Biquinolino-Modified β-Cyclodextrin Dimers and Their Metal Complexes as Efficient Fluorescent Sensors for the Molecular Recognition of Steroids

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Abstract: A series of bridged β-cyclodextrin (β-CyD) dimers possessing functional tethers of various lengths was synthesized in moderate yield by the treatment of 2,2′-biquinoline-4,4′-dicarboxylic dichloride with β-CyD or mono[6-oligo(ethylenediamino)-6-deoxy]-β-CyDs. The products were 2,2′-biquinoline-4,4′-dicarboxy-bridged bis(6-*O*-β-CyD) (**8**), *N*,*N*′-bis(2-aminoethyl)-2,2′-biquinoline-4,4′-dicarboxamide-bridged bis(6-amino-6-deoxy-β-CyD) (**9**), and *N*,*N*′-bis(5-amino-3-azapentyl)-2,2′-biquinoline-4,4′-dicarboxamide-bridged bis(6-amino-6-deoxy-β-CyD) (**10**). The reaction of **8**–**10** with copper perchlorate give their copper(II) complexes **11**–**13** in satisfactory yields of over 77%. All the bis(β-CyD)s **8**–**13** act as efficient fluorescent sensors and display remarkable fluorescence enhancement upon addition of optically inert steroids. The inclusion complexation behaviors of **8**–**13** when treated with the representative steroids cholate (**14**), deoxycholate (**15**), and glycocholate (**16**) in aqueous solution at 25 °C were investigated by means of UV/Vis spectroscopy, conductivity and fluorescence measurements, circular dichroism spectroscopy, and 2D NMR spectroscopy. The tether length of bis(β-CyD) **9** allows it to adopt a cooperative host–tether–guest binding mode in which the spacer and guest are co-included in the two CyD cavities. As a result of this cooperation, **9** has a stability constant (K_s) about 2×10^2 times higher than that of monomodified β-CyD **4** for inclusion complexation with cholate. Metallooligo(β-CyD)s with four β-CyD units have enhanced binding abilities compared with monomodified β-CyDs. These metallo compounds have binding affinities for guest steroids that are up to 50–4.1×10^3 times higher than those of CyDs **2**–**4**. The guest-induced fluorescence enhancement of bis(CyD)s opens a new channel for the design of sensor materials. The complex stability constants of these compounds are discussed from the viewpoint of induced-fit interaction and cooperative multiple binding between host and guest.

Keywords: cyclodextrins · fluorescent sensors · molecular recognition · steroids · supramolecular chemistry

Introduction

Cyclodextrins (CyDs) are a class of cyclic oligosaccharides with six, seven, or eight D-glucose units linked by α-1,4-glucose bonds. These three types of CyD are named α-, β-, and γ-CyD, respectively.[1] The truncated cone-shaped hydrophobic cavities of these compounds have a remarkable ability to include various guest molecules, either in aqueous solution or in the solid state.[1–4] This property has led to the wide application of cyclodextrins in various fields, such as analytical chemistry, enzymology,[5] pharmaceuticals,[6] the food industry,[7] and so on. Within the last decade, molecular sensors based on the guest-induced response of fluorescent-labeled CyDs have attracted increasing attention.[8] For example, Ueno et al. have investigated tens of monosubstituted CyDs modified with chromogenic groups that are sensitive to changes in the binding state of the CyDs.[9–14] A chromogenic aromatic group originally accommodated in the CyD cavity suffers substantial conformational changes upon guest inclusion, with accompanying appreciable spectral changes. The binding ability of a fluorescent-labeled CyD can be quantitatively assessed by analysis of the spectral changes induced by guest inclusion. Bridged bis-CyDs are known to exhibit greatly enhanced binding abilities compared with those of other native and synthetic CyDs as a result of the cooperative binding of one guest molecule with two hydrophobic CyD cavities located in close proximity. Much effort has been devoted to the design and synthesis of bis-CyDs with considerable structural diversity and the investigation of their inclusion complexation behavior with model substrates.[15–19] Metal ions have also been introduced as additional recognition sites to further enhance the binding abili-

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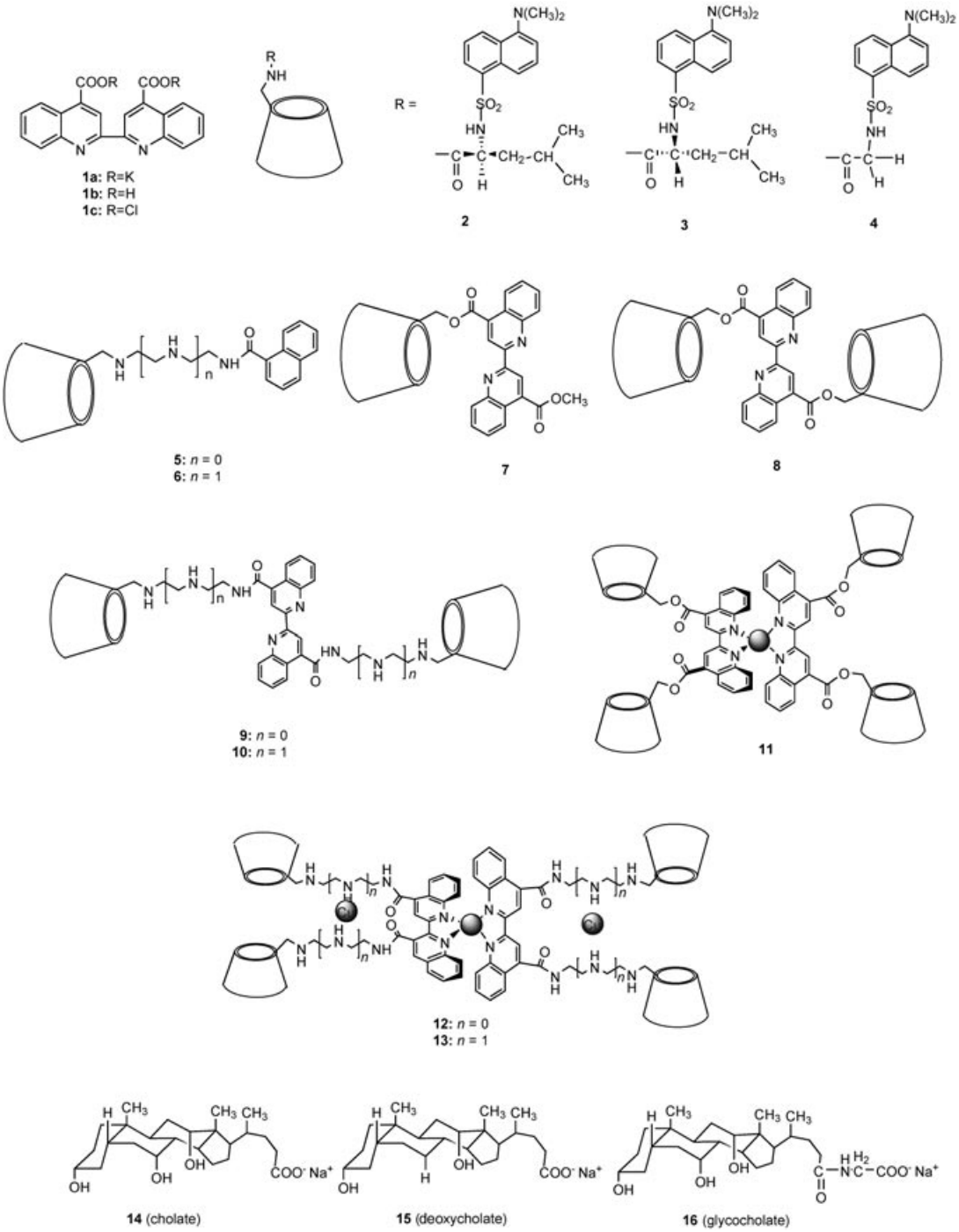
ty and selectivity of CyD derivatives.[20–22] However, to the best of our knowledge, the use of dimeric CyDs linked by a chromogenic bridge and/or the metal-coordinated complexes of such CyDs as fluorescence probes that recognize optically inert guest molecules has rarely been reported so far. In the study reported herein, we synthesized a series of bis(β-CyD)s containing a fluorescent biquinolino tether (**8**–**10**), along with the Cu^{II} complexes of these compounds (**11**–**13**). We investigated the inclusion complexation behavior of these CyDs with some optically inert guest molecules. We chose these steroids as model substrates simply because they are known to possess a great variety of biological functions in many eukaryotic organisms[23] and studies on the molecular recognition of steroids by small synthetic hosts such as CyDs ideally complement biological investigations with large protein receptors and supramolecular transporting assemblies. These studies together will improve our insight into steroid recognition processes and may ultimately lead to new potential therapeutic approaches.[24] The stability constants (K_s) determined for the complexation of steroids with CyDs are described below in terms of cooperative host–tether–guest interactions and a molecular multiple recognition mechanism for the interaction between the guest steroids and CyD hosts **8**–**13**. We are also particularly inter-

