Bridged Bis(β-cyclodextrin)s Possessing Coordinated Metal Center(s) and Their Inclusion Complexation Behavior with Model Substrates: Enhanced Molecular Binding Ability by Multiple Recognition

Yu Liu,* Yong Chen, Li Li, Heng-Yi Zhang, Shuang-Xi Liu, and Xu-Dong Guan

Department of Chemistry, Nankai University, Tianjin, 300071, China

yuliu@public.tpt.tj.cn

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To investigate quantitatively the cooperative binding ability of several β-cyclodextrin oligomers bearing single or multiligated metal center(s), the inclusion complexation behavior of four bis(β-cyclodextrin)s (**2**−**5**) linked by 2,2′-bipyridine-4,4′-dicarboxy tethers and their copper(II) complexes (**6**−**9**) with representative dye guests, i.e., methyl orange (MO), acridine red (AR), rhodamine B (RhB), ammonium 8-anilino-1-naphthalenesulfonic acid (ANS), and sodium 6-(*p*-toludino)-2-naphthalenesulfonate (TNS), have been examined in aqueous solution at 25 °C by means of UV−vis, circular dichroism, fluorescence, and 2D NMR spectroscopy. The results obtained indicate that bis(β-cyclodextrin)s **2**−**5** can associate with one or three copper(II) ion(s) producing 2:1 or 2:3 bis(β-cyclodextrin)−copper(II) complexes. These metal-ligated oligo(β-cyclodextrin)s can bind two model substrates to form intramolecular 2:2 host−guest inclusion complexes and thus significantly enhance the original binding abilities of parent β-cyclodextrin and bis(β-cyclodextrin) toward model substrates through the cooperative binding of two guest molecules by four tethered cyclodextrin moieties, as well as the additional binding effect supplied by ligated metal center(s). Host **6** showed the highest enhancement of the stability constant, up to 38.3 times for ANS as compared with parent β-cyclodextrin. The molecular binding mode and stability constant of substrates by bridged bis- and oligo(β-cyclodextrin)s **2**−**9** are discussed from the viewpoint of the size/shape-fit interaction and molecular multiple recognition between host and guest.

Introduction

Possessing dual hydrophobic cavities in a close vicinity and a nucleophilic or electrophilic tether with good structural variety in a single molecule, bridged cyclodextrin dimers can greatly enhance the original binding ability and molecular selectivity of parent cyclodextrin through the potential cooperative binding of two adjacent cyclodextrin units[1−8] and therefore be successfully employed in several areas of science and technology as an excellent model system mimicking substrate-specific interaction of enzymes.[9,10] Moreover, their metal complexes can further extend the binding affinity of bis(β-cyclodextrin)s through the electrostatic interaction and/or electron transfer between ligated metal and accommodated guest molecule. Consequently, much work has been devoted to the design and syntheses of novel cyclodextrin dimers with considerable structural diversity and their metal complexes in order to examine and compare the molecular binding abilities of cyclodextrin, bis(β-cyclodextrin)s, and their metal complexes, to elucidate the recognition mechanism controlled by the simultaneous operation of available weak interactions and to gain insights into factors governing the inclusion complexation behavior from the viewpoint of multiple recognition and induced-fit interaction between host and guest.[11−14] Unfortunately, studies on the molecular recognition behavior of cyclodextrin oligomers possessing

* To whom correspondence should be addressed. Phone +86-22-23503625; Fax +86-22-23504853.

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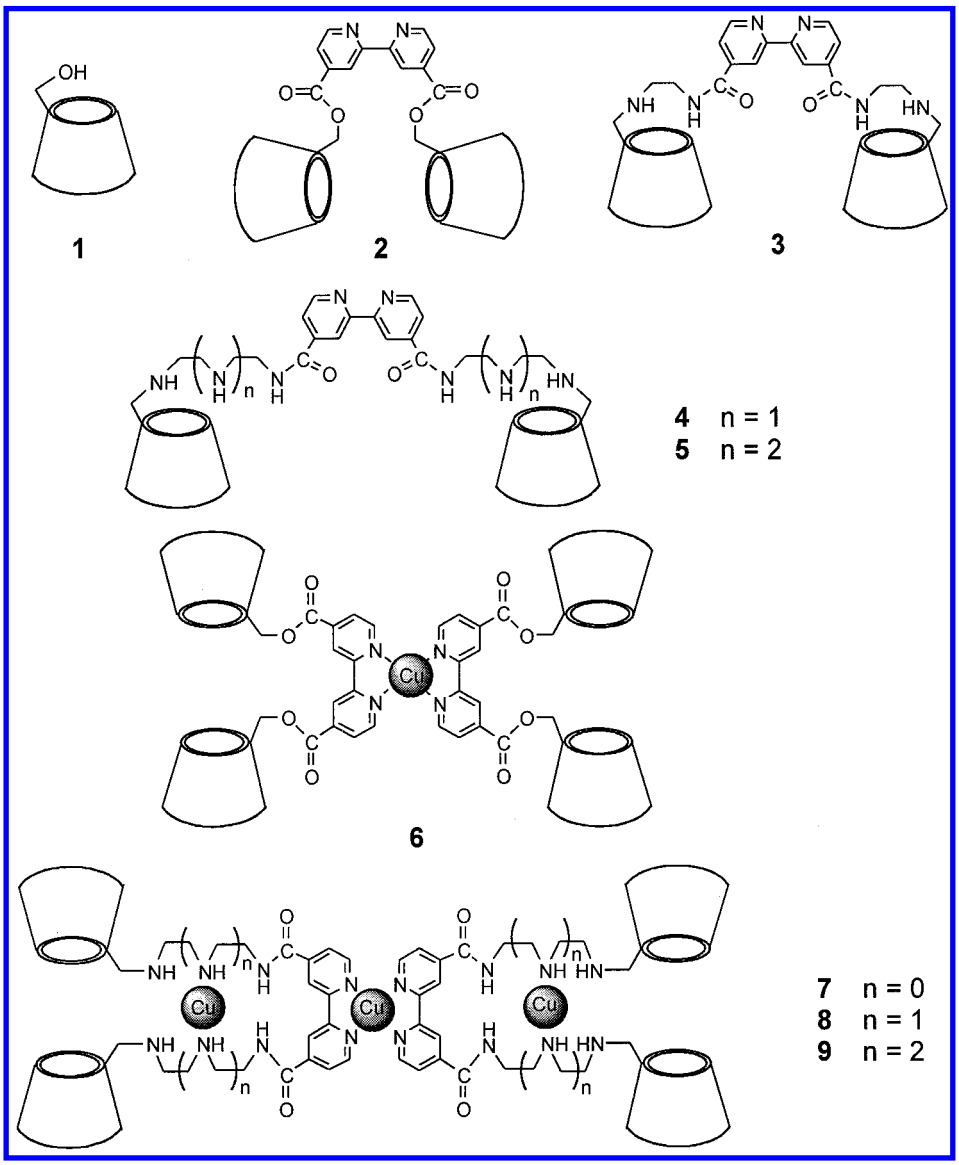
Chart 1



ligated metal center(s) are still rare.[8a,11] We have recently reported a study on the molecular recognition behavior of some dyes by several bridged bis(β-cyclodextrin)s with 2,2′-bipyridine-4,4′-dicarboxy tethers, which shows that rhodamine B displays a unique fluorescence behavior upon inclusion complexation with bis(β-cyclodextrin-6-yl) 2,2′-bipyridine-4,4′-dicarboxylate, attributed to the formation of the host−guest sandwich inclusion complex.[15,16] This research advanced our understanding of the intramolecular sandwich complexation behavior of bis(β-cyclodextrin)s with model substrates as well as multiple recognition and the induced-fit interaction hypothesis proposed for the binding of specific substrates by receptors. There is an inherent advantage for the flexible bipyridine dicarboxy tethers incorporated in these cyclodextrin dimers, since both the bipyridine and the oligo(ethylenediamine) moiety in the tether group can ligate to transition metal ions, therefore enabling us to modify and potentially switch the original binding ability through the metal ligation and the cooperative multiple recognition.

