# Artificial Receptors for Amino Acids in Water. Local Environmental Effect on Polar Recognition by 6*A*-Amino-6*B*-carboxy- and 6*B*-Amino-6*A*-carboxy-β-cyclodextrins

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**Abstract:** Cyclodextrins having both positive and negative charges on C6 carbons of A and B (or B and A) glucose rings, respectively, **1** (or **2**), were newly synthesized in order to investigate their molecular recognition capacities toward hydrophobic L- and D-α-amino acids based on triple recognition. These host–guest couples allow us to study the magnitude of polar interactions in an aqueous solution by use of fluorescent ANS as a competitive inhibitor. Among couples studied, strongest association was observed for the **1Y**·D-Trp complex for which the estimated equilibrium constant is (54 ± 8) $M^{-1}$ at 25 °C, and the weakest interaction was observed for the **2X**·L-Trp complex for which the equilibrium constant is (15 ± 10) $M^{-1}$ at 25 °C. From the observed temperature dependence of the host–guest equilibrium constants, host–guest interaction enthalpies were estimated. From comparison of these values with the parent cyclodextrin–guest interaction enthalpy, polar interaction ($\Delta H_{pol}$) between ionic groups on the host and those of the guest was estimated. The polar interaction between D-Trp and **2** having more hydrophilic environment around ionic groups is −0.9 kcal/mol, being much weaker than the London's dispersion force ($\Delta H_{disp}$ = −10 kcal/mol) estimated for the CD–guest complexes. On the other hand, for **1Y** having more hydrophobic environment around ionic groups, the polar interaction operating in **1Y**·D-Trp complex becomes stronger (−4.6 kcal/mol), suggesting the significance of hydrophobic environment for enhancement of polar interaction. Even for this couple, however, the magnitude of polar interaction is still smaller than the London's dispersion force.

Recently, much attention has been paid for elucidation of the driving force which drives complexation between a cyclodextrin and a guest molecule.[1] The latest studies are more oriented toward investigation of possible change in binding capacity caused by functionalization of the parent cyclodextrins.[2] In these approaches, understanding of inclusion mechanism is mostly attained based on molecular thermodynamics.

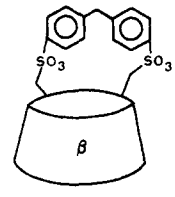
For the complexation with *apolar* guests, we have presented a model[3] for calculation of free energy change upon inclusion in water (eq 1 and 2) where $\Delta H_{disp}$ is the London's dispersion energy[4]

$$\Delta H_{inclusion} = \Delta H_{disp} + \Delta H_{conf} + \Delta H_{host\ solv} + \Delta H_{guest\ solv} \quad (1)$$

$$\Delta S_{inclusion} = \Delta S_{motional\ freedom} + \Delta S_{host\ solv} + \Delta S_{guest\ solv} \quad (2)$$

between a cyclodextrin and a guest; $\Delta H_{host\ solv}$ is the enthalpy change required for stripping water molecules from the cyclodextrin cavity, $\Delta H_{guest\ solv}$ is the enthalpy change upon the loss of water assembly around the bound guest molecule, and $\Delta S_{motional\ freedom}$ is the entropy change due to the restriction of motional freedom of the bound guest. $\Delta S_{host\ solv}$ and $\Delta S_{guest\ solv}$ are the entropy changes associated with the same process as mentioned for $\Delta H$.

Estimation of each term involved in eq 1 and 2 indicated that $\Delta H_{disp}$ and $\Delta S_{guest\ solv}$ are sensitive to the structure of the guest molecules, and these two terms are among main driving forces of inclusion. This simplified model for the inclusion process was ascertained to be valuable for design of functionalized cyclodextrins based on rough estimation of $\Delta G_{inclusion}$. For example, capping of cyclodextrin with aromatic moiety is expected to enhance both the London's dispersion energy ($\Delta H_{disp}$) and the entropy gain caused by host dehydration ($\Delta S_{host\ solv}$). In fact, capped β-cyclodextrins, **9** and **10**, bind 1-anilino-8-naphthalenesulfonate (ANS) by 24 and 11 times stronger (1.9 and 1.4 kcal/mol more favorable), respectively, than the parent β-cyclodextrin.[5]

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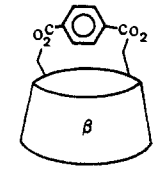


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In order to design a cyclodextrin derivative bearing polar substituents as recognition elements, it may be necessary to take polar interaction into calculation of free energy change. Polar interaction (point charge–point charge, point charge–dipole, dipole–dipole interactions, etc.) operating in the inclusion complex is the subject of controversy in the recent studies on the guest binding to cyclodextrins.[2,6] Meanwhile polar interaction ($\Delta G°$) between a charged cyclodextrin derivative and a charged guest is reported to be small, ranging between −1 and −4 kcal/mol depending on host–guest combination. This fact suggests that the polar interaction is indeed not very important. Under the circumstances, more explicit experiments for determination of

(1) (a) Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344–362. (b) Tabushi, I.; Kuroda, Y. *Adv. Catal.* **1983**, *32*, 417–466. (c) Tabushi, I. *Acc. Chem. Res.* **1982**, *15*, 66–72. (d) Tabushi, I. *Tetrahedron* **1984**, *40*, 269–292. (e) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer Verlag: New York, 1978. (f) Szejtli, J. *Cyclodextrins and Their Inclusion Complexes*; Akademiai Kiado: Budapest, 1982.

