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Organic Anion Recognition of Naphthalenesulfonates by Steroid-Modified β-Cyclodextrins: Enhanced Molecular Binding Ability and Molecular Selectivity

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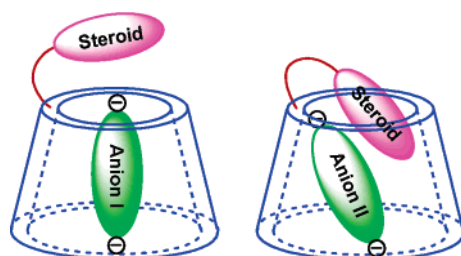
Two β-cyclodextrin (β-CD) derivatives bearing steroid groups (**1** and **2**) were synthesized by the condensation of mono(6-aminoethylamino-6-deoxy)-β-CD with cholic acid and deoxycholic acid, respectively, and their original conformations and binding behavior to the organic anion of naphthalenesulfonate derivatives were investigated by using ^{1}H NMR spectroscopy and spectrofluorometric titration in combination with computational methods. The 2D NMR experiments reveal that the steroid groups attached to the β-CD rim could be deeply embedded in the β-CD cavity to form the intramolecular (for **1**) or intermolecular (for **2**) inclusion complexes in aqueous solution. Upon complexation with naphthalenesulfonate derivatives, modified β-CDs display two obviously different binding modes, that is, the competitive inclusion mode and the induced-fit inclusion mode, which is consistent with the results of molecular modeling study. The two modes and the strict size/shape fitting relationship between the hosts and guests reasonably explain the different binding behaviors and molecular selectivity of host β-CDs **1** and **2** toward the naphthalenesulfonate guests. Therefore, the cholic acid- or deoxycholic acid-modified β-CDs could effectively recognize the size/shape of guest molecules as compared with the parent β-CD, giving good molecular selectivity up to 24.9 for the disodium 2,6-naphthalenedisulfonate/disodium 1,5-naphthalenedisulfonate pair by the host **1**.

Introduction

In the field of host−guest chemistry, the progress in synthetic receptors for anions has attracted considerable attention in the past two decades due to the fact that a large number of biological processes involve molecular recognition of anionic species.[1−7]

As one of the successful receptors for the molecular recognitions, cyclodextrins (CDs), a class of cyclic oligosaccharides with 6−8 D-glucose units, and their derivatives have been widely applied due to their hydrophobic cavities capable of binding guest molecules through the simultaneous contributions of several noncovalent interactions.[8−12] Simultaneously, the CDs

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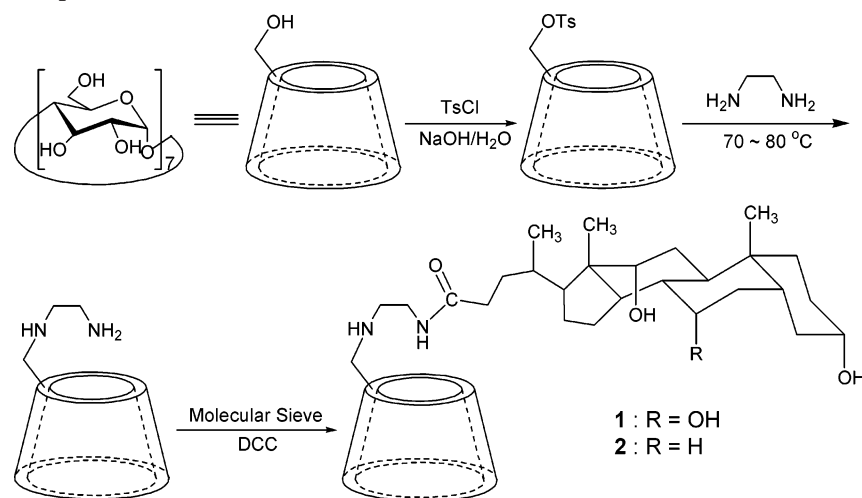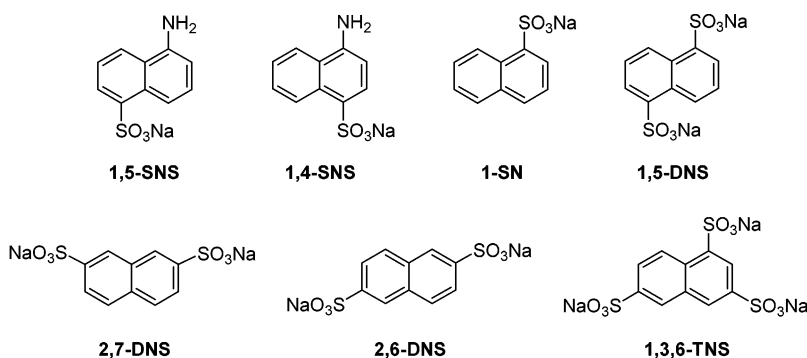**SCHEME 1. Schematic Representation of the Formation of Hosts 1 and 2**

1 : R = OH
2 : R = H

CHART 1. Naphthalenesulfonate Guests

1,5-SNS   **1,4-SNS**   **1-SN**   **1,5-DNS**

2,7-DNS   **2,6-DNS**   **1,3,6-TNS**

also acted as anionic recognition receptors to bind inorganic[13−15] or organic[16−22] anions. As a general rule, the guest molecules not well hydrated in water but of the correct complementary size to fit into the hydrophobic cavity will associate with the CDs. In line with this expectation, some inorganic anions such as ClO_4^-, I^-, and SCN^- but not well-hydrated species (CH_3COO^-, Cl^-, and SO_4^{2-}) form weak complexes ($K_S = 10-50$ M^{-1}) with α- or β-CDs.[13,14] Furthermore, the chemically modified CDs, which are tethered by some functional groups, have been designed and synthesized to enhance the original molecular binding ability and selectivity of parent CDs, through the induced-fit interaction and the complementary geometrical relationship between the host and guest. Prominent in this respect was the introduction of amino groups in the 6-position of CDs, which after protonation could interact by salt bridging with anionic substructures of the guest.[16] Somewhat weaker synergism of binding interactions was observed when β-CD was modified with two imidazole heterocycles and reacted with zinc to form a coordinatively unsaturated zink complex. Binding of the zink complex with cyclohexane-1,4-dicarboxylate was found to outmatch complexation by the parent ligand by a factor of 6.6.[17] These studies are not only directed toward an understanding of the binding ability of CDs, but also provide valuable information on the effects of changes in functionality of guests, which attempt to elucidate the nature of anionic recognition of CDs with anion guests.

