



One-Pot Synthesis of a Red-Fluorescent Chemosensor from an Azacrown, Phloroglucinol and Squaric Acid: A Simple In-Solution Construction of a Functional Molecular Device.

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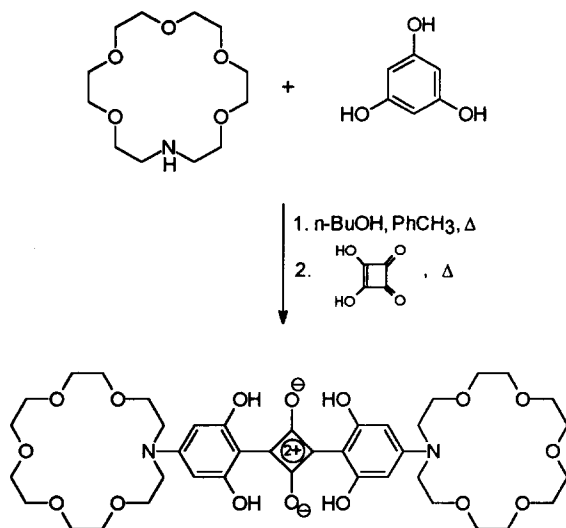
Abstract: A long wavelength fluorescent, squaraine-based chemosensor has been synthesized in one step from commercially available precursors. The resulting squaraine derivative absorbs maximally at 635 nm ($\epsilon = 260,000$) and the emission peak is at 665 nm. This azacrown-appended squaraine signals alkaline and earth-alkaline metal ions in millimolar concentrations in acetonitrile. © 1997 Elsevier Science Ltd.

Fluorescent chemosensors have received considerable attention¹ in recent years as the potential of these molecules that signal molecular recognition events by a fluorescence signal is realized in many practical applications. Sensors for both charged species and neutral molecules have emerged. Especially for metal ions which are highly regulated in biological systems or those implicated in such regulatory mechanisms, there have been a number of proposals as potential chemosensors.^{2,3,4} As the field matures, the characteristics for a desirable chemosensor have become apparent. Among these, the requirement for long wavelength excitation and emission stands out as the principal requirement^{2,3,4,5} for a successful chemosensor. This is needed for low background signals in any biological application, as the endogenous fluorophores typically fluoresce brightly when excited at short wavelengths.

Squaraines are a group of fluorescent dyes and pigments derived from squaric acid and dialkylanilines, well-known in applications such as photoreceptors,⁶ organic solar cells,⁷ optical recording media⁸ and non-linear optics.⁹ Their very promising spectral properties,¹⁰ long wavelength absorption and emission, high absorptivity and quantum yields have not been exploited so far in relation to chemosensor design. This may be partly due to their solubility characteristics; most are sparingly soluble in all solvents except CH_2Cl_2 and CHCl_3 and tend to form various aggregate structures in other solvents.¹¹ But, we observed that with appropriate modifications on the amine function, solubility and aggregation behavior can be modulated to a great extent.

The chemosensor is designed as an intrinsic chemosensor, i.e.; the ligand is an integral part of fluorophore π -system. It is well-established that fluorescence signal to be obtained using such probes is stronger and/or carry more information by creating a different absorbing species¹² on metal ion binding, enabling ratioing of the emission signals thereby cancelling concentration dependent artifacts. One important drawback has been the synthetic challenge of assembling such a molecular device, especially one to absorb at the red-end of the visible spectrum. But, here we report an extraordinarily simple way of synthesizing such an

ion-sensing molecular device functioning at long wavelengths, starting from simplest possible synthons of the molecule: Two azacrown ligands, two phenolic moieties (phloroglucinol) and a squaric acid molecule. The



Scheme 1. One-pot synthesis of the azacrown-squaraine

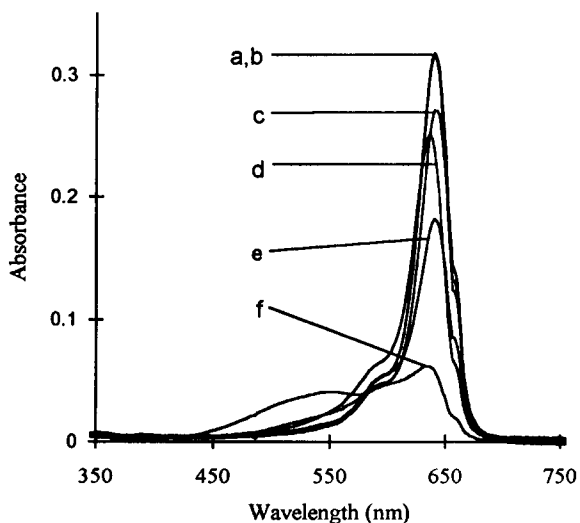


Figure 1. Absorption spectrum of the chemosensor (1.1 μM) in acetonitrile in the presence of 2 mM metal ions. (a) Na^+ , (b) K^+ , (c) no added metal ion, (d) Ca^{+2} , (e) Mg^{+2} , (f) Ba^{+2} .

