

Spectrophotometric Study of Selective Binding Behaviors of Dye Molecules by Pyridine- and Bipyridine-Modified β -Cyclodextrin Derivatives with a Functional Tether in Aqueous Solution

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Four β -cyclodextrin (β -CD) derivatives bearing pyridine or bipyridine linkers, i.e., mono[6-(3-pyridinecarboxamide)ethyleneamino-6-deoxy]- β -CD (**2**), mono[6-(4-pyridinecarboxamide)ethyleneamino-6-deoxy]- β -CD (**3**), *N,N'*-bis(2-aminoethyl)-2,2'-bipyridine-4,4'-dicarboxamide-bridged bis(6-amino-6-deoxy- β -CD) (**4**), *N,N'*-bis(2-aminoethyl)-2,2'-bipyridine-3,3'-dicarboxamide-bridged bis(6-amino-6-deoxy- β -CD) (**5**), and their copper(II) complexes (**6** and **7**) were selected as molecular receptors to explore the conformation–function relationship of oligo(β -CD)s. The original conformations of hosts **4**–**7** and their inclusion complexation behaviors with some guest molecules, i.e., ammonium 8-anilino-1-naphthalenesulfonate (ANS), sodium 6-(*p*-toluidino)-2-naphthalenesulfonate (TNS), and rhodamine B (RhB), were comprehensively investigated by means of UV–vis, 2D NMR, and fluorescence spectroscopy. The results indicated that these oligo(β -CD)s, especially bis(β -CD) **5** and its copper(II) complex **7**, exhibited the significantly enhanced binding abilities toward guest molecules as compared with native β -CD. Typically, hosts **5** and **7** efficiently enhanced the original binding ability of native β -CD toward ANS by a factor of 38–42 times. These increased binding abilities of oligomeric hosts were discussed from the viewpoint of the size/shape-fit and multipoint recognition between host and guest.

Introduction

It is well known that cyclodextrins (α -, β -, and γ -CDs) are a class of cyclic oligosaccharides with six to eight D-glucose units linked by α -1,4-glucose bonds which can accommodate various guest molecules in their truncated cone-shaped hydrophobic cavity either in aqueous solution or in the solid state.^{1–3} Possessing dual hydrophobic cavities in a close vicinity and a nucleophilic or electrophilic linker with a good structural variety in a single molecule, bridged CD dimers can greatly enhance the original binding ability and molecular selectivity of parent CD through the potential cooperative binding of two adjacent CD units^{4–12} and therefore be successfully employed in several areas of science and technology as an excellent model system mimicking substrate-specific interaction of enzymes.^{13,14} Moreover, there is another advantage for CD dimers upon inclusion complexation with model substrates. That is, besides the cooperative sandwich binding of two CD cavities with substrates, the linker groups can also provide the additional binding interactions toward substrates through several noncovalent interactions such as van der Waals, hydrophobic, hydrogen-bond, electrostatic, or electron-transfer interactions. To understand the role of linker group in the molecular recognition, a number of functional groups, such as alkanedioates,^{15,16} disulfides,^{17,18} bipyridines,^{19,20} and imidazole,^{21,22} have been selected as the linkers between two CD units. We recently reported the molecular recognition behaviors of a series of azacyclodextrins,²³ especially the bis(β -CD)s with 2,2'-bipyridine-4,4'-dicarboxylic bridges.²⁴ In this work, we find that fine-tuning the structure of

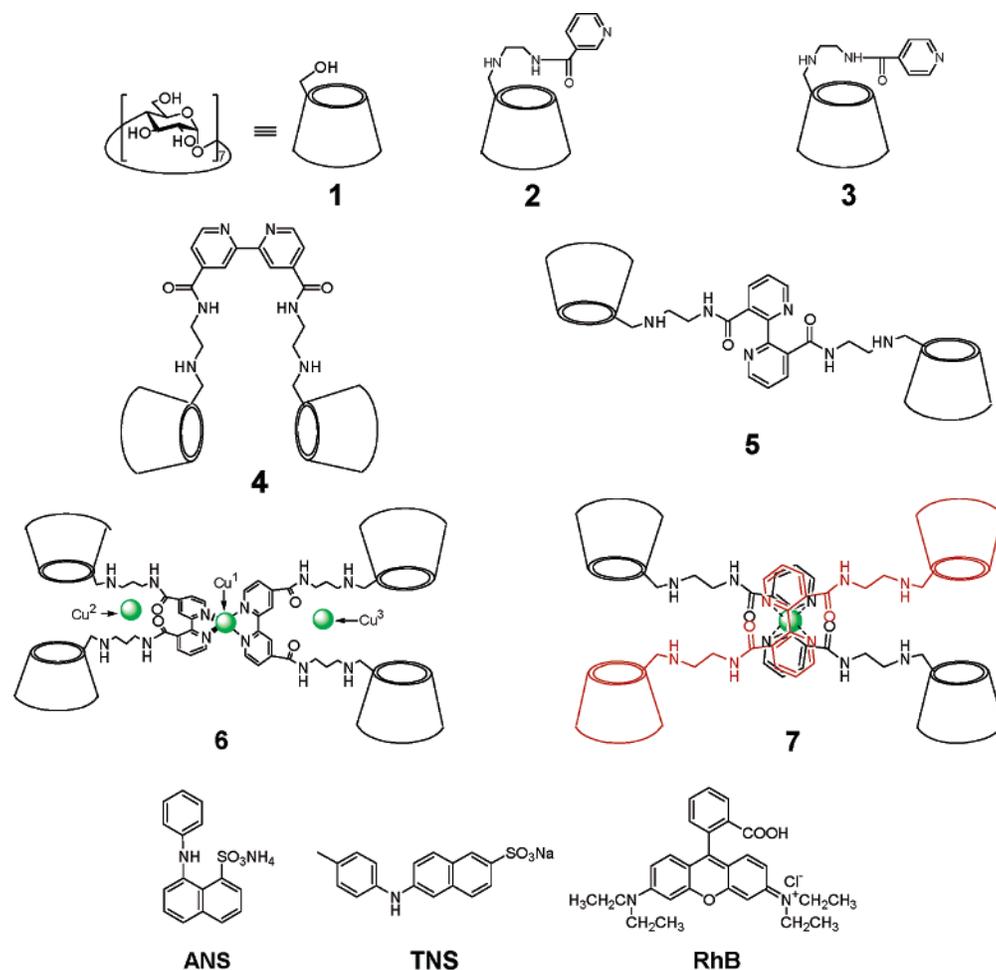
the linker group can significantly alter the conformation and binding behavior of the resultant bis(β -CD)s. Although this concept is drawn from a rather limited variation of bis(β -CD) species, we ensure that it should be extended more generally to a wide variety of synthetic receptors. Therefore, we wish to report contrastive studies on the conformation and binding behavior of bis(β -CD)s with 2,2'-bipyridine-4,4'-dicarboxamide and 2,2'-bipyridine-3,3'-dicarboxamide linkers as well as their copper(II) complexes (Chart 1). It is our special interest to explore how the bridge group affects the cooperative binding of bis(β -CD)s and further understand the factors governing the molecular multiple recognition mechanism.