In previous work, we have studied the inclusion behavior of a series of naphthalenesulfonates by β-CD in aqueous solution.[19] The binding interaction in these complexes is primarily a result of the hydrophobic effect, with the naphthalene residue displac-

ing water molecules from the internal cavity of the CD. The anionic sulfonate group remains outside the cavity in contact with the solvent, thereby controlling the orientation of the naphthalene group within the cavity. However, the molecular recognition of modified CDs to anion guests, especially naphthalene derivatives, has not been well investigated so far, although these studies are very important to discuss the anionic recognition mechanism and control the binding behavior of

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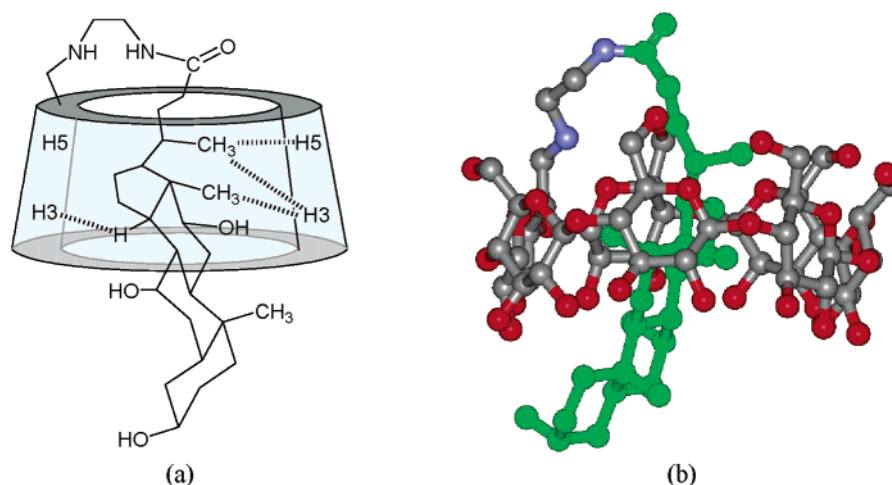
FIGURE 1. (a) Possible structure of **1** in solution based on the ^{1}H ROESY NMR experiment and (b) the optimized structure of **1** with ball-and-stick representation based on the molecular modeling study. The hydrogen atoms were omitted and the structure was colored by atom type: gray, carbon atoms; red, oxygen atoms; pale blue, nitrogen atoms in the β-CD moiety; and green, cholic acid moiety.

biological receptors. On the basis of valuable application of steroids in biological and life sciences,[23–40] we have recently

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**CHART 2. The Molecular Structures of Cholic Acid and Deoxycholic Acid**

Cholalic Acid: R = OH
Deoxycholic Acid: R = H
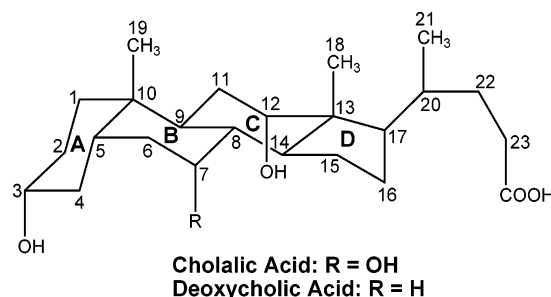
shown that the amphiphilic cholic acid-modified β-CD (**1**) could form a supramolecular porous nanosphere triggered by guest sodium 1-naphthylamino-4-sulfonate.[41] In the present paper, we wish to report our investigation results on the binding behavior of steroid-linked β-CDs **1** and **2** (Scheme 1) with some representative naphthalenesulfonate derivatives (sodium 1-naphthylamino-5-sulfonate (1,5-SNS), sodium 1-naphthylamino-4-sulfonate (1,4-SNS), sodium 1-naphthalenesulfonate (1-SN), disodium 1,5-naphthalenedisulfonate (1,5-DNS), disodium 2,7-naphthalenedisulfonate (2,7-DNS), disodium 2,6-naphthalenedisulfonate (2,6-DNS), and trisodium 1,3,6-naphthalenetrisulfonate (1,3,6-TNS), Chart 1) by using the spectrofluorometric titration method in phosphate buffer solution (pH 7.2). The results obtained indicated that hosts **1** and **2** significantly enhanced the original binding abilities of parent β-CD toward naphthalenesulfonate derivatives. On the basis of the investigation of ^{1}H ROESY (Rotating frame Overhause Effect SpectroscopY) NMR spectroscopy and computational methods, the molecular binding modes and complex formation constants (K_S) of substrates with hosts **1** and **2** were discussed from the viewpoints of the size/shape matching, induced-fit, and electrostatic interactions between the hosts and guests. It is our special interest to examine the anionic recognition mechanism and effects of number, position, and type of substituent groups in naphthalene molecules by modified CDs, which will serve

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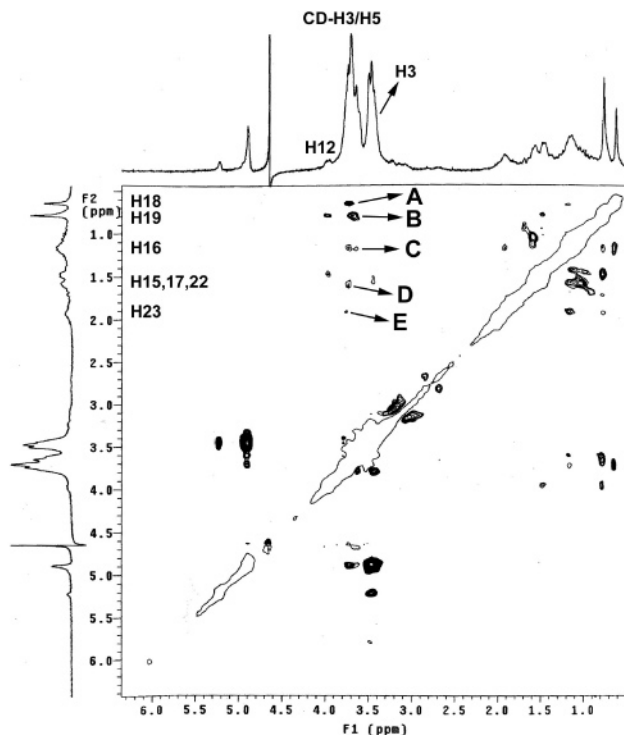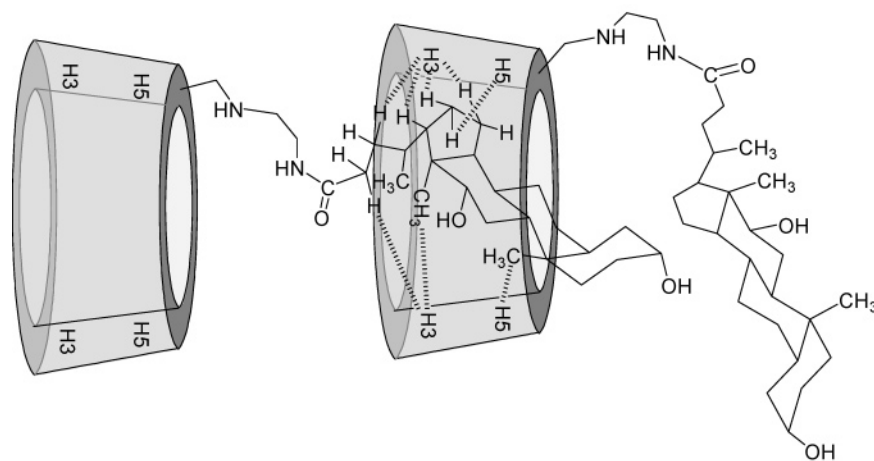
FIGURE 2. (a) ROESY spectrum of **2** (1.7×10^{-3} mol dm^{-3}) in D$_2$O at 25.0 °C with a mixing time of 300 ms and (b) possible structure of **2** in solution.

