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Disrupting Aggregation of Tethered Rhodamine B Dyads through Inclusion in Cucurbit[7]uril

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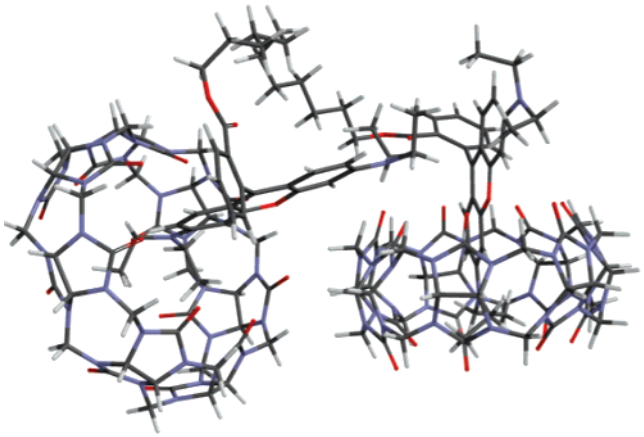

Hexano- and dodecano-tethered diesters of rhodamine B were prepared. The absorption and fluorescence spectra of these flexibly tethered dyads were compared with those of the rhodamine 3B ethyl ester. Increased J- and H-type dimer formation and decreased fluorescence emission were observed for the tethered dyads. Complexation of the cationic chromophoric units in cucurbit[7]uril (CB7) hosts decreased H-dimer aggregation, especially for the dodecano-tethered dyad. The monomeric dye and both dye dyads exhibited enhanced fluorescence upon addition of CB7.

Xanthenes such as the various rhodamines are important not only as laser dyes[1] but increasingly as components of fluorescent probes in aqueous media.[2,3] Concerns regarding the aggregation of rhodamines and the effects of aggregation on the photophysical properties of these dyes are part of larger questions regarding interactions between tethered chromophores in multichromophoric light harvesting systems[4−6] and fluorescent probes.[1,7−9] When aggregated or tethered to one another, chromophores can exhibit potentially undesirable changes in absorption and fluorescence properties due to dipole−dipole interactions and additional relaxation pathways.[10−12] Applications involving light harvesting or bright long-lived molecular probes could benefit from creating tethered packets of chromophores only if these aggregates still exhibit good photostability and low rates of quenching.[13−17] Efforts to control interactions between tethered chromophores have focused primarily on the use of rigid linking groups that hold the chromophoric units at fixed distances.[18,19] The approach described herein successfully isolates flexibly tethered rhodamine B ester dyads by binding the cationic chromophoric units in cucurbit[7]uril (CB7, shown in Figure 1) hosts resulting in enhanced fluorescence of these tethered dyads.[20]

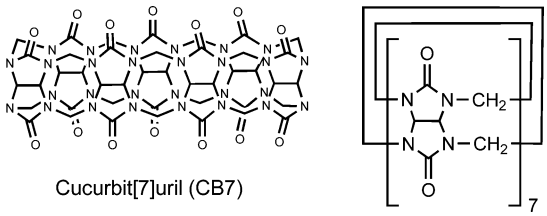

FIGURE 1. Line drawings of CB7.

Cucurbiturils are pumpkin shaped cyclic oligomers of glycoluril that have carbonyl-rimmed portals and a very nonpolar interior cavity.[21,22] Cationic dyes such as alkylviologens,[23,24]

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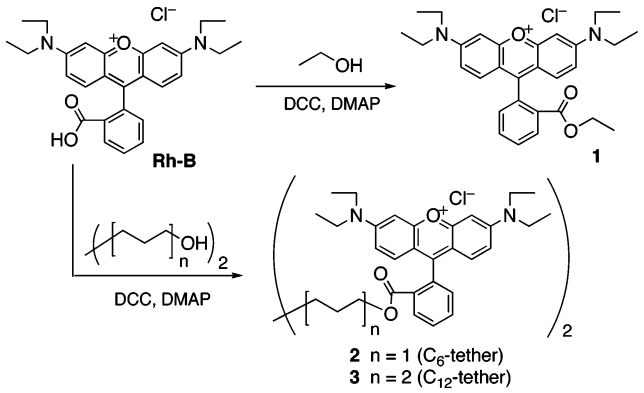
SCHEME 1. Synthesis of Rhodamine B Esters



xanthenes,[25] and brilliant green[26] not only bind tightly to CB7 in aqueous solution, but typically exhibit enhanced fluorescence and photostability due to the unique solvation properties of the cucurbiturils nonpolarizing interior.[25,27−29] We chose to study rhodamine B ester dyads since rhodamines are widely utilized chromophores whose ability to form dimeric aggregates in solution[7,30−33] and whose binding to CB7 in solution[25] and on surfaces[34] has been established.

