Chinese Science Bulletin 2004 Vol. 49 No. 2 115—118

Synthesis of L-cystine bridged bis(β-cyclodextrin) and its cooperative binding toward guest molecules

LI Li, LI Xiaoyun & LIU Yu

Department of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, China
Correspondence should be addressed to Liu Yu (e-mail: yuliu@pulic.tpt.tj.cn)

Abstract **A novel β-cyclodextrin derivative, L-cystine bridged bis(β-cyclodextrin) 1 has been synthesized and characterized by ^{1}H NMR, ^{13}C NMR, IR, Raman and combustion analyses. Spectrofluorometric titrations have been performed in aqueous phosphate buffer solution (pH=7.20) at 25℃ to give the complex stability constants (K_s) and Gibbs free energy changes ($-\Delta G^\circ$) for the stoichiometric 1∶1 inclusion complexation of 1 with four dyes. The binding abilities and molecular selectivities are discussed from the viewpoint of size/shape-fit and electrostatic interactions between hosts and guests. 2D ROESY investigation for the complexation between host and guest further confirms the cooperative binding of bridged cyclodextrin 1 with guest.**

Keywords: L-cystine, birdged bis(cyclodextrin), dye, cooperative binding.

DOI: 10.1360/03wb0170

Native and chemical modified cyclodextrins (CDs), served as synthetic receptors (hosts) of supramolecular systems, display dramatically different binding behaviors toward guest molecules[1—5]. As a new family of CD derivatives, the bridged CD dimers are known to greatly enhance the original binding ability and molecular selectivity of native CD by the cooperative interaction of its two nearly located hydrophobic cavities[6—8]. The introduction of special chiral substituents can improve the original chiral microenvionment of CD cavity, and consequently contribute to the chiral recognition ability of CD. In our previous works, we have reported the synthesis and molecular recognition of some amino acid modified CDs, and obtained some interesting results[9,10].

In the present work, a novel CD derivative, L-cystine bridged bis(β-CD) **1** has been synthesized and characterized by ^{1}H NMR, ^{13}C NMR, IR, Raman and combustion analyses. The inclusion complexation behaviors of **1** with some dyes are investigated by means of spectrofluorometric titrations in aqueous phosphate buffer solution (pH=7.20) at 25℃. The results indicate that the binding abilities and molecular selectivities of **1** are greatly influenced by size/shape-fit and electrostatic interactions between hosts and guests. 2D ROESY spectra for the host-guest complexes further confirm the cooperative binding of bridged CD **1** toward guest. The cooperative binding mode of **1** with TNS is deduced from the ROESY results.

1 Experimental

(ⅰ) Materials. β-CD of reagent grade (Shanghai Reagent Works) was recrystallized twice from water and dried for 24 h *in vacuo* at 95℃ prior to use. All guest dyes, i.e. ammonium 8-anilino-1-naphthalenesulfonate (ANS), sodium 6-(*p*-toluidino)-2-naphthalenesulfonate (TNS), Acridine Red (AR) and Neutral Red (NR) (Fig. 1) were commercially available and used without further purification. Disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in distilled, deionized water to make a 0.1 mol • dm^{-3} phosphate buffer solution of pH 7.20 for spectral titration.

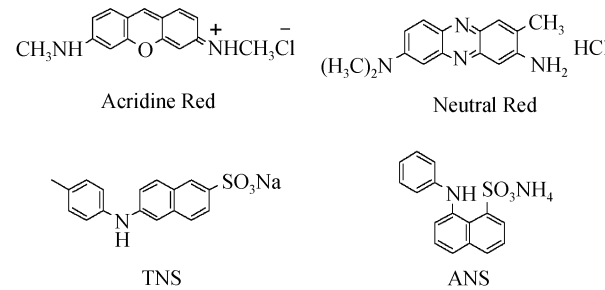

Fig. 1. The structures of guest dyes.

