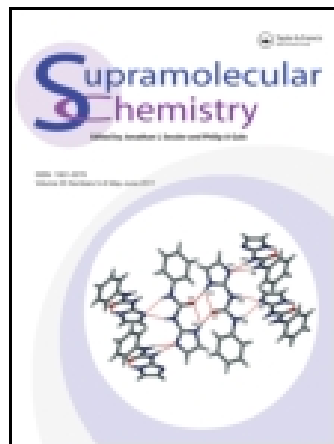


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### Molecular Recognition Studies on Supramolecular Systems 34. Synthesis of Aromatic Diamino-bridged Bis( $\beta$ -cyclodextrin)s and their Inclusion Complexation with Dye Molecules

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# Molecular Recognition Studies on Supramolecular Systems 34. Synthesis of Aromatic Diamino-bridged Bis( $\beta$ -cyclodextrin)s and their Inclusion Complexation with Dye Molecules

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A series of 6,6'-bis( $\beta$ -cyclodextrin)s with rigid aromatic diamino tethers, i.e. *p*-phenylenediamino-bridged-bis(6-amino-6-deoxy- $\beta$ -cyclodextrin) (3), 4,4'-biphenyl-bridged-bis(6-amino-6-deoxy- $\beta$ -cyclodextrin) (4) and 3,3'-biphenyl-bridged-bis(6-amino-6-deoxy- $\beta$ -cyclodextrin) (5), have been synthesized by the reaction of mono[6-*O*-(*p*-toluenesulfonyl)]-( $\beta$ -cyclodextrin) with corresponding materials. The inclusion complexation behavior of native  $\beta$ -cyclodextrin (1), mono-(6-anilino-6-deoxy)- $\beta$ -cyclodextrin (2), and novel bis( $\beta$ -cyclodextrin) 3–5 with some representative dyes, i.e. ammonium 8-anilino-1-naphthalenesulfonate (ANS), Brilliant Green, Methyl Orange, Acridine Red and Rhodamine B, was investigated at 25°C in aqueous phosphate buffer solution (pH 7.20) by means of fluorescence, ultraviolet, circular dichroism spectrometry as well as fluorescence lifetime measurement. The spectrophotometric titrations gave the complex stability constants ( $K_S$ ) and Gibbs free energy changes ( $\Delta G^0$ ) for the stoichiometric 1:1 inclusion complexation of hosts examined with dye molecules. As compared with 1 or 2, bridged bis( $\beta$ -cyclodextrin)s displayed significantly enhanced binding abilities towards these dyes. Typically, dimer 3 showed the highest binding ability upon inclusion complexation with acridine red affording 17 times higher  $K_S$  for 3 than for 1. The molecular binding abilities and selectivities of dyes by bridged bis( $\beta$ -cyclodextrin)s have been discussed from the viewpoint of induced-fit interaction and multipoint recognition mechanism.

**Keywords:** Molecular recognition; Bridged bis( $\beta$ -cyclodextrin); Cooperative binding; Inclusion complexation; Dye molecule

## INTRODUCTION

Possessing two hydrophobic cavities, the bridged bis( $\beta$ -cyclodextrin)s with simple tethers can remarkably enhance the original molecular binding ability of the parent  $\beta$ -cyclodextrin through the cooperative binding of one molecule in the closely located two  $\beta$ -cyclodextrin cavities [1–4], which provide an excellent model system mimicking the substrate-specific interaction of enzymes [5,6]. Recently, a variety of bridged bis( $\beta$ -cyclodextrin)s have been designed and synthesized in order to examine and compare the molecular recognition behavior between the parent  $\beta$ -cyclodextrin and bridged bis( $\beta$ -cyclodextrin)s and also to investigate the inclusion complexation mechanism from the viewpoints of multiple recognition and induced-fit interaction between host bis( $\beta$ -cyclodextrin)s and guest molecules, including contribution of the distance controls of bis(cyclodextrin)s [7–16]. We have recently shown that organoselenium and oligoethylenediamine bridged  $\beta$ -cyclodextrin dimers form more stable complexes with some representative dyes through the cooperative binding of one guest molecule by two  $\beta$ -cyclodextrin moieties. Furthermore, platinum (IV) or copper (II) complexes of these  $\beta$ -cyclodextrin dimers showed yet stronger binding abilities [17,18]. These results advanced our understanding of the cooperative effect of the several weak interactions working between two adjacent receptor units (host) and a substrate molecule (guest) and also prompted us to

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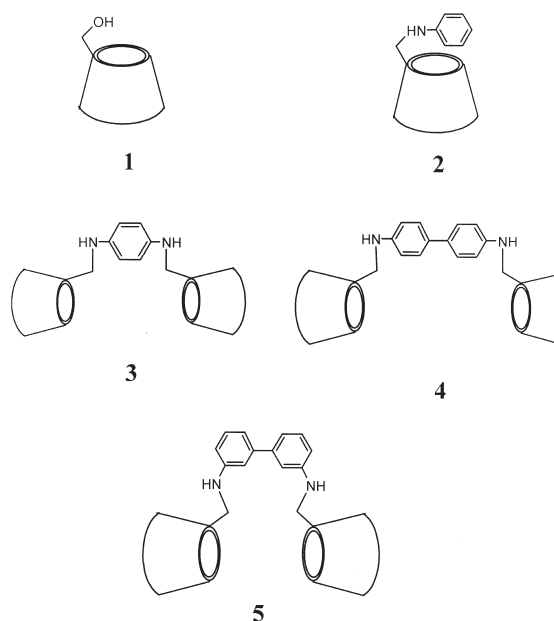


CHART 1

further investigate the inclusion complexation behavior of bis( $\beta$ -cyclodextrin)s.

