

# Cooperative molecular recognition of dyes by dyad and triad cyclodextrin–crown ether conjugates

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Received 24th February 2004, Accepted 25th March 2004  
First published as an Advance Article on the web 26th April 2004

Three  $\beta$ -cyclodextrin ( $\beta$ -CyD) derivatives with crown ether units, that is *N*-(4'-benzo-15-crown-5)-6-imino-6-deoxy- $\beta$ -CyD (**2**), 6,6'-[*N*-(4,4'-dibenzo-18-crown-6)-imino]-bridged bis( $\beta$ -CyD) (**3**), and 2,2'-[*O*-(4',5'-benzo-15-crown-5)-ethyl]-bridged bis( $\beta$ -CyD) (**5**), were synthesized as cooperative recognition receptor models. Their molecular binding behavior with four representative fluorescent dyes, *i.e.*, ammonium 8-anilino-1-naphthalenesulfonate (ANS), sodium-6-toluidino-2-naphthalene-sulfonate (TNS), Acridine Red (AR) and Rhodamine B (RhB), was investigated in buffer solutions (pH = 7.20) at 25 °C by means of circular dichroism, NMR and fluorescence spectroscopy. 2D-ROESY experiments showed that dyad host **2** and triad host **3** adopted a CyD–guest–crown ether binding mode, while triad host **5** adopted a CyD–guest–CyD binding mode, upon inclusion complexation with guest molecules. Therefore, hosts **2** and **3** showed high molecular recognition ability towards charged guests, giving an enhanced binding ability up to 115 times for ANS by **3** and fairly high molecular selectivity up to 1450 times for the ANS/AR pair by **2** as compared with native  $\beta$ -CyD in an aqueous phosphate buffer solution. On the other hand, host **5** was found to be able to effectively recognize the shape of a guest molecule, showing significantly higher binding ability towards linear guests. The binding affinities and molecular recognition abilities of these CyD–crown ether conjugates towards guest molecules are discussed from the viewpoint of electrostatic and/or hydrophobic interactions, size/shape-fit concept, and multiple recognition mechanism between host and guest.

## Introduction

Selective binding of guest molecules by synthetic receptors in an aqueous medium is a fascinating topic in science and technology,<sup>1</sup> and how to obtain highly selective functional systems is still the major goal in molecular recognition field. In the past few decades, both cyclodextrins (CyD) and crown ethers have been successfully employed as effective guest selectors.<sup>2–10</sup> Recently, several efforts on CyD–crown ether conjugates, which simultaneously exhibit the superiority of these two kinds of supramolecular hosts, have been made by several research groups.<sup>4,11–17</sup> Crown ether capped  $\beta$ -CyDs were reported to be able to mimic the receptor sites of enzymes,<sup>12</sup> and thus used as excellent artificial enzyme-mimetic systems to accelerate the hydrolysis of *p*-nitrophenyl ester in the presence of transition metal cations.<sup>13</sup> Also they were used as stationary-phase selectors for chromatography.<sup>14</sup> Diaza-18-crown-6 tethered  $\beta$ -CyDs exhibited good binding ability toward *p*-nitrophenolate in the presence of alkali metal cations<sup>12</sup> and were utilized for realizing energy transfer from benzene accommodated in a CyD cavity to transition metal cations bound by an azacrown unit.<sup>15</sup> Suzuki *et al.* reported the strong binding of tryptophan by crown ether-tethered CyDs, attributing to the superiority of the CyD secondary side modification.<sup>16</sup> Moreover, Lincoln and co-workers reported the inclusion complexation of Brilliant Yellow tetraanions and their sodium analogues with diaza-coronand linked  $\beta$ -CyD dimer.<sup>17</sup> These investigations mostly focused on the synthesis of azacrown ether-tethered mono-modified CyDs and their complexation towards some organic small molecules, revealing an enhanced molecular binding ability and ion pairing interaction mechanism.<sup>11–17</sup> However, less attention has been paid to the molecular recognition of benzocrown ether-tethered mono and bridged  $\beta$ -CyDs,<sup>16</sup> to the best of our knowledge. Compared with azacrown ether, using a benzocrown ether unit as the modifying group can exclude any ambiguity exerted by charged ammonium nitrogen atoms of azacrown ethers upon complexation with a guest molecule.

Now, we wish to report the results of our investigation into the syntheses of benzocrown ether-tethered mono  $\beta$ -CyD (**2**) and bridged bis( $\beta$ -CyD)s (**3** and **5**) (Chart 1) and their molecular recognition behavior with fluorescent dyes (Chart 2), *i.e.* ammonium 8-anilino-1-naphthalenesulfonate (ANS), sodium-6-toluidino-2-naphthalene-sulfonate (TNS), Acridine Red (AR) and Rhodamine B (RhB), in an aqueous NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub> buffer and a Tris–HCl buffer solution (pH = 7.20) at 25 °C. It is significant to note that, in the NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub> buffer, the crown ether moiety can coordinate with Na<sup>+</sup> forming a cationic electrostatic cap near the CyD rim and thus affect the binding ability of CyD–crown ether conjugates through cooperative attracting or anti-cooperative repulsive interactions toward a charged guest molecule, which will add a new dimension to the molecular recognition of such potentially ternary systems. Moreover, the comparative study on these dyad and triad hosts

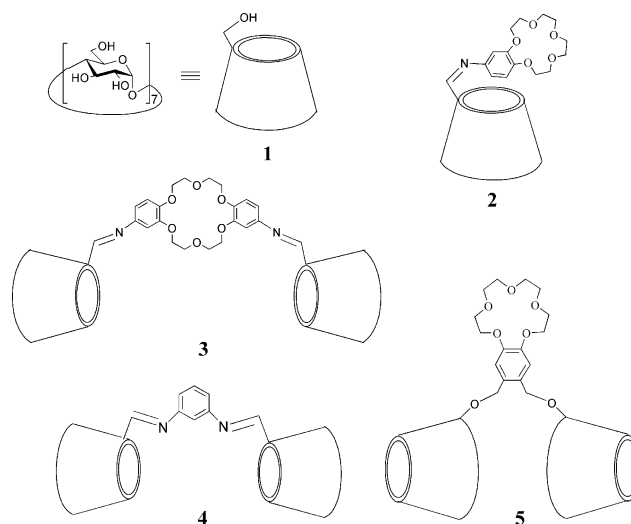


Chart 1 Host structures.

