

Original Article

## Molecular Recognition Thermodynamics of Steroids by Novel Oligo(aminoethylamino)- $\beta$ -cyclodextrins Bearing Anthryl: Enhanced Molecular Binding Ability by Co-inclusion Complexation

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### Abstract

Three  $\beta$ -cyclodextrin ( $\beta$ -CD) derivatives bearing anthracene group (**2–4**) were synthesized by the condensation of 9-anthracenecarboxylic acid with the corresponding oligo(aminoethylamino)- $\beta$ -CDs in 33–36% yields and their original conformations and binding behavior with steroid molecules were investigated by using spectroscopic techniques and isothermal calorimeter. The combination of induced circular dichroism (ICD) and 2D NMR spectra reveals that the anthryl group attached to  $\beta$ -CD is itself included in cavity and the chain length of oligo(aminoethylamino) decides the orientation of the anthryl located in the cavity to some extent, directly affecting the binding ability with guest molecules. Calorimetric titration has been performed at buffer aqueous solution (pH 7.2) at 25 °C to give the binding constants ( $K_S$ ) and thermodynamic parameters for 1:1 inclusion complexation of modified  $\beta$ -CDs **2–4** and representative steroids, i.e., cholate, deoxycholate, glycocholate, and taurocholate. Possessing the sidearm with appropriate length, **3** gives the highest stability constant of  $22485 \pm 15 \text{ M}^{-1}$  for the complexation with deoxycholate molecule, which may be ascribed to the co-inclusion interactions between the host and guest. As compared with parent  $\beta$ -CD **1** upon complexation with steroids, hosts **2–4** with different chain lengths enhanced the binding ability and significant molecular discrimination, which are discussed comparatively and globally from the viewpoint of thermodynamics. Furthermore, we establish the correlation between the conformation of the resulting complexes and the thermodynamic parameters obtained.

### Introduction

Possessing the hydrophobic cavity and additive binding size, modified cyclodextrins (CDs) can significantly alter the original molecular binding ability and selectivity of the parent CDs, and thus investigations on molecular recognition with CDs and modified CDs have recently received much attention in supramolecular chemistry [1–3]. Many works have been concentrated on the design and syntheses of cyclodextrin derivatives in order to study their molecular/chiral recognition behavior with various guest molecules and control inclusion complexation phenomena by CDs. It has been demonstrated that besides the several weak intermolecular non-covalent forces, involving dipole–dipole (ion), hydrophobic, electrostatic, van der Waals, and hydrogen-bonding interactions on the basis of size/shape matching concept in the host–guest systems, the solvent, environment, and conformation for both the host compounds and guest molecules will definitely change during the molecular

recognition process [4–7]. Therefore, investigations of the structure of modified CDs and their inclusion complexation thermodynamics are of great importance in elucidating the origin of selective binding to a specific guest. In recent years, a lot of thermodynamic studies focused mainly on inclusion complexation of native and modified CDs with conventional guests [8–11], but less attention has been paid to the molecular recognition thermodynamics of chemically modified CDs with different chain length [12–14].

In the present paper, we wish to report our investigation results on the synthesis of three anthracene-modified  $\beta$ -CDs **2–4** (Scheme 1) and their binding behavior with guest steroids. One important reason for choosing anthracene-modified CD as hosts is that anthracene and its derivatives possess special properties such as the photodimer, chemically switched DNA intercalator, and so on [15–19]. The original conformations of modified  $\beta$ -CDs **2–4** and their binding models with steroids have been studied by induced circular dichroism (ICD) and 2D NMR spectroscopy. At the same time, microcalorimetric titration has been

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performed in phosphate buffer solutions (pH 7.20) at 25 °C to calculate thermodynamic quantities for the inclusion complexation of four steroid molecules (Chart 1) with native and modified  $\beta$ -CDs (**1–4**). On the basis of the relationship between the host conformation and the guest structure, binding thermodynamics with structurally related guests for **1–4** can be elucidated from the viewpoints of the size/shape matching, induced-fit, and competitive inclusion/co-inclusion interaction. The thermodynamic studies of such systems are expected to serve us with a further understanding of the factors governing the supramolecular complexation through cooperative multiple intermolecular interactions.

## Experimental

### Materials

$\beta$ -CD of reagent grade (Shanghai Reagent Factory) was recrystallized twice from water and dried *in vacuo* at 95 °C for 24 h prior to use. *N,N*-Dimethylformamide (DMF) was dried over calcium hydride for 2 days and then distilled under a reduced pressure prior to use. Dicyclohexylcarbodiimide (DCC) and 9-anthracenecarboxylic acid were commercially available (Shanghai Reagent Factory) and used without further purification. All steroid guests, i.e., cholate (CH), deoxycholate (DC), glycocholate (GC), and taurocholate (TC) were purchased from Sigma and used as received. 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 spectral measurements and microcalorimetric titrations.

