Thermodynamic Origin of Molecular Selective Binding of Bile Salts by Aminated β-Cyclodextrins

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To elucidate quantitatively the sidearm effects on the molecular selective binding of aminated β-cyclodextrins (β-CD), microcalorimetry titration has been performed in aqueous phosphate buffer solution (pH = 7.20) at 298.15 K to give the complex stability constants (K_s) and the standard free energy ($\Delta G°$), enthalpy ($\Delta H°$), and entropy changes ($\Delta S°$) for the 1:1 inclusion complexation of β-CD (**1**), mono(6-amino-6-deoxy)-β-CD (**2**), mono(6-carboxymethylamino-6-deoxy)-β-CD (**3**), and mono[6-(*R*(−)-1-hydroxymethylpropylamino)-6-deoxy]-β-CD (**4**) with representative bile salts, deoxycholate, cholate, glycocholate, and taurocholate. The results obtained indicate that the aminated β-CDs could alter significantly the original molecular binding ability and selectivity of parent β-CD through the cooperative electrostatic interaction, van der Waals, and hydrophobic interactions between hosts and guests. As compared with parent **1** and aminated β-CD **2**, glycine-modified β-CD **3** possessing a hydrophilic carboxylic group at the sidearm shows a lower binding ability toward bile salts, attributed to the relatively weaker hydrophobic interactions and the electrostatic repulsion between host and guest to some extent. However, the *R*(−)-2-amino-1-butanol-modified β-CD **4** possessing additional binding sites at the chiral sidearm could significantly orient the guest molecules to be included in cavity and thus evidently enhances the molecular binding ability and selectivity through steric interactions. Thermodynamically, the higher complex stability for inclusion complexation of aminated β-CDs is mainly resulting from enthalpy gain with smaller entropy loss. The combination of calorimetric titration experiments and ROESY spectra establishes the correlation between the thermodynamic parameters and the conformation of the resulting complex, and reveals the factors governing the molecular binding ability and selectivity of bile salts by aminated β-CDs.

Introduction

Modified cyclodextrins (CDs) possessing the diverse functional groups, such as pyridinio and picolinio groups with positive charge,[1] phosphorus,[2] amino acids,[3] chromophoric groups,[4] amino,[5] and organoselenium[6] as additional binding sites, have been known to alter significantly the molecular binding ability and selectivity toward a variety of guests in comparison with parent CDs through the simultaneous operation of available weak interactions.[7] Therefore, much work has been devoted to the design and synthesis of novel CD derivatives with diverse functional substituting groups in order to investigate their molecular recognition behavior and inclusion complexation mechanism in recent years.[1−12] Kano et al. studied the chiral recognition of α-amino acid derivatives by charged mono- and peraminated β-CDs through Coulombic interaction between host and guest by means of ^{1}H NMR spectroscopy, demonstrating the advantage of the use of Coulombic interaction for chiral recognition in host−guest chemistry.[5a] Recently, Inoue and co-worker had reported the chiral recognition thermodynamics of the simple amino-modified β-CDs with amino acid derivatives, indicating that the resulting complexes of host and the L/D-isomers exist counterbalance between van der Waals and Coulombic interactions despite only a tiny difference in complex stability.[5c] Unfortunately, the thermodynamic origin of molecular recognition of aminated β-CDs possessing chiral functional groups has not been investigated so far to our best knowledge.

On the other hand, bile salts are important surfactant-like biological amphipathic compounds possessing a steroid skeleton, which have distinctive detergent properties and play an important role in the metabolism and excretion of cholesterol in mammals.[13] Hence, the studies on the molecular recognition of bile salts by CDs are an attractive topic of host−guest chemistry. Recently, the interactions of some bile salts with CDs have been well studied.[14−20] Brown et al.[14] reported the studies on the thermodynamics and kinetics of the inclusion complexation of some bile salts with native β-CD by NMR spectroscopy. Tato and co-workers[15] studied the complex geometry of β-CD and its derivatives in D_2O by ROESY experiments, exhibiting different binding modes for inclusion complexation with bile salts. Breslow,[16] Cooper,[17] and Ollila[18] et al. separately reported the thermodynamics on the inclusion complexation of some steroids by native and/or commercially available methyl, hydroxypropyl β-CDs under different experimental conditions. Reinhoudt et al.[19] reported the cooperative binding of bile salts by CD dimers in 1 mM sodium hydroxide aqueous solution by microcalorimetry, showing enhanced binding ability toward cholate and deoxycholate by CD dimers. More recently, we have reported the complexation and sensing behavior upon inclusion complexation with bile salts by bridged β-CD possessing fluorescent spacer by means of fluorescence spectroscopy.[20] Apparently, the inclusion complexation of bile salts as repre-

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SCHEME 1: Synthetic Routes to Hosts 2−4

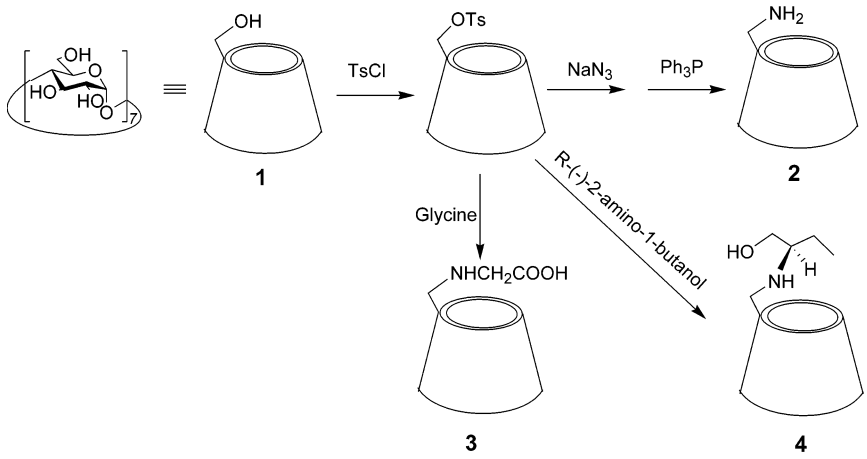


CHART 1: Structures of Host Compounds

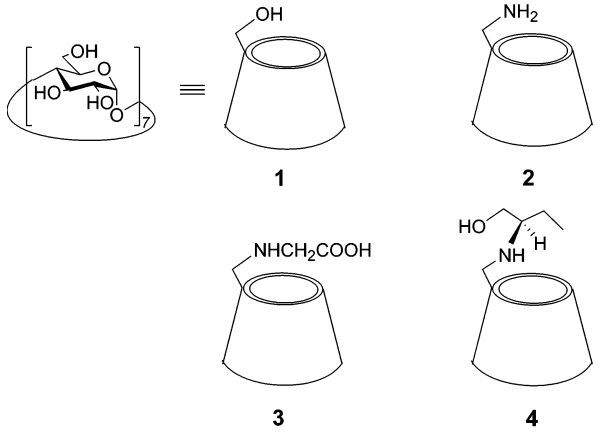
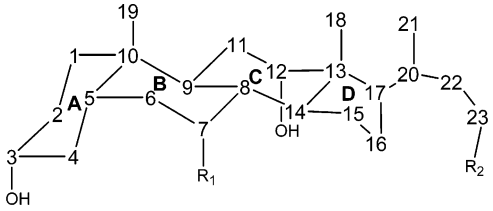