Results and Discussion

Synthesis. As illustrated in Scheme 1, bipyridine-bridged bis(β -CD) **5** is synthesized in a yield of 20% by the reaction of 2,2'-bipyridine-3,3'-dicarboxylic dichloride with mono[6-(2-aminoethyleneamino)-6-deoxy]- β -CD, while two mono-modified β -CDs, i.e., mono[6-(3-pyridinecarboxamide)ethyleneamino-6-deoxy]- β -CD (**2**) and mono[6-(4-pyridinecarboxamide)ethyleneamino-6-deoxy]- β -CD (**3**), are synthesized in relatively high yields (56% for **2**, 50% for **3**) as reference compounds. The further reactions of bis(β -CD) **5** with copper(II) perchlorate give the copper(II) complex **7** in a moderate yield (40%). In our previous report,²³ we demonstrated that coordination of bis(β -CD) **4** with copper(II) perchlorate displays a 2:3 stoichiometry, which indicates that besides each bis(β -CD) unit associating with one copper(II) ion, two bis(β -CD) components participate in the binding of the third copper(II) ion. However, when altering the 2,2'-bipyridine-4,4'-dicarboxamide linker in bis(β -CD) **4** to the 2,2'-bipyridine-3,3'-dicarboxamide group, the

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CHART 1



resultant bis(β -CD) **5** will adopt a different coordination stoichiometry with copper(II) ion. Figure 1 shows a representa-

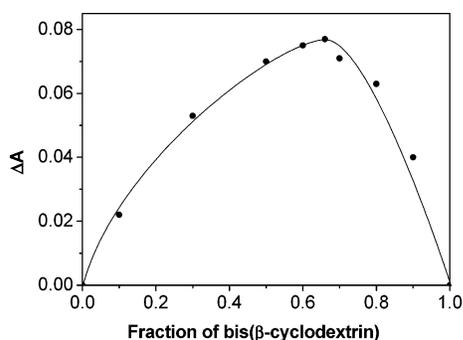


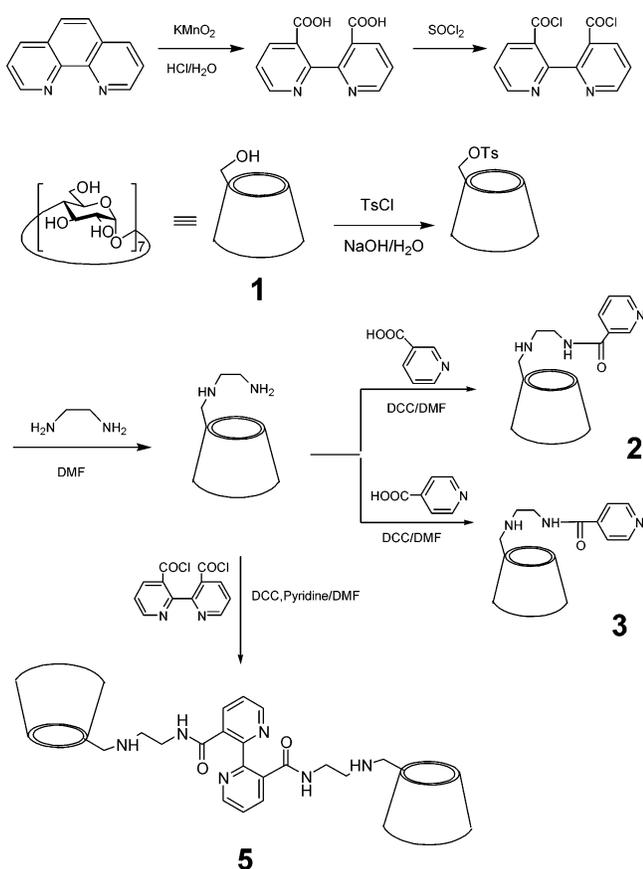
Figure 1. Job's plot of **5**/ Cu(II) ($[\text{5}] + [\text{Cu}^{2+}] = 1 \times 10^{-4} \text{ mol dm}^{-3}$) system by UV-vis spectra.

tive spectrophotometric titration curve to determine the coordination stoichiometry of **5** with copper(II) ion. As can be seen in Figure 1, the Job's plot for the **5**/ Cu(II) system displays a maximum at 0.67 which corresponds to a 2:1 **5**/ Cu(II) stoichiometry. This result indicates that although possessing four $-\text{NH}-$ fragments and one bipyridine group in each bis(β -CD) **5**, two bis(β -CD)s **5** can only coordinate with one copper(II) ion. This 2:1 stoichiometry for **5**/ Cu(II) complex is also verified by elemental analysis.

Conformation. It is well known that elucidation of the crystal structure is one of the most convincing methods of unequivocally illustrating the geometrical structure of CD derivatives.

Unfortunately, our repeated attempts to prepare single crystals of β -CD dimers and their metal complexes were unsuccessful. Therefore, to reveal the reason for the different coordination stoichiometry of bis(β -CD)s **4** and **5** with copper(II) ion, we perform a molecular modeling study with the CAChe 3.2 program (Oxford Molecular Co., 1999) and obtain the energy-optimized structures of bis(β -CD)s **4** and **5**. The initial geometry of β -CD used in these calculations is taken from the crystal structure described in the literature,²⁵ and the energy of these structures is minimized using the MM2 force field. Although the computed structures may not taken as direct evidence of the actual structures, these computed models can still provide some useful information about the possible geometries of bis(β -CD)s.²⁶ As shown in Figure 2, the bipyridine group in bis(β -CD) **4** is coplanar and exists in a cis conformation. Further investigations with a Corey-Pauling-Koltun (CPK) molecular model demonstrate that three copper(II) ions in metallooligo(β -CD) **6** adopt two types of four-coordinate geometry upon coordination with bipyridine dicarboxamide bridge (Chart 1). That is, the copper(II) ion located between two bis(β -CD) components (Cu^1) adopts a tetrahedral geometry upon coordination with two bipyridine groups, while either of the other two copper(II) ions (Cu^2 and Cu^3) adopts a planar geometry when coordinated with four $-\text{NH}-$ fragments in the linker group. These two factors jointly result in a perpendicular conformation of metallooligo(β -CD) **6** as shown in Chart 1. However, bis(β -CD) **5** is found to adopt a trans conformation as shown in Figure 2, which may be attributed to the steric hindrance between two carbonyl groups at the 3,3'-positions of bipyridine.

