Thermodynamics of Molecular Recognition of Bile Salts by 3,6'-(Oligoethylenediamino-Bridged) β-Cyclodextrin Dimers

Yu Liu*, Shu Kang, Yong Chen, Rui Cao and Jun Shi

Department of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, P.R. China

Abstract: The molecular recognition behaviors of some representative bile salts by three 3,6'-bridged β-cyclodextrin dimers with oligo(ethylenediamino) linkers in different lengths, i.e. 3,6'-(ethylenediamino-bridged) β-cyclodextrin dimer (**1**), 3,6'-(diethylenetriamino-bridged) β-cyclodextrin dimer (**2**), and 3,6'-(triethylenetetraamino-bridged) β-cyclodextrin dimer (**3**), were investigated in aqueous phosphate buffer solution (pH 7.20) at 25°C by means of 2D NMR spectroscopy and isothermal titration microcalorimetry. Owing to the cooperative host-linker-guest binding mode between host and guest, these 3,6'-bridged β-cyclodextrin dimers showed significantly enhanced binding abilities and molecular selectivities as compared with native β-cyclodextrin through the simultaneous contributions of hydrophobic, hydrogen bond, and electrostatic interactions. Thermodynamically, the inclusion complexations of these β-cyclodextrin dimers with bile salts were mainly driven by large enthalpic gain, accompanied by slight to moderate entropic loss. An enthalpy-entropy compensation analysis demonstrated that these β-cyclodextrin dimers experienced large conformational changes and extensive desolvation effect upon inclusion complexation with guest molecules.

Keywords: Cyclodextrin, bile salt, calorimetry, inclusion phenomena, synthetic receptors, host-guest modeling systems, molecular recognition, thermodynamics.

INTRODUCTION

Possessing a hydrophobic cavity capable of binding a variety of hydrophobic or amphipathic molecules, cyclodextrins (CDs) are widely used as hosts to form inclusion complexes with small- and medium-sized organic/biological substrates [1]. Among the various CD derivatives, CD dimers have been known to significantly enhance the binding abilities and molecular selectivities toward model substrates in comparison with parent CDs through the cooperative binding of a single model substrate by two hydrophobic cavities located in close proximity [2]. Generally, CD dimers can be divided into three groups depending on the side of CD where the linking is carried out: head-to-head, tail-to-tail, and head-to-tail, where the head and tail are the primary and secondary hydroxyl sites of CD [3]. In the past three decades, many CD dimers have been synthesized and their molecular binding behaviors with guest molecules are widely investigated [2, 4-10]. However, studies on the molecular recognition of head-to-tail β-CD dimers with guests are still rare, to the best of our knowledge. On the other hand, bile salts are important biological amphiphiles possessing many attractive functions. For example, they cannot only assist the digestion of fats by the formation of micelles and micellar aggregates but also can be widely applied as anionic surfactants [11]. Moreover, they can also interact with anti-hyperlipidemia drugs such as neomycin, clidamycin, kanamycin, and lincomycin [12]. Earlier studies showed that β-CD derivatives were efficient steroid receptor molecules, because the hydrophobic cavity of β-CD well matched the size of steroid molecules such as cholesterol and bile salts [13, 14]. Tato *et al.* studied the inclusion complexation behaviors of two bile salts with several head-to-head β-CD dimers by NMR techniques [14]. Reinhoudt *et al.* reported that tail-to-tail β-CD dimers could be used as receptor molecules for steroid sensors [15]. Recently, we reported the molecular selective binding of bile salts by a series of head-to-head β-CD dimers, demonstrating that the length, flexibility, and structure of linker groups not only determine the binding modes but also significantly affect the binding abilities of β-CD dimers [16]. In the present work, we synthesized three head-to-tail β-CD dimers with oligo(ethylenediamino) linkers (Scheme **1**) and investigated their inclusion complexation behaviors with some representative bile salts (Chart **1**), i.e. cholate (CA), deoxycholate (DCA), glycocholate (GCA), and taurocholate (TCA), by means of 2D NMR and isothermal titration calorimetry (ITC). It is our particular interest to establish the structure-energetics relationship for the molecular recognition of head-to-tail β-CD dimers towards steroid substrates, which will be helpful for the design of drug carriers involving β-CD dimers.

MATERIALS AND METHODS

Materials

All bile salts were purchased from Sigma and used as received. β-CD of reagent grade (Shanghai Reagent Factory) was recrystallized twice from water and dried in vacuo at 95 ºC for 24 h prior to use. *N*,*N*-Dimethylformamide (DMF) was dried over calcium hydride for two days and then distilled under reduced pressure prior to use. Mono[6-*O*-(*p*-toluenesulfonyl)]-β-CD (6-OTs-β-CD) [17] and mono[2-*O*-(*p*-toluenesulfonyl)]-β-CD (2-OTs-β-CD) [18] were respectively prepared by the reaction of *p*-tosyl chloride with β-CD according to the literature methods. Mono(6-ethylenediamino-6-deoxy)-β-CD, mono(6-diethylenetriamino-6-deoxy)

*Address correspondence to this author at the Department of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, P.R. China; Tel: 86-22-23503625; Fax: 86-22-23503625; E-mail: yuliu@nankai.edu.cn

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-β-CD and mono(6-triethylenetetraamino-6-deoxy)-β-CD were prepared by the reaction of 6-OTs-β-CD with the corresponding oligoethylenediamines [19]. 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.