(ⅱ) Synthesis. β-CDI[11] was prepared from the mono-[6-O-(*p*-tolylsulfonyl)]-β-CD and NaI (1∶10) in DMF(40 mL) at 90℃ for 5 h. After evaporating the DMF, the crude product was dissolved in water, and then acetone was added to the solution to give a precipitate, which was collected by filtration to give the pure product.

The synthesis of L-cystine bridged bis-CD **1** (Fig. 2): β-CDI (2.0 g, 1.5 mmol) was dissolved in 20 mL DMF and stirred under nitrogen. Then the L-cystine (0.074 g, 0.31 mmol) in water solution (25 mL) containing Na_2CO_3 (0.13 g, 1.2 mmol) was added to this solution. The mixture was kept at 70℃ for 21 h. Then the solvent was evaporated under a reduced pressure to dryness. The residue was dissolved in water, and then acetone was added to the solution to give a precipitate. The crude product was purified by column chromatography over Sephadex G-25 with distilled, deionized water to give a pure sample (0.12 g, yield 16%). ^{1}H-NMR (D_2O, 300 MHz, TMS, ppm) δ: 3.20—3.81, (90H, C_2-C_6—H, CH, CH_2), 4.88—5.0, (7H, C_1-H). ^{13}C-NMR (D_2O, 300 MHz, ppm) δc: 109.9, 101.9, 82.3, 81.1, 79.3, 77.7, 73.0, 72.1, 71.8, 69.0, 64.6, 60.3, 55.2, 53.5, 40.0. FT-IR (KBr, ν /cm^{-1}): 3401, 2931, 1638, 1415, 1371, 1302, 1261, 1157,

1081, 1032, 945, 855, 796, 755, 708, 608, 580, 527. Anal. Calcd. for $C_{90}H_{148}O_{72}N_2S_2 \cdot 4H_2O$: C 42.45, H 6.18, N 1.10, S 2.52; Found: C 42.40, H 6.20, N 0.71, S 2.64. Raman (ν /cm^{-1}): 2911, 2534, 1600, 1337, 1126, 1082, 852 (C-S), 480 (S-S).

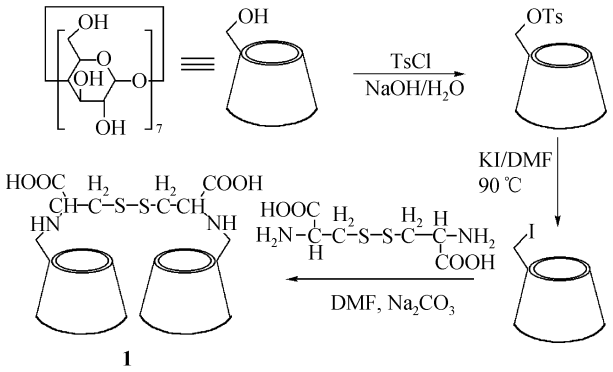

Fig. 2. The synthetic route of L-cystine bridged bis-CD.

(iii) Measurement. Fluorescence spectra were measured in a conventional quartz cell (10 mm×10 mm×45 mm) at 25℃ on a JASCO FP-750 spectrometer equipped with a temperature controller and with excitation and emission slits of 10 nm width. The relative fluorescence intensity of guest dyes will sequentially change with the addition of native and modified CDs, which could be used to calculate the stability constants (K_s) of the inclusion complex formed. In the titration experiments, the fluorescence intensity of dyes (2.5×10^{-6}—1×10^{-5} mol • dm^{-3}) gradually enhances upon the addition of varying concentrations of hosts (Fig. 3) and the stability constants (K_s) are calculated using a nonlinear least-squares curve-fitting method (Fig. 4).