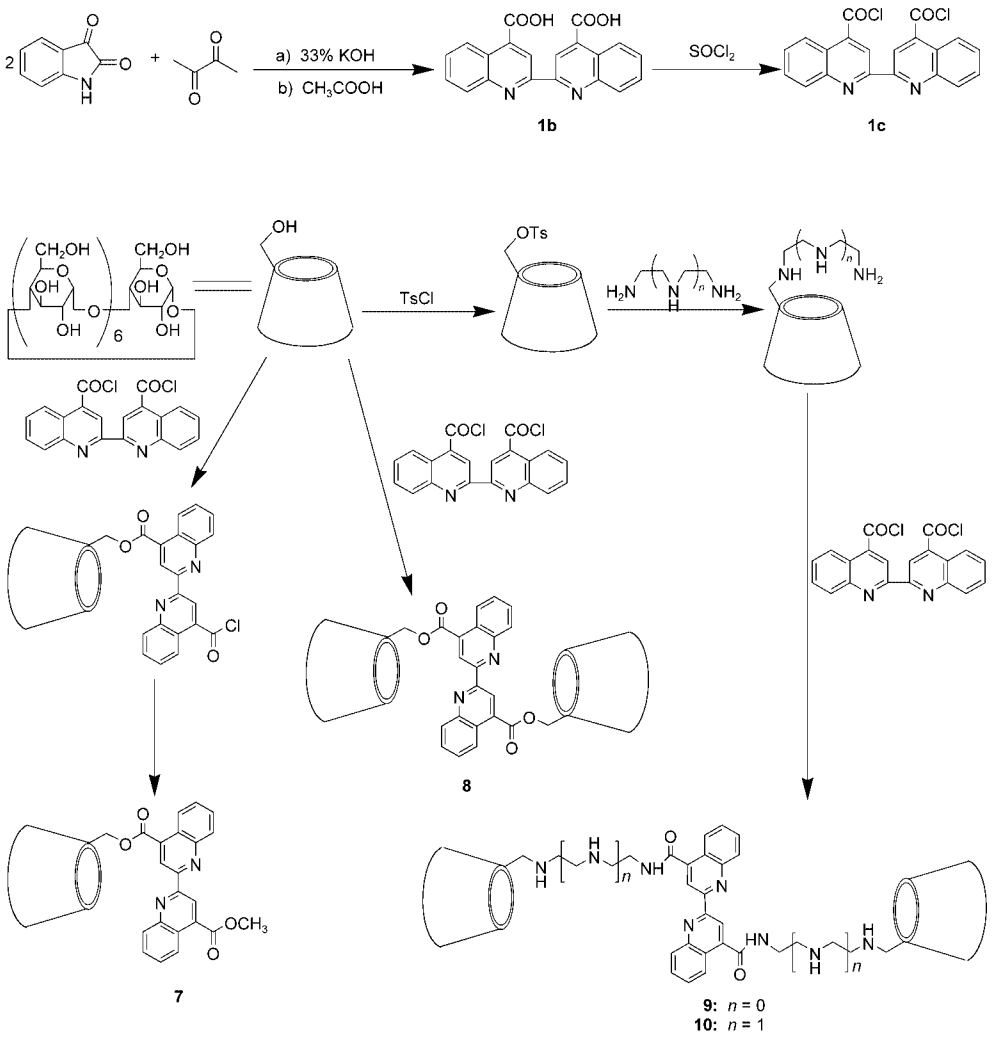


ested in examining the molecular recognition of biological steroid guests by bis(CyD)s through a fluorescence-sensing mechanism. This approach could serve to further our understanding of this developing but little-investigated area in the field of CyD chemistry.

Results and Discussion

Synthesis: Scheme 1 illustrates the synthesis of CyD derivatives **7**–**10**. Mono[6-(2,2′-biquinoline-4′-carboxymethylester-4-carboxy)-6-deoxy]-β-CD (**7**) was synthesized by treatment of 2,2′-biquinoline-4,4′-dicarboxylic dichloride with β-CyD, followed by esterification with methanol. A large excess of 2,2′-biquinoline-4,4′-dicarboxylic dichloride over β-CyD was used to ensure the monosubstituted product was obtained. A dimethylformamide solution of β-CyD was added dropwise to a highly diluted solution of 2,2′-biquinoline-4,4′-dicarboxylic dichloride over 2 h to enhance the reaction yield. Biquinoline-dicarboxylate-bridged bis(β-CyD) **8** was synthesized in moderate yield by treating 2,2′-biquinoline-4,4′-dicarboxylic dichloride with an excess amount of β-CyD, while bis(β-CyD)s **9** and **10** were synthesized from the corresponding mono[6-oligo(ethylenediamino)-6-deoxy]-β-CyDs.[25] Care was taken to keep the mixture anhydrous and at low temperature during these reactions, particularly at the initial stage, to achieve a smooth and clean reaction without undesirable product(s). Metallooligo(β-CyD)s **11**–**13** were prepared in satisfactory yields from the relevant bridged bis(β-CyD)s **8**–**10** through a coordination reaction with $Cu(ClO_4)_2 \cdot 6H_2O$ in aqueous solution. The quantities of reactants used were in accordance with the reaction stoichiometry, as determined by conductivity titration. The compositions of the products were verified by elemental analysis.

Metal-binding behavior and stoichiometry of CyD complexes: Spectrophotometric titrations were performed at 25 °C in aqueous solution to investigate the coordination behavior of bis(β-CyD)s **8**–**10** in the presence of copper(II) ions. A typical example of a titration curve obtained by titration of bis(β-CyD) **9** with copper(II) ions is shown in Figure 1. The absorption intensity of bis(β-CyD) **9** at 339 nm gradually decreased, while the maximum at 264 nm gradually increased with increasing copper(II) ion concentration. These changes were accompanied by an appreciable bathochromic shift in the absorption peaks (5–9 nm for the peak



Scheme 1. Synthesis of CyD derivatives **7**–**10**.

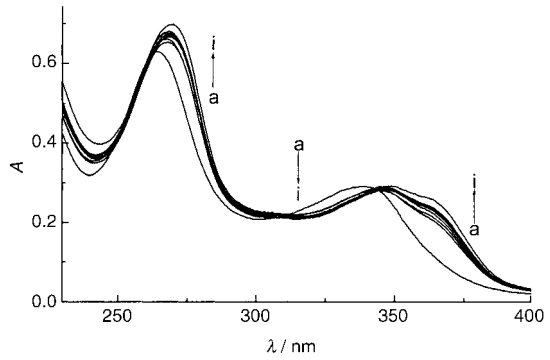


Figure 1. UV/Vis spectral changes of **9** upon addition of copper(II) ions in aqueous solution ($[\mathbf{9}]=3.4\times10^{-5}$ mol dm^{-3}; $[Cu^{2+}]=0$ and, from a to i, 0.103, 0.205, 0.308, 0.41, 0.615, 0.82, 1.03, 1.23, and 1.64×10^{-4} mol dm^{-3}; counteranion: ClO_4^-).

at 339 nm, 4–6 nm for that at 264 nm). In a control experiment, the UV/Vis spectrum of copper(II) ions was found to undergo no change at 200–400 nm over the concentration range used for the titrations and under comparable experimental conditions. The three isobestic points observed in each of the UV/Vis titration plots for bis(β-CyD)s **8**–**10** titrated with copper(II) ions confirm that a simple one-step transformation from the free bis(β-CyD)s to the copper(II)-ligated species occurs. These phenomena jointly indicate that copper(II) ions coordinate bis(β-CyD)s to form metal-ligated species. In addition to elemental analysis data, conductivity measurements were used to explore the coordination stoichiometry of the metallooligo(β-CyD)s in aqueous solution since the conductivity of the system decreases upon complex formation. The plots show a minimum at a concentration ratio of 2.0 for **8**–Cu^{II} (Figure 2a) and 0.67 for the **9**–Cu^{II} and **10**–Cu^{II} systems (Figure 2b). These values correspond to 2:1 and 2:3 coordination stoichiometries, respectively.

