

Thermodynamic Origin of Selective Binding of β -Cyclodextrin Derivatives with Chiral Chromophoric Substituents toward Steroids[†]

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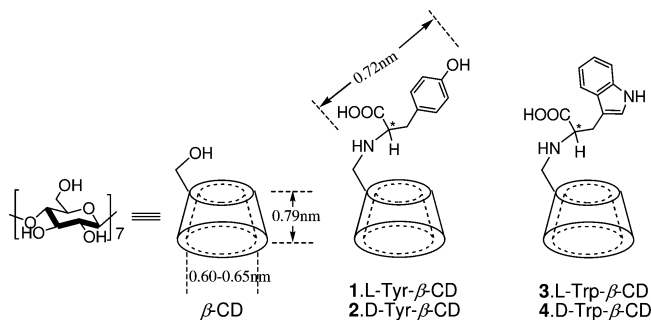
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Two β -cyclodextrin derivatives with chiral chromophoric substituents, that is, L- (**1**) and D-tyrosine-modified β -cyclodextrin (**2**), were synthesized and fully characterized. Their inclusion modes, binding abilities, and molecular selectivities with four steroid guests, that is, cholic acid sodium salt (CA), deoxycholic acid sodium salt (DCA), glycochoic acid sodium salt (GCA), and taurocholic acid sodium salt (TCA), were investigated by the circular dichroism, 2D NMR, and isothermal titration microcalorimetry (ITC). The results obtained from the circular dichroism and 2D NMR showed that two hosts adopted the different binding geometry, and these differences subsequently resulted in the significant differences of molecular binding abilities and selectivities. As compared with native β -cyclodextrin and tryptophan-modified β -cyclodextrin, host **2** showed the enhanced binding abilities for CA and DCA but the decreased binding abilities for GCA and TCA; however, host **1** showed the decreased binding abilities for all four bile salts. The best guest selectivity and the best host selectivity were $K_S^{2-DCA}/K_S^{2-TCA} = 12.6$ and $K_S^{2-CA}/K_S^{1-CA} = 10$, respectively, both exhibiting great enhancement as compared with the corresponding values of the previously reported L- and D-tryptophan-modified β -cyclodextrins. Thermodynamically, it was the favorable enthalpic gain that led to the high guest selectivity and host selectivity.

Introduction

In recent years, molecular recognition has received much attention in chemistry, biology, environmental science, and many other fields.^{1–3} Cyclodextrins (CDs), a class of macrocyclic oligosaccharides consisting of six, seven, or eight glucose units linked by α -1,4-glucose bonds, have been widely used as receptors in molecular recognition.⁴ It has been demonstrated that the formation of inclusion complexes between CDs and guest molecules is cooperatively governed by several weak forces, such as van der Waals, hydrophobic, electrostatic, dipole–dipole, and hydrogen-bonding interactions, and every weak force does its contribution to the complexation. The introduction of a suitable substituent may change the original balance of the weak forces between CDs and guests and thus influence the binding ability and selectivity. Therefore, a great deal of endeavor has been devoted to the design and synthesis of novel CD derivatives as well as their interactions with various guest molecules.⁵ Among the numerous guests researched, bile salts, a group of physiologically important steroids in vivo, play a crucial role in lipid digestion, transportation, and absorption.⁶ They are composed of a steroid skeleton and a hydrophilic tail, and the size/shape of bile salts is well fit for that of β -CD cavity. Vazquez Tato and coworkers⁷ studied the complex geometry of β -CD and its derivatives in D₂O by ROESY experiments, observing different binding modes upon inclusion complexation with bile salts. Ueno et al.⁸ employed the dansyl moiety as a fluorescence probe to investigate the complexation of CD–amino acid–chromophore triad systems with bile salts and alcohols, demonstrating that the molecular recognition behavior of these sensors was delicately affected by the chirality of the amino

CHART 1: Structures of Host Molecules



acid. Reinhoudt et al.⁹ reported a microcalorimetric study on the cooperative binding of bile salts by CD dimers in a basic environment, showing the enhanced binding abilities toward CA and DCA. Recently, we reported the molecular recognition behavior of L- and D-tryptophan- β -CDs¹⁰ (L- and D-Trp- β -CD, **3** and **4** in Chart 1) toward bile salts, and a satisfactory CA/TCA selectivity was obtained as 4.6 by D-Trp- β -CD. More recently, we also reported the molecular recognition of several permethylated β -CD derivatives¹¹ toward bile salts, showing the enhanced binding abilities. In the present work, we synthesized a pair of β -CD derivatives (**1** and **2**) with L- and D-tyrosine substituents (Chart 1). Their molecular recognition and binding behavior toward four bile salts (CA, DCA, TCA, and GCA) were investigated by the circular dichroism, 2D NMR, and ITC experiments and compared with those of L- and D-Trp- β -CDs. Significantly, both a good guest selectivity as $K_S^{2-DCA}/K_S^{2-TCA} = 12.6$ and a good host selectivity as $K_S^{2-CA}/K_S^{1-CA} = 10$ were obtained. The factors that resulted in the great enhancement of selectivity were discussed from the viewpoint of the binding

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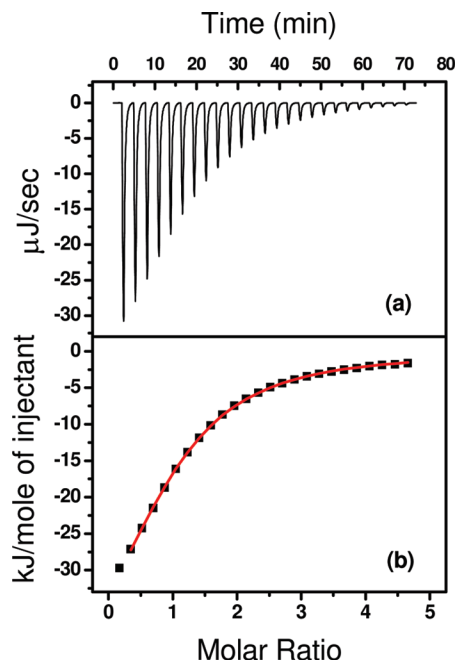


Figure 1. Calorimetric titration curve of **2** with DCA in pH 7.2 phosphate buffer solution ($I = 0.1$ M) at 25 °C. (a) Raw data for sequential 10 μ L injections of solution **2** (3.90 mM) into solution DCA (0.16 mM) (b) Heats of reactions, as obtained from the integration of the calorimetric traces.

geometry and the binding thermodynamics. It is our special interest to compare the binding abilities and molecular selectivities of β -CD derivatives with the aromatic amino acid substituents in different size, which will help us deeply understand the factors governing the molecular recognition of amino-acid-modified CDs as well as their thermodynamic origin.