CHART 2: Structures of Bile Salts



Guests	R_1	R_2
Cholate (CA)	OH	COONa
Deoxycholate (DCA)	H	COONa
Glycocholate (GCA)	OH	$CONHCH_2COONa$
Taurocholate (TCA)	OH	$CONHCH_2CH_2SO_3Na$

sentative guest molecules with CDs could be taken as an excellent model system mimicking the substrate-specific interaction of enzymes and could be used to reveal the recognition mechanism of size/shape and chiral of guest molecules by synthetic acceptors. It is well-known that aminated β-CDs, possessing positive charge at neutral to acidic pHs,[5d] could enhance the original molecular binding ability and selectivity of parent β-CD through the electrostatic interaction, but the effects of hydrophobic/hydrophilic and steric selectivity of amino sidearms attached to β-CD upon inclusion complexation with guest molecules are still unknown. In the present work, we wish to report our investigation results on the complexation thermodynamics of the aminated β-CDs **2**−**4** (Chart 1) with amino, carboxymethylamino, and *R*(−)-1-hydroxymethylpropylamino and bile salts (Chart 2). It is of our special interest to examine the contributions of electrostatic, van der Waals, and hydrophobic interactions as well as the additional binding sites upon the inclusion complexation with aminated β-CDs. Combining the calorimetric titration experiments and ROESY spectra, we could establish the correlation between the thermodynamic parameters and the conformation of the resulting complex, which will serve our understanding of the factors governing the molecular binding ability and selectivity of bile salts by aminated β-CDs.

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. All bile salts, i.e., deoxycholate (DCA), cholate (CA), glycocholate (GCA), and taurocholate (TCA) were purchased from Sigma and used as received. *N*,*N*-Dimethylformamide (DMF) was dried over calcium hydride for 2 days and then distilled under a reduced pressure before use. Other chemicals, i.e., dicyclohexylcarbodiimide (DCC), triethanolamine, glycine, and *R*(−)-2-Amino-1-butanol, were commercially available of high quality (Sigma) and used without further purification, unless noted otherwise. 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.[21] Mono(6-amino-6-deoxy)-β-CD (**2**) was prepared according to the reported procedure.[22] Disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in distilled, deionized water to make a 0.1 M phosphate buffer solution of pH 7.20 for microcalorimetric titrations. Synthetic routes to hosts **2**−**4** are given in Scheme 1.

Measurements. Elemental analyses were performed on a Perkin-Elmer-2400C instrument. ^{1}H NMR and ^{13}C NMR spectra and rotating-frame Overhauser effect spectroscopy (ROESY) experiments were recorded on a Varian Mercury VX300 instrument. All NMR experiments were carried out in D_2O.

Synthesis of Mono(6-carboxymethylamino-6-deoxy)-β-CD (3). Glycine (0.8 g) and 6-OTs-β-CD (4.0 g) were dissolved in water (30 mL) containing triethanolamine (20 mL), and the stirred mixture was heated to reflux under a nitrogen atmosphere for 24 h. After evaporation of most solvent under reduced pressure, the resulting solution was poured into vigorously stirred anhydrous ethanol (500 mL), and the resultant mixture was stored in a refrigerator to produce a slight yellow precipitate.

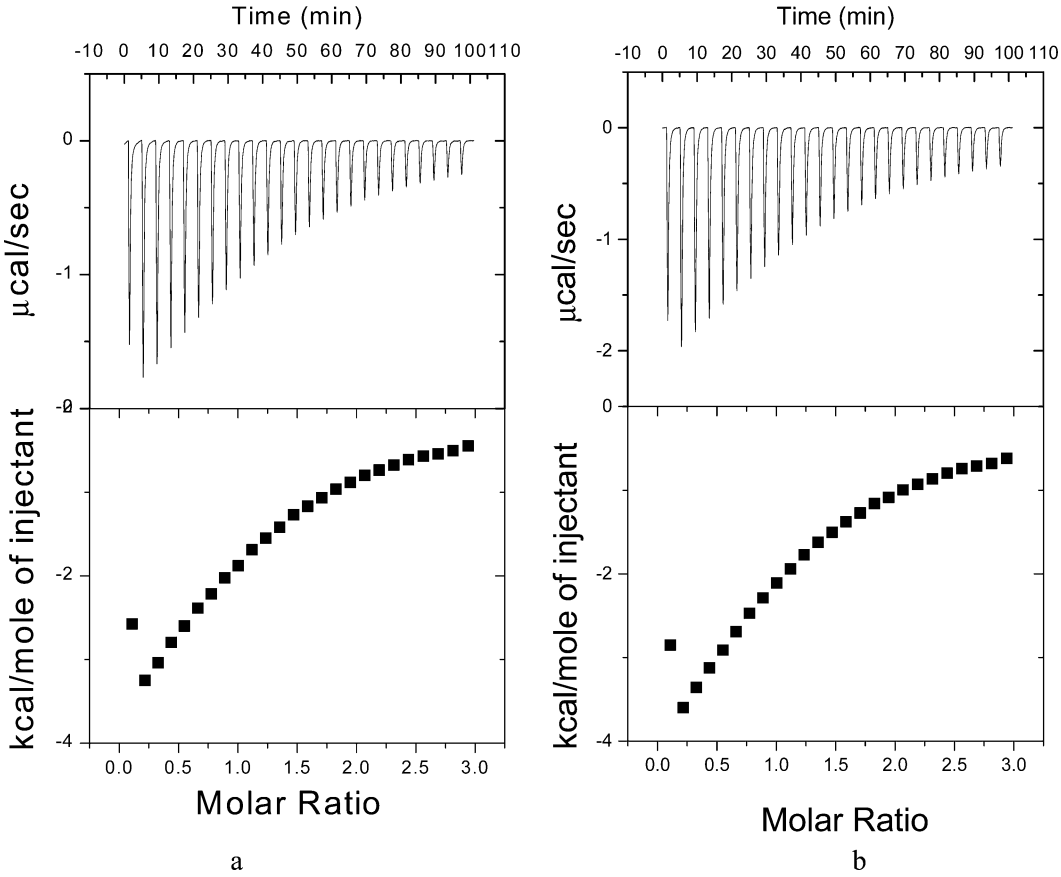


Figure 1. Calorimetric titrations of host **2** with CA (left) and DCA (right) in phosphate buffer (pH 7.20) at 25 °C. (a) Raw data for sequential 10 μL injections of CD solution (2.00 mM) into bile salt solution (0.13 mM). (b) Heats of reaction as obtained from the integration of the calorimetric traces.

