Synthesis and Molecular Recognition of Novel Oligo(ethylenediamino) Bridged Bis(β-cyclodextrin)s and Their Copper(II) Complexes: Enhanced Molecular Binding Ability and Selectivity by Multiple Recognition

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Abstract: Four bridged bis(β-cyclodextrin)s tethered by different lengths of oligo(ethylenediamine)s have been synthesized and their inclusion complexation behavior with selected substrates elucidated by circular dichroism spectroscopy and fluorescence decay. In order to study their binding ability quantitatively, inclusion complexation stability constants with four dye guests, that is, brilliant green (BG), methyl orange (MO), ammonium 8-anilino-1-naphthalenesulfonic acid (ANS), and sodium 6-(*p*-toluidino)-2-naphthalenesulfonate (TNS), have been determined in aqueous solution at 25 °C with spectrophotometric, spectropolarimetric, or spectrofluorometric titrations. The results obtained indicate that the two tethered cyclodextrin units might cooperatively bind to a guest, and the molecular binding ability toward model substrates, especially linear guests such as TNS and MO, could be extended. The tether length plays a crucial role in the molecular recognition, the binding constants for ANS and TNS decrease linearly with an increase in the tether length of dimeric cyclodextrin. The Gibbs free energy changes ($-\Delta G^\circ$) for the unit increment per ethylene are 0.99 kJ mol^{-1} for ANS and 0.44 kJ mol^{-1} for TNS, respectively. On the other hand, the presence of a copper(II) ion in metallobis(β-cyclodextrin)s oligo(ethylenediamino) tethers enhances not only the original binding ability, but also the molecular selectivity through triple or multiple recognition, as compared with the parent bis(β-cyclodextrin)s.

Keywords: cyclodextrins · dyes · molecular recognition · cooperative effects · supramolecular chemistry

Introduction

Cyclodextrins, a class of cyclic oligosaccharides with six to eight D-glucose units linked by α-1,4-glucose bonds, are well known to accommodate various guest molecules into their truncated cone-shaped hydrophobic cavity in aqueous solution.[1–3] This fascinating property enables them to be successfully used as drug carriers,[4] separation reagents,[5] enzyme mimics,[6] and photochemical sensors[7, 8] in science and technology. However, the binding constants of natural cyclodextrins and their simple derivatives with model substrates are generally in the range of 10^2 to 10^4 dm^3 mol^{-1}, and this limits their application as enzyme mimics and to a greater extent as antibody mimics. In this context, introduction of an additional cyclodextrin unit to the rim of a cyclodextrin seems attractive, since the potential cooperative work of two adjacent cyclodextrin units should greatly enhance their binding ability with model substrates through hydrophobic interactions. Much work has been devoted to the design and syntheses of novel cyclodextrin dimers since the 1980s, and a strong binding constant (K_S) as high as 10^{11} dm^3 mol^{-1},[9] has been obtained, and this is the same magnitude as the antigen–antibody interaction.

Cyclodextrin dimers tethered by the spacer (or linker) of different sizes and shapes may afford distinctly different binding abilities and molecular selectivities. Hence, diverse functional groups such as alkanedioates,[10, 11] disulfides,[12, 13] dipyridines,[14, 15] and imidazole,[16, 17] have been used as the linker between two cyclodextrin units. Unexpectedly, cyclodextrin dimers tethered with oligo(ethylenediamine) units have rarely been synthesized and therefore their molecular recognition behavior has not been extensively investigated, except for the study of Tabushi et al.[18] and a short report by Liu et al.[19] There is an inherent advantage for the oligo(ethylenediamine) tether incorporated in bis(cyclodextrin), since the tether group can ligate to transition metal ions, thus enabling us to modify, and potentially switch the original binding ability through the metal ligation. In the preliminary work,[19] we have investigated the molecular binding and recognition of triethylenetetraamino tethered β-cyclodextrin with fluorescent dyes ANS and TNS, and found that the copper(II) introduced onto the oligo(ethylenedi-

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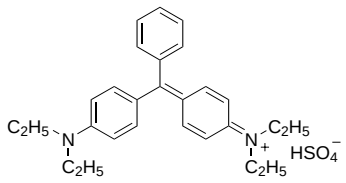
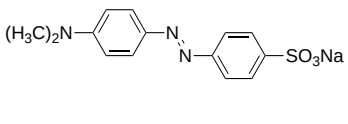
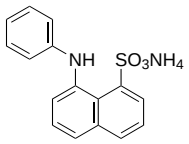
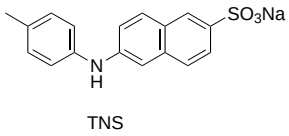








amine) tether enhances both the original binding ability and the molecular selectivity.