our further understanding of this recently developing, but less investigated, area of anionic recognition of CDs.

Results and Discussion

Synthesis. Possessing a characteristic skeleton, the cholic acid and deoxycholic acid (Chart 2) possess a side chain at C17, methyl groups at C10, C13, and C20, and a carboxylic group at C23, but their disparity in the presence and absence of a hydroxyl group at C7 adapts to different physical/chemical behavior. The four rings of cholic acid or deoxycholic acid are labeled A, B, C, and D in Chart 2. According to a good adaptation of these steroid molecules in the CD cavity, the thermodynamics, kinetics, and conformations of the resulting inclusion complexes of steroid molecules with CDs have been studied by using spectroscopy and microcalorimetry.[28,37] In the present work, we have linked the cholic acid and deoxycholic acid to the rim of β-CD in moderate yields, which would likely enhance the binding ability and molecular selectivity of the parent β-CD. The included conformations of hosts **1** and **2** are validated by the ^{1}H ROESY NMR spectroscopy described below.

Conformation Analysis of Hosts 1 and 2. The conformations of many monomodified CDs in solution have been carefully studied, and most studies indicate that the hydrophobic substituent prefers to be self-induced to the cavity of parent CD forming an intramolecular complex.[42–47] 2D NMR spec-

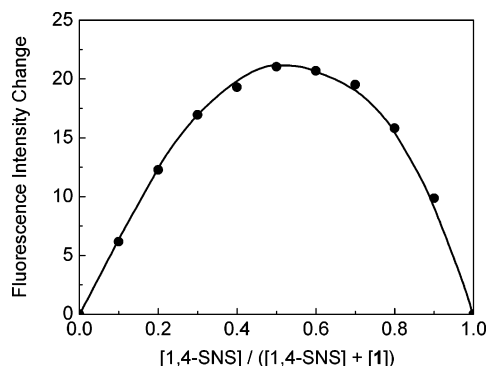(42) May, B. L.; Kean, S. D.; Easton, C. J.; Lincoln, S. F. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3157–3160.

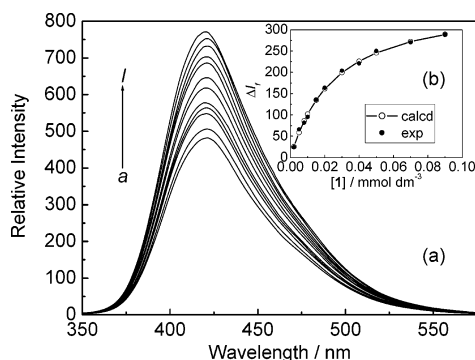**FIGURE 3.** Job's plot of **1**/1,4-SNS systems at 421.0 nm ([**1**] + [1,4-SNS] = 3.0×10^{-6} mol·dm^{-3}).

FIGURE 4. (a) Fluorescence spectral changes of 1,4-SNS (3.15×10^{-6} mol·dm^{-3}) upon addition of **1** (from *a* to *l* = 0, 0.002, 0.005, 0.008, 0.01, 0.015, 0.02, 0.03, 0.04, 0.05, 0.07, 0.09 $\times 10^{-3}$ mol·dm^{-3}) in phosphate buffer aqueous solution (pH 7.2) at 25.0 °C, $\lambda_{ex} = 322.0$ nm. (b) The nonlinear least-squares analysis of the differential intensity (ΔI_f) to calculate the complex formation constant (K_S).

troscopy has recently become an important method for the investigation of not only the interaction between host CDs and guest molecules, but also the self-included mode between the CD cavity and its substituting groups, while the substituent group is included into the β-CD cavity, the NOE correlations between the protons of the substituent group and the inner protons of the β-CD cavity (H3 and H5) will be measured. According to the relative intensity of these cross-peaks, it is possible to estimate the orientation of the substituent group within the β-CD cavity.[45,46] To confirm the original conformation of hosts **1** and **2** in aqueous solution, their 2D ROESY experiments were performed at 25 °C in D$_2$O. Although there was presented the superposition of some peaks in the ROESY spectrum of **1** between H3, H7, and H12 protons of cholic acid moiety and the β-CD's protons,[38b] we still could deduce the

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conformation of **1** according to the ROESY spectrum. The ROESY spectrum[41] of **1** (1.8×10^{-3} mol dm^{-3}) displayed clear NOE cross-peaks between the H3 protons of β-CD and H18 and H14 protons of cholic acid moiety, as well as between the H3/H5 of β-CD and H21 proton, which indicated distinctly that the cholic acid moiety in **1** was self-included into the hydrophobic cavity of β-CD, as illustrated Figure 1a. To elucidate the self-inclusion structure of host **1**, the molecular modeling study was performed by using the InsightII program. The obtained result (Figure 1b) showed the cholic acid moiety could be self-included in the β-CD's cavity, which was compatible with the NMR experimental results.

In the case of **2** (Figure 2a), the ROESY spectrum of **2** (1.7×10^{-3} mol dm^{-3}) in D$_2$O solution showed the NOE cross-peaks between the H3 protons of β-CD and the H18 (peak A), H15, H17, H22 (peaks D), and H23 (peak E) protons of the deoxycholic acid moiety, between the H5 protons of β-CD and the H19 protons (peak B), as well as between the H3/H5 protons of β-CD and the H16 protons (peaks C), which indicated distinctly that the deoxycholic acid moiety was included into the hydrophobic cavity from the secondary side of another β-CD to form the intermolecular inclusion complexes. A possible conformation for host **2** was shown in Figure 2b. Therefore, the results of the 2D NMR experiments could serve to establish the correlation between the conformational features of modified β-CDs **1** and **2** and their organic anion recognition abilities.

