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Inclusion complexation behavior of dyestuff guest molecules by a bridged bis(cyclomaltoheptaose)[bis(β-cyclodextrin)] with a pyromellitic acid diamide tether

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Abstract

A novel bridged bis(β-cyclodextrin) with a pyromellitic acid 2,5-diamide tether (2) has been synthesized by reaction of 6^{I}-(2-aminoethyleneamino)-6-deoxycyclomaltoheptaose [mono 6-(2-aminoethyleneamino)-6-deoxy-β-cyclodextrin] with 1,2,4,5-benzene-tetracarboxylic dianhydride. Its inclusion complexation behavior with some representative dyestuffs, i.e., Acridine Red (AR), Rhodamine B (RhB), Neutral Red (NR), Brilliant Green (BG), was studied by using UV-absorption, fluorescence, and 2D NMR spectroscopy. Fluorescence titrations have been performed at 25 °C in pH 7.2 buffer solution to calculate the binding constants of resulting complexes. These results obtained indicated that bis(β-cyclodextrin) 2 exhibits the strongly enhanced binding ability with all dye molecules examined compared with natural cyclodextrins. The binding modes of 2 with dye molecules have been deduced by 2D NMR experiments to establish the correlations between molecular conformations and binding constants of inclusion complexation. It is found that the improved binding ability and molecular selectivity of 2 could be attributed to double-cavity cooperative inclusion interaction and the size/shape matching between the host and guest.
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Keywords: Bridged bis(β-cyclodextrin); Molecular recognition; Binding mode; Stability constant; Dye molecule

1. Introduction

Seminatural artificial receptors built from cyclic oligosaccharides (cyclomaltooligosaccharide cyclodextrins) can bind a variety of inorganic, organic, and biological guest molecules inside their apolar cavities in aqueous solution to form host–guest complexes or supramolecular systems. Therefore, this fascinating property enables them to be successfully used as drug carriers,[1] separation reagents,[2] enzyme mimics,[3] and photochemical sensors[4] in science and technology. However, natural and mono-modified cyclodextrins possess relatively lower molecular binding ability and selectivity upon

inclusion complexation with guest molecules. It is interesting and significant that cyclodextrins can be linked together via a spacer to give a dimeric species possessing two hydrophobic cavities that can cooperate to bind one guest molecule and give much higher binding constants than either the native or modified cyclodextrin monomers. Therefore, much effort has been devoted to the design and synthesis of novel cyclodextrin dimers to significantly alter the original binding ability of parent cyclodextrins.[5–12] We have reported[13–17] that bridged bis(β-cyclodextrin)s with a variety of functional tethers not only can enhance the original binding ability, but also the molecular selectivity through the cooperative binding of one guest by the two closed cyclodextrin cavities.

In the present context, we wish to report our investigation results on the synthesis of (N,N′-bis(6^{I}-(2-ethylamino)-6^{I}-deoxycyclomaltoheptaose)benzene-

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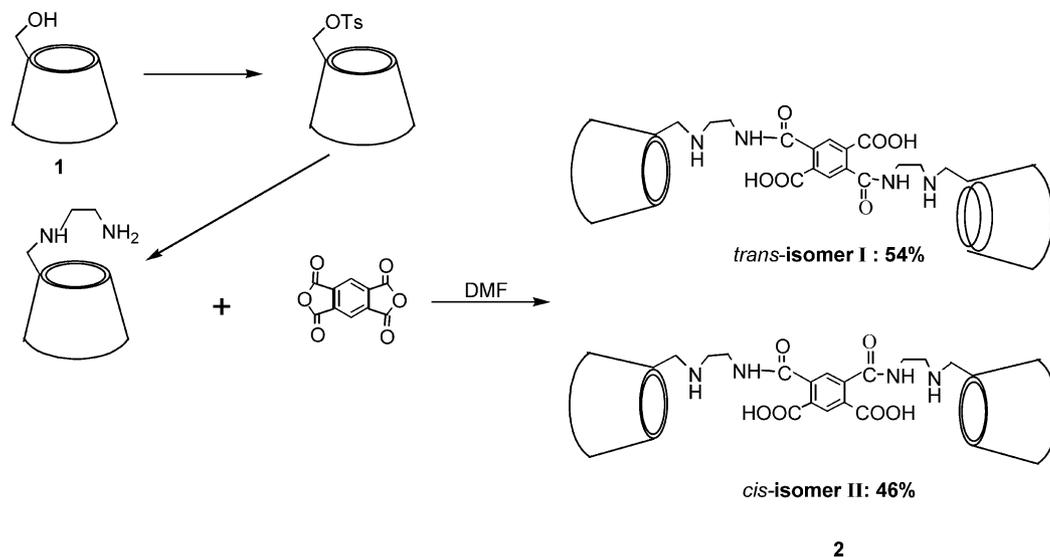
Scheme 1.