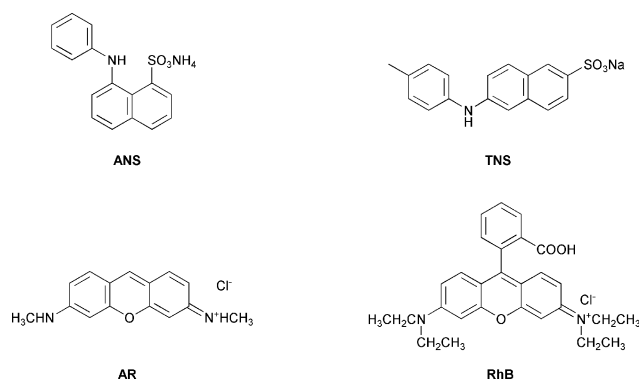


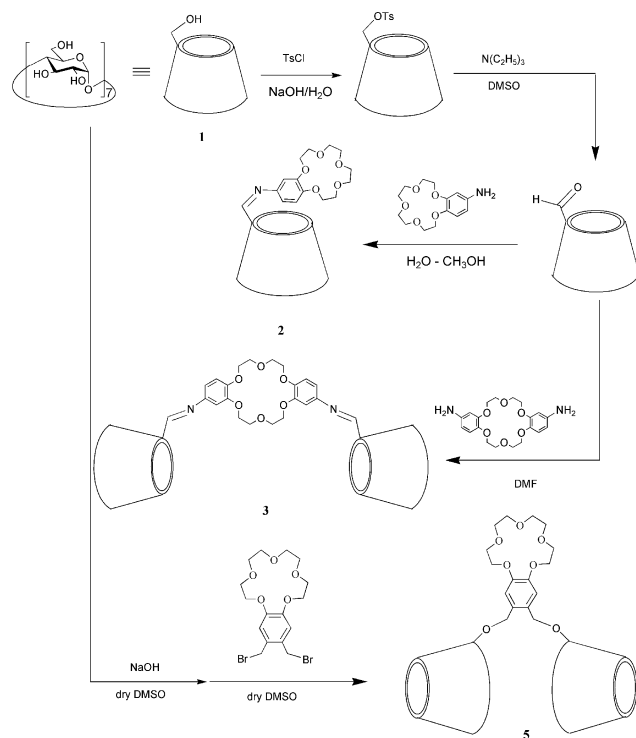
Chart 2 Guest structures.

containing the entirely different recognition sites of crown ether and  $\beta$ -CyD will certainly lead not only to enhancement of both affinity and selectivity for specific guests but also to deeper and more precise understanding of cooperative multiple interactions in natural and artificial supramolecular recognition systems.

## Results and discussion

### Syntheses

As shown in Scheme 1, the benzocrown ether-tethered mono- $\beta$ -CyD (**2**) and bridged bis( $\beta$ -CyD) **3** were synthesized in relatively low yields from 6-deoxy-6-formyl- $\beta$ -CyD. This is reasonable since the steric hindrance from the bulky CyD unit will be unfavorable to the condensation reaction between 6-deoxy-6-formyl- $\beta$ -CyD and amino-benzocrown ether to a large extent. Moreover, examination by NMR and UV-vis shows that no breakage of the C=N bond occurs during the binding process, which means that the imine linkers in **2** and **3** are stable upon complexation with guest molecules.



Scheme 1 Synthetic routes.

On the other hand, the secondary-linked CyD dimer **5** was synthesized through a procedure similar to the literature method for the xylene bridged secondary CyD dimers.<sup>18</sup> As explained in previous reports,<sup>19</sup> the substituted position of the

benzocrown ether bridge can be unambiguously determined by <sup>13</sup>C NMR spectrum (Fig. 1). The upfield-shifted signal at 100.8 ppm is assigned to the C1' position, and the large downfield-shifted signal at 79.5 ppm is assigned to the C2' position. However, the C3' position is not assigned. The C3' position overlaps the C5 position due to a small upfield shift. Generally, a downfield shift of the  $\alpha$ -carbon and an upfield shift of the  $\beta$ -carbon are caused by alkylation of a hydroxyl group of the C2 position.<sup>18,19</sup> Moreover, three signals at the aromatic region indicate the benzocrown ether bridge with the expected symmetric pattern. The integrated intensities from the <sup>1</sup>H NMR spectra are consistent with the number and type of protons in bis( $\beta$ -CyD) **5**. Microanalytical data of **5** are consistent with the proposed structures. From this evidence, we conclude that the benzocrown ether group is introduced at the C2 position of CyD.

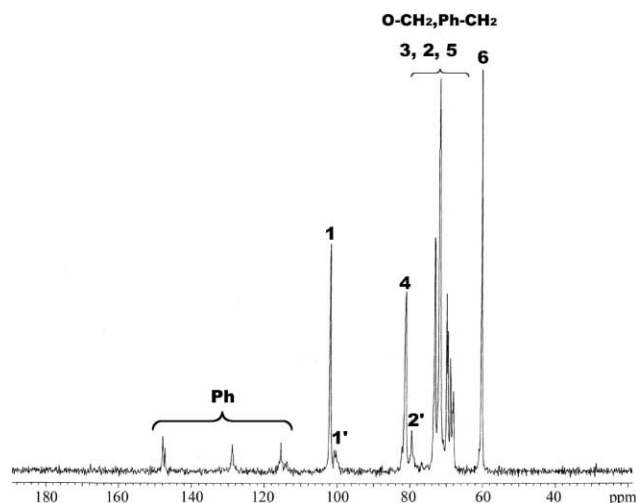


Fig. 1 <sup>13</sup>C NMR spectrum (both the carbohydrate region and aromatic region) of **5** in D<sub>2</sub>O at 298 K.