(2) (a) Tabushi, I.; Shimizu, N.; Sugimoto, T.; Shiozuka, M.; Yamamura, K. *J. Am. Chem. Soc.* **1977**, *99*, 7100–7102. (b) Boger, J.; Brenner, D. G.; Knowles, J. R. *Ibid.* **1979**, *101*, 7630–7631. (c) Boger, J.; Knowles, J. R. *Ibid.* **1979**, *101*, 7631–7633. (d) Tabushi, I.; Kuroda, Y.; Mizutani, T. *Tetrahedron* **1984**, *40*, 545–552.

(3) Tabushi, I.; Kiyosuke, Y.; Sugimoto, T.; Yamamura, K. *J. Am. Chem. Soc.* **1978**, *100*, 916–919.

(4) Eyring, H.; Walter, J.; Kimball, G. E. *Quantum Chemistry*; Wiley: New York, 1944; pp 351–355. We use the term "London's dispersion force" instead of "Van der Waal's force" because the latter usually involves the contribution from polar interactions such as dipole–dipole and dipole–induced dipole interactions. In the previous article[3] we discussed an apolar guest binding to unsubstituted α-cyclodextrin, in which contribution of polar interaction was nearly negligible. Therefore, "Van der Waal's" and "dispersion" were not explicitly distinguished. In the present case where polar interaction becomes much more important, "dispersion" term free from polar interaction is taken.

(5) Tabushi, I.; Shimokawa, K.; Shimizu, N.; Shirakata, H.; Fujita, K. *J. Am. Chem. Soc.* **1976**, *98*, 7855–7856.

(6) (a) Gelb, R. I.; Schwartz, L. M.; Cardelino, B.; Fuhrman, H. S.; Johnson, R. F.; Laufer, D. A. *Ibid.* **1981**, *103*, 1750–1757. (b) Bergeron, R. J.; Channing, M. A.; Gilbeily, G. J.; Pillor, D. M. *Ibid.* **1977**, *99*, 5146–5151. (c) Bergeron, R. J.; Channing, M. A.; McGovern, K. A. *Ibid.* **1978**, *100*, 2878–2883. (d) Bergeron, R. J.; Channing, M. A.; McGovern, K. A.; Roberts, W. P. *Bioorg. Chem.* **1979**, *8*, 263–281.

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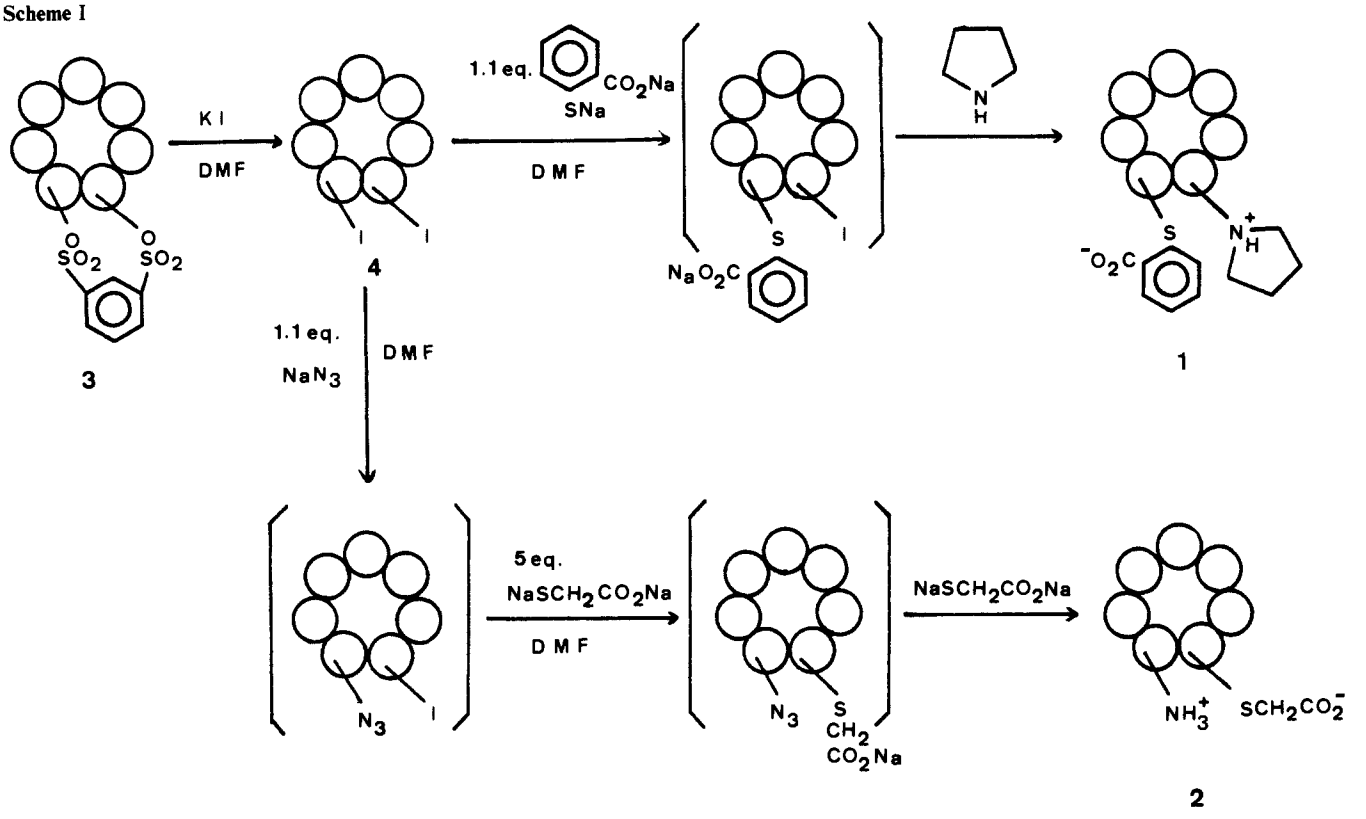
**Scheme I**

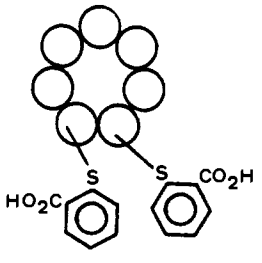



significance of the polar interaction are warranted.