In this paper we report our study on the synthesis and molecular recognition behavior of novel bridged bis( $\beta$ -cyclodextrin)s with rigid aromatic diamino linkers, shown in Chart 1. Their inclusion complexation behavior with some structurally related guest molecules (Chart 2) has been investigated by means of fluorescence, circular dichroism, ultraviolet spectroscopy as well as fluorescence lifetime measurement at 25°C in aqueous phosphate buffer solution (pH 7.20). The effect of tether length of two  $\beta$ -cyclodextrin moieties in the inclusion complexation according to the multipoint recognition and induced-fit concept between the dimeric host and model substrate is discussed.

## EXPERIMENTAL

### Instruments

Combustion analyses were performed on a Perkin-Elmer-240 instrument.  $^1\text{H-NMR}$  spectra were recorded in dimethyl sulfoxide- $d_6$  on a Bruker AC-P200 instrument operated at 200 MHz. FT-IR spectra were obtained on a Nicolet FT-IR 5DX spectrometer. Fluorescence spectra were measured in a conventional quartz cell ( $10 \times 10 \times 45 \text{ mm}^3$ ) 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. Circular dichroism (CD) spectra were measured in a conventional quartz cell ( $10 \times 10 \times 45 \text{ mm}^3$ ) on a JASCO J-720W spectropolarimeter equipped with a PTC-348WI temperature controller to keep the temperature at

25°C. UV-vis spectra were recorded in a conventional quartz cell ( $10 \times 10 \times 45 \text{ mm}^3$ ) at 25°C on a JASCO UV-550 spectrometer.

Fluorescence lifetimes were determined by the time-correlated single-photon-counting method using a Horiba NAES-550 instrument with a time resolution of 0.5 ns. A self-oscillating discharge lamp filled with hydrogen gas was employed as the pulsed light source, and the excitation light was made monochromatic by a 10 cm monochromator. The emission from the sample was passed through an appropriate filter (Toshiba UV-33) placed before the detector unit in order to eliminate scattered excitation light. Maximum counts of up to 10,000 were collected for each measurement. The accumulated signals were then processed and the lifetime determined by deconvolution with nonlinear least squares fit.

### Materials

ANS, Brilliant Green and Methyl Orange were purchased from Wako. Acridine red and rhodamine B were purchased from Tianjin Chemical Reagent Plant. All chemicals were reagent grade and used without further purification unless noted otherwise.  $\beta$ -Cyclodextrin of reagent grade (Shanghai Reagent Works) was recrystallized twice from water and dried in vacuo at 95°C for 24 h prior to use. *N,N*-dimethylformamide (DMF) was dried over calcium hydride for 2 days and then distilled under a reduced pressure prior to use. Mono[6-*O*-(*p*-toluenesulfonyl)]- $\beta$ -cyclodextrin was prepared by a reaction of  $\beta$ -cyclodextrin with *p*-toluenesulfonyl chloride in aqueous alkaline solution [19]. 6-Deoxy-6-formyl- $\beta$ -cyclodextrin was prepared according to the procedure reported by Yoon *et al.* [20]. Mono-(6-anilino-6-deoxy)- $\beta$ -cyclodextrin **2** was prepared according to our previous report [21]. Disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in distilled, deionized water to make a 0.10 M aqueous phosphate buffer solution of pH 7.20, which was used in the spectral measurements.

### Synthesis Of *p*-Phenylenediamino-Bridged Bis(6-amino-6-deoxy- $\beta$ -cyclo Dextrin) (3)

*p*-Phenylenediamine (0.11 g, 1 mmol) and 6-deoxy-6-formyl- $\beta$ -cyclodextrin (2.4 g, 2.1 mmol) were dissolved in dry DMF. The resultant mixture was stirred at 80–90°C for 48 h under nitrogen atmosphere. The solvent was evaporated under a reduced pressure to dryness. The residue was dissolved in a minimum amount of hot water, and then acetone was poured to the solution to give a dark brown solid. After drying, the solid was suspended in anhydrous methanol. Sodium borohydride (0.16 g, 4 mmol) was added to

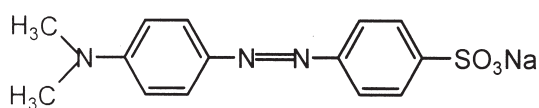
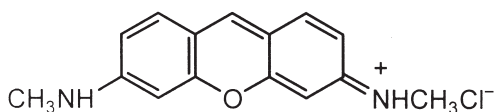
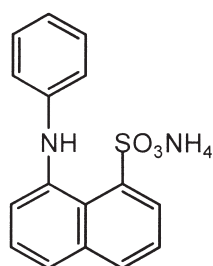
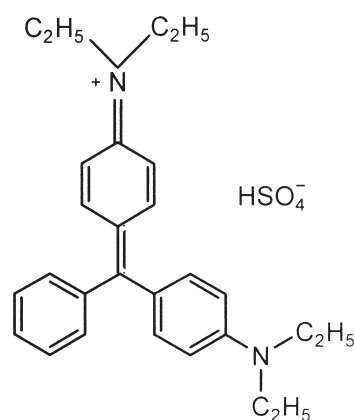
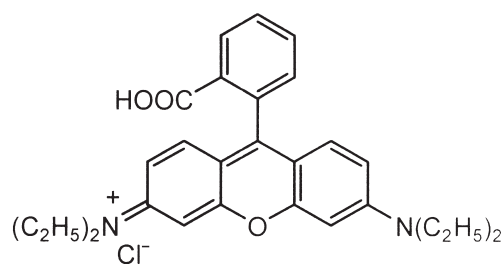
**Methyl Orange****Acridine Red****ANS****Brilliant Green****Rhodamine B**