### Circular dichroism (CD) spectra

To examine the original conformation of CyDs **2–5**, their CD spectra were performed in a dilute aqueous phosphate buffer solution (pH = 7.20). As can be seen from Fig. 2, hosts **2–5** display obviously different CD spectra in the absence of a guest, indicating that significant but different interactions exist between the aromatic tether and the chiral CyD cavity. Bis( $\beta$ -CyD) **4** exhibits a moderate positive Cotton effect peak ( $\Delta\epsilon = +1.1 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ) at 219 nm and a weak negative Cotton effect peak ( $\Delta\epsilon = -0.62 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ) at 288 nm, which belong to the <sup>1</sup>L<sub>a</sub> band and <sup>1</sup>L<sub>b</sub> band respectively, indicating that the aromatic chromophore of CyD dimer **4** shallowly perches over the rim of  $\beta$ -CyD cavity. Interestingly, benzocrown

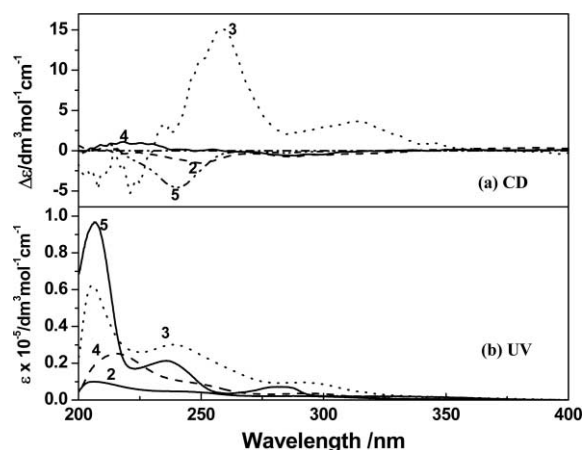
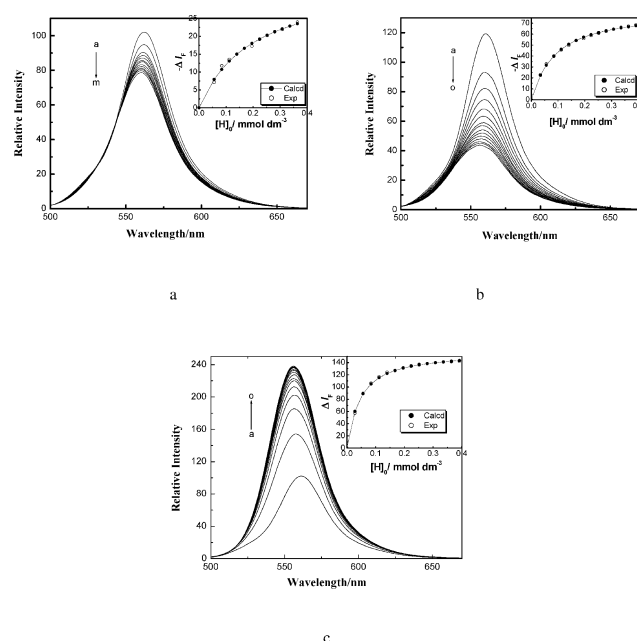


Fig. 2 Circular dichroism and UV-vis spectra of modified  $\beta$ -CyDs **2–5** (0.1 mM) in an aqueous phosphate buffer solution (pH = 7.20) at 25 °C.

ether modified mono (**2**) and dual CyDs (**3** and **5**) display quite different CD signals. Both **2** and **5** give two negative Cotton effect peaks for the  $^1L_a$  and  $^1L_b$  bands of phenyl chromophore(s). The  $\Delta\epsilon$  values are  $-1.541 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  at 250 nm for the  $^1L_a$  band of **2**,  $-0.638 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  at 281 nm for the  $^1L_b$  band of **2**,  $-4.572 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  at 240 nm for the  $^1L_a$  band of **5**, and  $-0.281 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  at 285 nm for the  $^1L_b$  band of **5**. However, host **3** presents two positive Cotton effect peaks at 259 nm ( $\Delta\epsilon = +15.070 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ) and 313 nm ( $\Delta\epsilon = +3.611 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ) as well as a negative Cotton effect peak at 222 nm ( $\Delta\epsilon = -4.858 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ). According to the empirical rules proposed by Kajtar *et al.*,<sup>20</sup> Harata and Uedaira,<sup>21</sup> and Kodaka<sup>22</sup>, we can deduce that two phenyl chromophores of dibenzo-18-crown-6 linker in host **3** may be partly self-included into the  $\beta$ -CyD cavities. On the other hand, the negative Cotton effects observed in the CD spectra of **2** or **5** indicate that the phenyl group in the tether of host **2** or **5** is not embedded in the CyD cavity, but shallowly capping the entrance of the CyD. This conformation is further confirmed by the ROESY spectrum, in which no NOE cross-peaks between the aromatic protons of **2** or **5** and H-3 and/or H-5 of the CyD cavity can be found.

### Fluorescence behavior

We have recently reported the contrasting fluorescence behavior of linear guest molecule AR upon inclusion complexation with  $\beta$ -CyD and calixarenesulfonate, where the former host induces a marked enhancement in fluorescence intensity, while the latter causes a reduction in fluorescence intensity.<sup>23</sup> More recently, calix[4]arene-tethered mono- and bis( $\beta$ -CyD), which possess both CyD and calixarene moieties in a single host molecule, are also found to be able to induce the contrasting fluorescent behavior of AR upon inclusion complexation.<sup>24</sup> In the present case, hosts **2–5** also induce the contrasting fluorescent behavior of AR upon inclusion complexation. Under our experimental conditions using diluted AR, the fluorescence of AR is gradually quenched by the stepwise addition of host **2** or **3**, as shown in Fig. 3. In sharp contrast, the gradual addition of host **4** or **5** to a diluted solution of AR significantly enhances the fluorescence of AR. Moreover, the addition of the CyD–crown ether conjugates causes appreciable hypochromic shifts in the fluorescence band position upon quenching or enhancement of AR emission. This may be due to the enhanced microenvironmental hydrophobicity around the AR fluorophore, which indicates that the residue of AR is embedded in the CyD cavity apart from the bulk water. In the control experiment, we find that AR shows similar fluorescence behavior with the addition of hosts **2–5** in Tris–HCl buffer solution (pH 7.20) or pure water. Meanwhile, the fluorescence intensity of AR also decreases in  $\text{CH}_3\text{CN–H}_2\text{O}$  (1 : 4, v/v) upon addition of the parent 4'-aminobenzo-15-crown-5 or 4,4'-diaminodibenzo-18-crown-6. These phenomena indicate that the decreases in fluorescence intensity of AR are mainly attributed to the complexation of AR with benzocrown ether moiety and not just to the simple quenching effect of the solvent. Therefore, we can deduce that hosts **2** and **3** adopt a cooperative CyD–guest–crown ether binding mode upon complexation with AR, where the benzocrown ether unit is actively incorporated in the inclusion complexation, and the increased microenvironmental polarity or hydrophilicity around the AR chromophore arising from cation–dipole and/or hydrogen-bond interactions between the AR cation and the benzocrown ether moiety, results in the decreased fluorescence of AR. On the other hand, for CyD–crown ether conjugate **5**, the benzocrown ether moiety in **5** does not actively participate in the binding with AR but merely acts a rigid tether to link two CyDs. In this case, the AR fluorophore is more effectively protected from the deactivating water attack by the cooperative binding of two CyD cavities, which consequently leads to the enhanced fluorescence of AR. Simul-

