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# Unique Fluorescence Behavior of Rhodamine B upon Inclusion Complexation with Novel Bis(β-cyclodextrin-6-yl) 2,2′-Bipyridine-4,4′-dicarboxylate

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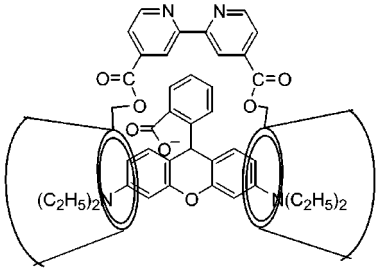
## ABSTRACT




**Newly synthesized bis(β-cyclodextrin-6-yl) 2,2′-bipyridine-4,4′-dicarboxylate was found to induce an unusual fluorescence enhancement of Rhodamine B (RhB) upon complexation. This effect is attributable to the equilibium shift of RhB to the highly fluorescent carboxylate ion form, which is induced by the cooperative binding by two appropriately preorganized cyclodextrin units in the bis(β-cyclodextrin). This sandwich complexation behavior was investigated by means of the fluorescence and 2D NMR spectroscopy.**

A variety of bridged bis(β-cyclodextrin)s have been synthesized as supramolecular hosts exhibiting higher binding abilities toward large-sized molecular guests than the parent and modified mono-β-cyclodextrins.[1−12] Enthalpically, the size/shape-fitted cooperative multiple recognition and the expanded pseudocavity created by two cyclodextrin moieties reasonably account for the enhanced binding abilities and selectivities of these cyclodextrin dimers for large-sized

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guests. Furthermore, the tethered bis(cyclodextrin)s are intrinsically advantageous in entropy through the prearranged conformation/orientation over the parent or corresponding monomeric cyclodextrins, which inevitably accompany a great loss of freedom upon intermolecular host−guest complexation. We have recently shown that organoselenium-bridged bis($\beta$-cyclodextrin)s form more stable complexes with some dyes than the native $\beta$-cyclodextrin through the cooperative binding of one guest by two cyclodextrin moieties in a single host molecule.[13] In the present communication, we wish to report unusual fluorescence behavior of Rhodamine B (RhB) upon inclusion complexation with a newly synthesized bipyridinedicarboxyl-bridged bis(6-*O*-$\beta$-cyclodextrin). Bis($\beta$-cyclodextrin-6-yl) 2,2′-bipyridine-4,4′-dicarboxylate (**5**) was synthesized by the reaction of dry $\beta$-cyclodextrin (**1**) with 2,2′-bipyridine-4,4′-dicarboxylic dichloride[14] in dry pyridine in the presence of dicyclohexylcarbodiimide. Chromatographic purification over a Sephadex G-25 column gave the pure product in 30% yield as a slightly reddish solid.[15] $\beta$-Cyclodextrin-6-yl isonicotinate (**2**) and 6,6′-trimethylene diseleno-bridged bis(6-deoxy-$\beta$-cyclodextrin)s (**4**) were prepared as reported.[13,16] 2,2′-Bipyridine-4,4′-dicarboxylic acid disodium salt (**3**) was prepared by mixing 2,2′-bipyridine-4,4′-dicarboxylic acid and solid sodium hydroxide (2 equiv) and dissolved in phosphate buffer solution (pH 7.20). The complex stability constants ($K_s$) and Gibbs free energy changes ($-\Delta G°$) for the 1:1 inclusion complexation of RhB were determined in phosphate buffer solution (pH 7.20) at 25 °C by the fluorometric titration method, using a JASCO FP-750 instrument.