1,3- or 1,4-dicarboxylic acid 4,6- or 2,5-diamine (N,N'-bis(2-ethylamino-6-deoxy-β-cyclodextrin)pyromellitic acid 2,4- or 2,5-diamide, **2** (Scheme 1) and its inclusion complexation behavior at pH 7.2 with dye molecules of different sizes and shapes, such as the dyestuffs Acridine Red (AR), Rhodamine B (RhB), Neutral Red (NR), Brilliant Green (BG) (Fig. 1).

2. Results and discussion

2.1. Spectral titration

Spectral titration is a convenient and efficient method to measure the complexation stability constant of supramolecular species. In this context, we employed fluorescence and absorption spectral titrations to quantitatively
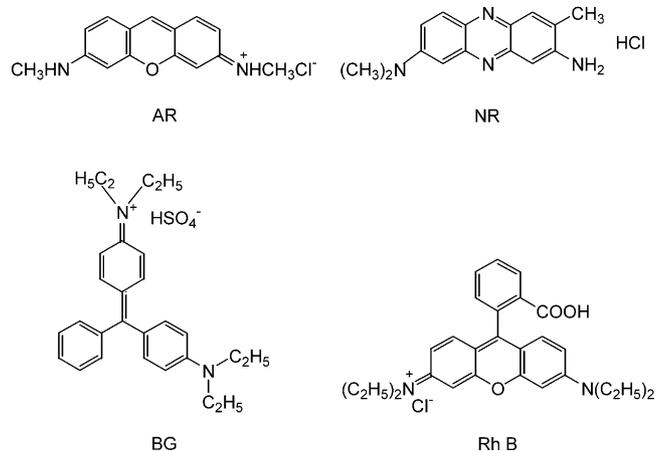
study the binding ability of hosts β-cyclodextrin **1** and the dimer **2** with guests, AR, NR, RhB, and BG (Fig. 1) at 25 °C in pH 7.2 buffer solution. In the fluorescence spectral titration experiments, the concentration of guest (spectrally active one) is kept constant, while the concentration of host is varied. The spectral changes depend critically on the formation of new species, i.e., host–guest inclusion complex, showing fluorescence enhancement. Representative spectral changes are shown in Fig. 2 for the inclusion complexation of dimer **2** with AR. However, the inclusion complexation behavior of RhB with dimer **2** showed the opposite spectral changes as compared with other guest mole-
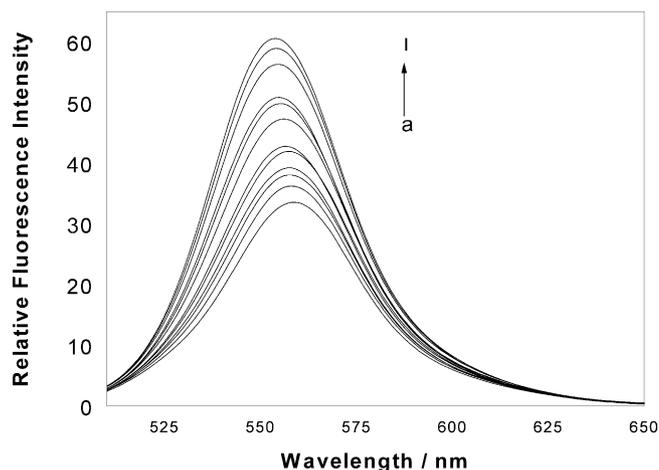

Fig. 1. Structures of dyestuffs evaluated.