Spectral Titration. As elucidated above, the cholic acid or deoxycholic acid moiety could be included in the β-CD cavity to form intramolacular (the case of **1**) or intermolecular (the case of **2**) inclusion complexes. Therefore, the linker group might suffer substantial conformational change upon guest inclusion and thus result in the relevant spectral changes which could obtain the binding constants between the host and guest in differential fluorescence spectrometry. Herein, the binding behaviors of representative naphthalenesulfonate derivatives (1,5-SNS, 1,4-SNS, 1-SN, 1,5-DNS, 2,7-DNS, 2,6-DNS, and 1,3,6-TNS) as organic anion guests with hosts **1** and **2** could be determined by the spectral titration experiments. It is noteworthy that the host **1** could self-assemble to form porous nanospheres with a critical concentration of about 5.0×10^{-4} mol dm^{-3} in the presence of guest 1,4-SNS.[41] Therefore, we performed the anion recognition experiments of hosts **1** and **2** under this concentration. Figure 3 illustrated a representative Job's plot for **1**/1,4-SNS systems in phosphate buffer aqueous solution (pH 7.2) at 25 °C. In the used concentration range, the plot for modified β-CD showed a maximum at a molar fraction of 0.5, indicating the 1:1 inclusion complexation between the host and guest. The same results were obtained in the cases of the inclusion complexation of hosts **1** and **2** with other selected guests.

Using the 1:1 host/guest stoichiometry, the complexation of modified β-CD hosts (CD) with the naphthalenesulfonate derivative guests (ND) could be expressed by eq 1.

$$\text{CD} + \text{ND} \underset{}{\overset{K_S}{\rightleftharpoons}} \text{CD·ND} \qquad (1)$$

The relative fluorescence intensity change of the guest unit (ΔI_f) upon addition of host molecule, where $\Delta I_f = I_f$(with host molecule) $- I_f$(without host molecule), was assumed to be proportional to the concentration of inclusion complex formed by the modified β-CD unit with a model substrate, i.e., $\Delta I_f = \alpha$[CD·ND]. The proportionality coefficient α was taken as a
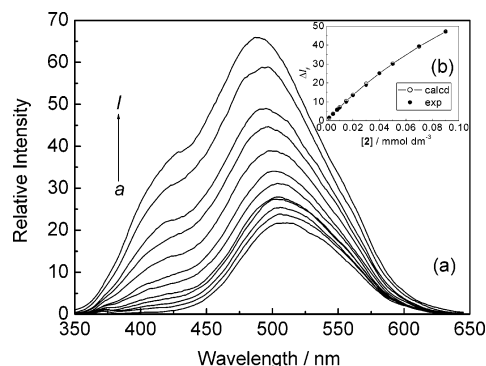
FIGURE 5. (a) Fluorescence spectral changes of 1,5-SNS (3.10 × 10^{-6} mol·dm^{-3}) upon addition of **2** (from *a* to *l* = 0, 0.002, 0.005, 0.008, 0.01, 0.015, 0.02, 0.03, 0.04, 0.05, 0.07, 0.09 × 10^{-3} mol·dm^{-3}) in phosphate buffer aqueous solution (pH 7.2) at 25.0 °C, λ_{ex} = 325.0 nm. (b) The nonlinear least-squares analysis of the differential intensity (ΔI_f) to calculate the complex formation constant (K_S).

sensitivity factor for the fluorescence change upon inclusion complexation. Then, the effective complex formation constant (K_S) can be expressed by eq 2:[48,49]

$$K_S = \frac{[\text{CD·ND}]}{[\text{CD}][\text{ND}]} =$$

$$\frac{[\text{CD·ND}]}{([\text{CD}]_0 - [\text{CD·ND}])([\text{ND}]_0 - [\text{CD·ND}])} =$$

$$\frac{\Delta I_f / a}{([\text{CD}]_0 - \Delta I_f / a)([\text{ND}]_0 - \Delta I_f / a)} \quad (2)$$

where $[\text{CD}]_0$ and $[\text{ND}]_0$ denoted the initial concentrations of modified β-CD host and naphthalenesulfonate derivatives, respectively. Subsequently, eq 2 can be solved for ΔI_f to give eq 3:

$$\Delta I_f = \{\alpha([\text{CD}]_0 + [\text{ND}]_0 + 1/K_S) -$$

$$\sqrt{\alpha^2([\text{CD}]_0 + [\text{ND}]_0 + 1/K_S)^2 - 4\alpha^2[\text{ND}]_0[\text{CD}]_0}\}/2 \quad (3)$$

Using the nonlinear least-squares curve-fitting method according to eq 3, we obtained the complex formation constant for each host−guest combination from the analysis of the sequential changes of fluorescence intensity (ΔI_f) at various host concentrations. Figures 4b and 5b illustrated a typical curve fitting plot for the titrations of **1** with 1,4-SNS and **2** with 1,5-SNS, respectively, which showed excellent fits between the experimental and calculated data obtained. A good correlation between the experimental and calculated result indicated the reliability of the obtained complex formation constants. The complex formation constants (K_S) and Gibbs free energy changes ($-\Delta G°$) obtained for the complexation of modified β-CDs **1** and **2** with naphthalenesulfonate derivatives were compiled in Table 1. When repeated measurements were made, the K_S values were reproducible within an error of ±6%.

