

Reduced Fluorescence Quenching of Cyclodextrin–Acetylene Dye Rotaxanes

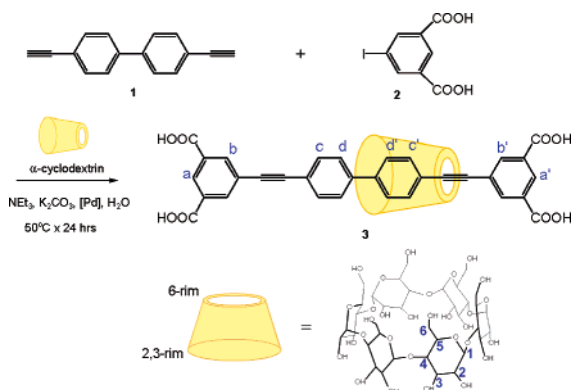
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Fluorescent dyes behave differently when trapped inside the cavity of cyclodextrins (CDs).^{1–5} When cyanine dyes, with extended conjugation, were encapsulated within CDs, their fluorescence efficiency and photostability increased.¹ Upon rotaxanation, the threaded cyanine dye also exhibited reversible redox processes, and its kinetic stability was increased.² The synthesis of fluorescent stilbene and tolane rotaxanes was reported; Suzuki coupling using a Pd catalyst in aqueous condition gave a dye rotaxane.³ PPV-based polyrotaxanes and monomeric [2]rotaxanes have been synthesized by the same method.⁴ Rotaxane encapsulation of fluorescent dyes, as in the above-mentioned examples, is a promising way of improving the stability and brightness of luminescent and electroluminescent materials in various applications, such as for organic light emitting diodes and biological probes.⁵

In an effort to access fluorescent rotaxanes, we present the synthesis of an acetylene dye rotaxane **3**, using a Pd-catalyzed reaction of the Heck–Cassar–Sonogashira–Hagihara-type.⁶ In the molecular structure, it corresponds to a monomer unit of the poly(*para*-phenyleneethynylene)s (PPEs), which are characterized by high fluorescence quantum yield and high oxidation potential due to the electron-withdrawing effect of the $\text{—C}\equiv\text{C—}$ unit. In our synthesis, 1,4-diethynylbiphenyl **1** was reacted with 5-iodo-isophthalic acid **2** in the presence of α -CD. A water-soluble palladium (Pd) salt⁷ and cuprous iodide were used as catalyst; triethylamine and potassium carbonate were added to maintain alkaline pH. After reaction, the target molecule **3** was isolated (13% yield) by removing residual starting materials and its uncomplexed congener using Soxhlet extraction.



The ROESY (Rotating Overhauser Effect Spectroscopy) spectrum of **3** is shown in Figure 1. Strong couplings are observed from the aromatic protons *d'* and *c'* of the dumbbell to protons H3 and

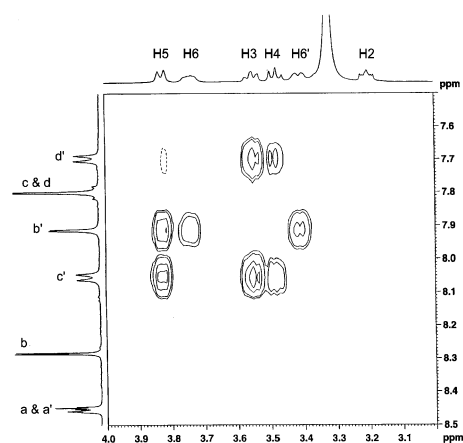


Figure 1. ¹H ROESY NMR (500 MHz) spectrum of acetylene dye rotaxane RD **3** (in DMSO-*d*₆, 298 K).

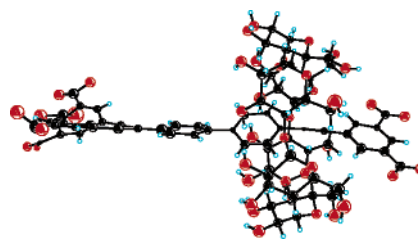


Figure 2. Side view (ORTEP) of an acetylene dye rotaxane RD **3**.

H4 of the α -CD, and from *b'* to H5 and H6, implying that the narrow 6-OH rim of α -CD is closer to the isophthalic acid blocker; the α -CD hovers over one end of the dye molecule, leaving the other end uncovered. Aromatic protons from the uncovered part show their peaks at the same positions as those of the free dye (FD).

Hydrated crystals of **3** suitable for X-ray analysis were isolated from a K_2CO_3 solution with 2 N HCl. An ORTEP (Oak Ridge Thermal Ellipsoid Plot) of **3** is shown (Figure 2). It is clear that the α -CD is displaced from the center of the threaded molecule, and the dye is almost planar, while its main axis exhibits a slight torsion.