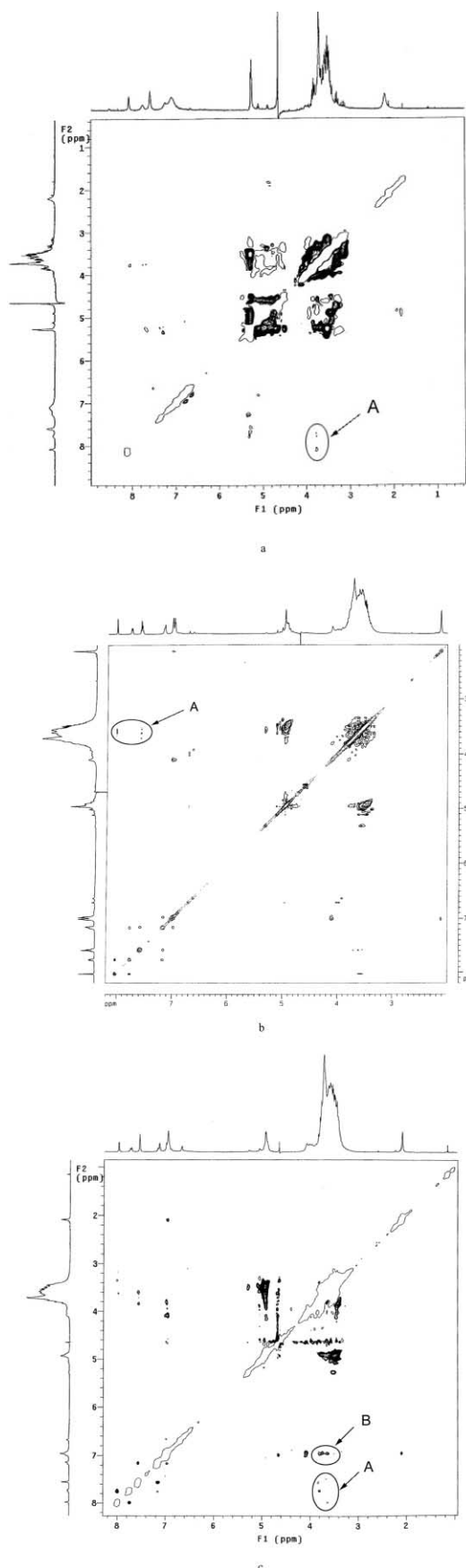


**Fig. 3** Fluorescence spectral changes of AR (12  $\mu\text{M}$ ) and the non-linear least-squares analysis (inset) of the differential intensity ( $\Delta I_F$ ) used to calculate the complex stability constant ( $K_S$ ) upon addition of (a) bis( $\beta$ -CyD) **3** (0–400  $\mu\text{M}$  from a to m) in an aqueous phosphate buffer solution (pH = 7.20); (b) bis( $\beta$ -CyD) **3** (0–400  $\mu\text{M}$  from a to o) in an aqueous Tris–HCl buffer solution (pH = 7.20); (c) bis( $\beta$ -CyD) **5** (0–400  $\mu\text{M}$  from a to o) in an aqueous Tris–HCl buffer solution (pH = 7.20).

aneously, the hypochromic shift of AR emission in the case of host **5** (8 nm) is larger than those for hosts **2** and **3** (less than 4 nm), which also indicates stronger microenvironmental hydrophobicity in the **5**/AR system than in the **2**/AR or **3**/AR systems.