SCHEME 1



In this conformation two pyridine rings in the linker group are not coplanar but twist to each other with a dihedral angle of about 30° and two ethylenediamino chains adopt a “tilt-out”

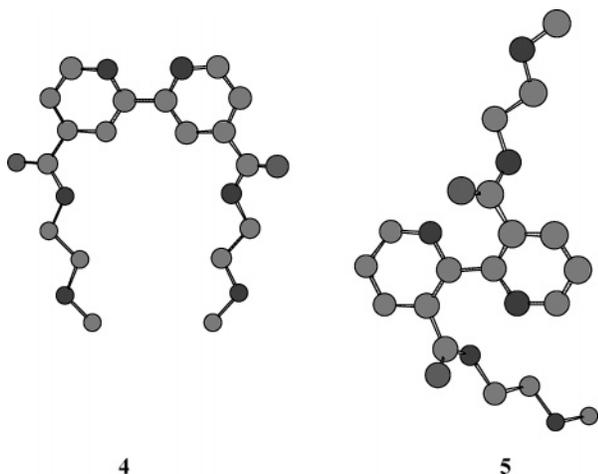


Figure 2. MM2-optimized structure of bis(β -CD)s **4** and **5** (β -CD cavities are omitted).

conformation relative to the bipyridine group. Therefore, these two ethylenediamino chains are too distant to enable coordination with copper(II) ion, which consequently results in the 2:1 **5**/Cu(II) coordination stoichiometry.

Another piece of evidence for the different conformations of bis(β -CD)s **4** and **5** comes from the UV spectra. Generally, the UV spectra of bipyridine and its derivatives have a great relationship with their conformations.²⁷ A coplanar bipyridine chromophore usually shows two absorption bands in the ultraviolet region. If two pyridine units twist along the central

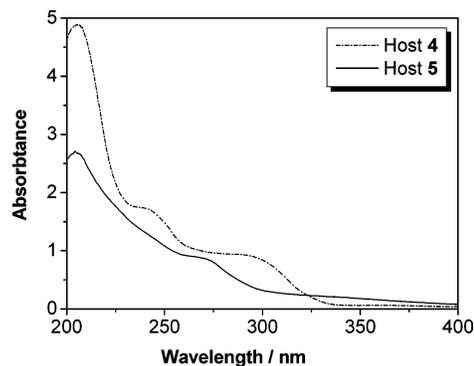


Figure 3. UV-vis spectra of bis(β -CD)s **4** and **5** in aqueous solution at 25°C ($[\mathbf{4}] = [\mathbf{5}] = 0.9 \times 10^{-4} \text{ mol}\cdot\text{dm}^{-3}$).

carbon-carbon bond, the absorption intensities of these two bands will quench, accompanied by hypsochromic shifts of the absorption maximums, and sometimes only one absorption band can be observed. As illustrated in Figure 3, bis(β -CD) **4** shows two absorption bands at 241 ($\epsilon = 191\,510 \text{ mol}^{-1} \text{ dm}^{-3} \text{ cm}^{-1}$) and 294 nm ($\epsilon = 10\,130 \text{ mol}^{-1} \text{ dm}^{-3} \text{ cm}^{-1}$), respectively, assigned to absorptions of the coplanar bipyridine chromophore. However, bis(β -CD) **5** only gives one absorption band at 270 nm ($\epsilon = 9710 \text{ mol}^{-1} \text{ dm}^{-3} \text{ cm}^{-1}$). This phenomenon further verifies the twist of the bipyridine group in bis(β -CD) **5**.

Interestingly, the absorption intensity of bis(β -CD) **4** at 294 nm obviously increases (ϵ from $10\,130$ to $12\,770 \text{ mol}^{-1} \text{ dm}^{-3} \text{ cm}^{-1}$) after coordination with copper(II) ion, accompanying a significant bathochromic shift (20 nm) of the absorption peak. Moreover, the other absorption peak of **4** at 241 nm changes to a shoulder after coordination with copper(II) ion, as illustrated in Figure 4a. These phenomena, together with the 2:3 coordination stoichiometry determined by the Job's plot, may indicate a relatively strong metal-ligand coordination involving two bis(β -CD)s and three copper(II) ions as shown in Chart 1. However, the UV spectrum of metallooligo(β -CD) **7** is quite similar to that of its precursor **5** (Figure 4b), except a slight increase of the absorption intensity at 270 nm (ϵ from 9710 to $10\,150 \text{ dm}^{-3} \text{ cm}^{-1}$), which indicates that the bipyridine units in **7** still remain in the trans conformation. These results will be greatly helpful to understand their binding mode with guest molecules.

Fluorescence Titrations. For a more qualitative assessment of the inclusion complexation behavior of bis(β -CD)s and their copper(II) complexes, the spectral titrations of hosts **1–7** with ammonium 8-anilino-1-naphthalenesulfonic acid (ANS), sodium 6-(*p*-toluidino)-2-naphthalenesulfonate (TNS), and rhodamine B (RhB) are performed at 25°C in aqueous solution by fluorescence spectroscopy. Figure 5 shows the typical spectral changes of ANS with the gradual addition of host **5**. The relative fluorescence intensity of ANS gradually enhances with an increase of the concentration of **5**. Because ANS barely fluoresces in aqueous solution but emits a strong fluorescence in nonpolar environment, the spectral changes observed indicate that the aromatic group of ANS is embedded into the hydrophobic β -CD cavities, forming a host-guest inclusion complex. Further study indicates that the pH value of the solution does not change significantly during the experimental procedure. These results lead us to deduce that the binding behavior is dependent on the individual structural features of host and guest.

The stoichiometry for the inclusion complexation of hosts **4–7** with guest molecules is determined through the Job's experiments by fluorescence spectroscopy. By treating each bis(β -CD) unit in hosts **4–7** as a host unit, all of the Job's plots show maxima at a molecular fraction of bis(β -CD) of 0.5, which

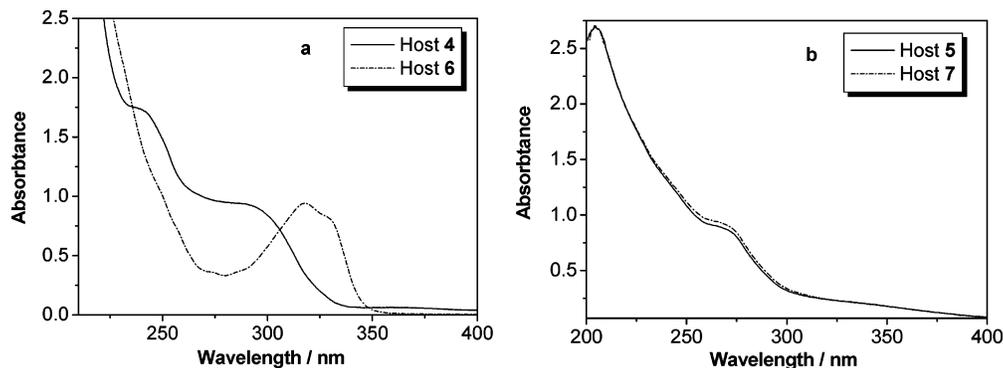


Figure 4. UV-vis spectra of (a) **4** and **6** ($[4] = [6] = 0.9 \times 10^{-4} \text{ mol}\cdot\text{dm}^{-3}$) and (b) **5** and **7** ($[5] = [7] = 0.9 \times 10^{-4} \text{ mol}\cdot\text{dm}^{-3}$).