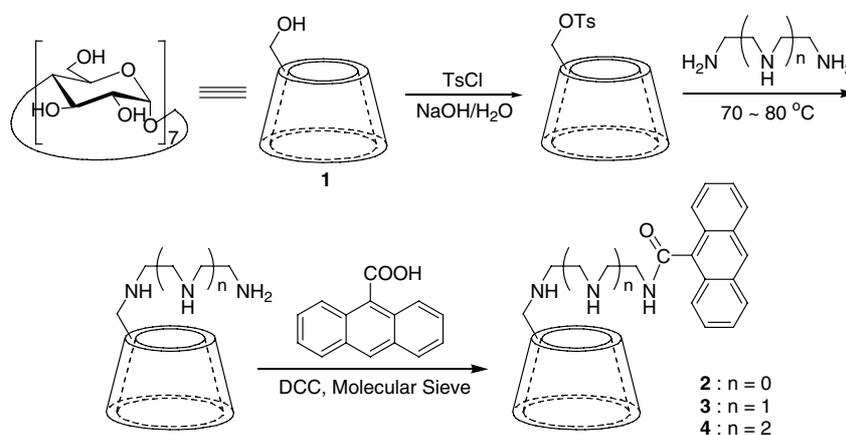
### *Mono[6-(9-anthrylformamido)ethyleneamino-6-deoxy]- $\beta$ -cyclodextrin (2)*

Mono[6-*O*-(toluene-*p*-sulfonyl)]- $\beta$ -cyclodextrin (6-OTs- $\beta$ -CD) was prepared by the reaction of *p*-toluenesulfonyl chloride with  $\beta$ -CD in alkaline aqueous solution [20].

Then, 6-OTs- $\beta$ -CD was converted to mono(6-aminoethylamino-6-deoxy)- $\beta$ -CD in 70% yield on heating in excess ethylenediamine at 70 °C for 7 h [21]. To a solution of DMF (50 mL) containing 2.4 g of mono(6-aminoethylamino-6-deoxy)- $\beta$ -CD and 0.62 g of DCC was added 0.67 g of 9-anthracenecarboxylic acid in the presence of a small amount of 4 Å molecular sieves. The reaction mixture was stirred for 2 days in an ice bath and another 2 days at room temperature, and then allowed to stand for 1 h. The precipitate was removed by filtration and the filtrate was poured into 300 mL of acetone. The precipitate was collected and subsequently purified on a Sephadex G-25 column with water as eluent. After the residue was dried *in vacuo*, a pure sample was obtained in 36% yield. UV/Vis  $\lambda_{\max}$  (H<sub>2</sub>O)/nm (log  $\epsilon$ ) 384.0 (4.04), 364.5 (4.07), 346.5 (3.90), 330.0 (3.62); <sup>1</sup>H NMR (D<sub>2</sub>O, TMS, ppm):  $\delta$  2.37–2.86 (m, 4H); 3.32–3.69 (m, 42H); 4.80–4.83 (m, 7H); 7.33–7.38 (m, 4H); 7.87–7.94 (m, 4H); 8.29 (s, 1H); <sup>13</sup>C NMR (D<sub>2</sub>O, TMS, ppm):  $\delta$  177.2, 137.3, 131.5, 128.1, 126.5, 125.8, 125.2, 101.7, 83.5, 81.4, 80.8, 73.2, 72.4, 68.2, 59.8, 48.7, 47.1, 45.6, 45.0, 38.2; Anal. Calcd for C<sub>59</sub>H<sub>84</sub>O<sub>35</sub>N<sub>2</sub>·2H<sub>2</sub>O: C, 50.00; H, 6.26; N, 1.98. Found: C, 49.80; H, 6.50; N, 2.20.

### *Mono[6-(9-anthrylformamido)diethylenediamino-6-deoxy]- $\beta$ -cyclodextrin (3)*

Modified  $\beta$ -CD **3** was prepared in 33% yield from 9-anthracenecarboxylic acid and mono[6-2-(2-aminoethylamino)ethylamino-6-deoxy]- $\beta$ -CD, according to similar procedures described above. UV/Vis  $\lambda_{\max}$  (H<sub>2</sub>O)/nm (log  $\epsilon$ ) 384.0 (3.32), 364.5 (3.35), 347.0 (3.20), 330.0 (2.98); <sup>1</sup>H NMR (D<sub>2</sub>O, TMS, ppm):  $\delta$  2.34–2.91 (m, 8H); 3.31–3.65 (m, 42H); 4.82–4.83 (m, 7H); 7.34–7.39 (m, 4H); 7.86–7.89 (m, 4H); 8.28 (s, 1H); <sup>13</sup>C NMR (D<sub>2</sub>O, TMS, ppm):  $\delta$  177.0, 137.0, 131.1, 127.9, 126.3, 126.0, 125.2, 101.9, 83.4, 81.1, 80.9, 73.2, 72.1, 68.2, 60.3, 48.4, 47.2, 45.5, 44.9, 38.5; Anal. Calcd for C<sub>61</sub>H<sub>89</sub>O<sub>35</sub>N<sub>3</sub>·8H<sub>2</sub>O: C, 46.71; H, 6.75; N, 2.68. Found: C, 46.82; H, 6.67; N, 2.52.