Microcalorimetric Titration. We performed ITC experiments at atmospheric pressure in aqueous phosphate buffer solution at 25 °C by using a Microcal VP-ITC titration microcalorimeter, which allows us to determine the enthalpy and equilibrium constant from a single titration curve simultaneously. We calibrated the instrument chemically by performing the complexation reaction of β -CD with cyclohexanol; the thermodynamic parameters obtained were in excellent agreement with the literature data.¹² All solutions were degassed and thermostatted using a Thermo Vac accessory before each titration. In each run, a phosphate buffer solution of the host molecule in a 250 μ L syringe was sequentially injected into the calorimeter sample cell containing a buffer solution of bile salt guests with a stirring speed at 300 rpm. The sample cell volume was 1.4227 mL in all experiments. Each titration experiment was composed of 25 successive injections (10 μ L per injection). The bile salt solutions that applied here were at a concentration range of 0.16 to 0.53 mM, which was below their critical micelle concentration (CMC). (The CMCs of the steroids employed here are >1 mM¹³.) Each addition of CDs to the sample cell gave rise to a heat of reaction, caused by the formation of inclusion complexes between bile salt molecules and CDs. The heats of reaction decrease after each injection of host CD because fewer and fewer bile salt molecules are available to form inclusion complexes. Typical titration curves are shown in Figure 1.

We performed a control experiment to determine the heat of dilution by injecting a host solution into a pure buffer solution containing no guest molecules. The dilution heat determined in the control experiment was subtracted from the apparent reaction

heat measured in the titration experiments to give the net reaction heat. The net reaction heat in each run was analyzed by using “one set of binding sites” model for all β -CD derivatives. The ORIGIN software (Microcal) allowed us to determine the binding constant (K_S) and reaction enthalpy (ΔH°) simultaneously with the standard derivation based on the scatter of data points from a single titration experiment. The binding stoichiometry was also given as a parameter when fitting the binding isotherm (panel b in Figure 2). Knowledge of the binding constant (K_S) and molar reaction enthalpy (ΔH°) enabled the calculation of the standard Gibbs free energy of binding (ΔG°) and entropy change (ΔS°) according to equation

$$\Delta G^\circ = -RT \ln K_S = \Delta H^\circ - T\Delta S^\circ$$

where R is the gas constant and T is the absolute temperature.

To check the accuracy of observed thermodynamic parameters, we carried out at least two independent titration experiments to afford self-consistency of the thermodynamic parameters, and their average values are listed in Table 1. For a comparison purpose, the thermodynamic quantities reported for L- and D-Trp- β -CDs¹⁰ are also included in Table 1.

Results and Discussion

Circular Dichroism Spectra. It is widely documented that the inclusion of a chromophoric group in a chiral cavity, such as CD cavity, will produce either positive- or negative-induced circular dichroism (ICD) signals according to the location and orientation of the transition dipole moment of the chromophore with respect to the CD axis.¹⁴ Therefore, the circular dichroism spectrometry becomes a convenient and widely employed method to elucidate the conformation of the CD-containing systems.¹⁵ As can be seen from Figure 3, the circular dichroism spectrum of host **1** displayed moderate positive Cotton effect peak at 225 nm ($\Delta\epsilon = 2.79$ M⁻¹ cm⁻¹) assigned to the ¹ L_a transitions of L-tyrosine substituent. Moreover, the circular dichroism signals of free L-tyrosine were relatively weak ($\Delta\epsilon < 0.8$ M⁻¹ cm⁻¹), whereas β -CD itself showed almost no appreciable signals in the wavelength range of 210–300 nm. In addition, the circular dichroism spectrum of the equimolar mixture of L-tyrosine with β -CD resembled the corresponding circular dichroism spectrum of free tyrosine. (See the Supporting Information.) In the control experiment, the corresponding circular dichroism signals of **1** resulting from displacement of the tyrosine substituent from the CD cavity by measuring the circular dichroism spectra in a water/alcohol mixture were also examined, which showed that the circular dichroism signals of **1** in a water/alcohol mixture were weaker than those of **1** in water. Therefore, we could deduce that the moderate circular dichroism signals of **1** should be partially attributed to the self-inclusion of the L-tyrosine substituent into the β -CD cavity with its ¹ L_a transition band nearly parallel to the C₇ axis of β -CD, although the L-tyrosine substituent was optically active and possessed the intrinsic circular dichroism signals.¹⁶ Upon inclusion complexation with DCA, the circular dichroism signal of **1** decreased to $\Delta\epsilon = 1.77$ M⁻¹ cm⁻¹ at 225 nm. This phenomenon indicated that the L-tyrosine moiety slightly moved to the external of β -CD cavity after complexation with DCA. The circular dichroism signal of host **2** was different from that of **1**, showing a moderate negative Cotton effect peak at 219 nm ($\Delta\epsilon = -2.27$ M⁻¹ cm⁻¹) for the ¹ L_a transition of D-tyrosine substituent. In the control experiment, the circular dichroism signals of free D-tyrosine were also very weak ($|\Delta\epsilon| < 0.8$ M⁻¹

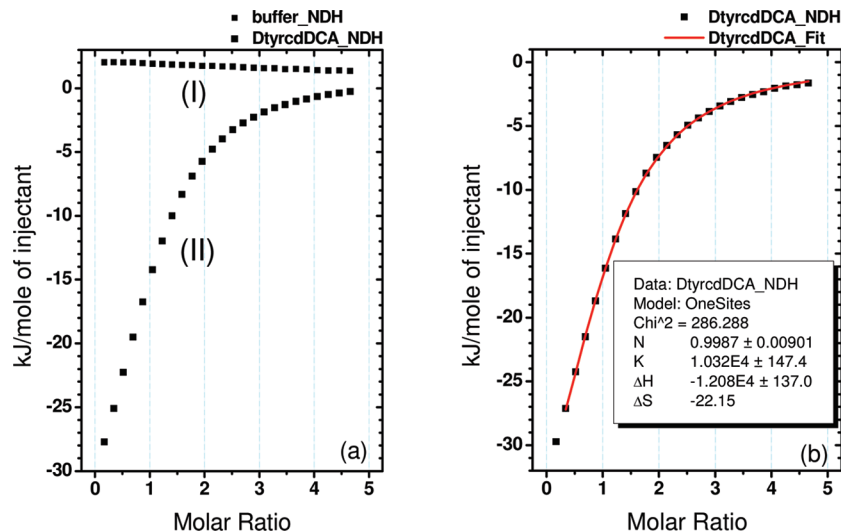


Figure 2. (a) Heat effects of dilution (I) and complexation (II) of **2** with DCA for each injection during microcalorimetric titration experiment. (b) “Net” heat effect obtained by subtracting the heat of dilution from the heat of reaction, which was analyzed by computer simulation using the “one set of binding sites” model.