In the present article, significance of the point charge–point charge interaction in the complex formation is investigated experimentally for the charged guests by use of two functionalized cyclodextrins bearing both the ammonium group and the carboxylate group as recognition elements.

## Results and Discussion

**Preparation of *A*-Amino-*B*-carboxy- and *B*-Amino-*A*-carboxy-β-cyclodextrin 1 and 2.**[7] Two functionalized cyclodextrins **1** and **2** were prepared from *A,B*-capped-β-cyclodextrin[8] **3** via the stepwise nucleophilic substitution (Scheme I).[9] Thus, 6*A*,6*B*-diiodo-6*A*,6*B*-dideoxy-β-CD (**4**) was treated with 1.1 equiv of disodium thiosalicylate in DMF, then DMF was evaporated, and the residue was heated in pyrrolidine to give a mixture of **1**, 6*A*,6*B*-bis((*o*-carboxyphenyl)thio)-6*A*,6*B*-dideoxy-β-CD (**5**), and 6*A*,6*B*-di-1-pyrrolidinyl-6*A*,6*B*-dideoxy-β-CD (**6**). The target



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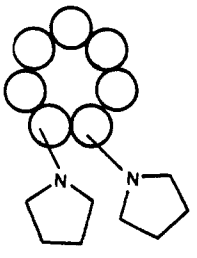
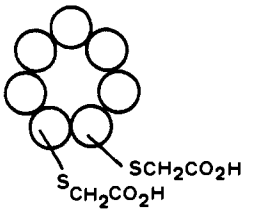


**6**

molecule **1**, as a regioisomer mixture, was successfully separated through reprecipitation and ion exchange column chromatography (CM-Sephadex C-25). Similarly, 6*A*,6*B*-diiodo-6*A*,6*B*-dideoxy-β-CD was treated with sodium azide in DMF followed by 5 equiv of disodium thioglycolate. The reduction of the azide function to amine was carried out in situ with disodium thioglycolate. From the reaction mixture containing **2**, 6*A*,6*B*-bis((carboxymethyl)-thio)-6*A*,6*B*-dideoxy-β-CD (**7**), and 6*A*,6*B*-diamino-6*A*,6*B*-dideoxy-β-CD (**8**), **2** was separated by reprecipitation and ion ex-



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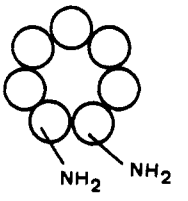

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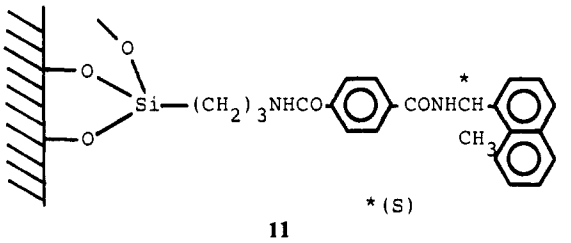
change chromatography (Amberlite CG-50 and CM-Sephadex C-25). The purified regioisomer mixtures of **1** and **2** were then applied to a "chiral" column packed with **11**.[10] Perfect separation



**11**

of *A,B*- and *B,A*-regioisomers was observed when the mixtures were eluted with 9/1 (v/v) water–ethanol for **1** or with water for **2**. The structures of **1** and **2** were confirmed by $^1H$ NMR, $^{13}C$ NMR, IR, UV spectra, and elemental analysis.[11]

**Binding of an Ionic Guest to Cyclodextrin Bearing Ionic Recognition Groups.** Association constants (*K*) between D-Trp and the cyclodextrin derivatives, **1X**, **1Y**, **2**, and β-CD, were determined by the competitive binding based on the measurements of ANS

$$\text{host} + \text{ANS} \rightleftarrows \text{host·ANS}$$

$$\text{host} + \text{D-Trp} \rightleftarrows \text{host·D-Trp}$$

(7) Nomenclature A and B means the relative location of glucose rings. See: Tabushi, I.; Nabeshima, T.; Fujita, K.; Matsunaga, A.; Imoto, T. *J. Org. Chem.* **1985**, *50*, 2638.

(8) Tabushi, I.; Nabeshima, T.; Fujita, K.; Matsunaga, A.; Imoto, T. *J. Org. Chem.* **1985**, *50*, 2638–2643.

(9) For the notation for glucose rings of β-cyclodextrin, see ref 8.

(10) Oi, N.; Nagase, M.; Doi, T. *J. Chromatogr.* **1983**, *257*, 111–117.

Table I. Association Constants ($K$) between Trp and Cyclodextrin Derivatives[a]

| host | guest | $K$ ($M^{-1}$) |
|---|---|---|
| **1X** | D-Trp | 45.5 ± 8.2 |
| **1X** | L-Trp | 34.5 ± 5.7 |
| **1Y** | D-Trp | 54.0 ± 7.6 |
| **1Y** | L-Trp | 42.5 ± 7.3 |
| **2**[b] | D-Trp | 15 ± 10 |
| β-CD | D-Trp | 13 ± 8 |

[a] 25 °C. [b] Observed differences in $K$ values between **2X** and **2Y** are in the range of experimental error.

