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Synthesis of L-cystine modified cyclodextrin monomers and dimers with primary-side *versus* secondary-side and their molecular binding behaviours

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A series of β-cyclodextrin (β-CD) derivatives modified by L-cystine, including 3,3′-L-cystine-bridged bis(β-CD) (**2**), 3-L-cystine-β-CD (3), 6,6′-L-cystine-bridged bis(β-CD) (**4**) and 6-L-cystine-β-CD (**5**), were synthesised in moderate yields by the reaction of L-cystine with mono-[2-*O*-(*p*-tolysulfonyl)]-β-CD (2-*O*-Ts-β-CD) or mono-[6-*O*-(*p*-tolysulfonyl)]-β-CD (6-*O*-Ts-β-CD). Their binding manners and inclusion abilities towards some dye guests (ANS, TNS, AR, NR, EY and FL) were, respectively investigated by the methods of 2D NMR spectrometry and fluorescence spectrometry in aqueous solution (pH 7.2). The results obtained show that the stoichiometric 1:1 complexes formed by L-cystine modified β-CD monomers and dimers **2**–**5** with dye guests give higher complex stability constants (K_S) than those of native β-CD. In addition, the difference of inclusion complexation between primary-side and secondary-side modified/bridged β-CDs was compared in detail and discussed from the viewpoint of inclusion orientation, size/shape fit, cooperative binding and hindrance of substituent.

Keywords: cyclodextrin; dye; L-cystine; molecular recognition

Introduction

It is well known that native and modified cyclodextrins (CDs), having fairly rigid and well-defined hydrophobic cavities, act as molecular receptors (hosts) to bind substrates (guests), forming host–guest complexes in aqueous solutions (*1–3*). Possessing dual hydrophobic cavities in close vicinity, bridged CD dimers are known to display many particular complexation properties through the cooperative inclusion of two CD cavities, which cannot be obtained by CD monomers (*4–6*). Therefore, a great deal of effort has been devoted to design and synthesise various CD dimers and to investigate their cooperative inclusion behaviours for special guests (*7–10*). Reinhoudt and co-workers synthesised several dansyl-modified CD dimers and investigated their binding abilities as receptor molecules for steroid sensors (*11*). CD dimers were used to cooperatively bind peptide side chains in water with sequence selectivity (*12*). Nolte's group reported the synthesis, conformation, and binding properties of CD homo- and heterodimers connected through their secondary sides (*13*). In our preliminary works, we have also reported the selective binding behaviours of oligoethylenediamine- (*14*), organoselenium- (*15*), biquinoline- (*16*), bipyridine- (*17, 18*), cystine- (*19*), and oxamidobisbenzoyl- (*20*) bridged bis(β-CD)s with some representative guest molecules in different sizes and shapes. The results showed that these bridged bis(β-CD)s adopted a multiple sandwich binding mode upon inclusion complexation with guest molecules. That is, in addition to the cooperative binding of two CD cavities with two side groups of the guest molecule, the linker group could also provide the additional binding interactions, such as hydrogen-bonding and electrostatic interactions, towards the accommodated guest molecule, which would significantly enhance the original binding ability of native β-CD, and even change the fluorescence behaviour of the guest molecule in some cases.

Owing to the difference of conjunctive orientations between primary-side bis(β-CD)s and secondary-side bis(β-CD)s, they provide the distinctly spacial pre-organised structures to include guests, and then there should be distinguishing selectivities of molecular recognition towards guests between them. However, to the best of our knowledge, the investigations concerning the close comparison of binding behaviours between primary-side and secondary-side bridged/mono-modified CDs have been studied less frequently (*21–23*). Therefore, in the present work, we synthesised two comparable cystine bridged bis(β-CD)s **2** (secondary sides) and **4** (primary sides) together with their mono-modified derivatives **3** and **5** (Figure 1), and studied systemically their binding abilities and molecular selectivities for a series of dye molecules (Figure 2). It is of our special interest to compare the binding abilities towards various model substrates between primary-side and secondary-side modified CDs, and further to elucidate the

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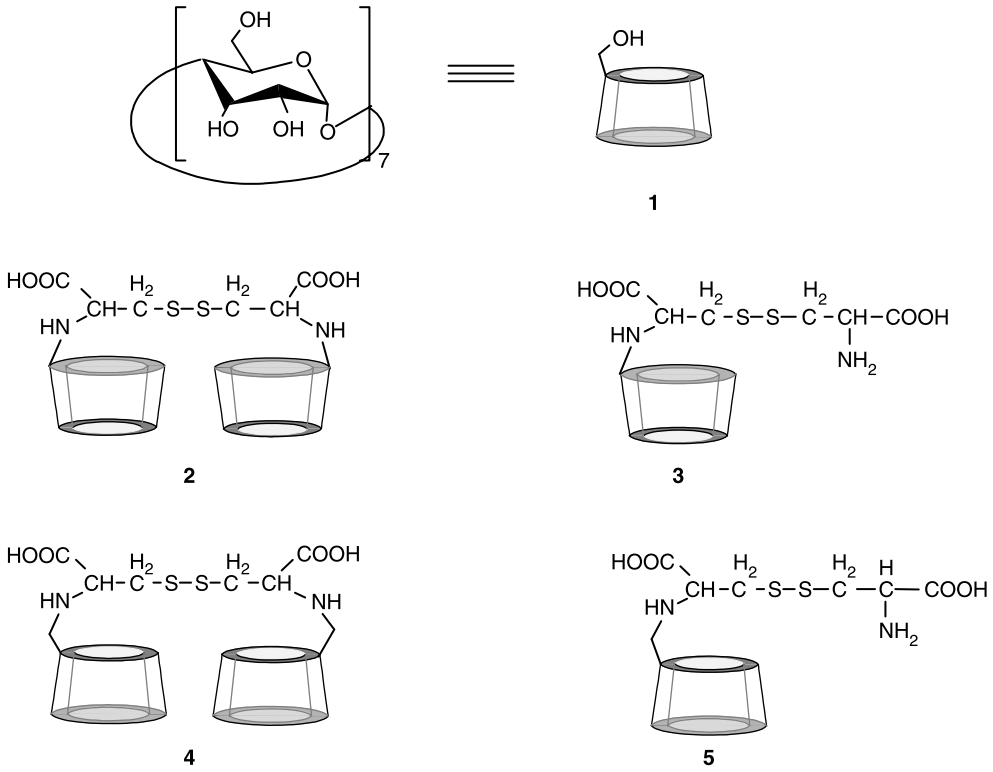