To further examine the molecular recognition behavior of bridged bis(β-cyclodextrin)s with 2,2′-bipyridine-4,4′-dicarboxy tethers (**2**−**5**) systematically, we synthesized a series of copper(II) complexes (**6**−**9**) of these bis(β-cyclodextrin)s (Chart 1) and subsequently investigated their molecular binding ability and selectivity with model substrates in different size and shapes, such as ammonium 8-anilino-1-naphthalenesulfonic acid (ANS), sodium 6-(*p*-toluidino)-2-naphthalenesulfonate (TNS), acridine red (AR), and rhodamine B (RhB) (Chart 2). The simple reason for choosing these dye molecules as spectral probe is that these fluorescent dyes are known to be very sensitive to environmental changes, which will enable us to investigate their inclusion complexation behavior with native and bridged cyclodextrins using fluorometric titration method. The experimental results obtained indicate that bis(β-cyclodextrin)s **2**−**5** can associate one or three copper(II) ions forming a β-cyclodextrin oligomer with single or multiligated metal center(s) and thus give a much stronger combination ability with model substrates than the parent monomeric and dimeric

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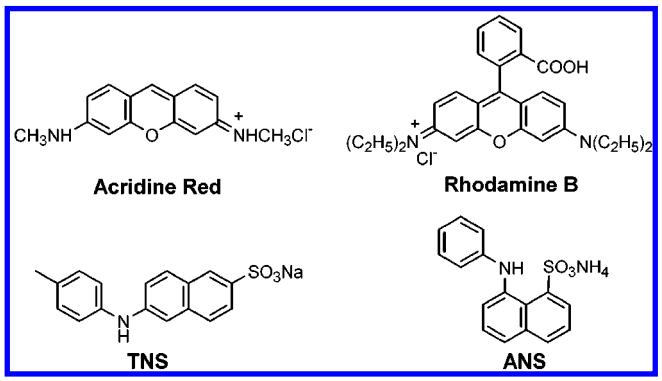
Chart 2



β-cyclodextrins. It is our special interest to examine the molecular recognition mechanism concerning the uncommon β-cyclodextrin oligomers bearing several cyclodextrin units and ligated metal center(s), which will serve our further understanding of this recently developing, but less investigated, area of bridged bis(β-cyclodextrin)s.

Experimental Section

General. Acridine red (AR) and rhodamine B (RhB) were commercially available from Chroma-Gesellschaft Schmid, and methyl orange (MO), ammonium 8-anilino-1- naphthalenesulfonic acid (ANS), and sodium 6-(*p*-toludino)-2-naphthalenesulfonate (TNS) from Tokyo Kasei, and all were used as received. β-Cyclodextrin (**1**) was purchased from Wako. 2,2′-Bipyridine-4,4′-dicarboxy-bridged bis(6-*O*-β-cyclodextrin) (**2**), *N*,*N*′-bis(2-aminoethyl)-2,2′-bipyridine-4,4′-dicarboxamide-bridged bis(6-amino-6-deoxy-β-cyclodextrin) (**3**), *N*,*N*′-bis(5-amino-3-azapentyl)-2,2′-bipyridine-4,4′-dicarboxamide-bridged bis(6-amino-6- deoxy-β-cyclodextrin) (**4**), and *N*,*N*′-bis(8-amino-3,6-diazaoctyl)-2,2′-bipyridine-4,4′-dicarboxamide-bridged bis(6-amino-6-deoxy-β-cyclodextrin) (**5**) were prepared according to our previous report.[15] Elemental analysis was performed on a Perkin-Elmer 2400C instrument. UV−vis spectra were recorded in a conventional quartz cell (10 × 10 × 45 mm) at 25 °C on a Shimadzu UV2401 spectrometer. NMR spectra were performed on a Varian INVOA 300 spectrometer. Fluorescence spectra were measured at 25 °C using a conventional quartz cell (10 × 10 × 45 mm) on a JASCO FP-750 spectrofluorometer with the excitation and emission slits of 5 nm width for all the fluorescent dyes. The excitation wavelengths for ANS, TNS, AR, and RhB were 350, 350, 490, and 520 nm, respectively. Deionized, distilled water was used as solvent in all spectral measurements.

Bis(β-cyclodextrin)−Copper(II) Complex 6. Bis(β-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 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 **6** as an azury solid in 40% yield. Anal. Calcd for $C_{96}H_{144}O_{72}N_2 \cdot 0.5Cu(ClO_4)_2 \cdot 14H_2O$: C 40.29, H 6.06, N 0.98. Found: C 40.09, H 5.99, N 1.20. IR (KBr) ν 3318.3, 2928.8, 1734.8, 1656.5, 1560.3, 1406.9, 1365.7, 1332.9, 1298.9, 1238.4, 1154.1, 1078.1, 1031.9, 944.6, 850.1, 758.3, 707.7, 607.9, 577.4 cm^{-1}. UV−vis (water): $\lambda_{max}(\epsilon)$ = 318.0 (7640 mol^{-1} dm^{-3} cm^{-1}).

Bis(β-cyclodextrin)−Copper(II) Complex 7. Bis(β-cyclodextrin)−Cu(II) complex **7** was prepared according to procedures similar to those in the synthesis of **6** as a blue greenish solid (yield 45%). Anal. Calcd for $C_{100}H_{156}O_{70}N_6 \cdot 1.5Cu(ClO_4)_2 \cdot 8H_2O$: C 38.74, H 5.59, N 2.71. Found: C 38.66, H 5.75, N 2.75. IR (KBr) ν 3300.6, 2931.4, 1658.6, 1553.4, 1406.8, 1367.0, 1333.1, 1299.0, 1238.5, 1153.3, 1078.3, 1032.3, 944.4, 845.5, 757.0, 705.9, 623.8, 580.1 cm^{-1}. UV−vis (water): $\lambda_{max}(\epsilon)$ = 314.0 (12770 mol^{-1} dm^{-3} cm^{-1}).

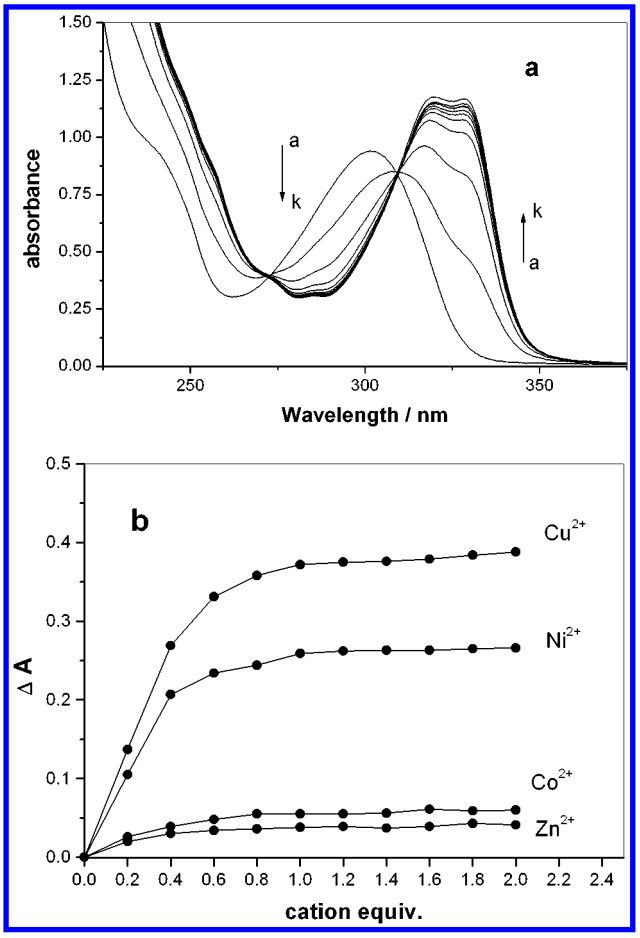


Figure 1. (a) UV−vis spectral change of **2** upon addition of copper(II) ion in aqueous solution. ([**2**] = 1.5 × 10^{-4} mol dm^{-3}, [Cu^{2+}] = 0−3.0 × 10^{-4} mol dm^{-3} from a to k) (b) spectrophotometric titration of **2** at 302 nm with four metal ions in aqueous solution. ([**2**] = 1.5 × 10^{-4} mol dm^{-3}; counteranion: NO_3^-).

