Binding Behavior of Aliphatic Oligopeptides by Bridged and Metallobridged Bis(*β*-cyclodextrin)s Bearing an Oxamido Bis(2-benzoic) Carboxyl Linker

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β-Cyclodextrin dimers bearing an oxamido bis(2-benzoic) carboxyl linker (**1**) or its metal complexes (**2** and **3**) were newly synthesized, and their inclusion complexation behavior with a series of representative aliphatic oligopeptides, i.e., Leu-Gly, Gly-Leu, Gly-Pro, Glu-Glu, Gly-Gly, Gly-Gly-Gly, and Glu(Cys-Gly), was elucidated by means of UV/vis, circular dichroism, fluorescence, and 2D NMR spectroscopy in Tris-HCl buffer solution (pH 7.4) at 25 °C. The results obtained indicated that metallobridged bis(*β*-cyclodextrin)s **2** or **3** could significantly enhance the original molecular binding abilities of parent bis(*β*-cyclodextrin) **1** toward model substrates through the cooperative binding of two cyclodextrin moieties and the additional chelation effect supplied by the coordinated metal centers. It is interesting that hosts **2** and **3** displayed an entirely different fluorescence behavior upon complexation with guest oligopeptides. Among the guest peptides examined, **3** showed the highest complex formation constant of 68 200 M^{-1} for Glu-Glu, up to 510-fold as compared with **1** (135 M^{-1}), while **1** gave excellent molecular selectivity for Glu(Cys-Gly)/Glu-Glu pair, up to 51-fold. The molecular binding ability and selectivity were discussed from the viewpoints of the induced-fit and multiple recognition mechanism between host and guest.

INTRODUCTION

Over the past three decades, numerous efforts have been devoted to molecular recognition studies of binding of biological molecules to various synthetic receptors, and among them, peptide recognition has become an exciting topic (*1*−*10*). These investigations potentially promote our insights into the selective binding between a designed molecular receptor (host) and a recognition target (guest). As one of the most successful receptors for peptide recognition and separation, cyclodextrins (CDs), a class of cyclic oligosaccharides with 6−8 glucopyranose units, and their derivatives have been widely applied due to their hydrophobic cavities capable of binding guest molecules through simultaneous contributions of several noncovalent interactions (*11*−*20*). Breslow et al. (*11*) studied the binding behavior of *β*-CD derivatives with some peptides using combinatorial chemistry, microcalorimetric titration, and a capillary electrophoresis approach, which showed that modified *β*-CDs could be used as sequence-selective peptide-cleaving reagents. Moroder et al. (*12*) linked the heptapeptide amide to mono(6-succinylamino-6-deoxy)-*β*-CD to analyze the effect of the bulky cyclic carbohydrate moiety on both the recognition of peptides by the G-protein-coupled CCK-B/gastrin receptor and their signal transduction potencies. Recently, Inoue et al. (*14*) reported chiral recognition thermodynamics of dipeptides with native *γ*-CD as studied by microcalorimetry, discussing the effects of length, bulkiness, and flexibility of the tether connecting the two aromatic moieties in a guest. These studies were not only directed toward an understanding of the thermodynamic aspects of binding and chiral/molecular discrimination of CDs, but also provided valuable information on the effects of changes in functionality and chirality, which attempt to elucidate the nature of molecular recognition between CDs and peptide guests.

It is interesting to note that a CD dimer (bis-CDs) connected with a linker of different length and structure, which possesses dual hydrophobic cavities in close proximity, can bind diverse molecules to form supramolecular sandwich complexes and thus give much higher binding abilities and molecular selectivities than those exhibited by parent CD. Furthermore, their metal complexes can extend the binding affinity of bis-CDs through the electrostatic interaction and/or cooperative chelation between ligated metal and accommodated guest molecule. Accordingly, a number of bis-CDs linked by functional tethers have recently been designed and synthesized to gain insights into the factors and mechanism controlling the inclusion complexation phenomena (*21*−*25*). However, the molecular recognition of metal-coordinated bridged bis-CDs to small peptides, especially aliphatic peptides, have not been well investigated so far (*11a*), although these studies are very important to elucidate the molecular recognition mechanism and control the binding behavior of biological receptors. In the present paper, we wish to report our investigation results on the binding behavior of bridged oxamide bis(2-benzoic) carboxyl linked bis(*β*-CD) (**1**) and its metal copper(II) (**2**) or nickel(II) (**3**) complexes (Scheme 1) with some representative oligopeptides (Chart 1) in Tris-HCl buffer solution (pH 7.4). The results obtained indicated that hosts **2** and **3** significantly enhanced the original binding abilities of parent bis(*β*-CD) (**1**) toward oligopeptides. Interestingly, **2** and **3** were found to induce different fluorescence behaviors upon addition of guest oligopeptides; that is, the fluorescence intensity of **2** or **3** gradually decreased upon addition of Glu(Cys-Gly) or Glu-Glu, but increased

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Scheme 1. Schematic Representation of the Formation of Hosts 1−3

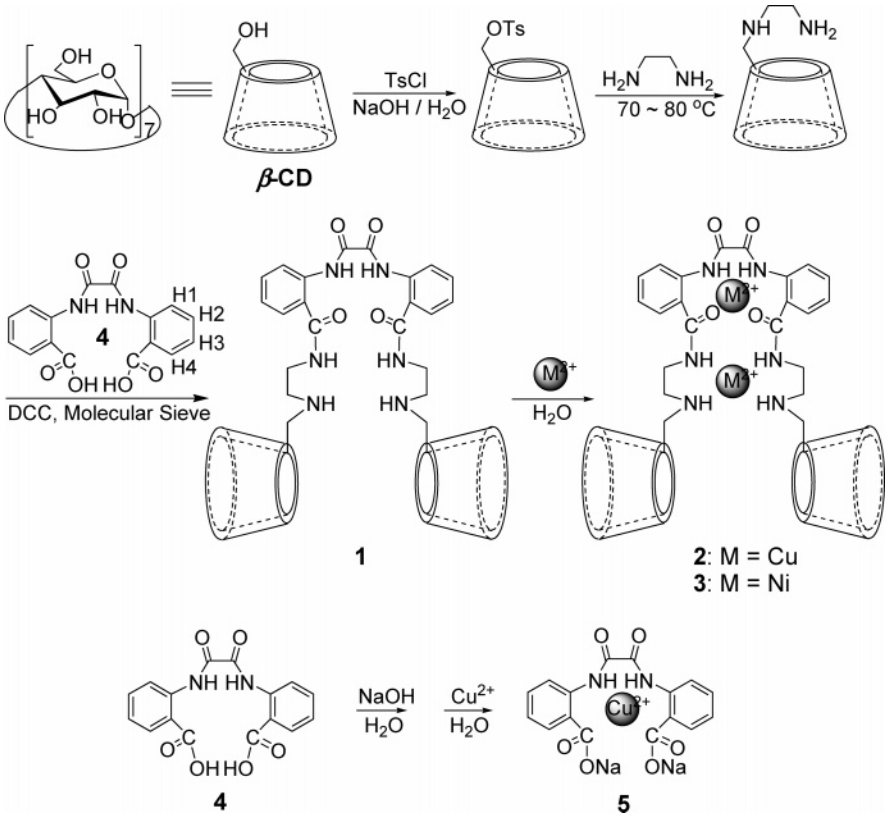
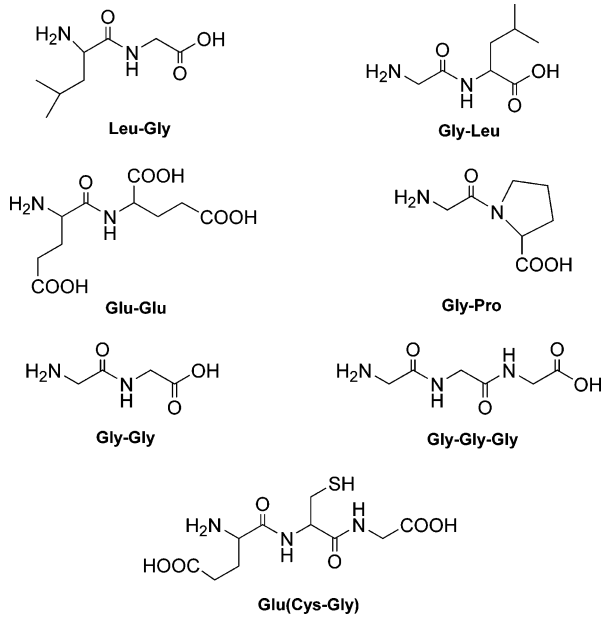