TABLE 1: Complex Stability Constants (K_S), Enthalpic Changes (ΔH°), Entropic Changes ($T\Delta S^\circ$), and Gibbs Free Energy Changes (ΔG°) for 1:1 Inclusion Complexation of Bile Salts with Host CDs in Phosphate Buffer Solution at 298.15 K

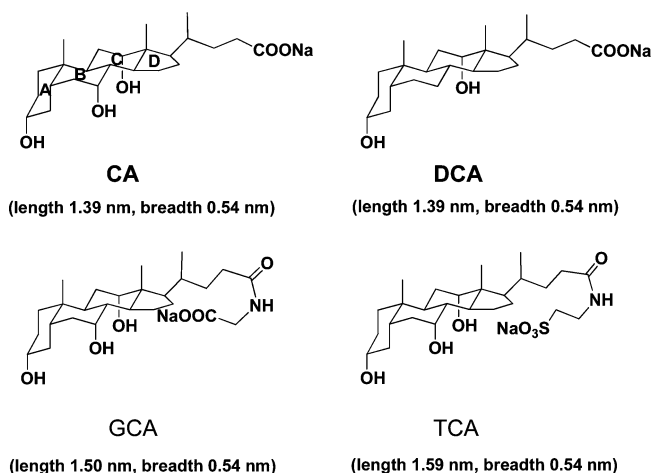
guests	hosts	K_S (M^{-1})	ΔG° (kJ mol $^{-1}$)	ΔH° (kJ mol $^{-1}$)	$T\Delta S^\circ$ (kJ mol $^{-1}$)
CA	β -CD ^a	4070 ± 80	−20.6	−23.0 ± 0.5	−2.4
	L-Tyr- β -CD (1)	871 ± 10	−16.8	−26.7 ± 0.5	−9.9
	D-Tyr- β -CD (2)	8689 ± 183	−22.5	−41.7 ± 0.6	−19.2
	L-Trp- β -CD (3) ^b	2020 ± 20	−18.9	−23.2 ± 0.1	−4.3
	D-Trp- β -CD (4) ^b	6680 ± 130	−23.4	−37.9 ± 0.3	−14.5
DCA	β -CD ^a	4840 ± 20	−21.0	−25.8 ± 0.0	−4.8
	L-Tyr- β -CD (1)	1087 ± 45	−17.3	−33.1 ± 2.5	−15.8
	D-Tyr- β -CD (2)	9962 ± 358	−22.8	−50.5 ± 0.0	−27.9
	L-Trp- β -CD (3) ^b	2310 ± 30	−19.2	−32.1 ± 0.2	−12.9
	D-Trp- β -CD (4) ^b	6770 ± 190	−21.9	−46.0 ± 0.3	−24.1
GCA	β -CD ^a	2350 ± 70	−19.3	−23.0 ± 0.1	−3.7
	L-Tyr- β -CD (1)	428 ± 1.4	−15.0	−28.3 ± 0.3	−13.3
	D-Tyr- β -CD (2)	1105 ± 3	−17.4	−30.5 ± 0.1	−13.1
	L-Trp- β -CD (3) ^b	1110 ± 20	−17.4	−23.4 ± 0.3	−6.0
	D-Trp- β -CD (4) ^b	1760 ± 40	−18.5	−24.9 ± 0.3	−6.4
TCA	β -CD ^a	2290 ± 10	−19.2	−23.8 ± 0.1	−4.6
	L-Tyr- β -CD (1)	391 ± 3	−14.8	−25.7 ± 0.2	−10.9
	D-Tyr- β -CD (2)	809 ± 9	−16.6	−26.7 ± 0.5	−10.1
	L-Trp- β -CD (3) ^b	1060 ± 20	−17.3	−23.1 ± 0.2	−5.8
	D-Trp- β -CD (4) ^b	1470 ± 10	−18.1	−24.3 ± 0.1	−6.2

^a Ref 19. ^b Ref 10.

cm $^{-1}$), whereas the circular dichroism spectrum of the equimolar mixture of D-tyrosine with β -CD resembled the corresponding circular dichroism spectra of free D-tyrosine. (See the Supporting Information.) This phenomenon indicated that the D-tyrosine substituent of **2** might be self-included in the β -CD cavity with the 1L_a transition band nearly perpendicular to the C_7 axis of β -CD. The circular dichroism signal of host **2** became more negative after complexation DCA, indicating that there may be a change of the angle between the 1L_a transition band and the C_7 axis of β -CD. It should be noted that the circular dichroism spectra could not provide us with the enough information about the relative position of substituent or guest molecule in the CD cavity, so the conformations of hosts **1–2** and their complexes with bile salts were further investigated by the 2D NMR experiments.

2D NMR. 2D NMR spectroscopy has become a powerful method to study the conformation of CD derivatives and their complexes because one can conclude that two protons are closely located in space (0.4 nm apart at most) if an NOE correlation is detected between the relevant proton signals in

CHART 2: Structures of Guest Molecules Employed



the NOESY or ROESY spectrum.^{17,18} Therefore, it is possible to estimate the orientation of substituent or guest molecule in

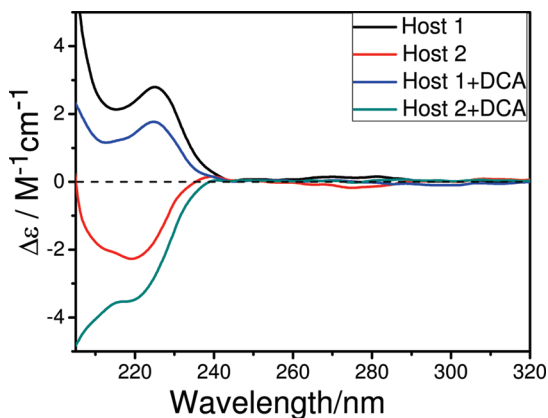


Figure 3. Circular dichroism spectra of **1** and **2** (1.0×10^{-4} M) in the presence and absence of DCA (1.0×10^{-3} M) in pH 7.20 phosphate buffer solution ($I = 0.1$ M).