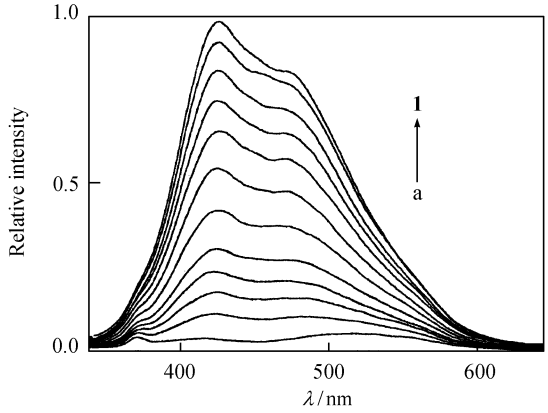

Fig. 3. Fluorescence spectral changes of ANS (12.6×10^{-6} mol • dm^{-3}) upon addition of bridged bis(β-CD) **1** in phosphate buffer solution (pH 7.20) at 25℃. The concentration of **1** (from a to **l**): (0—8.5)×10^{-4} mol • dm^{-3}, excitation at 350 nm.

As shown in Fig. 3, the fluorescence intensity of ANS was greatly enhanced upon stepwise addition of bridged bis(β-cyclodextrin) **1**, along with the pronounced hypsochromic shift of the original fluorescence maximum of guests, indicating that ANS transferred from the polar to the apolar microenvironment and formed the host-guest inclusion complex.

In the concentration range examined, the inclusion complexation of host **1** with all guest dyes gives the excellent curve fitting results, further indicating that the 1∶1 stoichiometry complexes are formed between host and guests. The typical curve-fitting analyses result for the inclusion complexation of host **1** with ANS is shown in Fig. 4, where no serious deviations are found. The complex stability constants (K_s) and the Gibbs free energy changes ($-\Delta G^\circ$) obtained are listed in Table 1. For comparison, the reported parameters for the complexation of native β-cyclodextrin with dyes are also listed in Table 1.

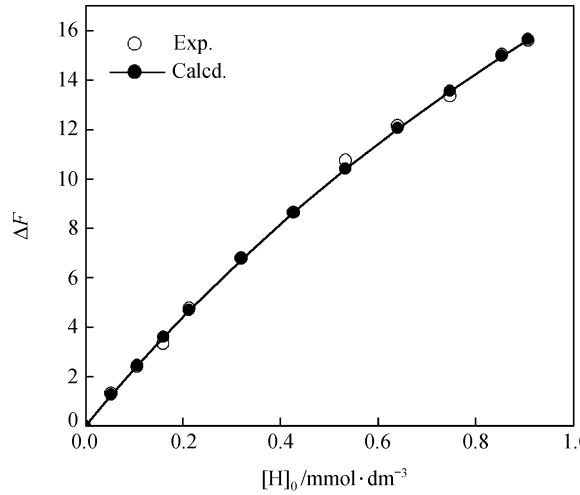

Fig. 4. Least-squares curve-fitting analyses for the inclusion complexation of 1 and ANS.

Table 1 Stability constant (K_s) and gibbs free energy change ($-\Delta G^\circ$) for the inclusion complexation of hosts **1** with guest dyes at 25℃ in phosphate buffer solution (pH 7.20)

Host	Guest	K_s	logK_s	$-\Delta G^\circ$	Ref.
β-CD	TNS	3670	3.56	20.35	[13]
	ANS	103	2.01	11.49	[14]
	AR	2630	3.42	19.50	this work
	NR	480	2.78	15.87	this work
1	TNS	9940	4.00	22.82	this work
	ANS	435	2.64	15.06	this work
	AR	3250	3.51	20.05	this work
	NR	4660	3.67	20.94	this work

2 Result and discussion

(i) Binding mode. It can be seen from Table 1 that bridged bisCD **1** always shows stronger binding abili-

ties for all guest dyes than native β-CD does, giving much larger complex stability constants ($K_1/K_{\beta\text{-CD}}$=2.71, 4.22, 1.24, 9.71). This should be mainly attributed to the cooperative binding of one guest molecule by the dual hydrophobic cavities of **1**. To validate the existence of this cooperative interaction, 2D ROESY spectroscopy for the complexation of CD **1** as well as β-CD with TNS is performed to investigation the interaction mode between host and guest (Figs. 5 and 6).