Table II. $\Delta G°$, $\Delta H°$, and $\Delta S°$ of Complex Formation of CD·D-Trp[a]

| host | $\Delta G°$ (kcal/mol) | $\Delta H°$ (kcal/mol)[14] | $\Delta S°$ (cal/deg mol) mol) |
|---|---|---|---|
| **1Y** | −2.3 ± 0.1 | −4.3 ± 1.8 | −6.7 ± 6.1 |
| **2**[b] | −1.6 ± 0.4 | −0.6 ± 1.1 | +3.4 ± 3.9 |
| β-CD | −1.5 ± 0.3 | +0.3 ± 1.3 | +6.0 ± 4.5 |

[a] 25 °C, pH 8.9 borate buffer solution containing 15.4 mM $Na_2B_4O_7$ and 34.6 mM $H_3BO_3$. [ANS] = $3.6 \times 10^{-5}$ M, [CD] = ca. $1 \times 10^{-3}$ M, [D-Trp] = $5 \times 10^{-3} - 2 \times 10^{-2}$ M. [b] By using approximately equimolar mixture of *A,B*- and *B,A*-isomers.

fluorescence intensities at 500 nm by changing the D-Trp concentration from $5 \times 10^{-3}$ to $2 \times 10^{-2}$ M at the 1-mM host concentration[5] (see eq 3 and Table I).

$K$ was calculated from eq 3 by using the host·ANS association constants ($K'$) which had been independently determined.

$$K = \left\{ \frac{K'}{\Delta I/\Delta\epsilon} \left( [\mathrm{CD}]_0 - \frac{\Delta I}{\Delta\epsilon} \right) \left( [\mathrm{ANS}]_0 - \frac{\Delta I}{\Delta\epsilon} \right) - 1 \right\} \frac{1}{[\mathrm{Trp}]}$$

$$[\mathrm{Trp}]_0 \gg [\mathrm{CD}]_0 \gg [\mathrm{ANS}]_0 \qquad (3)$$

where $\Delta I$, the increase in fluorescence intensity of $3.6 \times 10^{-5}$ M of the aqueous ANS solution by the addition of $5 \times 10^{-4}$ M of the host, was measured as a function of the Trp concentration ($5 \times 10^{-3} - 2 \times 10^{-2}$ M). $\Delta\epsilon$ was calculated from eq 4.

$$\Delta\epsilon = \frac{\Delta I_0(1 + K'[\mathrm{CD}]_0)}{K'[\mathrm{CD}]_0[\mathrm{ANS}]_0} \qquad ([\mathrm{CD}]_0 \gg [\mathrm{ANS}]_0) \qquad (4)$$

where $\Delta I_0$ is $\Delta I$ in the absence of Trp.

The association constants for the host·Trp complexes thus estimated (Table I) were in the range of 10–60 $M^{-1}$, being smaller than most of the reported values for β-CD·organic guest complexation ($K = 10^2 - 5 \times 10^3$ $M^{-1}$).[1e,2d,12] This small $K$ value for the β-CD·Trp complex is in accord with a general trend that hydrophilic guests (such as cyclohexane carboxylate or *trans*-cyclohexane dicarboxylate) are not bound strongly to cyclodextrins.[2d] The $K$ value for **2**·Trp is only 1.1 times larger than that for β-CD·Trp indicating that additional charge–charge interaction in **2**·Trp complex is weak, probably because the local environment is too hydrophilic around ionic groups of **2** to provide any reasonable Coulombic interaction.

In an interesting contrast, the association constants ($K$) for the **1X**·Trp (or **1Y**·Trp) complexes are 3 or 4 times larger, respectively, than that for the β-CD·Trp complex. The observation indicates that the hydrophobic local environment is essential for reasonable enhancement of the Coulombic interaction between ions in water. Even in this case, the Coulombic interaction free energy ($\Delta G_{pol}$) estimated from the ratio $K$(**1**·Trp)/$K$(β-CD·Trp) amounts to 0.7 kcal/mol. A tentative explanation for the weak Coulombic interaction is loss of one-dimensional rotational freedom of the guest in the host cavity due to multiple recognition since the freedom is kept at least partially in the CD–guest complex based on our preliminary measurements of $^1$H NMR relaxation time of the guests.[13]

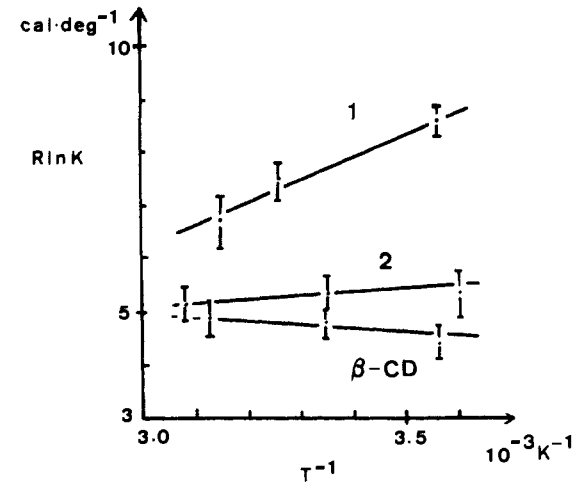



**Figure 1.** Plot of $R$ ln $K$ vs. $1/T$. The association constant between cyclodextrins and D-Trp ($K$) was determined by use of competitive binding method with ANS in a pH 8.9 borate buffer solution at 5–50 °C (see Experimental Section). $\Delta H°$ and $\Delta S°$ were calculated from the thermodynamic relations $d(R \ln K)/d(1/T) = -\Delta H°$, $\Delta G° = \Delta H° - T\Delta S° = -RT \ln K$.