Bis(β-cyclodextrin)−Copper(II) Complex 8. Bis(β-cyclodextrin)−Cu(II) complex **8** was prepared according to procedures similar to those in the synthesis of **6** as a blue solid (yield 40%). Anal. Calcd for $C_{104}H_{166}O_{70}N_8 \cdot 1.5Cu(ClO_4)_2 \cdot 10H_2O$: C 38.77, H 5.82, N 3.48. Found: C 38.61, H 5.89, N 3.41. IR (KBr) ν 3316.1, 2931.2, 1656.1, 1552.9, 1407.1, 1366.1, 1333.5, 1299.2, 1237.6, 1153.1, 1078.5, 1032.7, 944.6, 846.4, 756.1, 706.0, 624.0, 579.5 cm^{-1}. UV−vis (water): $\lambda_{max}(\epsilon)$ = 311.0 (5810 mol^{-1} dm^{-3} cm^{-1}).

Bis(β-cyclodextrin)−Copper(II) Complex 9. Bis(β-cyclodextrin)−Cu(II) complex **9** was prepared according to procedures similar to those in the synthesis of **6** as a blue solid (yield 40%). Anal. Calcd for $C_{108}H_{176}O_{70}N_{10} \cdot 1.5Cu(ClO_4)_2 \cdot 8H_2O$: C 39.64, H 5.91, N 4.28. Found: C 39.39, H 6.14, N 4.48. IR (KBr) ν 3289.6, 2930.9, 1655.7, 1555.4, 1406.0, 1368.1, 1333.9, 1297.9, 1238.6, 1153.3, 1078.4, 1032.6, 944.5, 846.3, 756.1, 705.0, 623.6, 578.2 cm^{-1}. UV−vis (water): $\lambda_{max}(\epsilon)$ = 311.0 (14080 mol^{-1} dm^{-3} cm^{-1}).

Results and Discussion

Metal Coordination Behavior and Stoichiometry. To compare the coordination behavior of bis(β-cyclodextrin)s with metal ions, spectrophotometric titrations of bis(β-cyclodextrin)s **2**−**5** with different metal ions have been performed at 25 °C in aqueous solution. A typical titration curve of bis(β-cyclodextrin) **2** with copper(II) ion was illustrated in Figure 1a. As can be seen in Figure

1a, the absorption intensity of bis(β-cyclodextrin) **2** at 302 nm gradually decreased with the addition of varying amounts of copper(II) ion, accompanying obvious bathochromic shift of absorption peak. In the control experiment, UV−vis spectra of copper(II) ion within measurement concentration range display no appreciable change at 200−400 nm under comparable experimental conditions. Moreover, the isobestic point observed in each UV−vis titration plot of bis(β-cyclodextrin)s **2**−**5** with copper(II) ion further confirm the simple one-step transformation from the free bis(β-cyclodextrin) to the copper(II)-ligated species. These phenomena jointly indicate that copper(II) is coordinated to the bis(β-cyclodextrin) to form metal-ligated species. The spectrophotometric titration curves of bis(β-cyclodextrin) **2** with $Cu(NO_3)_2$, $Ni(NO_3)_2$, $Co(NO_3)_2$, and $Zn(NO_3)_2$ were shown in Figure 1b, respectively, which display different coordination behavior of **2** with Cu(II), Ni(II), Co(II), and Zn(II) ions. Bis(β-cyclodextrin)s **3**−**5** also show the same metal coordination behavior sequence toward these metal ions. These results may be helpful to select the appropriate metal ion as the coordination center in the design and synthesis of new functional β-cyclodextrin dimers and their metal complexes.

The complexes of bis(β-cyclodextrin)s with copper(II) were prepared in moderate yield by the coordination reaction of copper(II) perchlorate with corresponding bis(β-cyclodextrin) in aqueous solution. In addition to the elemental analysis data, IR and UV−vis spectra will provide additional evidence about the formation of the metallooligo(β-cyclodextrin)s. In the IR spectra of metallooligo(β-cyclodextrin) **6**, the vibration bands assigned to the carbonyl group in the bipyridine dicarboxylate shift to high wavenumber region as compared unligated bis(β-cyclodextrin) **2** (from 1725.8 to 1734.8 cm^{-1}). Additionally, a weak absorption at ca. 624 cm^{-1}, which may be assigned to a weakly coordinated perchlorate, is also observed in the IR spectra of bis(β-cyclodextrin)-Cu(II) complexes. The UV−vis spectra of bis(β-cyclodextrin)−Cu(II) complexes **6**−**9** also display obvious bathochromic shift of absorbance peak (15.7−20 nm), along with the distinct enhancement of ϵ_{max} values (444−2642 mol^{-1} dm^3 cm^{-1}) as compared with the corresponding parent bis(β-cyclodextrin)s. On the other hand, since paramagnetic disturbance caused by the ligated copper(II) make the ^1H NMR of bis(β-cyclodextrin)−copper(II) complex unavailable to measure, we check the ^1H NMR of bis(β-cyclodextrin) **2** and its nickel(II) complex. From the spectra shown in Figure 2, we can see that bipyridine protons of **2** display appreciable downfield shifts (4.5−7.2 Hz) upon introduction of nickel(II), indicating the complex between bis(β-cyclodextrin) and nickel(II) was formed. Furthermore, UV−vis measurements were also performed to explore the bis(β-cyclodextrin)−Cu(II) coordination stoichiometry. Figure 3a shows a representative spectrophotometric titration curve to determine the complex stoichiometry for the coordination of bis(β-cyclodextrin) **2** with copper(II) perchlorate. As can be seen in Figure 3a, the plot for the **2**/Cu(II) system displays a maximum at 0.67 which corresponding to 2:1 **2**/Cu(II) stoichiometry. This result indicates that two bis(β-cyclodextrin) components participate in the binding of one Cu(II) ion, as illustrated in Chart 1. Typically, a spectrophotometric Job measurement to explore the complex stoichiometry for the coordination of bis(β-cyclodextrin) **3** with copper(II) perchlorate (Figure 3b) shows that the plot for the **3**/Cu(II) system displays a maximum at 0.4 that corresponds to 2:3 **3**/Cu(II) stoichiometry, which indicate that, besides each bis(β-cyclodextrin) unit associating with one copper(II) ion, two bis(β-cyclodextrin) components participate in the binding of the third copper(II) ion. This 2:3 coordination stoichiometry was also obtained in the case of **4** (or **5**)/Cu(II) system. Somewhat unexpectedly, although possessing eight −NH− fragments in the tether group, the polyamino moiety in bis(β-cyclodextrin) **5** can only ligate one copper(II) ion, which may be attributed to the strong electrostatic repulsion force that precludes the coexistence of two copper(II) ions in a limited space, i.e., the pseudo cavity formed by the tether group.