The crude solid product was collected by filtration and then purified by column chromatography on a hydroxymethylcellulose column with an aqueous ammonium bicarbonate eluent (0.05 mol dm^{-3}), followed by chromatography on a Sephadex G-25 column with deionized water as eluent, to give white product (2.2 g) in 50% yield. ^{1}H NMR (D_2O, TMS, ppm): δ 2.55 (m, 2H), 2.83−3.08 (m, 1H), 3.25−3.79 (m, 41H), 4.85 (d, 7H). ^{13}C NMR (D_2O, ppm): δ 178.3, 101.5, 83.5, 81.1, 79.0, 73.0, 72.1, 70.2, 60.2, 58.8, 55.8, 51.9, 48.7, 42.0. Anal. Calcd for $C_{44}H_{73}O_{36}\cdot 7H_2O$: C, 40.09; H, 6.65; N, 1.06. Found: C, 40.05; H, 6.75; N 1.15.

Synthesis of Mono[6-(*R*(—)-1-hydroxymethylpropylamino)-6-deoxy]-β-CD (4). Well-dried 6-OTs-β-CD (1.0 g) was dissolved in 20 mL freshly distilled *R*(−)-2-amino-1-butanol. The mixture was stirring at room temperature under nitrogen for about 3 h and then the solution was allowed to warm and stir at 70−80 °C for 10 h. Then the reaction mixture was poured into acetone to give a precipitate. The crude product obtained by filtration was dissolved in water, and the resultant solution was poured into acetone to give a precipitate. The same procedure was repeated twice. The precipitate was collected and dried to give a pure white sample, in 71% yield. ^{1}H NMR (D_2O, TMS, ppm): δ 0.72 (t, 3H), 1.26 (m, 2H), 2.45 (s, 1H), 2.62 (t, 1H), 3.00 (d, 1H), 3.24−3.79 (m, 42H), 4.87−4.94 (m, 7H). ^{13}C NMR (D_2O, ppm): δ 101.9, 100.9, 82.9, 81.1, 80.3, 73.2, 72.0, 71.8, 70.3, 61.4, 60.2, 47.2, 22.8, 9.6. Anal. Calcd for $C_{46}H_{79}O_{35}N\cdot 8H_2O$: C, 40.92; H, 7.09; N, 1.04. Found: C, 41.08; H, 6.89; N, 1.18.

Microcalorimetric Titration. The microcalorimetric titrations were performed at atmospheric pressure and 25 °C in aqueous phosphate buffer solution (pH 7.20) by using Microcal VP-ITC titration microcalorimetry, which allows us to determine simultaneously the enthalpy and equilibrium constant from a single titration curve. The instrument was calibrated chemically by performing the complexation reaction of β-CD with cyclohexanol, which gave thermodynamic parameters in good agreement with the literature data.[23] All solutions were degassed and thermostated using a ThermoVac accessory before the titration experiment, and titrations were performed below the critical micelle concentration of the bile salt molecules.

In each run, a phosphate buffer solution of host in a 0.250 mL syringe was sequentially injected with stirring at 300 rpm into the calorimeter sample cell containing a buffer solution of bile salt guests. The sample cell volume was 1.4227 mL in all experiments. Each titration experiment was composed of 25 successive injections (10 μL per injection). Bile salt solutions were applied at a concentration in a range between 0.10 and 0.52 mM, which is below their critical micelle concentration (cmc).[17,18] Typical titration curves are shown in Figure 1. Each titration of CD into 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 CD because less and less bile salt molecules are available to form inclusion complexes.

A control experiment was performed to determine the heat of dilution by injecting a host buffer solution into a pure buffer solution, containing no bile salt molecules. The dilution enthalpy was subtracted from the apparent enthalpy obtained in each titration run, and the net reaction enthalpy was analyzed by using the "one set of binding sites" model, as exemplified in Figure 2 for the complexation of DCA with modified β-CD **4**.

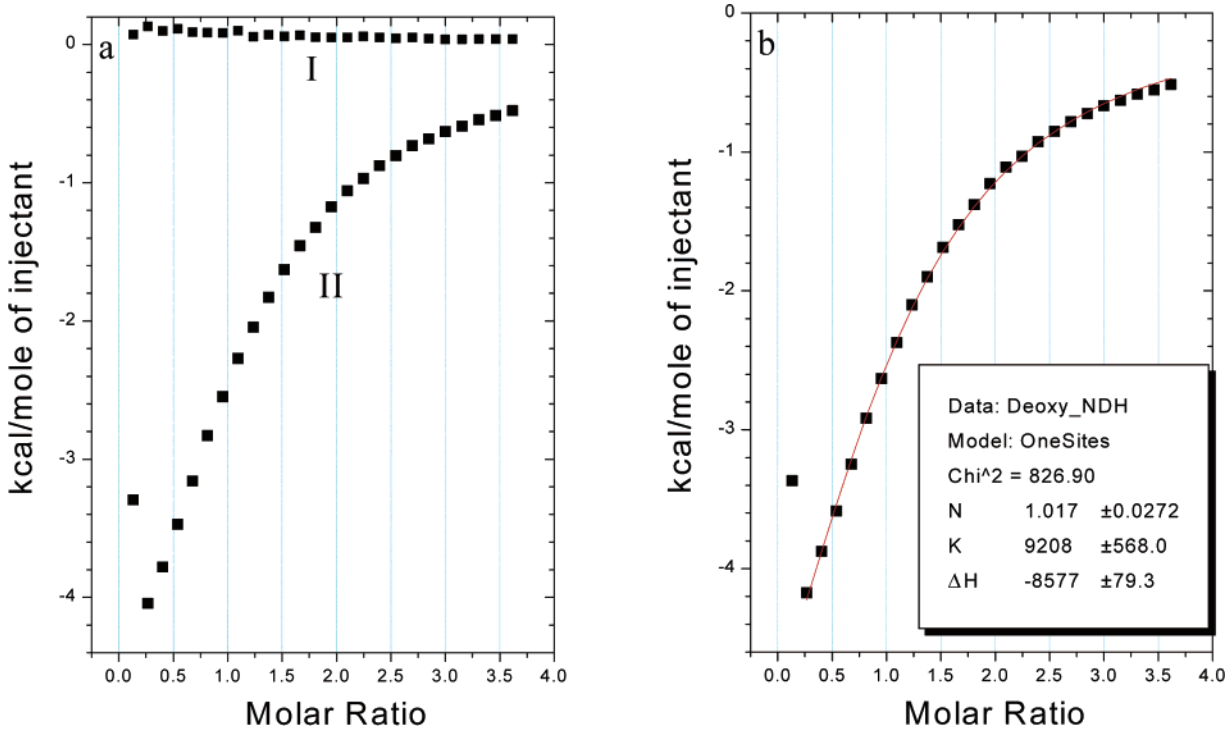


Figure 2. (a) Heat effects of dilution (I) and of complexation (II) of **4** with DCA for each injection during titration microcalorimetric experiment. (b) "Net" heat effect obtained by subtracting the heat of dilution from the heat of reaction, which was analyzed by computer simulation using the "one set of binding sites" model.