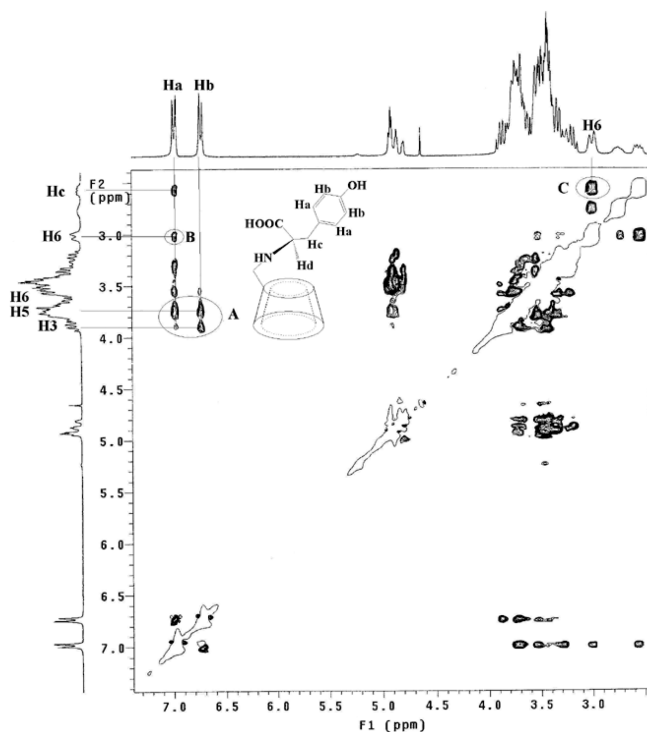


Figure 4. ROESY spectrum of host **1** with a mixing time of 250 ms at 298.1 K.

CD cavity with the aid of the assigned NOE correlations. On this basis, we performed ROESY experiments to obtain further information about the conformation of host CDs and their complexes. The ROESY spectrum of host **1** was shown in Figure 4. It is well known that only the H3, H5, and H6 protons of CDs could give the NOE cross-peaks for analyzing the conformation of host–guest complexes because H2 and H4 were facing the outer cavity. (Hn was used for host protons.) As seen from Figure 4, the cross-peak A was assigned to the NOE correlations between the H3/H5 protons of β -CD and the Ha/Hb protons of L-tyrosine substituent. A further comparison showed that Ha exhibited the stronger NOE correlations with the H5 protons than with the H3 protons of β -CD. Moreover, the cross-peaks B and C were assigned to the NOE correlations of the H6 protons of β -CD with the Ha/Hc protons of L-tyrosine substituent, respectively. Because the H5/H6 protons were located at the narrow opening, whereas the H3 protons were at the wide opening, of the β -CD cavity, these results unambiguously showed that the L-tyrosine moiety was self-included in

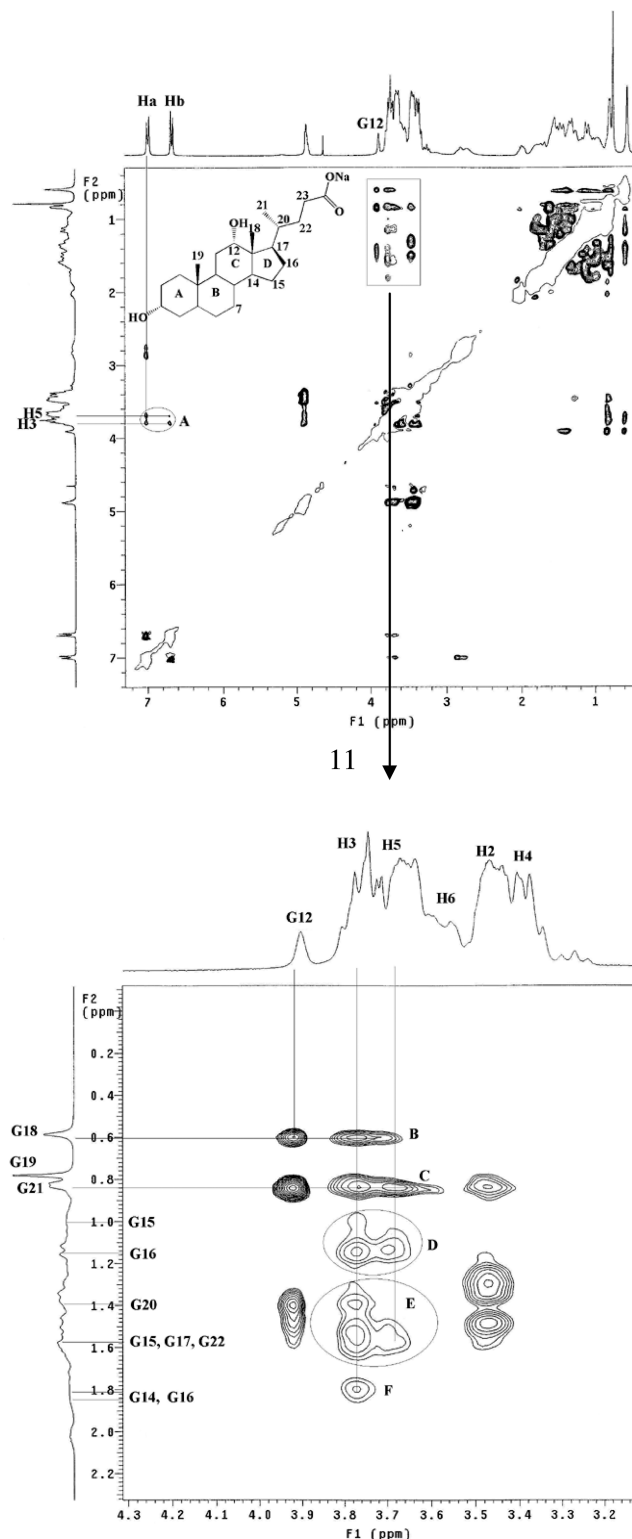


Figure 5. ROESY spectra of **1**/DCA (2.8 and 5.3 mM, respectively) with a mixing time of 250 ms at 298.1 K.

the β -CD cavity from the narrow opening. Moreover, the MALDI-MS experiments ruled out the possibility of the formation of oligomeric species. Furthermore, the ROESY experiment of the **1**/DCA system was also performed to investigate the binding geometry between L-Tyr- β -CD and bile salts. In Figure 5, the NOE correlations between the H3/H5 protons of β -CD and the Ha/Hb protons of L-tyrosine substituent (peak A) were also observed after the addition of DCA. This phenomenon indicated that the L-tyrosine moiety was not driven