In both Fig. 5 and Fig. 6, there exist strong NOE correlations between the aromatic protons of TNS and the

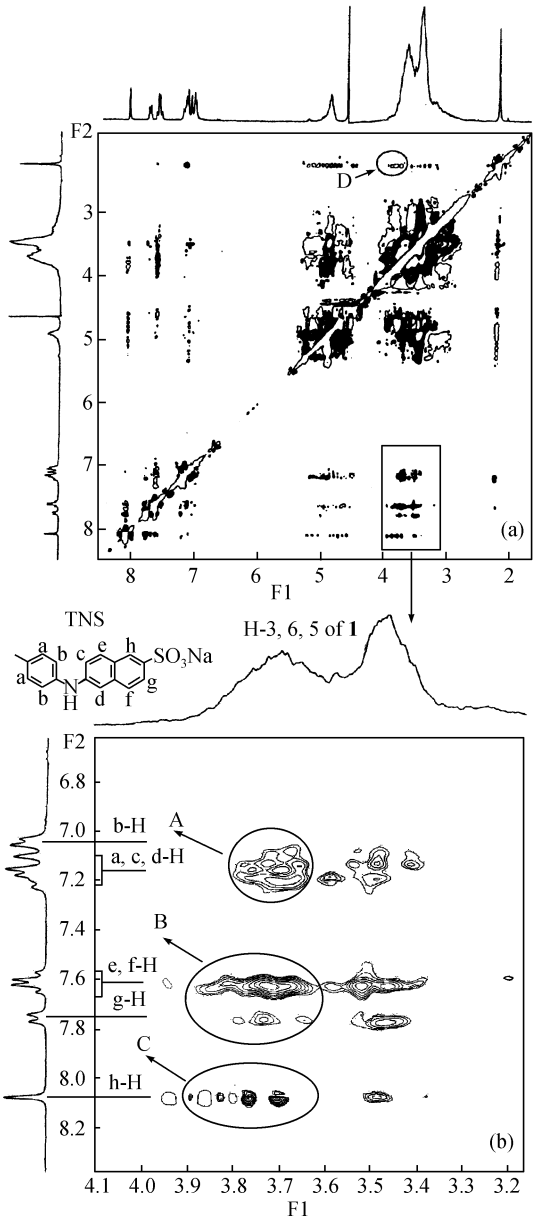

Fig. 5. ^{1}H ROESY spectra of a mixture of CD **1** (5 mmol/L) and TNS (5 mmol/L) in D_2O at 298 K with a mixing time of 400 ms.

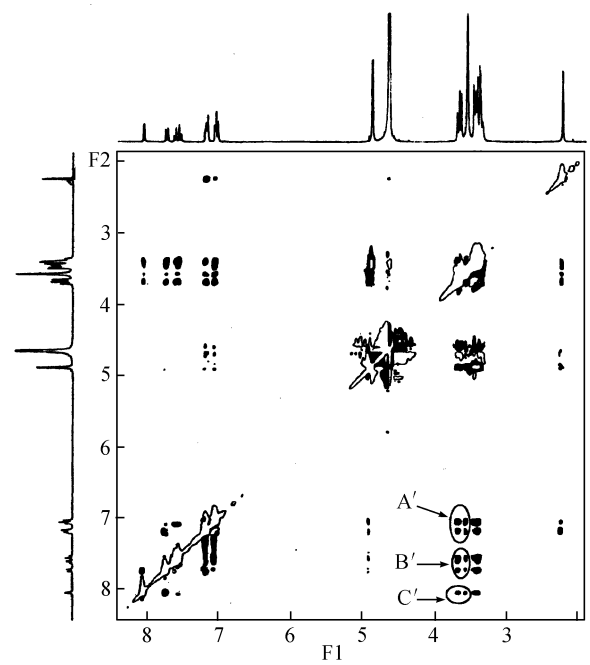

Fig. 6. ^{1}H ROESY spectra of a mixture of β-CD (5 mmol/L) and TNS (5 mmol/L) in D_2O at 298 K with a mixing time of 400 ms.