Synthesis of 3,6'-(Ethylenediamino-Bridged) β-CD Dimer (1)

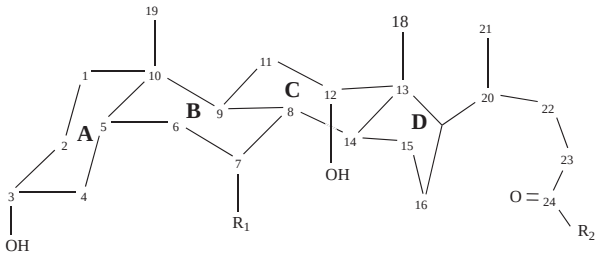
A mixture of mono(6-ethylenediamino-6-deoxy)-β-CD (320 mg) and 2-OTs-β-CD (330 mg) was allowed to react in DMF (30 mL) with stirring under nitrogen at 80°C for 3 d. The resulting solution was poured into acetone (200 mL), and the precipitate was collected by filtration. This procedure was repeated two times. The crude product thus obtained was subsequently purified on a CM Sephadex C-25 ionic column (with 1 mol·dm^{-3} aqueous ammonia as eluent) and a Sephadex G-25 column (with water as eluent), respectively. After the residue was dried *in vacuo*, a pure sample was obtained in 31% yield. ^{1}H NMR (300 MHz, D_2O, 25°C): δ = 4.93 (m, 14H), 3.89 - 3.44 (m, 84 H), 2.71 - 2.53 (m, 4H); ^{13}C NMR (300 MHz, D_2O, 25℃): δ = 101.9, 101.6, 81.1, 80.9, 73.1, 72.0, 71.8, 70.4, 60.3, 57.5, 49.3, 47.7; elemental analysis calcd (%) for $C_{86}H_{144}O_{68}N_2 \cdot 10H_2O$: C 41.75, H 6.68, N 1.13; found: C 41.97, H 6.85, N 1.33.

Synthesis of 3,6'-(Diethylenetriamino-Bridged) β-CD Dimer (2)

Compound **2** was prepared from 2-OTs-β-CD and mono(6-diethylenetriamino-6- deoxy)-β-CD in 34% yield according to a procedure similar to that in the synthesis of **1**.^{1}H NMR (300 MHz, D_2O, 25°C): δ = 4.94 (m, 14H), 3.86 - 2.94 (m, 84 H), 2.89 - 2.62(m, 8H); ^{13}C NMR (300 MHz, D_2O, 25℃): δ =101.9, 101.3, 81.1, 73.1, 72.2, 71.9, 71.2, 60.3, 60.0, 47.7; elemental analysis calcd (%) for $C_{88}H_{149}O_{68}N_3 \cdot 10H_2O$: C 41.99, H 6.77, N 1.67; found: C 42.11, H 6.93, N 1.72.

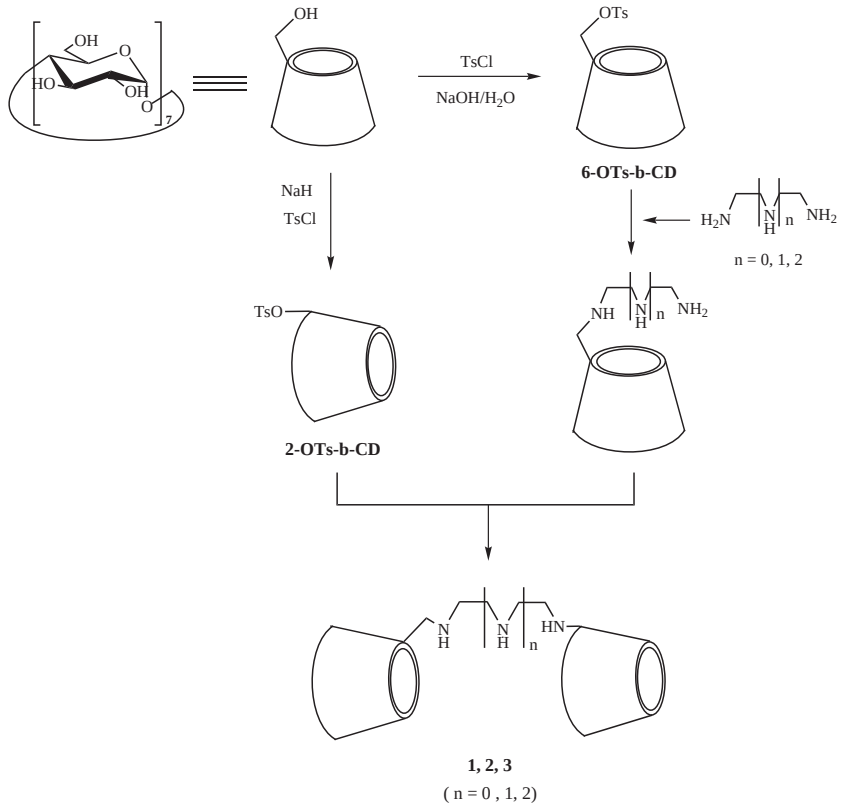
Synthesis of 3,6'-(Triethylenetetraamino-Bridged) β-CD Dimer (3)

Compound **3** was prepared from 2-OTs-β-CD and mono(6-triethylenetetraamino-6- deoxy)-β-CD in 31% yield according to a procedure similar to that in the synthesis of **1**. ^{1}H NMR (300 MHz, D_2O, 25°C): δ = 4.99 (m, 14H), 3.91 - 3.49 (m, 84 H), 2.84 - 2.61(m, 12H); ^{13}C NMR (300 MHz, D_2O, 25°C): δ = 101.9, 101.7, 81.8, 73.1, 72.1, 71.8, 71.6, 60.3, 59.8, 51.7, 49.1; elemental analysis calcd (%) for $C_{90}H_{154}O_{68}N_4 \cdot 8H_2O$: C 42.82, H 6.79, N 2.22; found: C 42.62, H 6.61, N 2.49.