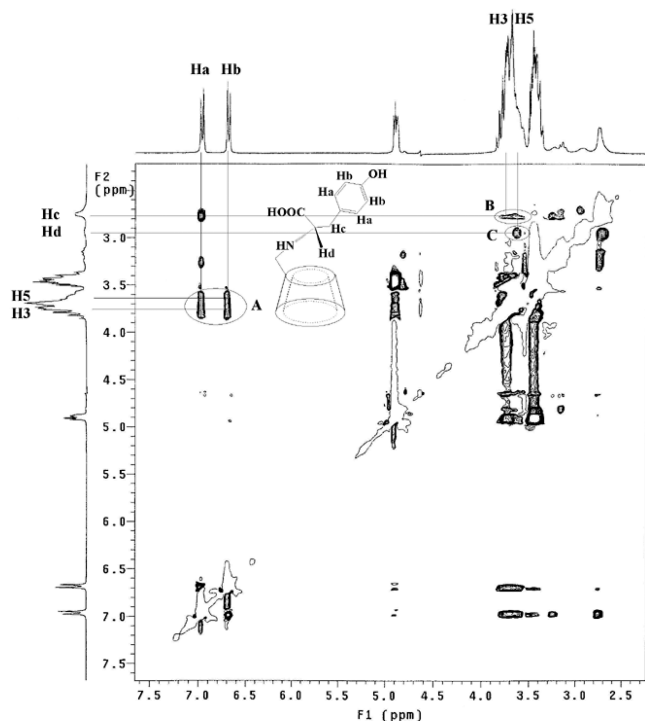


Figure 6. ROESY spectrum of host **2** with a mixing time of 250 ms at 298.1 K.

out of the β -CD cavity, even after the guest inclusion. In a general way, the bile salt molecule (Gn was used for DCA protons) was marked with A, B, C, and D rings according to the previous report.⁷ As seen in Figure 5, the peak B corresponded to the NOE correlations between the DCA's G18 protons and the β -CD's H3 protons, the peak C corresponded to the NOE correlations between the DCA's G21 protons and the β -CD's H3/H5 protons, and the peaks D, E, and F were assigned to the NOE correlations of the protons of the DCA's D ring and the hydrophobic tail with the β -CD's H3 or H5 protons. From the above information, we deduced that the DCA entered the β -CD cavity from the wide opening with the tail and the D ring and coexisted with the L-tyrosine substituent in the β -CD cavity to form a cooperative inclusion manner.

In the case of D-Tyr- β -CD (Figure 6), similarly, the cross-peak A was assigned to the NOE correlations between the H3/H5 protons of β -CD and the Ha/Hb protons of D-tyrosine substituent, and the correlation intensity of Ha with H3/H5 was basically the same as that of Hb. This implied that the distances between Ha/Hb and H3/H5 might be equal. At the same time, the cross-peak B corresponding to the NOE correlations of the D-tyrosine's Hc protons with the β -CD's H3/H5 protons and the cross-peak C corresponding to the NOE correlations of the D-tyrosine's Hd protons with the β -CD's H3 protons were also observed. Combining this information with the circular dichroism spectra results, we deduced that the D-tyrosine substituent was deeply self-included in the β -CD cavity and might be located in the center of the β -CD cavity. The ROESY spectra of **2**/DCA are shown in Figure 7. The NOE correlations between the β -CD's H3/H5 protons and the D-tyrosine's Ha/Hb protons still existed, but the NOE correlations of the D-tyrosine's Hc protons with the β -CD's H3/H5 protons and the NOE correlations of the D-tyrosine's Hd protons with the β -CD's H3 protons (peak B and C in Figure 6) disappeared, indicating that the D-tyrosine substituent of **2** partially moved out of the β -CD cavity. Similarly, the cross-peak B corresponded to the NOE correlations of the carboxylate tail and D ring protons of DCA

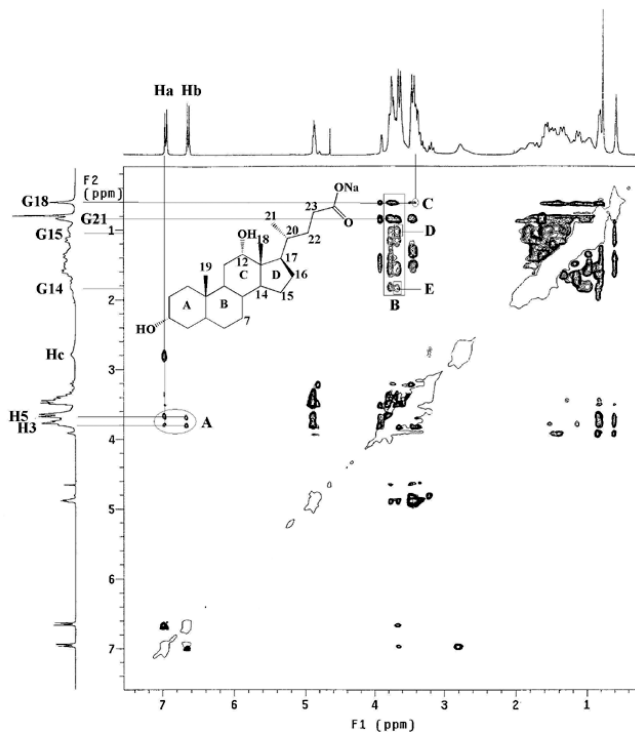


Figure 7. ROESY spectrum of **2**/DCA (2.6 and 5 mM, respectively) with a mixing time of 250 ms at 298.1 K.

with the β -CD's H3 or H5 protons. A close comparison of the NOE signals of **1**/DCA and **2**/DCA systems showed that for **2**/DCA the DCA's G15/G14 protons exhibited the NOE correlations with the β -CD's H3/H5 protons (peak D and E), but for **1**/DCA, the DCA's G15/G14 protons only exhibited the NOE correlations with the β -CD's H3 protons, and the NOE correlation intensity of **2**/DCA was stronger than that of **1**/DCA. Meanwhile, the peak C in Figure 7 should be assigned to the NOE correlation of the Hd proton of D-tyrosine side arm in **2** with the DCA's G18 protons. From this comparison, it is reasonable to come to the conclusion that DCA penetrated into the β -CD cavity of **2** more deeply than into that of **1**. These subtle differences in conformation between **1**/DCA and **2**/DCA systems may subsequently result in the significant differences of binding ability and molecular selectivity between hosts and guests, which would be discussed below.

Binding Stoichiometry. The ITC experiments of hosts **1** and **2** with bile salts (CA, DCA, GCA, and TCA) showed the typical titration curves of the 1:1 complex formation. The stoichiometric ratio "*N*" observed from the curve-fitting results was within the range 0.9 to 1.1, which clearly indicated that the majority of the inclusion complexes had a 1:1 binding mode. Moreover, the molecular modeling study demonstrated that hosts **1** and **2** could only accommodate one bile molecule in their hydrophobic cavity. Therefore, the possible conformations of hosts **1** and **2** and their complexes with DCA are illustrated in Figure 8, along with the reported conformations of hosts **3** and **4** and their DCA complexes. In addition, their computational structures are in the Supporting Information.