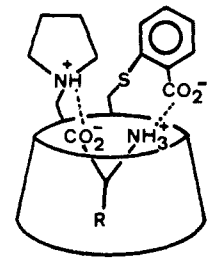



**Figure 2.** Polar interaction between **1Y** and D-Trp. Schematic representation of configuration in **1Y**·Trp complex, in which both hydrophobic interaction between indolylmethyl (R) and cyclodextrin cavity and polar interaction between ionic groups are operating.

Table III. Polar Interaction between CD and D-Trp[a]

| host | $\Delta G°_{pol}$[b] | $\Delta H°_{pol}$[b] | $\Delta S°_{pol}$[c] |
|---|---|---|---|
| **1Y** | −0.8 ± 0.3 | −4.6 ± 2.1 | −12.7 ± 7.6 |
| **2** | −0.1 ± 0.5 | −0.9 ± 1.7 | −2.6 ± 6.0 |

[a] Polar interaction was calculated from the equation by use of values listed in Table II: $\Delta G°_{pol} = \Delta G°$ (**1** or **2**) − $\Delta G°$(β-CD). See footnote of Table II. [b] kcal/mol. [c] cal/deg.mol.

Values of $\Delta H°$ and $\Delta S°$ for these complexation reactions were obtained from the measurement of $K$ as a function of temperature (5–50 °C) and listed in Table II. The plot of $R \ln K$ vs. $1/T$ is shown in Figure 1. Assuming that the difference in $\Delta G°$, $\Delta H°$, and $\Delta S°$ between the **1Y**·Trp (or **2**·Trp) complex and the β-CD·Trp complex is due to the polar interaction between ionic groups (Figure 2); the magnitude of polar interaction under the conditions was estimated experimentally, as listed in Table III. The ion–ion interaction ($\Delta G°_{pol}$) was thus estimated to be −0.1 to −0.8 kcal/mol. Considering that the dipole–dipole interaction in uncharged CD–guest complexation must be even smaller than the rather weak ion–ion interaction estimated in the present study, the dipole–dipole interaction in uncharged polar guest–cyclodextrin

(11) Our preliminary experiments on Taka-amylase hydrolysis of **1** also supported its structure.

(12) (a) Miyaji, T.; Kurono, Y.; Uekama, K.; Ikeda, K. *Chem. Pharm. Bull.* **1975**, *24*, 1155–1159. (b) Bergeron, R. J.; Meeley, M. P. *Bioorg. Chem.* **1976**, *5*, 197–202. (c) Harrison, J. C.; Effink, M. R. *Biopolymers* **1982**, *21*, 1153–1166.

(13) Relaxation time measurements for a variety of guests will be published.

(14) It is difficult to determine the formation enthalpies of inclusion complexes in the present case because of (1) small magnitude of $\Delta G°$ and (2) narrow temperature range (at most 30–40 K) for the measurement of K. It should also be noted that changes in water structure affect the estimated total $\Delta H°$, from which $\Delta H$ of host–guest interaction cannot be easily separated.

(15) Momany, F. A.; McGuire, R. F.; Burgess, A. W.; Scheraga, H. A. *J. Phys. Chem.* **1975**, *79*, 2361–2381.

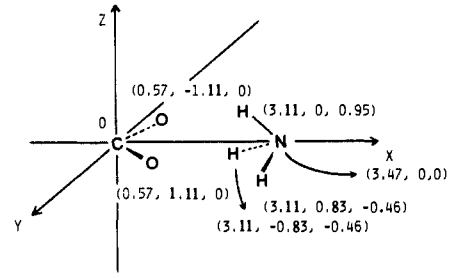



**Figure 3.** Polar interaction between $-NH_3^+$ and $-COO^-$. The distance between $-NH_3^+$ and $-COO^-$ was fixed so that the oxygen atoms of $-COO^-$ come into contact with the hydrogen atoms of $-NH_3^+$ with van der Waals radii. Partial charges on atoms of $Glu^-$, $Asp^-$, and $Lys^+$ reported by Scheraga et al.[15] were used and shown in parentheses in electronic charge unit: C(+0.5), O(−0.57), N(−0.32), H(+0.32). Geometry of each group was also taken from the same article.[15]

inclusion complexes seems to be of minor importance, although there are contradictory discussions.[2,6] It should also be noted that the polar interaction ($\Delta H_{pol}$) between Trp and the hydrophilic cyclodextrin with polar recognition groups (**2**, −0.9 kcal/mol) is considerably smaller than the usual London's dispersion energy, $\Delta H_{disp}$ = −10 kcal/mol[3]), for CD–guest complexes (vide infra), supporting the above conclusion. However, polar interaction becomes more important in hydrophobic environment, as seen for Trp·**1Y** complexation ($\Delta H_{pol}$ = −4.6 kcal/mol), although the magnitude is still smaller than the dispersion force.