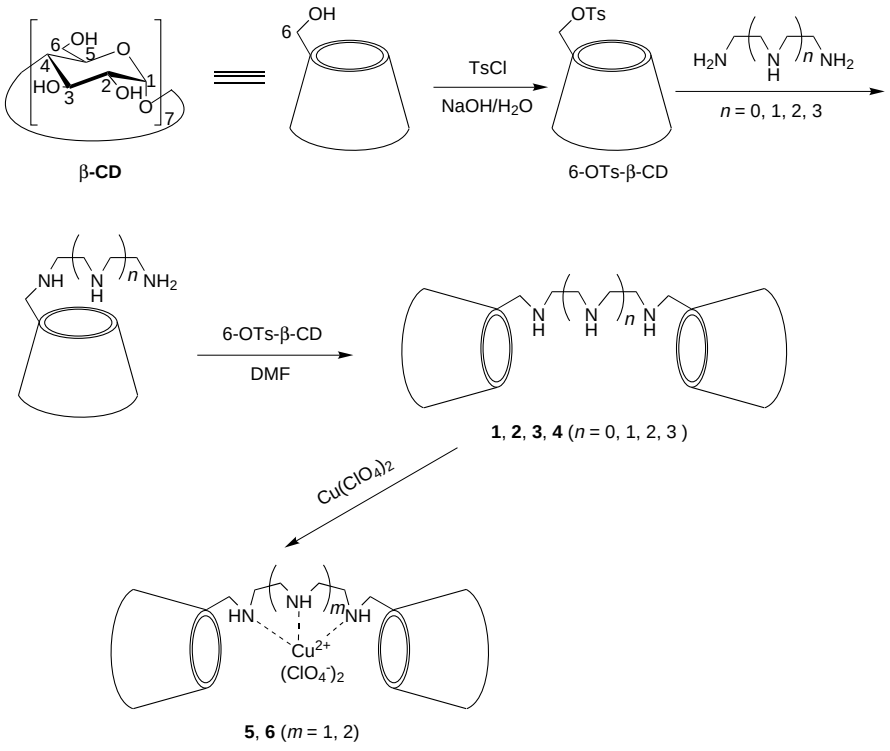
In order to examine the molecular recognition of oligo(ethylenediamino) tethered β-cyclodextrin systematically, we will report herein the syntheses of several novel β-cyclodextrin dimers with polyamino tethers and their molecular binding ability and selectivity with model dye guest molecules in different sizes and shapes. At the same time, we can also further explore the possibility of external modification of the binding ability through metal ligation. The results obtained indicate that the size/shape-matching between the host β-cyclodextrin dimer and the guest dye crucially dominates the complex stability. As another point of interest the role of the tether length in the molecular recognition of cyclodextrin dimers will be revealed. From the present results, it will be shown that the complex stability generally decreases with the increase of the tether length of dimeric β-cyclodextrin.

Results and Discussion

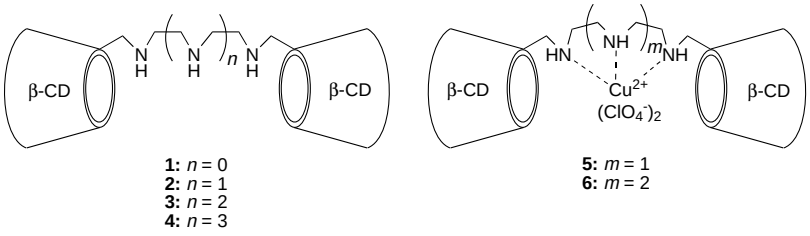
Synthesis: As shown in Scheme 1, the bridged bis(β-cyclodextrin)s **1**–**4** were synthesized in satisfactory yields starting from 6-*O*-monotosyl-β-cyclodextrin, while the metallobis(β-cyclodextrin)s **5** and **6** were prepared from diethylenetriamino-bridged bis(β-cyclodextrin) **2** and triethylenetetraamino-bridged bis(β-cyclodextrin) **3**, respectively, by the coordination reaction with copper(II) perchlorate in aqueous solution. In addition to the elemental analysis data, the IR spectra of **5** or **6** clearly indicate the presence of perchlorate, showing the vibration bands at 1114.2 and 939.2 cm^{-1}. Furthermore, a weak absorption at 624 cm^{-1}, which may be assigned to a weakly coordinated perchlorate, is also observed, and indicates that the Cu^{II} ion is coordinated to the oligo(ethylenediamino)-bridged bis(cyclodextrin) to form metallobis(β-cyclodextrin).

Conductivity measurements were also performed to explore the complex stoichiometry for the metallobis(β-cyclodextrin)s in aqueous solution as the conductivity of the system reduces with the complex formation. The results obtained indicate that the complex stoichiometry is 1:1 for both **2**-Cu^{II} and **3**-Cu^{II} complexes. A representative Job's plot for the 1:1 complexation of bis(β-cyclodextrin) **2** with copper(II) perchlorate is shown in Figure 1.

ICD spectra: Circular dichroism spectra have been widely employed to elucidate the absolute conformation of chiral compounds since this method was established at the end of the 1960s.[20] The achiral compounds located in a chiral environment produce induced circular dichroism (ICD) signal(s) in



Scheme 1. Syntheses of β-cyclodextrin derivatives **1**–**6**.



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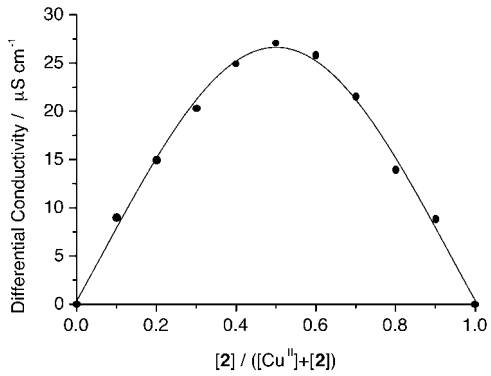

Figure 1. A Job plot of the complexation of bis(β-cylclodextrin) **2** with copper(II) perchlorate in aqueous solution. ($[\mathbf{2}]+[Cu^{II}]=50$ mmol dm^{-3}).

the corresponding transition band(s). Therefore, the inclusion behavior of the bridged bis(β-cyclodextrin)s with methyl orange was investigated using the ICD phenomena. As is shown in Figure 2, both native β-cyclodextrin and bridged