CHART 2

the mixture, and the resultant mixture was stirred at room temperature over night. The precipitate was collected by filtration and then washed with methanol to give the crude product. The crude product was dissolved in water and acetone was added to give a brown solid, which was subsequently purified on a column of Sephadex G-25 to give **3** (0.24 g, 0.1 mmol) in 10% yield as a bright brown solid. FAB-MS:  $m/z$  2343( $M^+ + H$ ).  $^1H$  NMR (DMSO- $d_6$ , TMS):  $\delta$  3.1–3.9 (m, 84H), 4.8–5.0 (m, 14H), 6.68 (s, Ar 4H). FT-IR (KBr)  $\nu$   $cm^{-1}$  3298.6, 2937.7, 2900.9, 2839.5, 1650.4, 1418.0, 1324.3, 1240.8, 1203.5, 1153.0, 1078.6, 1028.7, 935.9, 855.5, 759.2, 707.4, 606.2, 577.1. UV/vis (water)  $\lambda_{max}/nm$  ( $\epsilon$   $M^{-1}cm^{-1}$ ) 251.0 (10770), 207.5 (10340). Anal. Calcd for  $C_{90}H_{144}O_{68}N_2 \cdot 4H_2O$ : C, 44.78; H, 6.35; N 1.16. Found: C, 45.04; H, 6.63; N, 1.42.

#### Synthesis of 4,4'-Bianilino-Bridged Bis(6-amino-6-deoxy- $\beta$ -cyclodextrin) (**4**)

4,4'-Benzidine (0.18 g, 1 mmol) and mono[6-*O*-(*p*-toluenesulfonyl)]- $\beta$ -cyclodextrin (2.8 g, 2.1 mmol)

were dissolved in dry DMF. The resultant solution was stirred at 80–90°C for 24 h under nitrogen atmosphere. The solvent was then evaporated under a reduced pressure to dryness. The residue was dissolved in a minimum amount of hot water, and then acetone was poured to the solution to give the crude product as a yellow precipitate. After drying, the crude product was purified on a column of Sephadex G-25 to give **4** (0.27 g, 0.1 mmol) in 10% yield as a yellowish solid. FAB-MS:  $m/z$  2441( $M^+ + Na$ ).  $^1H$  NMR (DMSO- $d_6$ , TMS):  $\delta$  3.2–3.9 (m, 84H), 4.8–5.0 (m, 14H), 7.3–7.8 (m, Ar 8H). FT-IR (KBr)  $\nu$   $cm^{-1}$  3312.1, 2931.1, 1635.0, 1414.7, 1333.7, 1302.7, 1241.9, 1201.1, 1154.8, 1078.4, 1031.0, 1002.1, 944.4, 854.9, 756.1, 707.3, 607.7, 577.7. UV/vis (water)  $\lambda_{max}/nm$  ( $\epsilon/M^{-1}cm^{-1}$ ) 288.0 (1790), 206.0 (4720). Anal. Calcd for  $C_{96}H_{148}O_{68}N_2 \cdot 16H_2O$ : C, 42.61; H, 6.71; N 1.03. Found: C, 42.62; H, 7.00; N, 0.91.

#### Synthesis of 3,3'-Bianilino-Bridged Bis(6-amino-6-deoxy- $\beta$ -cyclodextrin) (**5**)

Bis( $\beta$ -cyclodextrin) **5** was similarly prepared in 10% yield from 3,3-benzidine and mono[6-*O*-(*p*-toluene-

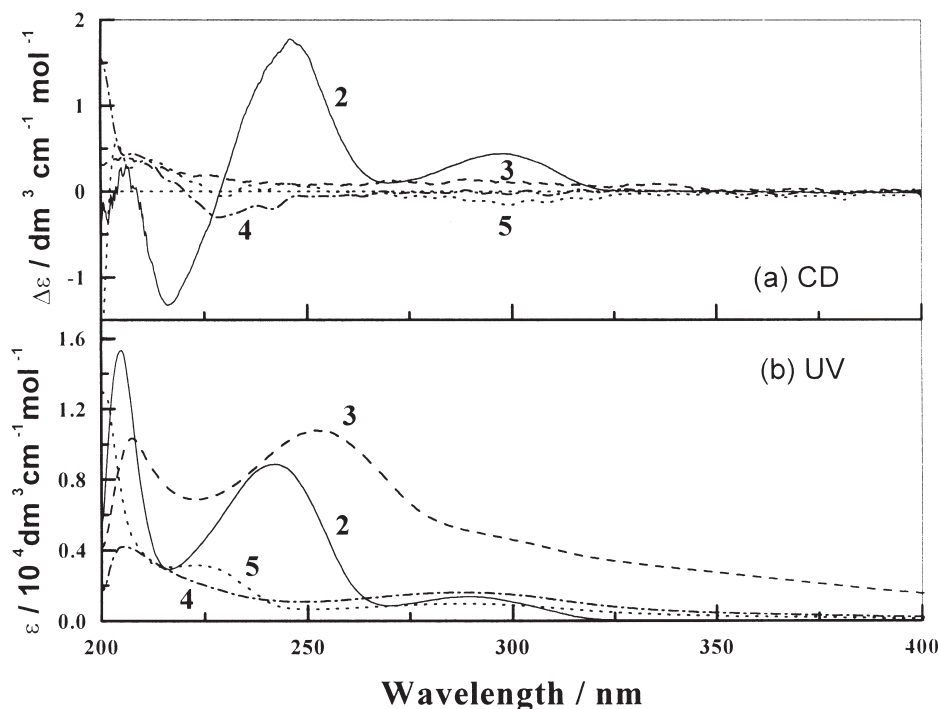


FIGURE 1 (a) Circular dichroism spectra and (b). absorption spectra of  $\beta$ -cyclodextrin derivatives 2–5 ( $1 \times 10^{-4}$  M) in aqueous phosphate buffer solution (pH 7.20) at 25°C.