Fig. 2. Fluorescence spectral changes of AR (3.1×10^{-6} mol dm^{-3}) upon addition of host **2** in aqueous phosphate buffer solution of pH 7.20. The concentration of **2** increases in the range 0–1.50×10^{-4} mol dm^{-3} from a to l. (Excitation wavelength was 490 nm.)
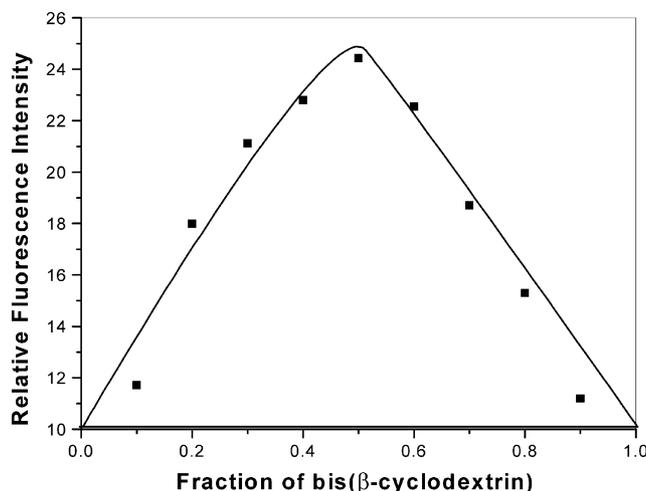
Fig. 3. Continuous variation plot of **2**/AR system. ([Host **2**] + [AR] = 5.0×10^{-5} mol dm^{-3}) in buffer solution of pH 7.2.

Fig. 4. The nonlinear least-squares analysis (inset) of the differential intensity (ΔI) to calculate the complex stability constant (K_S).

cules, which may be attributed to the balance between fluorescent acid-form and the colorless lactonic form.[18] In the present investigation, the interaction between the benzoate moiety in RhB and the bulk water is shielded in the resulting complex, which makes the equilibrium shift from the hydrophilic, fluorescent carboxylate ion form of RhB to the hydrophobic nonfluorescent lactone form, which leads to the fluorescence quenching.[19]

The stoichiometry for the inclusion complexation of the bis(β-cyclodextrin) **2** with representative guests was determined by the continuous variation method. Fig. 3 shows the representative plot of 1:1 stoichiometry for the inclusion complexation of **2** with guest AR. Treating two β-cyclodextrin moieties in **2** as a host unit, the inclusion complexation of a guest (G) with a host (H), is expressed by Eq. (1).

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

The effective stability constant (K_S)[20] 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 Eq. (2)[21]

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

where $[G]_0$ and $[H]_0$ 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 $[G]_0$ gave an excellent fit, and the experimental data do not show any significant deviations from the theoretical curve in each case. When
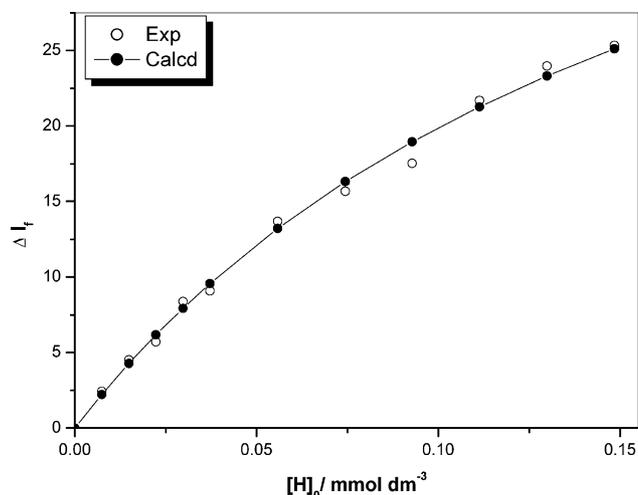
repeated measurements were made, the K_S value was reproducible within an error of ±5%, which corresponds to an estimated error of 0.15 kJ mol^{-1} in the free energy of complexation ($\Delta G°$). The curve-fitting analyses for the inclusion complexation of **2** with AR were shown in Fig. 4. The K_S values obtained are listed in Table 1, along with the free-energy changes ($-\Delta G°$) of complex formation.