Scheme 1

*Mono[6-(9-anthrylformamido)triethylenetriamino-6-deoxy]- $\beta$ -cyclodextrin (4)*

Modified  $\beta$ -CD **4** was prepared in 34% yield from 9-anthracenecarboxylic acid and mono{6-2-[2-(2-aminoethylamino)ethylamino]ethylamino-6-deoxy}- $\beta$ -CD, according to similar procedures described above. UV/Vis  $\lambda_{\max}$  (H<sub>2</sub>O)/nm (log  $\epsilon$ ) 384.0 (3.80), 364.5 (3.84), 346.5 (3.67), 329.5 (3.42); <sup>1</sup>H NMR (D<sub>2</sub>O, TMS, ppm):  $\delta$  2.38–2.92 (m, 12H); 3.31–3.70 (m, 42H); 4.82–4.83 (m, 7H); 7.34–7.37 (m, 4H); 7.86–7.95 (m, 4H); 8.33 (s, 1H); <sup>13</sup>C NMR (D<sub>2</sub>O, TMS, ppm):  $\delta$  177.3, 136.9, 131.3, 128.0, 126.4, 125.8, 125.3, 102.1, 83.7, 81.3, 80.7, 73.3, 71.9, 68.3, 60.2, 48.5, 47.3, 45.6, 45.1, 38.4; Anal. Calcd for C<sub>63</sub>H<sub>94</sub>O<sub>35</sub>N<sub>4</sub>·11H<sub>2</sub>O: C, 45.43; H, 7.02; N, 3.36. Found: C, 45.74; H, 7.47; N, 3.53.

*Microcalorimetric titration*

An isothermal calorimeter was used for all microcalorimetric experiments. The instrument was calibrated chemically by performing the complexation reaction of  $\beta$ -CD with cyclohexanol, which gave thermodynamic parameters in good agreement with literature data [12, 13]. The microcalorimetric titrations were performed at atmospheric pressure at 25 °C in aqueous phosphate buffer solution (pH 7.20). All solutions were degassed and thermostated using a ThermoVac accessory before the titration experiment. In each run, a buffer solution of host in a 0.250 mL syringe was sequentially injected with stirring at 300 rpm into a phosphate buffer solution of steroid guest in the sample cell (1.4227 mL volume). Each titration experiment was composed of 25 successive injections (10  $\mu$ L per injection). 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 guest. 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. The ORIGIN software (Microcal) allowed us to simultaneously determine the binding constant ( $K_S$ ) and reaction enthalpy ( $\Delta H^0$ ) with the standard derivation on the basis of the scatter of data points from a single titration experiment. All thermodynamic parameters reported in this work were obtained by using the one set of binding sites model. Two independent titration experiments were performed to afford self-consistent parameters, giving the experiment’s averaged values.

**Results and discussion**

*Investigations on the conformations of modified  $\beta$ -CDs 2–4*

It has been amply demonstrated that inclusion of chromophoric achiral guest in a chiral host such as

CDs produces ICD signals at the wavelengths absorbed by the guest chromophore [22, 23]. In order to confirm the original conformations of **2–4** in aqueous solution, their ICD spectra have been measured in a conventional quartz cell (light path 10 mm) on a JASCO J-715S spectropolarimeter equipped with a PTC-348WI temperature controller to keep the temperature at 25 °C. As can be seen from Figure 1, the circular dichroism spectrum of modified  $\beta$ -CD **2** ( $8.0 \times 10^{-5}$  mol dm<sup>-3</sup>) in phosphate buffer solutions (pH 7.20) showed a major positive Cotton effect peak around 253 nm ( $\Delta\epsilon = 2.70$  dm<sup>-3</sup> mol<sup>-1</sup> cm<sup>-1</sup>). Similarly, **3** ( $8.7 \times 10^{-5}$  mol dm<sup>-3</sup>) showed a positive Cotton effect peak around 255 nm ( $\Delta\epsilon = 0.19$  dm<sup>-3</sup> mol<sup>-1</sup> cm<sup>-1</sup>). Dramatically, **4** ( $7.1 \times 10^{-5}$  mol dm<sup>-3</sup>) showed a positive Cotton effect peak around 246 nm ( $\Delta\epsilon = 0.52$  dm<sup>-3</sup> mol<sup>-1</sup> cm<sup>-1</sup>) and a negative Cotton effect peak around 258 nm ( $\Delta\epsilon = -0.25$  dm<sup>-3</sup> mol<sup>-1</sup> cm<sup>-1</sup>). According to the sector rule of Kajtár *et al.* [24] on the ICD phenomena of CDs complexes, we can deduce that the anthracene moieties of hosts **2** and **3** are entered longitudinally into own  $\beta$ -CD cavity to form the self-inclusion complexes, but the anthracene moiety of **4** is included in the cavity of  $\beta$ -CD with an acclivitous orientation than that of **2** and **3**.

To obtain further evidence about the self-included models of the modified  $\beta$ -CDs **2–4**, 2D NMR spectroscopy experiments have been performed in D<sub>2</sub>O solution on a Varian INVOA 300 spectrometer. As shown in Figure 2a, the ROESY spectrum of **3** displays clear NOE cross-peaks between the H<sub>3</sub> and/or H<sub>5</sub> of  $\beta$ -CD and the H<sub>a</sub> and/or H<sub>d</sub> protons of anthracene moiety (peaks A), as well as between the H<sub>3</sub> and/or H<sub>5</sub> and the H<sub>b</sub> and/or H<sub>c</sub> protons (peaks B), but did not found that the NOE cross-peaks between  $\beta$ -CD and the H<sub>e</sub> protons of anthracene moiety, which indicated distinctly that the anthracene moiety in **3** is partially self-included into the hydrophobic cavity from the primary side of  $\beta$ -CD, a possible structure of **3** was showed at Figure 2b. At the same time, the conformations of **2** and **4** studied by 2D