Figure 1. Structures of host CDs: native β-CD (**1**), 3,3′-*L*-cystine-bridged bis(β-CD) (**2**), 3-*L*-cystine-β-CD (**3**), 6,6′-*L*-cystine-bridged bis(β-CD) (**4**), 6-*L*-cystine-β-CD (**5**).

recognition mechanism of CD derivatives towards model substrate molecules.

Experimental section

Materials

All guest dyes, i.e., ammonium 8-anilino-1-naphthalene-sulfonate (ANS), sodium 6-(*p*-toluidino)-2-naphthalene-sulfonate (TNS), acridine red (AR), neutral red (NR), eosin Y (EY) and fluorescein (FL) (Figure 2) were purchased from WaKo. β-CD of reagent grade (Shanghai Reagent Works) was recrystallised twice from water and dried for 24 h *in vacuo* at 100°C prior to use. All other chemicals were of reagent grade and used without further purification. 6,6′-*L*-cystine-bridged bis(β-CD) (**4**) was synthesised according to a reported procedure (*19*). Disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in distilled, deionized water to make a 0.1 mol dm^{-3} phosphate buffer solution (pH 7.20).

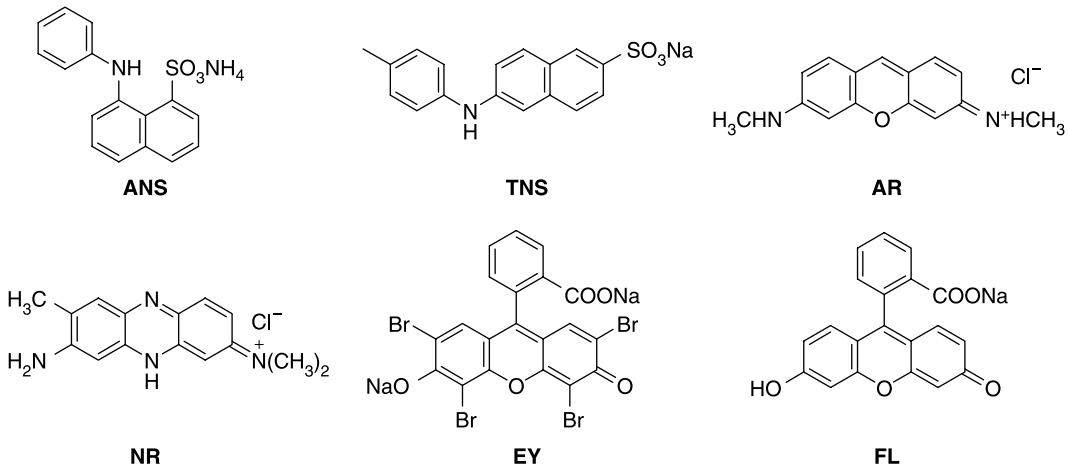


Figure 2. Structures of the selected guest dyes.

Measurements

Elemental analyses were performed on a Perkin–Elmer-2400C instrument. All NMR experiments were recorded on a Varian Mercury VX300 instrument. FT-IR spectra were obtained on a Nicolet FT-IR 5DX spectrometer. Mass spectra were performed on a Finnigan LCQ-Advantage LC-MS. Fluorescence spectra were measured in a conventional quartz cell (10 × 10 × 45 mm) at 25°C on a JASCO FP-750 spectrometer equipped with a constant-temperature water bath, with the excitation and emission slits of 5 nm width for all measurements.

Computational method

The initial geometry of β-CD was taken from the crystal structure (*24*). We drew the guest TNS and the modified β-CD molecules, then put TNS molecule into the CD cavity of hosts **1**–**5**. 5000 energy minimisation steps were performed to obtain the stable structure using the smart method, which started with the steepest descent method, followed by the adopted basis Newton Raphson method and quasi Newton methods, and ending with the accurate truncated Newton method. All the simulations use the Dreiding force field (*25*).

Synthesis of 3,3′-L-cystine-bridged bis(-CD) (2)

