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Thiosquaraine Rotaxanes: Synthesis, Dynamic Structure, and Oxygen Photosensitization

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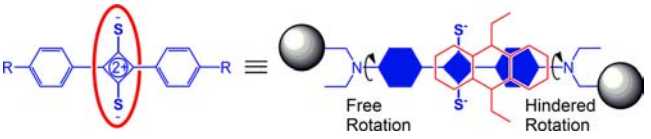
ABSTRACT



Thiosquaraine dyes have sulfur atoms instead of oxygens on the central squaraine core, and they are powerful singlet oxygen photosensitizers. Stability studies show that they are susceptible to attack by nucleophiles. This problem was circumvented by preparing a mechanically interlocked thiosquaraine rotaxane. NMR studies of the rotaxane indicate an unusual dynamic molecular structure due to a nonsymmetrical coconformation. Upon irradiation with red light, the thiosquaraine rotaxane generates the same amount of singlet oxygen as the known photosensitizer methylene blue.

Squaraine dyes have narrow and intense absorption/emission bands with deep-red or near-infrared wavelengths and high fluorescence quantum yields. Since their introduction in the 1960s, they have been investigated intensely for various imaging and photonic applications.[1] Most squaraine dyes exhibit low triplet state quantum yields, and thus they are poor oxygen photosensitizers.[2] Efforts to increase intersystem crossing efficiency, by modifying the chromophore or attaching heavy atoms to the structure, have produced promising deep-red photosensitizers for photodynamic therapy (PDT) of cancer, and ongoing studies have demonstrated phototoxicity in cell culture[3] and small animal models.[4] Very recently, a new design strategy was described using thiosquaraine dyes that have sulfur atoms instead of the oxygens on the central squaraine core.[5] This substitution leads to an inversion of the lowest energy singlet state from a $\pi-\pi^*$ transition to an $n-\pi^*$ transition. As a result, thiosquaraine dyes exhibit enhanced rates of intersystem crossing with near-unity triplet state quantum yields and excellent abilities to photogenerate singlet molecular oxygen ($^1O_2$).

In addition to efficient $^1O_2$ generation, a practically useful photosensitizer for PDT must also be endowed with other favorable molecular properties such as high chemical stability, good solubility, and synthetic flexibility.[2] A known stability limitation with certain classes of squaraine dyes is susceptibility to chemical attack by protic solvents and biological nucleophiles.[6] Here, we report that this is

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also a significant problem with thiosquaraine dyes. However, we find that this potential drawback can be circumvented by encapsulating the thiosquaraine dye inside a protective macrocycle to form a mechanically interlocked [2]rotaxane.[7] Specifically, we report the preparation of thiosquaraine rotaxane **1** by a synthetic method that permanently trapped dumbbell-shaped thiosquaraine **3** inside the tetralactam macrocycle **2** (Figure 1). We describe the dynamic molecular structure of rotaxane **1**, and we also demonstrate that it exhibits improved chemical stability and impressive oxygen photosensitization ability.

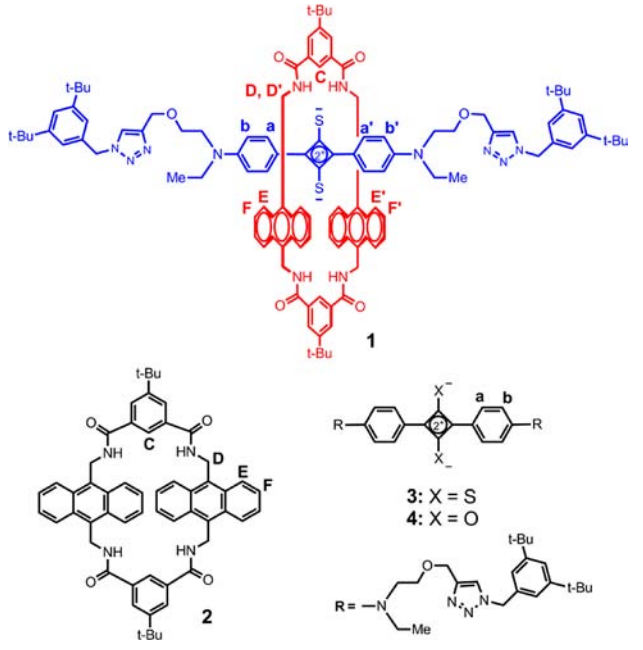

Figure 1. Chemical structures with atom labeling.