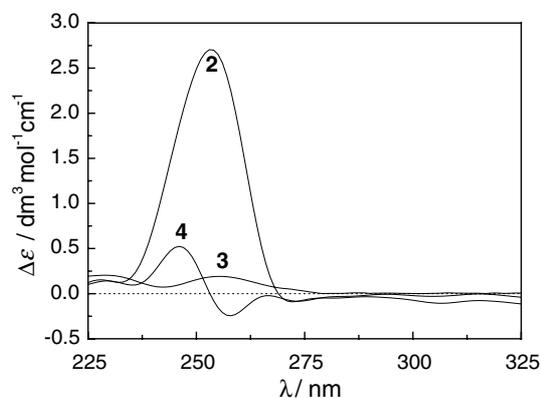


Figure 1. ICD spectra of **2** ( $8.0 \times 10^{-5}$  mol dm<sup>-3</sup>), **3** ( $8.7 \times 10^{-5}$  mol dm<sup>-3</sup>) and **4** ( $7.1 \times 10^{-5}$  mol dm<sup>-3</sup>) in phosphate buffer solutions (pH 7.20) at 25 °C.

NMR spectroscopy are also similar to that of host **3**. Therefore, the results of the ROESY experiments not only further support that of the ICD investigations on the conformations of modified  $\beta$ -CDs **2–4**, but also may be served to establish correlation between the initial conformations of modified  $\beta$ -CDs and their molecular recognition ability.

#### Binding ability and molecular selectivity toward steroids

Possessing a characteristic skeleton, steroid molecules hold a side chain at C17, methyl groups at C10, C13, C20, and a carboxylic derivative at C23, but their differences in the number and position of hydroxyl groups at C3, C7, and C12 adapt to examine the molecular recognition ability with native and modified CDs. Thus, the thermodynamics, kinetics, and conformations of the resulting inclusion complexes of some steroid molecules and native CDs have been studied by spectroscopy and microcalorimetry [14, 25–31]. Comparing the purpose, the thermodynamic parameters of inclusion complexation of native  $\beta$ -CD with steroid guests are also determined by calorimetric titrations. In

titration experiments, dilution experiments of the steroid molecules proved that the titrations with **1–4** were performed below the critical micelle concentration of the steroids. All complex stability constants ( $K_S$ ) and molar enthalpies ( $\Delta H^0$ ) reported in this paper were calculated successfully by using the one set of binding sites model. 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, indicating that the resulting complexes of bile salts and CDs are a 1:1 stoichiometry. Typically, calorimetric titration of host **3** with GC was shown in Figure 3. The averaged values of thermodynamic data obtained are listed in Table 1.

As can be seen from Table 1, the complex stability constants for **1** with steroids are variable according to the guest molecular structures. Distinctly, the  $\beta$ -CD **1** affords the highest stability constant of  $4844 \pm 16 \text{ M}^{-1}$  for inclusion complexation with DC, and the difference of the Gibbs free energy changes ( $\Delta\Delta G^0 = \Delta G_{\text{TC}}^0 (-19.18 \text{ kJ mol}^{-1}) - \Delta G_{\text{DC}}^0 (-21.03 \text{ kJ mol}^{-1})$ ) was the largest up to  $1.85 \text{ kJ mol}^{-1}$ , indicating that the cavity of  $\beta$ -CD **1** could encapsulate strongly the DC molecule. The relative good molecular selectivity ( $K_S^{\text{DC}}/K_S^{\text{TC}} = 2.11$ ) upon inclusion complexation with  $\beta$ -CD **1** should be attributed to the size/shape matching and hydrophobic interactions between host  $\beta$ -CD and guest molecule. According to previous studies [32] on the binding mode between  $\beta$ -CD and steroids, we can deduce that the aliphatic side chain folded toward the steroid skeleton can be included into the cavity of  $\beta$ -CD from the secondary side, and thus the highest affinity for DC is likely to arise from its more hydrophobic steroid skeleton, lacking the 7-hydroxyl group, and the polarity of anionic tail ( $\text{COO}^-$ ) in DC is much weaker than that of TC ( $\text{SO}_3^-$ ), for which the more hydrophobic steroid skeleton and the less polar carboxylic tail are jointly responsible.

As compared with parent  $\beta$ -CD, modified  $\beta$ -CDs **2–4** with different chain length not only enhanced molecular binding ability but also significant molecular selectivity upon inclusion complexation with homologous steroids, except for resulting complex of **4** with TC. The stability constants ( $K_S$ ) for the inclusion complexation of hosts **1–4** and the each steroid molecule decreased in the following order:  $\text{DC} > \text{CH} > \text{GC} > \text{TC}$ . Among them, the host **3** gave the highest stability constant of  $22485 \pm 15 \text{ M}^{-1}$  for the inclusion complexation with DC. The difference of the Gibbs free energy changes ( $\Delta\Delta G^0 = \Delta G_{\text{TC}}^0 - \Delta G_{\text{DC}}^0$ ) was the largest up to  $4.45 \text{ kJ mol}^{-1}$ . Apparently, the higher molecular selectivity for the steroid guests bearing same skeleton not only depend on the original conformation of host **3** but also the length and polarity of the steroid's side chain. The hydroxyl group at the C7 carbon atom of CH, GC and TC guests prevented deeper inclusion of the steroids in the  $\beta$ -CD cavity than that of DC guest [25]. Furthermore, DC with a shorter chain and an anionic tail ( $\text{CO}_3^-$ ) showed the highest  $K_S$ , whereas TC possessing a longer chain and a