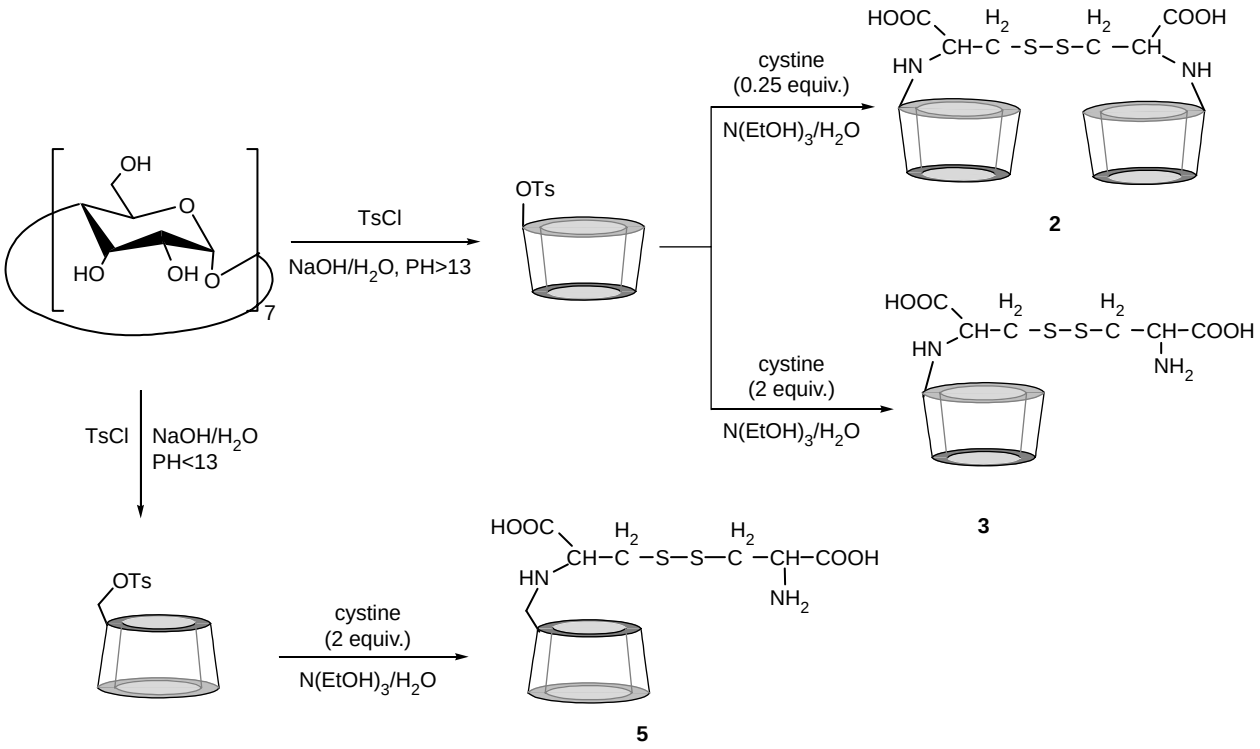
L-Cystine (0.12 g, 0.5 mmol) and mono-[2-*O*-(*p*-tolysulfonyl)]-β-CD (2-*O*-Ts-β-CD) (*26*) (2.6 g, 2 mmol) were dissolved in 20 mL of $N(EtOH)_3$ and 30 mL of H_2O. The mixture was stirred at 95°C under nitrogen for 4 days. Then, the reaction mixture was concentrated under a reduced pressure to ca. 20 mL, and poured into 200 mL of ethanol. After filtration, the precipitate was dissolved in a minimum amount of hot water, and then 200 mL of ethanol was added to give the crude product, which was subsequently purified by ion-exchange chromato graphy on a Sephadex CM-25 column with ammonia ($1\,mol\,dm^{-3}$) as an eluent and gel chromatography on a Sephadex G-25 column with distilled, deionized water as an eluent to give a pure sample (0.15 g, yield 12%). ESI-MS *m/z* calculated for $C_{90}H_{148}O_{72}N_2S_2 = 2472.7$, found 1237.4 for $[M + 2H]^{2+}$. ^{1}H-NMR (D_2O, 300 MHz, TMS, ppm): δ 2.50–4.00, (90H, 14 × C_2-H, 14 × C_3-H, 14 × C_4-H, 14 × C_5-H, 28 × C_6-H, 2 × CH, 2 × CH_2), 4.88–5.25, (14 × C_1-H). ^{13}C-NMR (D_2O, 75MHz, TMS, ppm): δ 172.2 (COOH of cystine), 103.4 (C-1′), 101.9 (C-1), 81.1 (C-4), 79.4 (C-4′), 76.8 (C-5′), 74.2 (C-2′), 73.1–71.9 (C-3, C-2, C-5), 60.4 (C-6), 59.5 (C-6′), 55.6 (CH of cystine), 53.0 (C-3′), 42.8 (CH_2 of cystine). FT-IR (KBr, ν_{max}/cm^{-1}): 3358, 2926, 2039, 1649, 1455, 1421, 1334, 1152, 1028, 941, 853, 754, 704, 577, 438. Anal. Calcd. for $C_{90}H_{148}O_{72}N_2S_2{\cdot}10H_2O$: C 40.72, H 6.38, N 1.06, S 2.42; Found: C 40.49, H 6.33, N 0.96, S 2.60.

Synthesis of 3-L-Cystine–CD (3)

L-Cystine (0.48 g, 2 mmol) and 2-*O*-Ts-β-CD (1.3 g, 1 mmol) were dissolved in 30 mL of $N(EtOH)_3$ and 30 mL of H_2O. The mixture was stirred at 95°C under nitrogen for 2 days. Then, the reaction mixture was concentrated under a reduced pressure to ca. 30 mL, and poured into 300 mL of ethanol. After filtration, the precipitate was dissolved in a minimum amount of hot water, and then 200 mL of ethanol was added to give the crude product, which was subsequently purified by ion-exchange chromatography on a Sephadex CM-25 column with ammonia ($1\,mol\,dm^{-3}$) as an eluent and gel chromatography on a Sephadex G-25 column with distilled, deionized water as an eluent to give a pure sample (0.30 g, yield 22%). ESI-MS *m/z* calculated for $C_{48}H_{80}O_{38}N_2S_2 = 1356.4$, found 1357.5 for $[M + H]^+$. ^{1}H-NMR (D_2O, 300 MHz, TMS, ppm): δ 2.30–3.95, (48H, 7 × C_2-H, 7 × C_3-H, 7 × C_4-H, 7 × C_5-H, 14 × C_6-H, 2 × CH, 2 × CH_2), 4.72–5.34, (7 × C_1-H). ^{13}C-NMR (D_2O, 75 MHz, TMS, ppm): δ 172.6 (COOH of cystine; there is only one kind of signal of COOH detected, which may be owing to that the ppm values of the two unequivalent COOH groups are close to each other as the NMR of 75 MHz used), 103.3 (C-1′), 102.0 (C-1), 81.0 (C-4), 79.1 (C-4′), 76.9 (C-5′), 74.3 (C-2′), 73.2–72.0 (C-3, C-2, C-5), 60.4 (C-6), 59.7 (C-6′), 56.0 (C'H of cystine), 53.8 (CH of cystine), 49.1 (C-3′), 44.2 (CH_2 of cystine), 43.0 (C'H_2 of cystine). FT-IR (KBr, ν_{max}/cm^{-1}): 3365, 2928, 2046, 1636, 1364, 1153, 1078, 1031, 944, 847, 755, 706, 576, 433. Anal. Calcd. for $C_{48}H_{80}O_{38}N_2S_2{\cdot}6H_2O$: C 39.34, H 6.33, N 1.91, S 4.38; Found: C 39.49, H 6.19, N 1.76, S 4.31.