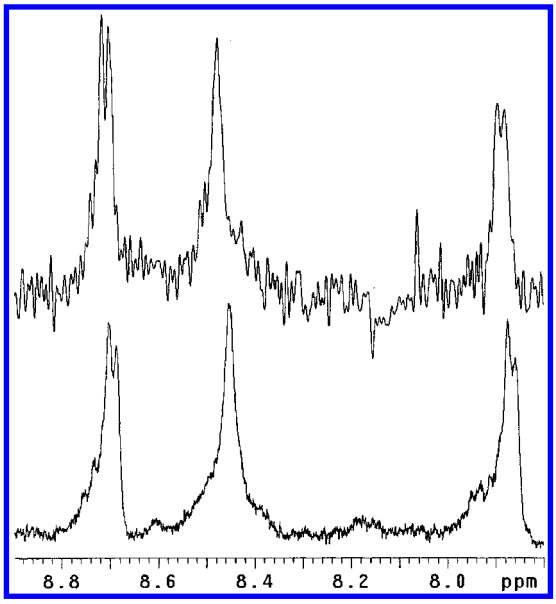


Figure 2. Partial ^1H NMR spectra (300 MHz, D_2O) of bis(β-cyclodextrin) **2** (the lower) and **2**−Ni(II) complex (the upper).

Circular Dichroism 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.[17] Herein, to examine the original conformation of the copper(II)-ligated oligo(β-cyclodextrin)s in dilute aqueous solution, the circular dichroism (CD) spectra of **6**−**9** were taken at a concentration of 1×10^{-4} mol dm^{-3} and compared with the CD spectra of parent bis(β-cyclodextrin)s **2**−**5** reported previously.[15] As can be seen from Figure 4, all of the metallooligo(β-cyclodextrin)s show analogous, but obviously weak circular dichroism signals as compared with the unligated bis(β-cyclodextrin)s **2**−**5**. The observed $\Delta\epsilon_{max}$ values for the ^1L_b (^1L_a) transition of the bipyridine chromophore are +0.122 dm^3 mol^{-1} cm^{-1} at 301 nm (−0.771 dm^3 mol^{-1} cm^{-1} at 226 nm) for **6**, +0.167 dm^3 mol^{-1} cm^{-1} at 309 nm (−1.51 dm^3 mol^{-1} cm^{-1} at 235 nm) for **7**, +0.361 dm^3 mol^{-1} cm^{-1} at 298 nm (+0.483 dm^3 mol^{-1} cm^{-1} at 217 nm) for **8**, +0.564 dm^3 mol^{-1} cm^{-1} at 302 nm (+1.04 dm^3 mol^{-1} cm^{-1} at 222 nm) for **9**. Similar to the experimental phenomena observed in UV−vis measurement, hosts **6**−**9** also display bathochromic shift (3−33 nm) of the Cotton effect peaks for the ^1L_b transition of bipyridine chromophore, accompanying the decreasing

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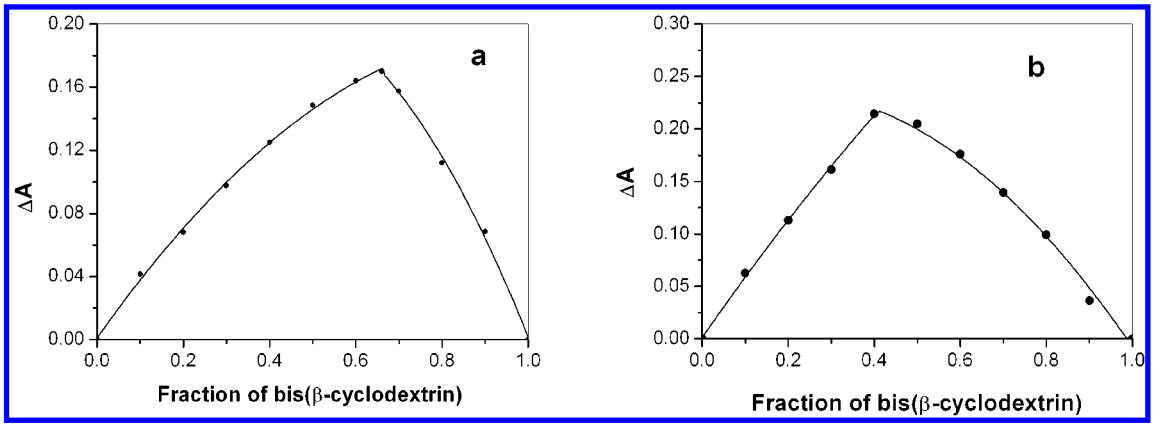

Figure 3. Continuous variation plot of (a) **2**/Cu(II) ([**2**] + [Cu^{2+}] = 1.1 × 10^{-4} mol dm^{-3}) and (b) **3**/Cu(II) system. ([**3**] + [Cu^{2+}] = 1.5 × 10^{-4} mol dm^{-3}).

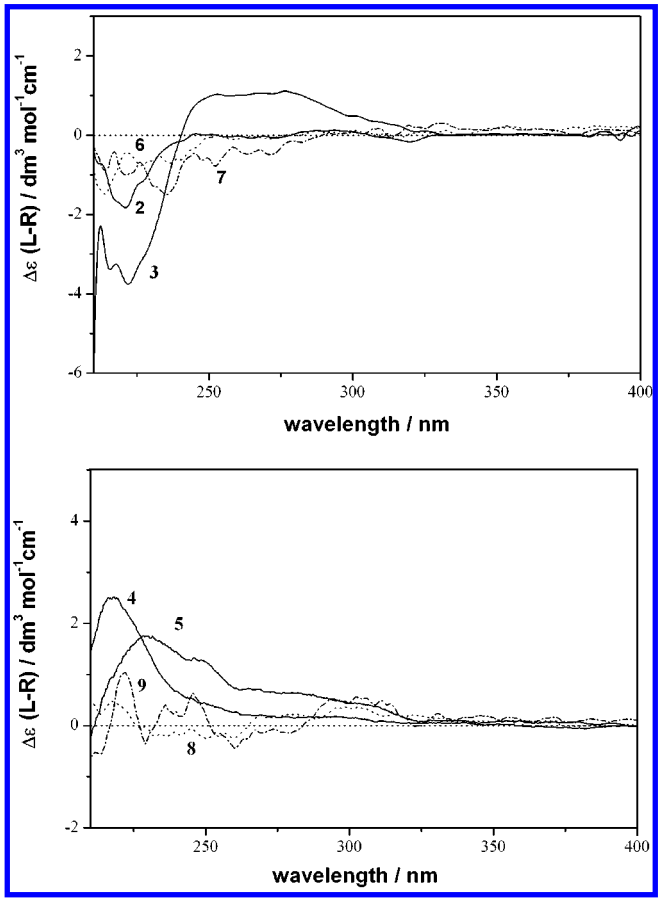

Figure 4. Circular dichroism spectra of hosts **2**−**9** (1 × 10^{-4} mol dm^{-3}) in aqueous solution at 25 °C.

$\Delta\epsilon_{max}$ values for both ^1L_b ($\Delta\Delta\epsilon_{max}$ = 0.004−0.945 dm^3 mol^{-1} cm^{-1}) and ^1L_a ($|\Delta\Delta\epsilon_{max}|$ = 0.482−2.25 dm^3 mol^{-1} cm^{-1}) transition bands except for **8**. According to the empirical rules proposed by Kajtar,[18] Harada,[19] and Kodaka[20] et al., we can deduce that the bipyridine carboxyl moiety in hosts **6**−**9** are distant from or shallowly perching on the edges of two chiral cavities, while are not embedded into the hydrophobic cavities. These conformations of hosts **6**−**9** will consequently favor the penetration of guest molecule into *β*-cyclodextrin cavities.

