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Squaraine rotaxane shuttle as a ratiometric deep-red optical chloride sensor†

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A new squaraine rotaxane molecular shuttle exhibits high chemical stability and acts as a deep-red, fluorescent and colorimetric sensor for Cl^- anion with reversible, ratiometric response. The molecular design encapsulates a dihydroxyl substituted squaraine dye inside an anthracene-containing tetralactam macrocycle and a "clicked capping" reaction was used to convert an appropriate pseudorotaxane precursor into a permanently interlocked rotaxane in high yield. Reversible binding of Cl^- to the rotaxane in solution, or on the surface of prototype dipsticks, causes lateral displacement of the surrounding macrocycle away from the central squaraine station and a substantial 30–40 nm shift in the squaraine absorption/fluorescence maxima that can be easily detected by the naked eye. The collective attributes of intense absorption/emission and ratiometric response at deep-red wavelengths is a significant advance in optical Cl^- sensor performance by an organic molecule.

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Introduction

An active topic in modern supramolecular chemistry is the development of optically active sensor molecules that report the presence of anions.[1] The ultimate goal is to produce synthetic receptors that can transduce anion binding events into detectable optical signals within a range of biological, environmental and industrial matrices, and often in a reversible manner that enables real-time monitoring of anion concentration levels.[2] The rational design of functional optical anion sensors is a significant supramolecular challenge, especially with weakly basic anions that do not form strong coordination bonds in competitive polar solvents.[3] A notable example in this regard is Cl^-, a ubiquitous and biomedically important anion.[4] Presently, there are a handful of commercially available fluorescent Cl^- sensors that have useful but not ideal sensing properties.[5] Most are fluorescent quinolinium or acridinium derivatives that emit blue/green light which means that they do not work well in biological samples that scatter the light or produce significant amounts of autofluorescence.[6] Furthermore, they are quenched by halide anions and thus they act as turn-off sensors with limited anion selectivity. Recent efforts to convert these systems into ratiometric sensors have modified the fluorophore structure or packaged the fluorophore inside nanoparticles, but these refined systems still lack specific Cl^- recognition ability.[4,7] The ongoing community effort to design synthetic receptors with suitable hydrogen bonding motifs for anion binding has produced increasingly impressive anion affinities, but to date very few of these molecules are optically active and able to undergo Cl^--induced changes in spectral properties.[8] Optical switching effects have been achieved using mechanically interlocked molecules, and rotaxanes are particularly attractive molecular architectures since dynamic shuttling processes can modulate the energy transfer between the surrounding macrocycle and encapsulated thread.[9] While recent progress on anion-binding interlocked molecules has been impressive,[10] continued advances are still needed to produce molecules that act as practically useful optical Cl^- sensors with performance properties that complement analogous sensing systems based on inorganic salts,[11] metal coordination complexes,[12] nanoparticles,[4,7,13] and fluorescent proteins.[14]

In 2010, we reported a first-generation rotaxane-based Cl^- sensor.[9i] The structure was comprised of a squaraine dye, with generic structure designated as Sq, encapsulated inside the tetralactam macrocycle **M1** (Scheme 1). The surrounding macrocycle partially quenched the dye's deep-red fluorescence, and we showed that binding of Cl^- to the rotaxane induced macrocycle translocation away from the central squaraine station producing an increase in the squaraine's narrow fluorescence emission maxima band at 655 nm. Thus, the rotaxane shuttle acted as a "turn-on" fluorescence sensor with light emission in the coveted 650–900 nm window that allows maximum sample penetration of the light, attenuated scattering, and diminished autofluorescence.[15] While these optical performance features were important advances towards an effective Cl^- sensor, the first-generation rotaxane exhibited some less desirable properties, including: (a) the fluorescence response was not ratiometric and thus susceptible to systematic artifacts that alter