Conformation of the CyD dimers and their metal complexes: It is well known that elucidation of the crystal structure is one of the most convincing methods of unequivocally illustrating the geometrical structure of CyD derivatives. Unfortunately, our repeated attempts to prepare single crystals of the CyD dimers and their metal complexes that were suitable for X-ray crystallography were unsuccessful. To elucidate the possible structures of the CyD dimers, we performed a molecular modeling study with the CAChe 3.2 program (Oxford Molecular Co., 1999) and obtained the energy-optimized structures of hosts **8**–**10**. The initial geometry of β-CyD used in these calculations was taken from the crystal structure described in the literature,[26] and the energy of this structure was minimized by using the MM2 force field. Although the computed structures of the hosts may not be taken as direct evidence of the actual structures of these CyD dimers, these computed models can still provide some useful information about the possible geometries of the hosts.[27]

As shown in Figure 3, the biquinoline chromophore in CyD dimer **9** is approximately planar and exists in a *trans*

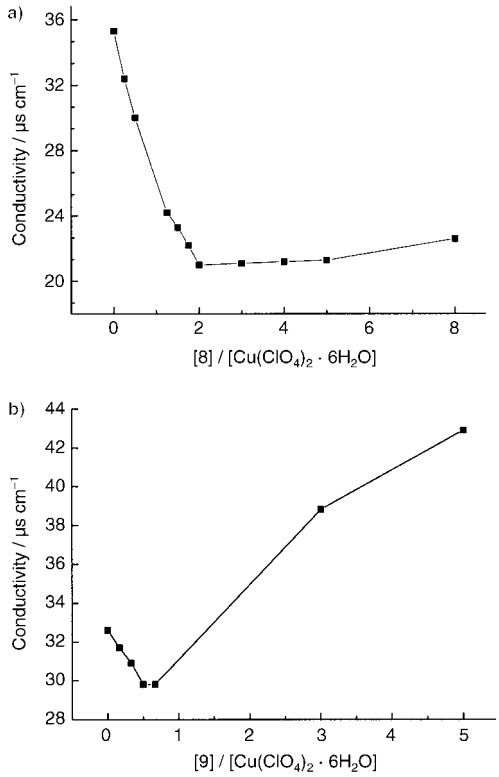


Figure 2. The dependence of the conductivity of a) $Cu(ClO_4)_2\cdot6H_2O$ (2.2×10^{-5} mol dm^{-3}) on the relative concentration of bis(β-CyD) **8** (0, 0.567, 1.13, 2.83, 3.39, 3.97, 4.53, 6.64, 8.42, 11.3, 18.1×10^{-5} mol dm^{-3}), and b) $Cu(ClO_4)_2\cdot6H_2O$ (3.4×10^{-5} mol dm^{-3}) on the relative concentration of bis(β-CyD) **9** (0, 0.571, 1.14, 1.71, 2.28, 10.3, 17.1×10^{-5} mol dm^{-3}) at 25 °C in aqueous solution.

conformation. A similar *trans* conformation is also observed in **8** and **10**. However, further investigations with a Corey–Pauling–Koltun molecular model demonstrated that the biquinoline chromophores in hosts **8**–**10** adopt the *cis* conformation after coordination with metal ion(s), as previously illustrated.[28]

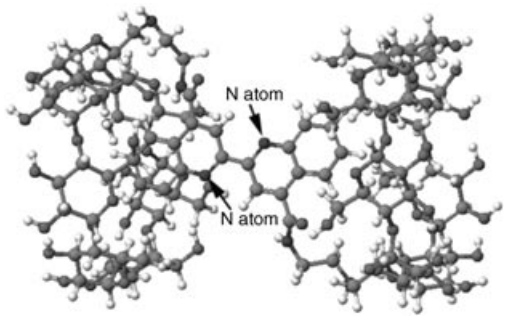


Figure 3. MM2-optimized structure of bis(β-CyD) **9**.

Proximity of an achiral chromophoric guest/moiety to the CyD cavity can give rise to induced circular dichroism (ICD).[29] In a control experiment, an aqueous solution of **1a** gave neither a circular dichroism (CD) signal nor a rotatory signal, which indicates that the biquinoline group is an achiral chromophore in aqueous solution. To obtain infor-

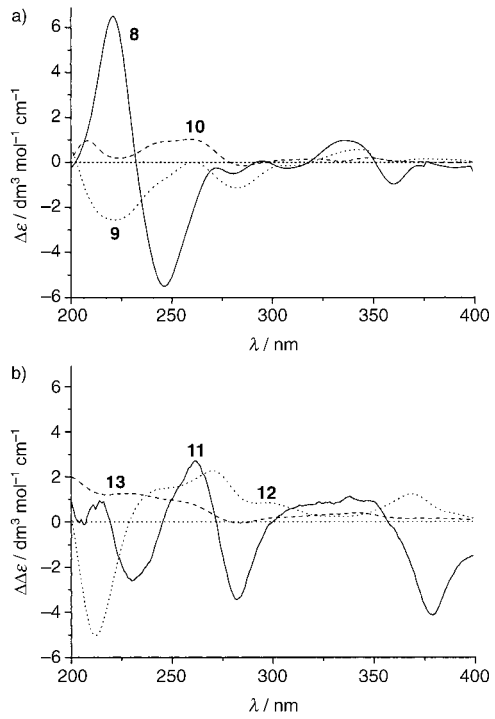


Figure 4. Circular dichroism spectra of hosts **8–13** (5×10^{-5} mol dm^{-3}) in aqueous solution at 25 °C.

mation about the conformations of the β-CyD dimers containing a chromophoric biquinoline-dicarboxy tether in dilute aqueous solution, CD spectra were recorded for **8–10** at a concentration of 5×10^{-5} mol dm^{-3}. As shown in Figure 4a, hosts **8–10** display clearly different CD spectra in the absence of a guest, which indicates that significant (but different degrees of) interaction between the aromatic tether and the two chiral cavities of the CyD dimers occurs.

Host **8** displays a strong negative Cotton effect indicated by a peak at 248 nm ($\Delta\varepsilon=-5.53$ M^{-1}cm^{-1}) for the ^1L_b transition, and a strong positive Cotton effect marked by a peak at 225 nm ($\Delta\varepsilon=6.82$ M^{-1}cm^{-1}) corresponding to the ^1L_a transition of the phenyl group in the biquinoline chromophore. Host **9**, which is a higher-order homologue of **8**, gives an absolutely different CD signal; the spectrum has two negative Cotton effect peaks with moderate intensities at 225 nm ($\Delta\varepsilon=-3.83$ M^{-1}cm^{-1}) and 283 nm ($\Delta\varepsilon=-2.22$ M^{-1}cm^{-1}) that may be assigned to the ^1L_a and ^1L_b bands, respectively. Host **10** displays only a weak positive Cotton effect peak for the ^1L_b band at 260 nm ($\Delta\varepsilon=1.05$ M^{-1}cm^{-1}). The generally accepted empirical rule[30–32] states that the sign of the ICD signal depends on the orientation of the transition dipole moment of the chromophore with respect to the dipole moment of the CyD. An electronic transition parallel to the CyD axis gives a positive ICD signal, whereas a perpendicular transition gives a negative signal. This situation is reversed for a guest located just outside the CyD cavity. We thus deduced that the biquinoline chromophore in **8** penetrates deeply, whilst that in **9** penetrates shallowly into the CyD cavities. The weak positive Cotton effect observed for **10** in the range 300–400 nm may indicate that the biquinoline chromophore is distanced from CyD cavity and this result is best interpreted as an ordinary CD signal induced by the two distant chiral CyD moieties.

Since the self-inclusion of tethers often decreases the binding ability of bis-CyDs toward guests, appropriate adjustment of the tether conformation is necessary in the design of functional bis-CyDs. We tried to introduce metal center(s) into the bridge chains of our bis(β-CyD)s to fix the conformation of the tether group and thus exclude disadvantageous self-inclusion. As can be seen from Figure 4b, all the metallooligo(β-CyD)s **11–13** show signals that can be assigned to the ^1L_b transition of the phenyl group in the biquinoline chromophore and are indicative of a positive Cotton effect. This result indicates that the biquinoline moiety in the tether group is located some distance from the CyD cavities after coordination of the metal ion.

Two-dimensional NMR spectroscopy has recently become an essential method for the study of the structures of CyDs and their complexes since one can conclude that two protons are closely located in space if an NOE cross peak is detected between the relevant proton signals in the NOESY or ROESY spectrum. It is possible to estimate the orientation of the biquinoline moiety in the CyD cavity by using the assigned NOE correlations; if the biquinoline moiety is self-included in the CyD cavity, NOE correlations between the protons of the biquinoline moiety and H3/H5 of the CyD should be observed.

