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Protecting a Squaraine near-IR Dye through Its Incorporation in a Slippage-Derived [2]Rotaxane

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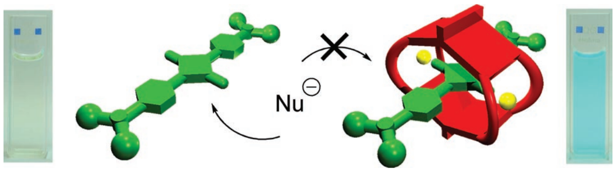
ABSTRACT



The photophysical properties and chemical stability of a squaraine derivative were enhanced after using Na$^+$ ions to template a high-yield slippage synthesis of a [2]rotaxane from the dye and a molecular cage.

Because of their potential use in biomedicine and in materials science, the search continues for new fluorescent near-IR dyes exhibiting narrow absorption, high quantum yield, and enhanced photo- and chemical stability.[1] Although squaraines are well-established near-IR dyes, their applications are limited by their inherent high electrophilicity, which disrupts their conjugated molecular structures and damages their favorable optical characteristics.[2] To solve this problem, the concept of encapsulating squaraine dyes has been investigated.[3] For example, a rotaxane synthesized using the clipping approach[4] in which a dumbbell-shaped squaraine moiety was used to template the formation of a Leigh-type amide-containing macrocycle—displayed improved chemical resistance of the squaraine dye.[5] Unfortunately, this clipping reaction afforded only moderate yields (ca. 28−35%) of the [2]rotaxane, possibly because of the unavoidable presence of nucleophiles that react with the chemically unstable squaraine-derived dumbbell during the reaction process. Intuitively, use of the slippage approach[6] toward rotaxane synthesis would minimize the squaraine dye's contact with nucleophiles during the rotaxane formation process. Previously, we reported that Na$^+$ ions could be used to template the formation of a [2]pseudorotaxane from a squaraine dye and a molecular cage in CH$_3$CN.[7] Herein, we report high-

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yield slippage syntheses of corresponding [2]rotaxanes from this unique Na^+ ion-templated recognition system and the photophysical properties and enhanced chemical stability of the encircled squaraine dye.

Previously, we reported that the molecular cage **1** and the threadlike squaraine derivative **2** formed pseudorotaxane complexes in the presence of templating Na^+ ions (Figure 1).[7] We sought suitable terminal groups for the dumbbell-shaped squaraine in the slippage reaction through a discovery process involving increasing the length of the *N*-alkyl side chains sequentially, i.e., taking advantage of the gradual increase in the energy barrier for the threading of the squaraine dye through the Na^+-ion-complexed molecular cage upon increasing the size and flexibility of the alkyl chains. Thus, we prepared the threadlike squaraine derivatives **3** and **4**.[8] Initially, the ^{1}H NMR spectrum (Figure 2) of a mixture of macrocycle **1**, squaraine **3**, and NaClO_4 (2:2:4 mM) in $\mathrm{CDCl}_3/\mathrm{CD}_3\mathrm{CN}$ (1:1) exhibited signals of free **3** and the Na^+-complexed macrocycle **1**; we detected a new set of signals after the solution had been heated at 313 K for 16 h.

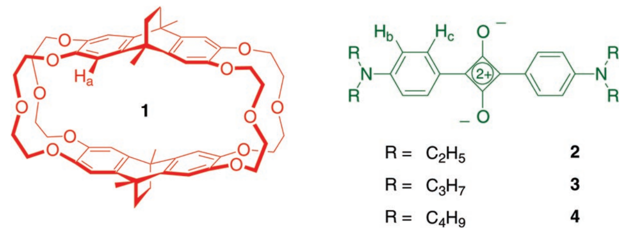


Figure 1.