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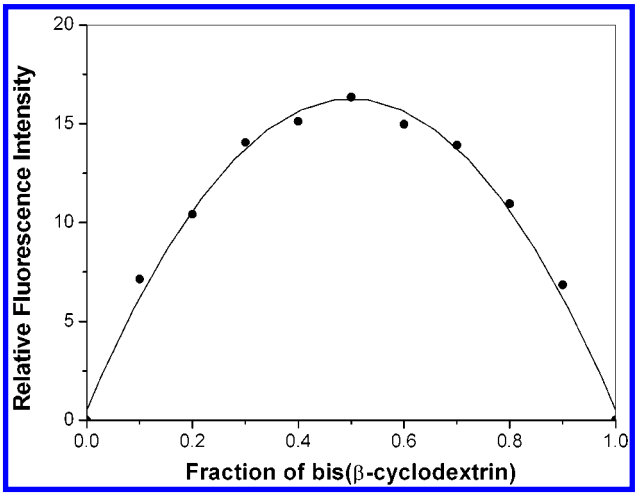

Figure 5. Continuous variation plot of **6**/AR system. ([bis(*β*-cyclodextrin) unit] + [AR] = 1.0 × 10^{-5} mol dm^{-3}).

Inclusion Complexation Stoichiometry. The stoichiometry for the inclusion complexation of metallooligo(*β*-cyclodextrin)s with representative guests, i.e., TNS, ANS, AR, and RhB, was determined by the continuous variation method. Figure 5 illustrates the continuous variation plot for **6**/AR system. In the concentration range, the plot for bis(*β*-cyclodextrin) unit shows a maximum at a molar fraction of 0.5, indicating either 1:1 or 2:2 inclusion complexation. Regarding that host **6** possesses two bis(*β*-cyclodextrin) units, each of which can form sandwich inclusion complex with one guest molecule,[16] we can draw a conclusion that a stoichiometry of the inclusion complexation should be intramolecular 2:2 for the **6**/AR system. The same results were obtained in the cases of the inclusion complexation of other metallooligo(*β*-cyclodextrin)s with selected guest molecules. These results are further supported by two-dimensional NMR spectroscopy described below.

Spectral Titration. Since copper can form the most stable species with bis(*β*-cyclodextrin)s, we select copper-ligated oligo(*β*-cyclodextrin)s as representative hosts to quantitatively compare their molecular binding abilities and selectivities with unmodified *β*-cyclodextrin and simply tethered bis(*β*-cyclodextrin)s by fluorescence spectral titrations. Figure 6a shows the typical spectral changes of TNS with gradual addition of host **5**. As illustrated in Figure 6a, the relative fluorescence intensity of TNS greatly enhances with the increase concen-

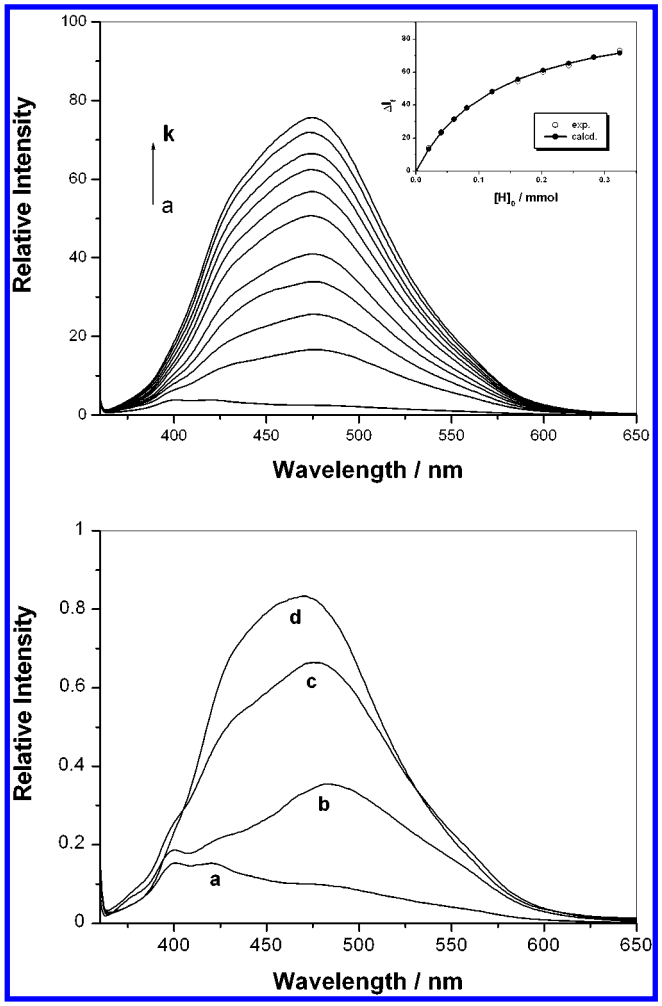

Figure 6. (a) Fluorescence spectral changes of TNS (9.4×10^{-6} mol dm^{-3}) and the nonlinear least-squares analysis (inset) of the differential intensity (ΔI_f) to calculate the complex stability constant (K_s) upon addition of **5** ($0-324 \times 10^{-6}$ mol dm^{-3} from a to k) in aqueous solution. (b) Fluorescence spectra of TNS (9.4×10^{-6} mol dm^{-3}) (a) in the absence and in the presence of (b) β-cyclodextrin **1** (450×10^{-6} mol dm^{-3}), (c) bis(β-cyclodextrin) **5** (20×10^{-6} mol dm^{-3}), and (d) metallooligo(β-cyclodextrin) **9** (20×10^{-6} mol dm^{-3}) in aqueous solution.

tration of the host **5**. Since TNS barely fluoresces in aqueous solution but emits strong fluorescence in a nonpolar environment, therefore, the spectral change observed indicates that the aromatic group of TNS is embedded into the hydrophobic cavity of cyclodextrins forming a host−guest inclusion complex. As can be seen from Figure 6b, the fluorescent sensitization of TNS with bis(β-cyclodextrin)s or oligo(β-cyclodextrin)s is much stronger than with native β-cyclodextrin, and this could be ascribed to the cooperative binding of the former upon inclusion complexation with guest molecules. Additionally, further study indicates that the solution's pH value shows no any significant changes in the experiment procedure. So we can deduce that the binding behavior should be dependent on the individual structural features of host and guest.

By treating one bis(β-cyclodextrin) moiety in host **2**−**9** as a host unit, the inclusion complexation of a guest molecule (G) with a host unit (H) is expressed by eq 1.

$$H + G \overset{K_s}{\rightleftharpoons} G{\cdot}H \qquad (1)$$

Herewith, the relative fluorescence intensity change of guest molecule (ΔI_f) upon addition of host unit, where $\Delta I_f = I_f$ (with host unit) $- I_f$ (without host unit), is assumed to be proportional to the concentration of inclusion complex formed by bis(β-cyclodextrin) unit with model substrate, i.e., $\Delta I_f = \alpha[H{\cdot}G]$. The proportionality coefficient α is taken as a sensitivity factor for the fluorescence change upon inclusion complexation. Then, the effective stability constant (K_s)[21] can be expressed by eq 2:

$$K_s = \frac{[H{\cdot}G]}{[H][G]} = \frac{\Delta I_f/\alpha}{([H]_0 - \Delta I_f/\alpha)([G]_0 - \Delta I_f/\alpha)} \qquad (2)$$

where $[G]_0$ and $[H]_0$ refer to the initial concentrations of organic dye and host unit, respectively. Subsequently, eq 2 can be solved for ΔI_f to give eq 3.

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

Employing a nonlinear least-squares method according to the curve-fitting eq 3,[22,23] we obtained the complexation stability constant for each host−guest combination from the analysis of the sequential changes of fluorescence intensity (ΔI_f) at various host unit concentrations. Figure 6a (inset) illustrates the typical curve-fitting plot for the titration of TNS with host **5**, which shows excellent fits between the experimental and calculated data. In repeated measurements, the K_s values were reproducible within an error of ±5%. The K_s values obtained are listed in Table 1, along with the free energy changes ($-\Delta G°$) of complex formation. To visualize the inclusion complexation behavior between host and guest, the changing profiles of the complex stability constants (K_s) are plotted in Figure 7.