TABLE 1: Complex Stability Constant (K_S) and Standard Enthalpy ($\Delta H°$) and Entropy Changes ($T\Delta S°$) for 1:1 Inclusion Complexation of Bile Salt Guests with β-CD 1 and Aminated β-CDs 2−4 in Phosphate Buffer Solution (pH 7.20) at T = 298.15 K

host[a]	guest[b]	N[c]	K_S/M^{-1}	$-\Delta G°$/kJ mol^{-1}	$-\Delta H°$/kJ mol^{-1}	$T\Delta S°$/kJ mol^{-1}
1	DCA	2	4844 ± 16	21.03 ± 0.01	25.79 ± 0.00	−4.76
	CA	4	4068 ± 84	20.60 ± 0.05	22.98 ± 0.45	−2.38
	GCA	2	2394 ± 69	19.29 ± 0.07	22.99 ± 0.08	−3.7
	TCA	2	2293 ± 13	19.18 ± 0.01	23.77 ± 0.08	−4.59
2	DCA	2	7705 ± 3	22.18 ± 0.00	32.16 ± 0.08	−9.98
	CA	2	11160 ± 75	23.10 ± 0.02	25.53 ± 0.25	−2.43
	GCA	4	2075 ± 19	18.93 ± 0.03	25.90 ± 0.03	−6.97
	TCA	2	2309 ± 82	19.20 ± 0.08	26.89 ± 0.07	−7.69
3	DCA	2	4034 ± 15	20.58 ± 0.01	38.91 ± 0.02	−18.33
	CA	2	4832 ± 79	21.03 ± 0.04	24.90 ± 0.16	−3.87
	GCA	2	2221 ± 24	19.10 ± 0.03	19.75 ± 0.05	−0.65
	TCA	2	1322 ± 51	17.82 ± 0.09	32.75 ± 0.69	−14.93
4	DCA	2	9382 ± 173	22.67 ± 0.05	35.78 ± 0.08	−13.11
	CA	2	16920 ± 330	24.13 ± 0.05	28.11 ± 0.12	−3.98
	GCA	2	3904 ± 4	20.50 ± 0.00	24.74 ± 0.23	−4.24
	TCA	2	2796 ± 72	19.67 ± 0.05	20.37 ± 0.19	−0.7

[a] [host] = 1.99−4.16 mM. [b] [guest] = 0.10−0.52 mM. [c] Number of titration runs performed.

The ORIGIN software (Microcal), used for the calculation of the binding constant (K_S) and standard molar reaction enthalpy ($\Delta H°$) from the titration curve, gave the relevant standard derivation on the basis of the scatter of data points in a single titration experiment. The binding stoichiometry was also given as parameters when fitting the binding isotherm (panel b in Figure 2). Knowledge of the binding constant (K_S) and molar reaction enthalpy ($\Delta H°$) enabled calculation of the standard free energy of binding ($\Delta G°$) and entropy changes ($\Delta S°$), according to

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

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

Multiple independent titration runs (N = 2−4) were performed to afford self-consistent thermodynamic parameters, and the averaged values are reported in Table 1. The uncertainties in the thermodynamic parameters reported for host−guest complexation are two standard deviations of the mean value unless stated otherwise.

Results and Discussion

Aminated β-CDs have been known to possess positive charge at pH = 7.2,[5d] which could enhance the binding ability toward negatively charged guest molecules through additional electrostatic interactions in the opposite charged host−guest complexation. As can be seen from Table 1, aminated β-CDs **2**−**4** with amino, carboxymethylamino, and R(−)-1-hydroxymethylpropylamino sidearms exhibit different binding behaviors and molecular selectivities upon inclusion complexation with bile salts. As compared with parent β-CD **1**, the positively charged monoamino-modified β-CD **2** and aminated β-CD **4** possessing an additional binding site in the chiral sidearm evidently enhance

the binding ability and molecular selectivity toward bile salts. In contrast, glycine-modified β-CD **3** possessing a hydrophilic carboxylic group at the sidearm shows a lower binding ability toward bile salts, attributed to the relatively weaker hydrophobic interactions between host and guest to some extent and the electrostatic repulsion between the anionic carboxylate at the sidearm of **3** and anionic carboxylate or sulfonate of bile salts. Thermodynamically, the binding behavior of bile salts by parent **1** and aminated β-CDs **2**−**4** was entirely driven by favorable enthalpy changes with accompanying small unfavorable entropy changes ($\Delta H° < 0$; $T\Delta S° < 0$), which are attributed to the predominant contribution of the van der Waals interactions arising from the size/shape fit and geometrical complement between host and guest and to the accompanying decreases in translational and structural freedoms upon complexation.[5c,24] To compare the contributions of electrostatic and hydrophobic interaction as well as the additional binding site upon the inclusion complexation with bile salts, the binding behavior and thermodynamic parameters are respectively discussed according to different hosts.

Binding Stoichiometry. The microcalorimetric experiments of β-CD **1** and aminated β-CDs **2**−**4** with bile salts, i.e., CA, DCA, GCA, and TCA, showed typical titration curves of 1:1 complex formation. The stoichiometric ratios (*N* value) that we observed from curve-fitting results of the binding isotherm fell within the range of 0.9−1.1:1. This clearly indicates that the majority of the inclusion complexes had a 1:1 stoichiometry of bile salts and CDs. Simultaneously, the 1:1 binding modes for the inclusion complexation of modified β-CD **4** with CA and DCA are also investigated by ROESY experiments, which further validates the above microcalorimetric experiment results.