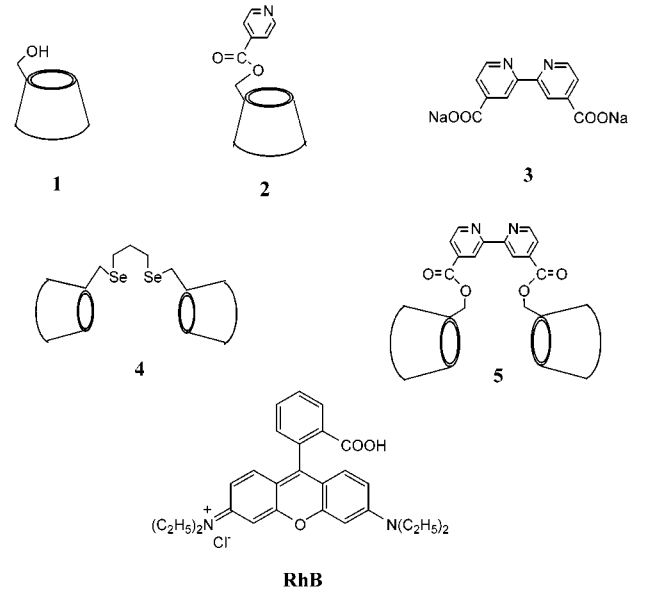


**Figure 1.** Molecular structure of host and guest.

It has been demonstrated that the fluorescence behavior of RhB upon addition of $\beta$-cyclodextrin (**1**) critically depends on the concentration of RhB, showing either fluorescence quenching or enhancement.[17] Under our experimental conditions using dilute RhB ($1.5-5.0 \times 10^{-6}$ M), the fluorescence was quenched by adding **1**, or its derivatives modified by nonaromatic amino acids, in concentrations up to $7 \times 10^{-4}$ M,[18] as exemplified for **1** in Figure 3. This phenomenon is reasonably accounted for in terms of the equilibrium shift from the hydrophilic, fluorescent carboxylate ion form of RhB to the hydrophobic, nonfluorescent lactone form upon inclusion complexation with cyclodextrin derivatives (Figure 2a). In sharp contrast, the gradual addition of **5** in concentrations up to $2 \times 10^{-4}$ M to a dilute solution of RhB ($4.9 \times 10^{-6}$ M) significantly enhanced the fluorescence intensity under comparable conditions, as illustrated in Figure 4. To elucidate the origin of this opposite fluorescence behavior of RhB observed upon inclusion complexation with **1** and **5**, the fluorometric titrations of RhB with **1**, **2**, and 2,2′-bipyridine-4,4′-dicarboxylic acid disodium salt (**3**) were performed under the same conditions. These experiments revealed that the fluorescence intensity of RhB is not appreciably affected by the addition of **2** or **3** in concentrations up to 2.0 or $7.1 \times 10^{-4}$ M but is quenched by the addition of **1** (Figure 3) in good agreement with the previous studies.[17,18]

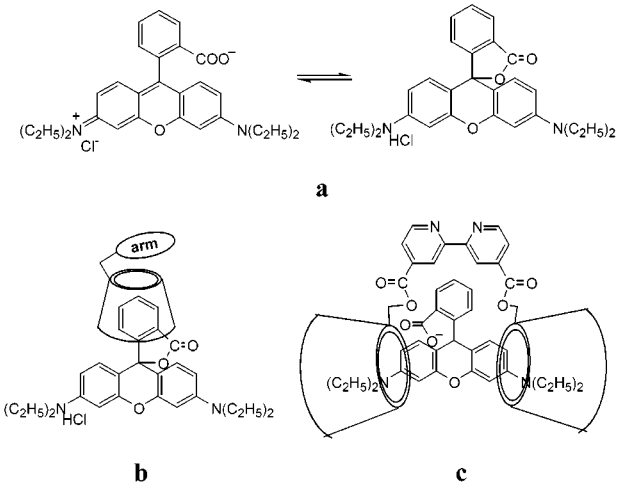


**Figure 2.** (a) Equilibrium between carboxylate ion and lactone form of RhB. (b) Inclusion complexation mode of RhB with monomeric cyclodextrin. (c) Inclusion complexation mode of RhB with **5**.

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(15) **Data for host 5:** mp ca. 180 °C (dec); FAB-MS *m/z* 2477 ($M + H^+$); $^1$H NMR ($D_2O$, TMS) $\delta$ 3.19−3.92 (m, 85 H), 5.06 (m, 14 H), 7.99 (m, 2 H), 8.55, (m, 2 H), 8.82 (m, 2 H); FT-IR (KBr) $\nu$/cm$^{-1}$ 3358.0, 2907.8, 1725.8, 1703.0, 1646.9, 1397.1, 1371.1, 1327.7, 1235.1, 1140.1, 1071.9, 1023.1, 941.1, 841.9, 747.7, 573.5, 541.3; UV−vis ($H_2O$) $\lambda_{max}$/nm ($\epsilon$/M$^{-1}$ cm$^{-1}$) 302.3 (6185). Anal. Calcd for $C_{96}H_{144}O_{72}N_2 \cdot 12H_2O$: C, 42.80; H, 6.29; N, 1.04. Found: C, 42.53; H, 6.20; N, 1.00.
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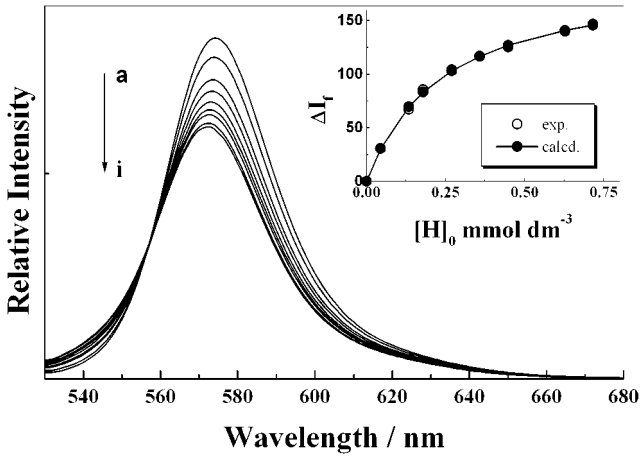