Binding Mode. It is very important to investigate the inclusion modes between the host β-CDs and guest molecules for elucidating the mechanism of organic anion recognition. To establish possible binding modes of modified β-CDs **1** and **2**

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TABLE 1. Complex Stability Constants (K_S) and Gibbs Free Energy Change ($-\Delta G°$) for the Inclusion Complexation of Modified β-CDs 1 and 2 with Naphthalenesulfonate Derivatives as Organic Anion Guests in Phosphate Buffer Aqueous Solution (pH 7.2) at 25.0 °C

hosts	guests	K_S	log K_S	$-\Delta G°$/kJ·mol^{-1}	method[a]	ref
β-CD	1,4-SNS	50	1.70	9.7	Cal	*b*
	1-SN	2512	3.40	19.4	Cal	*b*
	2,7-DNS	275	2.44	13.9	Cal	*b*
	2,6-DNS	1950	3.29	18.8	Cal	*b*
1	1,5-SNS	16700	4.22	24.1	Fl	*c*
	1,4-SNS	41900	4.62	26.4	Fl	*d*
	1-SN	8040	3.90	22.3	Fl	*c*
	1,5-DNS	4170	3.62	20.7	Fl	*c*
	2,7-DNS	25500	4.41	25.2	Fl	*c*
	2,6-DNS	103900	5.02	28.6	Fl	*d*
	1,3,6-TNS	7790	3.89	22.2	Fl	*c*
2	1,5-SNS	6380	3.80	21.7	Fl	*c*
	1,4-SNS	117000	5.07	28.9	Fl	*c*
	1-SN	10230	4.01	22.9	Fl	*c*
	1,5-DNS	7600	3.88	22.2	Fl	*c*
	2,7-DNS	5270	3.72	21.2	Fl	*c*
	2,6-DNS	59800	4.78	27.3	Fl	*c*
	1,3,6-TNS	11970	4.08	23.3	Fl	*c*

a Cal: microcalorimetric titration. Fl: spectrofluorometric titration. *b* Reference 19. *c* This work. *d* Reference 41.

with naphthalenesulfonate derivatives, in the present paper, ^{1}H ROESY experiments of hosts **1** and **2** with representative guests 1,4-SNS, 2,6-DNS, and 1,5-DNS were performed in D$_2$O at 25.0 °C, respectively. The ROESY spectrum[41] of **1** (2.1 × 10^{-3} mol dm^{-3}) with 1,4-SNS (2.4 × 10^{-3} mol dm^{-3}) showed the NOE cross-peaks between the H3 protons of β-CD in **1** and the Hd/He protons of the 1,4-SNS molecule, and between the H5 of β-CD and the Hd/He and Hc protons of the 1,4-SNS molecule, and no NOE cross-peaks between the H3/H5 and the protons of the cholic acid moiety in **1**, indicating that the 1,4-SNS molecule was included at the cavity of β-CD and excluded the cholic acid moiety to the outside of the hydrophobic cavity. According to the results of the 2D NMR experiment, a possible conformation of host **1** with 1,4-SNS is shown in Figure 6a. In further work, we performed the molecular modeling study of the **1**/1,4-SNS complex. The obtained result showed that the 1,4-SNS molecule could exclude the cholic acid moiety from the β-CD's cavity, which was almost similar with the result of the ^{1}H ROESY NMR experiment. An optimized structure of host **1** with 1,4-SNS is shown in Figure 6b.

In the case of **1** (2.0 × 10^{-3} mol dm^{-3}) with 2,6-DNS (2.6 × 10^{-3} mol dm^{-3}), the ROESY spectrum (Figure 7a) gave the clear NOE cross-peaks between the H3/H5 protons of β-CD in **1** and the Ha (peaks C) and Hb (peaks B) protons of the 2,6-DNS molecule and between the H3 of β-CD and the Hc (peak A) protons of the 2,6-DNS molecule, which indicated that the 2,6-DNS molecule was deeply included at the cavity of β-CD. Among these signals, the NOE cross-peaks (peaks D−K) between the protons of the cholic acid moiety and the H3/H5 protons of β-CD in **1** were clearly observed, suggesting that there was an inclusion equilibrium between the cholic acid moiety and the 2,6-DNS molecule at the cavity of β-CD. The induced-fit inclusion mode between the cholic acid moiety and the 2,6-DNS molecule might be an important reason for obtaining a higher binding affinity of host **1** with the 2,6-DNS molecule (K_S = 103 900 M^{-1}). The result of the molecular modeling study showed that it could occur as an inclusion equilibrium between the cholic acid moiety and the 2,6-DNS molecule at the β-CD's cavity with a minimum energy. On the
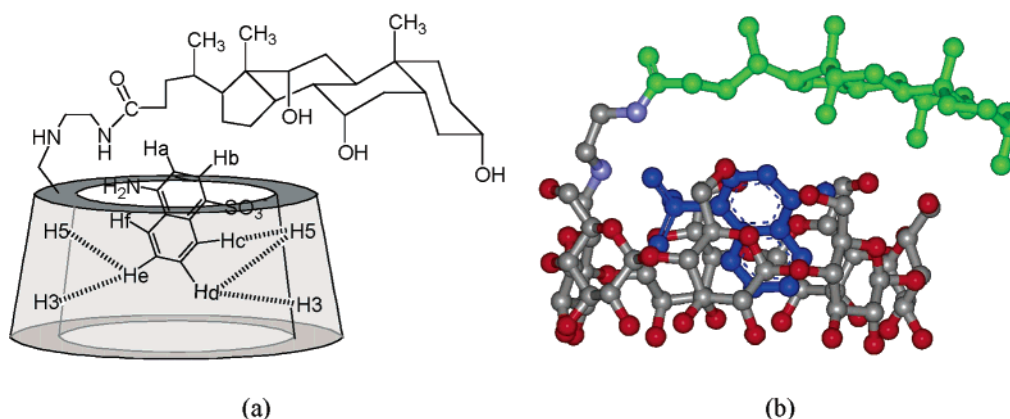
FIGURE 6. (a) Possible complex structure of **1** with 1,4-SNS based on the ^{1}H ROESY NMR experiment and (b) the optimized structure of **1**/1,4-SNS complex with ball-and-stick representation based on the molecular modeling study. Blue: 1,4-SNS molecule.

basis of the results of the 2D NMR experiment and the molecular modeling study, the possible conformation and optimized structure of host **1** with 2,6-DNS are shown in Figure 7, parts b and c, respectively.

The ROESY spectrum (Figure 8a) of **1** (2.1×10^{-3} mol dm^{-3}) with 1,5-DNS (2.1×10^{-3} mol dm^{-3}) also showed clear NOE cross-peaks between the H3 protons of β-CD in **1** and the Ha (peak A) and Hc (peak B) protons of the 1,5-DNS molecule and between the H3/H5 of β-CD and the protons (peaks C−G) of the cholic acid moiety, which indicated that there was an inclusion equilibrium between the cholic acid moiety and the 1,5-DNS molecule at the cavity of β-CD. According to the 2D NMR experimental results, a possible conformation of host **1** with 1,5-DNS was shown in Figure 8b. Because the sulfonate groups in the α position of 1,5-DNS could prevent 1,5-DNS from including effectively into the β-CD cavity,[19] the 1,5-DNS with host **1** gave a shallow inclusion mode with low binding affinity ($K_S = 4170$). This result indicated that the strict size/shape fitting relationship between the 1,5-DNS and β-CD's cavity in host **1** played a key role rather than the induced-fit inclusion mode.