sulfonyl)]- $\beta$ -cyclodextrin as a yellowish solid: FAB-MS  $m/z$  2441 ( $M^+ + Na$ );  $^1H$  NMR (DMSO- $d_6$ , TMS)  $\delta$  3.2–4.0 (m, 84H), 4.8–5.0 (m, 14H), 7.0–7.5 (m, Ar 8H). FT-IR (KBr)  $\nu$   $cm^{-1}$  3313.7, 2932.8, 2842.9, 1645.2, 1415.5, 1333.5, 1302.6, 1241.4, 1202.4, 1155.4, 1078.4, 1031.2, 1003.7, 944.6, 849.6, 756.6, 707.8, 607.7, 578.1. UV/vis (water)  $\lambda_{max}$  nm ( $\epsilon$   $M^{-1} cm^{-1}$ ) 291.0 (1080), 223.0 (3670). Anal. Calcd for  $C_{96}H_{148}O_{68}N_2 \cdot 16H_2O$ : C, 42.61; H, 6.71; N 1.03. Found: C, 42.63; H, 6.65; N, 0.97.

## RESULTS AND DISCUSSION

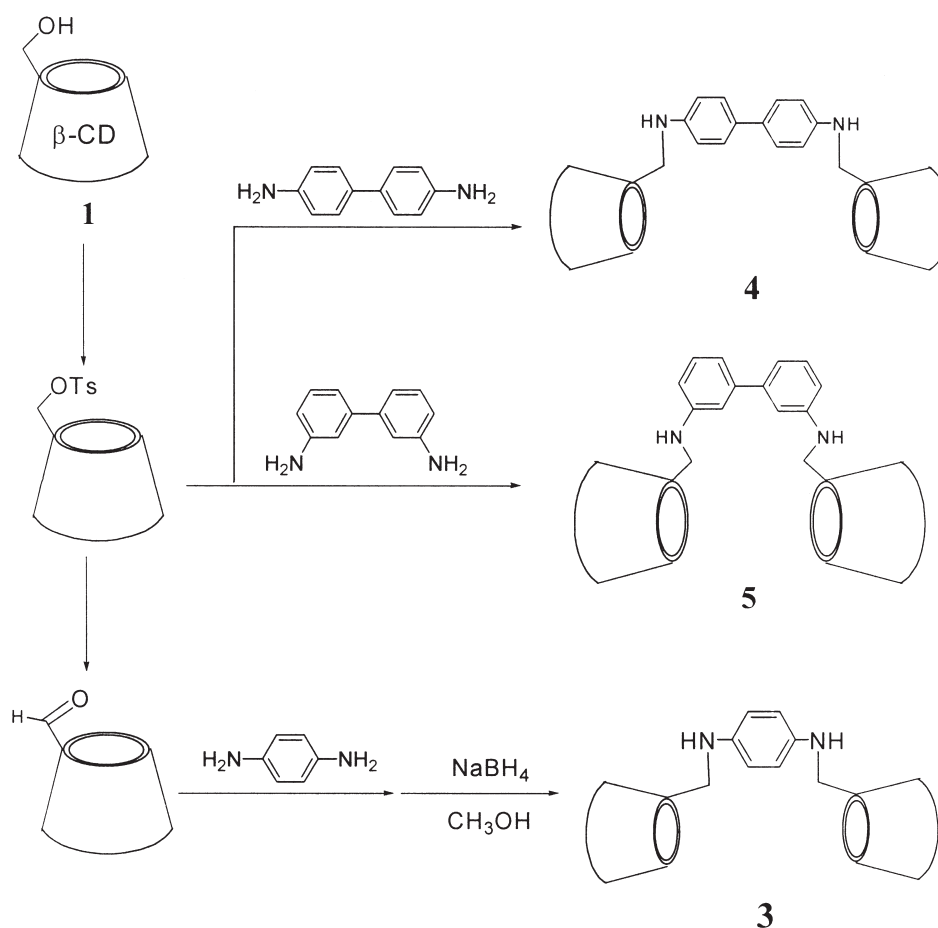
### Circular Dichroism Spectra

In order to obtain the information about the original conformation of bis( $\beta$ -cyclodextrin)s, induced circular dichroism (ICD) spectra of  $\beta$ -cyclodextrin dimers 3–5 were examined in aqueous phosphate buffer solution (pH 7.20). (Fig. 1) It is interesting and significant to note that, the ICD spectra of mono-(6-anilino-6-deoxy)- $\beta$ -cyclodextrin 2 displays a weak positive cotton effect peak ( $\Delta\epsilon = +0.454$ ) for the  $^1L_b$  transition at 297 nm and a strong positive cotton effect peak ( $\Delta\epsilon = +1.775$ ) for the  $^1L_a$  transition at 246 nm [22], while all the bis( $\beta$ -cyclodextrin)s show appreciate but fairly weak cotton effect peaks for both of  $^1L_a$  and  $^1L_b$  transitions of aromatic diamine chromophore; the observed  $\Delta\epsilon$  values are  $+0.133 M^{-1} cm^{-1}$  at 269 nm for the  $^1L_b$  transition

and  $+0.284 M^{-1} cm^{-1}$  at 215 nm for the  $^1L_a$  transition of 3,  $-0.302 M^{-1} cm^{-1}$  at 228 nm for the  $^1L_a$  transition of 4,  $-0.157 M^{-1} cm^{-1}$  at 299 nm for the  $^1L_b$  transition and  $0.093 M^{-1} cm^{-1}$  at 245 nm for the  $^1L_a$  transition of 5. According to the general rule for the ICD effects developed by Kajta [23], Harata [24] and Kodaka *et al.* [25], we can deduce that the aromatic diamine group is not embedded in the hydrophobic cavity of  $\beta$ -cyclodextrin, but shallowly perching over the rims of two  $\beta$ -cyclodextrin cavities, which will consequently favor the penetration of guest molecule into  $\beta$ -cyclodextrin cavities.