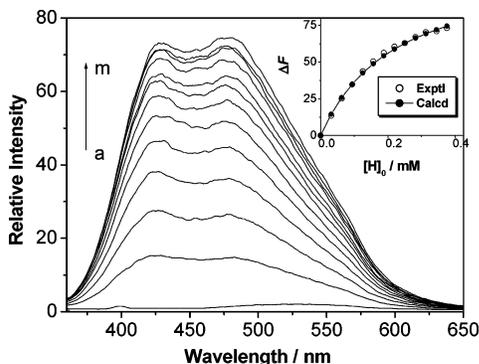


Figure 5. Fluorescence spectral changes of ANS ($1.0 \times 10^{-5} \text{ mol}\cdot\text{dm}^{-3}$) and the nonlinear least-squares analysis (inset) of the differential intensity (ΔF) to calculate the complex stability constant (K_s) upon addition of **5** ($0-375 \times 10^{-6} \text{ mol}\cdot\text{dm}^{-3}$ from a to m) in aqueous solution.

indicates 1:1 inclusion complexation stoichiometry between a host unit and a guest molecule. Moreover, the Job's experiments for inclusion complexation of mono-modified β -cyclodextrins **2** and **3** with guest molecules are also performed under comparable conditions to give 1:1 stoichiometry.

Validating the 1:1 stoichiometry, where each bis(β -cyclodextrin) moiety in **4-7** is treated as a host unit, inclusion complexation of a guest (Dye) with a host can be expressed by the following equation



Therefore, the effective stability constant (K_s)²³ could be calculated from analysis of the sequential changes in fluorescence intensity (ΔF) at varying host concentrations by using a nonlinear least-squares method according to the curve-fitting equation.²⁸ Figure 5 (inset) illustrates a typical curve-fitting plot for titration of ANS with host **5**, which shows excellent fits between the experimental and calculated data. In repeated measurements the K_s values are reproducible within an error of $\pm 5\%$. The K_s values obtained are listed in Table 1 along with the Gibbs free energy changes ($-\Delta G^\circ$).

Binding Mode. We demonstrated that the cis conformer **4** adopts a cooperative sandwich binding mode upon inclusion complexation with guest molecule,²³ where two side groups of the guest molecule are embedded into the hydrophobic cavities from the primary side of β -CD to form a sandwich inclusion complex, as illustrated in Figure 6a. However, for the trans conformer **5**, the CPK model studies give three possible binding modes, i.e., the intermolecular 2:2 binding mode (Figure 6b), the intramolecular 1:1 binding mode (Figure 6c), and the inter-

TABLE 1: Stability Constants (K_s) and Gibbs Free Energy Changes ($-\Delta G^\circ$) for the Inclusion Complexation of Guest Dyes with Hosts 1-7 in Aqueous Solution at 25 °C

| host | guest | $\lambda_{\text{ex}}/\text{nm}$ | K_s/M^{-1} | $\log K_s$ | $-\Delta G^\circ/\text{kJ mol}^{-1}$ | ref |
|----------|-------|---------------------------------|---------------------|------------|--------------------------------------|----------|
| 1 | ANS | 350 | 112 | 2.05 | 11.70 | <i>a</i> |
| | TNS | 350 | 3590 | 3.56 | 20.30 | <i>a</i> |
| | RhB | 520 | 4900 | 3.69 | 21.07 | <i>a</i> |
| 2 | ANS | 350 | 383 | 2.58 | 14.74 | <i>b</i> |
| | TNS | 350 | 4590 | 3.66 | 20.9 | <i>b</i> |
| | RhB | 520 | 3160 | 3.50 | 19.98 | <i>b</i> |
| 3 | ANS | 350 | 281 | 2.45 | 13.98 | <i>b</i> |
| | TNS | 350 | 3410 | 3.53 | 20.16 | <i>b</i> |
| 4 | RhB | 520 | 2560 | 3.41 | 19.45 | <i>b</i> |
| | ANS | 350 | 665 | 2.82 | 16.11 | <i>a</i> |
| | TNS | 350 | 7610 | 3.88 | 22.16 | <i>a</i> |
| 5 | RhB | 520 | 11 360 | 4.06 | 23.15 | <i>a</i> |
| | ANS | 350 | 4650 | 3.67 | 20.93 | <i>b</i> |
| | TNS | 350 | 13 100 | 4.12 | 23.50 | <i>b</i> |
| 6 | RhB | 520 | 6360 | 3.80 | 21.71 | <i>b</i> |
| | ANS | 350 | 3900 | 3.59 | 20.50 | <i>a</i> |
| | TNS | 350 | 17 300 | 4.24 | 24.19 | <i>a</i> |
| 7 | RhB | 520 | 14 300 | 4.16 | 23.72 | <i>a</i> |
| | ANS | 350 | 4230 | 3.63 | 20.70 | <i>b</i> |
| | TNS | 350 | 10 170 | 4.01 | 22.87 | <i>b</i> |
| | RhB | 520 | 13 500 | 4.13 | 23.58 | <i>b</i> |

^a Reference 24c. ^b This work.

molecular n:n binding mode (Figure 6d). To estimate the actual binding mode of bis(β -CD) **5** with guest molecule, we perform 2D NMR experiments to investigate the structure of the host-guest inclusion complex since two protons closely located in space can induce an NOE correlation between the relevant protons in the NOESY or ROESY spectrum. In a preliminary experiment the ROESY spectra of hosts **4** and **5** show no NOE correlations between the protons of 2,2'-bipyridine-dicarboxamide linker (aromatic protons and ethylene protons) and the interior protons of β -CD (H-3 and H-5), indicating that the linker group of bis(β -CD) is not self-included in the β -CD cavity. By comparing the ROESY spectra of **4** and **5** we can see that the ROESY spectrum of **4** is more complicated than that of **5** in the aromatic region (Figure 7). This phenomenon should be reasonable because the cis conformation of **4** enables close distances among the bipyridine protons and thus results in more complicated NOE signals. Unfortunately, the conformational features of hosts **6** and **7** are unable to be estimated by 2D NMR due to the paramagnetic disturbance of copper(II).