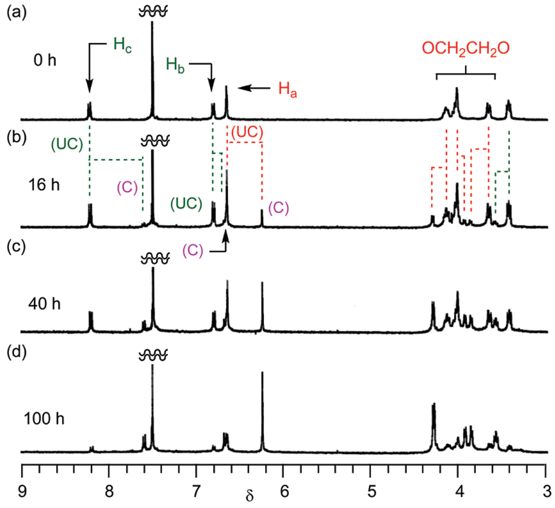
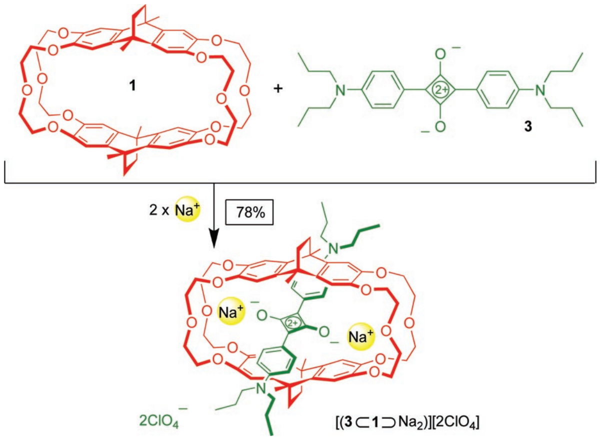


Figure 2. Partial ^{1}H NMR spectra [400 MHz, $\mathrm{CDCl}_3/\mathrm{CD}_3\mathrm{CN}$ (1:1), 313 K] indicating the extent of formation of the [2]rotaxane $[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$ from a mixture of the molecular cage **1**, squaraine dye **3**, and NaClO_4 (2:2:4 mM) at time intervals of (a) 0, (b) 16, (c) 40, and (d) 100 h.

The intensity of these new signals increased with time, at the expense of those of free **3** and the Na^+-complexed macrocycle **1**, and became predominant after 100 h. The similar appearance of this new set of signals with respect to those in the ^{1}H NMR spectra[7] of the pseudorotaxane $[\mathbf{2}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$ suggested that the product was the desired slippage rotaxane $[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$ (Scheme 1).

Scheme 1. Using Slippage To Form the [2]Rotaxane $[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$



Thus, we repeated this slippage experiment on a larger scale; after allowing the mixture to equilibrate for 7 days, we isolated (column chromatography) the corresponding [2]-rotaxane $[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$ in 78% yield.

We grew single crystals suitable for X-ray crystallography through liquid diffusion of isopropyl ether into a $\mathrm{CH}_3\mathrm{CN}$ solution of the [2]rotaxane $[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$.[9] The solid-state structure (Figure 3) confirms the expected rotaxane molecular geometry of $[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2]^{2+}$ with the squaraine unit of the dumbbell component located within the molecular cage; the complex was stabilized through interactions of the macrocycle's two [18]crown-6-like units with the two Na^+ ions, which were simultaneously bound to the two oxygen atoms of the squaraine unit.[10]

Because the ^{1}H NMR spectrum of a mixture of the macrocycle **1**, squaraine **4**, and NaClO_4 (2:2:4 mM) in $\mathrm{CDCl}_3/\mathrm{CD}_3\mathrm{CN}$ (1:1) did not exhibit any signals for a

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(9) Crystal data for $[(\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2)\cdot 4\mathrm{H}_2\mathrm{O}][2\mathrm{ClO}_4]$: $[\mathrm{C}_{80}\mathrm{H}_{104}\mathrm{O}_{18}\mathrm{N}_2\mathrm{Na}_2]$-$[\mathrm{ClO}_4]_2$, $M_r = 1626.53$, tetragonal, space group $I41/a$, $a = 34.7389(5)$ Å, $b = 34.7389(5)$ Å, $c = 14.3489(2)$ Å, $V = 17316.1(4)$ Å^3, $\rho_{\mathrm{calcd}} = 1.248$ g cm^{-3}, $\mu(\mathrm{Mo\ K}\alpha) = 0.160$ mm^{-1}, $T = 295(2)$ K, orange prisms; 9841 independent measured reflections, F^2 refinement, $R_1 = 0.0838$, $wR_2 = 0.2386$.