### Fluorescence Lifetime

It is well documented that the fluorescent dyes accommodated in the hydrophobic cavity of cyclodextrin will produce fluorescence enhancement and peak shifts as well as significantly elongated fluorescence lifetimes [17,26–28]. In the present case, we performed the nanosecond time-resolved fluorescence experiments with ANS in aqueous phosphate buffer solution (pH 7.20) in the presence or absence of  $\beta$ -cyclodextrin 1 or the bridged bis( $\beta$ -cyclodextrin)s 3–5 in order to assess the microenvironmental polarity around the included ANS and further understand the inclusion complexation behavior of bis( $\beta$ -cyclodextrin)s (Scheme 1).



SCHEME 1

Since the rate of complexation/decomplexation is much slower than that of the fluorescence decay, the decay profile of fluorescence intensity ( $F(t)$ ) can be described as the sum of unimolecular decays for all fluorescing species present in the solution

$$F(t) = \sum_{i=1}^n A_i \exp(-t/\tau_i) \quad (n = 1, 2, \dots) \quad (1)$$

where  $A_i$  and  $\tau_i$  represent the initial abundance and lifetime of the  $i$ th species. In the absence of the host, the fluorescence decay curve observed for ANS in aqueous phosphate buffer solution was

perfectly fitted to a single exponential function. On the contrary, the decay profile of ANS in the presence of β-cyclodextrin or bis(β-cyclodextrin)s could be analyzed only by a linear combination of two exponential functions. The short and long fluorescence lifetimes ( $\tau_S$  and  $\tau_L$ ) and relative quantum yields ( $\Phi$ ) observed for ANS in the presence of β-cyclodextrin or bis(β-cyclodextrin)s 3–5 are summarized in Table I. Judged from the two component decay obtained in the presence of native or dimeric β-cyclodextrins, we can deduce that the ANS molecule is located in two different environments, one of which is polar and the other nonpolar. Furthermore, the two different

TABLE I Fluorescence lifetime ( $\tau$ ) and relative quantum yield ( $\Phi$ ) of ANS in the absence and presence of native β-cyclodextrin and bis(β-cyclodextrin)s 3–5 in aqueous buffer solution (pH 7.20) at 25°C

ANS (μM)	Host	Equivalent	$\tau_S$ (ns)	$\Phi_S$ (%)	$\tau_L$ (ns)	$\Phi_L$ (%)	$\chi^2$
500	None		0.4	100			1.46
10	β-CD	40	0.5	96.5	3.1	3.5	1.00
250	β-CD	10	1.5	67.6	3.2	32.4	1.24
10	3	21	1.9	73.6	7.9	26.4	1.45
10	4	20	0.8	77.3	9.6	22.7	1.19
10	5	21	1.4	85.4	7.9	14.6	1.36

TABLE II Complex stability constant ( $K_S$ ) and Gibbs free energy change ( $-\Delta G^0$ ) for 1:1 inclusion complexation of organic dyes with  $\beta$ -cyclodextrin, mono(6-anilino-6-deoxy- $\beta$ -cyclodextrin) **2** and bis( $\beta$ -cyclodextrin)s **3–5** in aqueous phosphate buffer solution (pH 7.20) at 25°C

Host	Guest	$K_S$ ( $M^{-1}$ )	$\log K_S$	$-\Delta G^0$ ( $\text{kJ mol}^{-1}$ )	Method
$\beta$ -CD	Brilliant Green	2187	3.34	19.1	UV
	Methyl Orange	3560	3.55	20.3	CD
	Acridine Red	2630	3.42	19.5	FL
	Rhodamine B	4240	3.63	20.7	FL
	ANS	103	2.01	11.5	FL
<b>2</b>	Brilliant Green	$1600 \pm 80$	3.20	18.3	UV
	Methyl Orange	$3910 \pm 100$	3.59	20.5	CD
	Acridine Red	No inclusion			FL
	Rhodamine B	$2280 \pm 50$	3.36	19.2	FL
	ANS	$307 \pm 15$	2.49	14.2	FL
<b>3</b>	Brilliant Green	$4680 \pm 200$	3.67	20.9	UV
	Methyl Orange	$8970 \pm 400$	3.95	22.6	CD
	Acridine Red	$45000 \pm 1000$	4.65	26.6	FL
	Rhodamine B	$6720 \pm 300$	3.83	21.8	FL
	ANS	$1470 \pm 50$	3.17	18.1	FL
<b>4</b>	Brilliant Green	$6790 \pm 300$	3.83	21.9	UV
	Methyl Orange	$4380 \pm 200$	3.64	20.8	CD
	Methyl Orange	$4120 \pm 300$	3.61	20.6	UV
	Acridine Red	$8200 \pm 400$	3.91	22.3	FL
	Acridine Red	$8230 \pm 400$	3.91	22.3	UV
	Rhodamine B	$6770 \pm 300$	3.83	21.9	FL
	Rhodamine B	$7030 \pm 300$	3.85	22.0	UV
	ANS	$641 \pm 30$	2.81	16.0	FL
	ANS	$641 \pm 30$	2.81	16.0	FL
<b>5</b>	Brilliant Green	$16110 \pm 500$	4.21	24.0	UV
	Methyl Orange	$6100 \pm 300$	3.79	21.6	CD
	Acridine Red	$13150 \pm 500$	4.12	23.5	FL
	Rhodamine B	$9040 \pm 500$	3.96	22.6	FL
	ANS	$1050 \pm 50$	3.02	17.2	FL