Rhodamine 3B, the ethyl ester of rhodamine B, forms dimeric aggregates in solution at concentrations above 1 mM.[7,30] When adsorbed onto an ionic Montmorillonite surface rhodamine 3B forms nonassociated species as well as H-type and J-type aggregates depending upon experimental conditions.[35,36] Single molecule spectroscopy of tethered rhodamine dimers which have been spin-coated in a PVA matrix indicated two populations of self-associated species.[37] While these studies investigated changes in aggregation upon solvent changes or matrix isolation, data on the effects of inclusion are lacking.[38] We report here a comparison of absorbance and emission spectra of aqueous solutions of the rhodamine 3B monomer **1**, the rhodamine B hexano- and dodecano-diesters **2** and **3** and their enhanced fluorescence upon CB7 complexation.

The known ethyl ester **1** and the previously unreported hexano- and dodecano-diesters **2** and **3** were prepared through DCC-promoted esterifications as shown in Scheme 1. The esters could be purified as the salts by silica gel chromatography using initially 9:1 methanol/chloroform to elute rhodamine B, then 90:10:1 methanol/chloroform/acetic acid to elute the monoesters and finally 5:2:2:1 n-butanol/95% ethanol/water/acetic acid to elute the diesters. The solvents were removed under vacuum to provide the products as purple-red waxy solids.

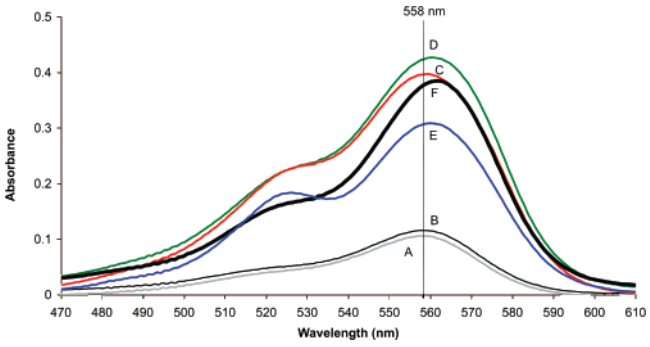


FIGURE 2. UV−vis absorption spectra: 5 μM Rhodamine B ethyl ester **1** (A, at 0 mM CB7 and B at 5 mM CB7), 2.5 μM hexano diester **2** (C at 0 mM CB7 and D at 5 mM CB7), and 2.5 μM dodecano diester **3** (E at 0 mM CB7 and F at 5 mM CB7).

The 1:1 binding of rhodamine B ethyl ester **1** by CB7 in water was established by absorption and NMR spectroscopy. An upfield shift of one set of broadened N,N-diethyl signals in the ^{1}H NMR spectrum of **1** (3.9 mM) and one equivalent of CB7 indicated a 1:1 inclusion of one end of the xanthene core into CB7 and borderline slow exchange on the NMR time scale. In the presence of two equivalents of CB7 broad averaged signals were seen for the ethyl groups which indicated borderline fast exchange at these relatively high concentrations. We speculate that the excess CB7 can promote faster exchange of the two N,N-diethyl ends of the xanthane core through an associative mechanism to form an unstable 2:1 CB7:xanthene intermediate. Formation of an initial strong 1:1 complex was also indicated by a continuous variation Job's plot constructed from absorbance measurements. A fluorescence titration curve of **1** (5 μM) with CB7 (0 μM to 12 μM) in water and subsequent nonlinear regression analysis gave a binding constant (K) of 2.8×10^6 M^{-1}. (Figures and methods provided in supplemental information).