2.2. Binding mode

In order to establish the correlations between the molecular conformations and binding ability to deduce the molecular recognition mechanism of inclusion complexation with bridged bis(β-cyclodextrin)s in the buffer solution, we recorded the 300 MHz ROESY spectra of **2** in D$_2$O in the absence and presence of RhB, shown in Figs. 5 and 6, respectively. As can be seen from Fig. 5, the cross-peaks (peaks A) between the protons of benzyl ring in spacer and H-3/H-5 of cyclodextrin in *trans*-isomer **I** together with the peaks B between the protons of benzyl ring in spacer and H-3/H-5 of cyclodextrin in *cis*-isomer **II** indicated that aromatic moieties of bridged chain in both *trans*-isomer **I** and *cis*-isomer **II** must be self-included into the hydrophobic cavity of cyclodextrin, resulting in similar conformations between trans-/cis-bis(β-CD). On the other hand, the ROESY spectrum shown in Fig. 6 displays clear cross-peaks (peaks A′) between the H-3 and H-5 of cyclodextrin and the methyl protons of diethylamino groups in RhB, and the other significant corresponding cross-peaks (peaks B′) between the aromatic protons of diethylaminophenyl in RhB and the H-5 of cyclodextrin, suggesting that the alkyl group in RhB must be deeply included into the cavity of the cyclodextrin. Therefore, we could conclude that RhB is included into the hydrophobic cavity of

Table 1
Complex stability constant (K_S) and Gibbs free energy change ($-\Delta G°$) for 1:1 inclusion complexation of various guest with β-cyclodextrin **1** and bis(β-cyclodextrin) **2** in pH 7.2 aqueous buffer solution at 25 °C

Host	Guest	K_S (**2**)/K_S (**1**)	K_S/M^{-1}	Log K_S	$-\Delta G°$ (kJ mol^{-1})	Reference
1	AR	≡1	2630	3.42	19.5	b
	RhB	≡1	4240	3.63	20.7	b
	NR	≡1	480	2.68	15.3	d
	BG	≡1	2190	3.34	19.1	a
2	AR	2.2	5810	3.76	21.5	c
	RhB	2.9	12,490	4.10	23.4	c
	NR	5.0	2410	3.38	19.3	c
	BG	3.6	7760	3.89	22.2	c

^a Ref. 1.
^b Ref. 2.
^c This work.
^d Ref. 3.