There is no significant difference in the electronic absorption and emission spectra of free and rotaxanated dyes. However, **3** exhibits higher relative fluorescence quantum yields (for FD and **3**, 0.55 and 0.67 in methanol; 0.55 and 0.63 in aqueous pH 9.6 buffer, respectively). This is consistent with previous results that CD encapsulation reduces the quenching effect of the environment and the flexibility of the threaded chromophore, leading to an increased quantum yield.^{1,4}

To investigate the sensing ability, the fluorescence intensities of both dyes were measured in the presence of various metal ions as quenchers. The Stern–Volmer equation (eq 1) is useful in the

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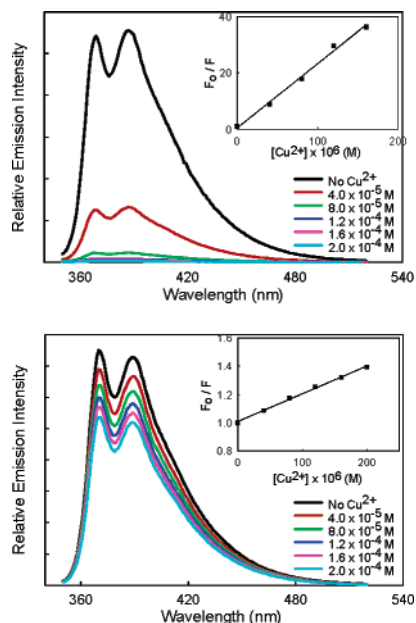


Figure 3. Fluorescence emission spectra of FD (upper) and **3** (lower) during quenching with CuSO_4 in aqueous buffer (HEPES pH 7.2 with 10 mM buffer strength). Concentrations of both dyes are 1.0×10^{-6} M. The insets show the Stern–Volmer plot. The Stern–Volmer constants, K_{SV} , were calculated to be $2.5 \times 10^5 \text{ M}^{-1}$ (for FD) and $2.0 \times 10^3 \text{ M}^{-1}$ (for RD).

Table 1. K_{SV} (M^{-1}) Values Measured with the Addition of Various Metal Ions

quenchers	FD	RD
CuSO_4	2.5×10^5	2.0×10^3
$\text{Hg}(\text{OOCCH}_3)_2$	7.8×10^3	3.7×10^3
PbCl_2	2.6×10^5	5.6×10^4
$\text{Pb}(\text{NO}_3)_2$	4.7×10^5	1.7×10^5
$\text{Mg}(\text{OOCCH}_3)_2$	NQ ^a	NQ ^a
ZnCl_2	<10	<10
methyl viologen	5.9×10^3	2.4×10^3

^a NQ: no quenching observed.

quantitative measurement of fluorescence quenching; F_0 is the initial fluorescence intensity measured without any quencher, F is the fluorescence intensity at a given concentration of the quencher $[Q]$, and K_{SV} is the Stern–Volmer constant.⁸

$$F_0/F = 1 + K_{\text{SV}}[Q] \quad (1)$$

Plotting (F_0/F) versus $[Q]$ yields K_{SV} value as the slope. The more sensitive system gives a steeper plot, leading to higher K_{SV} values. Linear Stern–Volmer plots were observed for both dyes when Cu^{2+} was used as a quencher (Figure 3). We observe that FD is effectively quenched by copper ions, and its K_{SV} was $2.5 \times 10^5 \text{ M}^{-1}$. By comparison, **3** shows a much smaller K_{SV} , $2.0 \times 10^3 \text{ M}^{-1}$, indicating that **3** is less sensitive to Cu^{2+} ions in solution than FD. Similar behavior is observed for Hg^{2+} , Pb^{2+} , and methyl viologen hydrate. The K_{SV} values for these quenchers are calculated and listed in Table 1.

The photoluminescence quenching will aid in the design of highly sensitive chemical and biological sensors.⁹ Proton dissociation ($-\text{COOH} \rightarrow -\text{COO}^- + \text{H}^+$) makes the negatively charged, fluorescent dye water-soluble and allows the binding of cationic electron acceptors, such as metal ions (M^{2+}), via electrostatic interactions. Often, monomeric fluorophores are less sensitive, giving a low K_{SV} , when compared to their corresponding conjugated polymeric form,^{8,9} which is normally able to amplify, synchronize, and play multivalency effects in its quenching ability. However,

the present FD exhibits an exceptionally high sensitivity, showing almost the same K_{SV} as that of conjugated polymers. This is attributed to the presence of the four carboxylate units in FD, implying that even monomeric acetylene dyes can exhibit a high degree of sensitivity and function in metal sensing applications. Obviously, once rotaxanated, **3** experiences much less fluorescence quenching with lower K_{SV} 's. Table 1 shows that all quenchers exhibit a higher quenching effect on the FD; **3** does show some quenching by these metals since its molecular structure is still the same as that of FD, but its degree of quenching is much smaller. The K_{SV} difference is >100 in the case of Cu^{2+} and >4 for Pb^{2+} , to which FD is highly sensitive.

There are two possible processes for fluorescence quenching, namely, static and dynamic quenching.^{8,9} From its high sensitivity, FD is thought to form aggregates with metal ions even in very dilute solutions. With the addition of quenchers, the spectral shapes of both dyes remain unchanged, even though the overall fluorescence intensity is decreased. The generally short emission lifetimes of phenyleneethynylene chromophores (see Table S3) suggest that static quenching, that is, formation of a ground state complex, is operative in the cases of FD and the rotaxane **3**. Dynamic quenching, that is, complex formation of the excited state molecule with the quencher, is improbable in our case.

In summary, an acetylene dye rotaxane with α -CD and its FD analogue have been synthesized using a Heck–Cassar–Sonogashira–Hagihara-type reaction. The free dye with four carboxylic acids is highly sensitive to various metal ions, showing high Stern–Volmer constants, K_{SV} . As CD encapsulation protects and stabilizes the threaded chromophore against outside quencher ions, metal-insensitive biological tags are an obvious application for this class of molecules.

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Supporting Information Available: Dye synthesis and experimental details for fluorescence measurements. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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