Fluorescence Sensing and Selective Binding of a Novel 4,4′-Sulfonyldianiline-Bridged Bis(β-cyclodextrin) for Bile Salts

by **Yan Zhao***[a]), **Yong Cun Yang**[a]), **Shao Ming Chi**[a]), **Hao Shi**[a]), **Yong Zhao**[a]), **Hong You Zhu**[b]), **Qi Lin Li**[a]), and **Yu Fei Wang**[a])

[a]) College of Chemistry and Chemical Engineering, Yunnan Normal University, Kunming 650092, P. R. China (phone/fax: +86-871-5516061; e-mail: zhaooyann@163.com)

[b]) School of Chemical Science and Technology, Yunnan University, Kunming 650091, P. R. China

A novel 4,4′-sulfonyldianiline-bridged bis(β-cyclodextrin (CD)) **2** was synthesized, and its complex stability constants (K_s) for the 1:1 inclusion complexation with bile salts, *i.e.*, cholate (CA), deoxycholate (DCA), glycocholate (GCA), and taurocholate (TCA) have been determined in phosphate buffer (pH 7.2) at 25° by fluorescence spectroscopy. The result indicated that **2** can act as efficient fluorescent sensor and display remarkable fluorescence enhancement upon addition of optically inert bile salts. Structures of the inclusion complexes between bile salts and **2** were elucidated by 2D-NMR experiments, indicating that the anionic tail group and the *D* ring of bile salts penetrate into one CD cavity of **2** from the wide opening deeply, while the phenyl moiety of the CD linker is partially self-included in the other CD cavity to form a host–linker–guest binding mode. As compared with native β-CD **1** upon complexation with bile salts, bis(β-CD) **2** enhances the binding ability and molecular selectivity. Typically, **2** gives the highest K_s value of 26200 M^{-1} for the complexation with CA, which may be ascribed to the simultaneous contributions of hydrophobic, H-bond, and electrostatic interactions. These phenomena are discussed from the viewpoints of multiple recognition and induce-fit interactions between host and guest.

1. Introduction. – Cyclodextrins (CDs) are cyclic oligosaccharides, involving six or more D-glucopyranose units, which form truncated cone-shaped molecules with a hydrophobic cavity. They form inclusion complexes with a variety of organic compounds in aqueous solution and are largely studied for their host–guest interaction properties, and as building blocks for supramolecular structures [1]. It is well known that molecular recognition by modified CD-based chemosensors is a significant topic in supramolecular chemistry [2]. Introduction of a fluorophore sidearm to a CD host not only alters the original binding ability and selectivity, but also provides us with a spectral probe for investigating the inclusion complexation behavior with some optically silent guests, as the sidearms originally accommodated in the CD cavity suffers substantial conformation changes upon guest inclusion, accompanying appreciable spectral changes [3–6]. Among the various CD derivatives, bridged bis(β-CD)s have been known to significantly enhance the binding ability and molecular selectivity for specific guests in comparison with native CD through the cooperative binding of one guest with two hydrophobic cavities located in a close vicinity [7–10]. However, works on the molecular recognition of bis(β-CD)s with fluorescent linkers are concentrated mostly on the inclusion complexation of rather simple organic guests and amino acids [11–14], and less attempts have been made on the recognition of optically

inert bile salts [15][16], despite their importance in understanding the chiral and multiple recognition mechanisms of CDs. Bile salts, on the other hand, are involved in one of the most important pathways for the metabolism and excretion of cholesterol in mammals and represent an example of the liver capacity to convert lipid-soluble material into excretable H_2O-soluble products [17]. Because of their amphipatic nature, they behave as biosurfactants and are used as drugs in gallstone disease treatments [18]. In this article, we report the synthesis of bis(β-CD) **2** possessing a fluorescent 4,4′-sulfonyldianiline linker and its selective binding behavior with a series of structurally related bile salt guests (*Fig. 1*) by fluorescence and 2D-NMR spectroscopy. The reason for choosing the 4,4′-sulfonyldianiline group as a linker is that the fluorescence intensity of the phenyl moiety is sensitive to the microenvironment change. Moreover, 4,4′-sulfonyldianiline was reported to be used as a drug to treat leprosy or tuberculosis [19]. We believe the novel bis(β-CD) **2** will exhibit some interesting properties. It is of special interest to compare the binding abilities of β-CD **1** and bis(β-CD) **2** with bile salts, and to discuss the molecular multiple recognition of bis(β-CD) **2** through a fluorescence-sensing mechanism. This approach could serve to further understanding of this developing but little-investigated area in the field of CD chemistry.