Chart 1. Structures of the Di- and Tripeptides Employed



with addition of other guests, which enabled these metallobridged bis(β-CD)s to be efficient fluorescence probes for the recognition of peptides. On the basis of the investigation of circular dichroism and 2D NMR spectroscopy, the molecular binding mode and complex formation constants (K_S) of substrates with bridged bis(β-CD)s **1**−**3** were discussed from the viewpoints of cooperative chelation and molecular multiple recognition between the host and guest. It is our special interest to examine the molecular recognition mechanism of oligopeptides by β-CD oligomers with ligated metal centers, which will serve our further understanding of this recently developing, but less investigated, area of metallobridged bis(β-CD)s.

EXPERIMENTAL PROCEDURES

Materials. β-CD of reagent grade was recrystallized twice from water and dried in vacuo at 95 °C for 24 h prior to use. *N*,*N*-Dimethylformamide (DMF) was dried over calcium hydride for 2 days and then distilled under reduced pressure prior to use. The oligopeptides Leu-Gly, Gly-Leu, Gly-Pro, Glu-Glu, Gly-Gly, Gly-Gly-Gly, and Glu(Cys-Gly) were commercially available and used without further purification. Oxamido bis(2-benzoic) acid (**4**) and its Cu(II) complex (**5**) were prepared according to the procedure reported by Kahn et al. (*26*). Mono[6-*O*-(*p*-toluenesulfonyl)]-β-cyclodextrin (6-OTs-β-CD) was prepared by the reaction of *p*-toluenesulfonyl chloride with β-CD in alkaline aqueous solution (*27*). Then, 6-OTs-β-CD was converted to mono(6-aminoethylamino-6-deoxy)-β-CD in 70% yield upon heating in excess ethylenediamine at 70 °C for 7 h (*28*, *29*).

Instruments. Circular dichroism and UV/vis spectra were recorded in a conventional quartz cell (light path 10 mm) on a JASCO J-715S spectropolarimeter or a Shimadzu UV-2401PC spectrophotometer equipped with a PTC-348WI temperature controller to keep the temperature at 25 °C. Fluorescence spectra were recorded in a conventional quartz cell (10 × 10 × 45 mm) at 25° C on a JASCO FP-750 fluorescence spectrometer with excitation and emission slits of 10 nm width. Elemental analysis was performed on a Perkin-Elmer 2400C instrument. NMR spectra were recorded on a Varian Mercury VX300 spectrometer.

[Oxamido bis(2-benzoic)acylamide]ethyleneamino-6,6′-deoxy-bis(β-CD)s 1. Mono(6-aminoethylamino-6-deoxy)-β-CD (1 mmol) was dissolved in DMF (30 mL) in the presence of a small amount of 4 Å molecular sieves, and then DCC (1 mmol) and **4** (0.5 mmol) were added.

The mixture was stirred for 2 days in an ice bath and another 2 days at room temperature and then allowed to stand for 5 h until no more precipitate deposited. The precipitate was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in a minimum amount of hot water and then poured into 150 mL of acetone, and the precipitate formed was collected by filtration. This procedure was repeated several times. The crude product obtained was purified on a column of Sephadex G-25 with water as eluent. After the residue was dried in vacuo, a pure sample **1** was obtained in 27% yield. UV/vis λ_{max} (H_2O)/nm (log ϵ): 305.8 (3.62). ^{1}H NMR (D_2O, TMS, ppm): δ 2.54∼2.95 (m, 8H); 3.34∼3.79 (m, 84H); 4.85∼4.87 (m, 14H); 7.15∼7.20 (m, 2H); 7.43∼7.48 (m, 2H); 7.88∼8.04 (m, 2H); 8.39∼8.42 (m, 2H). IR (KBr, cm^{-1}): ν 3336.0, 2927.8, 2362.7, 2061.9, 1672.5, 1584.4, 1503.7, 1434.0, 1365.5, 1202.8, 1155.2, 1078.6, 1029.7, 944.9, 846.7, 756.7, 705.6, 578.2. Anal. Calcd for $C_{104}H_{160}O_{72}N_6 \cdot 8H_2O$: C, 44.76; H, 6.36; N, 3.01. Found: C, 44.65; H, 6.47; N, 2.87.

Bis(β-CD)−Copper(II) Complex 2. Bis(β-CD) **1** was added dropwise 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 solution was evaporated under reduced pressure, and 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 complex **2** as a blue solid in 63% yield. UV/vis λ_{max} (H_2O)/nm (log ϵ): 306.8 (3.55). IR (KBr, cm^{-1}): ν 3351.4, 2930.4, 2051.0, 1637.0, 1581.1, 1449.5, 1426.1, 1364.2, 1333.6, 1152.1, 1118.4, 1080.2, 1029.0, 944.5, 846.0, 757.2, 706.3, 627.5, 577.8, 529.6, 415.3. Anal. Calcd for $C_{104}H_{160}O_{72}N_6 \cdot 2Cu(ClO_4)_2 \cdot 11H_2O$: C, 37.07; H, 5.44; N, 2.49. Found: C, 37.31; H, 5.23; N, 2.61.

Bis(β-CD)−Nickel(II) Complex 3. Bis(β-CD)−nickel(II) complex **3** was prepared, according to procedures similar to those in the synthesis of **2**, as a green solid (yield 58%). UV/vis λ_{max} (H_2O)/nm (log ϵ): 307.4 (3.55). ^{1}H NMR (D_2O, TMS, ppm): δ 2.96∼3.20 (m, 8H); 3.35∼3.86 (m, 84H); 4.94 (s, 14H); 7.21∼7.24 (m, 2H); 7.48∼7.52 (m, 2H); 8.01∼8.05 (m, 2H); 8.40∼8.43 (m, 2H). IR (KBr, cm^{-1}): ν 3336.0, 2931.0, 2044.0, 1665.9, 1587.6, 1506.9, 1439.9, 1334.1, 1299.5, 1240.9, 1152.7, 1079.6, 1029.8, 946.7, 843.6, 756.7, 706.6, 626.8, 578.2, 411.0. Anal. Calcd for $C_{104}H_{160}O_{72}N_6 \cdot 2Ni(ClO_4)_2 \cdot 13H_2O$: C, 36.78; H, 5.52; N, 2.47. Found: C, 36.96; H, 5.38; N, 2.56.