fluorescent species resulting in the short- and long-lived fluorescence lifetimes of ANS may be assigned to free and included ANS, respectively. Another interesting fact is, although the  $\tau_S$  for native or bis( $\beta$ -cyclodextrin)s (0.8–1.9 ns) approximately agrees with that of free ANS (0.4 ns), the  $\tau_L$  for bis( $\beta$ -cyclodextrin)s (7.9–9.6 ns) is far larger than that for parent  $\beta$ -cyclodextrin (3.1 ns), which may indicate that bis( $\beta$ -cyclodextrin)s provide a significantly stronger hydrophobic environment than native  $\beta$ -cyclodextrin. This hydrophobic difference between parent  $\beta$ -cyclodextrin and bis( $\beta$ -cyclodextrin)s will subsequently result in the dramatically difference in the complex stabilities upon inclusion complexation with guest molecules. Independent fluorescence titrations also show that bis( $\beta$ -cyclodextrin)s display significantly enhanced affinities towards ANS as compared with native  $\beta$ -cyclodextrin. (Table II).

### Spectrophotometric Titration

In order to determine the molecular binding abilities of bis( $\beta$ -cyclodextrin)s for various guests quantitatively, spectrophotometric titrations of native  $\beta$ -cyclodextrin **1**, mono-(6-anilino-6-deoxy)- $\beta$ -cyclodextrin **2** and bis( $\beta$ -cyclodextrin)s **3–5** with representative guests, i.e. ANS, Brilliant Green, Methyl Orange, acridine red and rhodamine B, were performed at 25°C in aqueous phosphate buffer solution (pH 7.20)

by means of circular dichroism, ultraviolet and fluorescence spectrometry. As shown in Fig. 2, the relative fluorescence intensity of Acridine Red significantly enhances upon the gradual addition of a known amount of bis( $\beta$ -cyclodextrin) **4**, accompanying appreciable hypsochromic shifts of the fluorescence peak. Validating the 1:1 complex stoichiometry, where the two  $\beta$ -cyclodextrin moieties in bis( $\beta$ -cyclodextrin) are treated as a unit [29], the inclusion complexation of guest (G) with host (H) is expressed by Eq. (2).



The complex stability constant ( $K_S$ ) can be calculated from the analysis of the sequential changes in fluorescence intensity ( $\Delta I_f$ ) at various host concentration, using a non-linear least squares method according to the curve fitting Eq. (3) [28].

$$\Delta I_f = \{\alpha([H]_0 + [G]_0 + 1/K_S) \pm \sqrt{\alpha^2([H]_0 + [G]_0 + 1/K_S)^2 - 4\alpha^2[H]_0[G]_0}\}/2 \quad (3)$$

where  $[G]_0$  and  $[H]_0$  refer to the initial concentrations of organic dye and bis( $\beta$ -cyclodextrin), respectively, and  $\alpha$  is the proportionality coefficient, which may be taken as a sensitivity factor for the fluorescence change upon complexation. For each host examined, the  $\Delta I_f$  values were plotted as a function of  $[G]_0$  to