H-3, H-5 protons located in the cavity of CD. Through the ascription of these interactions, we can find that correlation peaks A(A′) correspond to the correlations between the protons in toluene moiety of TNS and the interior protons of CD, while peaks B(B′) and C(C′) correspond to correlations between the protons in the naphthalene ring moiety of TNS and the interior protons of CD. Simultaneously, it should be observed from peaks B and C that the proton near the sulfonic group only shows strong interactions with the H-3 protons of CD but weak interaction or no interaction with H-5 protons. The same observation can also be found in peaks B′ and C′. This means that the naphthalene ring moiety deeply insert into the CD cavity from the primary side. On the other hand, it is found from Fig.6 that there is no correlations between the methyl protons of TNS and interior protons of β-CD, which indicate that the methyl of TNS is located outside the β-CD cavity, inferring the possible binding mode for native β-CD with TNS (Fig. 7(a)). However, the strong interactions between methyl protons of TNS and interior protons of CD **1** (Fig. 5, peak D) indicate that the toluene moiety of TNS is included into another CD cavity of **1**; that is to say, host **1** achieves the cooperative binding of TNS by its two hydrophobic cavities. The possible binding mode for host **1** and TNS is shown in Fig. 7(b).

(ii) Molecular recognition. In the present experiment conditions (pH = 7.20), the carboxyl groups in the bridge of **1** will exist as anions. Therefore, two positively charged (AR and NR) and two negatively charged (TNS and ANS) guests are chosen to investigate the effect of

electrostatic interaction in the inclusion complexation between host and guest.

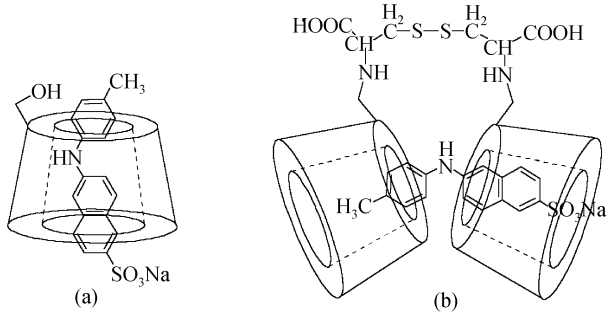

Fig. 7. Possible binding modes of TNS with (a) β-CD and (b) host **1**.

Bearing the negative charges, the inclusion complexations of ANS and TNS with CD **1** mainly dependly on the hydrophobic interactions. Though TNS and ANS possess the similar frameworks (naphthalene ring moiety), TNS is substituted at 2- and 6-positions but ANS at 1- and 8-positions. Upon complexation with CDs, ANS suffers the relatively large steric hindrance for penetrating into the CD cavity. Therefore, the size/shape fit between host and guest becomes the pivotal factor influencing the binding behaviors of CDs toward ANS and TNS. It can be seen clearly that, in the case of either native β-CD or bridged CD **1**, TNS molecule with the structural advantage is always easier than ANS to be included into the hydrophobic cavities forming stable supramolecular complexes. So both of these two hosts show the stronger binding abilities to TNS than to ANS. However, due to the duple recognition by the additional cavity, host **1** shows the greatly enhanced binding ability toward ANS, giving the large complex stability constant as 4.2 times higher than that of native β-CD.

Although AR and NR possess the similar linear structures with a positively charged heterocyclic anthracene center, native β-CD shows remarkably different binding abilities toward these guests. The stronger binding ability of β-CD toward AR ($K_{AR\text{-}\beta\text{-}CD}/K_{NR\text{-}\beta\text{-}CD}$ = 5.5) is probably attributed to the relative small substituents of AR making it easy to penetrate into the hydrophobic cavity of CD. However, host **1** shows greatly enhanced binding ability toward NR ($K_{NR\text{-}\mathbf{1}}/K_{NR\text{-}\beta\text{-}CD}$ = 9.7) and the reversed binding ability sequence for AR and NR ($K_{NR\text{-}\mathbf{1}}/K_{AR\text{-}\mathbf{1}}$=1.4). This result indicates that, upon complexation with these two guest dyes, the size/shape fit is not the most important factor for host **1** like the case of β-CD. It is thought that the electrostatic attract might exist between the positive charge center of AR/NR and the carboxyl anions in the bridge of CD **1**, which cancels the substituents effect to some extent, and therefore leads to the proximate K_s values for AR and NR.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant No. 20272028), the Tianjin Natural Science Fund (Grant No. 013613511), and the Special Fund for the Doctoral Program from the Ministry of Education of China (Grant No. 20010055001).