The absorption spectra for the ethyl ester **1** and the tethered dyads **2** and **3** in the absence of and presence of excess CB7 in water are shown in Figure 2.[39] The ethyl ester at 5 μM exhibited the expected absorption spectrum of the nonaggregated monomeric form showing a λ_{max} around 558 nm and a lower energy broad monomer shoulder near 510 nm as previously described for dilute aqueous solutions of rhodamine 3B. An increase in the concentration of CB7 resulted in only a slight and uniform increase in absorbance with no indication of altered aggregation. The minimal effect of CB7 on the wavelength and extinction coefficient of a nonaggregated rhodamine dye is consistent with literature data. Rhodamines are known to form face-to-face H-dimers in which the phenyl side groups are oriented in opposite directions and thus could only be tethered in such a head-to-tail conformation by a sufficiently long tether. The short C_6-tether length was chosen to restrict the ability of dyad **2** from achieving an intramolecular face-to-face H-dimer while still enabling an oblique edge-to-face J-dimer to form.[30,31,40,41] The

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(39) A note of clarificaiton: we refer to the tethered compounds as "dyads" and to the aggregated chromophores as "dimers."

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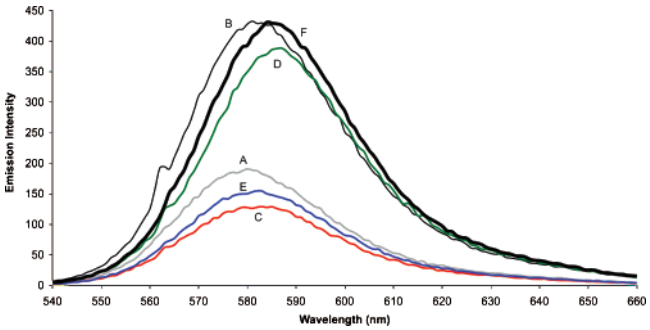

FIGURE 3. Fluorescence emission spectra: 5 μM Rhodamine B ethyl ester **1** (A, at 0 mM CB7, and B at 5 mM CB7), 2.5 μM hexano diester **2** (C at 0 mM CB7, and D at 5 mM CB7), and 2.5 μM dodecano diester **3** (E at 0 mM CB7, and F at 5 mM CB7).

longer C_{12}-tether should enable not only J-dimer formation but should also allow the two xanthane cores to adopt a face-to-face H-dimer form. The absorption spectra of high concentration, aggregated rhodamines, including rhodamine 3B show a new band at 525 nm that has been assigned to an H-type dimer as well as a much more intense J-dimer band that is shifted a few nanometers bathochromically from the 558 nm band in the nonaggregated form. The three and 4-fold increase in the absorbance at 560 nm of the dyads **2** and **3** support the presence of more strongly absorbing J-dimer aggregated forms. The presence of a pronounced band at 525 nm in the C_{12}-dyad **3** along with a decrease in the band at 560 nm supports the presence of more H-dimer and less J-dimer form than in the monoester **1**.[30] The hexano-tethered dyad **2** exhibited less H-dimer form than seen for the longer dodecano-tethered **3**. The striking decrease of this 525 nm band and the increase in the size of the band at 560 nm upon addition of CB7 to the C_{12}-dyad **3** supports a conversion of the H-dimer into the J-dimer in this C_{12}-tethered dyad.

As is typical for a rhodamine dye, the fluorescence emission for the ethyl ester increased 2.4 fold upon addition of excess CB7 (Figure 3).[27] Although the C_6- and C_{12}-dyads exhibited about a 3-fold absorbance enhancement compared to ethyl ester **1**, their fluorescence *decreased* about 1.2−1.5 fold in the absence of CB7. Upon addition of CB7 dyads **2** and **3** each exhibited a fluorescence enhancement of about 3.1 fold (Figure 4).

These results support the conclusion that we observed a J-dimer form in the C_6-dyad and a form of this type was maintained upon complexation by CB7. Notably in the C_{12}-dyad in the absence of CB7 we observed a significant amount of H-dimer but in the presence of CB7 this H-dimer portion was apparently converted to the J-dimer form. As a visualization aid locally minimized structures for this conversion were obtained and are depicted in Figure 4.[42]

In summary, we have demonstrated the ability to convert a tethered rhodamine B dyad from an H-dimer form to a J-dimer form upon addition of CB7. The strong fluorescence enhancement of the monomeric rhodamine B ethyl ester is preserved in CB7 complexes of C_6- and C_{12}-tethered rhodamine dyads.