Moreover, the ROESY spectrum of an equimolar mixture of host **5** with T-shaped guest RhB (Figure 8) displays the clear NOE correlations between the H-3 and H-5 protons of β -CD and the methyl protons of diethylamino groups in RhB (peaks b) as well as the NOE correlations between the H-5 protons of β -CD and the aromatic protons of diethylaminophenyl group

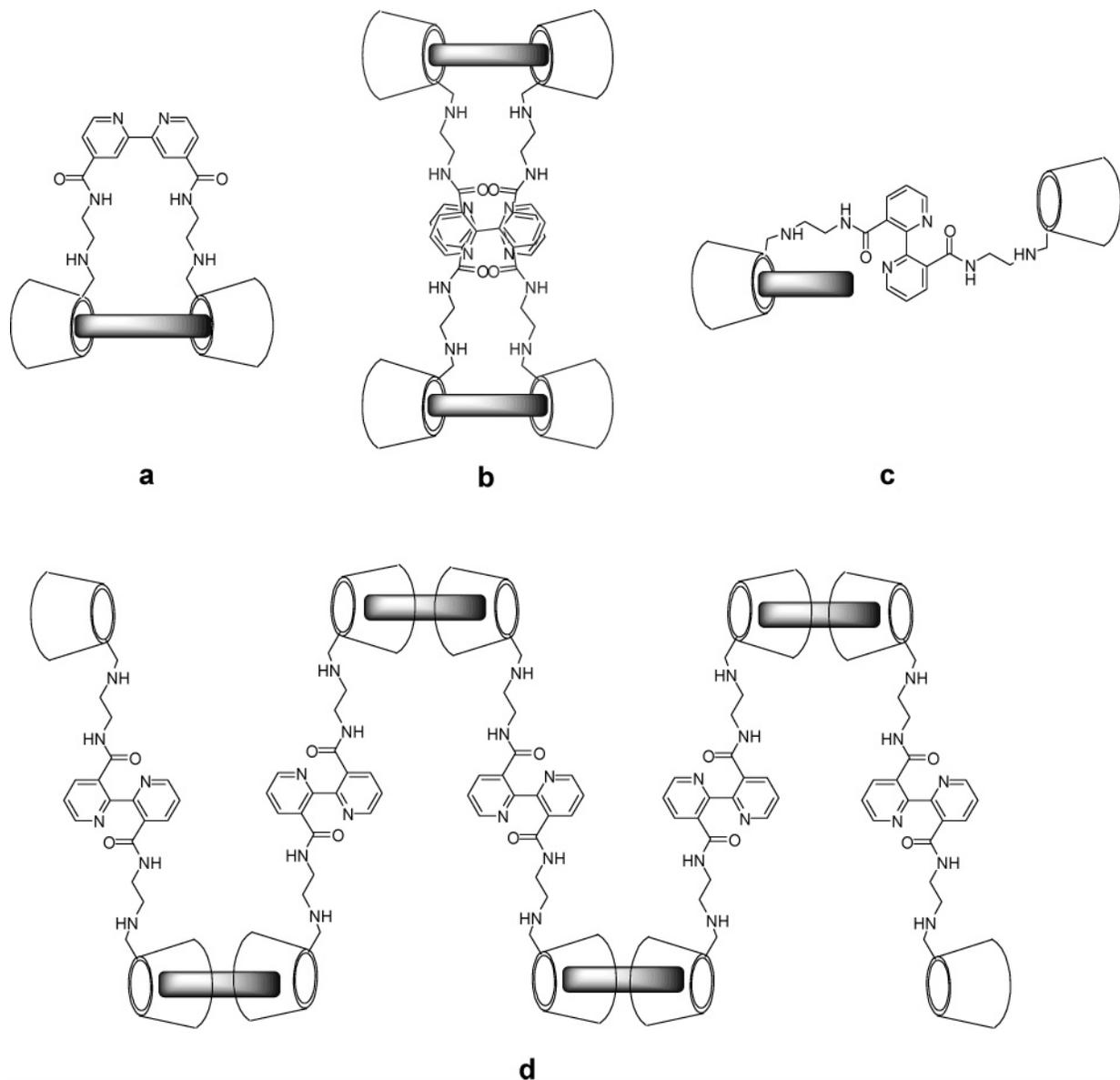


Figure 6. Possible binding modes of bis(β -CD)s 4 and 5 with guest molecule.

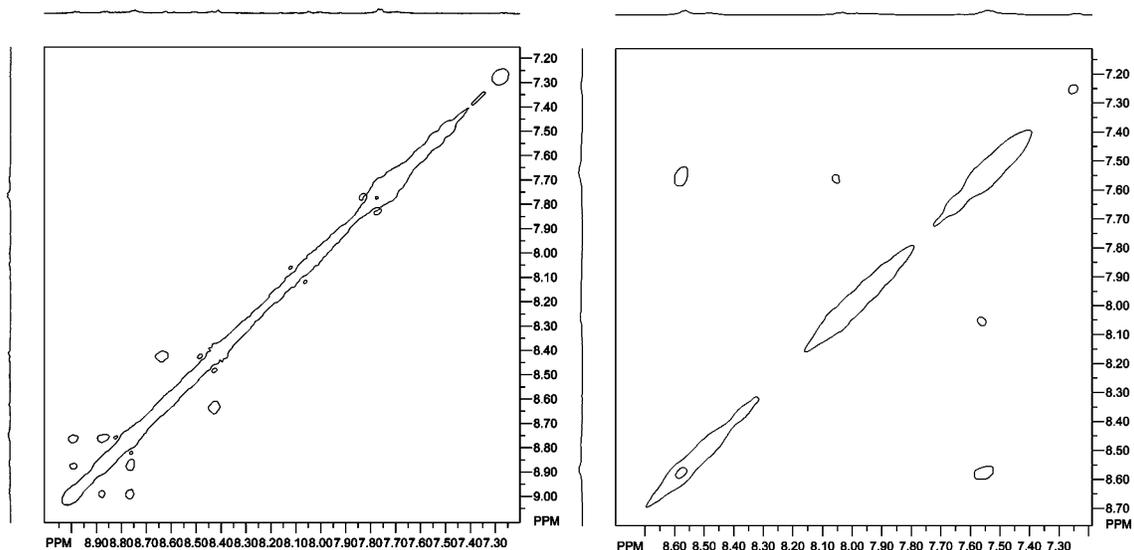


Figure 7. Partial ROESY spectra of bis(β -CD)s 4 (left) and 5 (right) in D_2O with a mixing time of 200 ms at 298 K.

in RhB (peaks a). Further examination of the intensities of these correlations indicates that the correlations between the H-5

protons of β -CD and the methyl protons of diethylamino groups in RhB are stronger than those between the H-3 protons of β -CD