CA R1 = OH R2 = O^-Na^+
DCA R1 = H R2 = O^-Na^+
GCA R1 =OH R2 = $NH\text{-}CH_2\text{-}COO^-Na^+$
TCA R1 =OH R2 = $NH\text{-}CH_2\text{-}CH_2\text{-}SO_3^-Na^+$

Chart 1. Structures of bile salts.



Scheme 1. Syntheses of β-CD dimers **1-3**.

Microcalorimetric Titration

The microcalorimetric titrations were performed at 25°C under atmospheric pressure in aqueous phosphate buffer solution of pH 7.20 by using a Microcal VP-ITC titration microcalorimeter, which allow us to simultaneously determine the enthalpic change 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 the thermodynamic parameters in good agreement with the literature data [20]. All solutions were degassed and thermostated using a ThermoVac accessory before the titration experiment, and the titrations were performed below the critical micelle concentration of steroid molecules. In each run, a phosphate buffer solution of host molecule in a 0.250 mL syringe was sequentially injected into the calorimeter sample cell containing a buffer solution of steroid guests with stirring at 300 rpm. The sample cell volume was 1.4227 mL in all experiments. Each titration experiment was composed of 25 successive injections (10 μL per injection). The steroid solutions were applied at a concentration of ca. 0.2 mM, which is below their critical micelle concentration (CMC). (The CMC of steroids employed here are >1 mM [21]). A control experiment was performed to determine the heat of dilution by injecting a host buffer solution into a pure buffer solution containing no steroid molecules. The dilution enthalpies determined in control experiments were subtracted from the enthalpies measured in the titration experiments to obtain the net reaction heat.

The ORIGIN software (Microcal Inc.), which was used to simultaneously compute the equilibrium constant (K_S) and standard molar enthalpy of reaction ($\Delta H°$) from a single titration curve, gave a standard deviation based on the scatter of the data points in the titration curve. The net reaction heat in each run was calculated by the "one set of binding sites" model. Additionally, the first point was removed from the titration curve acknowledging that the concentration of host in the cell far exceeded the concentration of the guest. To check the accuracy of the observed thermodynamic quantities, two independent titration experiments were carried out; the average values and standard deviations of the two independent titration experiments are listed in Table **1**.

RESULTS AND DISCUSSION

Synthesis

The 3,6'-connected β-CD dimers were synthesized in satisfactory yields from 2-OTs-β-CD (18) and 6-oligoethylenediamino-β-CD. Herein, the former starting material, 2-OTs-β-CD, firstly was converted to *manno*-2,3-epoxy-β-CD through the elimination of the tosyl group at the 2-position by the hydroxyl groups at the 3-position in a basic environment [22]. Subsequently, the nucleophiles 6-oligoethylenediamino-β-CDs reacted with *manno*-2,3-epoxy-β-CD to give the target products **1-3**. Generally, the ^{13}C NMR spectra of N- or S-substituted sugar derivatives are known to show a larger upfield shift of the α-carbon and smaller upfield shifts of the β-carbon and γ-carbon relative to the parent sugar [23,24]. In the ^{13}C NMR spectra of **1-3**, both C-3 (as a α-carbon of 3-substituted) and C-6 carbon (as a α-carbon of 6-substituted) showed the appreciable upfield shifts (1.5-2.7 ppm for C-3, 0.3-2.8 ppm for C-6), while C-1 (as a γ-carbon of 3-substituted), C-2 (as a β-carbon of 3-substituted), C-4 (as a β-carbon of 3-substituted and a γ-

Table 1. Complex Stability Constant (K_S), Standard Enthalpic Change ($\Delta H°$), and Entropic Changes ($T\Delta S°$) for the 1:1 Inclusion Complexation of Bile Salts with Native β-CD and β-CD Dimers 1-3 in Phosphate Buffer Solutions of pH 7.20 at T = 298.15 K

Host[a]	Guest[b]	K_S	$\Delta G°$/kJ mol^{-1}	$\Delta H°$/kJ mol^{-1}	$T\Delta S°$/kJ mol^{-1}	Ref.
β-CD	CA	4068±84	-20.6±0.1	-23.0±0.5	-2.4	c
	DCA	4844±16	-21.0±0.0	-25.8±0.0	-4.8	c
	GCA	2394±69	-19.3±0.1	-23.0±0.1	-3.7	c
	TCA	2293±13	-19.2±0.0	-23.8±0.1	-4.6	c
1	CA	21065±1105	-24.7±0.1	-32.8±0.3	-8.1	d
	DCA	22780 ±340	-24.9±0.0	-42.7±0.1	-17.9	d
	GCA	9707±433	-22.8±0.1	-23.0±0.0	-0.3	d
	TCA	6848±370	-21.9±0.1	-22.4±0.1	-0.5	d
2	CA	5868±129	-21.5±0.0	-39.3±0.4	-17.7	d
	DCA	7017±130	-22.0±0.0	-47.4±0.4	-25.5	d
	GCA	4031±140	-20.6±0.1	-25.8±0.5	-5.2	d
	TCA	2947±101	-19.8±0.1	-26.9±0.6	-7.1	d
3	CA	5606±81	-21.4±0.0	-41.0±0.4	-19.6	d
	DCA	5511±64	-21.4±0.0	-52.1±0.4	-30.7	d
	GCA	2847±74	-19.7±0.1	-26.5±0.6	-6.9	d
	TCA	1877±53	-18.7±0.1	-29.0±0.7	-10.3	d

[a][host] = 3.74-3.82 mM. [b][guest] = 0.201-0.205 mM. [c]Ref 25. [d]This work.

carbon of 6-substituted), and C-5 (as a β-carbon of 6-substituted) showed the smaller upfield shifts compared with the corresponding α-carbons (0.2-0.6 ppm for C-1, 0-0.2 ppm for C-4, no appreciable shifts for C-2 and C-5). Therefore, we conclude that the oligoethylenediamino bridge was introduced at the C-3 and C-6' positions of β-CD. This result is consistent with the reports that the ring opening reactions of *manno*-2,3-epoxy-CD by alkylamino, aqueous ammonia, and hydroxylamine produce the 3-substituted CDs [22].