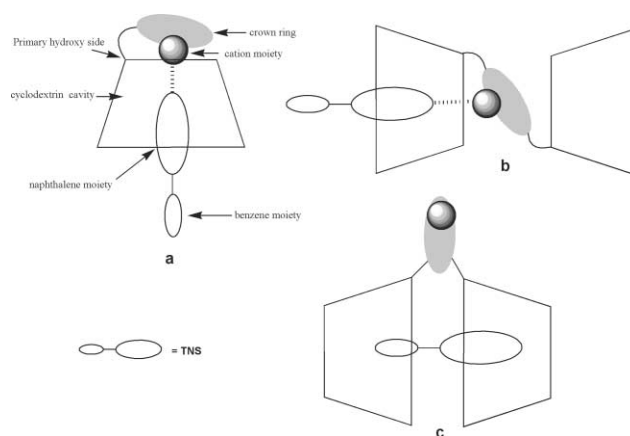
### ROESY spectra

Since two protons located closely in space can induce an NOE cross-peak between the relevant protons in the NOESY or ROESY spectrum, we performed 2D NMR experiments to further investigate the CyD–guest–crown ether or CyD–guest–CyD binding mode between CyD–crown ether conjugate and guest molecule. Fig. 4 illustrates the ROESY spectra of hosts **2**, **3** and **5** in the presence of guest TNS. The simple reason for choosing TNS as the guest to examine the binding geometry of CyD–crown ether conjugate with a guest molecule is that TNS possesses two different aromatic fragments, *i.e.* phenyl and naphthalene groups, and their chemical shifts can be easily recognized in the  $^1\text{H}$  NMR spectrum. As shown in Fig. 4a, the ROESY spectrum of an equimolar mixture of host **2** and TNS (2 mM each) displays clear NOE cross-peaks between the aromatic protons of naphthalene in TNS and the H-3/H-5 of CyD (peaks A), while no NOE cross-peak is found between the methylphenyl protons of TNS and any protons of **2**. In addition, examination on a Corey–Pauling–Koltun (CPK) molecular model clearly demonstrates that the naphthalene group can be well accommodated in the CyD cavity, which is consistent with the 2D NMR experimental results. Similar ROESY results are observed in the case of **3** with TNS (Fig. 4b). From the above NMR results, together with the 1 : 1 **2**/TNS or **3**/TNS inclusion complexation stoichiometry obtained from Job experiments, we can confirm that the CyD–crown ether conjugates **2** or **3** adopt a cooperative CyD–guest–crown ether binding mode upon complexation with TNS (Figs. 5a and 5b), where the  $\text{Na}^+$  ligated benzocrown ether, acting as a positively charged cap near the primary rim of CyD, attracts the anionic naphthalenesulfonate tail of TNS deeply penetrating into the CyD cavity through the electrostatic interactions, while the methylphenyl fragment of TNS is located outside.



**Fig. 4** ROESY spectra of hosts (a) **2**, (b) **3** and (c) **5** in the presence of TNS in a phosphate buffer solution ( $[\text{Host}] = [\text{TNS}] = 2 \text{ mM}$ ) with a mixing time of 400 ms at 298 K.

However, in the ROESY spectrum of the **5**/TNS system, both the naphthalene and phenyl protons of TNS show the clear NOE correlations with the interior protons (H-3/H-5) of

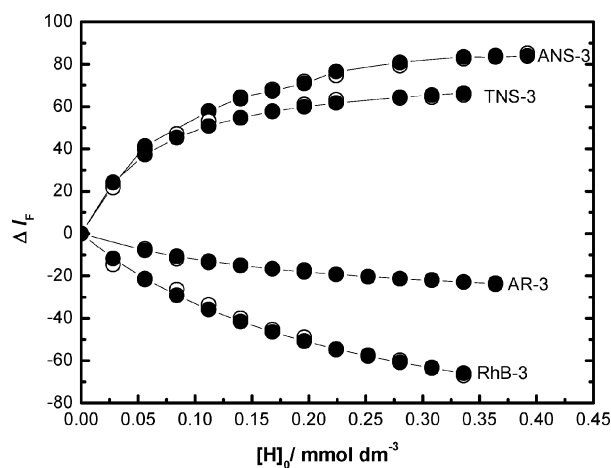


**Fig. 5** Schematic illustration of different binding modes of CyD-crown ether conjugates upon complexation with a guest.

CyD. That is, peak A represents the NOE correlations between the naphthalene protons of TNS and H-3/H-5 of CyD, and peak B represents the NOE correlations between the phenyl protons of TNS and H-3/H-5 of CyD. This ROESY information demonstrates that the TNS molecule is cooperatively bound by two CyD cavities, which unambiguously confirms the CyD-guest-CyD binding mode as illustrated in Fig. 5c.

#### Binding ability and molecular selectivity

For a more qualitative assessment of the inclusion complexation behavior of these CyD-crown ether conjugates, spectral titration experiments of hosts **1–5** with selected guest molecules are performed at 25 °C in an aqueous phosphate or a Tris-HCl buffer solution (pH 7.20). Some representative spectra of fluorometric titrations are illustrated in Fig. 3. Validating 1 : 1 stoichiometry for all host-guest complexations by Job experiments, the complex stability constants ( $K_S$ ) can be calculated using a non-linear least square curve-fitting method.<sup>25</sup> The representative curve-fitting analyses of fluorescence titration of organic dyes with host **3** in an aqueous phosphate buffer solution at pH 7.20 are shown in Fig. 6. In the repeated measurements, the  $K_S$  values are reproducible within an error of  $\pm 5\%$ . The  $K_S$  values of complex formation obtained by the curve fitting are listed in Table 1.



**Fig. 6** Curve-fitting analyses of fluorescence titration of organic dyes with bridged bis( $\beta$ -CyD) **3** in an aqueous phosphate buffer solution at pH 7.20.

As can be readily recognized from Table 1, the binding constant for the complexation of each organic dye by native  $\beta$ -CyD **1** and  $\beta$ -CyD derivatives **2–5** in phosphate buffer and Tris-HCl buffer increases in the following order:

**Table 1** Complex stability constants ( $K_S$ ) for 1 : 1 inclusion complexation of organic dyes with hosts **1–5** in aqueous buffer solution (pH = 7.20) at 25 °C

Host	Guest	$\lambda_{\text{ex}}^{\text{F}}/\text{nm}$	${}^c K_S$	${}^d K_S$	Ref.
<b>1</b>	ANS	350	103 <sup>b</sup>	103 ± 5	<i>a</i>
<b>1</b>	TNS	366	3670 <sup>b</sup>	3670 ± 50	<i>a</i>
<b>1</b>	AR	490	2630 <sup>b</sup>	2630 ± 30	<i>a</i>
<b>1</b>	RhB	525	4240 <sup>b</sup>	4240 ± 50	<i>a</i>
<b>2</b>	ANS	350	9020 ± 300	5580 ± 150	<i>a</i>
<b>2</b>	TNS	366	10500 ± 400	8950 ± 200	<i>a</i>
<b>2</b>	AR	490	156 ± 5	1010 ± 30	<i>a</i>
<b>2</b>	RhB	525	910 ± 30	2020 ± 60	<i>a</i>
<b>3</b>	ANS	350	11800 ± 600	19000 ± 500	<i>a</i>
<b>3</b>	TNS	366	18000 ± 500	22000 ± 200	<i>a</i>
<b>3</b>	AR	490	5300 ± 200	12300 ± 500	<i>a</i>
<b>3</b>	RhB	525	4160 ± 100	4150 ± 100	<i>a</i>
<b>4</b>	ANS	350	3600 ± 150 <sup>b</sup>	3600 ± 200	<i>a</i>
<b>4</b>	TNS	366	9020 ± 400 <sup>b</sup>	9020 ± 300	<i>a</i>
<b>4</b>	AR	490	9420 ± 400 <sup>b</sup>	9420 ± 350	<i>a</i>
<b>4</b>	RhB	525	5180 ± 200 <sup>b</sup>	5180 ± 300	<i>a</i>
<b>5</b>	ANS	350	5000 ± 200	4710 ± 80	<i>a</i>
<b>5</b>	TNS	366	42800 ± 500	26500 ± 200	<i>a</i>
<b>5</b>	AR	490	27500 ± 400	26300 ± 400	<i>a</i>
<b>5</b>	RhB	525	5010 ± 100	8850 ± 400	<i>a</i>