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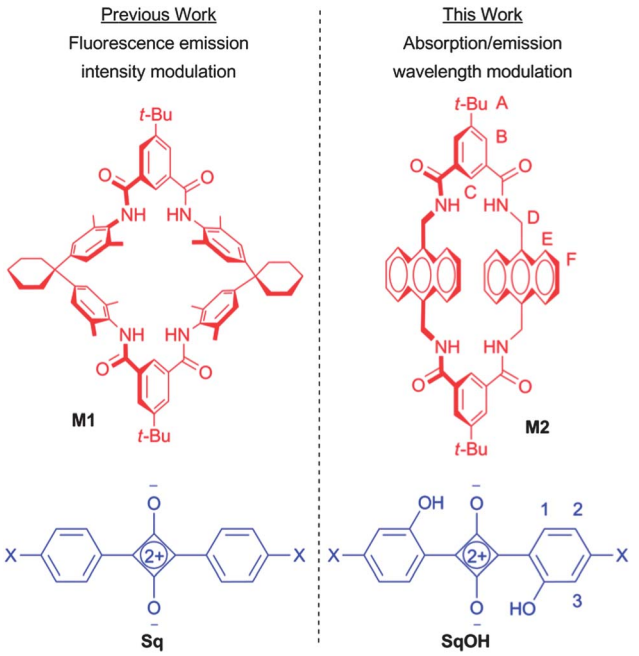
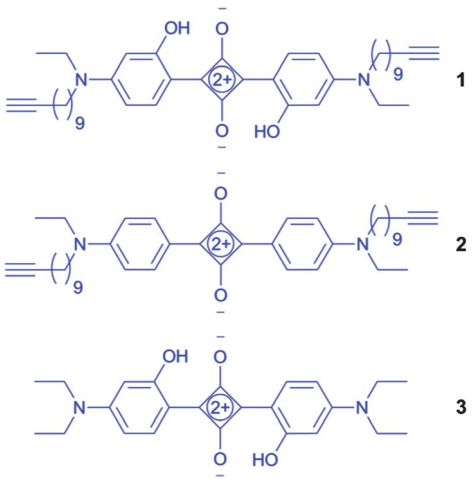


Scheme 1



Scheme 2

emission intensity and detection accuracy, and (b) the Cl^--induced rotaxane shuttling process exposed the relatively reactive Sq structure to the solvent, accelerating its chemical decomposition. In this current report, we describe the rational design of an improved next-generation rotaxane system that mitigates these two performance limitations. Our new system employs the anthracene-containing tetralactam macrocycle **M2** which we have previously shown can encapsulate squaraine dyes and produce a significant red-shift in squaraine absorption and emission maxima.[16] We expected that rotaxane shuttles with **M2** as the surrounding macrocycle would act as ratiometric optical sensors. Furthermore, light excitation of the macrocycle's anthracene side-walls would produce efficient energy transfer to the encapsulated squaraine. The choice of **M2** as the rotaxane macrocycle meant that Sq dyes were not suitable as the rotaxane thread component for two important reasons: (a) exposed Sq dyes are susceptible to decomposition in nucleophilic solvents as described above, and (b) affinity of a Sq dye inside **M2** was likely to be so strong that it could not be displaced by a competing Cl^-. Therefore, we had to find a modified squaraine dye structure that was chemically more resistant to nucleophilic solvents and also not held as tightly by the surrounding **M2** macrocycle. We reasoned that dihydroxyl substituted squaraine dyes with generic structure SqOH would solve both problems. A collection of scattered reports indicated that SqOH dyes are inherently more stable than Sq dyes,[17] and that the internal hydrogen bonding within SqOH dyes would weaken the ability of the two squaraine oxygen atoms to form hydrogen bonds with the amide NH residues of **M2**,[18] thus enabling displacement by Cl^-. Herein, we describe studies that test this next-generation design. We have investigated pseudorotaxane stability using appropriate molecular building blocks and converted the pseudorotaxanes into rotaxane shuttles. We find that the rotaxane comprised of SqOH dye and **M2** macrocycle permits ratiometric optical sensing of Cl^- in solution and allows fabrication of color-changing dipsticks that enable naked-eye detection of Cl^- in aqueous environments (Scheme 2).