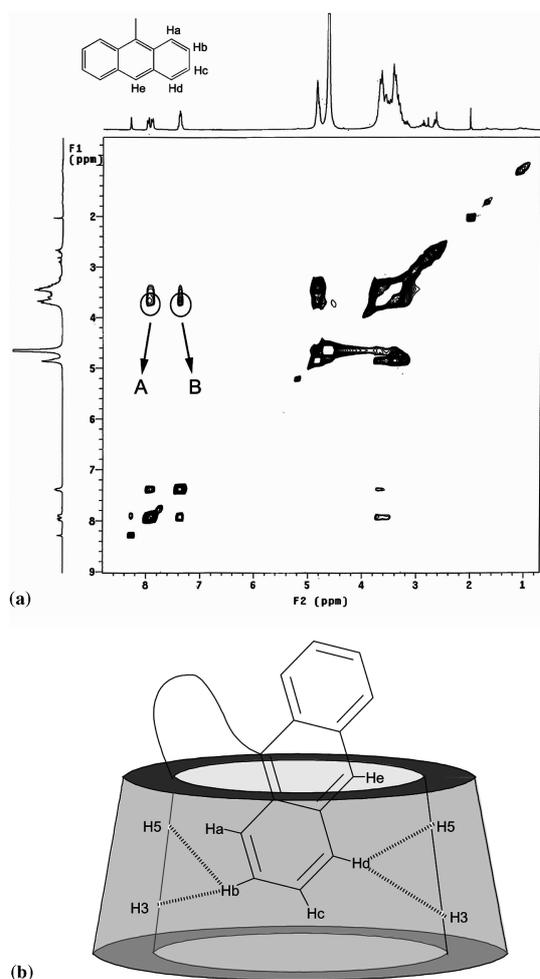
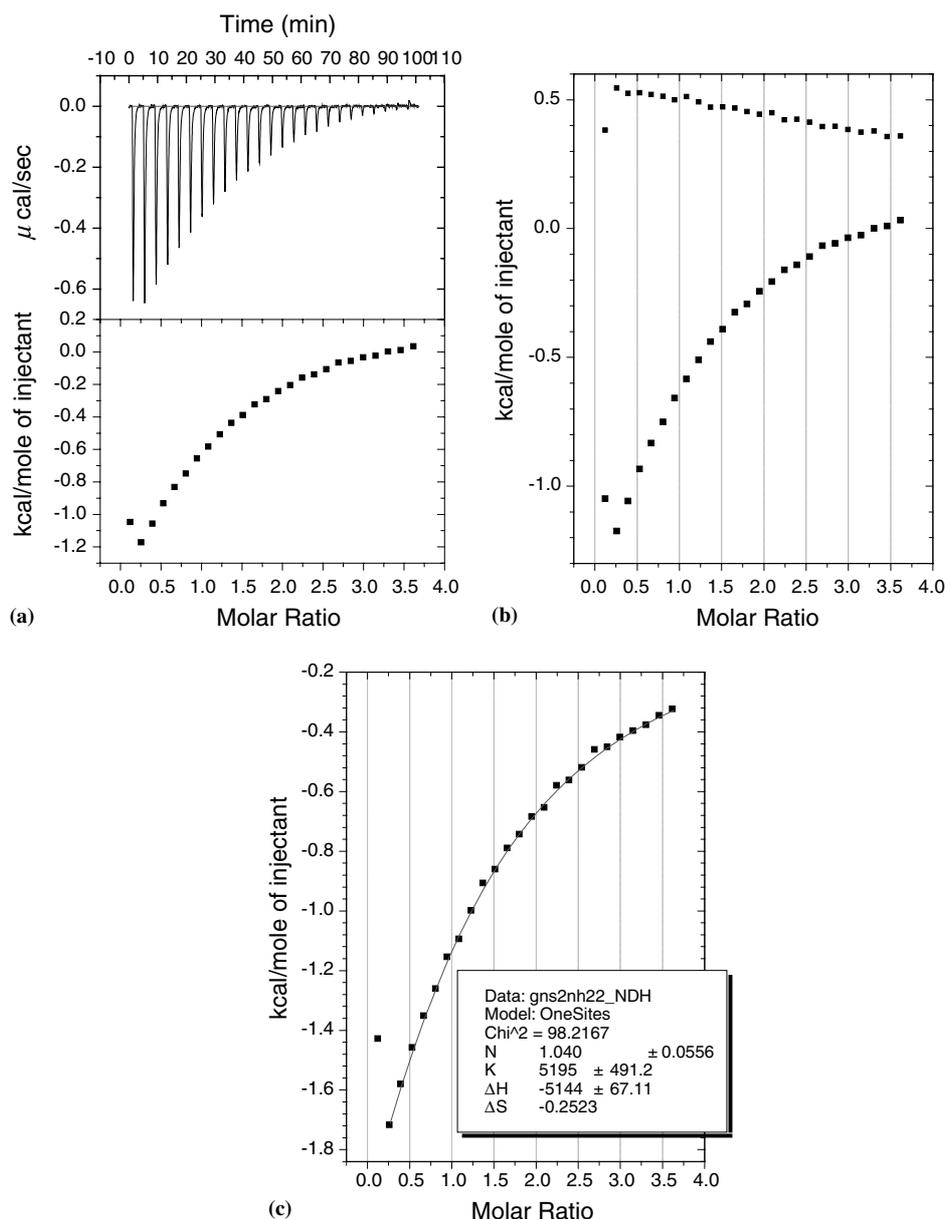