Synthesis of 6-L-Cystine–CD (5)

Compound **5** was synthesized from L-cystine and mono-[6-*O*-(*p*-tolysulfonyl)]-β-CD (6-*O*-Ts-β-CD) (*27*) according to the procedures described above (yield 20%). ESI-MS *m/z* calculated for $C_{48}H_{80}O_{38}N_2S_2 = 1356.4$, found 1357.5 for $[M + H]^+$. ^{1}H-NMR (D_2O, 300 MHz, TMS, ppm): δ 2.54–4.08, (48H, 7 × C_2-H, 7 × C_3-H, 7 × C_4-H, 7 × C_5-H, 14 × C_6-H, 2 × CH, 2 × CH_2), 4.78-5.10, (7 × C_1-H). ^{13}C-NMR (D_2O, 75 MHz, TMS, ppm): δ 172.5 (COOH of cystine), 102.0 (C-1), 100.9 (C-1′), 81.2 (C-4), 80.0 (C-4′), 73.2–71.9 (C-3, C-3′, C-2, C-2′, C-5), 68.9 (C-5′), 60.3 (C-6), 56.8 (C-6′), 55.8 (C'H of cystine), 53.2 (CH of cystine), 44.0 (CH_2 of cystine), 42.9 (C'H_2 of cystine). FT-IR (KBr, ν_{max}/cm^{-1}): 3346, 2926, 2045, 1649, 1366, 1155, 1078, 1031, 946, 845, 755, 706, 572, 447. Anal. Calcd. for $C_{48}H_{80}O_{38}N_2S_2{\cdot}4H_2O$: C 40.34, H 6.20, N 1.96, S 4.49; Found: C 40.33, H 5.97, N 2.07, S 4.52.



Scheme 1. Synthesis routes of host CDs.

Results and discussion

Synthesis

As shown in Scheme 1, hosts **2** and **3** were synthesised in moderate yields from 2-*O*-Ts-β-CD and L-cystine by choosing the proper proportion between them, and host **5** was synthesised from 6-*O*-Ts-β-CD and excessive L-cystine. In the process of synthesising secondary-side hosts **2** or **3**, 2-*O*-Ts-β-CD was converted to 2,3-anhydro-β-CD firstly, and then the amino-functionalised group (L-cystine) was introduced at the 3-position of β-CD. The amination of a methenyl group of the C3 position caused a large upfield shift of C3, a moderate upfield shift of C4 as well as a moderate downfield shift of C1 and C2 in the ^{13}C NMR spectra of host **2** or **3**, and meanwhile, the chemical shifts were in accordance with the literatures (*28, 29*).

Fluorescence titration

It is well known that several dye molecules are very sensitive to environmental changes, which enables us to use the fluorescent dyes as spectral probes to investigate the inclusion complexation with native β-CD **1** and the L-cystine modified β-CDs **2**–**5**. To compare quantitatively the binding abilities of hosts **2**–**5**, fluorescence titrations using ANS, TNS, AR, NR, EY and FL as representative guest molecules were performed at 25°C in aqueous phosphate buffer solution (pH 7.20). Typical titration spectral changes are shown in Figure 3 for the inclusion complexation of host **2** with ANS, and the stepwise addition of a known amount of **2** to a dilute ANS solution (10 μM) caused significant enhancement in fluorescence

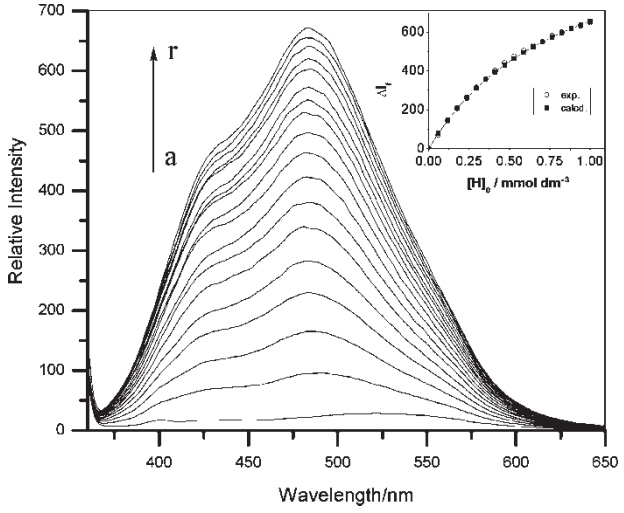


Figure 3. Fluorescence spectral changes of ANS (10 μM) and the nonlinear least-squares analysis (inset) of the differential intensity (I_f) to calculate the complex stability constants (K_S) upon addition of **2** in aqueous buffer solution (pH 7.20, (**2**) = 0, 59, 118, 176, 235, 294, 353, 412, 470, 529, 588, 647, 706, 764, 823, 882, 941, 1000 μM from a to r) at 25°C. Excitation wavelength was 350 nm.