Binding Behavior. ITC is becoming a widely accepted method to determine the thermodynamic parameters associated with the noncovalent interactions of two (or more) molecules.²⁰ According to the data presented in Table 1, thermodynamically, the binding of all CDs with the bile salt guests was entirely driven by the favorable enthalpic changes accompanied by the unfavorable entropic changes ($\Delta H^\circ < 0$; $T\Delta S^\circ < 0$). The different host–guest binding ability directly led to the huge differences

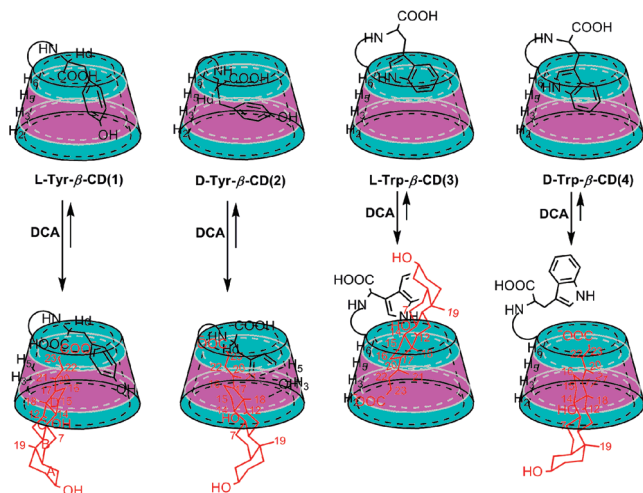


Figure 8. Conformation of hosts 1–4 and their possible binding modes with DCA.

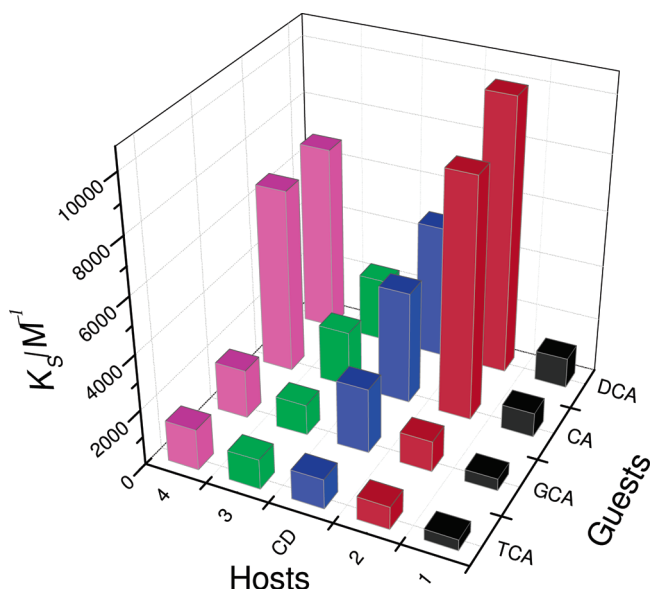


Figure 9. Complex stability constants (K_s) of bile salts with hosts 1–4 in phosphate aqueous buffer solution at 298 K.

of the host–guest selectivity. To visualize and easily discuss the binding behavior and molecular selectivity between hosts 1–4 and bile salt molecules, we plotted the changing profiles of the complex stability constants (K_s) as well as the enthalpic changes (ΔH°) and the entropic changes ($T\Delta S^\circ$) in Figures 9 and 10, respectively. Thermodynamically, the large negative enthalpic changes should mainly be attributed to the strong van der Waals interactions between host and guest. It was reported that aminated β -CDs would be positively charged at pH 7.2.¹⁹ Therefore, the electrostatic interactions between the positively charged side arm and the negatively charged bile salt would lead to the negative enthalpic changes. Moreover, the release of the high-energy water in the β -CD cavity,²¹ the C–H \cdots O hydrogen bond between the tyrosine group and the carboxyl group of bile salt,²² and the O–H \cdots O hydrogen bond between the –OH groups of CD cavity and the carboxyl group of bile salt also contributed to the favorable enthalpic gain. Before the host–guest binding, both the host CD and the guest molecule were highly solvated, and the solvent molecules around the host and the guest were highly ordered. During the host–guest binding, the solvation shells of both the host and the guest underwent reorganization. This process created disorder in the

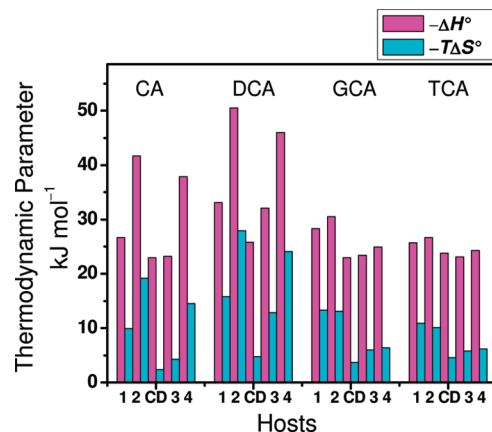


Figure 10. Enthalpic (ΔH°) and entropic changes ($T\Delta S^\circ$) for the inclusion complexation of bile salts with hosts 1–4 in phosphate aqueous buffer solutions at 298 K.

system and thus led to the favorable entropic changes. With the tyrosine side arm located at the narrow opening of the CD cavity, the shape of hosts 1 and 2 was like a barrel with a bottom, and the close location of “bottom” to the bile salt guest made the accommodated bile salt more immovable in the CD cavity. This large loss of conformational freedom upon host–guest binding consequently resulted in the big entropic loss, which overwhelmed the entropic gain arising from the solvent reorganization. This was consistent with the widely reported enthalpy/entropy compensation effect that the large enthalpic changes always accompanied by the large entropic changes.²³

Complexation of Bile Salts by D-Tyr- β -CD (2). As can be seen from Table 1, host 2 displayed the distinct binding abilities toward four bile guests. Although β -CD, D-Trp- β -CD, and 2 all formed the 1:1 inclusion complexes with bile guests, 2 gave the higher bind ability toward CA and DCA than β -CD and D-Trp- β -CD (4). This might be attributed to the introduction of D-tyrosine substituent and the conformational difference between 2 and 4. According to the binding geometry deduced from the circular dichroism spectra and the 2D NMR experiments, after complexation with guest molecules, the D-tyrosine moiety of host 2 was not expelled out of the β -CD cavity. This conformation decreased the effective volume of the β -CD cavity to some extent, and thus led to the higher size-fit efficiency between host 2 and guest molecules. For D-Trp- β -CD (4), the D-tryptophan substituent was expelled out of CD cavity¹⁰ after complexation with the bile salt, which would lead to the relatively weak size-fit efficiency as compared with that of the 2/bile salt system. Thermodynamically, the large entropic losses of the 2/bile salt system ($T\Delta S^\circ = -19.2$ kJ mol^{−1} for CA and -27.9 kJ mol^{−1} for DCA) could be compensated by the larger negative enthalpic gain ($\Delta H^\circ = -41.7$ kJ mol^{−1} for CA and -50.5 kJ mol^{−1} for DCA), which directly resulted in the high binding constant ($K_s^{2-CA} = 8689$ M^{−1} and $K_s^{2-DCA} = 9962$ M^{−1}). In addition, the bind constant of 2 for DCA was slightly bigger than that for CA. Possessing a more hydrophobic structure due to the absence of the C-7 hydroxyl group as compared with CA, DCA was easier to bind to the β -CD cavity than CA, which consequently led to the more favorable hydrophobic interactions between hosts and guests.