RESULTS AND DISCUSSION

Metal Coordination and Stoichiometry. As shown in Scheme 1, the metallobridged bis(β-CD)s **2** and **3** were prepared from bis(β-CD)s **1** by the coordination reaction with copper(II) and nickel(II) in aqueous solution, respectively. In addition to the elemental analysis data, the ^{1}H NMR, IR, and UV/vis spectra provided additional evidence about the formation of the **2** and **3**. Since the paramagnetic disturbance caused by the ligated copper(II) complex makes the ^{1}H NMR spectrum of **2** unavailable to measure, we compared the ^{1}H NMR spectra of **1** and its nickel(II) complex **3**. Some changes in the chemical shift of the aromatic protons of **3** were observed relative to those in **1**: the H1, H2, H3, and H4 protons shifted downfield ca. 0.07, 0.05, 0.05, 0.01 ppm, respectively, which indicated that a complex had formed between the **1** and nickel(II). The IR spectra of **1**−**3** showed that the stretching vibration of carbonyl groups

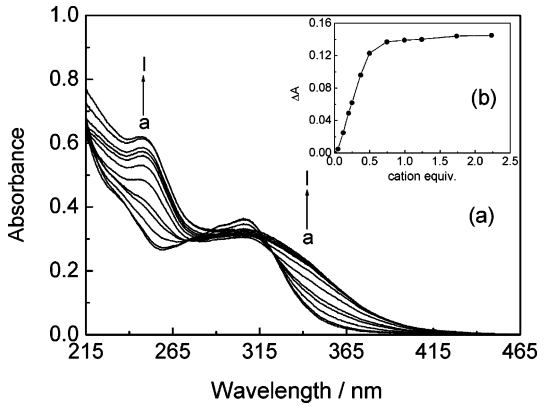


Figure 1. (a) UV/vis spectral change of **1** upon addition of copper(II) perchlorate in aqueous solution. ([**1**] = 7.8 × 10^{-5} mol dm^{-3}, [Cu^{2+}] = 0 to 1.7 × 10^{-4} mol dm^{-3} from *a* to *l*). (b) Absorbance changes of **1** at 350 nm with the addition of copper(II) ion in aqueous solution.

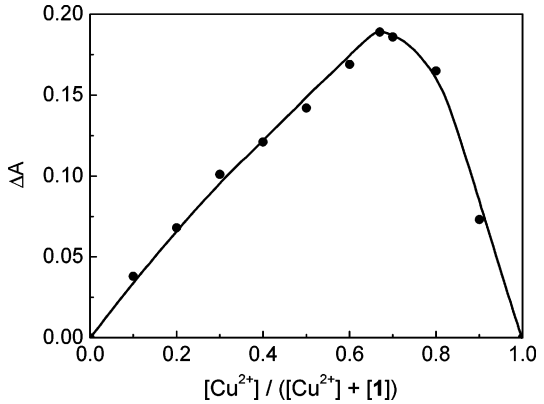


Figure 2. Job's plot of **1**/Cu(II) system at 350.0 nm. ([**1**] + [Cu(II)] = 2.0 × 10^{-4} mol dm^{-3}).

shifted from 1672.5 cm^{-1} for **1** to 1637.0 cm^{-1} for **2** and 1650.9 cm^{-1} for **3**, which could also be ascribed to the coordination of metal ions. Additionally, a weak absorption at 627.5 cm^{-1} for **2** (626.8 cm^{-1} for **3**), which may be assigned to a weakly coordinated perchlorate, was also observed, and indicated that the copper(II) or nickel(II) ion was coordinated to the bridged bis(β-CD) to form metallobridged bis(β-CD)s.

To compare the coordination behavior of bis(β-CD)s **1** with metal ions, spectrophotometric titrations of **1** with copper(II) or nickel(II) ions have been performed at 25 °C in aqueous solution. A typical titration curve of **1** with copper(II) ion was illustrated in Figure 1a. As can be seen in Figure 1a, the absorption intensity of **1** at 259 and 350 nm gradually increased with the addition of varying amounts of copper(II) perchlorate, accompanying an obvious bathochromic shift of absorption peaks. In the control experiments, UV/vis spectra of copper(II) ion within measurement concentration range displayed no appreciable change at 200−400 nm under comparable experimental conditions. Figure 1b showed the absorbance changes of **1** at 350 nm with the addition of copper(II) perchlorate. Furthermore, Job's experiments were also performed to explore the coordination stoichiometry of the bis(β-CD)−metal(II) complex in aqueous solution. The results obtained indicated that the complex stoichiometry was 1:2 for both **2** and **3**. A representative Job's plot for the coordination of **1** with copper(II) perchlorate was shown in Figure 2. The plot for the **1**/Cu(II) system showed a maximum at 0.67 which corresponded to the 1:2 **1**/Cu(II) stoichiometry. This result indicated that one

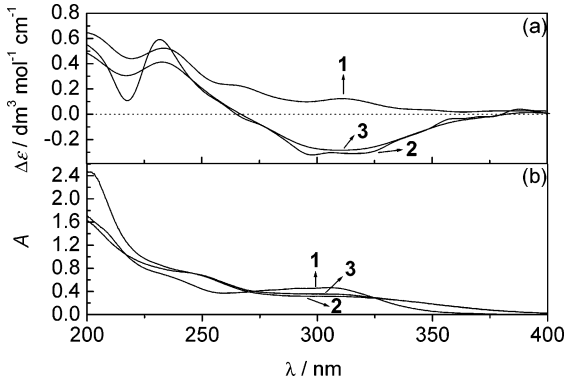


Figure 3. Circular dichroism (a) and absorption (b) spectra of **1** (1.1×10^{-4} mol dm^{-3}), **2** (8.9×10^{-5} mol dm^{-3}), and **3** (1.0×10^{-4} mol dm^{-3}) in aqueous solution at 25 °C.

bis(β-CD) **1** could bind two copper(II) ions, as illustrated in Scheme 1. According to the spectrophotometric titration and the Job's experiments, we can deduce that the coordination of **1** with Cu(II) should be a two-step process. The spectra from *a* to line *f* (Figure 1), which gave two isosbestic points at 273.8 and 322.2 nm, might refer to the coordination process of **1** with the first metal ion, while the spectra from line *g* to line *l* (Figure 1) might point to the coordination process of the second metal ion. Therefore, as an overall illustration of this two-step process, the spectra of absorption titration of **1** with Cu(II) did not give clear isosbestic points.

Conformations of Hosts 1−3. Achiral organic compounds can show an induced circular dichroism (ICD) signal around their corresponding transition bands in cases where there is a chiral microenvironment. Therefore, to deduce the conformation of interaction between the achiral chromophoric compound and the β-CD chiral cavity, ICD spectra of **1**−**3** were measured at 25 °C in aqueous solution. As can be seen from Figure 3, the circular dichroism spectrum of **1** showed two positive Cotton effects around 233.0 nm ($\Delta\epsilon = 0.52$ dm^{-3} mol^{-1} cm^{-1}) and 311.0 nm ($\Delta\epsilon = 0.12$ dm^{-3} mol^{-1} cm^{-1}), respectively. Interestingly, host **2** gave a positive Cotton effect around 232.0 nm ($\Delta\epsilon = 0.59$ dm^{-3} mol^{-1} cm^{-1}) and a negative Cotton effect around 298.0 nm ($\Delta\epsilon = -0.32$ dm^{-3} mol^{-1} cm^{-1}), while **3** gave a positive Cotton effect around 233.0 nm ($\Delta\epsilon = 0.41$ dm^{-3} mol^{-1} cm^{-1}) and a negative Cotton effect around 310.0 nm ($\Delta\epsilon = -0.28$ dm^{-3} mol^{-1} cm^{-1}). According to the pioneering studies (*24*, *30*, *31*) on the ICD phenomena of cyclodextrin complexes, we can deduce that the oxamide bis(2-benzoic) carboxyl group of **1** was shallowly included in the β-CD cavity with an acclivous orientation to form the self-included complexes. After the metal coordination, the linker group was located outside the β-CD cavity, leading to an opposite ICD signal around 300 nm for **2** or **3**. In addition to the ICD experiments, the 2D NMR experiments gave the primary evidence of inside−outside movement of the linker group, which is described below.