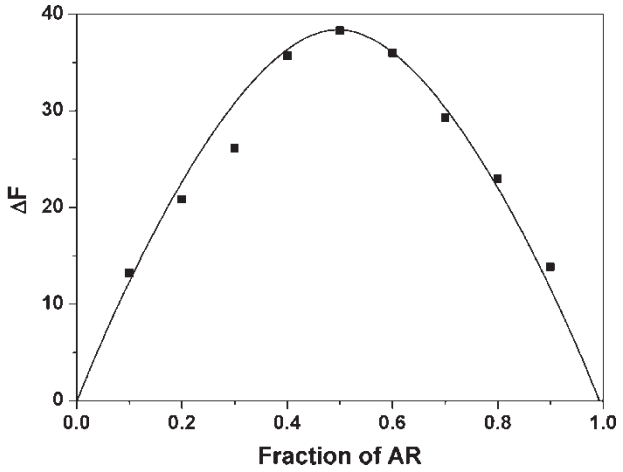


Figure 4. Job's plot of **2**/AR system by fluorescence spectra ($(\mathbf{2}) + [\mathrm{AR}] = 5.0 \times 10^{-5}\,\mathrm{mol\,dm^{-3}}$) ($\Delta F$ represents the relative fluorescent intensity).

intensity, indicating that ANS transferred from the polar to the apolar microenvironment and formed the host–guest inclusion complex.

The stoichiometry for each of the inclusion complexation of L-cystine modified β-CDs with dye molecules is determined by the Job's method of continuous variation to the stoichiometry of host–guest complexes. Figure 4 illustrates the Job's plot for **2**/AR system. In the concentration range examined, the plot for AR shows a maximum at a molar fraction of 0.5, indicating 1:1 sandwich complexation. The same results are obtained in the cases of the inclusion complexation of other hosts with selected guest molecules.

Validating the 1:1 host–guest inclusion complexation stoichiometry by Job's method, the inclusion complexation of a guest (G) with a host (H) was expressed by Equation (1).

$$\mathrm{H} + \mathrm{G} \overset{K_S}{\rightleftharpoons} \mathrm{H{\cdot}G} \qquad (1)$$

The effective stability constant (K_S) (*30*) can be obtained from the analysis of the sequential changes of fluorescence intensity (ΔF) at various host concentrations, using a nonlinear least-squares method according to the curve fitting Equation (2) (*15, 31*).

$$\Delta F = \{\alpha([\mathrm{H}]_0 + [\mathrm{G}]_0 + 1/K_S) \pm \sqrt{\alpha^2([\mathrm{H}]_0 + [\mathrm{G}]_0 + 1/K_S)^2 - 4\alpha^2[\mathrm{H}]_0[\mathrm{G}]_0}\}/2 \qquad (2)$$

Where $[\mathrm{G}]_0$ and $[\mathrm{H}]_0$ refer to the total concentrations of the guest and host, and α the proportionality coefficient, which may be taken as a sensitivity factor for the fluorescence change. For each host examined, the plot of ΔF as a function of $[\mathrm{G}]_0$ gave an excellent fit, verifying the validity of the 1:1 complex stoichiometry assumed above. Figure 3 illustrates the typical curve-fitting plots for the titrations of ANS with host **2**. There are no serious diversions between the experimental and calculated data of host **2**/ANS system, indicating 1:1 complexation only throughout the concentration range of host **2** (0–1 mM). The complex stability constants (K_S) obtained are listed in Table 1, along with the free energy change of complex formation ($-\Delta G^\circ$). In the repeated measurements, most of the K_S values are reproducible within an error of ± 5%.

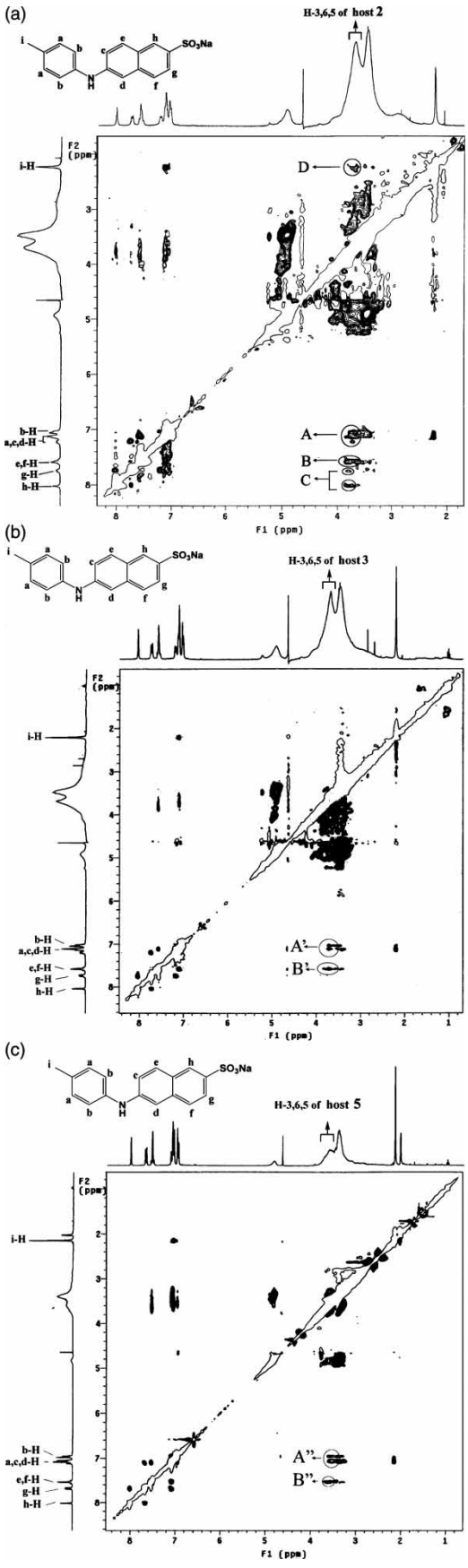
Binding mode

The 2-dimensional NMR spectroscopy has become an indispensable method to study the structures and binding geometries of host–guest inclusion complexation, since it can be concluded that two protons are closely located in space if NOE cross-peak is detected between the relevant protons in the ROESY (rotating frame Overhauser effect spectroscopy) or NOESY (nuclear Overhauser effect spectroscopy) spectrum. To get further information about