The 2D NOESY spectrum of **9** in D_2O (Figure 5a) shows six cross peaks between the aromatic protons of the spacer and the CyD protons. Only cross-peak interactions with H3, H5, and H6 of a CyD must be considered when such results are analyzed because H2 and H4 do not face the inner cavity of the CyD and H1 is affected by D_2O. The cross peaks A, B, and C, respectively, were assigned to the NOEs between the 8,8′, 7,7′, and 6,6′ protons of the biquinoline moiety and the H5 proton of the CyD. These results unambiguously show that the spacer moiety is embedded in the cavity of the CyD. Since the H5 protons are located at the primary side of the cavity, we concluded that the biquinoline spacer penetrates into the CyD cavity from the narrower opening. However, no cross peaks were found between the 3,3′ or 5,5′ protons of the biquinoline tether and the interior protons of the CyD. It is therefore likely that the unsubstituted rings of the biquinoline moiety penetrate shallowly into the CyD cavity from the primary side. The paramagnetic disturbance caused by a ligated copper(II) ion makes the 2D NMR spectrum of the bis(β-CyD)–copper(II) complex impossible to measure. Instead, we examined the 2D NMR spectrum of the **9**–nickel(II) complex, which has a similar coordination stoichiometry and conformation to the **9**–Cu^{II} complex and could be used to investigate the change in location of the tether group upon metal ion coordination despite the fact that the copper(II) ion tends to prefer a square planar coordination whilst the nickel(II) ion favors an octahedral geometry. Figure 5b shows that the biquinoline protons display no correlation with the H3/H5 protons of CyD after coordination with a nickel(II) ion. These phenomena, together with the ICD results, verify the exclusion of the tether group from the CyD cavity upon metal coordination. This conformation change favors the sequential penetration

of guest molecules into CyD cavities by inclusion complexation.

Fluorescence sensor: The emission intensity of a fluorophore attached to the CyD rim is usually stronger than that of the parent fluorophore because of the increased microenvironmental hydrophobicity introduced by self-inclusion. As can be seen from Figure 6, the fluorescence intensities of hosts **8** and **9** are much larger than that of reference compound **1a**, while the fluorescence intensity of **10** is relatively close to the reference value under the same conditions. Among the bis-CyDs examined, host **8** exhibits the largest fluorescence intensity. Since the fluorescence intensity of the biquinoline moiety is sensitive to changes in its microenvironment and is greater in a hydrophobic microenvironment than in a hydrophilic one, the above results suggest that the biquinoline moiety of **8** is located in the most hydrophobic environment, that is, deep inside the CyD cavity.

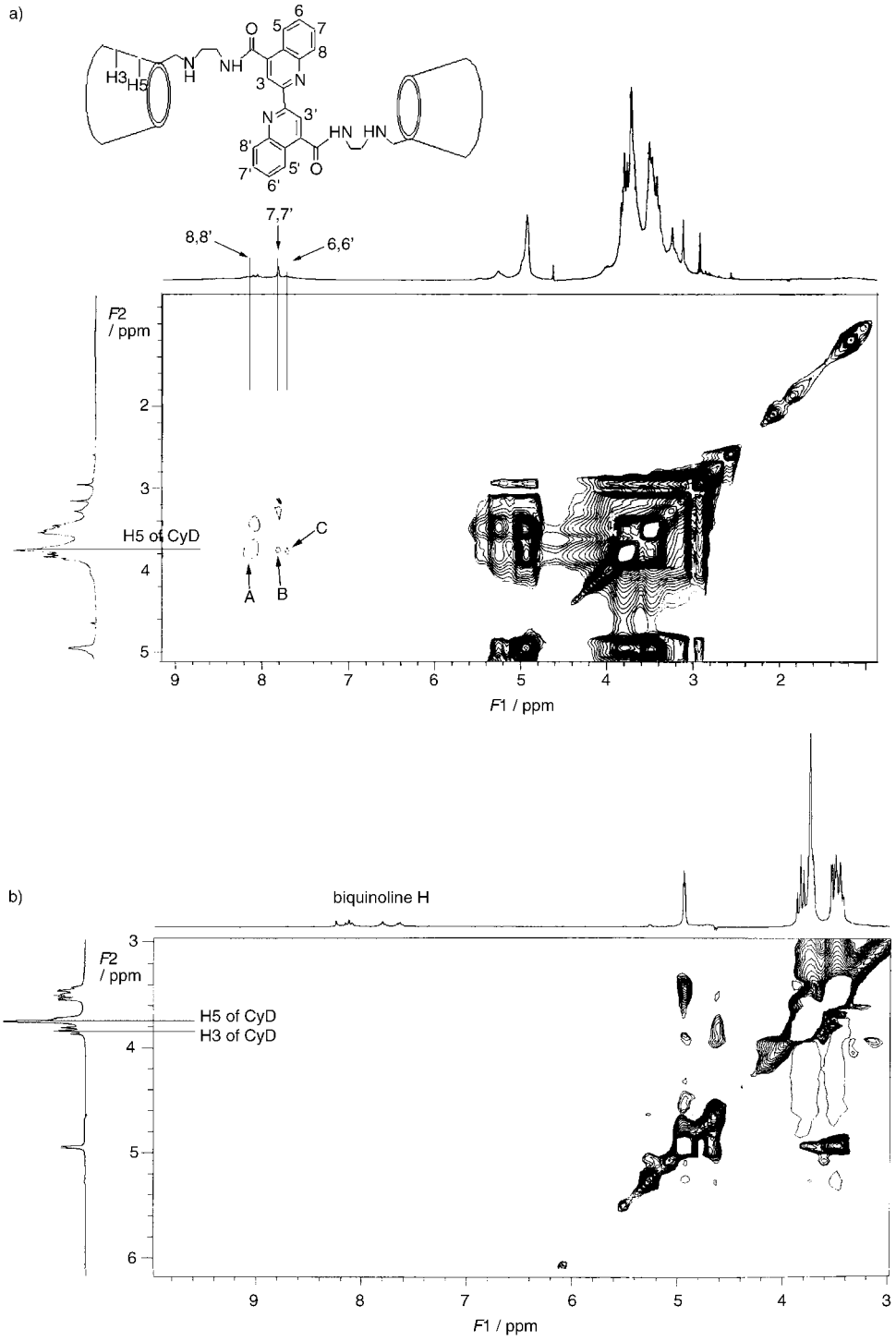


Figure 5. Sectional ^{1}H NOESY spectra (300 MHz) of a) CyD dimer **9**, and b) a **9**–Ni^{II} complex (1.0×10^{-3} M) in D_2O at 298 K; mixing time, 800 ms.

The rank order of the maximum intensities, **8**>**9**>**10**, may be regarded as roughly the order of the hydrophobicities of the environments surrounding the biquinoline moieties of the fluorescent bis-CyDs. Our results suggests that, for a bis-CyD with a short linker, self-inclusion of the tether efficiently shields the fluorescent biquinoline moiety from a deactivating attack by water, whilst elongation of the spacer leads to exclusion of the biquinoline moiety from the CyD cavities. This outcome is consistent with the ICD and 2D NMR results described above.

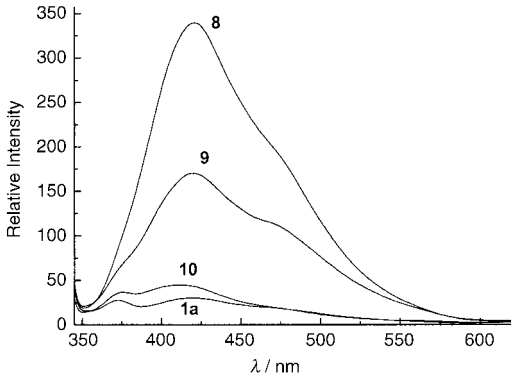


Figure 6. Fluorescence spectra of **1a** and bis(β-CyD)s **8**–**10** (2.5×10^{-6} M) in aqueous solution at 25 °C; excitation wavelength (λ_{ex}), 330 nm.

Fluorescence titrations of the bis(β-CyD)s and their copper(II) complexes with optically inert steroids were performed at 25 °C in aqueous solution to quantitatively assess the inclusion complexation behavior of these compounds. As shown in Figure 7, the fluorescence intensities of both the bis(β-CyD)s and the metallooligo(β-CyD)s gradually increase with increasing steroid concentration. Further study indicated that the pH value of the solution did not change significantly during the experimental procedure. These results led 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 **8**–**13** with representative guests was determined by Job's method. Figure 8 shows a Job's plot for the **12**/cholate system. Within the examined concentration range, the plot shows a maximum in fluorescence intensity at a molar fraction of bis(β-CyD) of 0.5, which indicates either 1:1 or 2:2 inclusion complexation. We have previously reported that biquinolino-bridged bis(β-CyD) **9** can form a 1:1 sandwich inclusion complex with a steroid guest through cooperative binding of the two cavities of the bis(β-CyD) unit with a guest molecule.[33] Since host **12** possesses two biquinolino-bridged bis(β-CyD) units, each of which can form a sandwich inclusion complex with one guest molecule, we conclude that the stoichiometry of the inclusion complex formed by the **12**/cholate system is likely to be 2:2, with intramolecular complexation. Stoichiometries of 1:1 (for bis(β-CyD)) or 2:2 (for metallooligo(β-CyD)) were obtained in other similar cases of host–guest inclusion complexation.