2D NMR spectroscopy has recently become an important method for the investigation of not only the interaction between host CDs and guest molecules, but also the self-included mode between the CD cavity and its substituent groups, since the NOE cross-peaks between the protons that are closer than 0.4 nm in space will be observed in the NOESY or ROESY spectrum and the relative intensities of these cross-peaks depend on the spaces between the corresponding protons. The height and the diameter of the β-CD cavity are about 0.79 ± 0.01 nm and 0.60−0.65 nm, respectively. Therefore, while

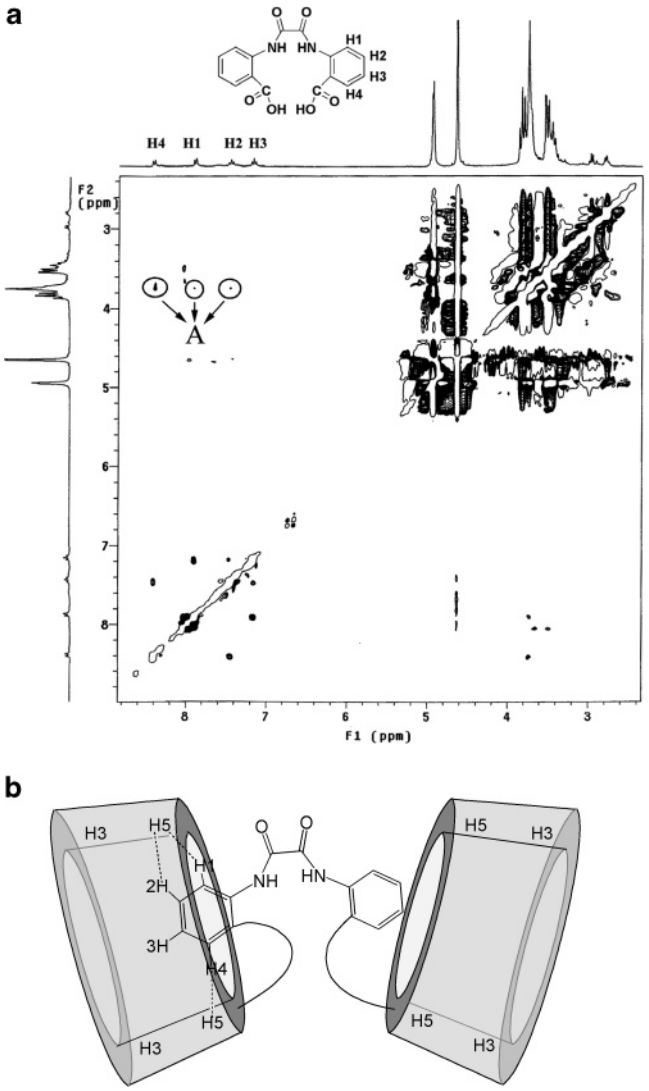


Figure 4. (a) ^{1}H ROESY spectrum of **1** (3.8×10^{-3} mol dm^{-3}) in D_2O at 25 °C with a mixing time of 400 ms, (b) possible conformation of **1**.

the substituent group is included into the β-CD cavity, the NOE correlations between the protons of the substituent group and the inner protons of the β-CD cavity (H-3 and H-5) will be measured. On the other hand, according to the relative intensity of these cross-peaks (*32*), it is possible to estimate the orientation of the substituent group within the β-CD cavity. To obtain further evidence about the original conformation of **1**−**3**, 2D NMR spectroscopy experiments were performed in D_2O solution. As shown in Figure 4a, the ROESY spectrum of **1** displayed clear NOE cross-peaks between the H5 of β-CD and the H1, H2, and H4 protons of oxamide bis(2-benzoic) carboxyl group (peaks A), which indicated distinctly that the linker group of **1** was shallowly self-included into the hydrophobic cavity from the primary side of β-CD. According to these results, a possible conformation of **1** was shown in Figure 4b. At the same time, the NOE cross-peaks between the β-CD's H5 and/or H3 and the protons of the linker group were not found in the ROESY spectrum of **3** (Figure 5), which indicated that the linker group of **3** should be located outside the β-CD cavity. Therefore, the results of the ROESY experiments not only further supported the ICD investigation results on the conformations of bridged bis(β-CD)s **1**−**3**, but also may serve to establish correlation

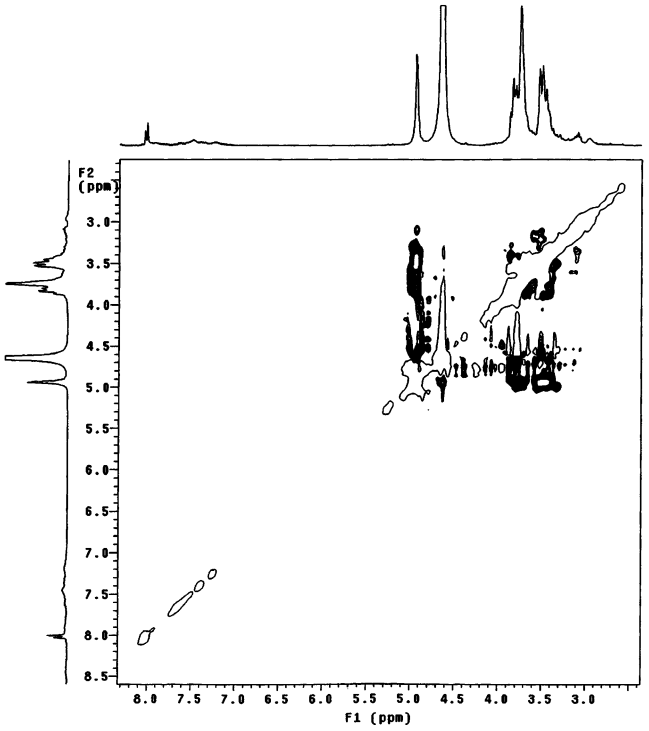


Figure 5. ^{1}H ROESY spectrum of **3** (3.5×10^{-3} mol dm^{-3}) in D_2O at 25 °C with a mixing time of 400 ms.

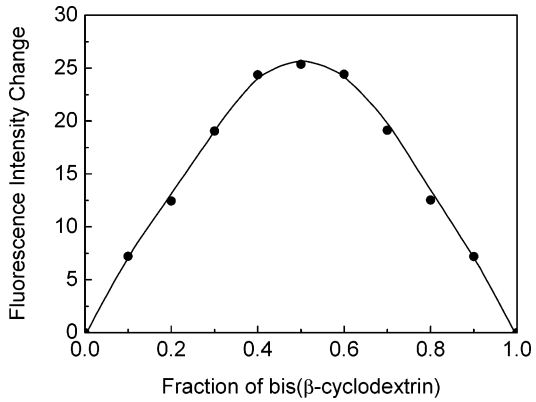


Figure 6. Job's plot of **2**/Glu-Glu system at 408.0 nm. ([**2**] + [Glu-Glu] = 1.0×10^{-5} mol dm^{-3}).

between the original conformation of modified β-CDs and their molecular recognition ability.