[a] Ref. 1.
[b] Ref. 2.
[c] This work.
[d] Ref. 3.
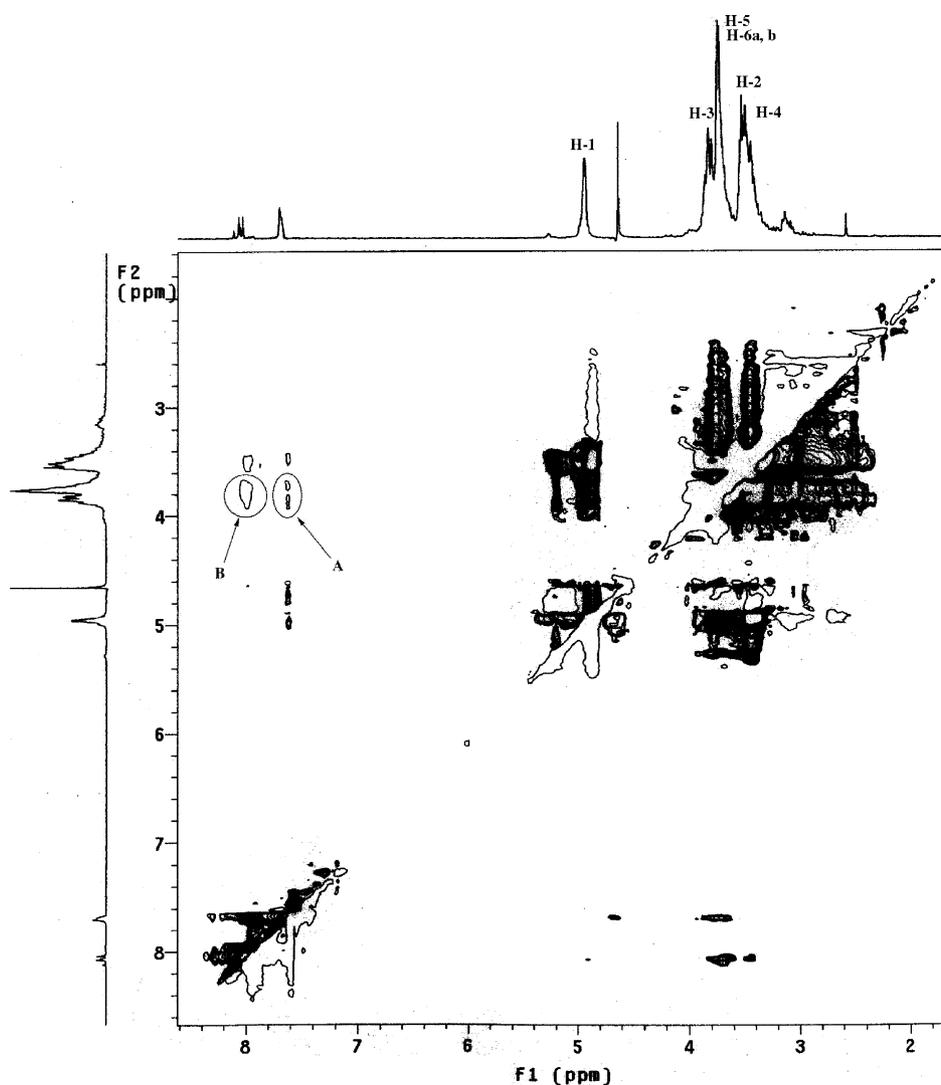
Fig. 5. ^{1}H ROESY spectrum (300 MHz) of **2** ([**2**] = 1.0 × 10^{-3} M) in D$_2$O at 298 K with a mixing time of 400 ms.
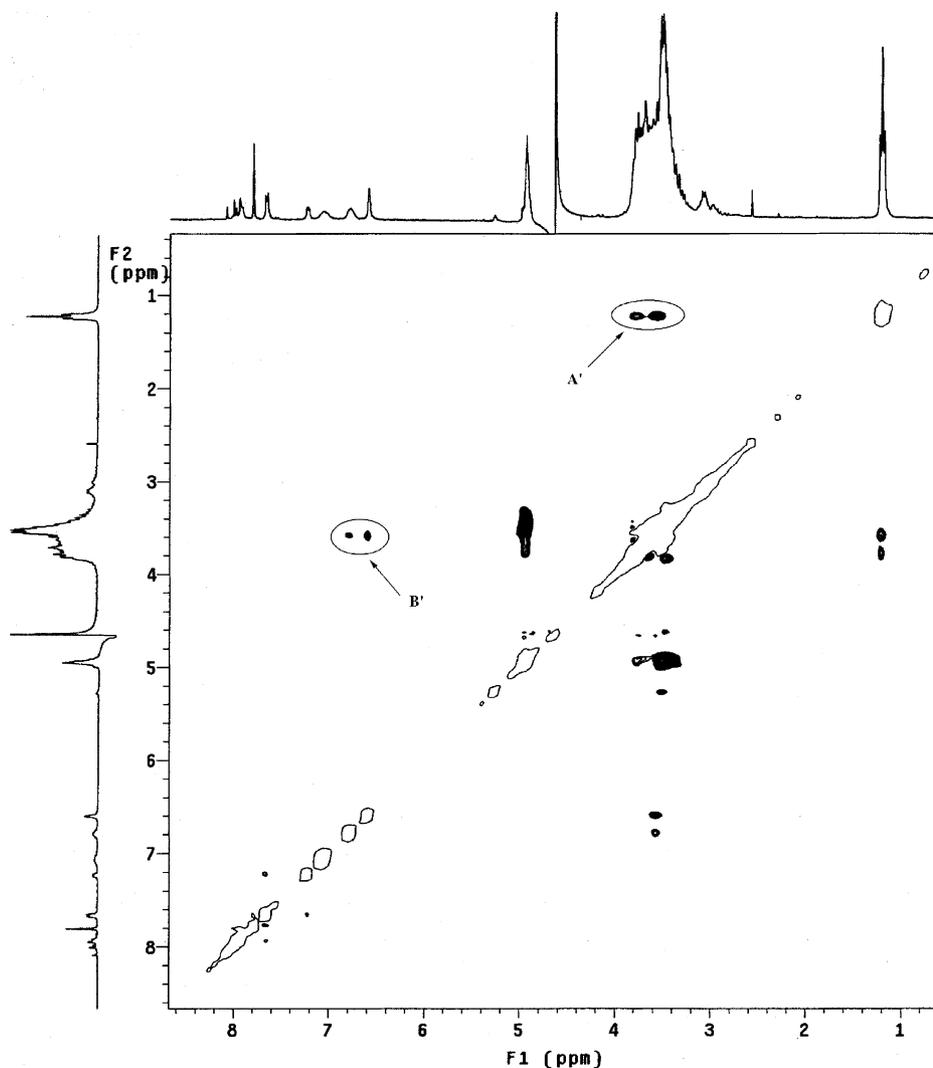
Fig. 6. ^{1}H ROESY spectrum (300 MHz) of a mixture of **2** with RhB ([**2**] = [RhB] = 1.0×10^{-3} M) in D$_2$O at 298 K with a mixing time of 400 ms.