The effective stability constants of hosts **8**–**13** can be obtained by treating each bis(β-CyD) unit as a host unit and

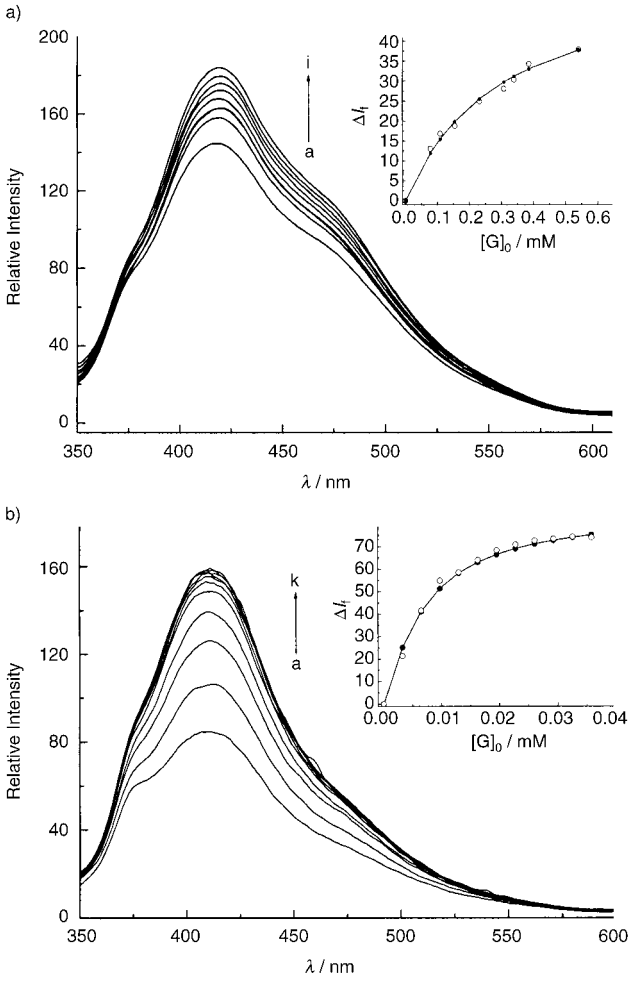


Figure 7. Changes in the fluorescence spectra of a) bis(β-CyD) **10** (8.6×10^{-6} M) upon addition of cholate (0 and (a–i) 0.77, 1.1, 1.5, 2.3, 3.1, 3.4, 3.9, 5.4×10^{-4} M), and b) metallooligo(β-CyD) **12** (4.16×10^{-6} M) upon addition of cholate (0 and (a–k) 0.33, 0.65, 0.98, 1.3, 1.6, 1.9, 2.3, 2.6, 2.9, 3.3, 3.6×10^{-5} M) in aqueous solution at 25 °C; λ_{ex} = 330 nm; insets: ● calcd, ○ exptl.

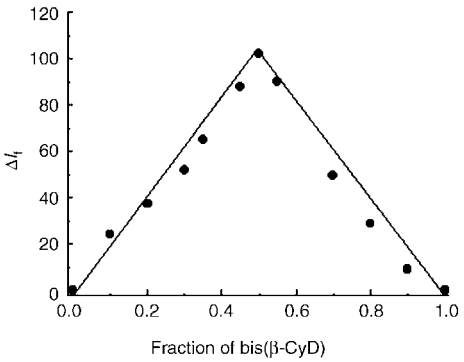


Figure 8. Job's plot for the complexation of **12** with cholate at 25 °C in aqueous solution ([bis(β-CyD) unit] + [cholate] = 1.5×10^{-5} M) produced with data taken from fluorescence spectra (λ_{ex} = 330 nm).

analyzing the sequential changes in fluorescence intensity (ΔF) that occur with changes in guest concentration. This analysis was carried out by using a nonlinear least-squares curve-fitting method.[20c] Treatment of each bis(β-CyD) unit

as a host unit results in a relatively gross approximation of the effective stability constants of the tetrameric Cu^{II} complexes. The calculated K_s values are listed in Table 1, along with the free energy changes of complex formation.

Table 1. Stability constants (K_s) and Gibbs free energy changes ($-\Delta G^0$) for the inclusion complexation of steroids by hosts **8**–**13** in aqueous solution at 25 °C.

Host	Guest	K_s [M^{-1}]	$\log K_s$	$-\Delta G^0$ [kJ mol^{-1}]	ref.
2	**14**	1650	3.2	18.4	[a]
	15	2660	3.4	19.5	[a]
3	**14**	588	2.8	15.8	[a]
	15	1520	3.2	18.2	[a]
4	**14**	60.4	1.8	10.2	[a]
	15	1030	3.0	17.2	[a]
5	**14–16**	[d]	[d]	[d]	[b]
6	**14–16**	[d]	[d]	[d]	[b]
7	**14–16**	[d]	[d]	[d]	[b]
8	**14**	5380 ± 50	3.7	21.3	[b]
	15	2790 ± 40	3. 5	19.7	[b]
	16	[d]	[d]	[d]	[b]
9	**14**	11300 ± 150	4.1	23.1	[c]
	15	21730 ± 250	4.3	24.8	[c]
	16	11040 ± 100	4.0	23.1	[c]
10	**14**	3380 ± 50	3.5	20.1	[b]
	15	3710 ± 50	3.6	20.4	[b]
	16	[d]	[d]	[d]	[b]
11	**14**	30500 ± 800	4.5	25.6	[b]
	15	529000 ± 1000	5.7	32.7	[b]
	16	1745000 ± 1500	6.2	35.6	[b]
12	**14**	196000 ± 800	5.3	30.2	[b]
	15	283700 ± 1000	5.5	31.1	[b]
	16	13000 ± 500	4.1	23.5	[b]
13	**14**	246000 ± 1000	5.4	30.8	[b]
	15	54000 ± 700	4.7	27.0	[b]
	16	891000 ± 800	5.9	34.0	[b]

[a] Ref. [12]. [b] This work. [c] Ref. [33]. [d] The guest-induced variations in the excimer emission are too small for these values to be determined.

Fluorescence enhancement and the host–tether–guest binding mode: Many researches have demonstrated that the emission of fluorophore-appended mono-CyDs is quenched upon inclusion complexation with a guest (such as a steroid)[9–14] as a consequence of decomplexation of the initially self-included fluorophore moiety (Figure 9a). We found that the fluorescence intensities of hosts **8**–**13** increase upon addition of guest steroids. This unique fluorescence behavior suggests that the biquinoline moieties in these hosts may undergo changes in location and orientation upon complexation, which consequently leads to increased microenviromental hydrophobicity and/or steric shielding around the fluorophore. Our 2D NMR investigation results support this hypothesis that the conformation of a host CyD changes upon complexation. As illustrated in Figure 10, the NOESY spectrum of an equimolar mixture of bis(β-CyD) **9** and **15** (0.5 mM each) displays clear NOE cross peaks between CyD H5 and the aromatic protons of the biquinoline moiety. Peaks A indicates that the included biquinoline moiety is not driven out of the CyD cavity by guest inclusion. Furthermore, the protons of **15** give not only the cross peaks B with the biquinoline protons, but also the cross peaks C with CyD H5, which confirms the cooperative co-inclusion of the fluorophore tether and guest steroid in the two CyD cavities. These results, together with the 1:1 host–guest inclusion complexation stoichiometry indicated by Job's experiment, indicate that a cooperative "host–tether–guest" binding mode is operative in the association of bis(β-CyD) with a guest molecule; upon complexation with bis(β-CyD), the guest steroid is embedded into one hydrophobic CyD cavity from the primary side, while the tether group is partly self-included in the other cavity (Figure 9b). In the metallooligo(β-CyD)s, the tether group is entirely excluded from the CyD cavities as a result of metal coordination. This arrangement allows two side groups of the guest molecule to be embedded into the hydrophobic CyD cavities from the primary side of the CyD to form a sandwich host–guest inclusion complex (Figure 9c). The biquinoline fluorophore in this structure is efficiently protected from deactivating water attack through steric shielding by the two closely located hydrophobic steroid molecules. In addition, the introduction of two guests will certainly increase the microenviromental hydrophobicity around the biquinoline fluorophore, which also contributes to the enhanced fluorescence of metallooligo(β-CyD).