The straightforward synthesis of rotaxane **1** from readily available building blocks is described in the Supporting Information. The rotaxane-forming reaction was a "clicked capping" procedure that self-assembled a thiosquaraine bis(alkyne) precursor inside macrocycle **2** and covalently capped each end of the dye with a bulky stopper group using a copper-catalyzed azide/alkyne cycloaddition reaction.[8] Samples of the free thiosquaraine thread component **3** and its oxygen-containing squaraine analogue **4** were also prepared as control dyes for comparative studies.

The molecular structure of **1** was confirmed by mass spectrometry and NMR spectroscopy. In Figure 2 are comparisons of ^{1}H NMR spectra for rotaxane **1** and the free components (macrocycle **2** and thiosquaraine **3**) at 23 °C. A distinctive feature of the spectrum for rotaxane **1** is loss of lateral planar symmetry for both rotaxane components, a spectral property not exhibited by analogous rotaxanes with oxygen-containing squaraines.[9] More specifically, the lateral protons within rotaxane **1** are split into pairs with the partner signals having equal intensity but substantial differences in chemical shifts and line widths.

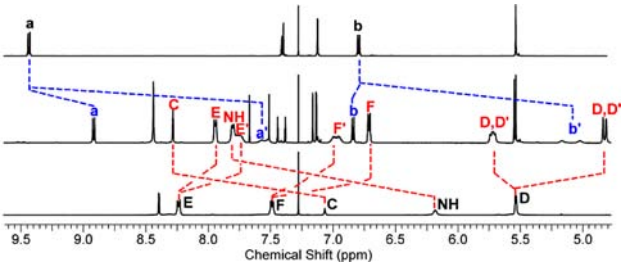

Figure 2. Partial ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) spectra of thiosquaraine **3** (top), rotaxane **1** (middle), and macrocycle **2** (bottom). See Figure 1 for atom labeling and color correlation.

The spectrum for rotaxane **1** is rationalized by the dynamic molecular structure in Figure 3. There are two notable coconformational features. The first is due to the larger atomic radius of sulfur (1.8 Å) compared to oxygen (1.5 Å), which prevents complete symmetrical encapsulation of the central thiosquaraine core by the surrounding macrocycle. Instead, the tetralactam macrocyle adopts a boat-like conformation that allows it to form hydrogen bonds with one side of the thiosquaraine core.[7,10,11] Consistent with this offset coconformation is the chemical shift of 8.25 ppm for rotaxane macrocycle proton *C* which is 1.25 ppm upfield of the chemical shift exhibited by the analogous rotaxane having an oxygen-containing squaraine.[9] In the case of **1**, the offset coconformation moves proton *C* away from the anisotropic deshielding zone of the thiosquaraine core.

The second coconformational feature that is illustrated in Figure 3 is a difference in activation energies for rotation of the two C−N bonds at either end of the thiosquaraine dye. The C−N bond that is located inside the surrounding macrocycle is sterically restricted due to close packing against the anthracene side walls of the surrounding macrocycle and thus has a higher rotational barrier.

At room temperature, the NMR signals for rotaxane protons that are proximal to the site of hindered C−N bond rotation are relatively broad. For example, a

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(11) Even at high temperature, macrocycle translocation to the other side of the encapsulated dye is slow on the NMR time scale. As discussed in ref 10, the same translocation motion with oxygen containing squaraine rotaxanes is much faster.