2.3. Binding ability and molecular selectivity

cyclodextrin from the primary hydroxyl side to form the sandwich inclusion complex. The binding mode of inclusion complexation could be used to explain the enhanced molecular binding ability of bridged bis(β-cyclodextrin) with guest molecules.

As can be seen from the Table 1, the complexation stability constants for parent β-cyclodextrin and bridged bis(β-cyclodextrin) with dye guests are effected by the structure, size and shape of guest. The K_S value for the complexation of each dye by parent **1** increases in the order: RhB > AR ≈ BG > NR in the buffer solution at pH 7.2. On the other hand, the parent β-cyclodextrin affords only limited binding ability probably due to the weak hydrophobic interaction. However, bridged bis(β-cyclodextrin)s can enhance the original binding ability to some extent through the cooperative binding of one guest molecule into two adjacent cavities. It is noted that the binding constants of the guest molecules with dimer **2** are higher than those of the native cyclodextrin **1** by a factor of 2.2–5.0. The binding ability of host **2** with a triangular guest is higher than that with a linear guest. The 2D NMR experiment indicates that the two cyclodextrin units of the host **2** can bind a triangular guest, giving the relative higher effective binding constants according to the 1:1 stoichiometry. Although both native β-cyclodextrin **1** and bridged bis(β-cyclodextrin) **2** give lower K_S values for NR, the effect of the cooperative binding by dimeric β-cyclodextrin is more remarkable for NR, showing the higher enhanced binding ability upon inclusion complexation with host **2** as compared with parent

β-cyclodextrin (K_S (**2**)/K_S (**1**) = 5.0). One possible explanation for enhanced binding ability is that the size or shape of the NR is probably not matching that of β-cyclodextrin cavity; therefore, the cooperative interaction of the second cavity can greatly enhance the original molecular binding ability.