Complexation of Bile Salts by Native β-CD 1. Investigations on molecular binding behavior of steroids by CDs indicated that DCA and CA possessing A, B, C, and D rings are able to enter and bind to the asymmetric CD from either the primary or the secondary side[25] and the bile salts can also enter into the cavity of CD either with the A-ring of the steroid body or with the carboxylate group (tail).[14a,15a,26] Apparently, the conformation and binding behavior of the resulting complex of bile salts and CDs are affected by the structures of hosts and guests as well as the environments employed. In this text, the stoichiometric 1:1 binding behavior was observed for the inclusion complexation of bile salts and native β-CD. The obtained thermodynamic parameters are used to compare with data for aminated β-CDs **2**−**4** in order to elucidate the sidearm effects and the role of the electrostatic interaction between protonated amino of **2**−**4** and the negative charge of the carboxyl or sulfonic acid group of the bile salts as well as the potential binding mechanism. As can be seen from Table 1, the enthalpy change for the complexation of β-CD with DCA (25.79 ± 0.00 kJ mol^{-1}) is higher than that with CA (22.98 ± 0.45 kJ mol^{-1}), which directly contributes to the increased complex stability. Indeed, the binding ability ($K_s = 4844 \pm 16$) of β-CD with DCA is higher than that with CA ($K_s = 4068 \pm 84$). This is reasonable, since DCA possesses a more hydrophobic structure due to the absence of C-7 hydroxyl group as compared with CA, that it is easier to bind into native β-CD's cavity than CA, which should lead to more favorable hydrophobic and van der Waals interactions giving larger enthalpic and entropic changes. However, the enhanced favorable entropy gain by the desolvation effect may be canceled by the unfavorable entropy change caused by the structural freezing of the resulting complexes of host β-CD and guest DCA. Therefore, the stronger interaction between native β-CD and DCA only shows the larger negative enthalpic change, directly contributing the relatively larger complex stability constant. Meanwhile, β-CD shows a lower binding ability upon complexation with GCA and TCA. From the thermodynamic point of view, the complexation of β-CD with GCA and TCA exhibit similar enthalpic changes but much more unfavorable entropic changes as compared to that of β-CD with CA, which maybe due to the more polar side chains (tails) of GCA and TCA than CA.

Complexation of Bile Salts by Modified β-CD 3. Possessing the hydrophilic carboxylic group in the sidearm, modified β-CD **3** decreased the microenvironment hydrophobicity of natural β-CD cavity to some extent, and at the same time there should exist electrostatic repulsion between the anionic carboxylate at the sidearm of **3** and anionic carboxylate or sulfonate of bile salts. Thus, the binding constants of **3** upon inclusion complexation with DCA, GCA, and TCA are less than that with natural β-CD. On the other hand, the electrostatic interactions between the positively charged amino group of **3** and the carboxylic group and/or sulfonic acid group of bile salts should result in higher heat enthalpies than in the case of natural β-CD except for complexation with GCA. However, the higher heat enthalpy does not directly imply a higher complex stability for the inclusion complexation of **3** with DCA and TCA because the entropy loss caused by the fixation of host−guest structures partly counteracts the enthalpy gain. Unexpectedly, the resulting complex stability of aminated β-CD **3** with CA is higher than that of native β-CD **1,** which is mainly attributed to the more favorable enthalpy ($-\Delta\Delta H° = 1.92$ kJ mol^{-1} > $\Delta T\Delta S° = 1.49$ kJ mol^{-1}). This may be rooted in the enhanced cooperative van der Waals, hydrogen-bonding, and electrostatic interactions exceeding the decreased hydrophobicity of the interior of glycine-modified β-CD **3**. It is considered that the driving force of the inclusion complexation of bile salts with **3** must be a counterbalance between van der Waals, electrostatic interaction, and hydrophobic interaction.

Complexation of Bile Salts by Aminated β-CDs 2 and 4. As compared with parent β-CD **1** and modified β-CD **3** possessing a hydrophilic carboxylic group in the sidearm, positively charged monoamino-modified β-CD **2** and modified β-CD **4** possessing an additional binding site in the chiral arm, i.e., hydroxyl group, evidently enhance the molecular binding ability and selectivity toward CA and DCA through electrostatic, steric interactions, and could orient the guest molecules to be included in cavity. Thermodynamically, the inclusion complexation of DCA and CA by aminated β-CDs **2** and **4** exhibits more negative $\Delta H°$ and more unfavorable $\Delta S°$ compared to those for native β-CD **1**. The more negative enthalpy change is likely arise from the disturbance of the originally well-optimized van der Waals between host−guest interactions in the β-CD cavity, which is caused by attractive electrostatic interaction.[5c] Our previous study[27] also indicated the effective electrostatic interaction between host and guest usually leads to a more exothermic reaction enthalpy. Hence, it is reasonable that we assume that the "net" experimentally observed enthalpic increases most likely originate from effective electrostatic interactions upon complexation with amino β-CD **2** rather than β-CD **1**. As compared with parent **1**, the effective electrostatic interactions may be about 2.55 kJ mol^{-1} for the complexation of amino β-CD **2** with CA. The less negative $\Delta H°$ for the complexation of CA with **3** vs **2** (about 0.63 kJ mol^{-1}) may be due to the relatively smaller hydrophobicity of the CD cavity resulting from the hydrophilic carboxylic group at the sidearm and the electrostatic repulsion between the anionic carboxylate at the sidearm of **3** and anionic carboxylate or sulfonate of bile

salts. In contrast, the more negative $\Delta H°$ for the complexation of CA with **4** vs **2** (about 2.58 kJ mol^{-1}) suggests that the additional binding site, hydroxyl group, in the chiral tether moiety of **4** plays an important role in the complexation with CA. In addition, the more negative $\Delta S°$ observed for modified β-CDs **2** and **4** than for native β-CD **1** is likely to originate from the conformation fixation of host and guest and the rigid complex formation upon complexation. It is interesting that the chiral tether possessing an additional binding site may be favored and may ease the conformation fixation of host and guest upon complexation to afford the highest binding ability toward CA and DCA up to 16 920 and 9382 M^{-1}, which is 4.2 and 2 times the binding constants for the inclusion complexation of CA and DCA with native β-CD **1**, respectively. Though the inclusion complexation of aminated β-CDs **2** and **4** with DCA gives more exothermic reaction enthalpies compared to that with CA, the complex stability is not enhanced but decreased. Apparently, the strong interaction between host and guest leads to the more favorable negative $\Delta H°$, which is counteracted by the relatively more unfavorable negative $\Delta S°$ caused simultaneously by more rigidity in the structure, giving moderate binding constants. Therefore, we can deduce that the introduction of an oppositely charged group and an additional binding site to the CD rim can significantly enhance the binding ability of parent CD toward opposite charged guests, which can be used as a rule to design and synthesize receptors with a specific functional group to control the binding behavior toward guests.