<sup>a</sup> This work. <sup>b</sup> Reference 26. <sup>c</sup> In phosphate buffer (pH 7.20). <sup>d</sup> In Tris–HCl buffer (pH 7.20).

in phosphate buffer solution:

ANS: **1 < 4 < 5 < 2 < 3**

TNS: **1 < 4 < 2 < 3 < 5**

AR: **2 < 1 < 3 < 4 < 5**

RhB: **2 < 3 ≤ 1 < 5 < 4**

in Tris–HCl buffer solution:

ANS: **1 < 4 < 5 < 2 < 3**

TNS: **1 < 2 < 4 < 3 < 5**

AR: **2 < 1 < 4 < 3 < 5**

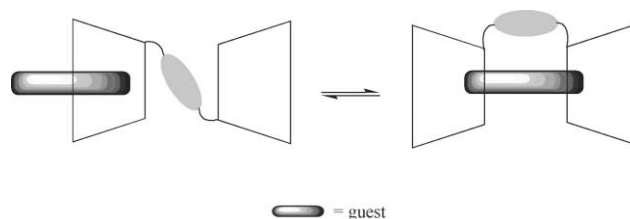
RhB: **2 < 3 ≤ 1 < 4 < 5**

From Table 1, we can see that CyD–crown ether conjugates **2** and **3**, both of which adopt a CyD–guest–crown ether binding mode upon complexation, display distinctly different binding ability, and hence significant discrimination, towards positively (AR and RhB) and negatively (ANS and TNS) charged guests in an aqueous phosphate buffer solution; that is, **2** and **3** show much higher  $K_S$  values toward ANS and TNS, but lower  $K_S$  toward AR and RhB than native  $\beta$ -CyD except for the complexation of **3** with AR. Although the ion strength of the buffer solution may affect the host–guest binding affinity to some extent, the very similar  $K_S$  values of  $\beta$ -CyD **1** or bis( $\beta$ -CyD) **4** for the selected guests in an aqueous phosphate or a Tris–HCl buffer solution indicate that the ion strength is not the main factor leading to the appreciable differences in the binding ability and molecular selectivity of CyD hosts in different buffer solutions. Hence, the exciting molecular recognition behavior of hosts **2** and **3** in a phosphate buffer solution may be mainly attributed to the positive contribution from the  $\text{Na}^+$  coordinated benzocrown ether unit in the CyD–guest–crown ether binding mode. Through an approximative calculation based on the relatively high concentration of  $\text{Na}^+$  ion ( $[\text{Na}^+] = 0.172 \text{ M}$ ) in a phosphate buffer solution and the low host concentration employed in the spectral titrations ( $[\text{host}] = 0 - 400 \mu\text{M}$ ) as well as the reported association constant between  $\text{Na}^+$  and benzo-15-crown-5 or dibenzo-18-crown-6 in water,<sup>27</sup> we deduce that the benzocrown ether unit in host **2** or **3** is mostly coordinated with  $\text{Na}^+$  ion forming a positively charged cap close to the primary side of CyD. This crown ether cap can subsequently provide the certain electrostatic attraction or repulsion interactions with charged guests and thus affect the host–guest binding abilities. For example, owing to the cooperative binding of two CyD cavities, hosts **4** and **5** effectively enhance the original binding ability of  $\beta$ -CyD for ANS by 35 and 48 times respect-

ively. However, benefiting from the electrostatic attraction between cationic crown ether cap and anionic ANS skeleton, hosts **2** and **3** display much higher  $K_S$  values for ANS; that is 88 and 115 times higher than  $\beta$ -CyD, respectively. On the other hand, the capping effect of crown ether attached to the CyD rim does not always favor the binding of CyD–crown ether conjugates with guests. For example, the inclusion complexation of host **2** with positively charged AR and RhB molecules gives negative results. As can be seen from Table 1, host **2** shows greatly decreased binding affinities toward AR and RhB as compared with native  $\beta$ -CyD **1**. One possible reason for the decreased binding ability is that, the electrostatic repulsion between the  $\text{Na}^+$  ligated crown ether unit and the positively charged guest (AR or RhB) skeleton prevents the penetration of the guest into the CyD cavity to some extent, which will consequently lead to reduced van der Waals and/or hydrophobic interactions between the CyD cavity and the guest, giving poor host–guest inclusion complexation. A further comparison of the binding abilities of **2** and **3** towards the selected guests shows that host **3** gives stronger binding affinities than **2** upon complexation. This should be reasonable, since dibenzo-18-crown-6 (the linker group of **3**) is reported to display stronger association with  $\text{Na}^+$  than benzo-15-crown-5 (the linker group of **2**) in water.<sup>27</sup>

Some control experiments gave additional evidence for the cap effect of the benzocrown ether unit. Firstly, we repeated the spectral titration experiments in a Tris–HCl buffer solution (pH 7.20). According to the literature, the association between  $\text{Na}^+$  (or  $\text{NH}_4^+$ ) and benzo-15-crown-5 (or dibenzo-18-crown-6) is quite weak in water ( $< 1.0 \times 10^2$ ).<sup>27,28</sup> Therefore, under our experimental conditions in spectral titrations ( $[\text{host}] = 0 - 400 \mu\text{M}$ ,  $[\text{dye}] = 12 \mu\text{M}$ ), only a negligible amount ( $< 0.1\%$ ) of CyD–crown ether host is coordinated with  $\text{Na}^+$ , which will greatly decrease the electrostatic capping effect of benzocrown ether units in hosts **2** and **3** upon complexation with charged guests. As can be seen from Table 1, host **2** displays lower  $K_S$  values for anionic guests (ANS and TNS) in a Tris–HCl buffer solution than in a phosphate buffer solution due to the decreased electrostatic attraction. On the other hand, lacking the unfavorable electrostatic repulsion between host and guest, host **2** exhibits obviously higher  $K_S$  values for cationic guests (AR and RhB) in Tris–HCl than in phosphate buffer solution. In another control experiment, the binding abilities of host **2** for anionic guests in a Tris–HCl buffer increases, but decreases in the case of cationic guests, by adding an excess amount of NaCl to the Tris–HCl system. These results jointly confirm the active impact of the charged crown ether cap on the charge recognition ability of CyD–crown ether conjugates.