Results and discussion

Squaraine dye studies

The squaraine dyes **1–3** were each synthesized using standard methods that condensed squaric acid with two molar equivalents of the appropriate aniline derivative (ESI, Schemes S1 and S2†).[19] Squaraines **1** and **2** were equipped with long alkyl chains and terminal alkyne groups to enable subsequent covalent conversion to rotaxanes. In chloroform the compounds exhibit the expected spectral properties of squaraine dyes with narrow and intense absorption/emission bands and high fluorescence quantum yields (ESI, Fig. S1 and S2†). The solution-state chemical stabilities of **1** and **2** in hydrolytic solvents were conveniently measured by monitoring the intensity of the dye absorption maxima bands. Decay profiles in a mixed acetone–water solution were found to obey pseudo first-order kinetics, and the half-lives at 25° C were calculated to be 130 h for **1** and 20 h for **2** (see ESI, Fig. S5 and Table S3†). Thus, as predicted, the SqOH dye **1** was substantially more resistant to hydrolytic decomposition and a good candidate for conversion into a robust squaraine rotaxane shuttle with optical activity.

Pseudorotaxane studies

In a prior study, we showed that an admixture of **M2** and Sq dye self-assembled with high affinity ($K_a \sim 2 \times 10^5\ M^{-1}$ in chloroform) to produce a pseudorotaxane inclusion complex whose absorption and emission maxima were red-shifted by 30–40 nm.[16] Since rates of squaraine pseudorotaxane self-assembly are quite sensitive to the size of the squaraine *N*,*N*′-dialkyl groups,[20] it was not obvious at the beginning of this study if the long chains at both ends of squaraine structures **1** and **2** would sterically prevent pseudorotaxane formation. In the event,

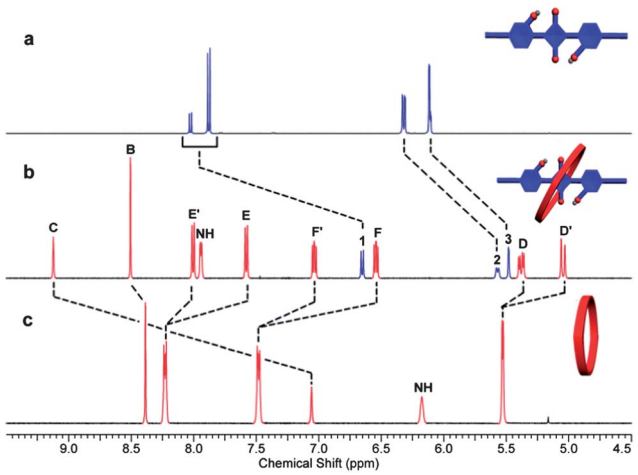


Fig. 1 Partial ^{1}H NMR spectra ($CDCl_3$) of: (a) **1** as 1 : 4 mixture of *cis* and *trans* rotamers; (b) pseudorotaxane **M2⊃1**; (c) free **M2**. For atom assignments, see Scheme 1.

separate samples of **M2** and the corresponding squaraine were stirred in $CDCl_3$ at 30 °C for 24–48 h and NMR analysis showed essentially complete formation of pseudorotaxanes **M2⊃1** and **M2⊃2**. Both pseudorotaxanes were stable enough for isolation in 30–50% yield using column chromatography with relatively nonpolar eluents. The ^{1}H NMR spectra in Fig. 1 show several diagnostic changes in chemical shift that indicate formation of **M2⊃1**. The strong anisotropic deshielding of the internal isophthalamide proton C is especially distinctive. As expected, the free squaraine **1** exists as a mixture of two rotamers,[17] with the relative positions of the two OH groups either *cis* or *trans*. Only the C_2 symmetric *trans* rotamer is encapsulated inside the macrocycle, as confirmed by the macrocycle spectral pattern (Fig. 1b shows four anthracene peaks and one peak for proton C). Another notable spectral feature is the pair of diastereotopic chemical shifts for the macrocycle benzylic protons D and D′. The NMR data indicate that the surrounding macrocycle adopts a rigid chair conformation and does not undergo rapid pirouetting around the encapsulated dye. Further insight was gained by calculating the structure of the analogous pseudorotaxane **M2⊃3** (energy minimized in Gaussian09 using DFT, B3LYP/6-31G* basis set). The low energy structure in Fig. 2 concurs with the NMR data in Fig. 1 and also with the literature X-ray structure of a structurally related squaraine (SqOH) rotaxane that has a surrounding macrocycle with phenylene side-walls.[18]