Figure 2. (a)  $^1\text{H}$  ROESY spectrum of **3** ( $7.8 \times 10^{-4} \text{ mol dm}^{-3}$ ) in  $\text{D}_2\text{O}$  at 298 K with a mixing time of 600 ms, (b) possible structure of **3**.



**Figure 3.** Calorimetric titration of host **3** with GC in phosphate buffer (pH 7.2) at 25 °C. (a) Upper panels: Raw data for sequential 10  $\mu$ L injections of host **3** solution (2.03 mM) into GC solution (0.11 mM); Lower panel: Heats of reaction as obtained from the integration of the calorimetric traces. (b) Heat effects of dilution and of complexation of GC with host **3** for each injection during titration microcalorimetric experiment. (c) “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.

highly polar anionic tail ( $\text{SO}_3^-$ ) gave the lowest  $K_S$ . Thus, the highest molecular selectivity ( $K_S^{\text{DC}}/K_S^{\text{TC}}$ ) of up to 7.29 was observed for host **4**, which was higher than that of  $\beta$ -CD **1**.

On the other hand, the tether length of the host and induced-fit interactions also played crucial roles in the selective molecular binding process of modified  $\beta$ -CD **2–4** with guests. From Table 2, we may see that the hosts show different selectivity for steroid guests, i.e., **3** > **4** > **2** > **1** for DC, CH and GC guests, **3** > **2** > **1** > **4** for TC, which indicated that host **3** possessing suitable tether length could encapsulate more tightly the steroid guests than the other, through the

size/shape-matching and the induced-fit interactions between the host and guest.

Thermodynamically, the inclusion complexation of **1–4** with steroid guests is entirely driven by favorable enthalpy contribution with negative or minor positive entropy change. Typically, the stronger interaction of DC with **3** gave the highest enthalpy ( $\Delta H_3^0$ ) value up to  $-36.48 \text{ kJ mol}^{-1}$  with the negative entropy loss ( $T\Delta S_3^0 = -11.64 \text{ kJ mol}^{-1}$ ). Comparable to that of **1** ( $\Delta H_1^0 = -25.79 \text{ kJ mol}^{-1}$ ,  $T\Delta S_1^0 = -4.76 \text{ kJ mol}^{-1}$ ), the larger enthalpic gain ( $\Delta H_3^0 - \Delta H_1^0 = -10.69 \text{ kJ mol}^{-1}$ ) is partially counteracted by the larger entropic loss ( $T\Delta S_3^0 - T\Delta S_1^0 = -6.88 \text{ kJ mol}^{-1}$ ). In contrast, the

Table 1. Complex stability constant ( $K_S$ ) and standard enthalpy ( $\Delta H^0$ ) and entropy changes ( $T\Delta S^0$ ) for 1:1 inclusion complexation of steroid guests with  $\beta$ -CD **1** and modified  $\beta$ -CDs **2–4** in phosphate buffer solution (pH 7.20) at  $T = 298.15$  K

Host <sup>a</sup>	Guest <sup>b</sup>	$N^c$	$K_S/M^{-1}$	$-\Delta G^0/kJ\ mol^{-1}$	$-\Delta H^0/kJ\ mol^{-1}$	$T\Delta S^0/kJ\ mol^{-1}$
<b>1</b>	DC	2	4844 ± 16	21.03 ± 0.01	25.79 ± 0.00	-4.76 ± 0.01
	CH	4	4068 ± 84	20.60 ± 0.05	22.98 ± 0.45	-2.38 ± 0.50
	GC	2	2394 ± 69	19.29 ± 0.07	22.99 ± 0.08	-3.70 ± 0.15
	TC	2	2293 ± 13	19.18 ± 0.01	23.77 ± 0.08	-4.59 ± 0.09
<b>2</b>	DC	2	15030 ± 425	23.85 ± 0.07	42.72 ± 0.59	-18.87 ± 0.66
	CH	2	11760 ± 160	23.24 ± 0.05	42.70 ± 0.07	-19.47 ± 0.12
	GC	2	3870 ± 220	20.47 ± 0.14	25.23 ± 1.05	-4.75 ± 1.19
	TC	2	2647 ± 308	19.52 ± 0.29	20.99 ± 0.14	-1.47 ± 0.43
<b>3</b>	DC	2	22485 ± 15	24.84 ± 0.00	36.48 ± 0.18	-11.64 ± 0.18
	CH	2	18965 ± 285	24.42 ± 0.04	32.37 ± 0.05	-7.95 ± 0.09
	GC	2	4888 ± 307	21.05 ± 0.16	21.61 ± 0.08	-0.56 ± 0.24
	TC	2	3755 ± 434	20.39 ± 0.29	19.15 ± 0.79	0.7 ± 0.54
<b>4</b>	DC	2	13365 ± 115	23.54 ± 0.02	39.57 ± 0.01	-16.20 ± 0.22
	CH	2	11850 ± 240	23.25 ± 0.05	33.23 ± 0.03	-9.98 ± 0.02
	GC	2	4254 ± 205	20.71 ± 0.12	20.07 ± 0.68	0.65 ± 0.56
	TC	2	1833 ± 117	18.62 ± 0.16	26.58 ± 0.20	-7.96 ± 0.36

<sup>a</sup>[Host] = 1.99–4.16 mM.