β-CD derivatives **2** and **4** give a lower binding ability upon complexation with GCA and TCA as compared to the complexation with CA and DCA, which shows a tendency similar to that for the complexation of β-CD **1** and derivative **3**. The universal decreased binding ability toward GCA and TCA must relate to the structural differences from CA and DCA. GCA and TCA possess the same steroid skeleton as CA. On the other hand, GCA and TCA are the resulting compounds of the conjugation of chololic acid with glycine and taurine, respectively. Thus, the more polar side chains at C23 for GCA and TCA remarkably affect their binding thermodynamics. The mechanism of GCA and TCA binding to CD is more straightforward, since GCA and TCA are much less likely to enter the interior of CD with their "tail" first. That is also the reason for the nearly nonselectivity of β-CD toward GCA and TCA. From Table 1, we can see that β-CD **2** possessing a charged amino group at the rim of the CD cavity does not enhance the binding ability toward GCA and TCA, giving only similar binding constants with native β-CD. These results indicate that the amino group nearly does not operate in the complexation. Therefore, we can deduce that GCA and TCA most likely bind CD with their A-ring (OH) and not with their tail (side chain). While for host **3**, the interaction of an aminoethyl acid group and CD cavity changes the intrinsic chirality of the CD and the hydrogen binding system, which therefore affect the hydrophobicity of cavity and limits the depth of A-ring of GCA and TCA into the CD cavity, affording a decrease in binding ability toward GCA and TCA, especially for TCA (0.58 times the binding constant for β-CD). Interestingly, modified β-CD **4** gives enhanced binding ability as compared with **1**−**3**, which is understandable because the chiral tether of CD provides not only the positive amino group but also the additional binding sites, i.e., the hydroxyl group, to participate in the electrostatic attraction and hydrogen binding in the complexation between β-CD **4** and bile salt molecules, making for easier and stronger interaction with bile salts. For instance, the complexation of GCA and TCA with modified β-CD **4** possessing chiral tether gives 1.63 and 1.22 times the binding constants (3904 ± 4 and 2796 ± 72 M^{-1}) as compared with native β-CD **1** (2394 ± 69 and 2293 ± 13 M^{-1}), respectively. Therefore, the structures, properties, and size/shape fit of guest and host molecules are the primary and fundamental factor governing the inclusion complexation of host−guest. At the same time, we can draw the conclusion that the cooperative van der Waals, electrostatic, hydrogen bonding, and hydrophobic interactions are the main factors governing the molecular binding ability and selectivity of bile salts by aminated β-CDs, combining the front experimental results on the weaker complex stability of bile salts by modified β-CD **3** possessing the hydrophilic carboxylic group in the sidearm.

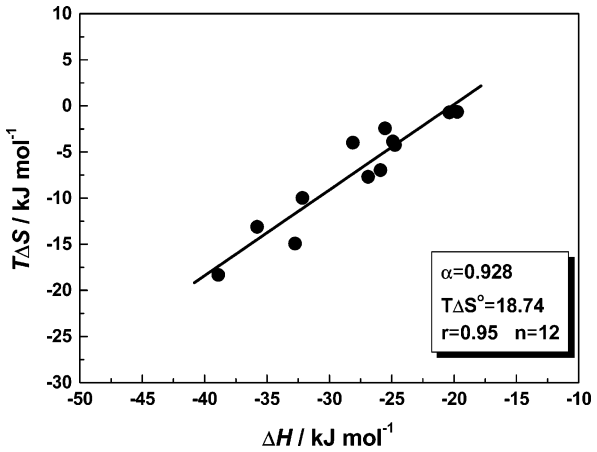


Figure 3. Enthalpy−entropy compensation plot for the inclusion complexation of various bile salts with aminated β-CDs **2**−**4** obtained in the present work in aqueous buffer solution at 298.15 K.

Molecular Binding Ability and Molecular Selectivity toward Bile Salts. Native β-CD **1** and glycine-modified β-CD **3** afford relatively small binding constants probably due to the weak van der Waals, hydrophobic interactions, and the host structure features, which have been discussed in the above paragraphs. Amino-modified β-CD **2** only enhances the binding ability toward CA and DCA. However, since it possesses a chiral tether, the aminated β-CD **4** exhibits a significantly enhanced molecular binding ability through the cooperative electrostatic, van der Waals, and hydrogen-bonding interactions. Experimental data indicate that the chiral tether as the additional binding site plays an important role in the complexation of **4** and bile salts.

It is also interesting to compare the "host selectivity" sequence obtained for each bile salt. The binding constant for the complexation of each bile salt by native β-CD **1** and aminated β-CDs **2**−**4** increases in the following order:

DCA: **3** < **1** < **2** < **4**

CA: **1** < **3** < **2** < **4**

GCA: **2** < **3** < **1** < **4**

TCA: **3** < **1** ≤ **2** < **4**

As can be seen from Table 1, the binding constants of aminated β-CDs **2**−**4** with guest CA molecule are larger than those of native β-CD, that is, the K_S values for the modified CDs are enhanced by factors of 2.74 for **2**, 1.19 for **3**, and 4.16 for **4**, respectively. Meanwhile, modified CD **4** gives the largest guest selectivity for CA/TCA up to 6.1 ($K_S^{(4-CA)}/K_S^{(4-TCA)}$) and 1.8 times guest selectivity for CA/DCA. Amino β-CD **2** gives 5.4 times selectivity for CA vs GCA. The difference of the Gibbs free energy changes for the complexation of **4** with CA and TCA ($\Delta\Delta G° = \Delta G°_{CA} - \Delta G°_{TCA}$) was the largest up to 4.46