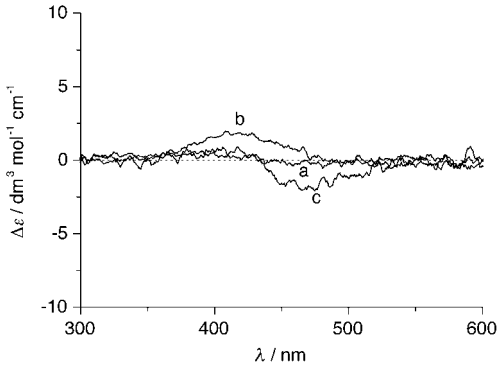

Figure 2. Circular dichroism spectra of methyl orange (10 μmol dm^{-3}) a) without and b) with β-cyclodextrin (1.6 mmol dm^{-3}) and c) with ethylenediamino-bridged bis(β-cyclodextrin) **1** (50 μmol dm^{-3}).

bis(β-cyclodextrin) **1** induce appreciable CD at the π–π* transition band of the azo group in methyl orange, while no CD is seen in the absence of cyclodextrin (Figure 2, trace a). These results clearly indicate that the methyl orange molecule is included in the chiral cyclodextrin cavity. The ICD spectrum of methyl orange (10 μmol dm^{-3}) with β-cyclodextrin (1.6 mmol dm^{-3}) shows a positive Cotton effect peak at 428 nm ($\Delta\varepsilon = +1.86$ dm^3 mol^{-1} cm^{-1}). With the dual hydrophobic cavities, the bridged bis(β-cyclodextrin) **1** at an even lower concentration (50 μmol dm^{-3}) induces a somewhat stronger Cotton effect at 465 nm ($\Delta\varepsilon = -2.00$ dm^3 mol^{-1} cm^{-1}). This may be attributed to the enhanced binding ability and/or to the more efficient ICD upon inclusion by **1** compared with β-cyclodextrin. The induced molar circular dichroism ($\Delta\varepsilon$) gradually increased upon further addition of β-cyclodextrin or bis(β-cyclodextrin) **1**.

From the geometrical requirement, the methyl orange molecule is inferred to be incorporated longitudinally into the β-cyclodextrin cavity.[21, 22] However, the ICD signals of methyl orange induced by β-cyclodextrin and bis(β-cyclodextrin)s are distinctly different. From the pioneering studies of Harata, Kajtár, and Shimizu et al.[23–26] on the ICD phenomena of cyclodextrin complexes, an empirical rule was proposed: If the transition moment of the guest chromophore is parallel to the axis of symmetry of cyclodextrin (that is, the axis of the cavity), then the sign of the ICD signal for that transition will be positive, whereas if the moment axis is aligned perpendicular to the cavity axis, the sign of ICD will be negative. From this rule, it can be concluded that the azo group of methyl orange is included in the cyclodextrin cavity, as is shown in Figure 3a. However, the negative ICD signal of

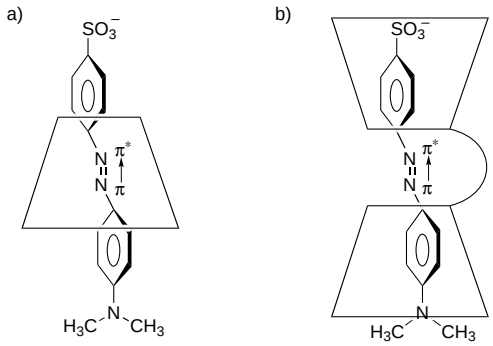

Figure 3. Possible structures of inclusion complexes between methyl orange and a) β-cyclodextrin, ICD >0; and b) dimeric β-cyclodextrins, ICD <0.

the methyl orange–bis(cyclodextrin) **1** complex at 465 nm can not be interpreted rationally by using the above rule. In this aspect, Kodaka's results[27–29] seem attractive, since he proposed that the sign of the ICD will be opposite to the expectation of the above rule, if the chromophore is located outside the cyclodextrin cavity on the basis of the Kirkwood–Tinoco theoretical calculation. We can deduce that the two aromatic groups in methyl orange are included into the adjacent cavities of bis(cyclodextrin) **1**, respectively, while the azo group is exposed outside the cyclodextrin cavity, as shown in Figure 3b. From the ICD results, it is proposed that the two cyclodextrin cavities cooperatively work to encapsulate an appropriate geometric guest.

Fluorescence spectra and fluorescence lifetimes: ANS and TNS barely fluoresce in aqueous solution, but emit strong fluorescence in a nonpolar environment, therefore, they have been widely used as fluorescent probes in both biological and organic chemistry. We can use these two fluorescent dyes as probes to examine the inclusion complexation of bridged bis(β-cyclodextrin)s **1**–**6**. As can be seen from Figure 4, the fluorescent intensity of ANS gradually increases upon addition of bis(β-cyclodextrin) **2**. At the same time, a large hypochromic shift of emission maximum from 522 to 477 nm is observed. These results clearly indicate that the aromatic group of ANS is embedded into the hydrophobic cavity of cyclodextrins. The fluorescent sensitization of bis(cyclodextrin)s is much stronger relative to native β-cyclodextrin, and this could be ascribed to the cooperative interaction of the former upon inclusion complexation with guest dyes.