It is also interesting to discuss the binding ability of host **3** for the selected guest in different buffer solutions. From Table 1, we can see that, different from the case of **2**, host **3** gives stronger binding affinities towards the selected guest molecules except for RhB in Tris–HCl buffer solution. This phenomenon reasonably accounts for the equilibrium between the CyD–guest–CyD and CyD–guest–crown ether binding mode of host **3** in Tris–HCl buffer solution (Fig. 7). That is, in a buffer solution without  $\text{Na}^+$  (in this work Tris–HCl), the crown ether unit of **3** may exist mostly in an uncoordinated form and its electrostatic cap effect seems to disappear. In this case, a part of benzocrown ether



**Fig. 7** Equilibrium between two binding modes of host **3** in Tris–HCl buffer solution.

bridged bis( $\beta$ -CyD) may tend to adopt a CyD–guest–CyD binding mode. In this binding mode, the guest molecule is cooperatively bound by two adjacent CyD cavities, which consequently results in strong host–guest hydrophobic interactions. As a joint result of these two binding modes, host **3** exhibits higher binding affinities in Tris–HCl than in a phosphate buffer solution. On the other hand, we have demonstrated in the previous section that the CyD–guest–crown ether binding mode may lead to decreased fluorescence of AR, while the CyD–guest–CyD mode may increase the fluorescence of AR upon complexation. Therefore, we can deduce that the CyD–guest–crown ether may predominate in the equilibrium in a Tris–HCl buffer solution judging from the quenched fluorescence of AR.

It is demonstrated that secondary-hydroxy side modified CyDs are superior to primary-modified CyDs in binding some guest molecules.<sup>16</sup> In the present case, secondary-bridged bis( $\beta$ -CyD) **5** also displays stronger binding ability than primary-bridged **3** and **4** in most of the host–guest inclusion complexations. This should be reasonable, since the number of secondary hydroxy groups is double that of the primary ones, which will lead to stronger hydrogen-bond interactions between host and guest. Another interesting point is that, differently from hosts **2** and **3**, CyD–crown ether conjugate **5** displays relatively poor charge recognition, but good shape recognition ability in either phosphate or Tris–HCl buffer solution. Among the hosts examined, host **5** gives the highest binding abilities for linear guests (TNS and AR). This may be attributed to the CyD–guest–CyD binding mode of **5** upon complexation with the guest molecules and the size/shape-fit concept between host and guest. According to this mode, linear guests such as TNS and AR, rather than bent guest ANS or T-shaped RhB, are able to fully enjoy the cooperative binding of two CyD cavities, giving stronger host–guest hydrophobic interactions. For example, host **5** displays the highest  $K_s$  value, up to 42800 M<sup>-1</sup> for TNS in a phosphate buffer solution. Examinations on CPK molecular models indicate that the longer guest TNS fits to the size of bis( $\beta$ -CyD) **5** better than other guests, and the hydrophobic phenyl and naphthalene fragments of TNS can be embedded deeply into the two  $\beta$ -CyD cavities of **5** upon complexation, which also contributes to stronger association of TNS with host **5**.

In summary, ascribed to the electrostatic capping effect of the crown ether unit, benzocrown ether-modified  $\beta$ -CyDs **2** and **3** as ditopic receptors significantly extend the original molecular recognition ability of parent  $\beta$ -CyD for charged guest molecules, and the ANS/AR selectivity is greatly enhanced from 0.04 by  $\beta$ -CyD to 58 by **2** in an aqueous phosphate buffer solution. On the other hand, the secondary-linked bis( $\beta$ -CyD) **5**, which adopts a CyD–guest–CyD binding mode upon inclusion complexation with guest molecules, exhibit strong binding abilities toward linear guests because of the cooperative binding by two CyD cavities. Although this is a preliminary study dealing with a limited number of host–guest pairs, the present results should be of particular interest and importance in designing functional molecular receptors with high binding ability and/or specific molecular selectivity. Based on the cooperative binding of crown ether and CyD, further studies are currently in progress concerning the cooperative, multipoint/multimode recognition of sophisticated systems.

## Experimental

### General

Mono[6-*O*-(*p*-toluenesulfonyl)]- $\beta$ -CyD was prepared by the reaction of  $\beta$ -CyD with *p*-toluenesulfonyl chloride in aqueous alkaline solution.<sup>29</sup> 6-deoxy-6-formyl- $\beta$ -CyD, 4'-aminobenzo-15-crown-5, 4,4'-diaminodibenzo-18-crown-6 and 4,4'-bis-(bromomethyl)benzo-15-crown-5 were prepared according to the reported procedure.<sup>30</sup> *m*-Phenylenediiminobridged bis-

(6-imino-6-deoxy- $\beta$ -CyD) (**4**) was synthesized according to our previously reported procedure.<sup>26</sup> Elemental analyses were performed on a Perkin-Elmer-2400C instrument. NMR spectra were obtained on a Varian Mercury VX300 and/or a Bruker AV600 instrument. Circular dichroism (CD) and UV-vis spectra were recorded in a conventional quartz cell (light path 10 mm) on a JASCO J-715S spectropolarimeter and a Shimadzu UV-2401PC spectrophotometer equipped with a PTC-348WI temperature controller to keep the temperature at 25 °C, respectively. 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. Excitation and emission slits of 5 nm were used for all the fluorescent dyes. The excitation wavelengths for ANS, TNS, AR, and RhB were 350, 366, 490 and 525 nm, respectively. In the fluorescence titration experiments, the concentration ranges of dyes and CyDs were 2–12  $\mu$ M and 28–450  $\mu$ M, respectively. Disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in deionized, distilled water to make a 0.10 M aqueous phosphate buffer solution of pH 7.20. Tri(hydroxymethyl)aminomethane (Tris) and HCl were dissolved in deionized, distilled water to make a pH 7.20 aqueous Tris–HCl buffer solution ( $I = 0.05$  M).