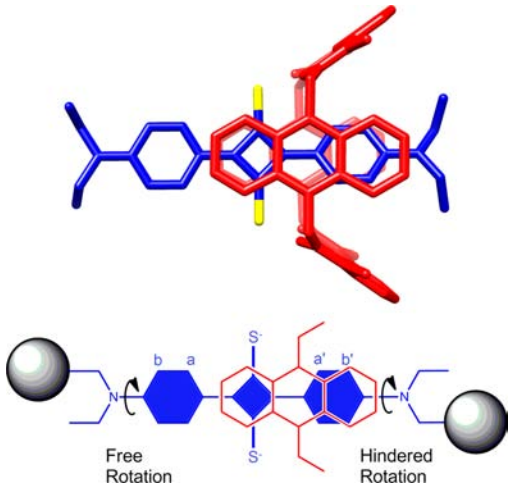

Figure 3. (Top) Calculated structure (minimized in Gaussian09, DFT, B3LPY/6-31G* basis set) of a mimic of **1** showing the surrounding macrocycle in a boat conformation. The structural mimic lacks peripheral groups on the macrocycle and dye thread, which were deleted to expedite the calculation. (Bottom) Schematic picture of **1** showing that rotation of the thiosquaraine C−N bond located inside the surrounding macrocycle is sterically restricted.

comparison of rotaxane thiosquaraine protons *a* and *b* with partners *a*′ and *b*′ reveals that the latter are strongly shielded and broadened by the surrounding macrocycle (Figure 2). The spectra in Figure 4 further illustrate this difference in dynamic NMR behavior. Squaraine C−N bonds are known to exhibit double bond character that hinders bond rotation.[12] A low temperature ^{1}H NMR study of **1** provided a value of $\Delta G^{\ddagger} = 15.0 \pm 0.2$ kcal/mol for rotation of the thiosquaraine C−N bond inside the surrounding macrocycle (see Supporting Information, Table S2), and a value of $\Delta G^{\ddagger} \approx 12$ kcal/mol was estimated for unencumbered C−N bond rotation at the other end of the thiosquaraine (see Supporting Information, Figure S6). A similar value was observed for the free dye, **3**. Thus, the off-center coconformation for thiosquaraine rotaxane **1** induces a substantial 3 kcal/mol difference in rotational barrier for the two C−N bonds at either end of the encapsulated thiosquaraine dye.

The photophysical and stability properties of thiosquaraine rotaxane **1** also support an offset coconformation. Previously, we have shown that complete encapsulation of oxygen-containing squaraine dyes inside macrocycle **2** produces about a 30 nm red shift in squaraine absorption.[9] In contrast, we find here that the absorption maxima for rotaxane **1** is 676 nm and only 4 nm longer than that of free thiosquaraine dye **3** (λ_{abs} 672 nm), consistent with a picture of partial dye encapsulation by the rotaxane macrocycle. As expected, the free thiosquaraine dye **3** is virtually nonfluorescent, but rotaxane **1** has a small but measurable

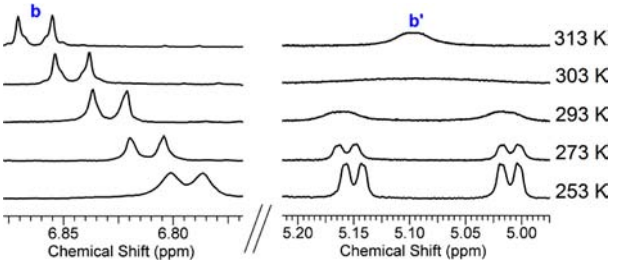

Figure 4. Partial ^{1}H NMR (600 MHz, $CDCl_3$) spectra of **1** at various temperatures. Unlike the signal for proton *b*, the signal for proton *b*′ splits into two doublets at low temperature.

fluorescence quantum yield of 0.03 (ex: 676 nm). Furthermore, excitation of the anthracene band (365 nm) in **1** leads to energy transfer and emission from the encapsulated thiosquaraine at 697 nm (see Supporting Information, Figure S2). To assess the chemical stability of these dyes, separate solutions of rotaxane **1** and free dye **3** in methanol were monitored by absorption spectroscopy. Nucleophilic solvents such as methanol are known to attack the electrophilic core of squaraine dyes and bleach the color. As reflected by the initial slopes in Figure 5, the degradation rate for **3** was four times faster than the rate for **1**. This result is consistent with partial encapsulation and moderate steric protection of the thiosquaraine dye inside rotaxane **1**.