To further investigate the inclusion complexation of bis(β-cyclodextrin)s, the fluorescence lifetimes of bis(β-cyclodex-

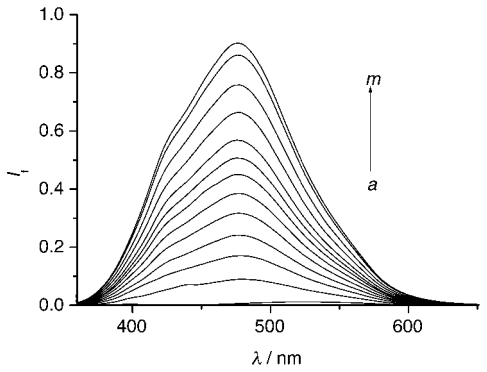

Figure 4. Fluorescence spectral changes of ANS (10 μmol dm^{-3}) upon addition of diethylenetriamino-bridged bis(β-cyclodextrin) **2**. The concentration of **2** (from a to m) is 0, 26, 53, 80, 106, 132, 159, 185, 212, 265, 318, 371, and 424 μmol dm^{-3}. Excitation wavelength was 350 nm.

trin)s with ANS were determined by the time-correlated single-photon-counting method. Since the rates of complexation/decomplexation are much slower than the fluorescence decay,[30] 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 [see Equation (1)].

$$F(t) = \sum_{i=1}^{n} \alpha_i \exp(-t/\tau_i \ (n = 1, 2, \text{etc.}) \quad (1)$$

In Equation (1) n represents the number of components, α_i the amplitude of the i-th component, and τ_i the decay time. In the absence of the host, the fluorescence decay curve observed for ANS in aqueous solution was fitted perfectly to a single exponential function.[32] In contrast, 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 (τ_S and τ_L) and relative quantum yields (Φ) observed for ANS in the presence of native β-cyclodextrin and bis(β-cyclodextrin)s **1**–**6** are summarized in Table 1.

From the data listed in Table 1, we can see that ANS itself exhibits a very short lifetime of 0.4 ns in aqueous solution, but in the presence of β-cyclodextrin exhibits two lifetimes of 0.5 ns and 3.1 ns, respectively. The shorter lifetime is consistent with the original lifetime, while the elongated lifetime of 3.1 ns in the presence of β-cyclodextrin clearly indicates that the environment around the ANS molecule is more hydrophobic than the bulk water. As we have demonstrated in previous reports,[33, 34] β-cyclodextrin is unfavorable for complexation with the naphthalene moiety with a substituent on the α-site. It is assumed that the anilino residue in ANS participates in the inclusion complexation with β-cyclodextrin. The lifetimes of aniline (2.7 ns) and *N*-methylaniline (3.7 ns) in the presence of β-cyclodextrin are consistent with the above long lifetime. On the other hand, the inclusion complex stabilities of aniline ($K_S = 61$ dm^3 mol^{-1}) and *N*-methylaniline ($K_S = 83$ dm^3 mol^{-1}) with β-cyclodextrin are also in accord with that of ANS.[35]

Interestingly, we see from Table 1 that ANS exhibits two longer lifetimes of 3.0 and 11.0 ns, in the presence of bis(β-cyclodextrin)s **1**–**6**. The shorter lifetime of 3.0 ns is ascribed to the interaction of the anilino moiety with the β-cyclodextrin cavity as described above. A probable explanation for the much longer lifetime of 11.0 ns is that the naphthalene group partly penetrates into an adjacent β-cyclodextrin cavity of the cyclodextrin dimers. In this context, the two β-cyclodextrin units, which are tethered through an oligo(ethylenediamino) spacer, cooperatively encapsulate a guest in the inclusion complexation. Therefore, it is not difficult to understand the strong binding ability of bis(β-cyclodextrin)s.

Spectral titration: Complex formation with cyclodextrin usually alters the original spectrum of the guest molecule. Figure 5 shows the spectral changes of brilliant green with gradual addition of diethylenetriamino tethered bis(β-cyclodextrin) **2**. As shown in Figure 5, the intensity at the absorption maximum around 625 nm is considerably decreased with the increase of the bis(β-cyclodextrin) (**2**) concentration.

If a 1:1 stoichiometry is assumed, where the two β-cyclodextrin moieties in **1**–**6** are treated as a unit, the inclusion complexation of a guest (Dye) with a host (bisCD) is expressed by Equation (2).

$$\text{Dye} + \text{bisCD} \overset{K_S}{\rightleftharpoons} \text{Dye}-\text{BisCD} \quad (2)$$

Table 1. The fluorescence lifetimes (τ) and relative quantum yields (Φ) of ANS in the absence and presence of polyamino bridged β-cyclodextrins (**1**–**6**) in aqueous solution at 25 °C.

Guest	Conc/μmol dm^{-3}	Host	Equiv	τ_S/ns	Φ_S/%	τ_L/ns	Φ_L/%	χ^2	Ref.
aniline	271.6	none		0.8	100			1.18	[a]
	271.6	β-CD	15	0.8	59	2.7	41	1.18	[a]
N-methyl-aniline	238	none		1.1	100			1.43	[a]
		β-CD	10	1.1	23	3.7	77	1.06	[a]
ANS	500	none		0.4	100			1.46	[b]
	10	none		0.4	100			1.42	[c]
	250	β-CD	10	1.5	67.6	3.2	32.4	1.24	[b]
	10	β-CD	40	0.5	96.5	3.1	3.5	1.00	[c]
	10	**1**	20	3.0	53.6	12.3	46.4	1.35	[c]
	10	**2**	22	3.8	58.4	11.1	41.6	1.49	[c]
	10	**3**	21	3.4	62.7	11.2	37.3	1.23	[c]
	10	**4**	20	3.1	76.5	10.7	23.5	1.45	[c]
	10	**5**	20	3.2	55.6	10.9	44.4	1.48	[c]
	10	**6**	20	2.7	58.0	10.8	42.0	1.37	[c]

[a] Ref. [31]; [b] ref. [32]; [c] this work.