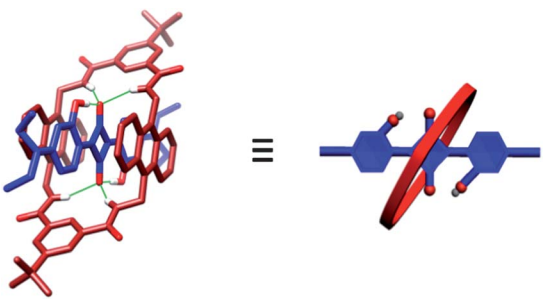


Fig. 2 Calculated structure of **M2⊃3** showing the macrocycle in a rigid chair conformation. Each squaraine oxygen forms bifurcated hydrogen bonds with two macrocycle NH residues and also an intramolecular hydrogen bond with the adjacent squaraine hydroxyl functionality.

Encapsulation of Sq or SqOH by macrocycle **M2** in chloroform produced a ~30 nm red shift in squaraine absorbance maxima (ESI, Table S4†),[16,21] and enabled absorption titration experiments to measure association constants. With squaraine **1** the rate of encapsulation was prohibitively slow at the micromolar concentrations required for absorption studies, so we employed squaraine **3** with smaller *N*,*N*′-diethyl groups and a much faster rate of association.[20] The squaraine absorption spectra in chloroform, produced by mixing different ratios of **M2** and **3** to produce **M2⊃3**, were analyzed by standard methods to give a value of $K_a = (1.5 \pm 0.2) \times 10^4\ M^{-1}$. Thus, we conclude that macrocycle **M2** binds SqOH templates an order of magnitude more weakly than Sq templates. This difference in relative affinity was confirmed by conducting pseudorotaxane dethreading studies. Stock solutions of pseudorotaxanes **M2⊃1** and **M2⊃2** were prepared in chloroform and then added to polar organic solvents that promoted dethreading. In acetone,