Complexation Thermodynamics

In order to investigate quantitatively the thermodynamic origin of the inclusion of β-CD dimers **1-3** with guests and establish the structure-energetics correlation, the isothermal titration calorimetry (ITC) titrations were performed at 298.15 K in aqueous phosphate buffer (pH 7.2), because the calorimetry measurement is the only method that directly measures the heat changes associated with intermolecular interactions. A representative titration curve is shown in Figs. **1**,**2** and the thermodynamic parameters obtained are listed in Table **1**. As can be seen in Table **1**, the inclusion complexations of bile salts with β-CD dimers **1-3** are driven by favorable enthalpic gain ($-\Delta H°$ = 22.4 - 52.1 kJ mol^{-1}), accompanied by slight to moderate entropic loss ($T\Delta S°$ = -0.3 - -30.7 kJ mol^{-1}). Interestingly, the enthalpic changes for the inclusion complexation of β-CD dimers **1-3** increased, while the entropic changes decreased, with the elongation of the linker group, giving an order of $-\Delta H°$ as **3** > **2** > **1** and an order of $T\Delta S°$ as **1** > **2** > **3**. On the other hand, the *K*s values show an order as **1** > **2** > **3**, which is the same as the order of $T\Delta S°$. This result indicates that the stronger binding of bile salts by the short-linked β-CD dimer is not thermodynamically accomplished by an increase of the originally favorable enthalpic gain ($-\Delta H°$), but by a reduction of the unfavorable entropic loss ($T\Delta S°$). Before the complex formation, both β-CD dimer and bile salt were solvated, and the solvent molecules around the solutes were highly ordered. During the complexation, before bile salt enters the β-CD cavity, it must lose its salvation shell and also, the solvent molecules have to leave the β-CD cavity to jointly make the disorder increases. Therefore the entropy of system considerably increases during this desolvation process. Then, bile salt enters the β-CD cavity to form a host-guest complex with an ordered conformation. This progress greatly decreases the rotational and structural freedom of both host and guest, and consequently leads to the unfavorable entropic loss. Therefore, we deduce that the short-linked β-CD dimer, with a better size and hydrophobicity match to bile salts, may experience more extensive desolvation upon complexation, and thus exhibits the less unfavorable entropic loss.

Binding Mode

2D NMR spectroscopy has become an essential method to study the host-guest inclusion complexation geometry since one can conclude that two protons are closely located in space if the NOE correlations are detected between the relevant protons in the NOESY or ROESY spectrum. Therefore, in order to elucidate the binding mode of β-CD dimers **1-3** with bile salts as well as to establish the correlation between the conformation of resulting complexes and the thermodynamic parameters, the ROESY experiments of **1-3**

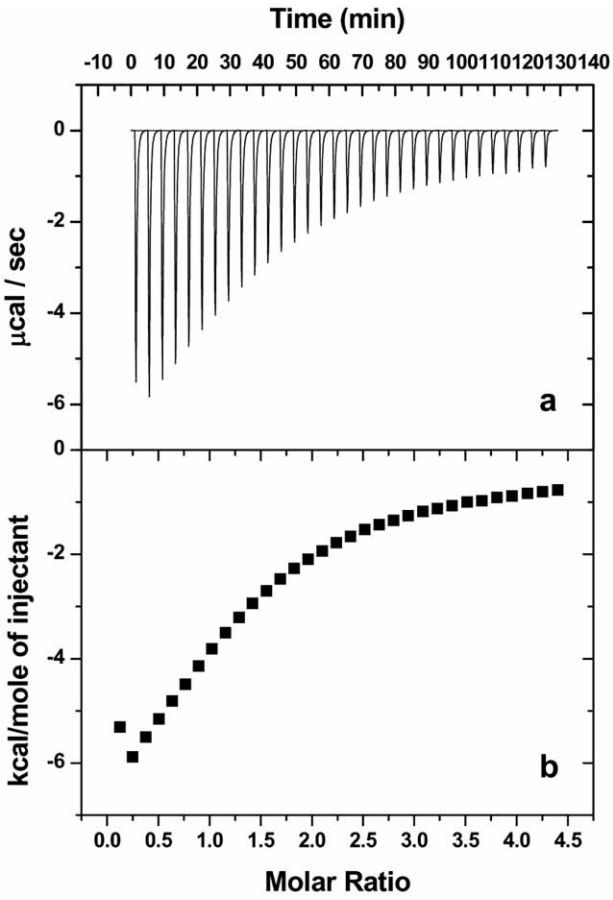


Fig. (1). Calorimetric titrations of β-CD dimer **2** with DCA in phosphate buffer solution of pH 7.20 at 25°C. (**a**) Raw data for sequential 10 µL injections of β-CD dimer solution (3.82 mM) into steroid solution (0.201 mM). (**b**) Heats of reaction as obtained from the integration of calorimetric traces.

