp and L-Trp in the complexes of **4**, no drastic difference was observed in 2D-ROESY spectra between D-Trp and L-Trp in the presence of **4**. This suggests that the conformational differences were large enough to be detected by circular dichroism spectroscopy, which is especially sensitive to subtle differences in the conformations of CyD complexes, but they are too small to be detected by 2D-ROESY spectroscopy. Thus, we discuss herein the major NOE signal differences between Trp complexes of **3** and **4** to establish the role of the crown ring.

NOE signals were stronger when Trp was complexed with **4** than **3**, as expected from the binding strength that **4** could bind Trp more strongly than **3**. For **3**, relatively strong NOEs were observed between the H7 proton of Trp and CyD protons, and weak NOEs were found between H4 and H5 protons of Trp and CyD protons in the **3**–D-Trp complex. Weak NOEs of H2 and H4 were also found in the **3**–L-Trp complex. No NOE signal was found for the H6 proton of Trp, indicating that the H6 proton does not exist close to the CyD protons. On the other hand, all the protons of Trp exhibited NOE signals with CyD protons of **4**. In addition, for instance, NOE signals of H7 of D- and L-Trp correlated with the **3** protons at 3.5–4.0 ppm, while those correlated with the **4** protons at 3.8–4.0 ppm. This difference indicates that the spatial location of the H7 of Trp was different between the complexes of **3** and **4**.

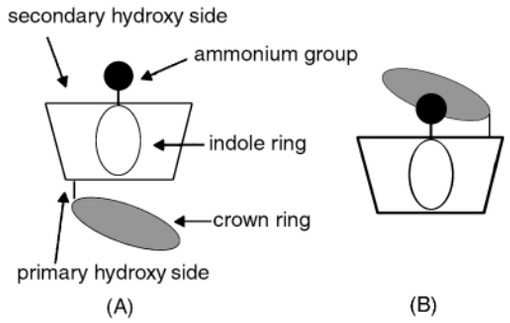


Fig. 6 Schematic illustrations of binding modes of the primary-hydroxy side-modified β-CyDs (A) and the secondary-hydroxy side-modified β-CyDs (B), focusing on the difference of the position of the crown ring.

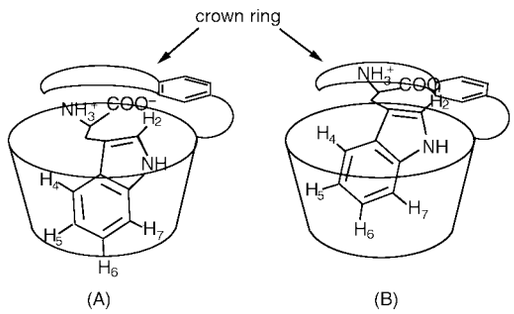


Fig. 7 Plausible binding conformations of **3** (A) and **4** (B) with Trp, focusing on the difference in the strength of the ammonium–crown interaction.

Combining the results of 2D-ROESY, binding strength, and Corey–Pauling–Koltun molecular model considerations, we drew up a possible arrangement with respect to the β-CyD derivatives and Trp, as depicted in Figs. 6 and 7. The benzo-crown moieties of **1** and **2** scarcely contribute to increasing the binding abilities of **1** and **2** for Trp, because the ammonium group of Trp would preferably exist at the secondary-hydroxy side of the CyD cavity, whereas the benzocrown moieties of **1** and **2** are attached at the primary-hydroxy side. On the other hand, both the ammonium moiety of Trp and the benzocrown moieties of **3** and **4** exist at the secondary-hydroxy side of **3** and **4** (Fig. 6). This mutual location causes ammonium–crown interaction, leading to the strong binding. Since the 18-crown-6 ring of **4** shows a much stronger preference for recognizing the ammonium group than the 15-crown-5 ring of **3**, the indole ring of Trp may be incorporated shallowly in the complex with **4** to maximize the ammonium–crown interaction. In contrast, in the complex with **3**, the ammonium–crown interaction is not strong enough to capture the ammonium group tightly. Therefore, the indole ring would be incorporated deep in the cavity of **3** to maximize the CyD cavity–indole interaction (Fig. 7). H5 and H6 protons of Trp exist close to the CyD protons in the complex of **4**, while these protons are nearly free from the CyD protons in the complex of **3**. These differences result in the difference of the pattern of the NOE signal appearance.

Conclusion

We described herein the superiority of the secondary-hydroxy side-modified β-CyD over the primary-hydroxy side-modified one in molecular recognition of Trp. Molecular recognition ability of CyD for Trp was improved by the cooperation of hydrophobic binding by the CyD cavity and ammonium cation binding by the benzocrown moiety. This cooperation was shown only by the benzo-18-crown-6 moiety appended at the secondary-hydroxy side of β-CyD. The benzocrown moiety appended at the primary-hydroxy side was not effective for binding Trp. Our results are helpful in designing and constructing molecular recognition devices acting in an aqueous medium.

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