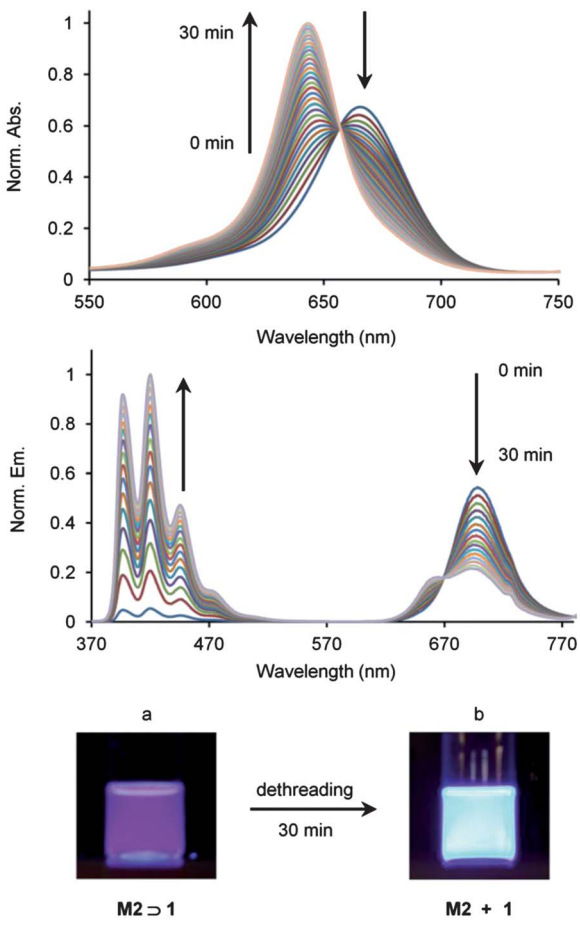


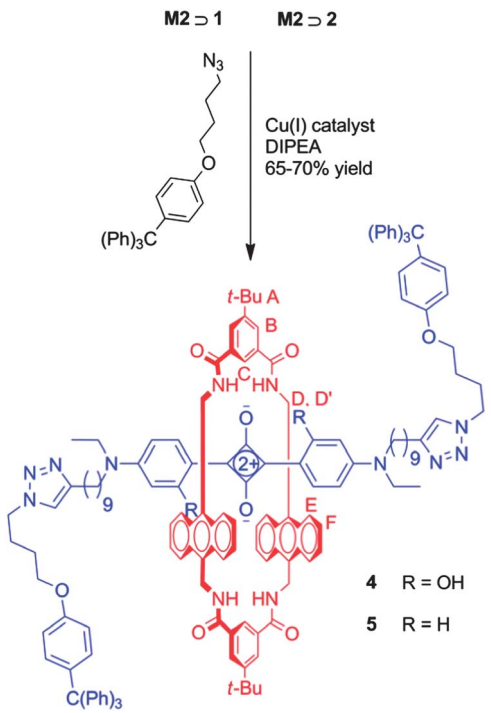
Fig. 3 Time-dependent dethreading of **M2⊃1** in methanol. (Top) Partial absorption spectra; (middle) fluorescence spectra, ex: 365 nm; (bottom) photographs of a vial illuminated with 365 nm light at two time points, (a) starting solution containing intact **M2⊃1**, (b) final solution containing dethreaded components **M2 + 1**.

the dethreading of **M2⊃1** was observed to be considerably faster than dethreading of **M2⊃2** (ESI, Fig. S6†). Indeed, the dethreading of **M2⊃1** in acetone was too rapid for accurate analysis but the process was slower in methanol and could be followed by repetitive full-spectrum fluorescence scans. The fluorescence spectra in Fig. 3 were generated by exciting the macrocycle anthracene band at 365 nm, and for intact **M2⊃1** there is efficient energy transfer from the surrounding anthracene to the encapsulated squaraine with consequent emission at 699 nm. Dethreading of **M2⊃1** produced two spectral effects; gradual restoration of the free squaraine emission band at 656 nm (Fig. 3, top) and loss of anthracene-to-squaraine energy transfer (Fig. 3, middle). Importantly, the dethreading process can be monitored by the naked eye when samples are illuminated with a hand-held lamp emitting 365 nm light (Fig. 3, bottom). The complex **M2⊃1** emits red light due to energy transfer, whereas, the binary mixture of the dethreaded components, **M2 + 1**, emits blue light. These observations encouraged us to covalently convert the pseudorotaxanes into rotaxanes and determine if they exhibited Cl^--induced shuttling behavior with concomitant ratiometric changes in optical spectra.

Rotaxane studies

The pseudorotaxanes **M2⊃1** and **M2⊃2** were converted into permanently interlocked rotaxanes by the "clicked capping" method described in Scheme 3.[22] Rotaxane **5** was obtained in 70% isolated yield when the alkyne/azide cycloaddition reaction in $CHCl_3$ contained 25 mol% copper catalyst and a stoichiometric amount of alkyl azide. Rotaxane **4** was obtained in lower yields (30–35%) using the same reaction conditions, due in part to partial dethreading of the **M2⊃1** pseudorotaxane over the long reaction times of 16–24 hours. Higher catalyst loadings of 40 mol% and an excess of alkyl azide (4 molar equiv.) produced a shorter reaction time (6 hours) and improved the isolated yield of **4** to 65%. The structures of rotaxanes **4** and **5** were characterized by mass spectrometry and NMR spectroscopy, and the ^{1}H NMR spectra showed very similar shielding patterns to the pseudorotaxanes (compare Fig. 1 and 6). Both rotaxanes exhibited nearly identical absorption and emission maxima in nonpolar solvents, such as chloroform, with the SqOH analogue **4** being slightly brighter than Sq analogue **5** (ESI, Fig. S3, S4 and Table S1†).

Solutions of rotaxanes **4** and **5** in different solvents (methanol, chloroform, and acetone) were titrated with tetrabutylammonium chloride (TBA^+Cl^-) and monitored by absorption and fluorescence spectroscopy. In the case of rotaxane **5** there were no significant spectral changes in any of the solvents, suggesting that Cl^- was unable to alter the rotaxane co-conformation (ESI, Fig. S8 and S9†). With rotaxane **4**, the addition of excess TBA^+Cl^- (10 mM) produced little optical effect when the solvent was methanol or chloroform.‡ In acetone, a color change from green to blue was easily distinguished by the naked-eye (Fig. 4).[23] The color change was due to a movement of the absorption maxima from 663 nm to 647 nm (ESI, Fig. S11†). The Cl^- association process was reversed by adding a Cl^- precipitating reagent ($AgPF_6$) which restored the rotaxane's original optical profile (Fig. 4c and S12†). In Fig. 5 are the results of a fluorescence titration experiment that added incremental amounts of TBA^+Cl^- to a solution of rotaxane **4** in acetone. There is progressive loss of the rotaxane emission at