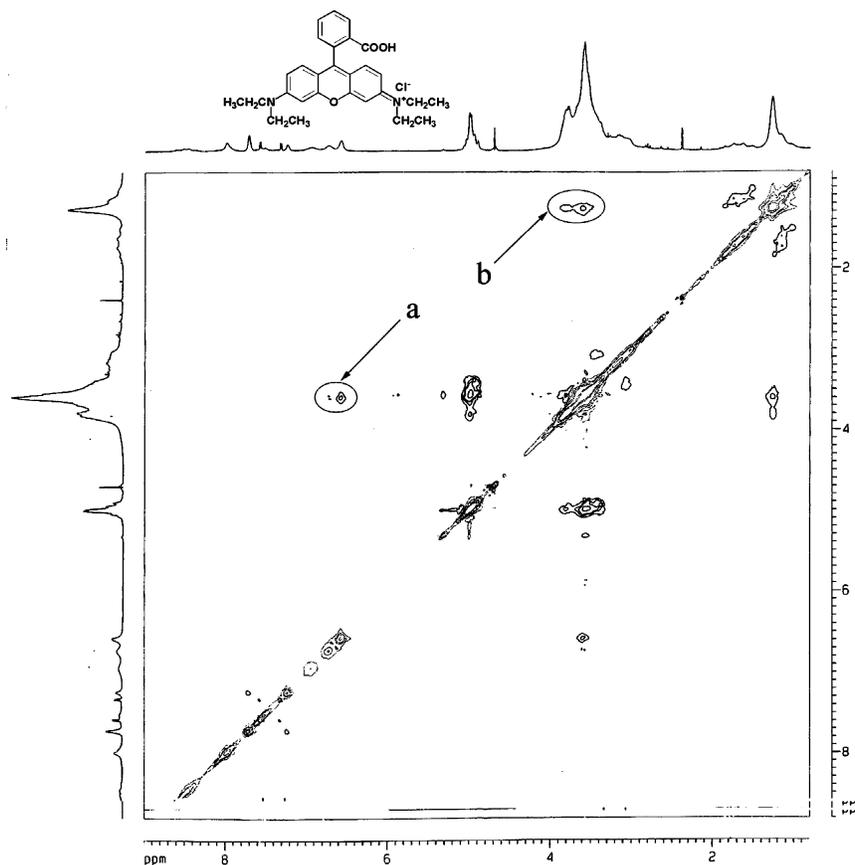


Figure 8. ROESY spectrum of a mixture of host **5** and RhB ($[5] = [\text{RhB}] = 3.0 \times 10^{-3} \text{ mol} \cdot \text{dm}^{-3}$) in D_2O with a mixing time of 200 ms at 298 K.

and the methyl protons of diethylamino groups in RhB. Since both the H-3 and H-5 protons are located at the interior of the β -CD cavity and the H-5 protons are located near the primary side (narrow side) of the cavity but the H-3 protons near the secondary side (wide side) of cavity, these NOE correlations indicate that the diethylaminophenyl group of RhB is accommodated in the β -CD cavities from the primary side. This result excludes the possibility of an intermolecular $n:n$ binding mode, where the guest molecule is included hypothetically in the β -CD cavities from the secondary side. Moreover, Figure 8 does not display the NOE correlations between the protons of the diethylaminophenyl group in RhB and the protons of the linker group in **5**, which excludes the possibility of the intramolecular 1:1 binding mode. According to this binding mode, one side group of the guest molecule should be located near the linker group of the host bis(β -CD) and give the corresponding NOE correlations. Therefore, we can deduce that bis(β -CD) **5** actually adopts an intermolecular 2:2 binding mode upon complexation with guest RhB, where two diethylaminophenyl groups of RhB are cooperatively included by two β -CD cavities from the primary side.

The intermolecular 2:2 binding mode is also verified by the ROESY spectrum of the **5**/ANS system. The simple reason for choosing ANS as the guest molecule to examine the binding geometry of bis(β -CD) is that ANS possesses two different aromatic fragments, i.e., phenyl and naphthyl groups, and their chemical shifts can be easily recognized in the ^1H NMR spectrum. As can be seen from Figure 9, the ROESY spectrum of an equimolar mixture of host **5** and ANS displays NOE correlations between the Ha protons of the phenyl group in ANS and the H-3/H-5 protons of β -CD (peaks a), the NOE correlations between the Hb protons of the phenyl group in ANS and

the H-3 protons of β -CD (peaks b), as well as the NOE correlations between the Hc protons of the naphthyl group in ANS and the H-3/H-5 protons of β -CD (peaks c). These results indicate that the phenyl and naphthyl groups of ANS are separately included by two β -CD cavities from the primary side. From the above 2D NMR results, together with the 1:1 host-guest inclusion complexation stoichiometry obtained from the Job's experiments for each bis(β -CD) unit, we can also confirm that bis(β -CD) **5** adopts an intermolecular 2:2 binding mode upon complexation with ANS.

On the basis of the conformations of metallooligo(β -CD)s **6** and **7**, the CPK model studies give their possible binding modes with guest molecules. As illustrated in Figure 10a, two bipyridine units in **6** are perpendicular to each other and each bis(β -CD) unit adopts a sandwich binding mode upon inclusion complexation with a guest molecule. However, metallooligo(β -CD) **7** adopts a double-helical conformation (Figure 10b), where two bipyridine units are nearly parallel to each other, and each bis(β -CD) unit provides a β -CD cavity to form the sandwich inclusion complex with a guest molecule. It is noteworthy that the bipyridine dicarboxamide bridge in these dual hosts acts as both a connector between two β -CD cavities and a versatile coordinating site for metal ions. Moreover, the copper(II) ion(s) introduced in the bridge not only adjusts and orients the β -CD cavities and the linker group to fit the size/shape of guest molecule, but also acts as an additional guest binding site(s) through the electrostatic and/or electron-transfer interactions with the accommodated guest molecules. These advantages, together with the cooperative binding of two β -CD cavities with one guest molecule, will significantly enhance the original binding ability of parent cyclodextrin, which will be discussed below.

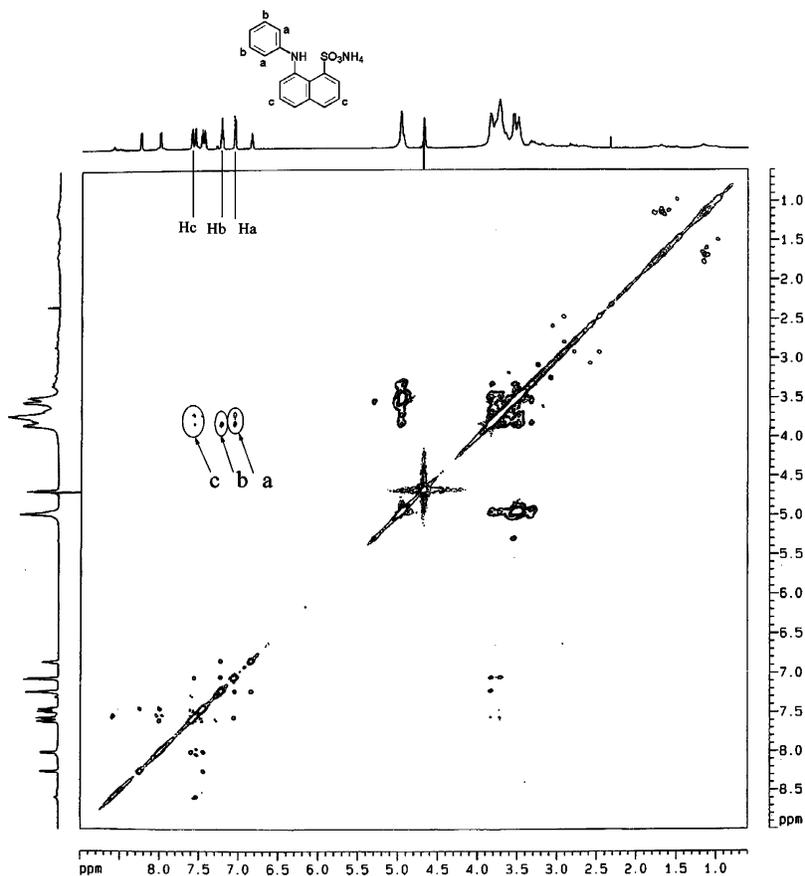


Figure 9. ROESY spectrum of a mixture of host **5** and ANS ($[5] = [\text{ANS}] = 3.0 \times 10^{-3} \text{ mol} \cdot \text{dm}^{-3}$) in D_2O with a mixing time of 200 ms at 298 K.