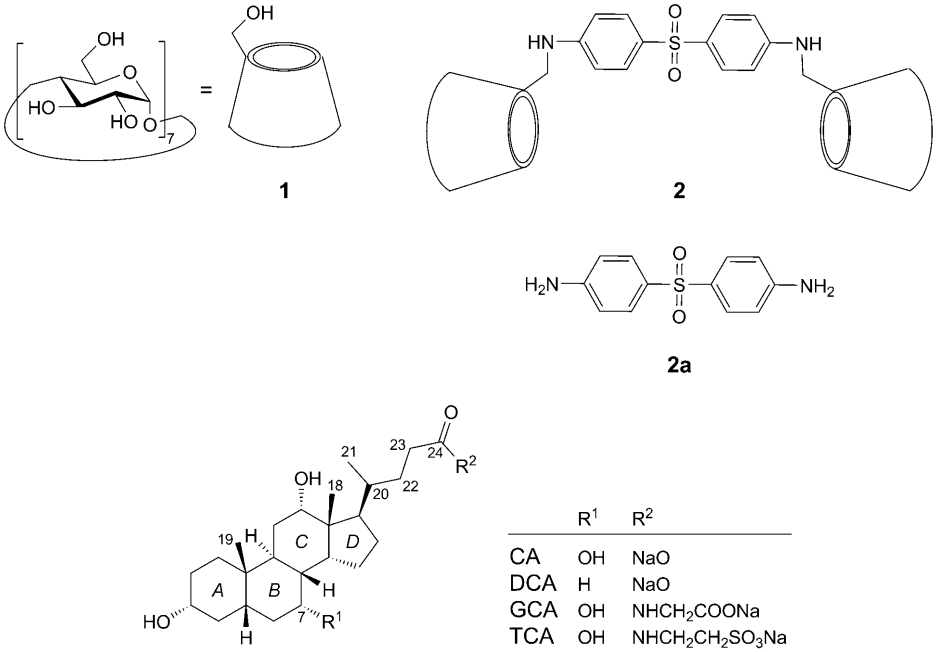
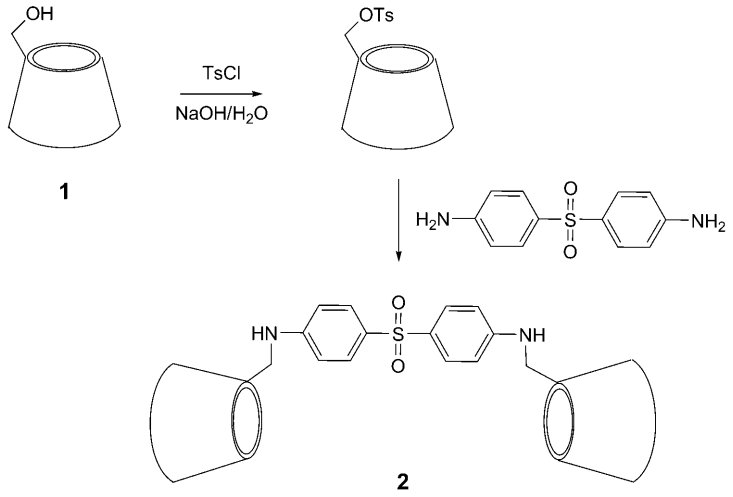


Fig. 1. *A series of structurally related bile salt guests*

2. Results and Discussion. – 2.1. *Synthesis.* As illustrated in the *Scheme*, 4,4′-sulfonyldianiline-bridged bis(β-CD) **2** was synthesized in a yield of 15% by the reaction of 4,4′-sulfonyldianiline with mono[6-*O*-(*p*-toluenesulfonyl)]-β-CD.

Scheme



2.2. *Original Conformation of Bridged Bis(β-CD)* **2**. 2D-NMR Spectroscopy has recently become an important method for the investigation of not only the interaction between host CDs and guest molecules, but also of the self-included mode between the CD cavity and its substituent groups, since the NOE cross-peaks between the H-atoms that are closer than 0.4 nm in space will be observed in the NOESY or ROESY spectrum, and the relative intensities of these cross-peaks depend on the space between the corresponding H-atoms. Therefore, it is possible to estimate the orientation of the linker moiety in the CD cavity using the assigned NOE correlations. It is well-known that only cross-peak interactions with H3, H5, and H6 of CDs are considered to analyze the results, because H2 and H4 are not facing the inner cavity, and H1 is affected by D_2O. If the linker moiety is self-included in the CD cavity, the NOE correlations between the H-atoms of the linker moiety and the H-atoms H3/H5/H6 of the CD should be observed. To obtain further evidence about the original conformation of **2**, 2D-NMR spectroscopy experiments were performed in D_2O solution. As shown in *Fig. 2*, the ROESY spectrum of bis(β-CD) **2** displays clear NOE cross-peaks between the H5/H6 of CD cavity and H_a of the aromatic H-atoms (peak *A*), as well as between the H5/H6 and the H_b H-atoms (peak *B*). Since the H5/H6 H-atoms are located near to the narrow opening of the CD cavity, while the H3 H-atoms are near to the wide opening, we can conclude that the phenyl moiety of the CD linker is not entirely but partially self-included into the hydrophobic cavity from the narrow opening. The results of the ROESY experiment may serve to establish a correlation between the initial conformations of host **2** and its molecular recognition ability.

2.3. *Spectral Titration.* Many investigations have demonstrated that a chromogenic group originally accommodated in the CD cavity may suffer substantial conformational change upon guest inclusion and result in the relevant spectral changes [20]. This property allows the fluorescent-labeled CD to function as a spectral probe to obtain binding constants in differential fluorescence spectrometry. As can be seen from *Fig. 3*,

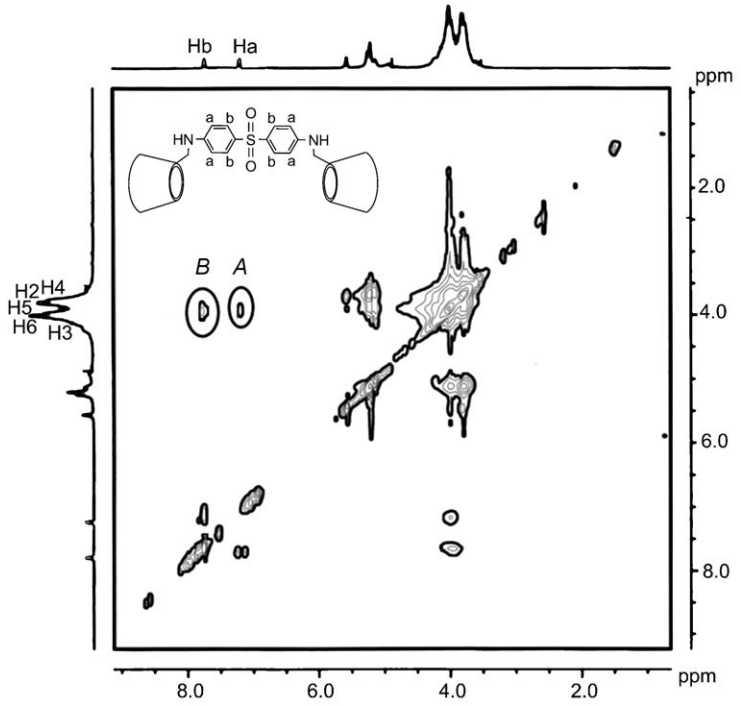


Fig. 2. *ROESY Spectrum of bis(β-CD)* **2** (5.0×10^{-3} M) *in* D_2O *at 25° with a mixing time of 400 ms*