Interestingly, the observed polar interaction free energies ($\Delta G°_{pol}$) are very small in magnitude, even for the hydrophobic host **1**. As clearly seen in Table III, remarkably large (unfavorable) entropy loss ($\Delta S_{pol}$) is involved in the triple recognition–complexation, compensating otherwise large enthalpy gain (negative $\Delta H_{pol}$) to a considerable extent. This is partly due to loss of rotational freedom of the guest in the host–guest complex according to our preliminary experiments, which show that a considerable rotational freedom of a singly charged guest still remains in the guest–singly charged CD complex (for double recognition) based on NMR relaxation studies.

**Computation of Polar Interaction between Tryptophan and Cyclodextrin–Ammonium–Carboxylate 1Y.** The electrostatic energy between the ammonium group and the carboxylate group was independently calculated for the geometry shown in Figure 3 with the aid of eq 5 where $e$ is 1.6 × $10^{-19}$ C, and $r_{ij}$ is the

$$\Delta H_{pol} = \frac{1}{4\pi\epsilon\epsilon_0}\sum\frac{q_iq_j}{r_{ij}} = \frac{332}{\epsilon}\sum\frac{(q_{i/e})(q_{j/e})}{r_{ij}/\text{Å}} \text{ (kcal/mol)} \qquad (5)$$

distance between two point charges $q_i$ and $q_j$. As a local dielectric constant ($\epsilon$), 54 and 18 are assumed. The former value is experimentally determined by use of the observed fluorescence intensity of the ANS·**1** complex. The observed intensity is interpolated from the correlation independently obtained for the observed fluorescence intensities of ANS in a series of water–methanol mixed solvent vs. the dielectric constants of the solvent system (see Figure 4). The latter value of 18 is taken from that of "isolocal" structure compound, isopropanol (for deep interior of cavity and in the absence of any other molecule around, it must give theoretically maximum polar interaction). Computation based on this simplified model afforded the polar interaction energy for **1Y**·Trp to be −1.0 kcal/ion pair or −2.0 kcal/two ion pairs by taking 54 as a local $\epsilon$ or −3.0 kcal/ion pair or −5.9 kcal/two ion pairs by taking 18 as a local $\epsilon$. The calculated values are in good agreement with the experimental value, $\Delta H°_{pol}$ = −4.6 kcal/mol, for the **1Y**·D-Trp complex.

## Conclusion

The polar interaction energies ($\Delta H°_{pol}$) estimated for the hydrophobic **1Y**·Trp complex and for the hydrophilic **2**·Trp complex are −4.6 and −0.9 kcal/mol, respectively, indicating the significance of hydrophobic environment for enhancement of the polar interaction. In the hydrophilic **2**·Trp complex, the ionic interaction, −0.9 kcal/mol, is far less important than the London's dispersion

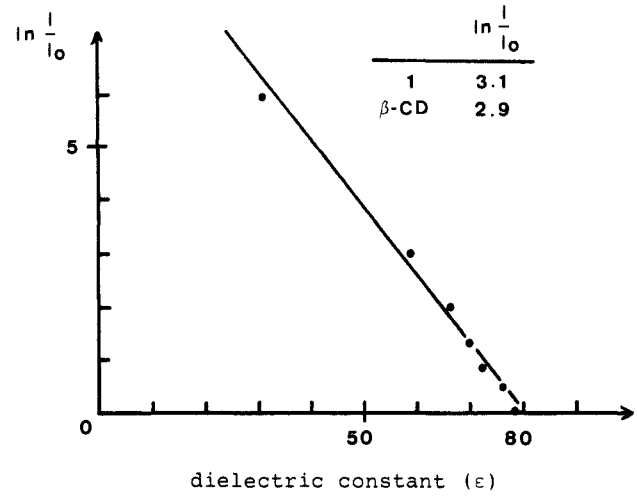



**Figure 4.** Correlation between the fluorescence intensity of ANS and the dielectric constant of the solvent in water–methanol mixture. $I$ is the fluorescence intensity of ANS in water–methanol mixtures and $I_0$ is the fluorescence intensity of ANS in water. Fluorescence intensity of ANS in the presence of cyclodextrins was corrected for the concentration of CD·ANS complex: [ANS] = 3.6 × $10^{-5}$ M, [CD] = 3.8 × $10^{-3}$ M, 25 °C.

energy, $\Delta H_{disp}$, of −10 kcal/mol. And even in the hydrophobic **1Y**·Trp complex, the ionic interaction, $\Delta H°_{pol}$, is still smaller than the usual London's dispersion interaction ($\Delta H_{disp}$ = −10 kcal/mol).

## Experimental Section

**Apparatus.** Recorded on a JEOL JMN-GX 400 were 400-MHz $^1H$ NMR spectra and 100-MHz $^{13}C$ NMR spectra. Fluorescence spectra were obtained on a Union FS 301 fluorescence spectrophotometer. UV–vis spectra were recorded on a Union SM 401 or a Hitachi 330 spectrophotometer. IR spectra were recorded on a Hitachi Model 215 spectrophotometer. High performance liquid chromatography (HPLC) was performed on a Waters Model 6000 instrument with an enantiomer separation column OA-1000A (25 cm × 4.6 mm i.d., Sumitomo Chem. Ind. Co.). Thin-layer chromatography (TLC) was performed by using Merck silica gel 60 F-254.