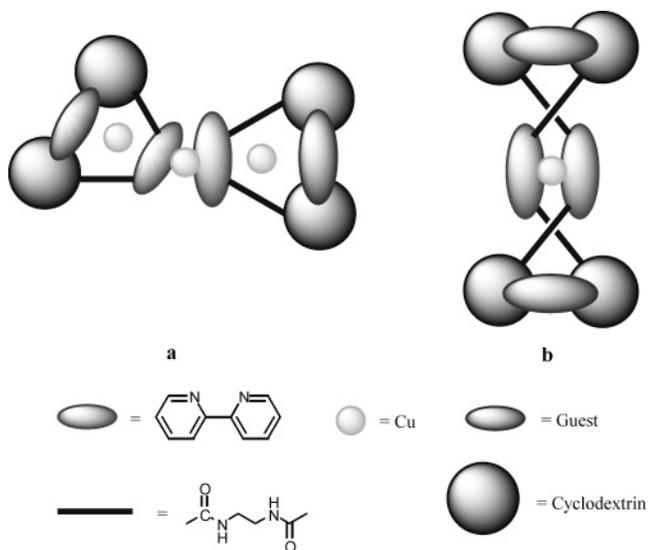


Figure 10. Schematic binding modes of metallooligo(β -CD)s **6** and **7**.

Molecular Binding Ability. It is demonstrated that native and simple modified β -CDs afford the limited binding ability with guest molecule due to the relatively weak host–guest hydrophobic interactions. However, β -CD dimers and their metal complexes can greatly enhance the original binding ability of parent β -CD through the cooperative binding of two adjacent cavities and the potential multiple recognition ability. As can be seen from Table 1, the K_s values for inclusion complexation of mono-modified β -CDs **2** and **3** with guest molecules are only 0.5–3.4 times as high as those for native β -CD.

However, bis(β -CD)s **4** and **5** and their copper(II) complexes **6** and **7** significantly enhance the original binding ability of parent β -CD toward guest molecules by a factor of 1.3–42. This result may be attributed to a multiple recognition behavior of these oligomeric hosts toward guest molecules. In addition to the cooperative binding of one guest molecule by two β -CD cavities, the linker group located near the accommodated guest also provides some additional interactions with guest molecule. These factors jointly contribute to the stronger host–guest association achieved by oligo(β -CD)s in comparison to monomeric hosts. Moreover, as can be readily recognized from Table 1, the K_s values for inclusion complexation of hosts **4**–**7** with guest molecules increase in the following order

4: ANS < TNS < RhB

5: ANS < RhB < TNS

6: ANS < RhB < TNS

7: ANS < TNS < RhB

We can see that the linear guest molecule TNS and the T-shaped guest RhB are better bound by oligo(β -CD)s than the bent guest ANS. This may be attributed to the fact that longitudinal incorporation of linear or T-shaped guest molecule with oligo(β -CD) fits better into the geometrical requirement than the bent guest, which consequently gives the strong van der Waals and hydrophobic interactions between host and guest. On the other hand, although both TNS and ANS possess a phenyl and naphthyl group, the former forms a more stable complex with oligomeric β -CD than the latter, which may be also attributed to the size–fit relationship between host and guest. The CPK

model studies show that the naphthyl group in ANS cannot penetrate deeply into the β -CD cavity owing to steric hindrance. Therefore, among the guest molecules used, the weakest binding ability for ANS by oligo(β -cyclodextrin)s should be reasonable. In addition, by comparing the enhancement effects for each guest, we can see that the oligo(β -CD) which gives the highest enhancement for each guest dye (with the observed enhancement factors shown in the parentheses) is **5** ($\times 42$) for ANS, **6** ($\times 4.8$) for TNS, and **6** ($\times 2.9$) for RhB, respectively. From a comparison of these enhancement factors, we can conclude that the bent guest ANS, rather than linear guest TNS and T-shaped guest RhB, is able to more fully exploit the cooperative multiple binding of oligo(β -CD) with the binding ability exhibiting more than 40-fold enhancement. Close examination of the binding abilities of hosts **4–7** toward ANS shows that the *cis* conformer **4** only increases the original binding ability of β -CD toward ANS by a factor of 6, but its copper(II) complex **6** enhances this value to 38. This may be attributed to the additional binding interactions provided by the coordinated copper(II) ions. However, the *trans* conformer **5** and its copper(II) complex **7** show the significantly enhanced binding ability toward ANS up to 38–42 times higher than that of β -CD. This result can be explained by the double-helical binding mode of hosts **5** and **7**, which results in a shorter distance between two β -CD cavities and thus improves the host–guest size fit.

Conclusion

In summary, a series of β -CD derivatives bearing pyridine and bipyridine linkers as well as their copper(II) complexes are synthesized in moderate to high yields. Comparative studies on the conformations and binding behaviors of these hosts indicate that *cis*-bis(β -CD) **4** and *trans*-bis(β -CD) **5** as well as their copper(II) complexes **6** and **7** exhibit different inclusion complexation geometry upon association with guest molecules, which consequently leads to the obvious difference in the binding abilities of these β -CD derivatives, giving relatively strong binding with linear and T-shaped guests and a significant enhancement effect on the binding ability toward bent guest as compared with native and monomeric β -CD. The present results provide a convenient and powerful method for controlling the conformation of synthetic receptors in aqueous solution, which will be useful for the design and synthesis of new supramolecular systems.