were performed in the presence of bile salts in pH 7.20 buffer solution. Fig. **3** shows a typical ROESY spectrum for the resulting complex between β-CD dimer **3** and CA. 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 **1**. As shown in Fig. **3**, the ROESY spectrum for the CA-3 complex displays the sophisticated NOE cross-peaks, which come from not only the intermolecular correlations between β-CD dimer and bile salt, but also the intramolecular correlations of **3** or CA. Among them, the cross-peak A corresponds to the NOE correlations between CA's P18 protons and CD's H3 protons, and the cross-peak B corresponds to the NOE correlations between CA's side-chain protons (P21) and CD's H3/H5 protons. Meanwhile, the cross-peaks C and D correspond to the NOE correlations between CA's D-ring protons (P14 to P17) and CD's H3 protons. In addition, the significant NOE correlations are found between CA's side-chain protons and steroid body (P23 with P16, P22 with P16), but no NOE correlations between CA's P19 protons and CD's H3/H5 protons can be observed. Because the H5 protons are located near the narrow opening of CD cavity while the H3 protons near the wide opening, we can deduce that the D-ring of CA is wholly included in the β-CD cavity from the wide opening, while the side-chain is located near

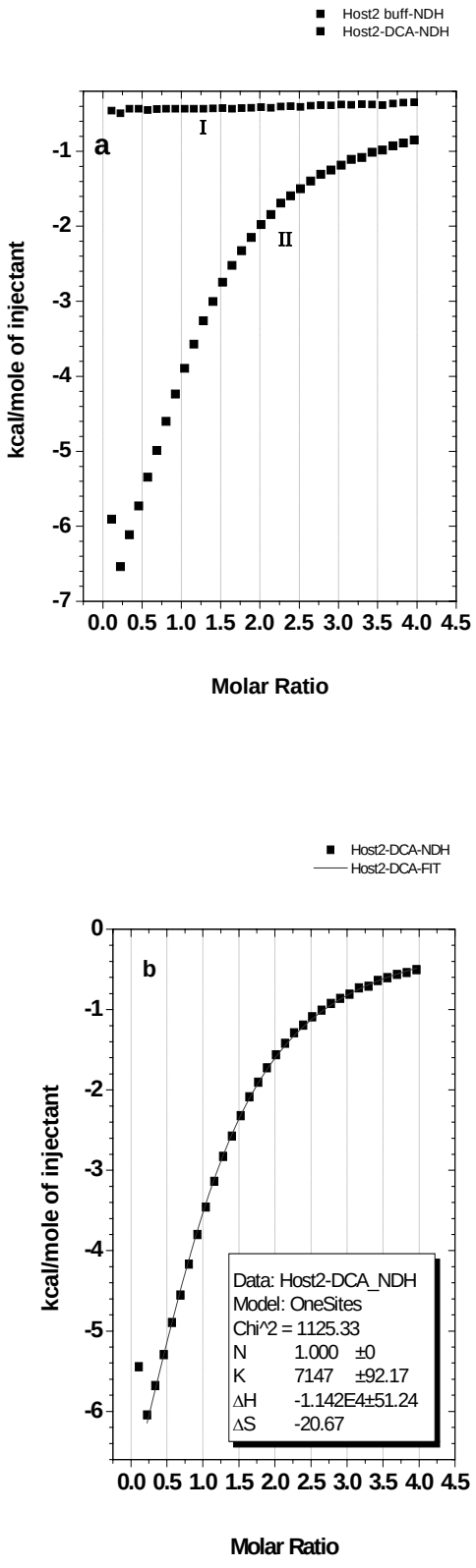


Fig. (2). (**a**) Heat effects of dilution (I) and of complexation (II) of host 2 with DCA for each injection during the titration microcalorimetric experiment. (**b**) "Net" heat effect obtained by subtracting the heat of dilution from the heat of reaction, which was analyzed by computer simulation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

the narrow opening of β-CD cavity and folded toward the steroid body. On the other hand, the obvious cross-peaks (peaks E) assigned to the NOE correlations between the methylene protons of oligoethylenediamino linker and CD's H3 protons are also observed, indicating the self-inclusion of linker group in the β-CD cavity from the wide opening. These results, along with the 1:1 binding stoichiometry between host and guest obtained from the microcalorimetric titration curve, jointly indicate a cooperative host-linker-guest binding mode between host and guest. That is, the D-ring and side-chain of bile salt guest enters one β-CD cavity from the wide opening, and the linker group is partially self-included in the other β-CD cavity, as illustrated in Fig. **4**. Similar binding mode and 1:1 binding stoichiometry are also observed in other cases of β-CD dimer/bile salt complexes.

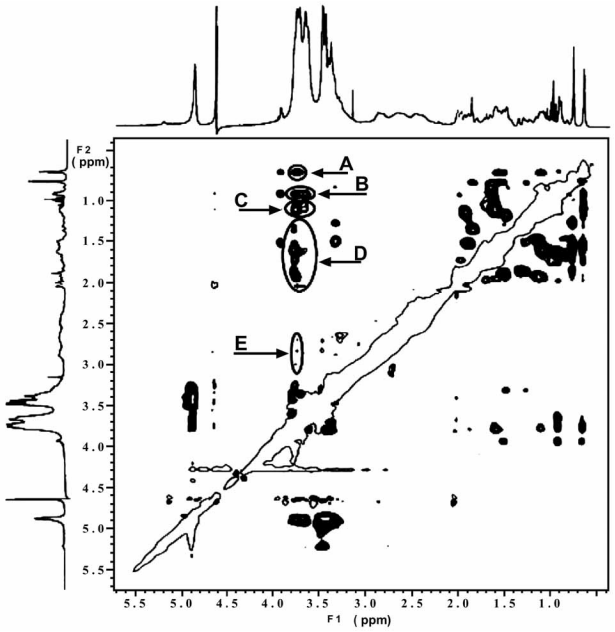


Fig. (3). ROESY spectrum of a complex of 3 with CA ([3] = [CA] = 2mM) with a mixing time of 250 ms.