Table 1. Complex stability constants (K_S) and Gibbs free energy changes ($-\Delta G^\circ$) for 1:1 inclusion complexation of guest molecules with host molecules in aqueous phosphate buffer solution of pH 7.20 at 25°C

Host	Guest	$K_S/\mathrm{M^{-1}}$	$-\Delta G^\circ/\mathrm{kJ\,mol^{-1}}$	Ref.
1	ANS	103	11.5	a
	TNS	3670	20.4	a
	AR	2630	19.5	a
	NR	480	15.9	a
	EY	28 ± 5	8.3	b
	FL	89 ± 8	11.1	b
2	ANS	1203 ± 52	17.6	b
	TNS	10040 ± 420	22.8	b
	AR	6300 ± 280	21.7	b
	NR	1262 ± 50	17.7	b
	EY	1194 ± 46	17.6	b
	FL	767 ± 32	16.5	b
3	ANS	1020 ± 45	17.2	b
	TNS	1480 ± 58	18.1	b
	AR	4330 ± 180	20.8	b
	NR	766 ± 35	16.5	b
	EY	1043 ± 42	17.2	b
	FL	253 ± 18	13.7	b
4	ANS	435	15.1	a
	TNS	9940	22.8	a
	AR	3250	20.0	a
	NR	4660	20.9	a
	EY	1070 ± 44	17.3	b
	FL	1220 ± 42	17.6	b
5	ANS	690 ± 32	16.2	b
	TNS	4980 ± 210	21.1	b
	AR	7720 ± 320	22.2	b
	NR	790 ± 30	16.5	b
	EY	473 ± 20	15.3	b
	FL	610 ± 30	15.9	b

[a] Reference [19], [b]this work.



the geometries of the inclusion complexation, 2D NMR measurements of complexes between the host molecules (**2**, **3**, **5**) and guest molecule TNS were performed. The obtained spectra are shown in Figure 5.

All these three ROESY spectra Figure 5 (a, b and c) present obvious NOE correlations between the protons of TNS and the H-3, H-5 protons located in the cavity of CD, which indicates that TNS should be included into the cavities of host **2**, **3**, and **5**. However, it can be found that the binding mode of dimer **2** is different from those of monomer **3** and **5** by closely analysing their ROSEY spectra, respectively. As can be seen from Figure 5(a), there are four clear crosspeaks (peaks A, B, C, and D) between the protons of TNS and the H-3, H-5 protons of CD **2**. Peak A represents the correlations between the aromatic protons of the toluene moiety in TNS and the H-3, H-5 protons of CD **2**, implying that the toluene moiety of TNS resides in the cavity of CD. On the other hand, the strong interactions between the naphthalic protons near the sulfonic group and the interior protons of CD **2** (peaks B and C) indicate that the naphthalene ring moiety should be included into another CD cavity of host **2**. Furthermore, peak D, representing the correlation between the methyl protons of TNS and the interior protons of CD **2**, provides the information that the methyl group of TNS is also included into the cavity of CD. Therefore, it could be deduced from these ROESY results that host **2** should achieve the cooperative binding of TNS by its two hydrophobic cavities. The deduced binding mode for host **2** and TNS is shown in Figure 6(a).

In the cases of mono-modified hosts **3** and **5** Figure 5(b) and 5(c), there are only two sets of obvious crosspeaks A′ (A″) and B′ (B″) between the protons of TNS and the H-3, H-5 protons of the CD monomers. Through the ascription of these interactions, we can find that crosspeaks A′,A″ correspond to the correlations between the protons in toluene moiety of TNS and the interior protons of CD; meanwhile, crosspeaks B′, B″ correspond to correlations between the protons in the naphthalene ring moiety of TNS and the interior protons of CD. Although there is an overlap between the H-3 and H-5 protons of the cyclodextrin, the signal of H-3 generally appears at downfield as comparison with that of H-5 according to the experience and the literature (*32*). It can be observed that peaks A′ and A″ show correlation at more upfield as comparison with peaks B′ and B″. This means the protons of the toluene moiety show stronger interactions with the H-5 protons of CD than those of the naphthalene ring moiety; on the contrary, the protons of the naphthalene

Figure 5. 2D ROESY spectra of the inclusion complexes of **2**-TNS (a), **3**-TNS (b) and **5**-TNS (c) with the concentration of 5 mmol/L in D_2O at 25°C with a mixing time of 300 ms.