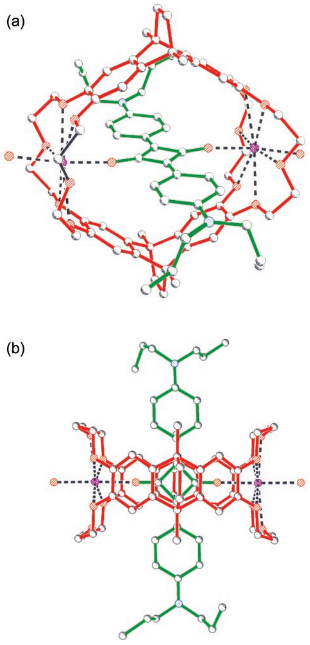

Figure 3. Ball-and-stick representations of the solid-state structure of the [2]rotaxane $[(\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2)\cdot 2\mathrm{H}_2\mathrm{O}]^{2+}$. Atom labels: C, gray; O, orange; N, blue; Na, pink. Noncoordinated H_2O molecules and anions have been omitted for clarity.

complex formed between these components, even after heating at 313 K for 190 h, it appears that *N*,*N*′-di-*n*-butylaniline termini are sufficiently sterically bulky to prevent threading of the squaraine derivative through the cavity of the Na^+-complexed molecular cage.

Table 1. Absorption and Emission Properties of the [2]Rotaxane $[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$ and Its Free Dumbbell-Shaped Component **3** in CH_3CN

compd	λ_{abs}	log ϵ	$\lambda_{\mathrm{em}}{}^{a}$	$\Phi_{\mathrm{f}}{}^{b}$
$[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$	634	5.04	643	0.38
3	640	5.57	656	0.13

[a] Solutions of $[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$ and **3** were excited at 634 and 640 nm, respectively. [b] Fluorescence quantum yields were determined using the squaraine dye **4** as the standard ($\Phi_{\mathrm{f}} = 0.15$ in CH_3CN; see ref 8b); error ±10%.

Table 1 lists the photophysical properties of the squaraine derivative **3** and the [2]rotaxane $[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$ in CH_3CN. Although the absorption and emission maxima of the [2]rotaxane both shifted to slightly shorter wavelengths relative to those of **3**, the quantum yield increased substantially, presumably because the relatively nonpolar molecular cage partially protected the squaraine dye from solvation by polar CH_3CN molecules, reducing the degree of nonradiative relaxation of the squaraine. This hypothesis is supported by our observation that increasing the amount of CH_3CN in a 1.8 μM solution of [2]rotaxane $[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$ in $CHCl_3$/CH_3CN decreased the emission intensity substantially (see the Supporting Information).

Because mercapto groups are good nucleophiles toward squaraine derivatives,[2,5] we investigated the additional chemical stability imparted upon the squaraine by the encircling molecular cage by treating both the free squaraine derivative **3** and its corresponding [2]rotaxane $[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$ with a large excess of octanethiol in CH_3CN. Figure 4 indicates that when a 1.5 μM solution of free **3** in CH_3CN was mixed with octanethiol (6 mM), both the emission and absorption intensities of the mixture decreased rapidly; in contrast, a mixture of the [2]rotaxane $[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$ and octanethiol was much more stable under the same conditions: the optical signals retained their high intensities after 5 min. This result suggests that the macrocyclic component of the [2]rotaxane $[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$ provided a steric shield that protected the squaraine dye from physical interaction with nucleophilic species.