Spectral Titration. As elucidated above, the oxamide bis(2-benzoic) carboxyl group was either shallowly included in the β-CD cavity to form the self-included complexes (the case of **1**) or located on the edges of the β-CD cavity (the case of **2** and **3**). Therefore, the linker group might suffer substantial conformational change upon guest inclusion and thus result in the relevant spectral changes, which allows the bis(β-CD) to function as a spectral probe to obtain binding constants in differential fluorescence spectrometry. Herein, we chose some representative aliphatic oligopeptides, i.e., Leu-Gly, Gly-Leu, Gly-Pro, Glu-Glu, Gly-Gly, Gly-Gly-Gly, and Glu(Cys-Gly), to determine their binding behavior with hosts **1**−**3** by spectral titration experiments. Figure 6 illustrated a representative Job's plot for **2**/Glu-Glu system in Tris-HCl buffer solution (pH 7.4) at 25 °C. In the used concentration range, the plot for metallobridged bis(β-CD) showed a maximum at a molar fraction of 0.5, indicating the 1:1 inclusion complexation between the host and guest. The same results were obtained in the

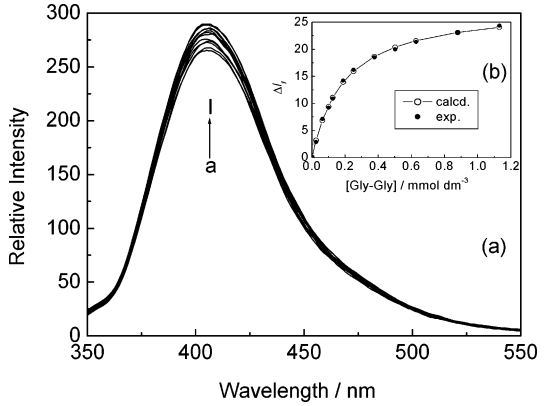


Figure 7. (a) Fluorescence spectral changes of host **3** (1.0×10^{-5} mol dm^{-3}) upon addition of Gly-Gly (0 to 1.13×10^{-3} mol dm^{-3} from *a* to *l*) in Tris-HCl buffer solution (pH 7.4) at 25 °C. (b) the nonlinear least-squares analysis of the differential intensity (ΔI_f) to calculate the complex formation constant (K_S). (λ_{ex} = 314.0 nm, λ_{em} = 406.0 nm).

cases of the inclusion complexation of other bis(β-CD)s with selected oligopeptide guests.

Using 1:1 host/guest stoichiometry, where the two β-CD moieties in **1**−**3** were treated as one unit, the complexation of the oligopeptide guest (OP) with the bis(β-CD) host (CD) can be expressed by eq 1.

$$\mathrm{CD} + \mathrm{OP} \overset{K_S}{\rightleftharpoons} \mathrm{CD{\cdot}OP} \qquad (1)$$

The relative fluorescence intensity change of host unit (ΔI_f) upon addition of guest molecule, where $\Delta I_f = I_f$ (with guest molecule) − I_f (without guest molecule), was assumed to be proportional to the concentration of inclusion complex formed by bis(β-CD) unit with model substrate, i.e., $\Delta I_f = \alpha[\mathrm{CD{\cdot}OP}]$. The proportionality coefficient α was taken as a sensitivity factor for the fluorescence change upon inclusion complexation. Then, the effective complex formation constant (K_S) (*33*, *34*) can be expressed by eq 2:

$$K_S = \frac{[\mathrm{CD{\cdot}OP}]}{[\mathrm{CD}][\mathrm{OP}]} = \frac{[\mathrm{CD{\cdot}OP}]}{([\mathrm{CD}]_0 - [\mathrm{CD{\cdot}OP}])([\mathrm{OP}]_0 - [\mathrm{CD{\cdot}OP}])} = \frac{\Delta I_f/a}{([\mathrm{CD}]_0 - \Delta I_f/a)([\mathrm{OP}]_0 - \Delta I_f/a)} \qquad (2)$$

where $[\mathrm{CD}]_0$ and $[\mathrm{OP}]_0$ denoted the initial concentrations of bis(β-CD) host and oligopeptide guests, respectively. Subsequently, eq 2 can be solved for ΔI_f to give eq 3:

$$\Delta I_f = \{\alpha([\mathrm{CD}]_0 + [\mathrm{OP}]_0 + 1/K_S) - \sqrt{\alpha^2([\mathrm{CD}]_0 + [\mathrm{OP}]_0 + 1/K_S)^2 - 4\alpha^2[\mathrm{OP}]_0[\mathrm{CD}]_0}\}/2 \qquad (3)$$

Using the nonlinear least squares curve-fitting method according to eq 3 (*35*), we obtained the complex formation constant for each host−guest combination from the analysis of the sequential changes of fluorescence intensity (ΔI_f) at various guest concentrations. Figure 7b illustrated a typical curve-fitting plot for the titration of Gly-Gly with **3**, which showed excellent fits between the experimental and calculated data obtained. A good correlation between the experimental and calculated result indicated the reliability of the obtained complex formation constants. The complex formation constants (K_S) and

Table 1. Complex Formation Constant (K_S) and Gibbs Free Energy Change ($-\Delta G°$) for the Inclusion Complexation of Bis(β-CD)s 1−3 with Oligopeptide Guests in Tris-HCl Buffer Solution (pH 7.4) at 25 °C

host	guest	K_S/M^{-1}	log K_S	$-\Delta G°$/kJ mol^{-1}
1	Leu-Gly	1450	3.16	18.04
	Gly-Leu	740	2.87	16.38
	Glu-Glu	135	2.13	12.16
	Gly-Pro	647	2.81	16.04
	Gly-Gly	890	2.95	16.83
	Gly-Gly-Gly	1400	3.15	17.96
	Glu(Cys-Gly)	6850	3.84	21.89
2	Leu-Gly	4470	3.65	20.83
	Gly-Leu	2160	3.33	19.03
	Glu-Glu	17900	4.25	24.27
	Gly-Pro	3820	3.58	20.45
	Gly-Gly	1260	3.10	17.70
	Gly-Gly-Gly	3770	3.58	20.42
	Glu(Cys-Gly)	12750	4.11	23.41
3	Leu-Gly	22000	4.34	24.79
	Gly-Leu	5260	3.72	21.24
	Glu-Glu	68200	4.83	27.59
	Gly-Pro	18100	4.26	24.30
	Gly-Gly	5370	3.73	21.29
	Gly-Gly-Gly	7270	3.86	22.04
	Glu(Cys-Gly)	42000	4.62	26.39

Gibbs free energy changes ($-\Delta G°$) obtained for the complexation of bis(β-CD)s **1**−**3** with oligopeptide guests were compiled in Table 1. When repeated measurements were made, the K_S values were reproducible within an error of ±5%.

Binding Mode. It is well-known that the competitive inclusion, cooperative binding, and induced-fit generally occur in the molecular binding process of modified β-CDs, and therefore it is very important to investigate the inclusion modes between host β-CDs and guest molecules for elucidating the mechanism of molecular recognition. In our previous research by means of ^{1}H NMR, circular dichroism, and fluorescence spectroscopy as well as CPK model examination (*24*), we found that a multiple sandwich binding mode was operative in the association of the guest molecule with bridged bis(β-CD); that is, upon inclusion complexation with bis(β-CD), two side groups of the guest molecule were embedded into the hydrophobic β-CD cavities from the primary side of β-CD to form a sandwich host−guest inclusion complex. In the present paper, we wish to establish the possible binding mode of bridged and metallobridged bis(β-CD)s **1**−**3** with oligopeptides according to the results of circular dichroism, ^{1}H NMR, and fluorescence spectral experiments.