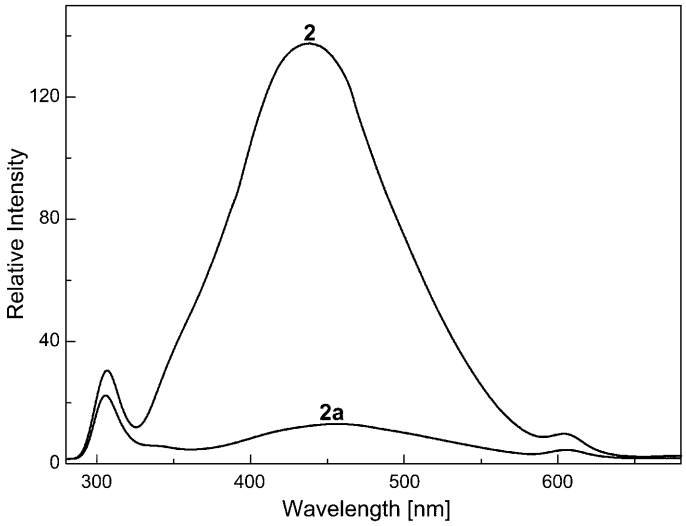


Fig. 3. *Fluorescence spectra of* **2a** *and bis(β-CD)* **2** *at the concentration of* 8.0×10^{-5} M *in phosphate buffer* (pH 7.2) *at 25°* ($\lambda_{ex} = 310$ nm)

the fluorescence intensity of bis(β-CD) **2** is much larger than that of reference compound **2a** under identical conditions. Moreover, the maximum emission wavelength of **2** shows obviously blue shift compared with **2a**. Since the fluorescence intensity of the phenyl moiety is sensitive to microenvironmental changes, being stronger in a hydrophobic microenvironment than in a hydrophilic one, the above results suggest that a phenyl moiety of bis(β-CD) **2** was included into the hydrophobic cavity, leading to a more effective shielding of the fluorophore from a deactivating H_2O attack. The result is in good agreement with the 2D-NMR experiment described above.

For a qualitative assessment of the inclusion complexation behavior of bis(β-CD) **2**, spectral titrations of host **2** with CA, DCA, GCA, and TCA were performed at 25° in phosphate buffer (pH 7.2) by fluorescence spectroscopy. *Fig. 4*,*a* and *b*, shows the typical spectral changes of host **2** upon gradual addition of CA and TCA, respectively. As can be seen, the relative fluorescence intensities of **2** present a continuous enhancement upon the addition of CA and TCA, which are distinct from most cases of CD appended with fluorescent groups [21][22]. In general, most of the fluorescent-labeled CDs show a decrease in fluorescence intensity upon addition of guest molecules resulting from the fact that the location of the chromophore is transferred from inside to outside of the cavity of CD. Some chromophore-modified γ-CDs are found to show an increase in fluorescence intensity in contrast to the behavior of the most cases. This phenomenon may be due to the large cavity of γ-CD that possesses enough space to accommodate the modified chromophore and the guest together [23]. The enhancement of the fluorescence intensity of **2** upon addition of bile salts may be phenyl fluorophore arising from cooperative guest–linker–host interactions. This hypothesis is supported by the 2D-NMR measurements. The unique fluorescence behavior may enable **2** to be an efficient fluorescence sensor for optically inserted molecules.

The stoichiometry for the inclusion complexation of bis(β-CD) **2** with bile salts was determined by the 'continuous variation' method. As shown in *Fig. 5*, the plot maximum point appears at a bis(β-CD) **2**'s molar fraction of 0.5, which obviously indicates that a 1:1 inclusion complex is formed between bis(β-CD) **2** and the CA guest. The same results were obtained in the other cases of inclusion complexation of bis(β-CD) **2** with bile salts.

With the 1:1 stoichiometry for the inclusion complexation of bile salts as guests (G) with CDs (H), where the two CD moieties in bis(β-CD)s are treated as a single unit, the inclusion complexation is expressed by *Eqn. 1*, and the complex stability constant (K_s) is given by *Eqn. 2*.

$$H + G \overset{K_s}{\rightleftharpoons} G \cdot H \tag{1}$$

$$K_s = \frac{[H \cdot G]}{[H][G]} \tag{2}$$

$$\Delta F = \Delta\varepsilon[H \cdot G] \tag{3}$$

where ΔF and $\Delta\varepsilon$ denote the sequential changes of fluorescence intensity and the differential molar extinction coefficient of host β-CDs in the absence and presence of