**Materials.** β-Cyclodextrin (Ando Kasei Industry) was recrystallized from water. *N,N*-Dimethylformamide (DMF) was dried over $P_2O_5$ for several days at room temperature. Then DMF was decanted, shaken with KOH pellets, and then distilled under reduced pressure. Disodium thioglycolate was prepared by mixing thioglycolic acid and sodium ethoxide in ethanol followed by evaporation of the solvent to dryness. Disodium thiosalicylate was obtained by mixing an ethanol solution of thiosalicylic acid and an ethanol solution of sodium ethoxide. The white precipitates were collected by filtration, washed with ethanol, and dried in vacuo. Sodium 8-anilino-1-naphthalenesulfonate (ANS) was obtained from Tokyo Kasei Kogyo Co. Ltd. Pyrrolidine, thioglycolic acid, and thiosalicylic acid were obtained from Wako Pure Chemical Industries. Sodium azide was obtained from Nakarai Chemicals Ltd. Fluorescamine was obtained from Sigma. CM-Sephadex C-25 and DEAE-Sephadex A-25, A-50 were obtained from Pharmacia Fine Chemicals, and Amberlite CG-50 was obtained from Rohm and Haas Co. *A,B*-Capped-β-cyclodextrin **3** was prepared according to our published method.[8]

**6*A*,6*B*-1-Pyrrolidinyl((*o*-carboxyphenyl)thio)-6*A*,6*B*-dideoxy-β-cyclodextrin (1).** 6*A*,6*B*-Diiodo-6*A*,6*B*-dideoxy-β-cyclodextrin (**4**) (2.16 g, 1.6 mmol) was dissolved in 20 mL of dry DMF, and into the solution 0.35 g of disodium thiosalicylate was added. The mixture was stirred at 50 °C for 30 min under argon. DMF was evaporated under reduced pressure, and 30 mL of pyrrolidine was added to the residue. The reaction mixture was heated to 80 °C for 2 h. The pyrrolidine was distilled off, and the residue was dried in vacuo. The crude mixture was dissolved in 5 mL of water, and the solution was added to 200 mL of ethanol. The white precipitates formed were filtered and washed with ethanol, and the solid was dissolved in 20 mL of water. The solution was acidified with 0.1 N HCl (pH 3.0), and then 3 mL of tetrachloroethylene was added to the solution. The mixture was stirred in an ice bath for 30 min. The precipitates were filtered off, and the filtrate was evaporated. The residue was then applied on a CM-Sephadex C-25 column ($Na^+$ form) and eluted with water. The desired product **1** was eluted after the top fraction bis((*o*-carboxyphenyl)thio)-β-cyclodextrin (**5**); yield of **1** was 0.28 g (13%): IR (KBr) 1580 $cm^{-1}$; UV ($H_2O$), 258 nm ($\epsilon$ 2600), 302 nm (500).

Table IV. $^1$H NMR of 1[a]

| protons | 1X | 1Y |
|---|---|---|
| β-protons of pyrrolidine | 1.7–2.0 (4 H, m) | 1.5–1.9 (4 H, m) |
| other protons | 2.9–4.1 (46 H, m) | 2.9–4.0 (46 H, m) |
| H-1 | 4.95–5.1 (6 H, m) | 4.95–5.08 (6 H, m) |
| H-1 | 5.2 (1 H, d, $J$ = 3.5 Hz) | 5.14 (1 H, d, $J$ = 3.5 Hz) |
| aromatic | 7.15 (1 H, t, J = 7 Hz) | 7.15 (1 H, t, $J$ = 7 Hz) |
| aromatic | 7.20 (1 H, t, J = 7 Hz) | 7.20 (1 H, t, $J$ = 7 Hz) |
| aromatic | 7.40 (1 H, d, J = 7 Hz) | 7.30 (1 H, d, $J$ = 7 Hz) |
| aromatic | 7.55 (1 H, d, J = 7 Hz) | 7.60 (1 H, d, $J$ = 7 Hz) |

[a] [1] = 0.035 M in $D_2O$, with a 5-mm sample tube, p$D$ = 8.5, 25 °C, δ (ppm from TSP).

Table V. $^{13}$C NMR of 1[a]

| carbons | 1X | 1Y |
|---|---|---|
| β-carbons of pyrrolidine | 24.7 | 23.6, 23.9 |
| C-6 carrying thiosalicyl | 36.5 | 36.2 |
| α-carbons of pyrrolidine | 57.1 | 56.4 |
| C-6 carrying pyrrolidine | 57.6 | 56.7 |
| C-6 | 62.1, 62.2 | 60.9–62.0 |
| C-5 closest to pyrrolidine | 72.0 | 69.5 |
| C-2, -3, -5 | 72.8–75.0 | 72.8–75.0 |
| C-4 | 83.0–83.4 | 81.9–82.7 |
| C-4 closest to pyrrolidine | 85.5 | 84.7 |
| C-4 closest to thiosalicyl | 86.5 | 86.3 |
| C-1 | 103.3, 103.8 | 102.7, 103.0 |
| C-1 | 104.0, 104.1 | 103.1, 103.2 |
| aromatic carbon | 127.2, 130.0 | 126.7, 127.6 |
| aromatic carbon | 130.8, 131.1 | 130.4, 130.7 |
| aromatic carbon | 135.5, 141.6 | 136.6, 139.6 |
| carbonyl | 177.0 | 175.9 |

[a] [1] = 0.02 M in $D_2O$ with a 10-mm sample tube, at 25 °C, ppm from external $Me_4Si$ in $CDCl_3$.

HPLC analysis on a OA-1000A column eluted with 9/1 (v/v) water–ethanol showed that the product was a mixture of two isomers, **1X** (elution volume 14 mL) and **1Y** (elution volume 16 mL). These isomers were separated on a DEAE-Sephadex A-25 ($Cl^-$ form) preparative column. The mixture of two isomers was applied on the column and eluted with water at the rate of 1 drop per 5 s. Each fraction was analyzed by HPLC on a OA-1000A column, and the fractions containing both **1X** and **1Y** were rechromatographed on the same column. Practically pure **1X** and **1Y** (purity of each was higher than 95% based on HPLC analysis) were obtained, from which pure regioisomers were obtained after recrystallizations.