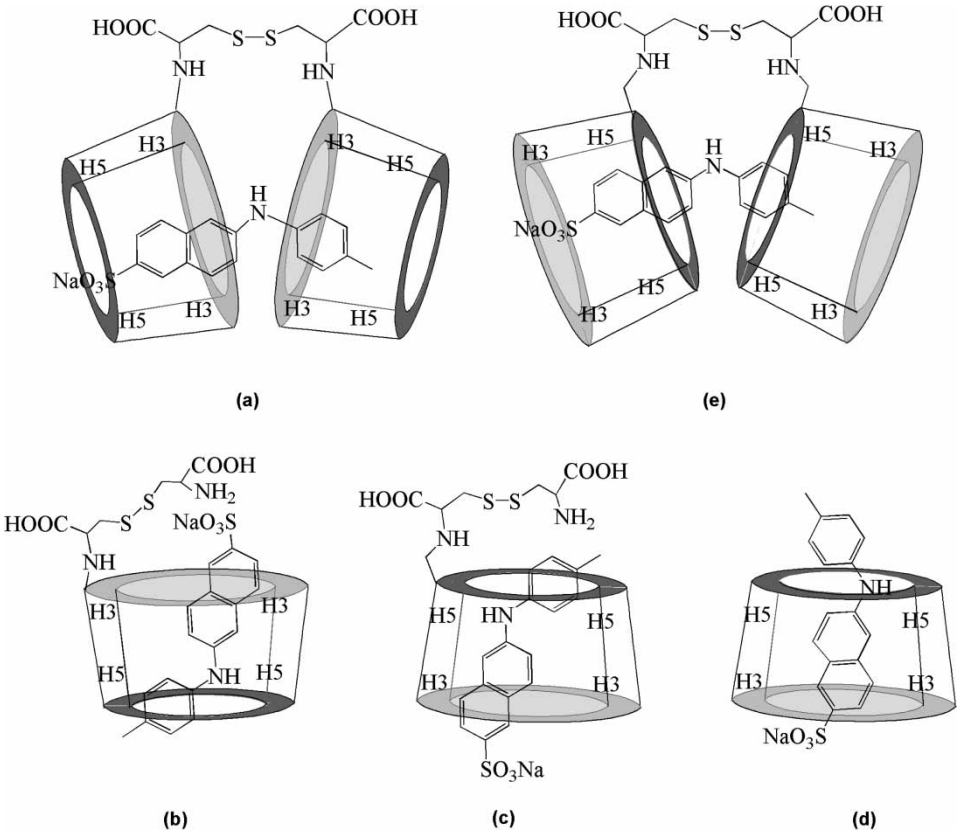


Figure 6. Inclusion complexation modes of TNS with host **2**(a), with host **3**(b), with host **5**(c), with host **1**(d) and with host **4**(e).

ring moiety show stronger interactions with the H-3 protons of CD than those of the toluene moiety. That is to say, the toluene moiety is close to the primary side and the naphthalene ring moiety is prone to reside at the secondary side. Much differently from dimer **2**, there is no correlation observed between the methyl protons of TNS and the interior protons of monomer **3** and **5**, which indicates that the methyl of TNS penetrates out of the CD cavity. Thus, the possible binding modes for hosts **3** and **5** with TNS were inferred as shown in Figure 6(b) and 6(c).

Furthermore, we minimised the complexes of TNS and hosts **1**–**5** using molecular mechanics method and got their corresponding minimum energy configurations as shown in Figure 7, which are coincident with the results obtained by 2D NMR experiments. Taking together the previous binding modes of native β-CD and bis(β-CD) **4** with TNS Figure 6(d, e) into account (*19*), it can be concluded that: (a) for native and mono-modified CDs (both primary-side **5** and secondary-side **3**), TNS is included into their cavities from the secondary side with the methyl group throughout CD's cavity; (b) for bridged bis(β-CD)s **2** and **4**, both of them provide two β-CD cavity to form the sandwich inclusion complexes with TNS, while differently, the toluene and naphthalene ring moiety of TNS are, respectively, included by the two β-CD cavities from the secondary sides in host **2** but from the primary sides in host **4**.

Binding ability and selectivity

As can be readily recognised from Table 1, for most dye molecules, all modified CDs (both mono-modified and bridged) show stronger binding abilities than native β-CD. Abnormally, the binding constant of host **3** with TNS is less than that of native β-CD with TNS. Taking account of the binding mode of host **3** and TNS, the L-cystine group at the edge of the CD cavity embarrasses TNS to penetrate into the CD cavity from the secondary side, and the electrostatic repulsion between the negatively charged sidearm cystine moiety (the isoelectric point of cystine (pI = 5.05 (*33*)) and the present pH = 7.20) and the sulfonate group of TNS decreases the binding ability of host **3** with TNS. Furthermore, it is noticed that the binding abilities of bis(β-CD)s with the dye molecules are mostly stronger than those of mono-modified CDs (host **2** versus **3** and host **4** versus **5**), because bis(β-CD)s can enhance the original binding abilities to some extent through cooperative binding of two adjacent cavities and potential multiple recognition. For example, host **2** shows the

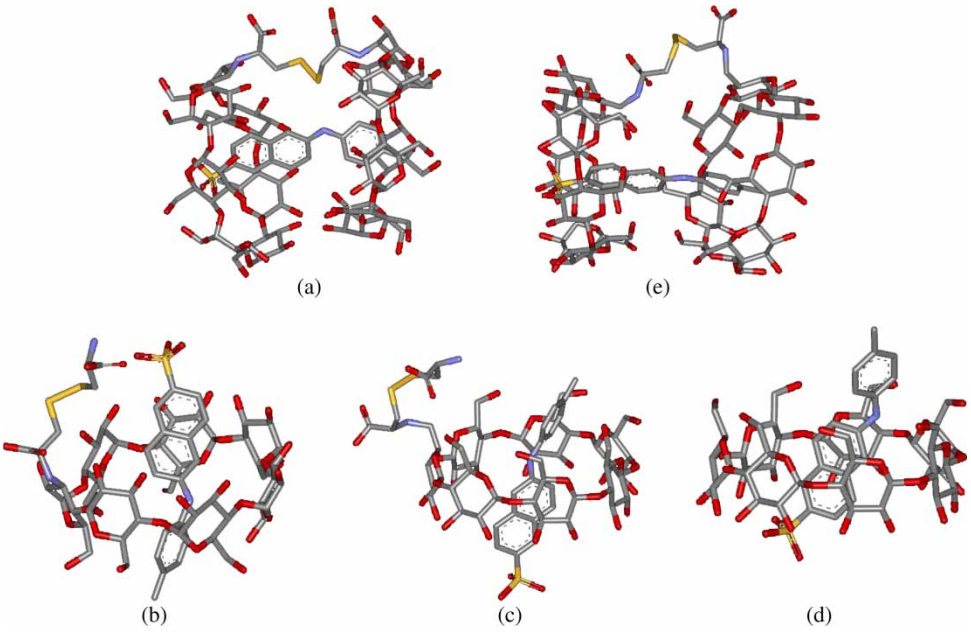


Figure 7. Possible structures of the inclusion complexes of TNS with host **2**(a), with host **3**(b), with host **5**(c), with host **1**(d) and with host **4**(e) constructed with molecular mechanics method.