Binding Ability and Molecular Selectivity

Among the various non-covalent weak interactions, van der Waals and hydrophobic interactions are the most crucial interactions contributing to the inclusion complexations of CDs with guest molecules, and the strength of these two interactions is mainly determined by the degree to which the size, shape and hydrophobicity of guest molecules match those of CDs. In addition, some other intermolecular interactions, such as hydrogen bonding and electrostatic interactions, can also contribute to the inclusion complexations of CDs to some extent. As can be seen in Table **1**, the stability constants (K_S) of the complexes formed by β-CD dimers 1-3 with bile salts (except 3/TCA complex) are larger than those of the complexes formed by native β-CD. For example, the inclusion complexation of 1 with CA gives a high K_S value up to 21065 M^{-1}, that is, 5.2 times higher than that of native β-CD. These enhanced binding abilities of β-CD dimers may be mainly attributed to the cooperative host-linker-guest binding mode between host and guest. We have demon-

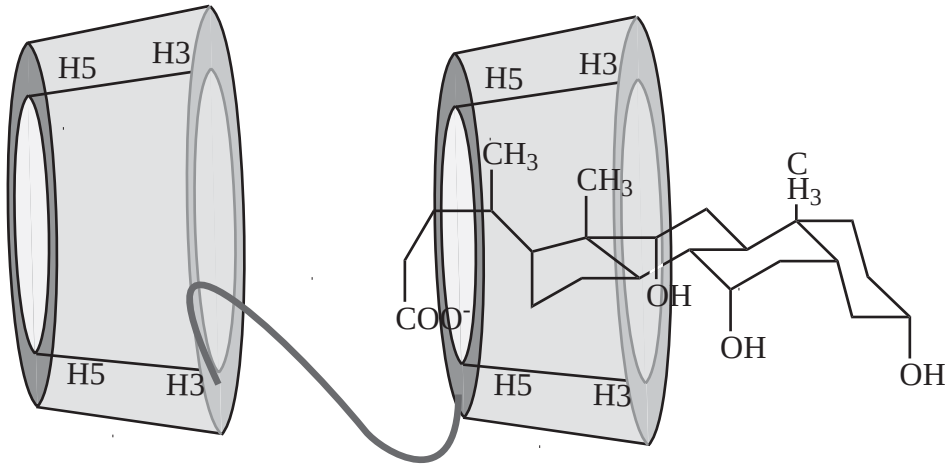


Fig. (4). Possible binding mode between β-CD dimer and bile salt.

strated that, upon complexation, the D-ring of CA is wholly included in one β-CD cavity from the wide opening, while the side-chain is located near the narrow opening of β-CD cavity. Meanwhile, the linker group is partially self-included in the other CD cavity. Under our experimental conditions, the carboxylate (or sulfonate) group in the side-chain of bile salt is not protonated and should exist as a carboxylate (or sulfonate) anion, and the -NH- fragments in the linker group of β-CD dimer should be partly protonated. Therefore, the electrostatic interactions between the protonated amino groups (-NH_2^+-) in the linker and the anionic carboxylate (or sulfonate) tail of bile salt may strengthen the inclusion complexations of these β-CD dimers with bile salts. Moreover, the hydrogen bond interactions of the hydroxyl groups of β-CD and the -NH- fragments of the oligo(ethylenediamino) linker with the carboxylate (or sulfonate) tail of bile salt also contribute to the enhanced binding abilities of β-CD dimers 1-3.

An interesting phenomenon observed is that the binding abilities of β-CD dimers **1-3** towards bile salts gradually decrease with the elongation of linker group, giving an order of K_S values as **1** > **2** > **3**. A possible reason for this phenomenon is the size and hydrophobicity match between host and guest. With the elongation of linker group, the protonated amino groups (-NH_2^+-) in the linker is located distant from the anionic carboxylate (or sulfonate) tail of bile salt, which consequently weakens the electrostatic interactions between the linker group and bile salt. Moreover, the increase of the number of -NH- fragments in the linker group decrease the hydrophobicity of β-CD dimer to some extent, which is also unfavorable to the hydrophobic interactions between host and guest. As a joint result of these factors, the long-linked β-CD dimer gives a lower K_S value upon inclusion complexation with bile salt than the short-linked one.

It is also interesting to compare the guest selectivities of these head-to-tail β-CD dimers. Compared with CA, GCA and TCA, DCA possesses a more hydrophobic structure due to the absence of C-7 hydroxyl group, which consequently leads to stronger hydrophobic interactions between host and guest. Therefore, DCA gives the highest binding abilities among the bile salts examined upon complexation with most host CDs. Possess more polar side-chains [26], GCA and TCA show weak binding abilities upon inclusion complexation with β-CD dimers due to the relatively poor hydrophobic interactions between host and guest. As a result of this hydrophobicity match, β-CD dimers **1-3** give the enhanced molecular selectivities towards bile salts as compared with that of native β-CD. As can be seen in Table **1**, the highest molecular selectivities among bile salts examined by β-CD dimers **1-3** are 3.3 by **1** for DCA/TCA pair, 2.4 by **2** for DCA/TCA pair, and 3.0 by **3** for CA/TCA pair, although the corresponding value is only 2.1 for DCA/TCA pair by native β-CD.