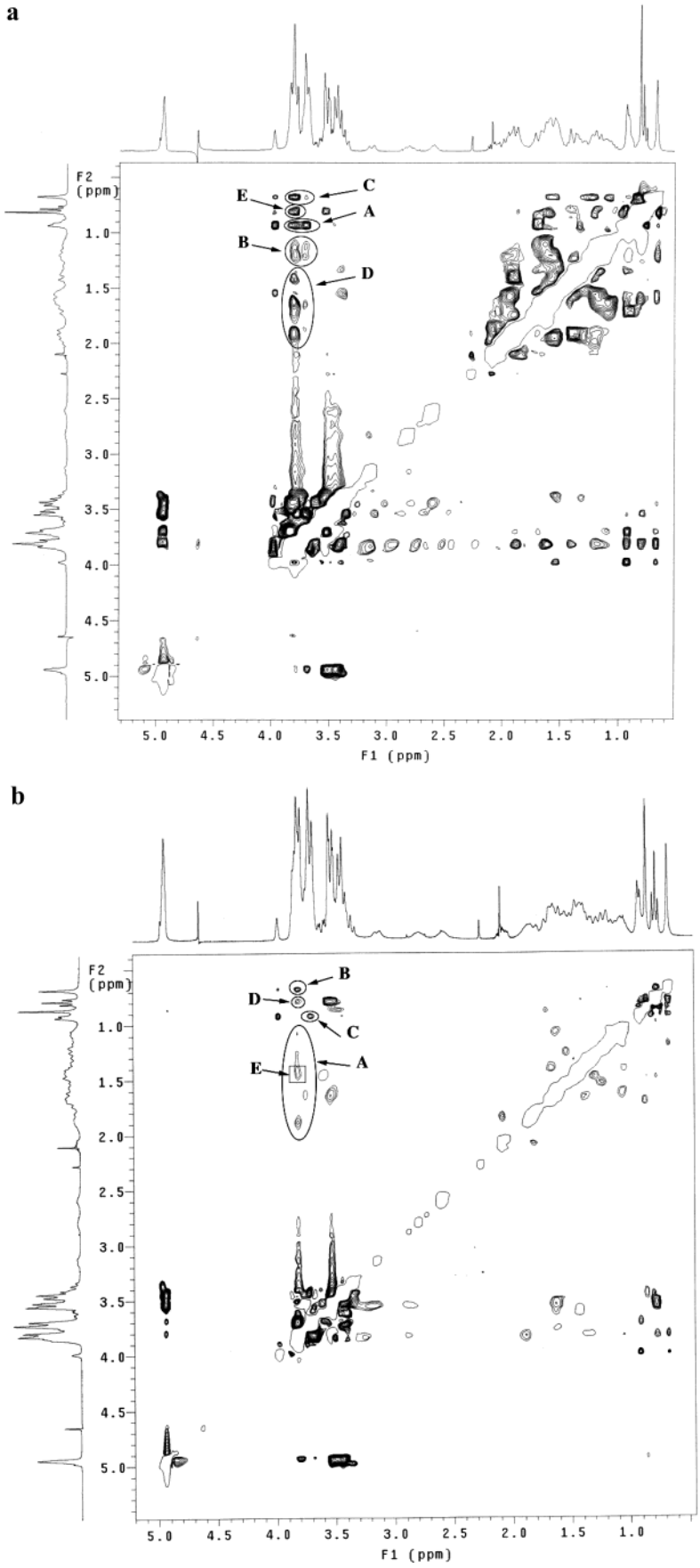


Figure 4. (a) ROESY spectrum of host **4** and CA. (b) ROESY spectrum of host **4** and DCA with a mixing time of 400 ms at 298.1 K.

kJ mol^{-1}. Thermodynamically, the complexation of TCA with **4** gives $\Delta H°$ and $T\Delta S°$ values comparable to those for native β-CD **1**, the enthalpic loss ($\Delta H°_{\mathbf{4}} - \Delta H°_{\mathbf{1}} = 3.4$ kJ/mol) is well compensated by the equally decreasing entropic loss ($T\Delta S°_{\mathbf{4}} - T\Delta S°_{\mathbf{1}} = 3.8$ kJ/mol). In contrast, the complexation of CA with **4** gives a larger enthalpic gain than that for β-CD

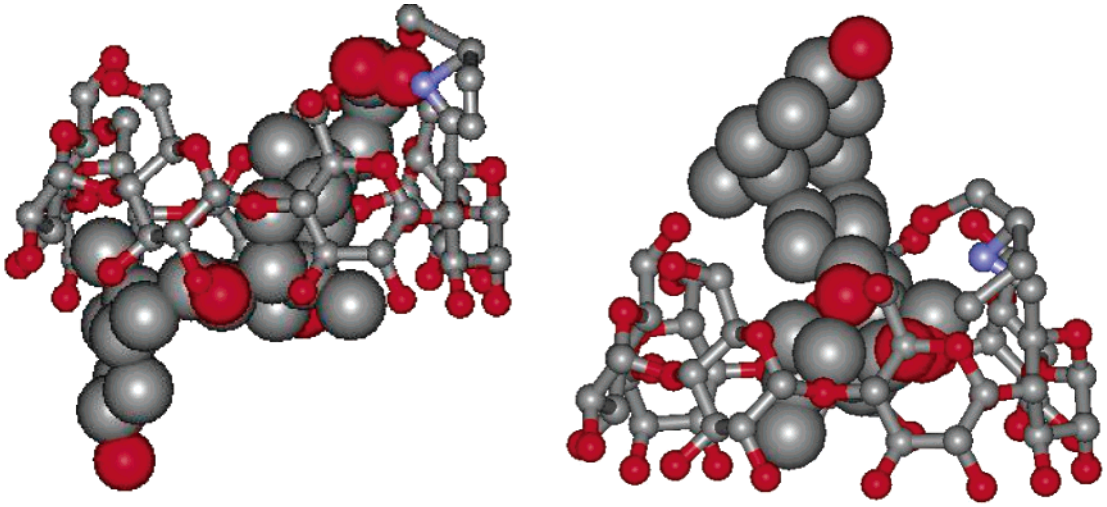


Figure 5. Molecular modeling of the inclusion complexation upon **4** with (a) CA and (b) DCA using the CS Chem 3D 6.0 and Web Labviewer 3.7 software.

($\Delta H°_4 - \Delta H°_1 = -5.13$ kJ/mol), which is not compensated by a small entropic loss ($T\Delta S°_3 - T\Delta S°_1 = -1.6$ kJ/mol). As a consequence of such opposite behavior of $\Delta H°$ and $T\Delta S°$, the negligible molecular selectivity of β-CD ($\Delta\Delta G° = \Delta G°_{TCA} - \Delta G°_{CA} = 1.42$ kJ/mol) is substantially enhanced to give a $\Delta\Delta G°$ value of 4.46 kJ/mol for host **4**.