Host 2 exhibited the obviously smaller binding abilities for GCA and TCA guests than β -CD and D-Trp- β -CD (4). This unusual phenomenon prompted us to explore the reasons that caused the decrease in binding constants. Thermodynamically, the values in Table 1 indicated that the decreased binding affinities of host 2 toward GCA and TCA arose from the entropy

rather than the enthalpy ($-\Delta\Delta H^\circ < -\Delta T\Delta S^\circ$). The large entropic loss might be attributed to at least two parts. First, GCA (or TCA) possessed a cholic acid skeleton and a glycine (or taurine) tail, and the side chain at C23 for GCA (or TCA) became more polar and more hydrophilic than that for CA (or DCA), which weakened the hydrophobic interactions between hosts and guests and thus affected their binding thermodynamics and binding constants. In addition, GCA (or TCA) possessed a longer side chain than CA (or DCA), and it might not exist in a serrated form but twist into a ring. This phenomenon would increase the volume of the side chain to some extent and thus lead to the relatively poor size-fit between host and guest because **2** had a smaller effective volume of β -CD cavity, as described above. Possessing a free β -CD cavity, β -CD and D-Trp- β -CD (whose substituent was expelled out of the β -CD cavity) could better bind the bigger GCA (or TCA) guest and thus gave higher binding constants than **2**.

Molecular Selectivity of **2 toward Bile Salts.** The sharp decrease in stability constants of **2** toward GCA and TCA seemed disappointing, but the high complex stability constants did not always refer to the high molecular selectivity. In general, both the increase and the decrease in the stability constants were important to the improvement of the molecular selectivity. As seen in Table 1, the selectivity of **2** toward four bile guests was DCA > CA > GCA > TCA, and K_S^{2-DCA}/K_S^{2-TCA} was up to 12.6, a sharp comparison with $K_S^{4-DCA}/K_S^{4-TCA} = 4.6$ for D-Trp- β -CD and $K_S^{\beta-CD-DCA}/K_S^{\beta-CD-TCA} = 2.1$ for native β -CD. Thermodynamically, the difference of the Gibbs free energy changes for the complexation of **2** with DCA and TCA ($\Delta\Delta G^\circ = \Delta G^\circ_{DCA} - \Delta G^\circ_{TCA}$) was up to -6.2 kJ mol^{-1} , and $\Delta\Delta H^\circ = \Delta H^\circ_{DCA} - \Delta H^\circ_{TCA} = -24.8 \text{ kJ mol}^{-1}$, $\Delta T\Delta S^\circ = T\Delta S^\circ_{DCA} - T\Delta S^\circ_{TCA} = -17 \text{ kJ mol}^{-1}$. In contrast, for the complexation of **4** with DCA and TCA, the enthalpic gain ($\Delta\Delta H^\circ = -21.7 \text{ kJ mol}^{-1}$) was less, but the entropic loss ($\Delta T\Delta S^\circ = -17.9 \text{ kJ mol}^{-1}$) was larger, than that of **2**. That means, the higher enthalpic gain and the less entropic loss together contributed to the great improvement of the guest selectivity.

Complexation of Bile Salts by L-Tyr- β -CD (1**).** We could also observe from Table 1 that compared with β -CD and L-Trp- β -CD (**3**), **1** showed clearly decreased binding abilities toward all four of the bile salts. In particular, for GCA and TCA, the binding constants were only 427 and 391 M^{-1} , respectively, and even its strongest binding was only up to 1087 M^{-1} toward DCA. These lower binding abilities of **1** might imply a change of the binding thermodynamics. Thermodynamically, the inclusion complexation of **1** with four bile salts exhibited the favorable negative ΔH° and unfavorable $T\Delta S^\circ$. The favorable enthalpic gain of **1** was slightly higher than those of β -CD and L-Trp- β -CD (**3**), but the entropic loss of **1** was much more than those of β -CD and L-Trp- β -CD (**3**) toward corresponding guests. Taking DCA as an example, the $\Delta\Delta H^\circ$ and $\Delta T\Delta S^\circ$ ($\Delta H^\circ_1 - H^\circ_3 = -1.0 \text{ kJ mol}^{-1}$, $T\Delta S^\circ_1 - T\Delta S^\circ_3 = -2.9 \text{ kJ mol}^{-1}$) data showed that the entropic changes mainly contributed to the decrease in the binding abilities. The similar results were also observed in the case of the three other bile salt guests. From the viewpoint of binding geometry, **1** showed a conclusion mode upon complexation with the bile salt guest. The bile salt guest entered β -CD cavity of **1** with its D-ring and tail from the wide opening of the β -CD cavity. Therefore, the relatively small volume of the D-ring and the tail gave the relatively poor size-fit with the β -CD cavity. In addition, the anionic carboxylate of L-tyrosine substituent that was self-included in the β -CD cavity of **1** would not only decrease the microenvironment hydrophobicity of β -CD cavity to some extent but also give

the electrostatic repulsion with the anionic carboxylate or sulfonate tail of the bile salt guest. These factors jointly led to the weaker interactions between host and bile salts and thus the lower binding ability. According to our previously report,¹⁰ DCA complexed L-Trp- β -CD (**3**) with the bigger A ring and B ring penetrating into the β -CD cavity from the narrow opening of the β -CD cavity, which consequently led to the better size-fit between β -CD cavity and the bile salt guest. Moreover, the L-tryptophan substituent of **3** also showed the NOE correlation with the B ring of the accommodated bile salt guest. This NOE correlation indicated that a possible $C-H\cdots\pi$ interaction resulted from the close location of these two groups. As a result, **3** gave stronger binding abilities toward the bile salt guest than **1**.

Diastereomeric Host Recognition. The diastereomeric host selectivity for the modified CDs with the enantiomeric side arms has rarely been discussed. We have reported the molecular recognition behaviors of L- and D-Trp- β -CDs toward bile salts, but the results showed only the moderate host selectivity. As the best result, CA gave a binding constant by D-Trp- β -CD (**4**) that was 3.3 times higher than that by L-Trp- β -CD (**3**) ($K_S^{4-CA}/K_S^{3-CA} = 3.3$). After the tryptophan substituent was changed to the smaller tyrosine substituent, the host selectivity got the satisfactory enhancement. Also, for CA, the binding constants for the hosts in Table 1 were in an order of **2** > **4** > β -CD > **3** > **1**, and the host selectivity of the **2/1** couple was up to 10 ($K_S^{2-CA}/K_S^{1-CA} = 10$). Thermodynamically, the higher $\Delta\Delta H^\circ$ value ($\Delta\Delta H^\circ_{2-1} = \Delta H^\circ_2 - \Delta H^\circ_1 = -15 \text{ kJ mol}^{-1}$, $\Delta T\Delta S^\circ_{2-1} = T\Delta S^\circ_2 - T\Delta S^\circ_1 = -9.3 \text{ kJ mol}^{-1}$ for CA) indicated that the favorable enthalpic gains, probably arising from the van der Waals forces, hydrogen bond, and the release of the high-energy water in the β -CD cavity, apparently contributed to the high selectivity.