Enthalpy-Entropy Compensation

Numerous studies show that the enthalpy-entropy compensation effect is widely existed in the inclusion complexations of CDs with guest molecules [27]. That is, the enthalpic and entropic changes for the inclusion complexations are mutually compensatory, giving a linear $T\Delta S°$ *vs* $\Delta H°$ plot, and the slope (α) and the intercept ($T\Delta S_0$) of the compensation plot can be used as the quantitative measures of the conformational changes and the extents of induced desolvation upon complex formation. Using the limited experimental data obtained in this work, the entropic changes ($T\Delta S^o$) for the 1:1 inclusion complexations of head-to-tail β-CD dimers are plotted against the corresponding enthalpic changes (ΔH^o), giving an excellent straight line with a correlation coefficient of 0.983 (Fig. **5**). The comparison of enthalpy-entropy compensation effect for native β-CD, mono-modified β-CDs and β-CD dimers is shown in Table **2**. As can be seen in Table **2**, the slope for head-to-tail β-CD dimers (α = 0.95) is obviously larger than those for native β-CD (α = 0.80) and head-to-head β-CD dimers (α = 0.87), but appreciably lower than that for mono-modified β-CDs (α = 0.99). This means that head-to-tail β-CD dimers experience large conformational changes upon inclusion complexation with guest molecules. On the other hand, head-to-tail β-CD dimers show the second largest $T\Delta S_0$ (19.85 kJ mol^{-1}) among the host CDs examined, which may be attributed to the extensive desolvation of host, especially two CD cavities, and guest.

CONCLUSIONS

In summary, we successfully synthesized a series of head-to-tail β-CD dimers and thermodynamically investi-

gated their selective binding behaviors towards some bile salts. Due to the cooperative host-linker-guest binding mode, these head-to-tail β-CD dimers were found to significantly enhance the original molecular binding ability and molecular selectivity of parent β-CD. A further comparative study on the molecular recognition of head-to-head, head-to-tail, and tail-to-tail β-CD dimers is still in progress.

Table 2. Enthalpy-Entropy Compensation Analyses of β-CD, Mono-Modified β-CDs, and β-CD Dimers

Host	α	$T\Delta S_0$ (kJ·mol^{-1})
native β-CD (27)	0.80	11
mono-modified β-CDs (27)	0.99	17
head-to-head β-CD dimers (16d)	0.87	23
head-to-tail β-CD dimers	0.95	19.85

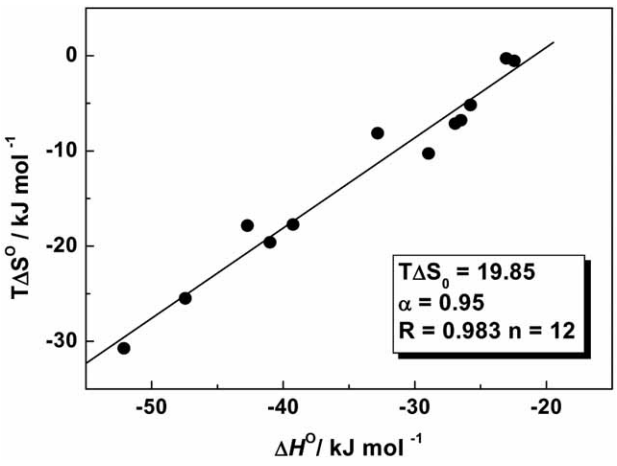


Fig. (5). Enthalpy-entropy compensation plot for the inclusion complexations of bile salts with hosts **1-3**.

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ABBREVIATIONS

CD = Cyclodextrin

CA = Cholate

DCA = Deoxycholate

GCA = Glycocholate

TCA = Taurocholate

ITC = Isothermal titration calorimetry

6-OTs-β-CD = Mono[6-*O*-(*p*-toluenesulfonyl)]-β-CD

2-OTs-β-CD = Mono[2-*O*-(*p*-toluenesulfonyl)]-β-CD

CMC = Critical micelle concentration

REFERENCES

[1] (a) Easton, C.J.; Lincoln, S.F. *Chem. Soc. Rev.*, **1996**, *25*, 163-170. (b) Szejtli, J.; Osa, T.; Atwood, J.L.; Davies, J.E.D.; MacNicol, D.D.; Vögtle, F. Eds., *Elsevier: Ixfird*, 1996, Vol. 3. (c) Connors, K.A. *Chem. Rev.*, **1997**, *97*, 1325-1358. (d) Wenz, G. *Angew. Chem. Int. Ed. Engl.*, 1994, *33*, 803-822.

[2] (a) Zhang, B.; Breslow, R. *J. Am. Chem. Soc.*, **1993**, *115*, 9353-9354. (b) Venema, F.; Rowan, A.E.; Nolte, R.J.M. *J. Am. Chem. Soc.*, 1996, *118*, 257-258. (c) French, R.R.; Wirz, J.; Woggon, W.-D. *Helv. Chim. Acta*, **1998**, *81*, 1521-1527. (d) Breslow, R.; Zhang, B. *J. Am. Chem. Soc.*, **1996**, *118*, 8495-8496. (e) Breslow, R.; Chung, S. *J. Am. Chem. Soc.*, **1990**, *112*, 9659-9660.

[3] Okabe, Y.; Yamamura, H.; Obe, K.; Ohta, K.; Kawai, M.; Fujita, K. *J. Chem. Soc. Chem. Commun.*, **1995**, 581-582,

[4] (a) Breslow, R.; Zhang, B. *J. Am. Chem. Soc.*, **1992**, *114*, 5882-5883. (b) Zhang, B.; Breslow, R. *J. Am. Chem. Soc.*, **1997**, *119*, 1676-1681. (c) Breslow, R.; Dong, S.D. *Chem. Rev.*, **1998**, *98*, 1997-2012.

[5] (a) Liu, Y.; You, C.-C.; Li, B. *Chem. Eur. J.*, **2001**, *7*, 1281-1288. (b) Liu, Y.; Chen, Y.; Li, B.; Wada, T.; Inoue, Y. *Chem. Eur. J.*, **2001**, *7*, 2528-2535. (c) Liu, Y.; You, C.-C.; Chen, Y.; Wada, T.; Inoue, Y. *J. Org. Chem.*, **1999**, *64*, 7781-7787.