Scheme 3

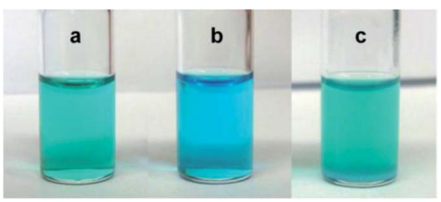


Fig. 4 Photographs of vials containing acetone solutions of: (a) **4** (50 μM), (b) **4** (50 μM) + TBA^+Cl^- (10 mM), (c) **4** (50 μM) + TBA^+Cl^- (10 mM) + $AgPF_6$ (100 mM).

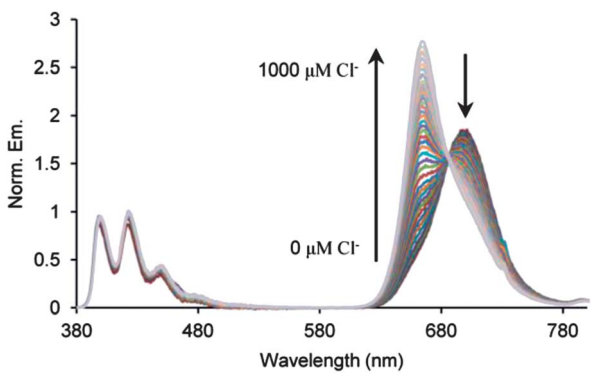


Fig. 5 Fluorescence spectra of **4** (3 μM, ex: 365 nm) in acetone during titration with TBA^+Cl^-.

698 nm and appearance of a new emission band at 665 nm with a clean isosbestic point. The intensity change at 665 nm was used to create a titration isotherm which fitted well to a 1 : 1 binding model and produced a Cl^- binding constant of $(1.9 \pm 0.2) \times 10^3\ M^{-1}$ (ESI, Fig. S10 and Table S5†). The halide selectivity of rotaxane **4** was assessed by additional titration studies in acetone using TBA^+Br^- and TBA^+I^-. A reliable association constant could only be calculated for Br^- ($(2.8 \pm 1.1) \times 10^2\ M^{-1}$) with no measurable response to I^-. The sensor selectivity of $Cl^- > Br^- > I^-$ is the reverse of what is generally observed with the quinolinium or acridinium sensors described in the introduction.[6c] The fluorescence spectra in Fig. 5 were generated by exciting the anthracene band at 365 nm, and it is notable that the Cl^- binding process induces very little change in energy transfer from the surrounding anthracene macrocycle to the encapsulated squaraine. In contrast, the fluorescence spectra for complete dethreading of analogous pseudorotaxane **M2⊃1** show a large decrease in anthracene-to-squaraine energy transfer (Fig. 3). Taken together, the absorption and fluorescence data indicate that Cl^- binding to rotaxane **4** induces a small-amplitude lateral displacement of the surrounding anthracene macrocycle away from the encapsulated squaraine station. The translocation distance is small enough to maintain efficient anthracene-to-squaraine energy transfer but far enough to eliminate the macrocycle's red-shift effect on the squaraine. The red-shift effect is caused, in part, by the macrocycle's ability to rigidify the structure of the encapsulated squaraine chromophore,[21] and partial displacement of the squaraine from the cavity is evidently enough to dissipate this effect.