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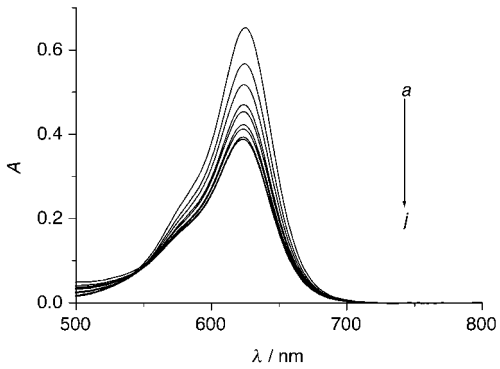

Figure 5. UV spectra of brilliant green (11 μmol dm^{-3}) in the absence and presence of various concentrations of diethylenetriamino-bridged bis(β-cyclodextrin) **2** (from a to j): 0, 50, 100, 150, 200, 250, 300, 350, 400, 450 μmol dm^{-3}.

Then, the binding constant (K_S) can be obtained from the analysis of the sequential changes of absorption (ΔA) at various cyclodextrin concentrations, with a nonlinear least-squares method according to the curve-fitting Equation (3).

$$\Delta A = \{\Delta\varepsilon([\mathrm{Dye}]_0+[\mathrm{bisCD}]_0+1/K_S) \pm \sqrt{\Delta\varepsilon^2([\mathrm{Dye}]_0 + [\mathrm{bisCD}]_0 + 1/Ks)^2 - 4\Delta\varepsilon^2[\mathrm{Dye}]_0[\mathrm{bisCD}]_0}\}/2 \quad (3)$$

where $[\mathrm{Dye}]_0$ and $[\mathrm{bisCD}]_0$ refer to the total concentrations of the guest and bis(cyclodextrin) and $\Delta\varepsilon$ is the differential molar extinction coefficient of dye guest in the absence and presence of bis(cyclodextrin). Similar equations for the spectropolarimetric or spectrofluorometric titrations can also be deduced.[32, 36]

Figure 6 illustrates the curve-fitting analyses result for the inclusion complexation of bis(β-cyclodextrin) **2** with ANS and BG. As shown in Figure 6, no serious deviations are found in the curve-fitting, and this confirms the 1:1 stoichiometry for the host–guest complexation assumed above. The complex stability constants (K_S) obtained are listed in Table 2, along with the free energy change of complex formation ($-\Delta G^\circ$). The data obtained in a similar way by the spectropolarimetric or spectroflurometric titrations are also listed in Table 2. When repeated measurements were performed, 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 (ΔG). In order to visualize the inclusion complexation behavior of bis(β-cyclodextrin)s with dye guests, the changing profiles of free energy change ($-\Delta G$) upon complexation with **1**–**6** are shown in Figure 7.

Molecular binding ability and selectivity: Native and simple modified cyclodextrins afford only very small binding constants probably due to the weak hydrophobic interactions. Dimeric cyclodextrins, however, afford much more stable inclusion complexes through cooperative binding of two adjacent cavities and potential multiple recognition ability. From Table 2, we can see that the binding constants of bis(cyclodextrin)s **1**–**6** with the guest dyes are larger than those of native β-cyclodextrin. As a result of cooperative binding, the binding constant of dimeric β-cyclodextrin (**2**)

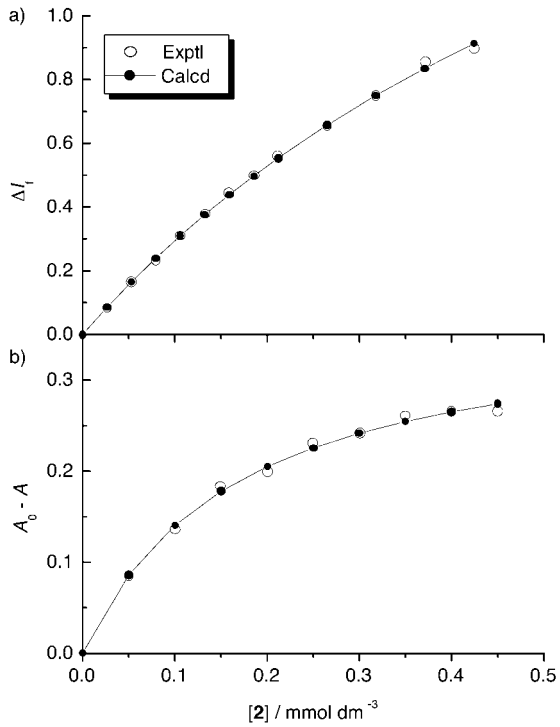

Figure 6. Curve-fitting analyses of a) fluorescence spectral titrations of ANS and b) UV/Vis spectral titrations of BG with diethylenetriamino-bridged bis(β-cyclodextrin) **2**.

Table 2. Stability constants (K_S) and Gibbs free energy changes (ΔG°) for the inclusion complexation of dye guests with polyamino bridged bis(β-cyclodextrin)s (**1**–**6**) in aqueous solution at 25.0 °C.