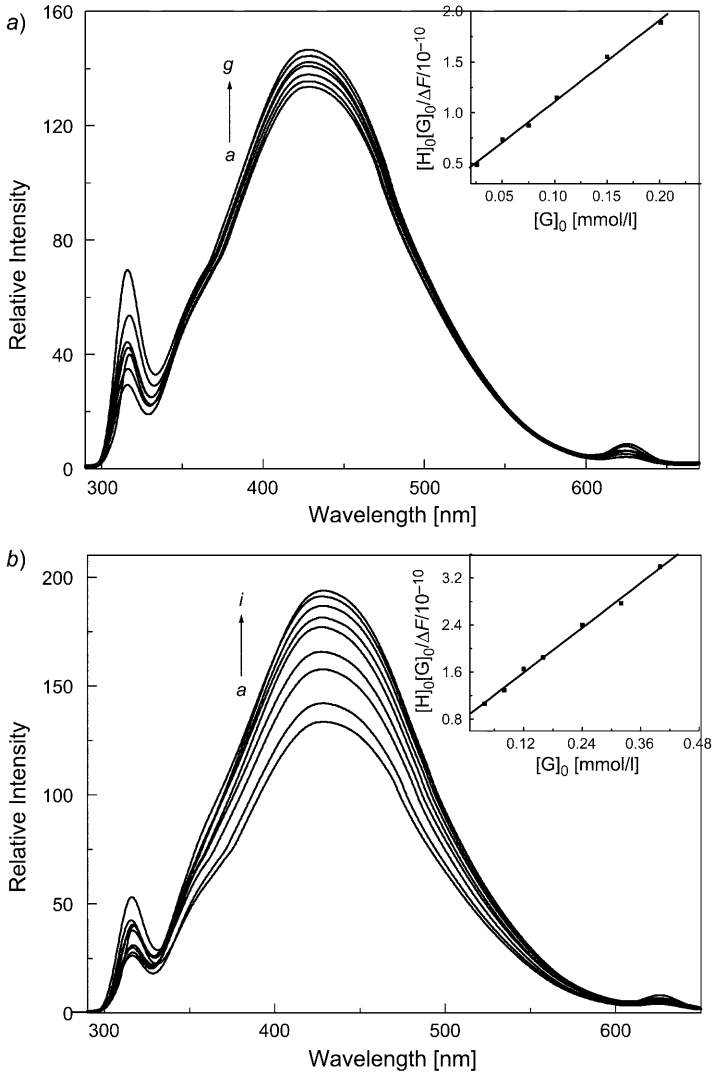


Fig. 4. *Fluorescence spectral changes of* a) *bis(β-CD)* **2** (5.3×10^{-6} M) *upon addition of CA* (from **a** to **g** = 0, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0×10^{-4} M), *and* b) *upon addition of TCA* (from **a** to **h** = 0, 0.4, 0.8, 1.2, 1.6, 2.4, 3.2, 4.0×10^{-4} M) *in phosphate buffer* (pH 7.2) *at 25°* ($\lambda_{ex} = 310$ nm). Insets: typical plots of $[H]_0[G]_0/\Delta F$ *vs.* $[G]_0$ for the inclusion complexation of bis(β-CD) **2** with CA and with TCA.

guest molecule. Under the conditions employed, the initial concentration of guest molecules is much larger than that of the host β-CD, *i.e.*, $[G]_0 \gg [H]_0$. Therefore, the combination of *Eqns. 2* and *3* leads to the extended *Benesi–Hildebrand* equation (*Eqn. 4*) [24], which is used to calculate the K_s (*Eqn. 2*) from the slope and intercept of $[H]_0[G]_0/\Delta F$ *vs.* $[G]_0$ plots.

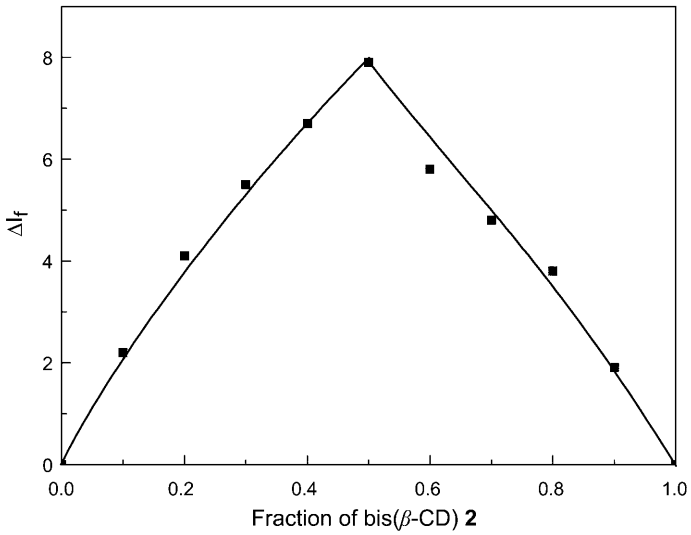


Fig. 5. *Continuous variation plot of the complexation of* **2** *with CA in phosphate buffer* (pH 7.2) *at 25°* ($[\mathbf{2}]+[CA]=2.0\times10^{-5}$ M) *produced with data taken from fluorescence spectra* ($\lambda_{ex}=310$ nm)

$$\frac{[H]_0[G]_0}{\Delta F}=\left(\frac{1}{K_s\Delta\varepsilon}\right)+\frac{[G]_0}{\Delta\varepsilon} \tag{4}$$

Fig. 4 (insets) illustrates the result of such a treatment for the inclusion complexation of bis(β-CD) **2** with CA and TCA, where the calculated $[H]_0[G]_0/\Delta F$ values were plotted against the $[G]_0$ values, generating an excellent linear curve. The complex stability constants (K_s) and the free energy changes ($-\Delta G^\circ$) calculated from the slope and intercept are listed in the *Table.*

Table. *Complex Stability Constant* (K_s) *and* Gibbs *Free Energy Change* ($-\Delta G^\circ$) *for the 1:1 Inclusion Complexation of Bile Salts with Native β-CD* **1** *and Bis(β-CD)* **2** *in Phosphate Buffer* (pH 7.2) *at 25°*