3. Conclusions

In summary, the newly synthesized bridged bis(β-cyclodextrin) **2** significantly extends the original molecular binding ability of parent β-cyclodextrin **1**, and the extended molecular recognition ability and selectivity can be understood by the correlation between the conformation of the resulting complexes of cyclodextrins with guest molecules and their stabilities.

4. Experimental

4.1. Measurements

The mass spectrum was obtained by using a REFLEX™ III instrument. Elemental analysis was performed on a Perkin–Elmer 2400C instrument. NMR spectra were determined on a Varian INVON 300 spectrometer. Fluorescence spectra were measured in a conventional quartz cell (5 × 5 × 45 mm) at 25 °C on a JASCO FP-750 fluorescence spectrometer equipped with a temperature controller and with excitation and emission slits of 5 nm width. UV–vis spectra were recorded in a conventional quartz cell (10 × 10 × 45 mm) on a Shimadzu UV-2401 spectrometer. In the spectral measurements, sodium dihydrogen phosphate and disodium hydrogen phosphate were dissolved in deionized water to make a buffer solution of pH 7.2.

4.2. Materials

All guest dyestuffs, i.e., Acridine Red (AR), Rhodamine B (RhB), Neutral Red (NR), Brilliant Green (BG), were commercially available and used without further purification. β-Cyclodextrin of reagent grade (Shanghai Reagent Factory) 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.

4.2.1. *N,N′*-Bis(6^I-(2-ethylamino)-6^I-deoxycyclo-maltoheptaose)benzene-1,3- or 1,4-dicarboxylic acid 4,6- or 2,5-diamide (2). To the solution of DMF (50 mL) containing 1,2,4,5-benzenetetracarboxylic dianhydride (0.4 mmol) was added 1.0 mmol of 6^I-(2-aminoethyl-eneamino)-6^I-deoxycyclomaltoheptaose.[22] The resulting

mixture was stirred for 4 days at 50 °C. The solvent was removed under reduced pressure. The residue was dissolved in a minimum amount of hot water and then poured into 150 mL of acetone. The precipitate formed was collected by filtration to obtain a crude powder. This procedure was repeated three times in order to remove unreacted small molecules. The crude compound was purified on a column of Sephadex G-25, with fractions monitored by UV-adsorption, to give a pure product (30% yield). MS (MALDI-TOF): m/z 2572 [M]$^+$; ^{1}H NMR (D$_2$O, TMS): δ 3.4–3.8 (m, 184 H), 4.9 (s, 28 H), 7.66 (s, 2 H), 7.96 (s, 1 H), 8.03 (d, 1 H). ^{13}C NMR (D$_2$O): δ 175.93 (COOH), 173.46 (CONH), 141.74, 141.43, 136.92, 128.98, 128.51, 125.39 (Aromatic carbons), 102.08 (C-1), 81.58 (C-4), 73.26 (C-5), 72.25 (C-3), 72.00 (C-2), 60.47 (C-6), 49.84, 48.45, 44.59, 41.85. Anal. Calcd for C$_{98}$H$_{154}$N$_4$O$_{74}$·9 H$_2$O: C, 43.05; H, 6.34; N, 2.05. Found: C, 42.73; H, 6.15; N 2.11. UV–vis (water) λ_{max}/nm (ε/M^{-1} cm^{-1}) 294 (1585). From this information (multiple, instead of singlet at the aromatic region), it was concluded that the product was a mixture of *trans*-isomer **I** and *cis*-isomer **II** derivatives. Assigning the aromatic region in the detail, we observe a single peak (7.66 ppm), another single peak (7.96 ppm) and a double peak (8.03 ppm) with an integration relation of 2:0.9:0.8. Considering that the first singlet belongs to *trans*-isomer **I** and the last singlet and doublets belong to *cis*-isomer **II** (asymmetric hydrogens), we can assume that our product is a mixture of both para and meta isomers in a 54:46 ratio. Although the isomers could not be separated, their apparent binding abilities with guest molecules could be determined.

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