Binding ability and selectivity: We have previously examined the inclusion complexation behavior of a variety of chemically modified CyDs with diverse guest molecules and found that several weak noncovalent forces, including van der Waals and hydrophobic interactions, hydrogen bonding, and dipole–dipole interactions, cooperatively contribute to the inclusion complexation of CyDs. The degree to which the size and shape of the host match those of the guest has a dominant effect on the stability of the complexes formed between bis- and/or oligo(β-CyD)s and model substrates. A good match leads to stronger van der Waals and hydrophobic interactions since the strength of these two types of interaction is closely related to the distance and contact surface area between host and guest. In accordance with this multiple recognition mechanism, native and modified monomeric CyDs display a relatively limited ability to associate with guest molecules, probably because of weak hydrophobic interactions. Dimeric CyDs, however, can have a greatly enhanced binding ability compared to the parent CyD as a result of cooperative binding of the two adjacent cavities and the potential of such a compound for multiple recognition. The introduction of metal ions to form metallooligo(β-CyD) complexes alters not only the original conformation of the tether group but also the distance between, and orien-

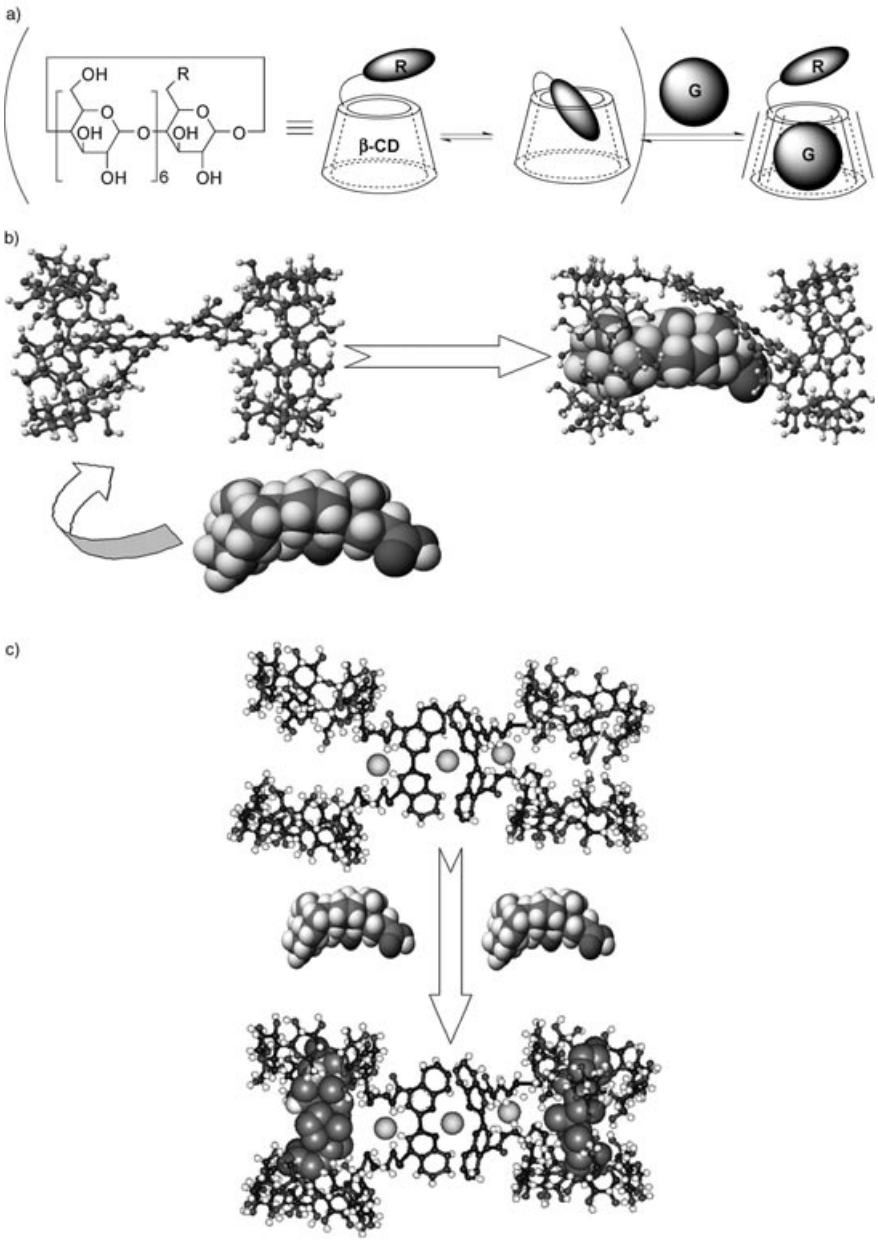
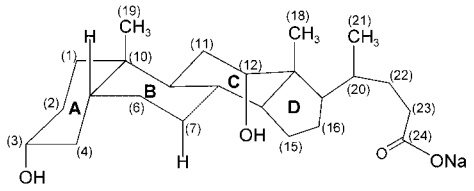


Figure 9. Schematic representation of the binding modes of fluorophore-appended a) mono-CyDs, b) bis(β-CyD)s, and c) metallobridged bis(β-CyD)s with guest molecules.

tation of, the two hydrophobic cavities in the CyD dimer. These changes affect the depth of penetration of the guest into the CyD cavity and allow additional binding interactions with the accommodated guest. Therefore, metallooligo(β-CyD)s can afford much more stable inclusion complexes than monomeric and dimeric CyDs owing to the cooperative association of four tethered hydrophobic cavities with model substrates, conformation fixation by metal ligation, and additional binding interactions between the metalligated tether group and the accommodated guest molecule. Table 1 shows that the stability constants of the complexes of bis(β-CyD)s **8**–**10** with guest molecules are larger than those of the complexes formed by monomodified β-CyDs **2**–**4** by a factor of about 1.1 to around 2×10^2. For example, cooperative binding leads to a stability constant for bis(β-CyD) **9** with guest **14** that is higher than that of monomodified β-CyD **4** by a factor of approximately 2×10^2. The K_s value of **9** with **15** is higher than that of **4** by a factor of around 20. In control experiments, the changes in the fluorescence spectra of **5**–**7** upon addition of guest steroids were too small to allow calculation of the stability constants, which may be attributed to strong self-inclusion of the substituted group preventing penetration of the guest into the CyD cavity. Comparison of the binding abilities of mono and bis(β-CyD)s highlights the inherent advantage of the cooperative host–tether–guest binding mode of bis(β-CyD)s **8**–**10**; in addition to inclusion complexation of the guest molecule within one hydrophobic CyD cavity, the tether group located near the accommodated guest provides some additional interactions with the guest. These factors jointly contribute to the stronger host–guest association achieved by dual CyDs in comparison to monomeric hosts. The data for the homologous steroids **14**/**15**, which possess slightly different skeletons but the same anionic tails, show that the mono- and bis-CyDs (except **8**) display higher binding affinities for **15** than for **14**. This stronger affinity for **15** is likely to arise from the more hydrophobic steroid skeleton of this compound compared with that of **14**. Compound **15** lacks the 7-hydroxy group and, as a result, hydrophobic interactions upon complexation are enhanced. The abilities of both the short-tethered compound **8** and the long-tethered host **10** to bind **14** and **15** are unexpectedly limited compared to the binding abilities of monomodified CyDs **2**–**4**. In the short-tethered bis(β-CyD) **8**, the low binding ability is the result of self-inclusion of the tether group, which means that the two CyD cavities are too close together to form a stable co-inclusion complex with the guest molecule. Compound **10**, which has a long tether, undergoes co-inclusion of the guest but steric hinderance from the relatively large 5-amino-3-azapentyl-2-quinoline-4-carboxyamide fragment on the exterior of the CyD cavity makes penetration of the guest molecule into the cavity from the primary side unfavorable and inevitably results in



15 (Deoxycholate)