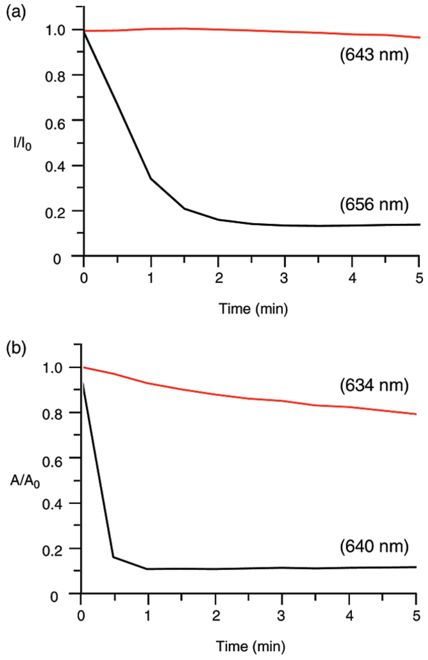

Figure 4. Time profiles of the (a) emission and (b) absorption intensities after the addition of octanethiol (6 mM) to 1.5 μM solutions of the threadlike squaraine derivative **3** (black line) and the [2]rotaxane $[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$ (red line) in CH_3CN at 298 K.

We suspected that the stability of the slippage [2]rotaxane $[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$ could be increased through enhancing the complexation of Na^+ ions to the [18]crown-6-like moieties of the molecular cage; thus, we added various amounts of $NaClO_4$ to a solution of $[\mathbf{3}\subset\mathbf{1}\supset\mathrm{Na}_2][2\mathrm{ClO}_4]$ (1.5 μM) and octanethiol (6 mM). When the concentration of Na^+ ions was increased to 7.5 μM, the corresponding decrease

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[10] A molecular cage possessing more sizable [24]crown-8-like units can complex bidentate ligands through the assistance of K^+ ions; see: Lin, C.-F.; Liu, Y.-H.; Lai, C.-C.; Peng, S.-M.; Chiu, S.-H. *Angew. Chem., Int. Ed.* **2006**, *45*, 3176−3181.

in intensity of the absorption band at 634 nm after 5 min was less than 2%; i.e., the presence of an excess of Na$^+$ ions stabilized the squaraine dye (Figure 5).[11] When more polar water molecules were added as a cosolvent to CH$_3$CN, the interactions between the crown ether moieties and the Na$^+$ ions weakened substantially, decreasing the molecular integrity of the [2]rotaxane and, hence, the stability of the squaraine units toward thiol-based nucleophiles. Figure 6 indicates that the [2]rotaxane [**3**$\subset$**1**$\supset$Na$_2$][2ClO$_4$] (0.6 μM) was destroyed rapidly in a solution of CH$_3$CN/H$_2$O (9:1) after we had added a large excess of cysteine (60 μM). When 1.2 mM Na$^+$ was present in the solution, 30% of the squaraine dye was destroyed within 5 min, according to the intensity of the emission band at 643 nm; when we increased the concentration of Na$^+$ ions to 3 mM, the corresponding decrease in intensity of this absorption band was less than 3% after 5 min, supporting the notion of Na$^+$-assisted stabilization of the squaraine dye under increasingly aqueous conditions.

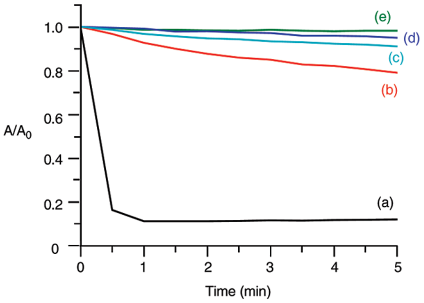

Figure 5. Changes in absorption spectra upon addition of octanethiol (6 mM) to 1.5 μM solutions (CH$_3$CN, 298 K) of (a) thread **3** (640 nm) and (b−e) [2]rotaxane [**3**$\subset$**1**$\supset$Na$_2$][2ClO$_4$] (634 nm) in the presence of Na$^+$ ions at concentrations of (b) 0, (c) 0.75, (d) 1.5, and (e) 7.5 μM.