Conclusions

In summary, we have successfully synthesized a pair of β -CD derivatives with L- and D-tyrosine as chiral side arms, and their binding behaviors and molecular selectivities toward four typical bile salts (CA, DCA, GCA, and TCA) were carefully studied. The results obtained from the circular dichroism and 2D NMR showed that the D-tyrosine moiety of **2** was more deeply self-included into the β -CD cavity than the L-tyrosine moiety of **1**. Upon inclusion complexation, bile salt guests inserted into the β -CD cavity of host **2** more deeply than into that of host **1**. The different binding geometry subsequently affected their binding abilities and selectivities. Thermodynamically, the binding behaviors of two hosts toward four bile salts were entirely driven by favorable enthalpic changes, accompanied by unfavorable entropic changes ($\Delta H^\circ < 0$; $T\Delta S^\circ < 0$). Host **2** displayed the enhanced binding ability for CA and DCA and decreased binding abilities for GCA and TCA; however, host **1** showed clearly decreased binding ability toward all four bile salts. The increase and the decrease in the stability constants cooperatively contributed to the high selectivities between hosts and guests. As a result, host **2** presented the best recognition ability for DCA/TCA pair ($K_S^{2-DCA}/K_S^{2-TCA} = 12.6$), and CA gave the best host selectivity ($K_S^{2-CA}/K_S^{1-CA} = 10$). Compared with their analogues L- and D-Trp- β -CD, L- and D-Tyr- β -CD with the smaller substituents adopted the different inclusion modes, which resulted in the better host-guest size-fit relationship and the higher molecular selectivities between hosts and guests.

Experimental Section

Materials. β -CD of reagent grade was recrystallized twice from water and dried in vacuo at 100 °C for 24 h prior to use. L- and D-tyrosine were purchased from Peptide Institute. All bile salt guests (CA, DCA, GCA, and TCA) were purchased from Sigma and used as received. Mono[6-O-(*p*-toluenesulfonyl)]- β -CD (6-OTs- β -CD) was prepared by the reaction of β -CD with *p*-toluenesulfonyl chloride in aqueous alkaline solution.²⁴ Phosphate buffer solution of pH 7.20 (*I* = 0.1 M) was used for circular dichroism spectral measurements and ITC experiments.

Instruments. Circular dichroism spectral measurements were performed in a conventional quartz cell (light path 1 cm) on a JASCO J-720W spectropolarimeter equipped with a temperature controller, and the temperature of the cell was kept constant at 25.0 °C. ¹H NMR experiments were recorded on a Bruker AV400 spectrometer, and 2D ROESY (rotating frame Overhauser effect spectroscopy) spectra were recorded on a Varian Mercury VX300 instrument. All NMR experiments were carried out in D₂O.

Synthesis of L-Tyr- β -CD (1). L-Tyrosine (0.7 g) and 6-OTs- β -CD (1.3 g) were dissolved in water (30 mL) containing triethanolamine (20 mL), and the resulting mixture was heated to reflux for 24 h with stirring under an argon atmosphere. After the evaporation of most of the solvent under a reduced pressure, the resulting solution was poured in anhydrous acetone (200 mL) with vigorous stirring to produce a pale-yellow precipitate. The crude solid was collected by filtration and then purified by Sephadex C-25 column chromatography with aqueous ammonia (1.0 M) as an eluent, followed by chromatography on a Sephadex G-25 column with deionized water as eluent to give a pale-yellow product (0.57 g, 44%). ESI-MS: *m/z* (relative intensity), 1298.1 ([M + H]⁺, 100%), 1320.1 ([M + Na]⁺, 7%). ¹H NMR (D₂O, δ): 2.54–2.64 (m, 1H), 2.72–2.84 (m, 1H), 2.99–3.12 (d, 2H), 3.21–3.98 (m, 4H), 4.84–5.01 (m, 7H), 6.71–6.85 (d, 2H), 6.98–7.09 (d, 2H). ¹³C NMR (100 MHz, D₂O, δ): 154.9, 129.6, 115.2, 101.8, 100.1, 82.9, 80.9, 73.1, 71.9, 60.0, 46.0, 35.5. Anal. Calcd for L-Tyr- β -CD: C₅₁H₇₉NO₃₇·6H₂O, C, 43.56%; H, 6.52%; N, 1.00%. Found: C, 43.43%; H, 6.59%; N, 0.85%. FT-IR (KBr) ν (cm⁻¹): 3404, 2928, 1635, 1516, 1246, 849. UV λ (ϵ): 224 nm (6.23 \times 10³ dm³ mol⁻¹ cm⁻¹), 277 nm (1.36 \times 10³ dm³ mol⁻¹ cm⁻¹).

Synthesis of D-Tyr- β -CD (2). D-Tyr- β -CD was synthesized in 41% yield using a similar method to the synthesis of 1. ESI-MS: *m/z* (relative intensity), 1298.3 ([M + H]⁺, 100%). ¹H NMR (D₂O, δ): 2.78–2.84 (m, 2H), 2.90–3.08 (m, 1H), 3.18–3.90 (m, 4H), 4.86–5.02 (m, 7H), 6.70–6.85 (d, 2H), 6.96–7.10 (d, 2H). ¹³C NMR (150 MHz, D₂O, δ): 154.9, 130.6, 129.1, 115.4, 102.0, 101.2, 83.35, 81.1, 73.3, 72.2, 65.8, 60.4, 48.7, 37.8. Anal. Calcd for D-Tyr- β -CD: C₅₁H₇₉NO₃₇·8H₂O, C, 42.47%; H, 6.64%; N, 0.97%. Found C, 42.62%; H, 6.54%; N, 0.95%. FT-IR (KBr) ν (cm⁻¹): 3388, 2927, 1635, 1547, 1517, 1241, 836. UV λ (ϵ): 224 nm (8.04 \times 10³ dm³ mol⁻¹ cm⁻¹), 275 nm (1.65 \times 10³ dm³ mol⁻¹ cm⁻¹).

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Supporting Information Available: UV-vis spectra, circular dichroism spectra, and computational structures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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