Binding Mode. Different from the general binding mode in inclusion complexation of monomeric cyclodextrin derivatives with guest molecule, upon complexation with bis(β-cyclodextrin), model substrate always penetrates into the two adjacent hydrophobic cavities from the primary side of the cyclodextrin. In our preliminary research by means of ^{1}H NOESY, circular dichroism, and fluorescence spectroscopy as well as CPK model examination,[15,16] we find that a multiple sandwich binding mode is operative in the association of the guest molecule with bridged bis(cyclodextrin); that is, upon inclusion complexation with bis(cyclodextrin), two side groups of the guest molecule are embedded into the hydrophobic cyclodextrin cavities from the primary side of the cyclodextrin to form a sandwich host−guest inclusion complex, as illustrated in Figure 8a. Thus, the guest molecule is more efficiently shielded from attack of the bulk water by the cooperative inclusion complexation with cyclodextrin cavities in bis(cyclodextrin)s and the formation of the sandwich host−guest complex. On the other hand, the tether group, located near the accommodated guest, would provide some additional interactions with guest. These factors jointly contribute to the much stronger host−guest interactions upon inclusion complexation of

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Table 1. Complex Stability Constants (K_s) and Gibbs Free Energy Changes ($-\Delta G°$) upon Inclusion Complexation of Some Fluorescent Dyes with Various Hosts 1−9 at 25 °C in Aqueous Solution

host	guest	K_s	log K_s	$-\Delta G°$ kJ mol^{-1}
1	AR	3090	3.49	19.92
	RhB	4900	3.69	21.07
	ANS	112	2.05	11.70
	TNS	3590	3.56	20.30
2	AR	30800	4.49	25.62
	RhB	27300	4.44	25.32
	ANS	1680	3.23	18.41
	TNS	10580	4.02	22.97
3	AR	3150	3.50	19.97
	RhB	11360	4.06	23.15
	ANS	665	2.82	16.11
	TNS	7610	3.88	22.16
4	AR	4850	3.69	21.04
	RhB	5430	3.73	21.32
	ANS	493	2.69	15.37
	TNS	4380	3.64	20.79
5	AR	16750	4.22	24.11
	RhB	15100	4.18	23.86
	ANS	1650	3.22	18.37
	TNS	8090	3.91	22.31
6	AR	60300	4.78	27.29
	RhB	44100	4.64	26.51
	ANS	4290	3.63	20.74
	TNS	29210	4.47	25.49
7	AR	51900	4.72	26.92
	RhB	14300	4.16	23.72
	ANS	3900	3.59	20.50
	TNS	17300	4.24	24.19
8	AR	20100	4.30	24.57
	RhB	16900	4.23	24.14
	ANS	1160	3.06	17.49
	TNS	11410	4.06	23.16
9	AR	28900	4.46	25.47
	RhB	66900	4.83	27.55
	ANS	2830	3.45	19.71
	TNS	12300	4.09	23.35

guest molecule with dual cyclodextrins than with monomeric hosts. Furthermore, upon complexation with triangular or T-shaped guest molecule, aside from the cooperative association of the two cyclodextrin cavities with the two side groups of model substrate, the tether group can form a well-organized pseudo cavity to accommodate the third branch group of guest (Figure 8b), which will significantly enhance the original binding affinity of unmodified cyclodextrin, and even change the fluorescence behavior of guest molecule in some case.[15,16] In this wok, examination with the Corey−Pauling−Koltun (CPK) molecular model clearly demonstrate that the diethylaminophenyl groups of RhB are well accommodated in the cavities from the primary side of cyclodextrin to form "face to face" sandwich inclusion complex, while the benzoate branch of RhB is located in the pseudo cavity formed by the linker groups of hosts **6**−**9** in part or in whole. Additionally, under our experimental condition, the carboxyl group of RhB in aqueous solution is not protonated and should exist as a carboxylate anion. Owing to the electrostatic attraction from the ligated copper(II) ion in tether group, we can deduce that the negatively charged benzoate component of RhB should penetrate into the pseudo cavity of bis(*β*-cyclodextrin), which consequently support the operation of the cooperative multiple binding mode in the complexation of RhB by bis(*β*-cyclodextrin) **6** (Figure 8c).

Additional evidence for this cooperative multiple binding mode comes from the induced circular dichroism (ICD) spectra of methyl orange in the absence and the

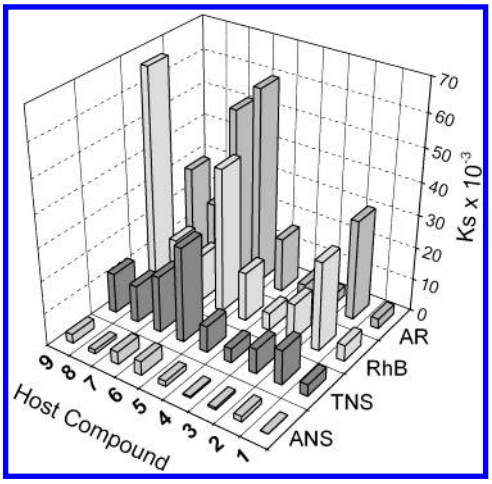

Figure 7. Complex stability constants (K_s) for the inclusion complexation of hosts **1**−**9** with guest molecules in aqueous solution.

presence of *β*-cyclodextrin, bis(*β*-cyclodextrin), and metallooligo(*β*-cyclodextrin). Since the achiral compound located in a chiral environment, such as the cyclodextrin cavity, produces ICD signal(s) at the wavelengths absorbed by the guest chromophore,[24] the inclusion complexation behavior of bis(*β*-cyclodextrin)s and metallooligo(*β*-cyclodextrin)s with methyl orange can be investigated using ICD spectrum. As illustrated in Figure 9, all of *β*-cyclodextrin **1**, bis(*β*-cyclodextrin) **2**, and metallooligo(*β*-cyclodextrin) **6** induce obvious ICD at the $\pi-\pi^*$ transition band of the azo group in methyl orange, while no appreciable ICD is seen in the absence of cyclodextrin hosts. Attributed to the cooperative binding of methyl orange by the two adjacent cyclodextrin cavities,[8,15] bis(*β*-cyclodextrin) **2** at an even lower (4.7 equiv of methyl orange) induces a stronger ICD signal than native *β*-cyclodextrin (150 equiv of methyl orange). Furthermore, metallooligo(*β*-cyclodextrin) **6** at the lowest concentration (4.5 equiv of methyl orange) induces the strongest ICD signal, which may benefit from the cooperative multiple binding mode of guest molecule with metallooligo(*β*-cyclodextrin).