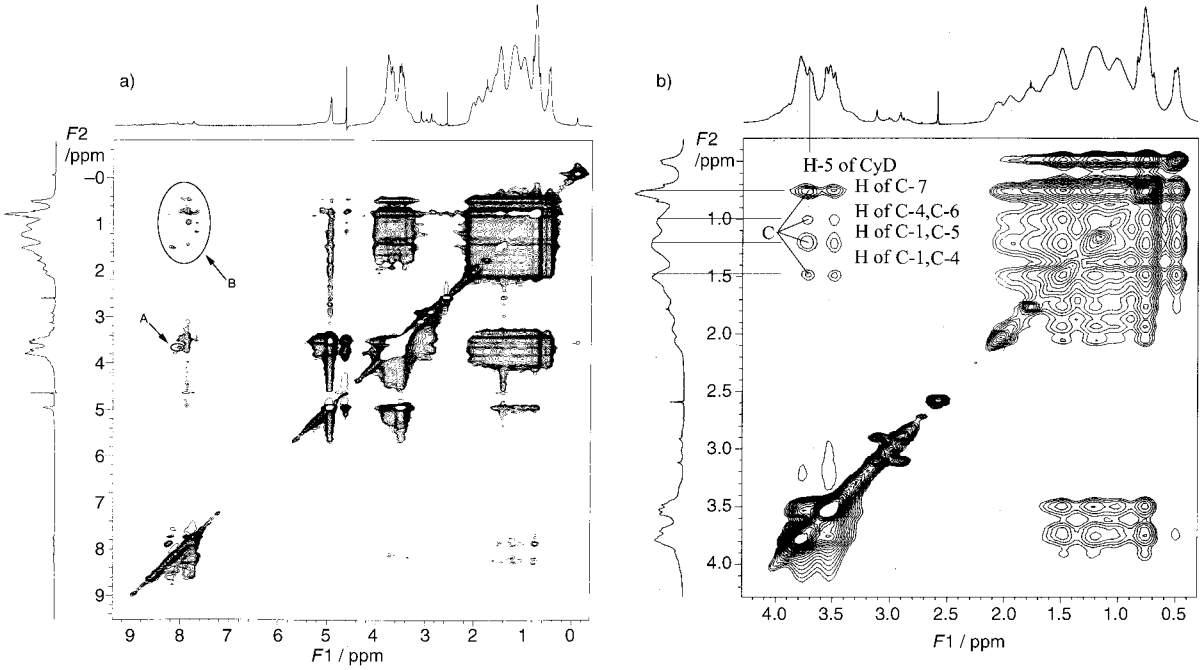


Figure 10. a) ^{1}H NOESY spectrum and b) sectional spectrum (300 MHz) of **9** with **15** (5.0×10^{-4} M each) in D_2O at 25 °C; mixing time, 600 ms.

a low binding ability. The metal-ligated oligomeric β-CyDs **11**–**13** have significantly enhanced (around 50–4.1×10^3 higher) binding affinities for the tested guest molecules compared with those of the monomodified β-CyDs. These results can be explained by considering a mechanism involving an uncommon multiple recognition behavior of metallooligo(β-CyD)s that is quite different from the mechanism of the parent biquinolino-bridged bis(β-CyD)s for binding model substrates. Only one CyD cavity and the tether group of a biquinoline-bridged bis(β-CyD) participate in association with the guest. In contrast, a metallooligo(β-CyD) affords four hydrophobic binding sites (four CyD cavities) and one (or three) metal coordination center(s), which jointly contribute to the cooperative binding of the oligomeric host with the guest molecule upon inclusion complexation. Each bis(β-CyD) unit cooperatively associates with one guest molecule to form the sandwich inclusion complex, and the metal-ligated tether group supplies further binding interactions to accommodate the guest. In addition, ligation of a Cu^{II} ion shortens the effective length of the tether to some extent and thus improves the size fit of the host with the guest. The cumulative result of these factors is that the metal-ligated β-CyD oligomers have binding abilities around 6–2×10^2 times higher than those of their parent bis(β-CyD)s, and **15**/**14** selectivity is enhanced from around 2 for **9** to approximately 17 for **11**. Of the host CyDs examined, metallooligo(β-CyD) **13** has the highest binding affinity for **14**, with a K_s value 4.1×10^3 times higher than that of monomodified CyD **4**. This difference in binding is a consequence of cooperative multiple binding by the metallooligo(β-CyD). More interestingly, metallooligo(β-CyD)s **11** and **13** display very high strong binding to **16** (1.7×10^6 M^{-1} for **11** and 8.9×10^5 M^{-1} for **13**). The corresponding values for the parent bis(β-CyD)s **8** and **10** are too small to determine. This result further supports the hypothesis that the introduction of copper(II) ions can greatly enhance the original binding ability of bis(β-CyD)s by allowing multiple recognition of the guest.

Conclusion

A series of biquinolino-bridged β-CyD dimers and their metal complexes were synthesized and used as efficient fluorescent sensors responsive to optically inert guests. The biquinoline–dicarboxy bridge introduced in the dual host can act as a positive binding site for host–guest complexation and as a versatile coordinating site for metal ions. Upon complexation with guests, biquinoline–dicarboxy-bridged bis(β-CyD)s adopt a host–tether–guest co-inclusion binding mode. The introduction of coordinated metal center(s) can alter the self-inclusion conformation of the host CyD. In a

rare example of multiple recognition, metal-ligated β-CyD oligomers give very high K_s values for guest molecules as a result of cooperative binding by four tethered hydrophobic CyD cavities and a metal-coordinated tether group. This system provides a convenient and powerful tool for enhancing guest-binding ability and selectivity. The cooperative host–tether–guest interactions described herein may also help us achieve a deeper understanding of the "multi-component, induced-fit" receptor–substrate interactions often observed in biological molecular recognition.

Experimental Section

General: Mono[6-(1-naphthyloxamino)ethyleneamino-6-deoxy]-β-CyD (**5**) and mono [6-(1-naphthyloxamino)diethylenediamino-6-deoxy]-β-CyD (**6**) were prepared as described previously.[34] Elemental analysis was performed on a Perkin–Elmer 2400C instrument. UV/Vis and circular dichroism spectra were recorded in a conventional quartz cell (10×10×45 mm) at 25 °C on a Shimadzu UV2401 and a JASCO-750 spectrometer, respectively. NMR spectra were performed on a Varian Mercury VX300 spectrometer. Fluorescence spectra were measured at 25 °C in a conventional quartz cell (10×10×45 mm) on a JASCO FP-750 spectrofluorometer at an excitation wavelength 330 nm.

2,2′-Biquinoline-4,4′-dicarboxylic acid (1b): A mixture of isatin (40 g, 0.27 mol), 2,3-butanedione (12.5 g, 0.14 mol), and potassium hydroxide (200 mL, 33 %) solution was heated for 24 h at 100 °C. After cooling, a gray precipitate was separated by filtration. The precipitate was redissolved in hot water, acidified with acetic acid, and dried in vacuo to give 2,2′-biquinoline-4,4′-dicarboxylic acid (3.2 g, 0.0089 mol). M.p. 367 °C;[35] UV/Vis (water): λ_{max} (ε) = 260.0 (46820), 332.2 nm (15140 $M^{-1}cm^{-1}$).

2,2′-Biquinoline-4,4′-dicarboxylic dichloride (1c): A mixture of 2,2′-biquinoline-4,4′-dicarboxylic acid (2.0 g, 5.6 mmol) and thionyl chloride (40 mL) was heated under reflux under nitrogen for 6 h. Removal of the residual thionyl chloride led to the diacyl chloride, which was obtained as a yellow solid in quantitative yield. The product was used in subsequent preparations without purification.

Mono[6-(2,2′-biquinoline-4′-carboxymethylester-4-carboxy)-6-deoxy]-β-CyD (7): Dry pyridine (25 mL) was added to a solution of DMF (140 mL) containing 2,2′-biquinoline-4,4′-dicarboxylic dichloride (0.4 g, 1.05 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (0.7 g, 3.4 mmol). A DMF (50 mL) solution containing β-CyD (0.7 g, 0.62 mmol) was added dropwise to the above-mentioned solution over 2 h. The resultant mixture was stirred for 18 h in an ice bath and another 48 h at room temperature. Anhydrous methanol (10 mL) was then added to the solution and the resultant mixture was stirred at 80 °C for 10 h. After cooling to room temperature, 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 acetone (300 mL). The precipitate formed was collected by filtration and purified twice on a column of Sephadex G-25 to give **7** as a light yellow solid (200 mg, 19 %). ^{1}H NMR ([D$_6$]DMSO), TMS): δ = 3.2–3.8 (m, 45H, $C_{2\text{-}6}$H of CyD, H of CH_3), 4.4–4.6 (m, 6H, O-6 H of CyD), 4.8–5.0 (m, 7H, C-1 H of CyD), 5.8–6.0 (m, 14H, O-2,3 H of CyD), 7.2–9.0 ppm (m, 10H, Ar); ^{13}C NMR ([D$_6$]DMSO): δ = 40.4, 60.6, 65.5, 69.3, 72.7, 73.7, 82.2, 102.3, 125.2, 126.1, 129.9, 130.8, 131.3, 136.8, 148.8, 154.8, 166.2 ppm; IR (KBr): $\tilde{\nu}$ = 3333, 2929, 1726, 1663, 1592, 1549, 1437, 1238, 1201, 1154, 1078, 1029, 944, 859, 757, 706, 662, 579, 529, 444 cm^{-1}; elemental analysis calcd (%) for $C_{63}H_{82}O_{38}N_2 \cdot 13H_2O$: C 44.26, H 6.36, N 1.63; found: C 44.10, H 6.26, N 1.35; UV/Vis (water): λ_{max} (ε) = 266.4 (43600), 341.8 nm (21500 $M^{-1}cm^{-1}$).