Experimental Section

General. Ammonium 8-anilino-1-naphthalenesulfonate (ANS), sodium 6-(*p*-toluidino)-2-naphthalenesulfonate (TNS), and Rhodamine B (RhB) were purchased from Wako. All chemicals were reagent grade and used without further purification unless noted otherwise. β -CD of reagent grade (Shanghai Reagent Works) was recrystallized twice from water and dried *in vacuo* for 12 h at 100 °C. *N,N*-Dimethylformamide (DMF) was dried over calcium hydride for 2 days and distilled under reduced pressure prior to use. Pyridine was refluxed over calcium hydride for 8 h and distilled prior to use. Mono[6-(2-aminoethyleneamino)-6-deoxy]- β -CD, *N,N'*-bis(2-aminoethyl)-2,2'-bipyridine-4,4'-dicarboxamide-bridged bis(6-amino-6-deoxy- β -CD) (**4**), and its copper(II) complex **6** were prepared according to our previous report.^{23,24} Elemental analyses were performed on a Perkin-Elmer-2400C instrument. NMR spectra were obtained on a Bruker AV600 instrument. UV–vis spectra were recorded in a conventional quartz cell (10 \times 10 \times 45 mm) at 25 °C on a Shimadzu UV2401 spectrometer. Fluorescence spectra were measured in a conventional quartz cell (10 \times 10 \times 45 mm) at

25 °C on a JASCO FP-750 spectrometer equipped with a constant-temperature water bath, with excitation and emission slits of 5 nm for all the fluorescent dyes. The excitation wavelengths for ANS, TNS, and RhB were 350, 350, and 520 nm, respectively.

Synthesis of Mono[6-(3-pyridinecarboxamide)ethyleneamino-6-deoxy]- β -CD (2**).** Dicyclohexylcarbodiimide (DCC, 0.49 g, 2 mmol) and nicotinic acid (0.3 g, 2 mmol) were dissolved in dry DMF (30 mL), and mono[6-(2-aminoethyleneamino)-6-deoxy]- β -CD (2.35 g, 2 mmol) was added to this solution at 0 °C under nitrogen atmosphere. After stirring the reaction mixture for 18 h in an ice bath and another 24 h at room temperature, the insoluble matter was removed by filtration and the filtrate evaporated under reduced pressure to dryness. The residue was dissolved in water, and then acetone was added to the solution to give a pale precipitate. The crude product obtained was purified by column chromatography over Sephadex G-25 with distilled deionized water as the eluent to give a pure sample as a yellow solid. Yield: 1.5 g, 1.1 mmol, 56%. ¹H NMR (600 MHz, D₂O, TMS): δ 2.6–3.0 (m, 4H), 3.1–4.0 (m, 42H), 4.8–5.0 (m, 7H), 7.2–7.4 (m, 1H), 8.0–8.1 (m, 1H), 8.3–8.6 (m, 1H), 8.7–8.8 (m, 1H). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3372, 3351, 3071, 2926, 2151, 1603, 1558, 1425, 1371, 1245, 1155, 1079, 1035, 943, 848, 758, 704, 654, 608, 580, 528, 426. Anal. Calcd for C₅₀H₇₈O₃₅N₃·3H₂O: C, 44.98; H, 6.34; N, 3.15. Found: C, 44.82; H, 6.53; N, 3.15. UV–vis (water): λ_{\max}/nm ($\epsilon/\text{M}^{-1} \text{cm}^{-1}$) 262 (3800 mol⁻¹ dm⁻³ cm⁻¹).

Synthesis of Mono[6-(4-pyridinecarboxamide)ethyleneamino-6-deoxy]- β -CD (3**).** Compound **3** was prepared in 50% yield from isonicotinic acid and mono[6-(2-aminoethyleneamino)-6-deoxy]- β -CD as a slight-yellow solid according to procedures similar to those employed in the synthesis of **2**. ¹H NMR (600 MHz, D₂O, TMS): δ 2.6–3.0 (m, 4H), 3.2–3.8 (m, 42H), 4.8–4.9 (m, 7H), 7.5–7.6 (m, 2H), 8.4–8.5 (m, 2H). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3358, 3049, 2085, 1603, 1547, 1452, 1371, 1301, 1243, 1206, 1155, 1079, 1034, 944, 849, 756, 708, 677, 658, 613, 581, 531, 449, 432. Anal. Calcd for C₅₀H₇₈O₃₅N₃·8H₂O: C, 42.14; H, 6.65; N, 2.95. Found: C, 42.20; H, 6.53; N, 3.02. UV–vis (water): λ_{\max}/nm ($\epsilon/\text{M}^{-1} \text{cm}^{-1}$) 266 (2060 mol⁻¹ dm⁻³ cm⁻¹).

Synthesis of *N,N'*-Bis(2-aminoethyl)-2,2'-bipyridine-3,3'-dicarboxamide-Bridged Bis(6-amino-6-deoxy- β -CD) (5**).** 2,2'-Bipyridine-3,3'-dicarboxylic dichloride (0.28 g, 1.0 mmol) was dissolved in dry pyridine (20 mL) containing DCC (0.7 g, 3.4 mmol). Dry mono[6-(2-aminoethyleneamino)-6-deoxy]- β -CD (4.1 g, 3.0 mmol) in DMF (30 mL) was added to this solution at room temperature under nitrogen atmosphere, and the resultant mixture was stirred for 24 h in an ice bath. The solution was allowed to warm and stirred for an additional 2 days at room temperature until no more precipitate was deposited. Then the precipitate was removed by filtration, and the filtrate was evaporated under reduced pressure to dryness. The residue was dissolved in water, and then acetone was added to the solution to give a reddish precipitate. The crude product obtained after drying was purified by column chromatography over Sephadex G-25 with distilled deionized water as the eluent to give a pure sample as a yellow solid. Yield: 0.8 g, 0.3 mmol, 30%. ¹H NMR (600 MHz, D₂O, TMS): δ 2.6–4.2 (m, 92H), 4.7–5.0 (m, 14H), 7.3–7.6 (m, 2H), 7.8–8.1 (m, 2H), 8.3–8.6 (m, 2H). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$ 3330, 2929, 1647, 1560, 1418.4, 1330, 1154, 1079, 1032, 944, 845, 755, 705, 580. Anal. Calcd for C₁₀₀H₁₅₆O₇₀N₆·8H₂O: C, 44.38; H, 6.41; N, 3.11. Found: C, 44.24; H, 6.40; N, 3.25. UV–vis (water): λ_{\max}/nm ($\epsilon/\text{M}^{-1} \text{cm}^{-1}$) 270 (9710 mol⁻¹ dm⁻³ cm⁻¹).

Synthesis of Bis(β -CD)–copper(II) Complex 7. Bis(β -cyclodextrin) **5** 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 days. 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(β -cyclodextrin)–Cu(II) complex **7** as a green solid in 40% yield. IR (KBr): $\nu_{\max}/\text{cm}^{-1}$ 3322, 2930, 1652, 1610, 1421, 1364, 1301, 1154, 1080, 1032, 944, 845, 757, 705, 580, 526, 430, 410. Anal. Calcd for $\text{C}_{100}\text{H}_{156}\text{O}_{70}\text{N}_6 \cdot 0.5\text{Cu}(\text{ClO}_4)_2 \cdot 8\text{H}_2\text{O}$: C, 42.33; H, 6.11; N, 2.96. Found: C, 42.61; H, 6.02; N, 2.89. UV–vis (water): λ_{\max}/nm ($\epsilon/\text{M}^{-1}\text{cm}^{-1}$) 270 (10 110 $\text{mol}^{-1}\text{dm}^{-3}\text{cm}^{-1}$).

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