For the self-included CD hosts, there should exist a competition between the substituent group of host and guest upon inclusion complexation with the CD cavity, and this competitive inclusion may be verified by the circular dichroism spectral studies. Typically, a series of ICD signals of **2** in the presence of different amounts of Gly-Gly were shown in Figure 8. As can be seen from Figure 8, the ICD intensity of host **2** around 310 nm increased gradually upon the addition of increasing amounts of Gly-Gly. However, the corresponding absorption intensity around 340 nm decreased gradually, accompanied by the appearance of an isobestic point at 309 nm. Since Gly-Gly displayed no ICD signal in the range of 210−400 nm, one possible explanation for these spectral phenomena was that, during the inclusion complexation, the guest molecule was cooperatively bound by two β-CD cavities from the primary side, which forced the linker group of bis(β-CD) away from the β-CD cavity and thus gave the weak ICD signals.

Interestingly, the **2** and **3** were found to show different fluorescence behaviors upon complexation of guest peptides. The gradual addition of Glu-Glu or Glu(Cys-Gly) to a dilute solution of **2** or **3** significantly decreased the fluorescence intensity (Figure 9). However, fluorescence intensity for **2** or **3** gradually enhanced with the stepwise addition of other peptides under comparable conditions. In a control experiment, neither disodium oxamidobis-(2-benzoxylate) nor its Cu(II) complex **5** exhibited appreciable fluorescence change upon the addition of oligopeptides. In additional control experiments, the formation constant (K_S) of disodium oxamido bis(2-benzoxylate) with Cu(II) ion was also determined by means of UV−vis spectral titration, and the obtained K_S value was 27930 ± 1390 M^{-1}, which indicated a strong association of oxamido bis(2-benzoxylate) with Cu(II). Moreover, we also compared the NMR spectra of disodium oxamido bis-(2-benzoxylate) ([**4**−Na] = 4.6 × 10^{-4} mol dm^{-3}), a 1:1 complex of **4**−Na with Ni(II) ([**4**−Na/Ni] = 3.8 × 10^{-4} mol dm^{-3}), and a 1:1 mixture of **4**−Na/Ni with Glu-Glu ([**4**−Na/Ni] = [Glu-Glu] = 3.9 × 10^{-4} mol dm^{-3}). The reason for choosing Ni(II) as metal ion was to avoid the paramagnetic disturbance caused by Cu(II). As can be seen in Figure 10, several changes of the δ values of the aromatic protons in the **4**−Na/Ni system were observed as compared with those in **4**−Na; that is, the δ values of H1, H2, H3, and H4 protons shifted downfield ca. 0.11, 0.08, 0.07, and 0.04 ppm, respectively, indicating that the Ni(II) ion was coordinated to the oxamido bis(2-benzoxy-

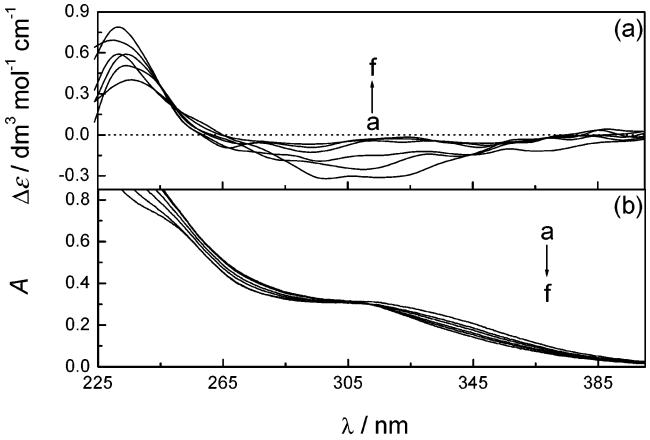


Figure 8. Circular dichroism (a) and absorption (b) spectra of **2** (8.9 × 10^{-5} mol dm^{-3}) upon addition of Gly-Gly (0 ∼ 9.13 × 10^{-3} mol dm^{-3} from *a* to *f*) in Tris-HCl buffer solution (pH 7.4) at 25 °C.

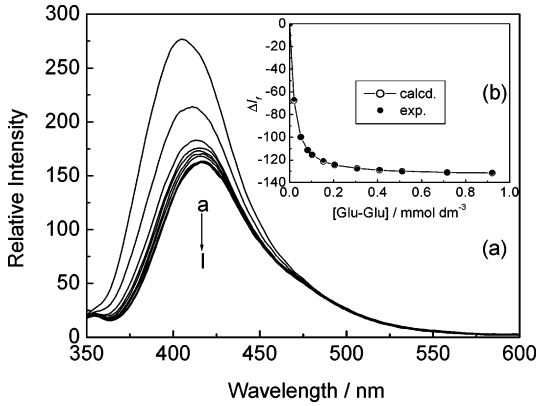


Figure 9. (a) Fluorescence spectral changes of host **3** (1.1 × 10^{-5} mol dm^{-3}) upon addition of Glu-Glu (0∼0.92 × 10^{-3} mol dm^{-3} from *a* to *l*) in Tris-HCl buffer solution (pH 7.4) at 25 °C and (b) the nonlinear least-squares analysis of the differential intensity (ΔI_f) to calculate the complex formation constant (K_S). (λ_{ex} = 314.0 nm, λ_{em} = 405.0 nm).

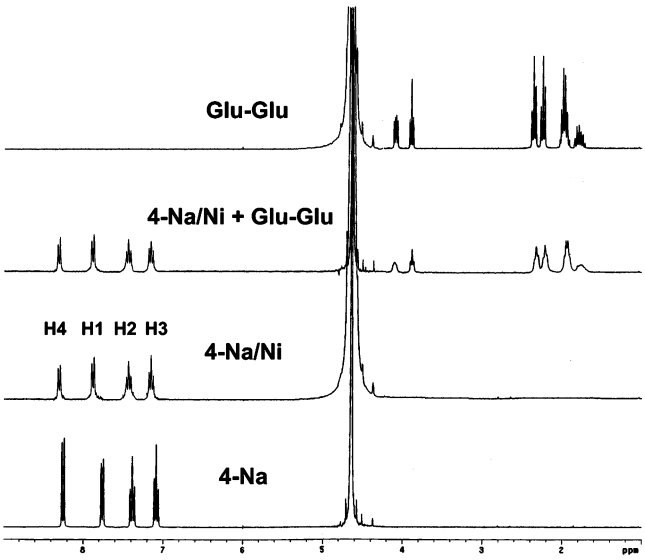


Figure 10. The ^{1}H NMR spectra of **4**−Na (4.6×10^{-4} mol dm^{-3}), a 1:1 complex of **4**−Na with Ni(II) ([**4**−Na/Ni] = 3.8×10^{-4} mol dm^{-3}), a 1:1 mixture of **4**−Na/Ni with Glu-Glu ([**4**−Na/Ni] = [Glu-Glu] = 3.9×10^{-4} mol dm^{-3}), and Glu-Glu (4.2×10^{-4} mol dm^{-3}) in D_2O at 25 °C.