Host	Guest	K_S	log K_S	ΔG°/kJ mol^{-1}	Method[a]	Ref.
β-CD	BG	2187	3.34	19.1	UV	[b]
	MO	3560	3.55	20.3	CD	[c]
	ANS	103	2.01	11.5	Fl	[c]
	TNS	3670	3.56	20.3	Fl	[c]
1	BG	12050	4.08	23.3	UV	[b]
	MO	24300	4.39	25.0	CD	[b]
	ANS	2430	3.39	19.3	Fl	[b]
	TNS	18800	4.27	24.4	Fl	[b]
2	BG	6260	3.80	21.7	UV	[b]
	MO	34300	4.54	25.9	CD	[b]
	ANS	1310	3.12	17.8	Fl	[b]
	TNS	14800	4.17	23.8	Fl	[b]
3	BG	20300	4.31	24.6	UV	[b]
	MO	6290	3.80	21.7	CD	[b]
	ANS	1040	3.02	17.2	Fl	[d]
	TNS	13900	4.14	23.6	Fl	[d]
4	BG	4570	3.66	20.9	UV	[b]
	MO	6520	3.81	21.8	CD	[b]
	ANS	702	2.85	16.2	Fl	[b]
	TNS	10700	4.03	23.0	Fl	[b]
5	BG	14250	4.15	23.7	UV	[b]
	MO	19500	4.29	24.5	CD	[b]
	ANS	1570	3.20	18.2	Fl	[b]
	TNS	21100	4.32	24.7	Fl	[b]
6	BG	56700	4.75	27.1	UV	[b]
	MO	34400	4.54	25.9	CD	[b]
	ANS	1350	3.13	17.9	Fl	[d]
	TNS	23000	4.36	24.9	Fl	[d]

[a] UV: Ultraviolet/Visible, CD: circular dichroism, Fl: fluorescence; [b] this work; [c] ref. [32]; [d] ref. [19].

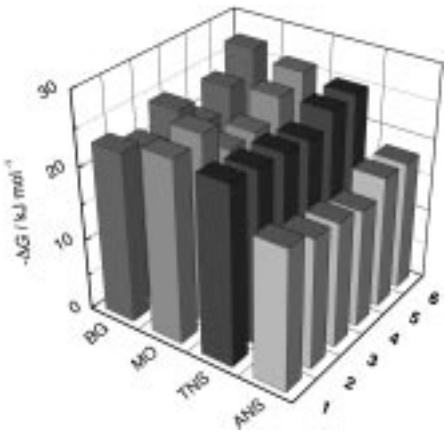

Figure 7. Gibbs free energy changes ($-\Delta G$) for the inclusion complexation of dimeric β-cyclodextrins **1**–**6** with some guest dyes.

with methyl orange is higher than that of native β-cyclodextrin by a factor of 10, while the binding constant of dimeric β-cyclodextrin **1** with ANS is even higher than that of native β-cyclodextrin by a factor of 24.

From Table 2 and Figure 7, we can see that the molecular selective profile of β-cyclodextrin is in the order TNS $\approx$ MO > BG > ANS. Dimeric β-cyclodextrins **1**, **2**, **4**, and **5** give a similar selective sequence, that is, MO > TNS > BG > ANS, or TNS > MO > BG > ANS. Triethylenetetraamino tethered β-cyclodextrin dimer **3** and its copper(II)-complex **6**, however, afford significantly different molecular selective profiles of BG > TNS > MO > ANS and BG > MO > TNS > ANS, respectively. In general, the linear guest dyes, that is, TNS and MO, afford the highest complexation stability constants with bis(β-cyclodextrin) hosts, because the longitudinally incorporation fits into the geometrical requirement. On the other hand, both TNS and ANS possess a phenyl and a naphthyl moiety, but the former forms a more stable complex with dimeric β-cyclodextrins than the latter. The examination of the CPK (Corey–Pauling–Koltun) molecular model reveals that the naphthalene moiety in ANS can not penetrate deeply into the β-cyclodextrin cavity owing to the steric hindrance. Therefore from the four guest dyes used, the weakest binding constants for ANS with bis(β-cyclodextrin)s together with the fluorescence lifetime results are not difficult to understand. Unexpectedly, brilliant green, a triangular molecule, may form very stable inclusion complexes with bis(β-cyclodextrin) hosts in some cases. A probable explanation is that the host conformations also affect the host–guest inclusion complexation.

We are especially interested in the effect of the tether length in the molecular recognition of dimeric β-cyclodextrins. As shown in Table 2 and Figure 7, the molecular binding constants for ANS and TNS are gradually decreased with the increase of the host tether length. Sikorski and Petter[37] have quantitatively studied the tether length effect of disulfide bridged bis(β-cyclodextrin)s with BNS, and found that the Gibbs free energy change ($-\Delta G$) decreases linearly with the increasing number of methylenes in the tether (N_C) and affords the unit decrement of complex stability per methylene ($-\mathrm{d}\Delta G^{\circ}/\mathrm{d}N_C$) as 2.4 kJ mol^{-1}(The original value of 0.25 kcal mol^{-1} quoted by Sikorski and Petter is incorrect. The data were recalculated from the original binding constants given in the paper.) In Figure 8, the Gibbs free energy

