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Water-Soluble Oligomer Dimers Based on Paracyclophane: A New Optical Platform for Fluorescent Sensor Applications

Janice W. Hong,[†] Brent S. Gaylord,[‡] and Guillermo C. Bazan*[,†,‡]

Department of Chemistry, Department of Materials, University of California, Santa Barbara, California 93106

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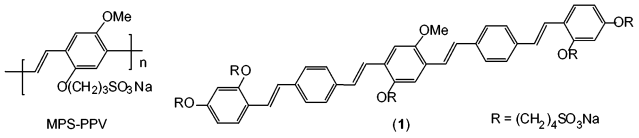
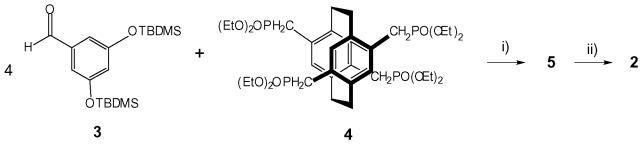
Conjugated polymers possess molecular attributes that enable their use as a highly sensitive optical platform for chemical and biological sensors.[1] Water solubility is required for biosensor applications, and this property can be achieved by the incorporation of charged functionalities along the polymer backbone.[2,3] Due to the hydrophobic conjugated framework, extensive aggregation occurs in solution.[4] Even in well-defined oligomers such as **1**, upon addition of the surfactant dodecyltrimethylammonium bromide (DTA) one observes a decrease in aggregation, resulting in decreased self-quenching and thus an increase in photoluminescence efficiency (Φ_{PL}).[5−7] More importantly, from an applications perspective, is that the quenching efficiency with acceptors such as methyl viologen (MV^{2+}) changes considerably when DTA is present.[1d,6,7] This process can be used to "report" a biological event; more efficient quenching leads to greater optical amplification and better sensitivity.[8] In the case of MPS-PPV one observes a *decrease* of 2 orders of magnitude in the Stern−Volmer quenching constant (K_{SV}).[7] For **1**, the opposite effect takes place, K_{SV} *increases* 1 order of magnitude upon DTA addition.[6] Indeed, it is possible to find conditions under which **1** is more sensitive than MPS-PPV, despite its more restricted conjugated framework.

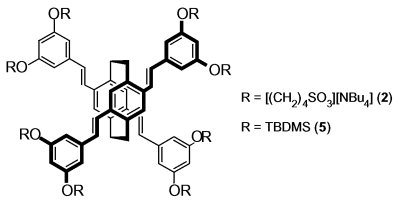


Surfactants are central to biosensor applications since they stabilize DNA/DNA and protein/protein interactions.[9] The effects of surfactants on the photophysics[10] of conjugated optical reporters thus need to be understood and controlled. It would be desirable to generate well-defined chromophores such as **1**, but with properties less sensitive to changes in aggregation. Our work on paracyclophane molecules with prebuilt interchromophore delocalization[11,12] suggested that this molecular platform could be useful for this purpose. The two chromophores are held in close proximity[13] so that they experience strong interchromophore delocalization[14] and are therefore expected to be less susceptible to electronic perturbation from adjacent molecules. Additionally, the less linear geometry is expected to disfavor close, efficient packing. In this communication we show that these simple design parameters are correct. Molecule **2** was synthesized, it shows less aggregation than the linear counterpart **1**, and its optical properties are nearly insensitive to the presence of DTA. These properties open the way to biosensor assays based on paracyclophane chromophores.

Scheme 1 [a]



[a] i) KOtBu/toluene; ii)TBAT, butane sultone/THF.



Solubility considerations of the intermediate products are important in devising a synthetic sequence. Scheme 1 shows our approach. The Horner−Emmons coupling of **3** with **4**[15] gave **5** in 53% yield. A novel method of generating the sulfoxide groups in a one-pot reaction involved cleaving the Si−O bond of the *tert*-butyldimethylsilyl (TBDMS) group with tetrabutylammonium triphenyldifluorosilicate (TBAT), followed by in situ treatment with excess butane sultone. Extraction with water gives **2** in 25% yield.[16] Resonance signals in the ^{1}H NMR spectrum of **2** in D_2O are broad at temperatures ranging from 25 to 96 °C. Purity was determined by HPLC, and the mass was confirmed by electrospray MS.

Light-scattering experiments[17] over a concentration range of 1.9×10^{-5} M to 4.5×10^{-4} M gave a molecular weight of 1.35×10^5 g/mol and a radius of gyration of ∼31 nm. Therefore, 30−40 molecules of **2** form each aggregate. For comparison, aqueous solutions of **1** show aggregates containing approximately 1000 molecules under similar conditions. Our thought is that the increased charge-to-molecule ratio, along with a nonlinear molecular shape and orientation of the charged groups in **2**, is responsible for shifting the equilibrium toward smaller aggregate units.

The fluorescence lifetimes (τ_f) of **2** in water and of **5**, the organic soluble analogue of **2**, in toluene were determined to be 12.5 ns and 2 ns, respectively. Previous studies[11] have shown that molecules such as **2** have two low-lying excited states with different properties. One is largely localized on the paracyclophane core and has a vanishing oscillator strength (the paracyclophane state). The second transition involves the "through-bond" conjugated fragment that is fully allowed and relates more closely to the symmetry of the individual chromophore (the chromophore state). The relative

* Corresponding author. Email: bazan @chem.ucsb.edu.
[†] Department of Chemistry.
[‡] Department of Materials.