**Figure 3.** Fluorescence spectral changes of RhB ($3.6 \times 10^{-6}$ M) and the nonlinear least-squares analysis (inset) of the differential intensity ($\Delta I_f$) to calculate the complex stability constants ($K_s$) upon addition of **1** ($0-716 \times 10^{-6}$ M from a to i) in aqueous buffer solution (pH 7.20).

Such contrasting fluorescence behavior indicates that distinctly different binding modes are operative in the complexations of RhB with monocyclodextrins and with bis(cyclodextrin) **5**. Thus, the conventional 1:1 binding of RhB by monocyclodextrins prefers the more hydrophobic lactone form (Figure 2b), whereas the fluorescent carboxylate ion form, possessing a positive charge highly delocalized over the bis(diethylamino)xanthene chromophore, is much more favored by ditopic host **5** to give a sandwich-type complex (Figure 2c) with the accompanying fluorescence enhancement. On the other hand, the fluorescent group of RhB can be efficiently shielded from the water hydrogen bond, which quenches fluorescence, by the inclusion complexation with cyclodextrin cavities in **5** and the formation of the sandwich

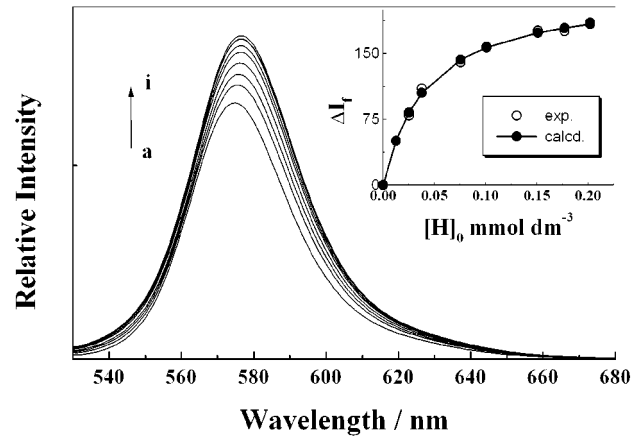


**Figure 4.** Fluorescence spectral changes of RhB ($4.9 \times 10^{-6}$ M) and the nonlinear least-squares analysis (inset) of the differnetial intensity ($\Delta I_f$) to calculate the $K_s$ value upon addition of **5** ($0-202 \times 10^{-6}$ M from a to i) in aqueous buffer solution (pH 7.20).

complex between host and guest, which will jointly contribute to the fluorescence enhancement of RhB. In this context, it is interesting and essential to examine the fluorescence behavior of RhB upon complexation with **4**, where two cyclodextrins are linked by a short trimethylenediseleno tether. However, the addition of **4** to a RhB solution did not enhance but rather quenched the fluorescence. Probably, the two cyclodextrin moieties in **4** are too closely located in space to form a stable sandwich-type complex with RhB. This idea is supported by examination of the CPK models, which indicates that the relatively rigid bipyridinedicarboxylate tether in **5** can align two cyclodextrin moieties in the right position and distance, whereas the short flexible tether in **4** does not allow such a favorable conformation.

Two-dimensional NMR spectroscopy has recently become an indispensable method in the study of interaction between cyclodextrin and guest molecules, since one can conclude that two protons are closely located in space if an NOE cross-peak is detected between the relevant protons in the NOESY or ROESY spectrum. To get further information about the geometry of the inclusion complex between **5** and RhB (Figure 2c), $^1$H NOESY experiments were performed on a Varian INOVA 600 spectrometer. As shown in Figure 5,