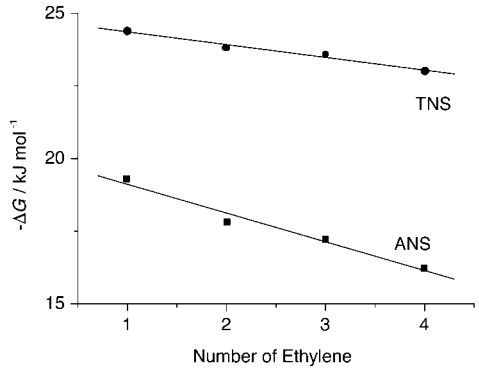

Figure 8. Plots of Gibbs free energy changes ($-\Delta G$) versus the ethylene number in the tether for the inclusion complexation of dimeric β-cyclodextrins **1**–**4** with ANS and TNS.

changes ($-\Delta G$) for the inclusion complexation of ANS and TNS were plotted against the number of ethylene units in the tether. As shown in Figure 8, the free energy changes ($-\Delta G$) decrease linearly, but the unit decrement of complex stability per ethylene are somewhat different, that is, 0.99 kJ mol^{-1} for ANS and 0.44 kJ mol^{-1} for TNS, respectively. The free energy decrease may be ascribed to the fact that the flexible tether is unfavorable for the cooperative binding of the two adjacent cyclodextrin cavities. On the other hand, Venma and Nolte et al.[38, 39] have revealed that the longer flexible tether of dimeric cyclodextrins, such as the octamethylene spacer, may penetrate into its own cavity to form a self-inclusion complex. The guest molecule must overcome much steric hindrance to fit in the host cavity. As the results of the two factors from above, bis(β-cyclodextrin) **4** always affords the weakest binding ability.

However, the binding constants for BG and MO do not always decrease with the increasing host tether length. The molecular binding ability for BG is in the order of **3** > **1** > **2** > **4**, while that for MO is **2** > **1** > **4** > **3**. These results indicate that the match of size/shape between the host and the guest dominates the stability of the inclusion complex formed to some extent.[40, 41] In this context, the tether length of host **3** is suitable for the cooperative binding of brilliant green, while host **2** gives the most stable complex with methyl orange.

There are many interesting reports that monomodified cyclodextrins with oligo(ethylenediamino) arms with copper(II) can alter not only the original binding ability,[42, 43] but also the chiral selectivity.[44–45] If there are appropriate functional groups in the tether of dimeric cyclodextrin, the tether may also participate in the molecular recognition procedure.[32, 46, 47] Therefore, we prepared the copper(II) complex of diethylenetriamino and triethylenetetraamino analogues and examined their molecular binding ability. It can be seen from Table 2 that the coordination of the ligand tether of **2** and **3** to Cu^{II} further enhances the binding ability of **5** and **6** for ANS,

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TNS, and BG. This further enhancement is attributable to the conformational fixation by metal ligation, electrostatic interaction with the ligated Cu^{II}, and/or ligation of the nitrogen of guest dyes to Cu^{II} in **5** and **6**. If one of the latter two mechanisms are operative, the metallobis(β-cyclodextrin)s **5** and **6** serve as the host with ternary recognition (two hydrophobic and one electrostatic/coordination) sites. Thus, the TNS/ANS selectivity is enhanced from 11 for **2** to 14 for **5**, and from 13 for **3** to 17 for **6**, respectively, which could be ascribed to the triple recognition of the guest by metallobis(β-cyclodextrin)s **5** and **6**. As for the methyl orange guest, we may note that **6** gives a binding constant similar to **1**, while **5** affords a binding constant in the same magnitude as **2**. It may be concluded that the ligation of Cu^{II} would shorten the effective length of the tethers, and further confirms the tether length's important role in the molecular recognition.

Conclusion

In conclusion, bridged bis(β-cyclodextrin)s **1**–**4** tethered by oligo(ethylenediamine) significantly extend the original molecular binding ability of the parent β-cyclodextrin, and the complex stability constants for the selected guests are larger than native β-cyclodextrin by factors from 2 to 24 through the cooperative interaction. Furthermore, the complex stability also depends greatly on the tether length of bis(β-cyclodextrin)s and the size and shape of guests. In particular, the coordination of copper(II) ion to the tether not only orientates two β-cyclodextrin cavities to fit the shape of the guest molecule, but also acts as an additional site of guest recognition through coordination and/or electrostatic interaction. As a result, a further enhanced molecular binding ability and selectivity could be observed for metallobis(β-cyclodextrin)s **5** and **6**.

Experimental Section

Materials: All guest dyes, that is, brilliant green (BG), methyl orange (MO), ammonium 8-anilino-1-naphthalenesulfonic acid (ANS), and sodium 6-*p*-toluidino-2-naphthalenesulfonic acid (TNS), 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 two days and then distilled under a reduced pressure prior to use.

Triethylenetetraamino bridged β-cyclodextrin dimer (**3**) was synthesized by the reaction of mono[6-*O*-(*p*-toluenesulfonyl)]-β-cyclodextrin (6-OTs-β-CD)[48] with mono(6-triethylenetetraamino-6-deoxy)-β-cyclodextrin in DMF according to the procedure reported previously.[19]. Ethylenediamino bridged bis(β-cyclodextrin) **1**, diethylenetriamino bridged bis(β-cyclodextrin) **2**, and tetraethylenepentaamino bridged bis(β-cyclodextrin) **4** were synthesized by the reaction of mono[6-*O*-(*p*-toluenesulfonyl)]-β-cyclodextrin with the corresponding oligo(ethylenediamino)-β-cyclodextrin in DMF according to a similar synthetic procedure described previously for β-cyclodextrin dimer **3**. A representative synthetic procedure was given as follows.