<sup>b</sup>[Guest] = 0.10–0.56 mM.

<sup>c</sup>Number of titration runs performed.

complexation of TC with **3** gave a smaller enthalpic gain than that for **1** ( $\Delta H_3^0 - \Delta H_1^0 = 4.62$  kJ mol<sup>-1</sup>), which is over-compensated by a larger entropic gain ( $T\Delta S_3^0 - T\Delta S_1^0 = 5.29$  kJ mol<sup>-1</sup>). As a consequence of such opposite behavior of  $\Delta H^0$  and  $T\Delta S^0$ , the molecular selectivity of  $\beta$ -CD ( $\Delta\Delta G^0 = \Delta G_{TC}^0 - \Delta G_{DC}^0 = 1.85$  kJ mol<sup>-1</sup>) is substantially enhanced to give a  $\Delta\Delta G^0$  value of 4.45 kJ mol<sup>-1</sup> for host **3**. The strong interaction between host and guest leads to the more favorable negative enthalpy ( $\Delta H^0$ ), which is counteracted by the relative more unfavorable negative entropic ( $\Delta S^0$ ), giving the moderate binding constants. Therefore, we can deduce that the introduction of anthracene group with different chain length, and additional binding site to CD rim can significantly enhance the binding ability of parent CD toward steroid guests, which can be used as a rule to design and synthesize receptors with specific functional group to control the binding behavior toward specific guests.

#### Enthalpy–entropy compensation

Enthalpy–entropy compensation effect has often been observed empirically in the kinetic and thermodynamic parameters determined for a wide variety of reactions and equilibria. Numerous experiment data in the original and review articles indicated that widely observed compensation enthalpy–entropy relationship is a powerful tool to understand and even to predict thermodynamic behavior. We have previously demonstrated that the enthalpy and entropy changes obtained for the inclusion complexation of various guests with native  $\alpha$ - to  $\gamma$ -CDs are mutually compensatory. We have also proposed that the slope ( $\alpha = 0.79$ – $0.97$ ) and intercept ( $T\Delta S^0 = 8$ – $15$  kJ mol<sup>-1</sup>) of the compensation plot

can be used as quantitative measures of the conformational changes and the desolvation extent upon inclusion complexation with CDs [33]. The relatively steep slopes of 0.79–0.97, obtained with  $\alpha$ - to  $\gamma$ -cyclodextrins, mean that only 3–23% of the enthalpic gains from complex formation are reflected in the free energy change or complex stability. This is probably due to the global reorganization of the original hydrogen-bonding network in CDs upon inclusion complexation. The compensatory enthalpy–entropy relationship for a wide variety of mono-modified  $\beta$ -CDs has also been reported, and gives a slope ( $\alpha = 0.99$ ) and larger intercept ( $T\Delta S^0 = 17$  kJ mol<sup>-1</sup>).

In this text, the correlation of enthalpy–entropy compensation is also performed by plotting  $T\Delta S^0$  versus  $\Delta H^0$  using current limited experimental data. As shown in Figure 4, a good straight line with a correlation coefficient ( $r$ ) of 0.98 has been obtained to give an intercept ( $T\Delta S^0 = 16.24$  kJ mol<sup>-1</sup>) and a slope ( $\alpha = 0.81$ ). From above results, we can see that intercept value obtained are larger than that of native CDs, and in agreement with those reported in the literature for complexes formed by modified CDs with flexible side-arms, indicating that the inclusion complexation of present modified  $\beta$ -CDs with steroids occur the larger conformational change and extensively desolvation effect.

#### ROESY experiments

It is well known that the size/shape-matching and induced-fit interaction occur in the molecular binding process of modified  $\beta$ -CDs, and therefore, it is very important to investigate the interaction binding models between host and guest molecules for elucidating the

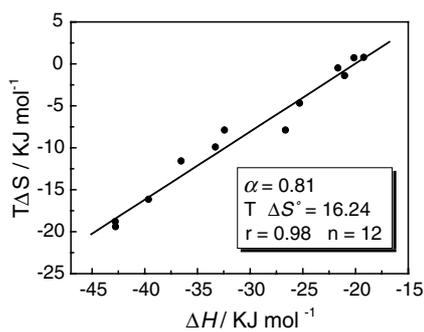
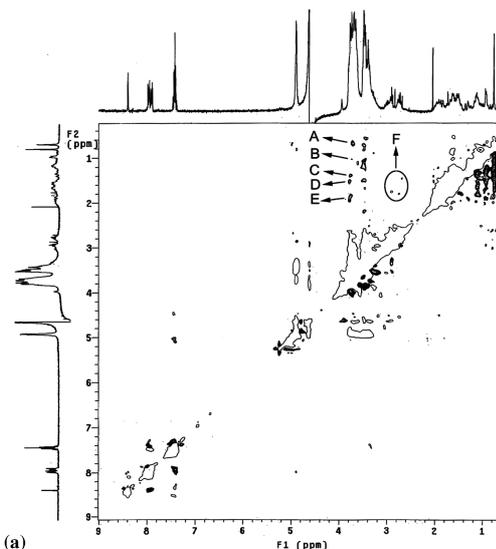
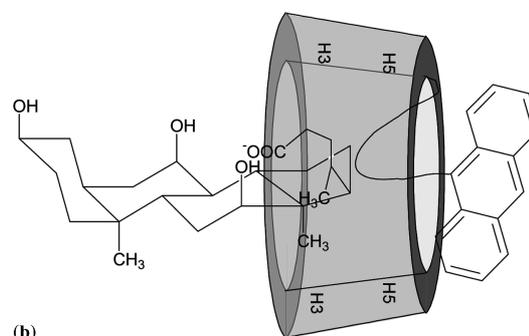