Host	Guest	K_s [M^{-1}]	Log K_s	$-\Delta G^\circ$ [kJ/mol]
1	CA	4068 ± 84 [25]	3.6	20.6
	DCA	4844 ± 16 [25]	3.7	21.0
	GCA	2394 ± 69 [25]	3.4	19.3
	TCA	2293 ± 13 [25]	3.4	19.2
2	CA	26200 ± 400	4.4	25.2
	DCA	10140 ± 200	4.0	22.9
	GCA	3150 ± 10	3.5	20.0
	TCA	7730 ± 40	3.9	22.2

2.4. *Binding Mode.* Previous investigations revealed that different binding modes are possible for the inclusion complexation of bile salts with CDs [26][27]. It should be noted that both CDs and bile salts possess two sides, that is, the primary and secondary OH side for CDs, as well as the *A* ring side and the carboxylate and sulfonate (tail)

group side for bile salts. The *A* ring or anionic tail group of bile salt can enter the CD cavity from either the primary or secondary side, which will result in dramatically different binding modes between host and guest. Such uncertain penetrating model and binding sites of guests may greatly affect the conformation and binding pattern of the resulting complex of bile salts with CDs, giving different binding constants. Therefore, it is very important to investigate the binding modes between bis(β-CD) **2** and bile salts for elucidation of the mechanism of molecular recognition. A typical ROESY spectrum of the bis(β-CD) **2** and CA in D_2O is shown in *Fig. 6,a*. The notations used are H*n* for CD H-atoms and P*n* for bile salt H-atoms, where *n* is the C-atom number in CD and bile salts. As shown in *Fig. 6,a*, the ROESY spectrum for the resulting CA/**2** complex in D_2O displays a complicated NOE cross-peak pattern, which does not only originate from the intermolecular correlations between the bis(β-CD) **2** and the bile salt, but also from the intramolecular correlations of **2** or CA. Among them, the cross-peak *C* represents the correlation of the signals of CA's P18 H-atoms and CD's H3 H-atoms, and the cross-peak *D* shows the correlation of the signals of CA's side-chain H-atoms (P21) and CD's H3/H5/H6 H-atoms. Meanwhile, the cross-peaks *E* and *F* exhibit the correlations between CA's *D* ring H-atoms (P14 to P22) and CD's H3 H-atoms. In addition, the significant correlation signals are found between CA's side-chain H-atoms and steroid body (P23 with P16, P22 with P16), but no NOE correlations between CA's P19 H-atoms and CD's H3/H5/H6 H-atoms can be observed. From the above information, we can deduce that the *D* ring of CA is wholly included in the CD cavity from the wide opening, while the side chain is located near the narrow opening of the CD cavity and folded toward the steroid body. On the other hand, the obvious cross-peaks *A* and *B* originate from the correlation signals between the phenyl H-atoms in the linker group and the H5/H6 H-atoms of CD, indicating that the phenyl moiety is not driven out of the CD cavity even after the guest inclusion. These correlation signals, along with the 1:1 binding stoichiometry, jointly indicate a host-linker-guest binding mode between the bis(β-CD) **2** and CA. That is, upon complexation with the bis(β-CD) **2**, the carboxylate tail and the *D* ring of CA penetrate into one CD cavity of **2** from the wide opening deeply, while the phenyl moiety of the CD linker is partially self-included in the other β-CD cavity (*Fig. 6,b*). Similar binding modes are also observed in other cases of bis(β-CD) **2**/bile salt complexes. According to this binding mode, several interactions will simultaneously be active between host and guest. Besides the *van der Waals* and hydrophobic interactions between CD cavity and guest bile salts, the OH group of CD and the amino fragments in the linker of bis(β-CD) **2** can form additional H-bond interactions with the carboxylate (or sulfonate) tail of bile salt. Moreover, under our experimental conditions, the carboxylate (or sulfonate) group in the side-chain of the bile salt is not protonated and should exist as a carboxylate (or sulfonate) anion, and the $-NH-$ fragments in the linker group of bis(β-CD) **2** 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 the bile salt may to some extent favor the inclusion complexations of **2** with bile salts.

The binding mode consequently rationalizes the enhanced fluorescence of **2** in the presence of the guest bile salts. Firstly, the phenyl chromophore may be efficiently protected from the deactivating H_2O attack by steric shielding by the closely located bulk bile salt guests. In addition, the introduction of a hydrophobic bile salt guest