Furthermore, it was found that there was an inclusion equilibrium between the deoxycholic acid moiety and 1,4-SNS (or 2,6-DNS and 1,5-DNS) at the β-CD's cavity of the host **2** by carefully determining the ROESY spectra of host **2** with guests. Among them, the ROESY spectrum (in the Supporting Information) of **2** (2.3×10^{-3} mol dm^{-3}) with 1,4-SNS (2.3×10^{-3} mol dm^{-3}) gave clear NOE cross-peaks between the H3 protons of β-CD in **2** and the Ha (peak A), Hb (peak B), and Hc (peak C) protons of the 1,4-SNS molecule and between the H3/H5 of β-CD and the protons (peaks E−I) of the deoxycholic acid moiety. Notably, the Hf proton of the 1,4-SNS molecule and the A ring protons of the deoxycholic acid moiety also showed a clear NOE cross-peak (peak D). The phenomenon was also presented in the ROESY spectrum (Figure 9a) of host **2** (2.2×10^{-3} mol dm^{-3}) with 2,6-DNS (2.3×10^{-3} mol dm^{-3}). Besides the NOE cross-peaks between the H3/H5 protons of β-CD in **2** and the Ha (peaks A) and Hb (peaks B) of 2,6-DNS, between the H5 protons of β-CD and the Hc (peaks C) of 2,6-DNS, and between the H3/H5 of β-CD and the protons (peaks H) of the deoxycholic acid moiety in the ROESY spectrum, the NOE cross-peaks (peaks D−G) between the Ha and Hb protons of the 1,4-SNS molecule and the A or B ring protons of the deoxycholic acid moiety could be observed clearly. These results indicated that the conformation of host **2** could be

switched from the original intermolecular inclusion complex to the self-inclusion complex by the induced-fit interaction between the host **2** and 2,6-DNS or 1,4-SNS, which gave higher binding affinity ($K_S = 59\,800$ for 2,6-DNS, $K_S = 117\,000$ for 1,4-SNS). In the case of **2** (2.1×10^{-3} mol dm^{-3}) with 1,5-DNS (2.2×10^{-3} mol dm^{-3}), the ROESY spectrum (in the Supporting Information) only showed the NOE cross-peaks between the H3 protons of β-CD in **2** and the Hb (peak A) protons of the 1,5-DNS molecule and between the H3/H5 of β-CD and the protons (peaks B−G) of the deoxycholic acid moiety. The phenomenon was similar with that of host **1** with 1,5-DNS, i.e., the strict size/shape fitting relationship between the β-CD cavity in host **2** and 1,5-DNS played a key role rather than the induced-fit inclusion mode, giving a shallow inclusion mode with lower binding affinity ($K_S = 7600$). Therefore, the results of ^{1}H ROESY NMR experiments and the molecular modeling study demonstrated jointly that the different inclusion conformations of modified β-CDs **1** and **2**, induced-fit, and strict size/shape fitting relationship between the hosts and guests could reasonably explain the different binding affinities and organic anion selectivity of hosts **1** and **2** toward the naphthalenesulfonate guests, which might find the potential application for designing the guest-controlled switch in the molecular or anion recognition.

Binding Ability and Organic Anion Recognition. Much research indicated that the host−guest size/shape matching and induced-fit interaction could dominate the stability of the complex formed between modified β-CDs and model substrates, leading to the stronger van der Waals and hydrophobic interactions.[50] A large guest could not be included at the β-CD cavity, and a small guest could not give stronger van der Waals and hydrophobic interactions between the host and guest, which could not result in a stable host−guest inclusion complex. According to the reported results, the naphthalene ring could match the β-CD cavity.[19] Therefore, we chose naphthalenesulfonate derivatives as organic anion guests to bind with modified β-CDs **1** and **2**.

When the hydrophobic group was substituted at the 6-position of β-CD, the modified β-CD could usually give higher binding ability for the model substrates as compared with the parent

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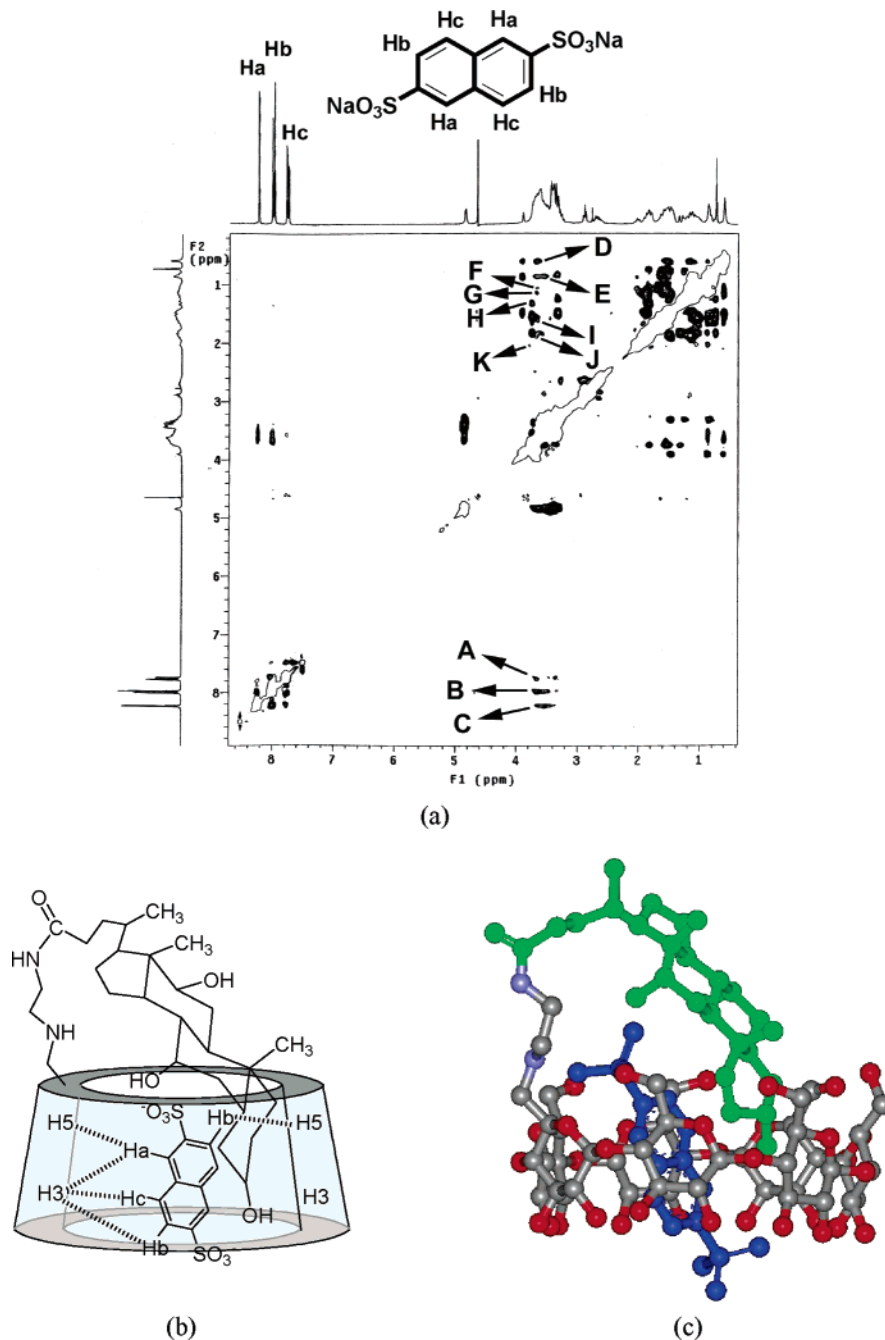
FIGURE 7. (a) ROESY spectrum of **1** (2.0 × 10⁻³ mol dm⁻³) with 2,6-DNS (2.6 × 10⁻³ mol dm⁻³) in D₂O at 25.0 °C with a mixing time of 300 ms, (b) possible complex structure of **1** with 2,6-DNS based on the ¹H ROESY NMR experiment, and (c) the optimized structure of the **1**/2,6-DNS complex with ball-and-stick representation based on the molecular modeling study. Blue: 2,6-DNS molecule.