Figure 4. Enthalpy–entropy compensation plot for the inclusion complexation of various steroids with anthracene modified  $\beta$ -CDs 2–4.

mechanism of molecular recognition. In the text,  $^1\text{H}$  ROESY experiment was performed to confirm the binding model of host **3** with the representative guest CH. As illustrated in Figure 5a, the ROESY spectrum of a mixture of host **3** ( $8.7 \times 10^{-4} \text{ mol dm}^{-3}$ ) with guest CH ( $9.4 \times 10^{-4} \text{ mol dm}^{-3}$ ) in  $\text{D}_2\text{O}$  solution displays sophisticated NOE cross-peaks, which come from not only intermolecular interaction between the  $\beta$ -CD host and the CH molecule, but also intramolecular interactions of **3** or CH. The ROESY spectrum displays clear NOE cross-peaks between the  $\text{H}_3$  protons of host **3** and the  $\text{H}_{18}$  (peaks A),  $\text{H}_{21}$  (peaks B) and  $\text{H}_{20}$  (peaks C) protons of CH molecule, between the  $\text{H}_3$  and the  $\text{H}_{15}$ ,  $\text{H}_{17}$ ,  $\text{H}_{22}$  (peaks D), as well as between the  $\text{H}_3$  and the  $\text{H}_{16}$  and/or  $\text{H}_{23}$  (peaks E) protons, which indicated distinctly that the CH molecule is included into the hydrophobic cavity from the secondary side of  $\beta$ -CD, with the side chain folded toward the steroid skeleton. The results obtained were similar to that of native  $\beta$ -CD with CH molecule [32]. On the other hand, no NOE cross-peaks between the  $\text{H}_3$  and/or  $\text{H}_5$  and the protons of anthracene group in



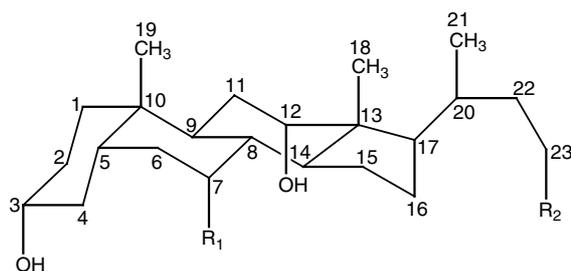
(a)



(b)

Figure 5. (a)  $^1\text{H}$  ROESY spectrum of **3** ( $8.7 \times 10^{-4} \text{ mol dm}^{-3}$ ) complex of CH ( $9.4 \times 10^{-4} \text{ mol dm}^{-3}$ ) in  $\text{D}_2\text{O}$  at 298 K with a mixing time of 600 ms; (b) plausible complex structures of CH with **3**.

host **3** were observed, indicating that the anthracene group is excluded outside the cavity of  $\beta$ -CD. It is interestingly noted that the correlation peaks F between



Guests	$\text{R}_1$	$\text{R}_2$
Cholate (CH)	OH	COONa
Deoxycholate (DC)	H	COONa
Glycocholate (GC)	OH	CONHCH <sub>2</sub> COONa
Taurocholate (TC)	OH	CONHCH <sub>2</sub> CH <sub>2</sub> SO <sub>3</sub> Na

Chart 1.

the protons of diethylenetriamine moiety in host **3** and the protons of CH appear, indicating that the CH molecule and the tether of  $\beta$ -CD can be co-included into the cavity through the induced-fit interaction between host and guest. The plausible conformation for host **3** and CH was shown in Figure 5b.

## Conclusions

In summary, three novel anthracene-contained  $\beta$ -CD derivatives **2-4** were synthesized and their binding behavior was investigated by using spectroscopic techniques and isothermal calorimeter. The results obtained indicated that the size/shape matching, hydrophobic and induced-fit interactions play crucial role in the molecular recognition process of the anthryl-modified  $\beta$ -CDs. The combination investigations of NMR spectroscopy and microcalorimetry established the correlation between the conformation of complexes and the thermodynamic parameters obtained, which will serve our further understanding of the molecular/chiral recognition mechanism of the special substrate by modified  $\beta$ -CDs.

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## Graphical Abstract

**Molecular Recognition Thermodynamics of Steroids by Novel Oligo(aminoethylamino)- $\beta$ -cyclodextrins Bearing Anthryl: Enhanced Molecular Binding Ability by Co-inclusion Complexation**

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