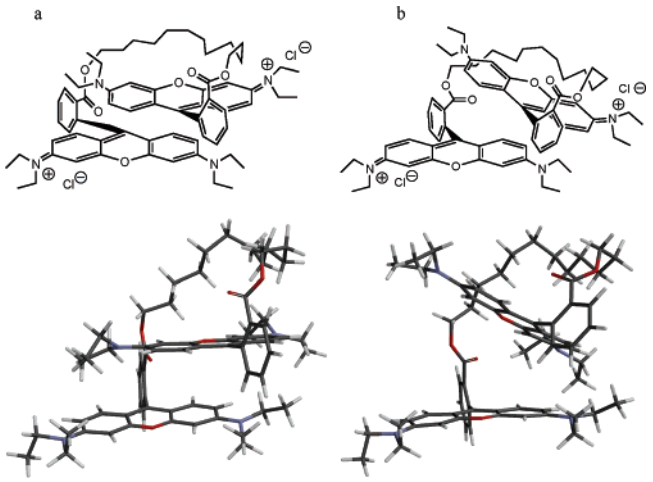

FIGURE 4. Line drawings and MM2 calculated three dimensional depictions: (a) C_{12}-Tethered **3** in a face-to-face H-dimer form. (b) C_{12}-Tethered **3** after complexation with CB7 in an oblique edge-to-face J-dimer form (CB7 omitted for clarity).

These results should facilitate the design of CB7-protected multichromophoric systems that retain desirable photophysical properties.

Experimental Section

Rhodamine B Ethyl Ester (1). A mixture of Rhodamine B (0.500 g, 1.04 mmol), methylene chloride (25 mL), 1,3-dicyclohexyl carbodiimide (0.258 g, 1.3 mmol), absolute ethanol (0.240 g, 5.2 mmol), and 4-dimethylaminopyridine (0.013 g, 0.10 mmol) was stirred under nitrogen at room temperature for 72 h. The crude dark-pink filtrate was collected via vacuum filtration and concentrated at room temperature under reduced pressure. A portion of the crude product (0.173 g) was purified via silica gel column chromatography (methanol/chloroform, 9:1), (methanol/chloroform/glacial acetic acid, 90:10:1), respectively, to give 0.094 g (0.185 mmol, 54%) of pure 1 as a purple-red waxy solid: ^{1}H NMR (300 MHz, CD_3CN): δ 8.27 (ddd, J = 0.5, 1.7, 5.4 Hz, 1H), 7.81 (m, 2H), 7.38 (ddd, J = 0.5, 1.7, 5.4 Hz, 1H), 7.07 (d, J = 9.5 Hz, 2H), 6.93 (dd, J = 2.5, 9.5 Hz, 2H), 6.84 (d, J = 2.5 Hz, 2H), 4.00 (q, J = 7.1 Hz, 2H), 3.61 (q, J = 7.1 Hz, 8H), 1.24 (t, J = 7.1 Hz, 12H), 0.96 (t, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, CD_3CN): δ 173.9, 159.3, 158.2, 156.1, 133.9, 133.3, 131.7, 131.4, 130.9, 130.7, 130.6, 114.7, 113.9, 96.3, 61.7, 46.1, 21.3, 13.4, 12.2. MS (ESI): m/z 471.33 $[M- Cl]^+$ (100%), 443.33 (25%), (M = $C_{30}H_{35}N_2O_3^+$ requires 471.26).

Rhodamine B Hexano diester (2). A mixture of Rhodamine B (2.533 g, 5.3 mmol), methylene chloride (40 mL), 1,3-dicyclohexyl carbodiimide (1.091 g, 5.3 mmol), 1,6-hexanediol (0.250 g, 2.1 mmol), and 4-dimethylaminopyridine (0.026 g, 0.21 mmol) was stirred under nitrogen at room temperature for 72 h. The crude dark-pink filtrate was collected via vacuum filtration and concentrated at room temperature under reduced pressure. A portion of the crude product (0.331 g) was purified via silica gel column chromatography (methanol/chloroform, 9:1), (methanol/chloroform/glacial acetic acid, 90:10:1) and (*n*-butanol/95% ethanol/distilled water/glacial acetic acid, 5:2:2:1), respectively, to give 0.049 g, (0.047 mmol, 15%) of **2** as a purple-red waxy solid: ^{1}H NMR (300 MHz, CD_3CN): δ 8.26 (dd, J = 1.1, 7.5 Hz, 2H), 7.81 (m, 4H), 7.37 (dd, J = 1.1, 7.5 Hz, 2H), 7.05 (d, J = 9.5 Hz, 4H), 6.89 (dd, J = 2.4, 9.5 Hz, 4H), 6.77 (d, J = 2.4 Hz, 4H), 3.87 (t, J = 6.3 Hz, 4H), 3.56 (q, J = 7.1 Hz, 16H), 1.20 (m, 32H). ^{13}C NMR (75 MHz, CD_3CN): δ 165.8, 159.1, 158.2, 156.0, 133.8, 133.4, 131.7, 131.6, 130.8, 130.7, 130.6, 114.7, 113.9, 96.3, 65.7, 46.1, 28.3, 25.6, 12.3.