References

1. Liu, Y., You, C. C., Zhang, H. Y., Supramolecular Chemisty-Molecular Recognition and Assembly of Synthetic Receptors (in Chinese), Tianjin: Nankai University Press, 2001, 207—248.
2. Rekharsky, M. V., Inoue, Y., Complexation and chiral recognition thermodynamics of 6-amino-6-deoxy-β-cyclodextrin with anionic, cationic, and neutral chiral guests: counterbalance between van der waals and coulombic interactions, J. Am. Chem. Soc., 2002, 124(5): 813—826.
3. Liu, Y., Zhang, Y. M., Qi, A. D. et al., Molecular recognition study on a supramolecular system (VIII)——The fluoroscopic study on inclusion complexation of adamantanol with cyclodextrin derivatives, Chinese Sci. Bull., 1998, 43(4): 286—289.
4. Wang, X. P., Pan, J. H., Shuang, S. M., Study on the supramolecular system of meso-tetrakis(4-sulfonatophenyl) porphyrin and cyclodextrins by spectroscopy, Spectrochimica Acta, Part A, 2001, 57(14): 2755—2762.
5. Chen, Y. M., He, B. L., Binding properties of amino-modified β-cyclodextrin derivatives with 1- and 2-naphthols, Chinese Sci. Bull., 1996, 41(8): 646—650.
6. Zhang, B., Breslow, R., Cholesterol recognition and binding by cyclodextrin dimers, J. Am. Soc. Chem., 1996, 118(35): 8495—8496.
7. Liu, Y., You, C. C., Molecular recognition studies on supramolecular systems——the binding ability and molecular selectivity of bridged bis(cyclodextrin)s with model substrates, Chem. J. Chinese Universities (in Chinese), 2001, 22(4): 591—597.
8. de Jong, M. R., Engbersen, J. F. J., Huskens, J. et al., Cyclodextrin dimers as receptor molecules for steroid sensors, Chem. Eur. J., 2000, 6(21): 4034—4040.
9. Liu, Y., Han. B. H., Sun, S. X. et al., Molecular recognition study on supramolecular systems. 20. Molecular recognition and enantioselectivity of aliphatic alcohols by l-tryptophan-modified β-cyclodextrin, J. Org. Chem., 1999, 64(5): 1487—1493.
10. Liu, Y., Jin, L., Sun, S. X., Molecular recognition studies on supramolecular systems. 26. Inclusion complexation of natural and amino acid modified β-cyclodextrins with model substances: ANS and RhB molecules, Microchem. J., 2000, 64: 59—65.
11. Tabushi, I., Kuroda, Y., Mochizuki, A., The first successful carbonic anhydrase model prepared through a new route to regiospecifically bifunctionalized cyclodextrin, J. Am. Chem. Soc., 1980, 102(3): 1152—1153.
12. Ren, X. J., Liu, J. Q., Luo, G. M. et al., A novel selenocystine-β-cyclodextrin conjugate that acts as a glutathione peroxidase mimic, Bioconjugate Chem., 2000, 11: 682—687.
13. Liu, Y., You, C. C., Chen, Y. et al., Molecular recognition studies on supramolecular systems. 25. Inclusion complexation by organoselenium-bridged bis(β-cyclodextrin)s and their platinum (IV) complexes, J. Org. Chem., 1999, 64(21): 7781—7787.
14. Liu, Y., Chen, Y., Li, B. et al., Cooperative multipoint recognition of organic dyes by bis(β-cyclodextrin)s with 2, 2′-bipyridine-4, 4′-dicarboxy tethers, Chem. Eur. J., 2001, 7(12): 2528—2535.

(Received September 26, 2003; accepted November 17, 2003)