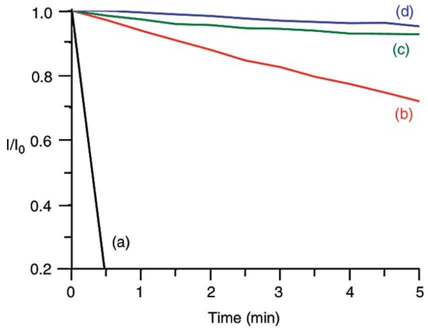

Figure 6. Changes in emission spectra at 643 nm upon addition of cysteine (60 μM) to a solution of the [2]rotaxane [**3**$\subset$**1**$\supset$Na$_2$]-[2ClO$_4$] (0.6 μM) in CH$_3$CN/H$_2$O (9:1) at 298 K in the presence of Na$^+$ ions at concentrations of (a) 0, (b) 1.2, (c) 2.4, and (d) 3.0 mM.

Figure 7 indicates that, among several physiologically important metal ions, only the presence of Na$^+$ ions helped to protect the squaraine dye (i.e., maintain its characteristic blue color) from attack by cysteine. This finding supports our previous observation that the formation of the [2]rotaxane was templated by Na$^+$ ions in a highly selective manner.[7] Thus, although the slippage [2]rotaxane [**3**$\subset$**1**$\supset$Na$_2$][2ClO$_4$] is relatively unstable in water, it might be suited to applications in biological systems containing sufficiently high concentrations of Na$^+$ ions (e.g., serum).

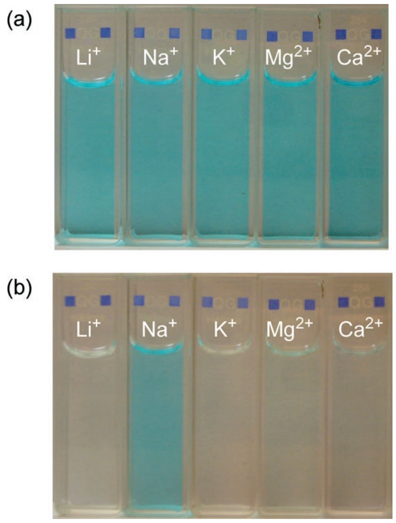

Figure 7. Photographic images of solutions of the [2]rotaxane [**3**$\subset$**1**$\supset$Na$_2$][2ClO$_4$] (6 μM) in CH$_3$CN/H$_2$O (9:1) containing various physiologically important metal ions (6 mM) in the (a) absence and (b) presence (after 5 min) of cysteine (2.4 mM).

We have demonstrated that the slippage approach can be used to efficiently encapsulate a squaraine dye in the form of a [2]rotaxane. The encircling molecular cage protected the squaraine moiety from polar solvents—enhancing its quantum yield substantially—and nucleophiles—increasing its stability toward chemical attack. Although the [2]rotaxane was more labile in aqueous solvents, the presence of a high concentration of Na$^+$ ions in solution improved its stability; other physiologically important metal ions—namely Li$^+$, K$^+$, Mg^{2+}, and Ca^{2+}—exhibited no such stabilizing effect.

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Supporting Information Available: Synthetic procedures and characterization data for the [2]rotaxane [**3**$\subset$**1**$\supset$Na$_2$]-[2ClO$_4$]. This material is available free of charge via the Internet at http://pubs.acs.org.

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[11] According to the intensity of the absorption band at 634 nm, ca. 60% of the encapsulated squaraine dyes were destroyed after 15 min in the absence of Na$^+$ ions. In the presence of Na$^+$ ions (7.5 μM), it took 90 min to destroy the same number of encapsulated squaraine dyes.