### Preparation of *N*-(4'-benzo-15-crown-5)-6-imino-6-deoxy- $\beta$ -CyD (**2**)

6-Deoxy-6-formyl- $\beta$ -CyD (0.5 g) and 4'-amino-benzo-15-crown-5 (0.14 g) were dissolved in 1 : 2 (v/v) H<sub>2</sub>O–CH<sub>3</sub>OH (30 mL), and several drops of acetic acid were added to catalyze the reaction. The reaction mixture was stirred at room temperature for 8 hours and then at 80 °C under a nitrogen atmosphere for 3 days. The solvent was evaporated under reduced pressure to dryness on a rotary evaporator. The residue was dissolved in a small amount of water, and subsequently the resultant solution was poured into acetone with vigorous stirring to produce a brown-yellow precipitate. After collection by filtration, the crude product was purified by column chromatography on Sephadex G-25 with distilled, deionized water as eluent to give a pure sample (0.11 g, yield 15%). Anal. Calcd for C<sub>56</sub>H<sub>87</sub>O<sub>39</sub>N·6H<sub>2</sub>O: C, 44.65; H, 6.62; N, 0.93. Found: C, 44.57; H, 6.14; N, 0.91%. <sup>1</sup>H NMR (D<sub>2</sub>O, 300 MHz, TMS, ppm):  $\delta$  3.0–4.0 (m, 58 H), 5.2 (m, 7 H), 6.0–6.9 (m, 3 H). <sup>13</sup>C NMR (D<sub>2</sub>O, 75 MHz, ppm):  $\delta$  189.5, 148.5, 146.8, 124.5, 112.0, 101.9, 99.7, 81.2, 76.7, 74.6, 74.1, 73.4, 72.9, 71.6, 71.2, 69.4, 60.5. UV/vis (H<sub>2</sub>O)  $\lambda_{\max}/\text{nm}$  ( $\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ): 285 (2120).

### Preparation of 6,6'-[*N*-(4,4'-dibenzo-18-crown-6)-imino]-bridged bis( $\beta$ -CyD) (**3**)

Bis( $\beta$ -CyD) **3** was prepared by the reaction of 6-deoxy-6-formyl- $\beta$ -CyD (1.05 g) and 4,4'-diamino-dibenzo-18-crown-6 (0.17 g) in anhydrous DMF (20 mL) in the presence of acetic acid. The reaction mixture was stirred at 100 °C under a nitrogen atmosphere for 5 days. The solvent was evaporated under reduced pressure to dryness on a rotary evaporator. The residue was dissolved in water (15 mL), and subsequently the resultant solution was poured into 2 : 1 (v/v) acetone–ethanol (500 mL) with vigorous stirring to produce a gray precipitate. The above procedure was repeated twice. After collected by filtration, the crude product was purified by column chromatography on Sephadex G-25 with distilled, deionized water as eluent to give a pure sample (0.18 g, yield 14%). Anal. Calcd for C<sub>104</sub>H<sub>160</sub>O<sub>74</sub>·N<sub>2</sub>·20H<sub>2</sub>O: C, 41.88; H, 6.75; N, 0.94. Found: C, 41.47; H, 6.27; N, 1.17%. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz, TMS, ppm):  $\delta$  3.4–3.7 (m, 84 H), 3.7–4.1 (m, 16 H), 4.1–4.6 (m, 12 H), 4.8–5.1 (m, 14 H), 5.2–5.7 (m, 28 H), 6.2–6.9 (m, 6 H), 8.1 (s, 2 H). <sup>13</sup>C NMR (D<sub>2</sub>O, 75 MHz, ppm):  $\delta$  189.7, 147.8, 147.0, 124.4, 112.1, 101.9, 99.7, 98.4, 95.9, 81.1, 76.5, 74.3, 73.4, 71.6, 69.5, 67.2, 65.5, 60.4, 57.5. UV/vis (H<sub>2</sub>O)  $\lambda_{\max}/\text{nm}$  ( $\epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ): 238.6 (30080), 292.4 (9520).

## Preparation of 2,2'-[O-(4',5'-benzo-15-crown-5)-ethyl]-bridged bis( $\beta$ -CyD) (5)

A DMSO solution (10 mL) containing native  $\beta$ -CyD (2 mmol) and finely grounded NaOH (20 mmol) was stirred at 55 °C under nitrogen atmosphere for 0.5 hours. Then, 4, 4'-bis(bromomethyl)benzo-15-crown-5 (1 mmol) in dry DMSO (5 mL) was added dropwise within 10 minutes, and the resultant mixture was stirred for 4 hours at 55 °C. After removal of the solvent under reduced pressure on a rotary evaporator, the residue was dissolved in water and added to vigorously stirred acetone to produce a precipitate. The above procedure was repeated twice. After collected by filtration, the crude product was purified by column chromatography on Sephadex G-25 with distilled, deionized water as eluent to give a pure sample (0.56 g, yield 20%). Anal. Calcd for  $C_{100}H_{160}O_{75} \cdot 14H_2O$ : C, 42.68; H, 6.73. Found: C, 42.87; H, 6.63%.  $^1H$  NMR ( $D_2O$ , 300 MHz, TMS, ppm):  $\delta$  3.3–4.0 (m, 88H), 4.7 (s, 14 H), 6.6–6.9 (m, 2 H).  $^{13}C$  NMR ( $D_2O$ , 75 MHz, ppm):  $\delta$  147.9, 128.9, 115.4, 101.9, 100.8, 81.1, 79.5, 73.2, 71.8, 69.9, 69.5, 68.9, 68.0, 60.2. UV/vis ( $H_2O$ )  $\lambda_{max}/nm$  ( $\epsilon/dm^3 mol^{-1} cm^{-1}$ ): 235.8 (21400), 281.2 (7200).

## Acknowledgements

This work was supported by NNSFC (Nos. 90306009, 20272028 and 20372038), and Special Fund for Doctoral Program from the Ministry of Education of China (No. 20010055001), which are gratefully acknowledged. We also thank the referees for their highly valuable suggestions regarding the revision.

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