2D NMR spectroscopy has recently become an important method to investigate the interaction between host cyclodextrins and guest molecules, since two protons, which are closely located in space, can produce an NOE cross-peak between the relevant protons in NOESY or ROESY spectrum. In the preliminary work, we have confirmed the cooperative sandwich binding mode of bis(*β*-cyclodextrin) with RhB[16] (Figure 8b). To obtain further evidence about the multiple binding mode between metallooligo(*β*-cyclodextrin) and model substrate, ^{1}H NOESY experiments have been performed on a Varian INVOA 300 spectrometer. Unfortunately, since the strong paramagnetic disturbance by the ligated copper(II), the relevant NOE cross-peaks between bis(*β*-cyclodextrin)−copper(II) and RhB cannot be detected. Therefore, we select the bis(*β*-cyclodextrin)−nickel(II) complex, which also shows 2:1 or 2:3 coordination stoichiometry similar to bis(*β*-cyclodextrin)−copper(II) complex, as host mol-

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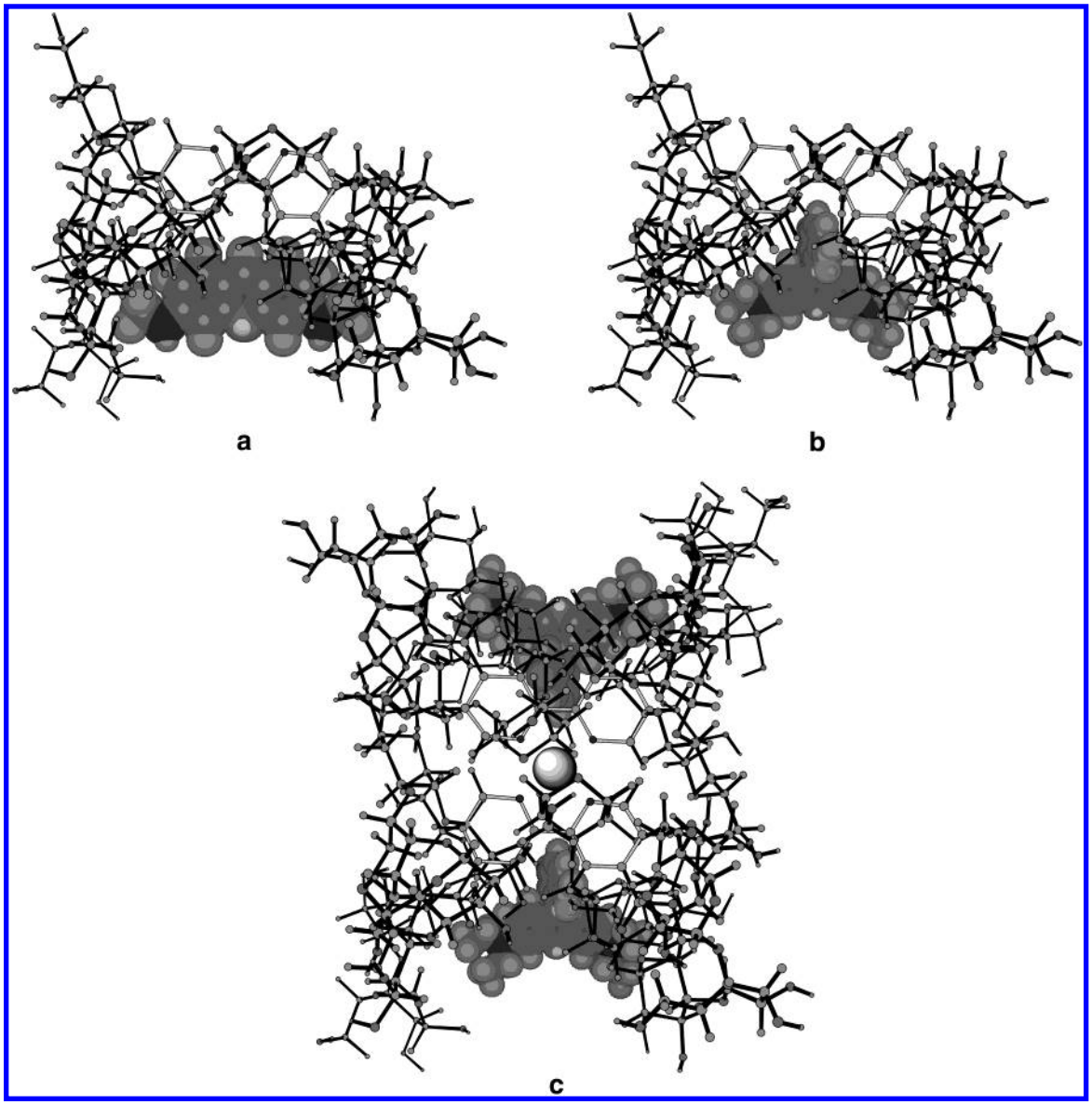

Figure 8. Inclusion complexation modes of guest molecule with bis(β-cyclodextrin) and metallooligo(β-cyclodextrin); model system: (a) host **2** + AR, (b) host **2** + RhB, (c) host **6** + RhB.

ecule to investigate the binding mode of its inclusion complexation with RhB, despite that copper(II) tends to prefer a square planar coordination whereas nickel(II) prefers octahedral. As illustrated in Figure 10, the NOESY spectrum of an equimolar mixture of **2**−Ni(II) with RhB (0.5 mM each) shows clear NOE cross-peaks between the H-3 and H-5 of cyclodextrin and the methyl protons of diethylamino fragments in RhB (peaks A), the cross-peaks between the H-3 and H-5 of cyclodextrin and the aromatic protons of diethylaminophenyl groups in RhB (peaks B), and the cross-peaks between the aromatic protons of bipyridine unit in **2**−Ni(II) and the aromatic protons of the benzoate moiety in RhB (peaks C). These relevant signals indicate that the diethylaminophenyl groups of RhB are accommodated in the cavities from the primary side of cyclodextrin, while the benzoate branch of RhB penetrates into the pseudo cavity of **2**−Ni(II) complex. Hence, the results of NOESY experiment strongly support the operation of the cooperative binding mode in the complexation of guest molecule by metal-ligated oligo(β-cyclodextrin).

Binding Ability and Molecular Recognition. In previous studies,[25] we examined the inclusion complexation of a variety of chemically modified cyclodextrins with diverse guest molecules and found that several noncovalent weak forces, including van der Waals, hydrophobic, hydrogen-bonding, and dipole−dipole interactions, cooperatively contributed to the inclusion complexation of cyclodextrins. In the present case, we found that the host−guest size/shape matching dominates the stability of complex formed between bis- and/or oligo(β-cyclodextrin)s and model substrates, leading to the stronger van der Waals and hydrophobic interactions, since these two interactions are closely related to the distance and contacting surface area between host and guest. It is significantly noted that the introduction of the copper(II) ions alters not only the original conformation of the tether group but also the distance and

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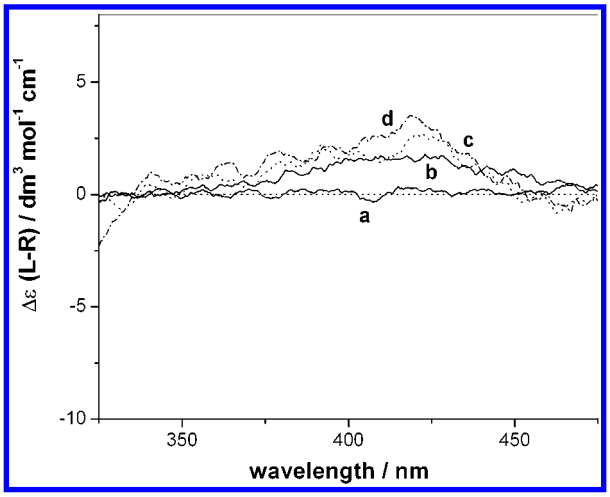

Figure 9. Circular dichroism spectra of methyl orange (10.7×10^{-6} mol dm^{-3}) (a) without and (b) with β-cyclodextrin (1600×10^{-6} mol dm^{-3}), (c) with bis(β-cyclodextrin) **2** (50.6×10^{-6} mol dm^{-3}), and d) with metallooligo(β-cyclodextrin) **6** (48×10^{-6} mol dm^{-3}).