Ethylenediamino bridged bis(β-cyclodextrin) 1: 6-OTs-β-CD was prepared by the reaction of *p*-tosyl chloride with β-cyclodextrin in alkaline aqueous solution according to literature reports.[48] Then, 6-OTs-β-CD was converted to mono(6-ethylenediamino-6-deoxy)-β-cyclodextrin in 70% yield on heating in excess triethylenetetraamine at 70 °C for 7 h.[49, 50] The mixture of mono(6-ethylenediamino-6-deoxy)-β-cyclodextrin (1.2 g) and 6-OTs-β-CD (1.3 g) was allowed to react in DMF (50 mL) with stirring under nitrogen at 80 °C for 3 d. The resultant solution was poured into acetone (300 mL), and the precipitate formed was collected by filtration. This procedure was repeated several times. The crude product thus obtained was subsequently purified on a CM Sephadex C-25 ionic column with 1 mol dm^{-3} aqueous ammonia as eluent and a Sephadex G-25 column with water as eluent, respectively. After the residue was dried in vacuo, a pure sample was obtained in 19% yield. ^{1}H NMR (300 MHz, D_2O, TMS): $\delta = 5.05$ (m, 14H), 4.1–3.6 (m, 56H), 3.6–3.2 (m, 28H), 2.9–2.5 (m, 4H); ^{13}C NMR (400 MHz, D_2O, TMS): $\delta = 104.66$, 103.98, 86.86, 83.99, 75.92, 74.87, 74.67, 72.10, 63.38, 47.87; MS(MALDI-TOF): m/z: 2294 $[M - 4H_2O]^+$; elemental analysis calcd (%) for $C_{86}H_{144}O_{68}N_2 \times 4H_2O$ (2366.1): C 43.66, H 6.48, N 1.18; found: C 43.53, H 6.60, N 1.36.

Diethylenetriamino bridged bis(β-cyclodextrin) 2: Compound **2** was prepared from mono[6-*O*-(*p*-toluenesulfonyl)]-β-cyclodextrin and mono[6-diethylenetriamino-6-deoxy]-β-cyclodextrin according to procedures similar to those in the synthesis of **1** (yield 20%). ^{1}H NMR (300 MHz, D_2O, TMS): $\delta = 4.9$ (m, 14H), 4.0–3.5 (m, 56H), 3.5–3.1 (m, 28H), 3.0–2.4 (m, 8H); ^{13}C NMR (400 MHz, D_2O, TMS): $\delta = 104.58$, 103.98, 86.86, 83.91, 76.30, 75.84, 74.82, 74.61, 72.28, 63.09, 61.90, 47.71; MS(MALDI-TOF): m/z: 2337 $[M - 2H_2O]^+$; elemental analysis calcd (%) for $C_{88}H_{149}O_{68}N_3 \times 2H_2O$ (2373.2): C 44.54, H 6.50, N 1.77; found: C 44.55, H 6.25, N 1.91.

Tetraethylenepentaamino bridged bis(β-cyclodextrin) 4: Compound **4** was prepared from 6-OTs-β-CD and mono[6-tetraethylenepentaamino-6-deoxy]-β-cyclodextrin according to procedures similar to those in the synthesis of **1** (yield 17%). ^{1}H NMR (300 MHz, D_2O, TMS): $\delta = 4.9$ (m, 14H), 4.0–3.6 (m, 56H), 3.6–3.2 (m, 28H), 2.9–2.6 (m, 16H); ^{13}C NMR (400 MHz, D_2O, TMS): $\delta = 104.71$, 103.91, 86.76, 83.99, 75.93, 74.82, 74.66, 63.04, 55.05, 47.90, 38.51; MS(MALDI-TOF): m/z: 2447 $[M+Na-2H_2O]^+$; elemental analysis calcd (%) for $C_{92}H_{159}O_{68}N_5 \times 2H_2O$ (2459.3): C 44.93, H 6.68, N 2.85; found: C 45.15, H 6.52, N 3.09.

Diethylenetriamino bridged bis(β-cyclodextrin) copper(II) complex 5: As for the preparation of metallobis(β-cyclodextrin) **6**,[19] bis(cyclodextrin) **2** was added portionwise to a dilute aqueous solution of slightly excess copper(II) perchlorate in an ice/water bath. Several drops of chloroform were further added, and the resultant solution was kept at 5 °C for 2 d. Then, the precipitate formed was collected by filtration, washed successively with a small amount of ethanol and diethyl ether, and then dried in vacuo to give bis(cyclodextrin) Cu^{II}-complex **5** in 40% yield. Elemental analysis calcd (%) for $C_{88}H_{149}O_{68}N_3 \times Cu \times 2ClO_4 \times 4H_2O$ (2635.6): C 39.56, H 5.92, N 1.57; found: C 39.32, H 5.82, N 1.56.

Measurements: CD and UV/Vis spectra were recorded in a conventional quartz cell (light path 10 mm) on a JASCO J-720S spectropolarimeter or a JASCO UV550 spectrophotometer equipped with a PTC-348WI temperature controller to keep the temperature at 25 °C. Fluorescence spectra were measured in a conventional rectangular quartz cell (10 × 10 × 45 mm) at 25 °C on a JASCO FP-750 fluorescence spectrometer with the excitation and emission slits of 5 nm width. Electronic conductivity was measured on a DDS-12A (Zhejiang) digital conductive instrument.

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 monochrometer. 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 10000 were collected for each measurement. The accumulated signals were then processed and the lifetime determined by deconvolution with nonlinear least squares fit.

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