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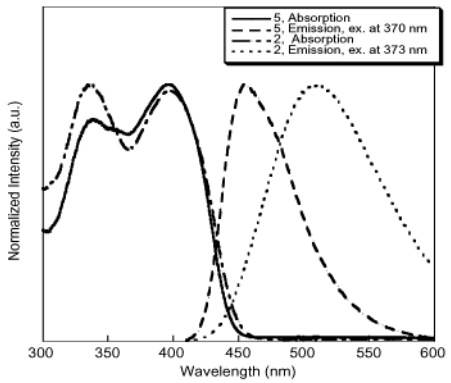

Figure 1. Absorption and emission spectra ($\sim 1.6 \times 10^{-6}$ M) of **2** (in water) and **5** (in toluene).

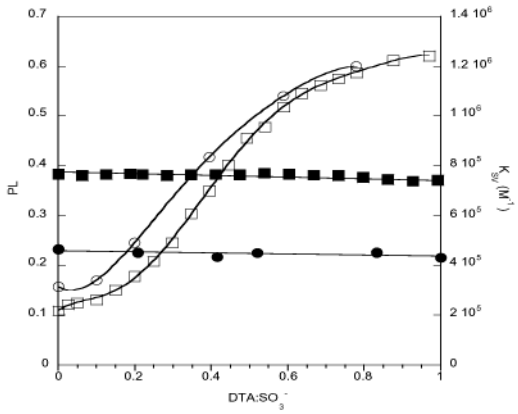

Figure 2. The effect of DTA concentration on: (a) the normalized integrated fluorescence intensity of **1** (□) and **2** (■) as a function of the DTA:SO_3^- ratio ($\lambda_{exc} = 400$ nm); (b) K_{SV} for **1** (○) and **2** (●) quenched by MV^{2+} as a function of DTA:SO_3^- ratio.

energies of these two states vary and depend on the orientation of the "chromophores" and their conjugation length. The longer lifetime in water indicates that the higher dielectric medium lowers the energy of the paracyclophane state and provides indirect evidence that the emissive state is dominated by the through-space delocalization of the two fragments.

At a concentration of 4.00×10^{-6} M, the absorption and emission maxima of **2** are at 399 and 511 nm, respectively. Figure 1 shows these spectra, along with the absorption and emission of **5**. The absorption spectra are nearly identical; however, the emission of **2** ($\lambda_{max} = 511$ nm) is considerably red-shifted relative to that of **5** ($\lambda_{max} = 455$ nm) and corresponds to the paracyclophane state. Calculated Φ_{PL} values[18] were 38% for **2** in water and 52% for **5** in toluene.

Fluorescence quenching of **2** ([**2**] $= 4.0 \times 10^{-6}$ M) with MV^{2+} gives a K_{SV} of 4.8×10^5 M^{-1}. Assuming a dynamic quenching mechanism, the quenching rate constant can be estimated to be $k_q = K_{SV}/\tau_f \approx 3.9 \times 10^{13} M^{-1}s^{-1}$, which is 3 orders of magnitude higher than for diffusion control.[1c] Static quenching via ion pairing between MV^{2+} and **2** dominates under dilute conditions.[19]

*Figure 2 shows that surfactant addition does not affect the Φ_{PL} of **2** or the quenching ability of MV^{2+}.*[20] At DTA:SO_3^- ratios of up to 1:1 for **2**, K_{SV} decreased by less than 8%, and Φ_{PL} decreased by less than 4%. For comparison we also include the influence of DTA on the properties of **1**. Note how for **1**, K_{SV} changes by an order of magnitude and Φ_{PL} increases nearly 7-fold under similar DTA:SO_3^- ratios. MPS-PPV experiences more dramatic changes at a ratio of 1:10 DTA:SO_3^- with a decrease in K_{SV} by 2 orders of magnitude and a 20-fold increase in Φ_{PL}.[7] Compound **2** is thus a stable optical reporter.

In summary, the TBAT deprotection/butane sultone ring-opening sequence is a new method for synthesizing water-soluble conjugated materials. Light-scattering experiments show that in the case of **2** one observes a decreased tendency toward aggregation, relative to **1**, probably as a result of increased charge per molecule and the nonlinear shape of the molecule. Within these aggregates *there is negligible perturbation of fluorescence efficiency and quenching with MV^{2+} by surfactants*. We attribute these effects to strong intramolecular delocalization, which eliminates perturbations by adjacent chromophores. Current efforts are geared toward the use of molecules such as **2** in fluorescent sensor applications.

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Supporting Information Available: Complete details for the synthesis, spectroscopy, and quenching studies of **2** (PDF). The material is available free of charge via the Internet at http://pubs.acs.org.

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(16) It is significant that the chromophore-building steps in Scheme 1 do not rely on a C−C bonding reaction in water. These tend to be problematic in circumstances were multiple attachments are required. See ref 6.

(17) Using the Wyatt EOS MALS software package.

(18) Relative to 9,10-diphenylanthracene. See: Maciejewski, A.; Steer, R. D. *J. Photochem.* **1986**, *35*, 59.

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(20) We have shown that the different counterions found in **1** and **2** (Na^+ vs NBu_4^+) do not affect these results. Quenching and surfactant studies were carried out with solutions containing an appropriate salt (i.e., studies of **1** with an equimolar amount of NBu_4Br and studies of **2** with an equimolar amount of NaBr), with results similar to those reported in Figure 2. See the Supporting Information.

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