highest affinity toward TNS (K_S(TNS-**2**) = 10040) in all of the inclusion complexes. This is attributable to the cooperative interactions of its two nearly located hydrophobic cavities. Naphthalene ring moiety and benzene ring moiety of TNS, respectively, penetrate into the two cavities of host **2** from the secondary side. There is also a reversed example for AR (K_S(AR-**4**) < K_S(AR-**5**)), and among all hosts **1**–**5**, host **5** gives the highest binding constant to AR as 7720 M^{-1}. Through further analyzing the isoelectric point of cystine (pI = 5.05), the negatively charged sidearm cystine moiety of host **5** can assistantly bind the protonated AR together with CD cavity at the present pH value (7.20), and therefore, AR fits with host **5** best of all. Meanwhile, host **3** also increases the binding ability with AR to a certain extent. However, the steric hindrance from the cystine moiety located at the secondary side of CD results in the relatively weaker combination of host **3** as comparison with host **5**. In the case of bridged bis(β-CD)s (host **2** and **4**), the cooperative binding by two hydrophobic CD cavities also makes them show stronger combination with AR than native β-CD.

We are especially interested in the comparison of the binding abilities between primary-side and secondary-side modified CDs. Host **2** shows stronger binding abilities for ANS, TNS, AR and EY. The two CD cavities of host **2** locate closer than those of host **4**, because the spacer L-cystine molecule in host **2** connects at 3-position methenyl at the edge of CD cavity and that in host **4** at 6-position methenyl outside of CD cavity. Host **2** is favor of cooperative binding appropriate size/shape guests penetrating into their two CD cavities from the wide sides. As a typical example, the binding constant of host **4** with ANS is higher than that of native β-CD by a factor of 4.2, while that of host **2** is greater by a factor of 11.7. It is deduced that naphthalene ring moiety and benzene ring moiety of ANS are easier to penetrate into the two cavities of host **2** from the wide sides, respectively, than those of host **4** from the narrow sides. In the case of mono-modified CDs (host **3** and host **5**), host **3** shows weaker binding abilities for TNS, AR, NR and FL. The imaginable reason for the higher binding abilities of host **5** is that most of the guests take precedence of penetrating into CD cavity from its secondary side without the steric hindrance.

On the other hand, although AR and NR possess the similar linear structures with a positively charged heterocyclic anthracene centre, hosts **1**, **2**, **3** and **5** show much stronger binding abilities towards AR than NR (K_S(AR-**1**)/K_S(NR-**1**) = 5.5, K_S(AR-**2**)/K_S(NR-**2**) = 5.0, K_S(AR-**3**)/K_S(NR-**3**) = 5.6, K_S(AR-**5**)/K_S(NR-**5**) = 2.6). This may be attributed to the relative small substituents of AR making it easy to penetrate into the hydrophobic cavity of CD. It is somewhat unexpected that host **4** shows the reversed binding ability sequence for AR and NR (K_S(AR-**4**)/K_S(NR-**4**) = 0.80). It is thought that there should be a model of ternary binding sites recognition (two CD cavities and the linker group). In addition to the aryl moiety and the two methyl moiety, respectively, penetrating into CD cavities from the narrow sides, the electrostatic and hydrogen-bonding interactions between the positively charged moiety of NR and the carboxyl anions in the L-cystine modified β-CDs should be another important reason for the reversed binding ability sequence

for host **4** with AR and NR. However, the L-cystine group of host **2** is linked at the wide rims of CDs, and therefore the L-cystine group of host **2** is relatively farther from guest molecules, which is unfavourable to cooperatively bind NR guest together with cavities of CDs. Therefore, host **2** displays the normal sequence selectivity for AR and NR. For the larger dye molecules (EY and FL), these four L-cystine modified β-CDs can all form more stable complexes than the native β-CD, but all CDs show relatively weak binding abilities to them. The possible reason is the relatively poor size-fit between CD cavity and the triangular fragment of EY and FL. In addition, the negatively charged carboxyl groups of EY and FL also decrease the binding abilities of L-cystine modified CDs to some extent.

Conclusion

In conclusion, all the four L-cystine modified CDs **2**–**5** present the enhanced binding abilities for most dye guests, and the bridged bis(β-CD)s **2** and **4** can form more stable complexes with dye guests than mono-modified CDs **3** and **5**. It is interesting that the secondary-side bridged bis(β-CD)s **2** displays stronger binding abilities than its primary-side analogue **4** because host **2** can cooperatively bind appropriate size/shape guests to penetrate into its two closer CD cavities from the wide sides. However, the primary-side modified mono-CD **5** displays stronger binding abilities than its secondary-side analogue **3** for most of the guests taking precedence of penetrating into CD cavity from its secondary side without the steric hindrance. To sum up, primary-side and secondary-side modified/bridged CDs all have their respective advantages to bind various types of guests. So far, the secondary-side modified/bridged CDs are less reported than the primary-side ones, and the present comparison results enlighten us to design more modified/bridged CDs at the secondary sides in order to provide stronger binding ability and better selectivity for model guest molecules.

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