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(42) Molecular mechanics minimization of provided arbitrary conformations performed using Spartan 02.

MS (ESI): *m*/*z* 484.40 $[M-2Cl]^{2+}$ (100%), 443.40 (26%), ($M = C_{62}H_{72}N_4O_6^{+}$ requires 968.55).

Rhodamine B Dodecanodiester (3). A mixture of Rhodamine B (1.480 g, 3.09 mmol), methylene chloride (40 mL), 1,3-dicyclohexyl carbodiimide (0.6373 g, 3.09 mmol), 1,12-dodecanediol (0.250 g, 1.2 mmol), and 4-dimethylaminopyridine (0.0151 g, 0.12 mmol) was stirred under nitrogen at room temperature for 72 h. The crude dark-pink filtrate was collected via vacuum filtration and concentrated at room temperature under reduced pressure. A portion of the crude product (0.2513 g) was purified via silica gel column chromatography (methanol/chloroform, 9:1), (methanol/chloroform/glacial acetic acid, 90:10:1) and (n-butanol/95% ethanol/distilled water/glacial acetic acid, 5:2:2:1), respectively, to give 0.0385 g (0.034 mmol, 15%) of 3 as a purple-red waxy solid: ^{1}H NMR (300 MHz, CD_3CN): δ 8.26 (d, $J = 7.0$ Hz, 2H), 7.81 (m, 4H), 7.37 (d, $J = 7.0$ Hz, 2H), 7.07 (d, $J = 9.3$ Hz, 4H), 6.93 (d, 9.3 Hz, 4H), 6.83 (s, 4H), 3.92 (t, $J = 6.1$ Hz, 4H), 3.60 (q, $J = 6.7$ Hz, 16H), 1.19 (m, 44H). ^{13}C NMR (75 MHz, CD_3CN): δ 165.9, 159.1, 158.2, 156.1, 133.7, 133.3, 131.7, 131.5, 130.9, 130.8, 130.7, 114.8, 113.9, 96.4, 65.9, 46.1, 29.7, 29.6, 29.374, 28.5, 26.0, 12.3. MS (ESI): *m*/*z* 627.47 $[M-2Cl]^{2+}$ (100%), 443.40 (29%) ($M = C_{68}H_{84}N_4O_6^{+}$ requires 1052.64).

Absorption Measurements. Absorption spectra were performed on a UV−vis scanning spectrophotometer using freshly prepared aqueous solutions in disposable polystyrene cuvettes of 10.0 mm path. The baseline was recorded with water in both sample and reference cuvettes. All measurements were performed at ambient temperature (ca. 20 °C).

Fluorescence Measurements. Steady-state measurements were performed on a spectrofluorophotometer, equipped with a Xenon lamp of 150 W as the excitation source using disposable polystyrene cuvettes of 10.0 mm path. The slit width was adjusted to 1.5 for all measurements. All measurements were performed at ambient temperature (ca. 20 °C).

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Supporting Information Available: Spectral data (^{1}H NMR and ^{13}C NMR) for compounds **1**−**3** and CB7. ^{1}H NMR spectra for the titration of **1** with CB7. Job's plot, absorption. and fluorescence titration spectra, titration curve, nonlinear regression. and Scatchard analysis and binding constant determination for **1**-CB7. Absorption and fluoresence titration spectra of **2**-CB7 and **3**-CB7. This information is available free of charge via the Internet at http://pubs.acs.org.

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