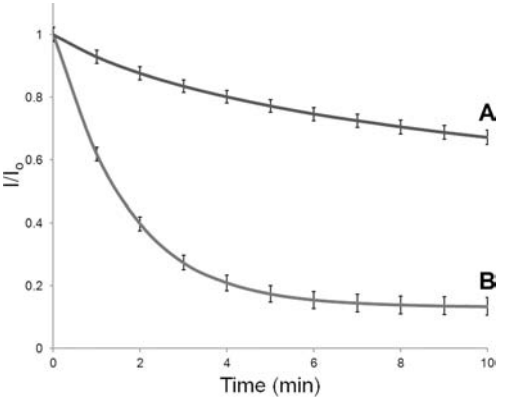

Figure 5. Stability of (A) rotaxane **1**; (B) thiosquaraine dye **3** in methanol (5.0 μM, 298 K) and in the dark.

The oxygen photosensitization ability of these dyes was evaluated by conducting well-established chemical trapping experiments using 1,3-diphenylisobenzofuran (DPBF) as a trap for highly reactive $^1O_2$.[13] The experiments directly compared thiosquaraine rotaxane **1**, thiosquaraine dye **3**, oxygen-containing squaraine dye **4**, and the well-known photosensitizer methylene blue (**MB**). In each case, a

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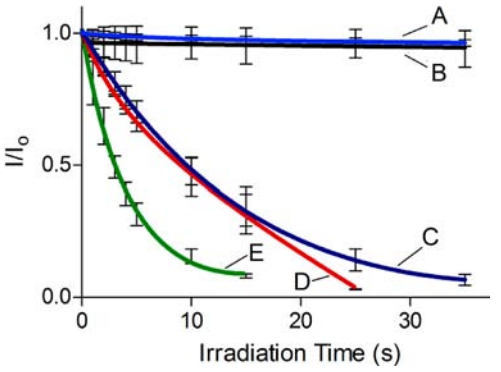

Figure 6. Rate of $^1O_2$ production as measured by the decrease in DPBF absorbance in $CHCl_3$ at 415 nm upon irradiation with red light. Samples also contained: (A) no dye; (B) **4**; (C) **MB**; (D) **1**; (E) **3**.

chloroform solution of the dye (5.0 μM) and excess DPBF (100 μM) was irradiated with red light and monitored periodically by absorption spectroscopy. The photogenerated $^1O_2$ reacts rapidly with the DPBF to produce a colorless product. The plots in Figure 6 show the decrease in DPBF absorption intensity at 415 nm and correspond to the rate of $^1O_2$ production. As expected, the oxygen-containing squaraine dye **4** exhibited essentially no ability to generate $^1O_2$, whereas thiosquaraine dye **3** was a powerful oxygen photosensitizer. The singlet oxygen quantum yield of **MB** is reported to be 0.57,[14] and analysis of the comparative data in Figure 6 gives values of 1.0 (**3**), 0.63 (**1**), and 0.03 (**4**). As shown in the Supporting Information (Figure S4), the **MB** absorption band remained unchanged throughout the experiment, while the absorption bands for **3** and **1** showed slow time-dependent decreases due to gradual reaction with the photogenerated $^1O_2$.[3a,15]

One of the main limitations with current PDT sensitizers is their tendency to remain photoactive for long periods of time after administration.[2,16] Our results indicate that thiosquaraine rotaxanes are potentially a flexible synthetic platform for fine-tuning chemical stability and creating powerful deep-red photosensitizers that survive physiological conditions long enough to reach a desired biomedical target but subsequently degrade quickly after PDT.

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Supporting Information Available. Synthesis, spectral data, and oxygen trapping experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.