β-CD.[51] In addition, the naphthalenesulfonate derivatives with anion sulfonate groups could participate in the formation of electrostatic interaction between the sulfonate groups and ethylenediamine moieties of hosts **1** and **2** during the molecular binding process, which might extend the binding ability of the hosts toward guests. As could be seen from Table 1, modified β-CDs **1** and **2** with 1,4-SNS, 1-SN, 2,7-DNS, and 2,6-DNS guests gave higher complex formation constants (K_S) as compared with that of β-CD, indicating that the introduction of cholic acid or deoxycholic acid could enhance the binding

ability and molecular selectivity of β-CD upon the inclusion complexation with naphthalenesulfonate derivatives. Among them, the **2**/β-CD ($K_S^2/K_S^{\beta-CD}$) pair afforded the highest molecular selectivity up to 2340 for 1,4-SNS guests.

The complex formation constants (K_S) for the inclusion complexation of hosts **1** and **2** with naphthalenesulfonate derivatives decreased in the following order:

1: 2,6-DNS > 1,4-SNS > 2,7-DNS > 1,5-SNS >
1-SN > 1,3,6-TNS > 1,5-DNS

2: 1,4-SNS > 2,6-DNS > 1,3,6-TNS > 1-SN >
1,5-DNS > 1,5-SNS > 2,7-DNS

(51) Wang, Y.-H.; Zhang, H.-M.; Liu, L.; Liang, Z.-X.; Guo, Q.-X.; Tung, C.-H.; Inoue, Y.; Liu, Y.-C. *J. Org. Chem.* **2002**, *67*, 2429−2434.
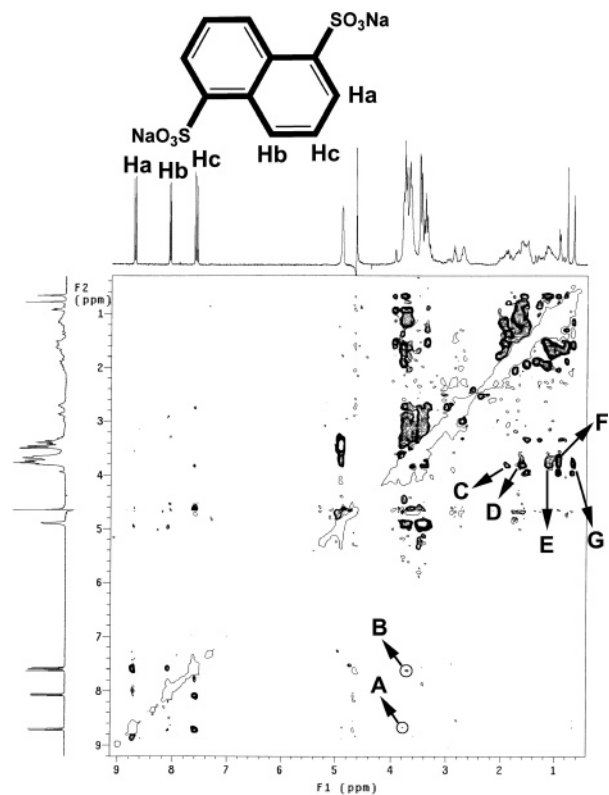
FIGURE 8. (a) ROESY spectrum of **1** (2.1×10^{-3} mol dm^{-3}) with 1,5-DNS (2.1×10^{-3} mol dm^{-3}) in D$_2$O at 25.0 °C with a mixing time of 300 ms and (b) possible complex structure of **1** with 1,5-DNS.

It could be seen that the hosts **1** and **2** afforded the higher complex formation constants for the inclusion complexation with 1,4-SNS or 2,6-DNS. According to the results of 2D NMR experiments and the molecular modeling study, the 1,4-SNS guest in the cavity located near the primary hydroxyl side of β-CD, and the substitute group of the host was excluded from the β-CD's cavity, which would make the hydrogen bonding interaction between the amino group of the 1,4-SNS guest and the hydroxyl groups of the primary side of β-CD possible.[43a] The naphthalene ring moiety of 2,6-DNS guests could be longitudinally included at the β-CD cavity by the strict size/shape fitting relationship between the host and guest, resulting in the stronger van der Waals and hydrophobic interactions. Furthermore, the host **1** gave the largest molecular selectivity for the 2,6-DNS/1,5-DNS pair ($K_S^{2,6-\text{DNS}}/K_S^{1,5-\text{DNS}} = 24.9$). As
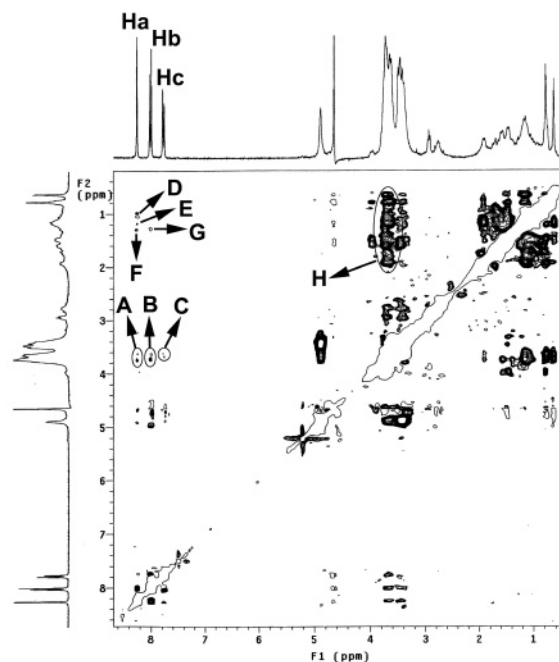
FIGURE 9. (a) ROESY spectrum of **2** (2.2×10^{-3} mol dm^{-3}) with 2,6-DNS (2.3×10^{-3} mol dm^{-3}) in D$_2$O at 25.0 °C with a mixing time of 300 ms and (b) possible complex structure of **2** with 2,6-DNS.