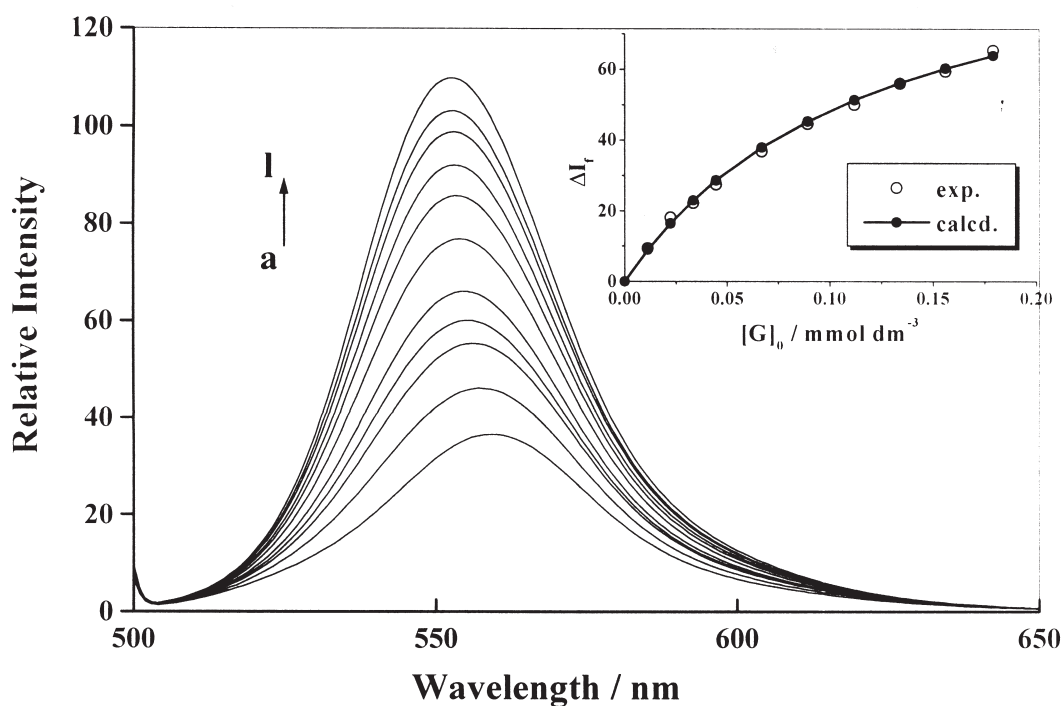


FIGURE 2 Fluorescence spectral changes of Acridine Red ( $2.4 \times 10^{-6}$  mol dm<sup>-3</sup>) and the non-linear least-squares analysis (inset) of the differential intensity ( $\Delta I_f$ ) to calculate the complex stability constant ( $K_S$ ) upon addition of bis( $\beta$ -cyclodextrin) 4 ( $0-179 \times 10^{-6}$  mol dm<sup>-3</sup> from a to l) at 25°C in aqueous phosphate buffer solution (pH 7.20).

give an excellent fit. The experimental data do not show any significant deviations from the theoretical curve in each case. In the repeated measurements, the  $K_S$  values were reproducible within an error of  $\pm 5\%$ . The  $K_S$  values obtained are listed in Table II, along with the free energy changes of complex formation ( $-\Delta G^0$ ). In order to visualize the inclusion complexation behavior between host and guest, the  $K_S$  values are also plotted against the hosts in Fig. 3.

### Molecular Binding Ability

Extensive studies of molecular recognition by cyclodextrins have revealed that the size/shape-fit concept plays a crucial role in the formation of inclusion complexes of host compounds with guest molecules of various structures. Therefore, weak intermolecular forces such as ion-dipole, dipole-dipole, dipole-induced dipole, van der Waals, electrostatic interaction, hydrogen bonding and hydrophobic interaction are known to cooperatively contribute to the inclusion complexation of guest molecules with bridged bis( $\beta$ -cyclodextrin)s according to the size/shape-fit concept. In the present case, the hydrophobic and van der Waals interactions are considered to determine the complex stability to a large extent.

As can be seen in Table II and Fig. 3, possessing two hydrophobic cavities, bis( $\beta$ -cyclodextrin)s 3–5

show significantly higher  $K_S$  values for model substrates than native  $\beta$ -cyclodextrin 1 or modified mono  $\beta$ -cyclodextrin 2 through the cooperative binding by dual  $\beta$ -cyclodextrin units with one guest molecule, which accounts for the inherent advantage of bis( $\beta$ -cyclodextrin)s in binding relatively large molecules. Unexpectedly, mono-(6-anilino-6-deoxy)- $\beta$ -cyclodextrin 2 does not give obviously enhanced complex stabilities towards selected guest molecules as compared with parent  $\beta$ -cyclodextrin. This phenomenon indicated that the self-inclusion of appended anilino group of 2 into the  $\beta$ -cyclodextrin cavity is unfavorable to the inclusion complexation with relatively large guest molecule, which consequently results in the less complex stability constants of 2 than native  $\beta$ -cyclodextrin. Another interesting fact is that the molecular binding abilities of bis( $\beta$ -cyclodextrin)s 3–5 towards linear guest molecules such as Methyl Orange and Acridine Red enhance with a sequence of  $3 > 5 > 4$ , which is well agreed with the decrease sequence of the tether length of these dimeric hosts. Among the  $\beta$ -cyclodextrin dimers examined, bis( $\beta$ -cyclodextrin)s 3 gives the highest complex stabilities up to 17 times for acridine red and 2.5 times for Methyl Orange as compared with parent  $\beta$ -cyclodextrin upon inclusion complexation. This phenomenon can be arising from the strict size-fit relationship between host and guest. On the other hand, possessing the hydrophilic sulfonyl group, Methyl Orange is more



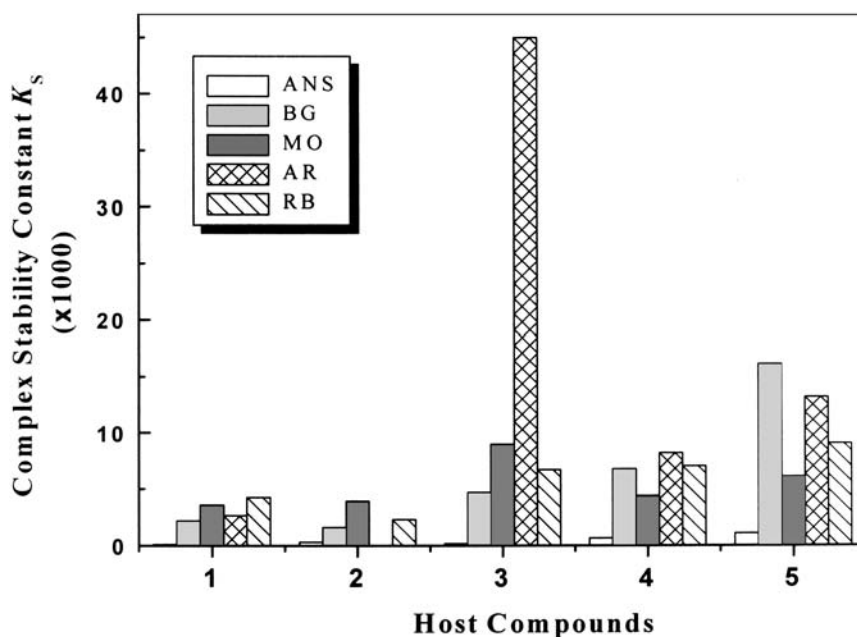


FIGURE 3 Complex stability constants  $K_S$  upon inclusion complexation of organic dyes with host 1–5 at 25°C in aqueous phosphate buffer solution (pH 7.20). BG: Brilliant Green; MO: Methyl Orange; AR: Acridine Red; RB: rhodamine B.

hydrophilic than acridine red, which will reduce the hydrophobic interaction and the extent of desolvation upon inclusion complexation with  $\beta$ -cyclodextrin units and consequently results in the relatively weaker affinities of bis( $\beta$ -cyclodextrin)s 3–5 towards Methyl Orange than towards acridine red.