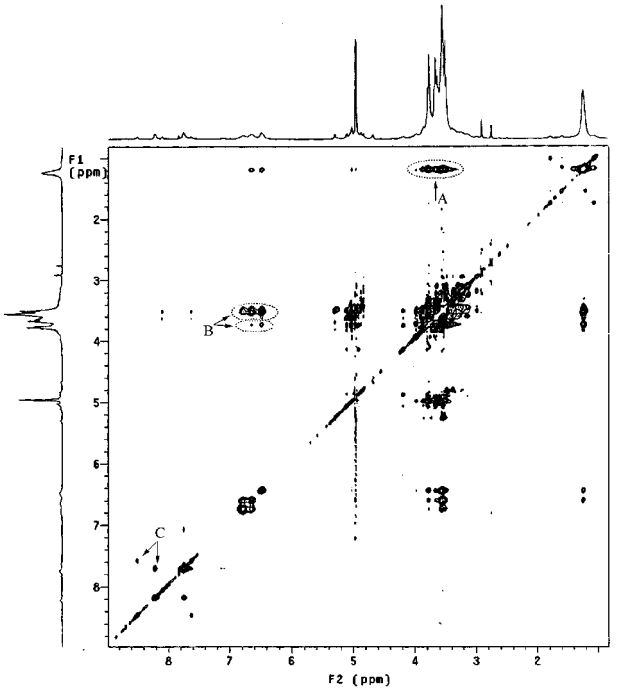


**Figure 5.** $^1$H NOESY spectrum (600 MHz) of a mixture of **5** with RhB ([**5**] = [RhB] = $5.0 \times 10^{-4}$ M) in $D_2O$ at 298 K with a mixing time of 800 ms.

the NOESY spectrum of an equimolar mixture of **5** with RhB (0.5 mM each) displays clear NOE cross-peaks between the H-3 and H-5 (comparable intensities) of cyclodextrin and the methyl protons of diethylamino groups in RhB (peaks A), as well as those between the H-3 (weak) and H-5 and the aromatic protons of diethylaminophenyl in RhB (peaks

B). These results nicely coincide with the complex structure illustrated in Figure 2c, where the diethylaminophenyl groups of RhB are accommodated in the cavities from the primary side of cyclodextrin. Furthermore, the cross-peaks C between the aromatic protons of bipyridine unit in **5** and the aromatic protons of the benzoate moiety in RhB confirm this complex structure. Hence, the results of the NOESY experiment are in excellent agreement with the proposed sandwich-type conformation and strongly support the operation of the cooperative binding mode in the complexation of RhB by bis($\beta$-cyclodextrin) **5**.

The values of complex stability constant ($K_s$) also supports this conclusion. As can be seen from Table 1, the $K_s$ value for **5** (26300 $M^{-1}$) is much larger (by a factor of >6) than those for the parent **1** (4240 $M^{-1}$) or amino acid substituted cyclodextrins (1280−2800 $M^{-1}$), while the $K_s$ for **4** (7950 $M^{-1}$) is only 1.9 times larger than that for **1**, probably as a consequence of the poor matching in distance between two cavities. These results concur with the preferential complexation of RhB by **5** through the cooperative binding by two cyclodextrins (Figure 2c), where the bipyridinedicarboxylate tether, located near the benzoate moiety of RhB (as indicated by the NOE experiment), would provide some additional interactions with the guest.

**Table 1.** Fluorescence Intensity Change, Complex Stability Constant ($K_s$) and Gibbs Free Energy Change ($-\Delta G°$) for 1:1 Inclusion Complexation of RhB with Mono- and Bis-$\beta$-cyclodextrins in Aqueous Buffer Solution (pH 7.20) at 25 °C

| host | fluorescence intensity | $K_s$ | $-\Delta G°$/kJ $mol^{-1}$ | ref |
|---|---|---|---|---|
| **1** | quench | 4240 | 20.7 | this work |
| L-Met-$\beta$-CD | quench | 2800 | 19.68 | 18 |
| L-Pro-$\beta$-CD | quench | 1579 | 18.26 | 18 |
| L-Ile-$\beta$-CD | quench | 1279 | 17.74 | 18 |
| **2** | no change | | | this work |
| **3** | no change | | | this work |
| **4** | quench | 7950 | 22.05 | this work |
| **5** | enhance | 26300 | 23.23 | this work |

Through the comparative study of the fluorescence behavior of RhB upon inclusion complexation with bis(cyclodextrin)s and the related cyclodextrin derivatives, it is clearly demonstrated that the preorganized host dimension with matching size, shape, conformation, and particularly intercavity distance is the most crucial factor for the efficient cooperative binding by bis(cyclodextrin) host. Although the present conclusion is drawn from a rather limited variation of host−guest combinations, this concept should be extended more generally to a wide variety of natural and synthetic supramolecular systems. Based on the binding model described above, further studies are currently in progress concerning the design and synthesis of $\beta$-cyclodextrin dimers with a functional tether, along with the elucidation of the detailed cooperative binding mechanism.

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