orientation of two hydrophobic cavities in cyclodextrin dimers, thus affecting the penetration depth of guest into cyclodextrin cavity upon inclusion complexation and supplying additional binding interactions toward accommodated guest. According to this multiple recognition mechanism, native and modified monomeric cyclodextrins display relatively limited association ability with guest molecules probably due to the weak hydrophobic interaction. Dimeric cyclodextrins, however, can greatly enhance the original binding ability of parent cyclodextrin through cooperative binding of two adjacent cavities and potential multiple recognition ability. As compared with monomeric and dimeric cyclodextrins, metallooligo(β-cyclodextrin)s can afford much more stable inclusion complexes with model substrates, owing to the cooperative association of four tethered hydrophobic cavities with model substrates, the conformation fixation by metal ligation, and the additional electrostatic interaction and/or electron transfer between ligated metal and accommodated guest molecule. From Table 1, we can see that the complex stability constants (K_s) of bis(β-cyclodextrin)s **2**−**5** with guest molecules are obviously or slightly larger than those of native β-cyclodextrin. For instance, as a result of cooperative binding, the binding constant of bis(β-cyclodextrin) **2** with AR is higher than that of native β-cyclodextrin by a factor of 10, while its K_s value with ANS is higher than that of native β-cyclodextrin by a factor of 15. Furthermore, possessing the metal ligation, the oligomeric β-cyclodextrins **6**−**9** can significantly enhance the original binding affinity of parent β-cyclodextrin toward these guest molecules by a factor of 2.9−38.3. The present result may point to a mechanism concerning an uncommon multiple recognition behavior of metallooligo(β-cyclodextrin)s toward model substrates. Upon inclusion complexation, metal-ligated β-cyclodextrin oligomer affords four hydrophobic binding sites (cyclodextrin cavities) and one (or three) metal coordination center(s), which jointly contribute to the cooperative binding of oligomeric host with guest molecule. Thus, as a cumulative result of these factors, metallo β-cyclodex-

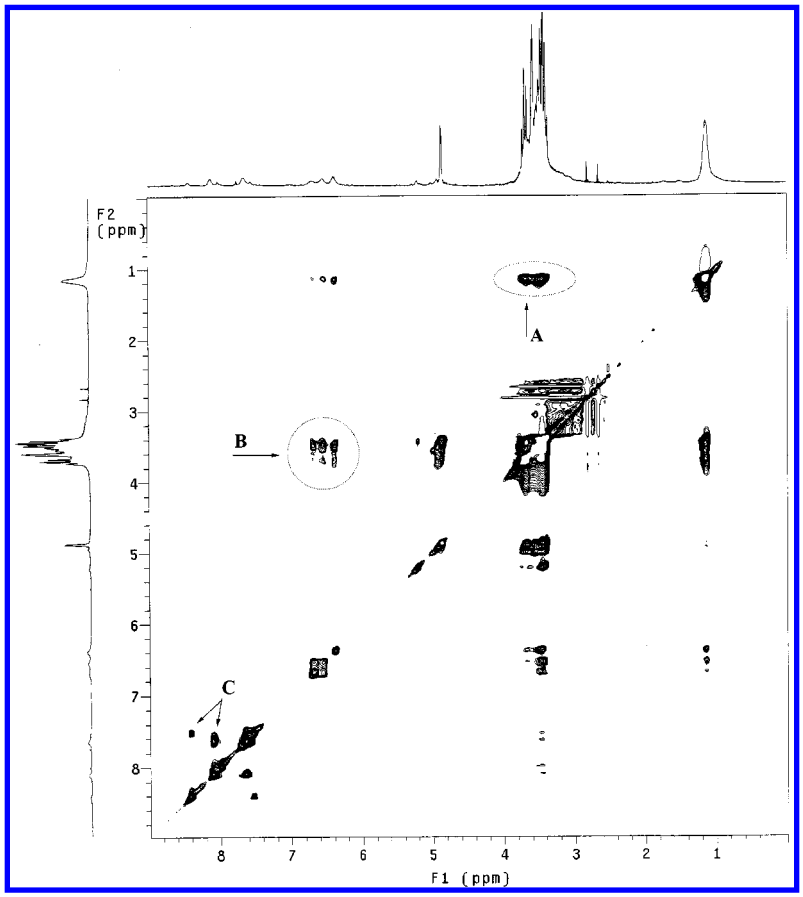

Figure 10. ^{1}H NOESY spectrum (300 MHz) of a mixture of **2**−Ni(II) with RhB ([**2**−Ni(II)] = [RhB] = 5.0×10^{-4} mol dm^{-3}) in D_2O at 298 K with a mixing time of 800 ms.

trin oligomers display significantly enhanced binding abilities toward model substrates as compared with parent monomeric and dimeric cyclodextrins. This enhanced binding is accomplished through the multiple hydrophobic interaction between four cyclodextrin cavities and included guest molecules, the conformational fixation by metal coordination, and electrostatic interaction and/or electron transfer between ligated metal and accommodated model substrates. By comparing the enhancement effect for each guest, we can see that the metallooligo(β-cyclodextrin) host which gives the highest enhancement for each guest dye (with the observed enhancement factors shown in the parentheses) is **6** (×19.5) for AR, (×8.1) for TNS, (×38.3) for ANS, and **9** (×13.7) for RhB, respectively. From a comparison of the enhancement factor, we may conclude that the bent guest ANS and T-shaped guest RhB are able to more fully exploit the cooperative binding of metallooligo(β-cyclodextrin)s rather than the linear guests TNS and AR.

It is also interesting to compare the "host selectivity" sequence for each guest dye. The stability constant (K_s) for the complexation of each dye molecule by metallooligo(β-cyclodextrin)s **6**−**9** decreases in the following order:

AR: **6** > **7** > **9** > **8**

RhB: **9** > **6** > **8** > **7**

TNS: **6** > **7** > **9** > **8**

ANS: **6** > **7** > **9** > **8**

The guest dyes AR, TNS, and ANS are better bound by **6** and **7**, which possess shorter linkers, than by long-tethered **8** and **9**. This may be attributed to the strict size-fit between these dyes and short-tethered metallooligo(β-cyclodextrin), which consequently gives strong van der Waals and hydrophobic interactions between host and guest. Whereas, the T-shaped RhB is best bound by the long-tethered **9**. One of the possible reasons for this contrasting host-selectivity sequence may be the size/shape matching between metallooligo(β-cyclodextrin) and RhB. In the preliminary work, we have reported that the tether group of bis(β-cyclodextrin) **2** can supply a well-organized pseudo cavity which in turn provides additional binding interaction with the benzoate branch of RhB by forming sandwich inclusion complex, and thus giving higher binding affinity toward RhB than simply tethered β-cyclodextrin dimers.[16] The present results indicate that the metallooligo(β-cyclodextrin) can also afford suitable pseudo cavities through the adjustment and orientation of flexible tethered group by the introduction of ligated metal, in which the branch fragment of T-shaped guests, such as RhB, can be appropriately accommodated. Among the oligomeric hosts examined, metallooligo(β-cyclodextrin)s **9** possesses the longest and most flexible linker and therefore can afford the most suitable pseudo cavity in which the ligated metal and the benzoate moiety of RhB may be best compatible, thus displaying the strongest binding with RhB. However, in the case of meatllooligo(β-cyclodextrin)s **7** and **8**, the benzoate branch of RhB can be only partly accommodated in the pseudo cavity, owing to the poor size/shape matching between guest branch and copper(II)-occupied pseudo cavity, which will in turn result in the relatively weak combination of hosts **7** and **8** with RhB. On the other hand, the pseudo cavity in host **6** is not occupied by copper(II), which will consequently lead to a higher binding constant with RhB than that of hosts **7** and **8**.

Conclusion

In conclusion, we wish to emphasize that the bipyridine dicarboxy bridge introduced in the dual host acts as both a positive binding site upon inclusion complexation with model substrates and a versatile coordinating site for metal ions. Additionally, the copper(II) ion(s) introduced in the bridge chain not only adjusts and orients β-cyclodextrin cavities and tether group to fit to the size/shape of guest molecule but also acts as an additional guest binding site(s) through coordination and/or electrostatic interaction. As an uncommon model of multiple recognition system, metal-ligated β-cyclodextrin oligomers give much higher K_s values for model substrates through the cooperative effect of the advantages mentioned above, which will in turn open an efficient channel to the design and synthesis of new supramolecular hosts with high molecular affinity and selectivity.

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