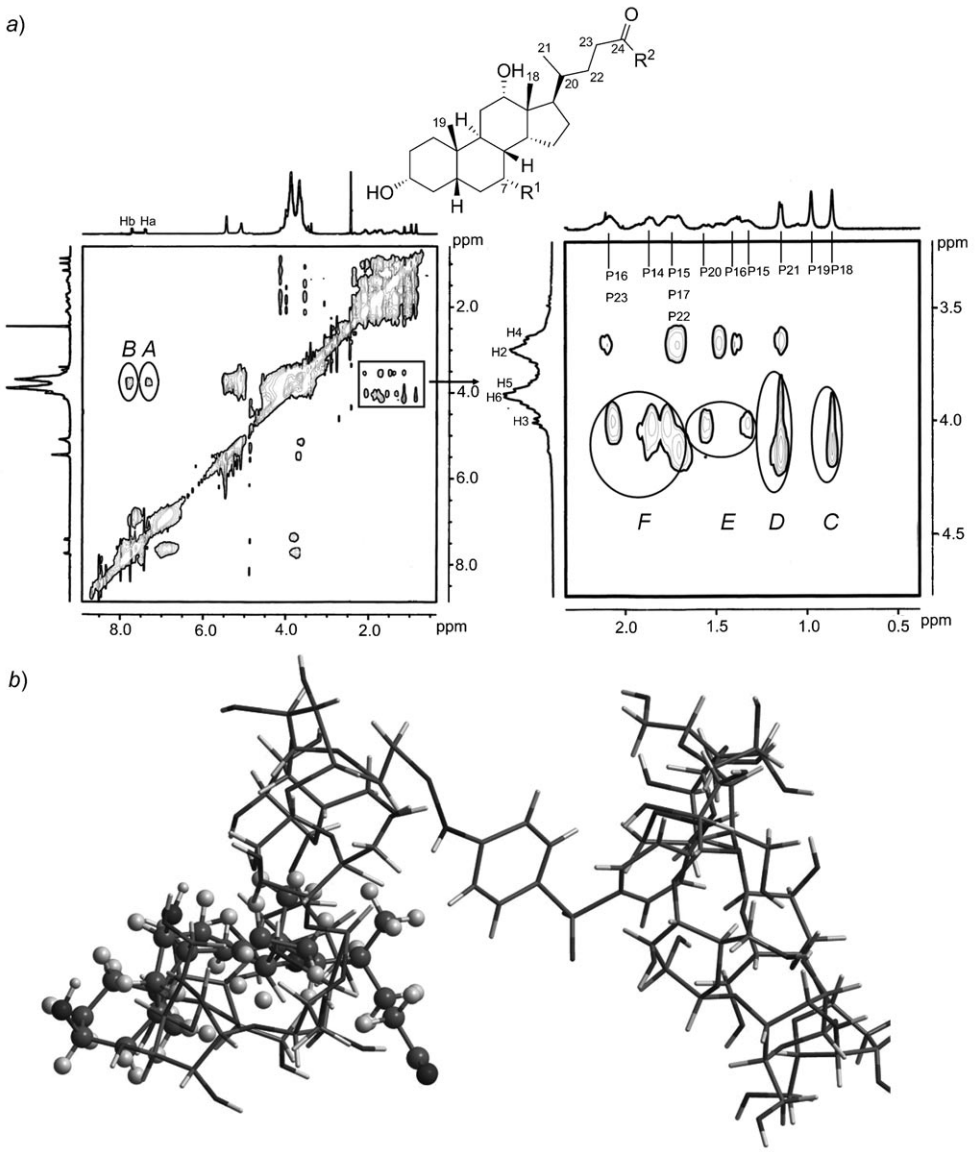


Fig. 6. a) *ROESY Spectrum of* **2** *with CA* (5.0×10^{-3} M each) *in* D_2O *at 25° with a mixing time of 400 ms,* b) *possible binding mode of* **2** *with CA*

should certainly increase the microenviromental hydrophobicity around the fluorophore, which consequently leads to enhanced fluorescence of bis(β-CD) **2**. As a joint result of these two factors, **2** shows enhanced fluorescence upon inclusion complexation with bile salts.

2.5. *Binding Ability and Molecular Selectivity toward Bile Salts.* It has been demonstrated that several possible weak interactions, such as *Van der Waals*, hydrophobic interactions, H-bond, as well as electrostatic interactions, simultaneously contribute to the inclusion complexation behavior of CDs and most of these interactions depend on the size/shape-fit relationship of host and guest. From the *Table*, we can see that the binding constants of bis(β-CD) **2** with bile salts are larger than those of native β-CD **1**. The K_s values for the inclusion complexation of bis(β-CD) **2** are enhanced by factors of 6.4 with CA, 2.1 with DCA, 1.3 with GCA, 3.4 with TCA, respectively, as compared with those of β-CD **1**. Native β-CD **1** affords relatively limited binding constants probably due to the weak *Van der Waals* and the hydrophobic interactions. The enhanced binding abilities of bis(β-CD) **2** may be mainly attributed to the cooperative host-linker-guest binding mode between host and guest. In addition to the association of the CD cavity with a guest molecule, the linker group provides some additional binding interactions towards the accommodate guest. These factors jointly contribute to the stronger binding ability achieved by bis(β-CD) **2** in relation to the native β-CD **1**.

Bile salts have a characteristic structure, with a side chain at C17, Me groups at C10, C13, and C20, and a carboxylic (or sulfonate) derivative at C23 of the steroid skeleton (*Fig. 1*). The bile salts CA and DCA only show a small difference in the structure of the C7 substituent (R^1), that is, a OH group for CA and a H-atom for DCA. However, this slight difference will lead to the great distinction in their hydrophobic nature and binding abilities with CDs. On the other hand, different from CA and DCA, guests GCA and TCA possess a more polar side chain (R^2), which will affect their binding abilities. As can be seen in the *Table*, the complex stability constants for hosts **1** and **2** with bile salts are variable according to the guest molecular structures. The K_s values for the complexation of each host with bile salt guests decreased in the following order:

1: DCA > CA > GCA > TCA

2: CA > DCA > TCA > GCA

Distinctly, the K value is significantly higher for DCA (4844 M^{-1}) binding to native β-CD **1** compared to CA (4068 M^{-1}), indicating that the cavity of native β-CD **1** can encapsulate strongly the DCA molecule. According to previous studies on the binding mode between β-CD and bile salts [18], we can deduce that the aliphatic side chain folded toward the steroid skeleton can be included into the cavity of β-CD from the secondary side. Therefore, possessing the more hydrophobic structure due to the absence of HO–C7 group as compared with CA, DCA is easier to bind into the β-CD cavity than CA, which should lead to the more favorable *van der Waals* interactions. However, different from native β-CD (**1**), bridged bis(β-CD) **2** reverses this binding selectivity, showing larger binding constants for CA ($K_s = 26200\ M^{-1}$) than DCA ($K_s = 10140\ M^{-1}$). One possible reason for the stronger affinity for CA may involve H-bond interactions between the HO–C7 group of CA and the HO–C2 and/or HO–C3 groups of CD. We have demonstrated that the carboxylate tail and the *D* ring of CA enter into the CD cavity through the wide opening. In this binding mode, the HO–C7 group of CA is located outside the CD cavity and close to the wide opening of CD, and

so can easily interact with the HO–C2 and HO–C3 groups of CD through H-bond interactions, which subsequently strengthen the host-guest association. Moreover, all the hosts, including native β-CD **1** and bridged bis(β-CD) **2**, show a weaker binding ability upon complexation with GCA and TCA than with CA and DCA. As can been seen from the *Table*, the highest binding constant towards GCA and TCA is 3150 and 7730 M^{-1} given by bis(β-CD) **2**, respectively. The universal decreased binding ability toward GCA and TCA must relate to the structure differences from CA and DCA. GCA and TCA possess the same steroid skeleton as CA, but GCA and TCA are the resulting compounds of the conjugation of chololic acid with glycine and taurine, respectively. Attributing to the more hydrophilic tail, which is attached to the end of the *D* ring, GCA and TCA are unfavorable to insert into the cavity from the second side of β-CD cavity with their *D* ring. Obviously, the hydrophobic interaction is the primary driving force for the inclusion complexation of bridged bis(β-CD) **2** and chosen bile salt guests. It is worthy to note that the binding ability of bis(β-CD) **2** is significantly larger for TCA, which possesses a longer chain and a highly polar anionic tail (SO_3^-), than for GCA, which is bearing a shorter chain and an anionic tail (CO_3^-), which leads to a relatively strong molecular selectivity of 2.5, while native β-CD **1** gives very similar binding constants for GCA (K_s 2394 M^{-1}) and TCA (K_s 2293 M^{-1}). Apparently, the higher molecular selectivity for the bile salts bearing the same skeleton not only depends on the structure of the host, but also on the length and polarity of the bile salt's side chain.

Hosts **1** and **2** show different guest selectivity upon binding with these four bile salts. For example, for native β-CD **1**, the best guest selectivity is 2.1 for DCA/TCA, but the situation is quite different for the bridged bis(β-CD)s due to the cooperative host-linker-guest binding mode and some additional binding interactions. As a result of this multiple recognition mechanism, bis(β-CD) **2** shows a higher molecular selectivity up to 8.3 for the CA/GCA and 3.4 for the CA/TCA pair, respectively.

3. Conclusions. – In the present study, a novel 4,4′-sulfonyldianiline-bridged bis(β-CD) **2** was synthesized. We have demonstrated that the novel bridged bis(β-CD) **2** can be used not only as efficient fluorescent sensor responsive to optically inert bile salt guests, but also as a convenient and powerful model of molecular receptors for enhancing their guest binding ability and selectivity. The guest-induced fluorescence enhancement of bis(β-CD) **2** opens a new channel for the design of materials. Furthermore, the host-linker-guest co-inclusion binding mode observed may share some similarities with biological molecular recognition involving the multicomponent, induced-fit receptor-substrate interactions.

Experimental Part

Instruments. UV-VIS Spectra: *Shimadzu UV2401 PC* spectrometer. FT-IR Spectra: *Bruker FL*-IR. Fluorescence spectra: measured in a conventional quartz cell ($10 \times 10 \times 45$ mm) at 25° on a *Hitachi F-4500* spectrometer equipped with a constant-temp. water bath, with the excitation and emission slits of 10 nm width at an excitation wavelength 310 nm. ^{1}H-NMR Spectra: *Bruker AV-DRX5* instrument operated at 500 MHz. Elemental analyses: *Elementar Vario EL III*.

Materials. All bile salt guests, *i.e.*, cholate (CA), deoxycholate (DCA), glycocholate (GCA), and taurocholate (TCA), were purchased from *Sigma* and used as received. Na_2HPO_4 and NaH_2PO_4 were

dissolved in dist., deionized H_2O to prepare a 0.1M phosphate buffer soln. of pH 7.2 for spectral measurements. β-CD of reagent grade (*Shanghai Reagent Works*) was recrystallized twice from H_2O and dried under vacuum at 95° for 24 h prior to use. *N,N*-Dimethylformamide (DMF) was dried over CaH_2 for 2 d and then distilled under reduced pressure. Mono[6-*O*-(*p*-toluenesulfonyl)]-β-cyclodextrin was prepared from β-cyclodextrin and *p*-toluenesulfonyl chloride in aq. alkaline soln. [28].

4,4′-Sulfonyldianiline-Bridged Bis(6-amino-6-deoxy-β-cyclodextrin) (**2**). As shown in the *Scheme*, 4,4′-sulfonyldianiline (1.49 mmol, 0.37 g) and mono[6-*O*-(*p*-toluenesulfonyl)]-β-cyclodextrin (3.1 mmol, 4.0 g) were dissolved in anh. DMF (30 ml), and the mixture was stirred at 95–100° under N_2 atmosphere for 4 d, followed by evaporation under reduced pressure to dryness. The residue was dissolved in a small amount of H_2O, and the resultant soln. was poured into acetone with vigorous stirring to obtain a yellow precipitate. The crude product was collected by filtration and chromatographed on a *Sephadex G-25* column with H_2O as eluent to give pure compound **2** (0.56 g, yield 15%). UV/VIS (H_2O): 259 (3641), 294 (4008). FT-IR (KBr): 3406, 2927, 1640, 1598, 1414, 1155, 1080, 1027, 939, 848, 758. ^{1}H-NMR (D_2O): 3.33–3.95 (*m*, 84 H, H2, H3, H4, H5, H6 of CD); 4.92–5.08 (*m*, 14 H, H1 of CD); 7.21 (*d*, $J = 7.80$, 4 arom. H); 7.68 (*d*, $J = 7.80$, 4 arom. H). FAB-MS: 2505 ($[M + Na]^+$). Anal. calc. for $C_{96}H_{148}O_{70}N_2S \cdot 8\ H_2O$: C 43.90, H 6.29, N 1.07, S 1.22; found: C 43.73, H 6.39, N 1.09, S 1.16.