[6] (a) Venema, F.; Nelissen, H.F.M.; Berthault, P.; Birlirakis, N.; Rowan, A.E.; Feiters, M.C.; Nolte, R.J.M. *Chem. Eur. J.*, **1998**, *4*, 2237-2250. (b) Nelissen, H.F.M.; Schut, A.F.; Venema, J.F.; Feiters, M.C.; Nolte, R.J.M. *Chem. Commun.*, **2000**, 577-578.

[7] (a) Baugh, S.D.P.; Yang, Z.; Leung, D.K.; Wilson, D.M.; Breslow, R. *J. Am. Chem. Soc.*, **2001**, *123*, 12488-12494. (b) Leung, D.K.; Yang, Z.; Breslow, R. *Proc. Natl. Acad. Sci. USA*, **2000**, *97*, 5050-5053. (c) Breslow, R.; Yang, Z.; Ching R. *J. Am. Chem. Soc.*, **1998**, *120*, 3536-3537. (d) Breslow, R.; Halfon, S.; Zhang, B. *Tetrahedron*, **1995**, *51*, 377-388.

[8] (a) Michels, J.J.; Huskens, J.; Reinhoudt, D.N. *J. Am. Chem. Soc.*, **2002**, *124*, 2056-2064. (b) Mulder, A.; Jukovic, A.; Lucas, L.N.; van Esch, J.; Feringa, B.L.; Huskens, J.; Reinhoudt, D.N. *Chem. Commun.*, **2002**, 2734-2735.

[9] Haskard, C.A.; Easton, C.J.; May, B.L.; Lincoln. S.F. *J. Phys. Chem.*, **1996**, *100*, 14457-14461.

[10] Liu, Y.; Chen, Y. *Acc. Chem. Res.*, **2006**, *39*, 681-691.

[11] Boulanger, C.; Giorgio, C.D.; Gaucheron, J.; Vierling, P. *Bioconjugate Chem.*, **2004**, *15*, 901-908.

[12] Setchell, K.D.R.; Street, J.M.; Sjovall, J. *The Bile Acids*, Setchell, K.D.R.; Kritcheveky, D.; Nair, P.P. Eds., *Plenum Press*: *New York*, London, 1988, Vol. 4, Chapter 12, p 543.

[13] Gonzalez-Gaitano, G.; Compostizo, A.; Sanchez-Martin, L.; Tardajos, G. *Langmuir*, **1997**, *13*, 2235-2241.

[14] Ramos, C.P.; Alvarez-Parrilla, E.; Meijide, F.; Seijas, J.A.; Rodriguez, N.E.; Vazquez, T.J. *Langmuir*, **1999**, *15*, 5489-5495.

[15] De Jong, M.R.; Engbersen, J.F.J.; Huskens, J.; Reinhoudt, D.N. *Chem. Eur. J.*, **2000**, *6*, 4034-4040.

[16] (a) Liu, Y.; Song, Y.; Wang, H.; Zhang, H.-Y.; Wada, T.; Inoue, Y. *J. Org. Chem.*, **2003**, *68*, 3687-3690. (b) Liu, Y.; Yang, Y.-W.; Yang, E.-C.; Gaun, X.-D. *J. Org. Chem.*, **2004**, *69*, 6590-6602. (c) Liu, Y.; Song, Y.; Chen, Y.; Li, X.-Q.; Ding, F.; Zhong, R.-Q. *Chem. Eur. J.*, **2004**, *10*, 3685-3696. (d) Liu, Y.; Li, L.; Chen, Y.; Yu, L.; Fan, Z.; Ding, F. *J. Phys. Chem. B*, **2005**, *109*, 4129-4134.

[17] Petter, R.C.; Salek, J.S.; Sikorski, C.T.; Kumaravel, G.; Lin, F.-T. *J. Am. Chem. Soc.*, **1990**, *112*, 3860-3868.

[18] Rong, D.; D'Souza, V.T. *Tetrahedron Lett.*, **1990**, *33*, 4275-4278.

[19] May, B.L.; Kean, S.D.; Easton, C.J.; Lincoln, S.F. *J. Chem. Soc. Perkin. Trans. 1*, **1997**, 3157-3160.

[20] Rekharsky, M.V.; Schwarz, F.P.; Tewari, Y.B.; Goldberg, R.N.; Tanaka, M.; Yamashoji, Y. *J. Phys. Chem.*, **1994**, *98*, 4098-4103.

[21] (a) Collo, A.; Meijide, F.; Rodriguez, N.E.; Vazquez, T.J. *J. Pharm. Sci.*, **1996**, *85*, 9-15. (b) Cooper, A.; Nutley, M.A.; Camilleri, P. *Anal. Chem.*, **1998**, *70*, 5024-5028.

[22] Khan, A.R.; Forgo, P.; Stine, K.J.; D'Souza, V.T. *Chem. Rev.*, **1998**, *98*, 1977-1996.
[23] Rao, K.R.; Srinivasan, T.N.; Bhanumathi, N.; Sattur, P.B. *J. Chem. Soc. Chem. Commun.*, **1990**, 10-11.
[24] Breitmaier, E.; Voelter, W. Carbon-13 NMR Spectroscopy;*VCH: Winheim*, **1987**.
[25] Liu, Y.; Yang, Y.-W.; Cao, R.; Song, S.-H.; Zhang, H.-Y.; Wang, L.-H. *J. Phys. Chem. B*, **2003**, *107*, 14130-14139.
[26] Ollila, F.; Pentikäinen, O.T.; Forss, S.; Johnson, M.S.; Slotte, J.P. *Langmuir*, **2001**, *17*, 7107-7111.
[27] Rekharsky, M.V.; Inoue, Y. *Chem. Rev.*, **1998**, *98*, 1875-1918.

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