Using triangular Brilliant Green and T-shaped rhodamine B, the inclusion complexation behavior of bis( $\beta$ -cyclodextrin)s 3–5 was quantitatively investigated under comparable experimental condition. As can be seen in Table II and Fig. 3, the complex stability constants  $K_S$  of dimeric  $\beta$ -cyclodextrins and

parent  $\beta$ -cyclodextrin with Brilliant Green and rhodamine B vary in an order of  $5 > 4 > 3 > \beta$ -cyclodextrin and bis( $\beta$ -cyclodextrin) 5 displays the highest  $K_S$  values up to 7.4 times for Brilliant Green and 2.1 times for rhodamine B as compared with parent  $\beta$ -cyclodextrin. This may be attributed to not only the cooperative binding of dual  $\beta$ -cyclodextrin units but also the additional binding site supported by the formation of the sandwich-type complex between host bis( $\beta$ -cyclodextrin) and guest molecule. According to our recent study [30], the bridged chain of  $\beta$ -cyclodextrin dimer can adjust the distance

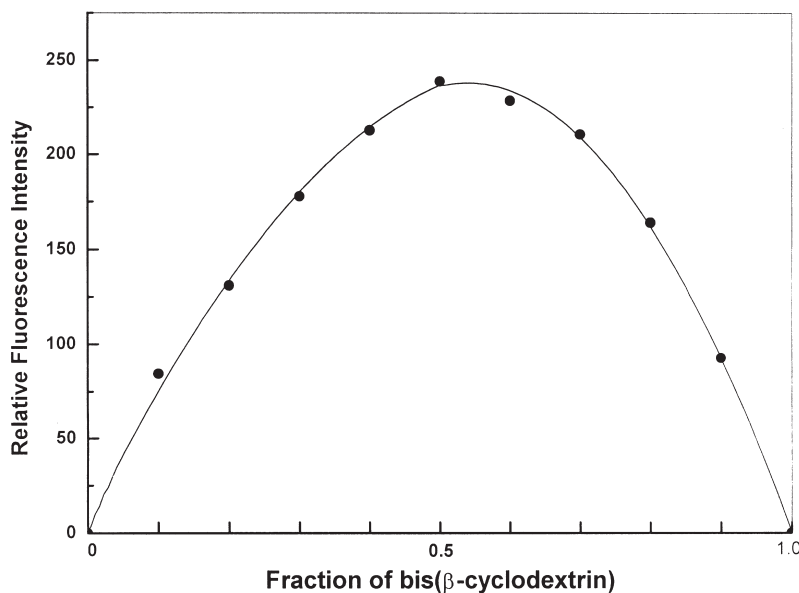


FIGURE 4 Continuous variation plot of bis( $\beta$ -cyclodextrin) 4+Acridine Red system:  $[\text{bis}(\beta\text{-cyclodextrin})]=1.00 \times 10^{-5} \text{ M}$ .

and the orientation of the two  $\beta$ -cyclodextrin units to form stable sandwich-type complex with guest molecule. In a closed comparison among bis( $\beta$ -cyclodextrin)s 3–5, the bridged chain of bis( $\beta$ -cyclodextrin) 5 possesses the most suitable size/shape to form sandwich-type complex with triangular Brilliant Green or T-shaped rhodamine B, so it gives the strongest binding towards these two substrates. In a sharp contrast, the two  $\beta$ -cyclodextrin moieties of bis( $\beta$ -cyclodextrin) 3 is too closely located in space to form stable sandwich-type complex with Brilliant Green or rhodamine B. As a result of such a poor matching in distance between two cavities, bis( $\beta$ -cyclodextrin) 3 shows the lowest  $K_S$  values upon inclusion complexation with Brilliant Green or rhodamine B. Bis( $\beta$ -cyclodextrin) 4 is located between these two extremes and gives moderate binding abilities.

Although the results described above are deduced from limited data, we still can conclude that the bridged bis( $\beta$ -cyclodextrin)s can remarkably enhance the original binding ability of parent  $\beta$ -cyclodextrin by the cooperative binding of one guest molecule in the two closely located  $\beta$ -cyclodextrin cavities, giving the highest binding ability towards acridine red up to 17 times higher than native  $\beta$ -cyclodextrin and the enhancement of molecular selectivity for acridine red/Methyl Orange pair by 8 times as compared with parent  $\beta$ -cyclodextrin for dimer 3. So we can deduce that bridged bis( $\beta$ -cyclodextrin)s possess the inherent advantage of orientating the two  $\beta$ -cyclodextrin moieties to fit to the size/shape of guest molecule, and the appropriate increase of tether chain length will favor the inclusion complexation of bis( $\beta$ -cyclodextrin)s with relatively larger guest molecules. Further studies on the effect of expansive different tether length in the inclusion complexation behavior of bis( $\beta$ -cyclodextrin)s with model substrates are still in progress.

## SUPPLEMENTARY MATERIALS

### Stoichiometry of Host:guest Inclusion Complexation

The stoichiometry was determined by the continuous variation method. Figure 4 shows the continuous variation plot for the bis( $\beta$ -cyclodextrin) 4+Acridine Red system. In the concentration range, the plot shows a maximum at a molar fraction of 0.5, indicating 1:1 inclusion.

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