The authors are grateful for the support of the *Natural Science Foundation of Yunnan Province* (Grant No. 2008ZC009M) and for a Grant for Scientific Research from *Yunnan Provincial Department of Education* (Grant No. 07Y10175).

REFERENCES

[1] J. Szejtli, T. Osa, 'Comprehensive Supramolecular Chemistry, (CDs)', Vol. 3, Eds. J. Szejtli, T. Osa, Pergamon, Oxford, 1996, p. 5.
[2] M. V. Rekharsky, Y. Inoue, *Chem. Rev.* **1998**, *98*, 1875.
[3] M. Narita, S. Mima, N. Ogawa, F. Hamada, *Anal. Sci.* **2001**, *17*, 379.
[4] H. Ikeda, Y. Iidaka, A. Ueno, *Org. Lett.* **2003**, *5*, 1625.
[5] Y. Liu, H.-M. Yu, Y. Chen, Y.-L. Zhao, *Chem. – Eur. J.* **2006**, *12*, 3858.
[6] Y. Liu, Y. Song, Y. Chen, X.-Q. Li, F. Ding, R.-Q. Zhong, *Chem. – Eur. J.* **2004**, *10*, 3685.
[7] F. Venema, A. E. Rowan, R. J. M. Nolte, *J. Am. Chem. Soc.* **1996**, *118*, 257.
[8] R. Breslow, B. Zhang, *J. Am. Chem. Soc.* **1996**, *118*, 8495.
[9] B. Zhang, R. Breslow, *J. Am. Chem. Soc.* **1993**, *115*, 9353.
[10] Y. Zhao, J. Gu, Y. C. Yang, H. Y. Zhu, R. Huang, B. Jing, *J. Mol. Struct.* **2009**, *930*, 72.
[11] Y. Liu, B.-H. Han, Y.-T. Chen, *J. Phys. Chem. B* **2002**, *106*, 4678.
[12] R. Breslow, Z. Yang, R. Ching, G. Trojandt, F. Odobel, *J. Am. Chem. Soc.* **1998**, *120*, 3536.
[13] Y. Zhao, X.-Q. Liu, J. Gu, L.-Q. Wang, H.-Y. Zhu, R. Huang, Y.-F. Wang, Z.-M. Yang, *J. Phys. Org. Chem.* **2008**, *21*, 440.
[14] Y. Zhao, Z. M. Yang, S. M. Chi, J. Gu, Y. C. Yang, R. Huang, B. J. Wang, H. Y. Zhu, *Bull. Korean Chem. Soc.* **2008**, *29*, 953.
[15] H. Wang, R. Cao, C.-F. Ke, Y. Liu, T. Wada, Y. Inoue, *J. Org. Chem.* **2005**, *70*, 8703.
[16] Y. Zhao, J. Gu, S. M. Chi, Y. C. Yang, H. Y. Zhu, Y. F. Wang, J. H. Liu, R. Huang, *Bull. Korean Chem. Soc.* **2008**, *29*, 2119.
[17] H. Danielsson, J. Sjövall, 'Sterols and Bile Acids', Elsevier Sci. Ltd., Amsterdam, The Netherlands, 1985.
[18] P. R. Cabrer, E. Alvarez-Parrilla, F. Meijide, J. A. Seijas, E. R. Núñez, J. V. Tato, *Langmuir* **1999**, *15*, 5489.
[19] W. T. Hughes, B. L. Smith, *Antimicrob. Agents Chemother.* **1984**, *26*, 436.
[20] A. Ueno, A. Ikeda, H. Ikeda, T. Ikeda, F. Toda, *J. Org. Chem.* **1999**, *64*, 382.
[21] K. Hamasaki, H. Ikeda, A. Nakamura, A. Ueno, F. Toda, I. Suzuki, T. Osa, *J. Am. Chem. Soc.* **1993**, *115*, 5035.
[22] H. Ikeda, M. Nakamura, N. Ise, N. Oguma, A. Nakamura, T. Ikeda, F. Toda, A. Ueno, *J. Am. Chem. Soc.* **1996**, *118*, 10980.

[23] J. J. Michels, J. Huskens, D. N. Reinhoudt, *J. Am. Chem. Soc.* **2002**, *124*, 2056.
[24] H. A. Benesi, J. H. Hildebrand, *J. Am. Chem. Soc.* **1949**, *71*, 2703.
[25] Y. Liu, Y.-W. Yang, E.-C. Yang, X.-D. Guan, *J. Org. Chem.* **2004**, *69*, 6590.
[26] F. Ollila, O. T. Pentikäinen, S. Forss, M. S. Johnson, J. P. Slotte, *Langmuir* **2001**, *17*, 7107.
[27] Z. J. Tan, X. X. Zhu, G. R. Brown, *Langmuir* **1994**, *10*, 1034.
[28] R. C. Petter, J. S. Salek, C. T. Sikorski, G. Kumaravel, F. T. Lin, *J. Am. Chem. Soc.* **1990**, *112*, 3860.

Received July 17, 2009