late) ligand. However, the δ values of the aromatic protons in **4**−Na/Ni did not show any appreciable changes after adding Glu-Glu. These unchanged δ values before and after adding guest oligopeptides may indicate that the metal ion was still bound to the oxamido bis(2-benzoxylate) ligand in the presence of guest oligopeptides, because the demetalation of **4**−Na/Ni by the guest would make the δ values of the aromatic protons in **4**−Na/Ni shift upfield to the original position. The results of these control experiments jointly indicated that the increased or decreased fluorescence of hosts **1**−**3** upon the addition of guests was mainly attributed to the inclusion complexation, not just to the simple enhancing/quenching effect of the linker group or the demetalation of metallobridged bis(β-CD) by the guest. That is to say, such contrasting fluorescence behavior indicated that the different binding modes were operative in the inclusion complexations of Glu-Glu with **1** and **3**. The increased fluorescence intensity could be rationalized by the increased microenvironmental hydrophobicity and/or steric shielding around the fluorophore arising from the cooperative interactions between the host and guest (*36*). Therefore, the fluorescent group of the host could be efficiently shielded from the deactivating water attack by the formation of a sandwich inclusion complex, which consequently contributed to the fluorescence enhancement of the host (Figure 11b). On the other hand, for guests Glu-Glu or Glu(Cys-Gly), their branch groups (amino and carboxyl groups for Glu-Glu, amino and sulfydryl for Glu(Cys-Gly)) could interact strongly with the coordinated metal center of host **2** or **3** through the cooperative chelation effect (Figure 12b) (*6*, *37*), which led to the degressive fluorescence intensity of **2** or **3** upon inclusion complexation.

To obtain direct evidence to further elucidate the origin of these opposite fluorescence behaviors, ^{1}H ROESY experiments of **1** and **3** with the representative guest Glu-Glu were performed in D_2O at 25 °C. As illustrated in Figure 11a, the ^{1}H ROESY spectrum of a mixture of host **1** with guest Glu-Glu in D_2O displayed sophisticated NOE cross-peaks, which came from not only the intermolecular interactions between β-CD and Glu-Glu, but also the intramolecular interactions of **1** or Glu-Glu. Among these

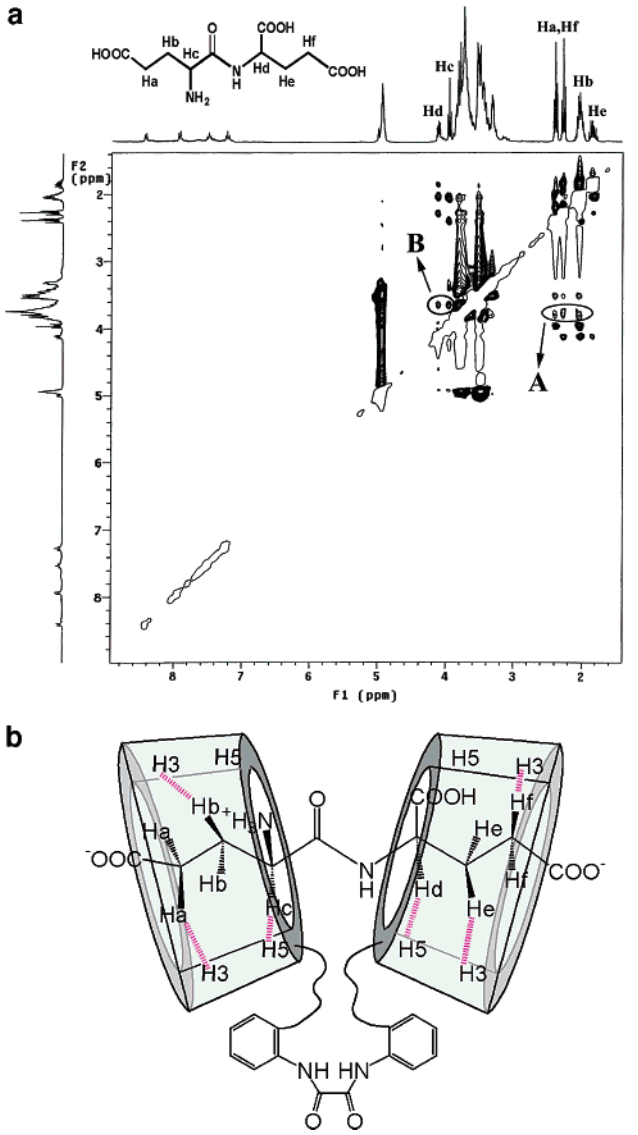


Figure 11. (a) ^{1}H ROESY spectrum of a mixture of **1** (2.5×10^{-3} mol dm^{-3}) with Glu-Glu (2.9×10^{-3} mol dm^{-3}) in D_2O at 25 °C with a mixing time of 400 ms; (b) possible complex structure of **1** with Glu-Glu.

signals, the NOE cross-peaks between the protons in the linker group of **1** and the H5/H3 protons of β-CD as well as the protons of Glu-Glu were not found, but the correlations between Ha, Hb, Hf protons of Glu-Glu and H3 protons of β-CD (peaks A), and the correlations between Hc, Hd protons of Glu-Glu and H5 protons of β-CD (peaks B), were clearly observed. These phenomena indicated that the Glu-Glu molecule was located in the β-CD cavity, while the linker group of **1** outside. According to the 2D NMR experimental results, a possible conformation of host **1** with Glu-Glu was shown in Figure 11b.

Figure 12a showed the ^{1}H ROESY spectrum of a mixture of **3** with Glu-Glu. Although the NOE correlation peaks between the H3/H5 of β-CD and the protons of Glu-Glu (peaks A) were not clear enough to estimate their relative intensity, we could deduce unambiguously that there did exist the corresponding NOE correlations. Simultaneously, the correlation peaks between the H3/H5 of β-CD and the protons of the linker group in **3** were not observed, which indicated that Glu-Glu was located in the β-CD cavities but the linker group outside the cavities (Figure 12b).