This physical picture was confirmed by NMR studies of rotaxane **4** in the presence of TBA^+Cl^-. In order to achieve useful solubility, the solvent was changed to a mixture of $CDCl_3$–acetone-d_6 (1 : 4 v/v). The ^1H NMR spectra in Fig. 6 (and also the ESI, Fig. S13†) show that addition of TBA^+Cl^- to **4** induced the appearance of a second set of peaks in slow exchange with the disappearing original set. The emerging signal pattern is consistent with a Cl^--induced asymmetric co-conformation that positions the macrocycle on one side of the central squaraine station. Diagnostic changes in chemical shift were observed for squaraine protons 2 and 3, which moved downfield from 5.63 ppm and 5.54 ppm and split into pairs of signals with equivalent intensities (Fig. 6b). These deshielded signals are similar to those for free thread component (Fig. 6c), indicating that the squaraine station in the Cl^--bound rotaxane is at least partially outside the shielding influence of the surrounding macrocycle. Additional chemical shift evidence is the upfield movement of macrocycle isophthalamide proton C to 8.51 ppm, which is consistent with Cl^--induced translocation of the macrocycle away from the deshielding influence of the squaraine station. But the macrocycle translocation distance does not reach the terminal stopper groups because there is essentially no change in chemical shifts for these protons.

The earlier studies of our first-generation rotaxane sensor included fabrication of prototype Cl^- sensing dipsticks and fluorescence imaging experiments showed Cl^--induced changes in fluorescence intensity. In similar fashion, rotaxane **4** was adsorbed onto C18-coated silica gel TLC plates to create dipsticks for immersion in aqueous environments. The top of Fig. 7 shows photographs of the same dipstick during the following sequence: (a) before immersion, (b) after brief immersion in an aqueous sample containing TBA^+Cl^- followed

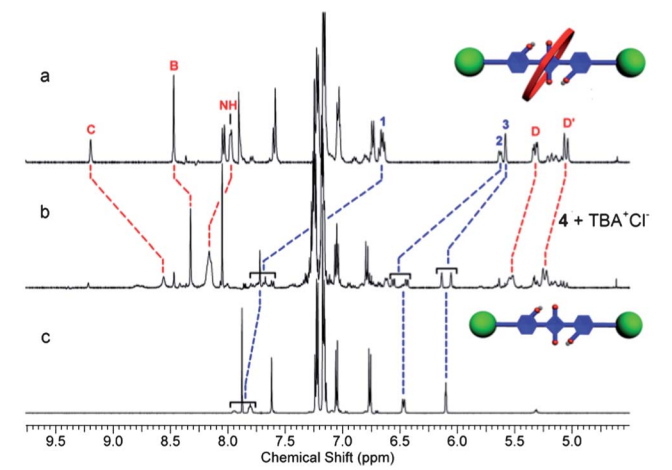


Fig. 6 Partial ^1H NMR spectra (1 : 4 $CDCl_3$–acetone-d_6) of: (a) **4**; (b) **4** + 50 molar equivalents of TBA^+Cl^-; (c) thread component of **4**. For atom assignments, see Scheme 1.

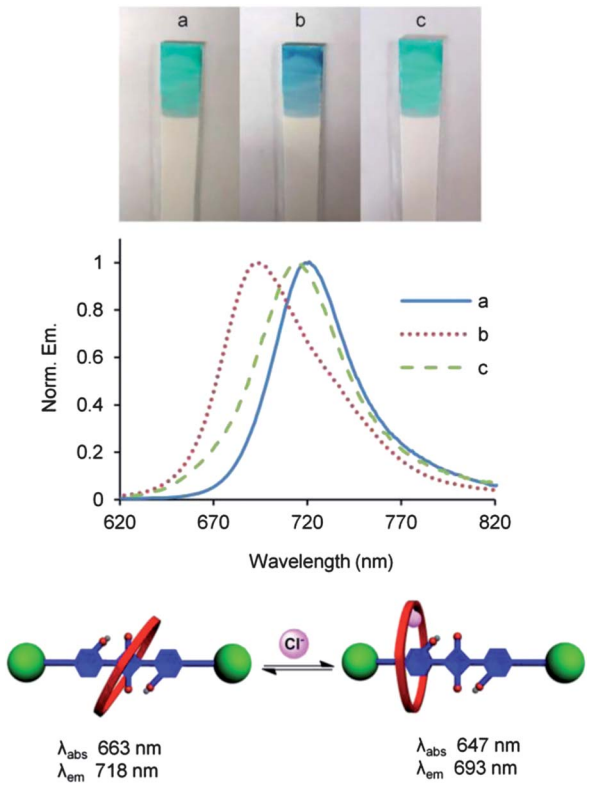


Fig. 7 (Top) Photographs of the same dipstick during the following sequence: (a) before immersion, (b) after immersion in aqueous TBA^+Cl^- (1 M), (c) after aqueous washing. (Middle) Fluorescence spectra (ex: 600 nm) of the dipstick surface at the same time points confirm the blue shift of emission maxima induced by TBA^+Cl^- and subsequent reversal after washing. (Bottom) Schematic summary of the optical sensing changes.