2,2′-Biquinoline-4,4′-dicarboxy-bridged bis(6-*O*-β-CyD) (8): 2,2′-Biquinoline-4,4′-dicarboxylic dichloride (0.30 g, 0.8 mmol) was dissolved in DMF (30 mL). Dry β-CyD (3.50 g, 3.08 mmol) in pyridine (25 mL) was added to this solution and the resultant mixture was stirred for 20 h in an ice bath. The solution was allowed to warm up and stirred for an additional two days at room temperature. The precipitate was removed by filtration and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in water then poured into acetone (300 mL) to give a light yellow precipitate. The crude product was purified by column chromatography over Sephadex G-25 with distilled, deionized water as the eluent. A pure sample of the desired product (0.4 g,20 %) was obtained as a yellow solid. ^{1}H NMR (300 MHz, [D$_6$]DMSO, TMS): δ = 3.0–4.0 (m, 84H, $C_{2\text{-}6}$H of CyD), 4.3–4.6 (m, 12H, O-6 H of CyD), 4.7–5.2 (m, 14H, C-1 H of CyD), 5.6–6.0 (m, 28H, O-2,3 H of CyD), 7.6–9.4 ppm (m, 10H, Ar); ^{13}C NMR (300 MHz, D_2O): δ = 62.5, 74.1, 74.4, 75.5, 83.4, 104.2, 122.4, 126.7, 132.1, 150.3, 168.4 ppm; IR (KBr): $\tilde{\nu}$ = 3342, 2930, 1725, 1693, 1641, 1593, 1550, 1426, 1330, 1302, 1237, 1196, 1153, 1078, 1030, 944, 857, 797, 777, 757, 706, 580 cm^{-1}; elemental analysis calcd (%) for $C_{104}H_{152}O_{72}N_2 \cdot 12H_2O$: C 44.63, H 6.34, N 1.00; found: C 44.63, H 6.56, N 1.04; UV/Vis (water): λ_{max} (ε) = 268.8 (48660), 342.8 nm (24520 $M^{-1}cm^{-1}$).

***N*,*N*′-Bis(2-aminoethyl)-2,2′-biquinoline-4,4′-dicarboxamide-bridged bis-β-CD (9)**:[33] Bis(β-CyD) **9** was obtained as a red solid in 35 % yield from 2,2′-biquinoline-4,4′-dicarboxylic dichloride and 6-(2-aminoethylamino)-6-deoxy-β-CD by using procedures similar to those employed in the synthesis of **8**. ^{1}H NMR (300 MHz, [D$_6$]DMSO, TMS): δ = 1.0–2.0 (m, 8H), 3.0–4.0 (m, 84H, $C_{2\text{-}6}$H of CyD), 4.4–4.7 (m, 12H, O-6 H of CyD), 4.8–5.2 (m, 14H, C-1 H of CyD), 5.4–6.2 (m, 28H, O-2,3 H of CyD), 7.6–9.4 ppm (m, 10H, Ar); ^{13}C NMR (300 MHz, D_2O): δ = 38.2, 45.5, 47.2, 50.9, 62.7, 69.7, 71.9, 74.2, 75.4, 78.4, 83.5, 85.4, 101.8, 104.2, 122.5, 126.7, 131.9, 152.0, 159.1, 170.4 ppm; IR (KBr): $\tilde{\nu}$ = 3343, 2929, 2056, 1708, 1661, 1642, 1592, 1549, 1427, 1331, 1238, 1202, 1153, 1078, 1031, 944, 850, 757, 706, 577 cm^{-1}; elemental analysis calcd (%) for $C_{108}H_{164}O_{70}N_6 \cdot 8H_2O$: C 46.22, H 6.32, N 2.99; found: C 46.35, H 6.31, N 3.10; UV/Vis (water): λ_{max} (ε) = 264.6 (37280), 339.0 nm (15480 $M^{-1}cm^{-1}$).

***N*,*N*′-Bis(5-amino-3-azapentyl)-2,2′-biquinoline-4,4′-dicarboxamide-bridged bis(6-amino-6-deoxy-β-CyD) (10)**: Bis(β-CyD) **10** was obtained as a bright red solid in 25 % yield from 2,2′-biquinoline-4,4′-dicarboxylic dichloride and mono[6-(5-amino-3-azapentylamino)-6-deoxy]-β-CyD by using procedures similar to those employed in the synthesis of **8**. ^{1}H NMR (300 MHz, [D$_6$]DMSO, TMS): δ = 2.6–4.0 (m, 100H), 4.3–4.6 (m, 12H, O-6 H of CyD), 4.8–5.1 (m, 14H, C-1 H of CyD), 5.5–6.0 (m, 28H, O-2,3 H of CyD), 7.6–9.2 ppm (m, 10H, Ar); ^{13}C NMR (300 MHz, D_2O): δ = 27.1, 32.0, 34.8, 40.8, 42.4, 44.7, 46.6, 47.6, 49.5, 50.7, 53.6, 59.8, 67.9, 74.3, 75.5, 83.5, 85.5, 104.2, 122.8, 126.6, 131.3, 141.2, 150.1, 153.4, 169.2 ppm; IR (KBr): $\tilde{\nu}$ = 3329, 2929, 2054, 1659, 1642, 1549, 1426, 1331, 1300, 1203, 1154, 1079, 1032, 944, 847, 756, 706, 578 cm^{-1}; elemental analysis calcd (%) for $C_{112}H_{170}O_{70}N_8 \cdot 16H_2O$: C 44.29, H 6.70, N 3.69; found: C 44.10, H 6.99, N 3.71; UV/Vis (water): λ_{max} (ε) = 261.0 (38900), 331.6 nm (12420 $M^{-1}cm^{-1}$).

Bis(β-CyD)–Cu^{II} complex 11: Complex **11** was synthesized by refluxing a mixture of bis(β-CyD) **8** and $Cu(ClO_4)_2 \cdot 6H_2O$ (0.55 equiv) in aqueous solution for 6 h. The complex obtained was purified by column chromatography over Sephadex G-25 with distilled, deionized water as the eluent. A pure sample of the desired product was obtained in 85 % yield. IR (KBr): $\tilde{\nu}$ = 3348, 2931, 1727, 1640, 1513, 1426, 1333, 1263, 1239, 1204, 1155, 1079, 1029, 946, 861, 798, 756, 707, 579, 529 cm^{-1}; elemental analysis calcd (%) for for $C_{104}H_{152}O_{72}N_2 \cdot 0.5Cu(ClO_4)_2 \cdot 12H_2O$: C 42.64, H 6.05, N 0.96; found: C 42.48, H 6.24, N 1.00; UV/Vis (water): λ_{max} (ε) = 272.2 (50240), 351.8 nm (24540 $M^{-1}cm^{-1}$).

Bis(β-CyD)–Cu^{II} complex 12: Bis(β-CyD) complex **12** was synthesized in 85 % yield from bis(β-CyD) **9** and $Cu(ClO_4)_2 \cdot 6H_2O$ (1.6 equiv) by using procedures similar to those employed in the synthesis of **11**. IR (KBr): $\tilde{\nu}$ = 3325, 3061, 2935, 2910, 1652, 1543, 1510, 1454, 1365, 1332, 1262, 1153, 1081, 1030, 945, 845, 757, 706, 627, 579, 525 cm^{-1}; elemental analysis calcd (%) for $C_{108}H_{164}O_{70}N_6 \cdot 1.5Cu(ClO_4)_2 \cdot 12H_2O$: C 39.16, H 5.84, N 2.54; found: C 39.75, H 5.57, N 2.83; UV/Vis (water): λ_{max} (ε) = 266.8 (39840), 348.6 nm (15360 $M^{-1}cm^{-1}$).

Bis(β-CyD)–Cu^{II} complex 13: Bis(β-CyD) complex **13** was synthesized in 77 % yield by using procedures similar to those employed in the synthesis of **11**. IR (KBr): $\tilde{\nu}$ = 3333, 3061, 2935, 2906, 1652, 1454, 1364, 1335, 1301, 1206, 1154, 1079, 1029, 945, 846, 757, 706, 625, 577, 531, 414 cm^{-1}; elemental analysis calcd (%) for $C_{112}H_{170}O_{70}N_8 \cdot 1.5Cu(ClO_4)_2 \cdot 24H_2O$: C 37.82, H 5.67, N 3.15; found: C 37.51, H 5.39, N 3.40; UV/Vis (water): λ_{max} (ε) = 264.4 (39800), 342.0 nm (12300 $M^{-1}cm^{-1}$).

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