Enthalpy—Entropy Compensation. Enthalpy−entropy compensation, which has often been observed empirically in the kinetic and thermodynamic parameters determined for a wide variety of reactions and equilibria, has long been an active topic in the chemical field. Numerous experimental data in the original and review articles indicate that the widely observed compensation enthalpy−entropy relationship is a powerful tool to understand and even to predict thermodynamic behavior.[23a,27,28]

The linear $\Delta H-\Delta S$ relationship observed experimentally leads to eq 1; when integrated, this gives us eq 2.

$$T\Delta\Delta S = \alpha\Delta\Delta H \quad (1)$$

$$T\Delta S = \alpha\Delta H + T\Delta S_0 \quad (2)$$

Thus, the slope (α) of the $T\Delta S$ vs ΔH plot (eq 2) indicates to what extent the enthalpic gain ($\Delta\Delta H$) is canceled by entropic loss, while the positive intercept indicates that the complex is stabilized even in the absence of enthalpic contributions, as far as the $T\Delta S$ term is positive. In this text, the correlation of enthalpy−entropy compensation is performed by plotting $T\Delta S$ vs ΔH using current limited experimental data. As shown in Figure 3, a good straight line with a correlation coefficient of 0.95 was obtained when reporting the limited CD−bile salt systems presently examined, having intercept $T\Delta S_0 = 18.7$ kJ mol^{-1} and slope = 0.93, respectively. However, a previous report on enthalpy−entropy compensation for the complexation of 1070 guest molecules with natural CDs gave a slope of $\alpha = 0.88$ and intercept of $T\Delta S_0 = 12$ kJ/mol. From the above results, we can see that these values we obtained are much larger than those relative to the natural CDs, and in agreement with those reported in the literature for complexes formed by modified CDs with flexible sidearms, they indicate that the inclusion complexation of aminated β-CDs with bile salt gives a larger conformational change and extensive desolvation effect.

ROESY Experiments. Since two protons located closely in space (the corresponding internuclear distance is smaller than 3−4 Å) can produce NOE cross-peaks between the relevant protons in the NOESY or ROESY spectra, two-dimensional NMR spectroscopy has recently become an important method for the investigation of the interaction between host CDs and guest molecules. This technique has been previously used to study the complexation of steroids and other compounds with CDs. Tato et al.[15] reported the ROESY study on the resulting complexes of CDs **1** and **2** with CA, respectively. The results indicated that in the 1:1 complex between β-CD **1** and CA the steroid body entered forward into the inner cavity of β-CD by the side of the secondary hydroxyl groups, with the side chain folded toward the steroid body, i.e., rings D and C are totally and partially included, respectively, while the ROESY spectrum of amino-modified β-CD **2** and CA exhibited different interactions of the side chain of CA with H5 and H6 of β-CD **2**. The facts indicated that the side chain was unfolded, with the negative carboxylate group moving toward the positive protonated amino group, and the side-chain elongation produced a deeper penetration of the steroid body in the inner cavity of β-CD **2**, which further proves the existence of electrostatic interaction. As a consequence, the stability constants for the inclusion complexation of amino CA with β-CD **2** should be larger than that with native β-CD **1**, which was in agreement with our thermodynamics results. To further investigate the mechanism of enhanced binding ability and the binding mode of interactions between **4** and bile salts as well as to establish the correlation between the conformation of the resulting complexes and the thermodynamics obtained for modified β-CD **4** tethered with chiral amino group, the ROESY experiments of modified β-CD **4** were performed in the presence of CA or DCA in D_2O. The spectra obtained are shown in Figure 4, parts a and b, respectively.

It is well known that only cross-peak interactions with H3, H5, and H6 of CDs were considered to analyze the results, because H2 and H4 are not facing the inner cavity and H1 is affected by D_2O. The notation used is H*n* for CD protons and P*n* for bile salts protons, where *n* is the carbon number indicated in Chart 2. As shown in Figure 4a, the spectrum for the resulting complex of CA-**4** exhibits clear NOE cross-peaks (peaks A) between the side chain protons (P21) and H3, H5 of CD. Meanwhile, cross-peaks B, C, and D present the interactions of the protons at D-ring of CA with H3 and/or H5 of CD cavity, describing that the D-ring of CA is accommodated shallowly in the cavity. Further observations show that the interactions

(peaks B) of P15, 16 of CA and H3 of CD are slightly stronger than that with H5 and the interaction (peaks C) of P18 and H3 of CD is much stronger than that with H5. From the above information, it is deduced that CA enters CD **4** from the second side of CD with the side chain and D-ring. At the same time, the ROESY experiment suggest that the side chain with the negative carboxylate group of CA moves toward the positive protonated amino group of **4**. On the basis of the above facts, together with the interaction of P19 of CA and H3 of CD (peak E), we propose the complex structure of CA-**4** (Figure 5a).

For the resulting complex of DCA-**4**, the ROESY spectrum exhibits entirely different NOE cross-peaks. Not only the cross-peaks (Peaks A and B) between D-ring of DCA and the H5, H6 of CD but also those (Peak C) between the side chain protons (P-21) and H3 of CD are observed, implying that the D-ring of DCA is included within the cavity of CD from the primary side of CD. Meanwhile, peaks D and E exhibit the interactions between the ethide protons of chiral tether and H6 of CD. The fixed structure of the complex DCA-**4** should be attributed to the cooperative van der Waals interaction and the electrostatic attractive interaction, as well as, the hydrogen bonding interactions between the hydroxyl group of chiral tether and the oxygen atom in the position 12 of DCA. On the basis of these facts, we propose the complex structure of DCA-**4** (Figure 5b).

From the above results of ROESY experiments, we can establish the correlation between the conformation of the resulting complexes and the thermodynamics obtained for modified β-CD **4** tethered with chiral amino group. Evidently, the deep inclusion of CA into β-CD **4** gave larger binding constants than the relatively shallow inclusion of DCA into β-CD **4**. From the thermodynamic point of view, the van der Waals interactions together with the cooperative additional electrostatic attractive interaction and the hydrogen bonding interactions between the hydroxyl group of chiral tether and the oxygen atom in the position 12 of DCA should lead to the large enthalpy gain, which was canceled by the more negative entropy caused by the weaker desolvation effect and the structural freezing of the resulting complexes of host **4** and guest DCA, giving the lower complex stability as compared with CA. Meanwhile, experimental results further demonstrated that the higher heat enthalpy does not directly imply a higher complex stability for the inclusion complexation of **4** with DCA because the entropy loss caused by the fixation of host−guest structures partly counteracts the enthalpy gain, giving a lower binding constant than that with CA. Structurally, the chiral tether and the positive amino group play an important role in the complexation between β-CD **4** and bile salt molecules.

Conclusion

In summary, we have demonstrated that β-CDs **2**−**4** tethered by a protonated amino group possessing different substituted groups can enhance not only the molecular binding ability toward bile salts by electrostatic interaction but also molecular selectivity by a chiral sidearm. Thermodynamically, the enhanced molecular recognition ability resulted from enthalpy gain with smaller entropy loss. The binding behavior inferred indirectly from the ROESY experiments shows that entirely different binding modes exist in the inclusion complexation of CA and DCA with aminated β-CD **4** possessing chiral tether, revealing that the cooperative electrostatic and van der Waals interactions of the protonated amino group, the chiral sidearm possessing an additional binding site, and the chiral cavity of aminated β-CD **4** are the factors governing the molecular binding ability and selectivity of bile salts by aminated β-CDs.

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