by mild heating until dry, (c) after aqueous washing followed by mild heating until dry. There is an obvious color change with each step in the sequence (with high resistance to color fading), and the colors match those observed in the solution experiment described in Fig. 4. As expected, fluorescence spectra of the dipstick surfaces showed that exposure to TBA^+Cl^- produced a 27 nm blue shift of emission maxima and the change was restored after washing the dipstick with water (Fig. 7, middle). Overall, the ratiometric, color-changing optical response and enhanced chemical stability of dipsticks composed of **4** represent substantial performance improvements over dipsticks composed of our first-generation rotaxane sensor. As expected, the dipsticks do not respond to more hydrophilic salts like Na^+Cl^- and K^+Cl^-. To achieve this practical outcome the dipstick's solid support would have to be modified by incorporating Cl^- phase transfer agents.[24]

Conclusions

Squaraine rotaxanes with thread components based on the non-hydroxyl Sq structure have limited potential as molecular shuttles due to slow chemical decomposition of the Sq dye when it is exposed during the shuttling process.[9i] We have overcome this drawback by encapsulating a more stable dihydroxyl substituted SqOH dye inside the anthracene-containing tetralactam macrocycle **M2** and forming a rotaxane shuttle with optical switching ability. Specifically, the squaraine rotaxane **4** was prepared in high synthetic yield using a "clicked capping" method and shown to act as an optical sensor for Cl^- with ratiometric response. Reversible binding of Cl^- to rotaxane **4** in solution, or on the surface of prototype dipsticks, induced a small-amplitude lateral displacement of the surrounding macrocycle away from the encapsulated squaraine station. The change in rotaxane co-conformation produced a 30 nm change in the squaraine absorption band that was detected by the naked eye, and a concomitant 40 nm change in the fluorescence emission wavelength. It is encouraging that such a significant ratiometric response was produced by only a small-amplitude shuttling process which is likely easier to reproduce in a next-generation design than a large-amplitude shuttle. It is worth noting, however, that efforts to create a large-amplitude shuttle version of **4** may be rewarded with access to molecules endowed with tremendous optical sensing potential. A large-amplitude shuttle would likely produce dramatic changes in emission color due to extensive modulation of the anthracene-to-squaraine energy transfer as reflected by the pseudorotaxane dethreading data in Fig. 3.

Synthetic organic receptors that exhibit a ratiometric fluorescent response to Cl^- are quite rare, with two previous studies utilizing bis-pyrene dimerization processes to alter emissions in the blue/green wavelength region.[8a,b] To the best of our knowledge there are no examples of ratiometric fluorescent Cl^- sensors that emit in the deep-red region and very few colorimetric systems with reversible binding behavior. Thus, rotaxane **4** with its collective attributes of intense, deep-red absorption/emission wavelengths and ratiometric response is a significant advance in optical Cl^- sensor performance by an organic molecule. Future efforts to translate the sensor design towards practical applications can take advantage of the rapidly growing portfolio of structural elements that are known to increase water solubility[25] and/or improve Cl^- affinity.[26] Typically, effective Cl^- sensors only require moderate association constants since Cl^- concentration levels in many analysis samples are quite high (*e.g.*, ~100 mM in blood, ~500 mM in seawater, 20–40 mM in epithelial cells);[4] thus, we are optimistic that future optimization efforts will bring success. From a broader perspective, it appears that the squaraine rotaxane building blocks reported here can be exploited to make various types of molecular shuttles with an array of attractive optical switching properties.

Acknowledgements

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Notes and references

‡ Addition of excess TBA^+Cl^- to the analogous pseudorotaxane **M2⊃1** in chloroform also produced little optical effect. Similar studies of **M2⊃1** in acetone and methanol were not possible due to spontaneous dethreading.

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