$^1$H and $^{13}$C NMR data are summarized in Tables IV and V. Anal. Calcd for **1**·8$H_2O$: H, 6.66; C: 43.35; S: 2.18. Found: H, 6.44; C, 43.17; S, 2.29.

**6*A*-Amino-6*B*-((carboxymethyl)thio)-6*A*,6*B*-dideoxy-β-cyclodextrin (2).** 6*A*,6*B*-Diiodo-6*A*,6*B*-dideoxy-β-cyclodextrin (**4**) (0.474 g, 0.35 mmol) was dissolved in 4 mL of dry DMF, and into the solution was added 25 mg of sodium azide. The mixture was stirred at 65 °C for 3 h, and then 0.428 g of disodium thioglycolate was added. The suspension was stirred at 65 °C for 3 h. To the reaction mixture was added 3 mL of water, and the mixture was stirred at 80 °C for 3 h. The solvent was removed by vacuum distillation, and the residue was dried in vacuo. The crude product was dissolved in 3 mL of water, and the solution was added to 130 mL of ethanol with stirring. White precipitates formed were filtered, washed with ethanol, and dissolved in the mixture of 2.5 mL of 0.6 N HCl and 2 mL of ethanol. The solution was applied to an Amberlite CG-50 column ($Na^+$ form, eluted with ethanol–water mixture, 1:1 (v/v)). The crude product, which still contained a small amount of 6*A*,6*B*-bis((carboxymethyl)thio)-6*A*,6*B*-dideoxy-β-CD (**7**), was chromatographed on a CM-Sephadex C-25 column ($Na^+$ form) eluted with water at 4 °C, giving 74 mg (17%) of **2** [TLC, $R_f$ 0.4 (*n*-propanol–ethyl acetate–water–aqueous ammonia, 5:2:3:1)]. The spot was fluorescent when stained with 0.1% fluorescamine in acetone and irradiated with 390 nm of light (primary $NH_2$ test), while bis((carboxymethyl)thio)-β-cyclodextrin (**7**) did not give any fluorescent spot: IR **2**·HCl (KBr) 1710 $cm^{-1}$. Anal. Calcd for $C_{44}H_{73}O_{35}NS·6H_2O$: C, 40.15; H, 6.51; N, 1.06; S, 2.44. Found: C, 40.16; H, 6.62; N, 1.08; S, 2.07.

Table VI. $^{13}$C NMR of 2

| carbons | 2X[c] | 2y[a] |
|---|---|---|
| α-carbons to S | 33.39, 34.75 | 33.93, 35.82 |
| C-6 carrying $NH_2$ | 39.96 | 40.42 |
| C-5 closest to $NH_2$ | 67.98 | 68.26 |
| C-5 closest to SR | 70.90 | |
| C-4 closest to $NH_2$ | 82.72 | 83.19 |
| C-4 closest to SR | 83.87 | 84.42 |
| COOH | 175.06 | 178.97 |
| | **2X**[b] | **2Y**[b] |
| α-carbons to S | 34.63 | 34.81 |
| | 35.71 | 36.04 |
| C-6 carrying $NH_2$ | | |
| C-6 | 60.99, 61.1 | 61.02 |
| C-5 closest to $NH_2$ | 68.82 | 68.80 |
| C-5 closest to SR | 71.80 | 72.34 |
| C-2, 3, 5 | 72.9–74.2 | 72.76–74.07 |
| C-4 | 82.26, 82.58, 82.73, 82.84 | 82.22–82.74 |
| C-4 closest to $NH_2$ | 82.52 | 84.21 |
| C-4 closest to SR | 85.21 | 85.65 |
| C-1 | 102.6–103.4 | 102.3–103.43 |
| carbonyl | 173.13 | 173.02 |

[a] [2] = 0.02 M in $D_2O$ with a 10-mm sample tube, at 25 °C, ppm from external $Me_4Si$ in $CDCl_3$. [b] [2] = 0.02 M in $Me_2SO$-$d_6$ with a 10-mm sample tube, at 25 °C, ppm from external $Me_4Si$ in $CDCl_3$.

HPLC analysis of **2** on a OA-1000A column (eluted with water) showed that the isolated product consisted of two components, one (**2X**) corresponding to the former peak (retention volume 7 mL), the other (**2Y**) corresponding to the latter peak (retention volume 9 mL). Thus, **2** was chromatographed on a DEAE-Sephadex A-25 column (1.5 cm × 85 cm) at 4 °C using water as an eluent. Since the separation of **2X** and **2Y** through single chromatography was not perfect, the chromatography was repeated until separation became satisfactory. The purity of each isomer in this stage was 95–98% based on HPLC analysis and after single recrystallization, pure isomers were obtained. $^{13}$C NMR data were listed in Table VI.

**Measurement of the Association Constants for Cyclodextrin–Trp Complexes.**[5] The association constant ($K$) between cyclodextrin (CD) and ANS in a pH 8.9 borate buffer solution containing 15.4 mM of $Na_2B_4O_7$ and 34.6 mM of $H_3BO_3$ was determined by following the fluorescence intensity of ANS ($3.6 \times 10^{-5}$ M) as a function of the concentration of CD ($5.0 \times 10^{-3}$, $1.2 \times 10^{-2}$ M).