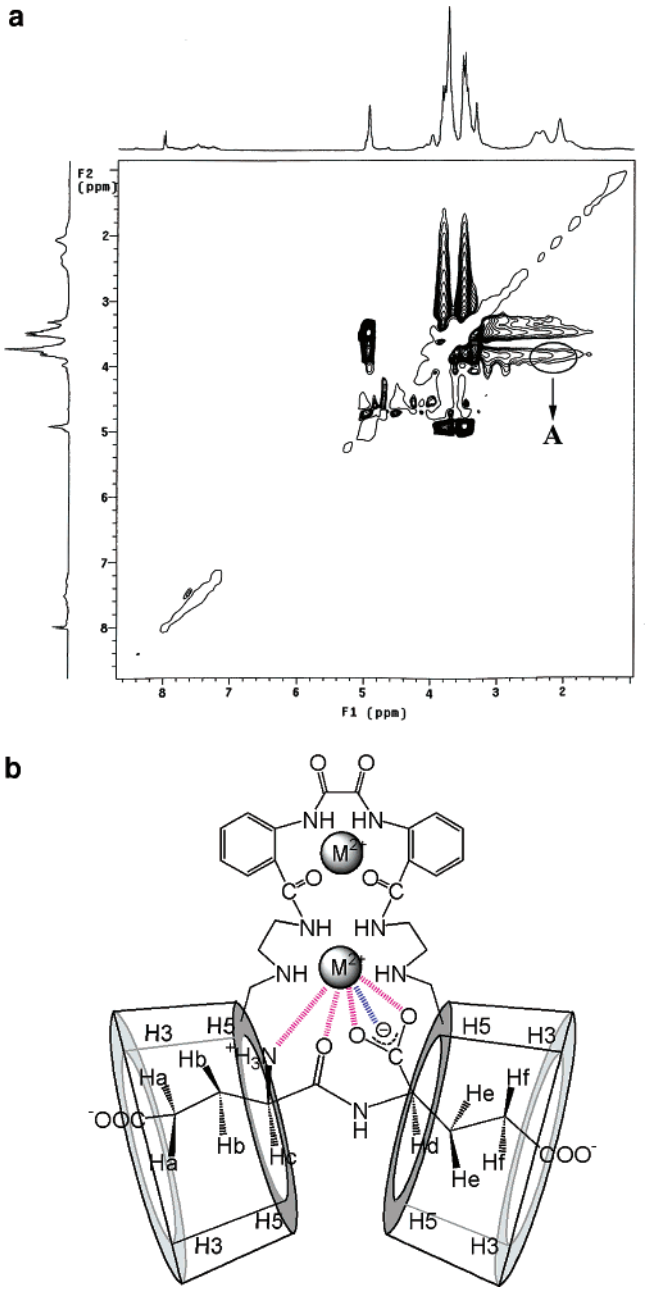


Figure 12. (a) ^{1}H ROESY spectrum of a mixture of **3** (2.7×10^{-3} mol dm^{-3}) with Glu-Glu (3.1×10^{-3} mol dm^{-3}) in D_2O at 25 °C with a mixing time of 400 ms; (b) possible complex structure of **3** with Glu-Glu.

Binding Ability and Molecular Selectivity. Native and simply modified CDs afford only very small binding constants to model substrates, probably due to the weak hydrophobic interactions between the host and guest. Bridged bis(β-CD), however, can enhance the original binding ability through cooperative binding of two adjacent cavities and potential multiple recognition ability. It is significantly noted that the introduction of the metal ions alters not only the original conformation of the linker group but also the distance and orientation of two β-CD cavities in bridged bis(β-CD). As compared with monomeric and dimeric CDs, metallobridged bis(β-CD)s can afford much more stable inclusion complexes with model substrates, owing to a fixed conformation by metal ligation, and additional cooperative chelation, electrostatic interaction, and/or electron transfer between ligated metal and accommodated guest. Furthermore, investigations on the inclusion complexation conformation of bridged and metallobridged bis(β-CD)s with guests also indicate that the cooperative binding and metal coordination can dominate the stability of complex formed between bis(β-CD)s and model substrate, leading to stronger van der Waals and hydrophobic interactions, since these interactions are closely related to the distance and contacting surface area between the host and guest. As can be seen from Table 1, the complex formation constants (K_S) for **1** with oligopeptides were variable according to the guest structures. Among the guest peptides examined, host **1** afforded the highest complex formation constant of 6850 M^{-1} for the inclusion complexation with Glu(Cys-Gly) and the largest molecular selectivity for Glu(Cys-Gly)/Glu-Glu pair ($K_S^{Glu(Cys-Gly)}/K_S^{Glu-Glu} = 51$). This may be attributed to the strict size/shape fitting relationship between the longest guest Glu(Cys-Gly) and the long-tethered bis(β-CD) **1**, which fully enjoyed the cooperative binding of two β-CD cavities with one guest molecule. However, possessing three carboxyl groups, Glu-Glu was more hydrophilic than other guests, which reduced the hydrophobic interactions as well as the extent of desolvation upon inclusion complexation and thus resulted in poor association with bis(β-CD).

Furthermore, metallobridged bis(β-CD)s **2** and **3** showed significantly enhanced binding abilities and molecular selectivities upon inclusion complexation with oligopeptides as compared with those of parent bis(β-CD) **1**. It can be seen from Table 1 that the coordination of the linker group to the metal ions further enhanced the binding abilities of **1** for guest peptides by a factor of 1.4−510. This further enhancement may point to a mechanism concerning a multiple recognition behavior of metallobridged bis(β-CD)s toward model substrates. Upon complexation with guest molecules, the metallobridged bis(β-CD)s not only afforded two hydrophobic binding sites (two β-CD cavities) cooperatively associating with a guest but also provided additional binding interactions between the heteroatoms of peptides and the coordinated metal center. Thus, as a cumulative result of these factors, metallobridged bis(β-CD)s displayed significantly enhanced binding abilities toward guest peptides as compared with parent bis(β-CD).

It is also interesting to compare the molecular selectivity sequence of hosts **1**−**3**. The complex formation constants (K_S) for the inclusion complexation of hosts **1**−**3** with oligopeptides decreased in the following order:

For **1**: Glu(Cys-Gly) > Leu-Gly > Gly-Gly-Gly > Gly-Gly > Gly-Leu > Gly-Pro > Glu-Glu

For **2**: Glu-Glu > Glu(Cys-Gly) > Leu-Gly > Gly-Pro > Gly-Gly-Gly > Gly-Leu > Gly-Gly

For **3**: Glu-Glu > Glu(Cys-Gly) > Leu-Gly > Gly-Pro > Gly-Gly-Gly > Gly-Gly > Gly-Leu

It can be seen that, different from parent bis(β-CD), metallobridged bis(β-CD)s showed the highest binding ability for Glu-Glu. This is because, besides the cooperative binding of two adjacent β-CD cavities and the heteroatom−metal chelation effects, the electrostatic interactions between the cationic metal center and the anionic carboxyl group located in the middle of Glu-Glu (blue line in Figure 12b) also contributed to the strong inclusion complexation of Glu-Glu with metallobridged bis(β-CD)s, since the carboxyl groups in peptides should exist as anions in this medium (pH = 7.4).

Another intriguing finding is the relatively high sequence selectivity of hosts **1**−**3** upon complexation of guest peptides. As can be seen in Table 1, hosts **1**−**3** could differentiate the sequence of dipeptide, with the selectivity as high as 2.0−4.2 for the Leu-Gly/Gly-Leu pair and

1.4−3.0 for the Gly-Gly-Gly/Gly-Gly pair. This sequence recognition ability of bis(β-CD)s could be accounted for in terms of the size/shape fit between host and guest. Possessing a quasi-linear structure, Leu-Gly was more favorable to the cooperative binding of two β-CD cavities than Gly-Leu that possessed a large isobutyl branch. On the other hand, the longer guest Gly-Gly-Gly was a better fit to the intercavity distance of bis(β-CD)s than Gly-Gly, which in turn led to stronger host−guest inclusion complexation.

As a whole, we can see that the host selectivity sequence of bis(β-CD)s **1**−**3** for each oligopeptide guests was **3** > **2** > **1**. This indicated that nickel(II)-coordinated bis(β-CD) **3** could give stronger binding abilities to oligopeptides than parent bis(β-CD) and its copper(II) complex, which may be helpful to select the appropriate metal ion as the coordination center in the design of the functional β-CD dimers and their metal complexes.

CONCLUSIONS

In summary, novel bridged bis(β-CD)s **1** can recognize the size and hydrophobicity of guest peptides through the cooperative binding of two CD cavities, having higher binding abilities toward relatively long peptides. Especially, its metal complexes effectively enhanced the original binding ability and sequence selectivity of parent **1** through a simultaneous contribution of the multiple chelation effects and/or the electrostatic interactions between the coordinated metal center and the guest peptide. On the other hand, the metal coordination can switch the fluorescence behavior of the parent bis(β-CD) for special substrates, which potentially enabled these novel bis(β-CD)s to function as fluorescence sensors for the molecular recognition of biological guests.

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