compared with 2,6-DNS, the sulfonate groups in the α position of 1,5-DNS could prevent 1,5-DNS from being included effectively into the cavity, which gave the lowest complex formation constant.[19] Similar cases also occurred at the inclusion complexation of hosts **1** and **2** with 2,7-DNS and 1,3,6-TNS guests, which afforded moderate complex formation constants. Therefore, van der Waals and hydrophobic interactions could dominate the stability of the complex formed between substituted β-CDs and model substrates, because these interactions were closely related to the distance and contacting surface area between the host and the guest.

On the other hand, the host **1** possessed a hydroxyl group at C7 of the cholic acid moiety as compared with host **2** with the deoxycholic acid moiety, adapting to their different molecular binding behavior for naphthalenesulfonate derivatives. From the data listed in Table 1, the **1/2** (K_S^1/K_S^2) pair gave the highest molecular selectivity up to 4.8 for 2,7-DNS guests.

Conclusions

In summary, two modified β-CD derivatives **1** and **2** were synthesized by the condensation reaction of mono(6-aminoet-

hylamino-6-deoxy)-β-CD with cholic acid and deoxycholic acid, respectively, and their original conformations and anion recognition behavior toward representative naphthalenesulfonate derivatives were investigated by using spectroscopic techniques and the molecular modeling method. The results obtained indicated that the hosts **1** and **2** could give intramolecular or intermolecular inclusion modes in aqueous solution. The diversity of the inclusion modes of **1** and **2** could lead to difference complex formation constants between the hosts and guests, which enhanced the recognition ability and molecular selectivity of β-CD. Furthermore, the size/shape matching, electrostatic, and induced-fit mechanisms played a crucial role in the anion recognition process of the modified β-CDs **1** and **2** with the anion guests. Therefore, the cholic acid or deoxycholic acid modified β-CD could act as an anion recognition probe to dominate the molecular and size/shape recognition of guests.

Experimental Section

Materials. β-CD of reagent grade 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), cholic acid, and deoxycholic acid were commercially available and used without further purification. All naphthalenesulfonate derivatives, i.e., sodium 1-naphthylamino-5-sulfonate (1,5-SNS), sodium 1-naphthylamino-4-sulfonate (1,4-SNS), sodium 1-naphthalenesulfonate (1-SN), disodium 1,5-naphthalenedisulfonate (1,5-DNS), disodium 2,7-naphthalenedisulfonate (2,7-DNS), disodium 2,6-naphthalenedisulfonate (2,6-DNS), and trisodium 1,3,6-naphthalenetrisulfonate (1,3,6-TNS), were commercially available and were used as received. Mono[6-*O*-(*p*-toluenesulfonyl)]-β-cyclodextrin (6-OTs-β-CD) was prepared by the reaction of *p*-toluenesulfonyl chloride with β-CD in alkaline aqueous solution.[38] Then, 6-OTs-β-CD was converted to mono(6-aminoethylamino-6-deoxy)-β-CD in 70% yield on heating in excess ethylenediamine at 70 °C for 7 h.[16,52]

Instruments. Fluorescence spectra were recorded in a conventional quartz cell (10 × 10 × 40 mm³) at 25.0 ± 0.1 °C on a fluorescence spectrometer with the excitation and emission slits of 5 nm width. Elemental analysis was performed on a standard instrument. ¹H NMR spectra were performed in D$_2$O on a standard spectrometer.

Mono[6-cholaminoethyleneamino-6-deoxy]-β-cyclodextrin (1).[41] Modified β-CD **1** was prepared in 25% yield from cholic acid and mono(6-aminoethylamino-6-deoxy)-β-CD, according to the reported results.

Mono[6-deoxycholaminoethyleneamino-6-deoxy]-β-cyclodextrin (2). To a solution of DMF (30 mL) containing 1.2 g of mono-(6-aminoethylamino-6-deoxy)-β-CD and 0.26 g of DCC was added 0.46 g of deoxycholic 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 30% yield. ¹H NMR (D$_2$O, TMS, ppm) δ 0.6–2.0 (m, 35H), 2.6–2.9 (m, 4H), 3.3–4.0 (m, 44H), 4.9–5.0 (d, 7H). ¹³C NMR (D$_2$O, TMS, ppm) δ 172.5, 102.6, 102.2, 83.5, 81.8, 73.3, 72.2, 71.2, 70.4, 67.8, 60.2, 57.3, 55.9, 48.6, 47.4, 46.5, 43.1, 41.2, 40.4, 38.4, 35.7, 35.5, 34.9, 34.5, 33.9, 32.7, 29.6, 28.2, 24.2, 22.9, 20.4, 19.4, 12.9, 12.3. Anal. Calcd for C$_{68}$H$_{114}$O$_{37}$N$_2$·13H$_2$O: C 45.74, H 7.90, N 1.57. Found: C 45.72, H 7.50, N 1.88. MS (FAB) *m/z* 1551.63 (M$^+$ + H − 13H$_2$O).

Computational Methods. The initial geometry of β-CD was taken from the crystal structure.[53] The starting structures of the host **1**, **1**/1,4-SNS complex, and **1**/2,6-DNS complex were assembled by using the Builder module of the InsightII program and energy minimized with the Discover program.[54] All simulations were performed by using a CVFF force field.[55]

Supporting Information Available: ROESY spectra and possible complex structures of host **2** with 1,4-SNS and 1,5-DNS, respectivaly. This material is available free of charge via the Internet at http://pubs.acs.org.

JO060677Q

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