synthesis (Scheme 1) is carried out by assembling three parts of the chemosensor in a one-pot reaction. The well-known reaction of phloroglucinol keto-tautomer with secondary amines is exploited using Toluene:n-Butanol (50:50) solvent mixture and removing water formed azeotropically, and the resulting 3,5-Dihydroxyphenyl-azacrown without isolation, was reacted in the same solvent system, with 0.5 equivalents of squaric acid. On cooling to RT, the squaraine product precipitated out of the solution and was obtained in analytically pure¹³ form, once it was washed with methanol. The overall yield is 75%.

Highly symmetrical nature of the chemosensor and intramolecular hydrogen bonding between aromatic OH's and squaryl oxygens is evident in the NMR data.¹³ The chemosensor obtained in this way has a λ_{max} of 640 nm in both acetonitrile and chloroform with an extinction coefficient of 240,000. The compound is also highly fluorescent in acetonitrile with an emission peak at 665 nm. The hydroxyl groups not only activate the phenyl azacrown in the squaraine formation reaction, but also increase the quantum yield and cause a small bathochromic shift in both absorption and emission spectra of the targeted molecule. These favorable spectroscopic properties is likely to place the squaraine based chemosensor in a different category as this molecule is the first example of laser diode excitable

fluoroionophore. As expected, the chemosensor successfully signals various alkali and earth-alkali metals at micro to millimolar concentrations. Ca^{2+} , Mg^{2+} and Ba^{2+} all lead to anti-auxochromic (a blue shift and hypochromic effect, Figure 1) shifts, reducing extinction coefficient, which is also reflected in the fluorescence emission intensity (Figure 2). K^+ and Na^+ produces small but reproducible auxochromic effects in both absorption and in emission spectra. Ba^{2+} seems to facilitate the formation of blue-shifted aggregate (H-aggregate)¹⁴ of the squaraine fluorophore leading to considerable peak broadening and formation of a new peak at 540 nm. This may be due to 1:2 Ba^{2+} :azacrown stoichiometry in the complex formation resulting in the stacking of squaraine molecules in solution. On standing overnight at RT, Mg^{2+} complex also slowly develops a similar aggregate spectrum. Considerable decrease in the extinction coefficient in the earth-alkaline metal binding is likely to be the result of deconjugation of azacrown nitrogen with the rest of the molecule. Examples of such interactions have been reported¹² and in donor-acceptor-donor (D-A-D) systems metal ions interacting with the electron donor parts of the molecule is known to cause such anti-auxochromic changes, whereas small increases with K^+ and Na^+ suggest an interaction with the squaryl center, as molecular mechanics calculations¹⁵ indicate that the squaryl-moiety is the "acceptor" part of the squaraines.

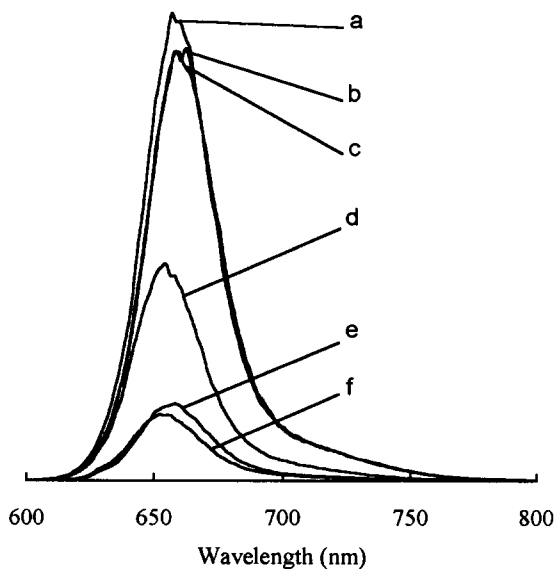


Figure 2. Emission spectrum of the chemosensor (1.1 μM) in acetonitrile in the presence of 2 mM metal ions. (a) Na^+ , (b) K^+ , (c) no added metal ion, (d) Ca^{2+} , (e) Mg^{2+} , (f) Ba^{2+} . Excitation was at 635 nm

The fluorescence signal obtained from this chemosensor is at 665 nm, close to the red-end of the visible spectrum, essentially freeing it from the interference of natural fluorophores.

This result is likely to stimulate the development of other squaraine-based chemosensors functioning in water and at conditions approximating intracellular conditions. Exceptionally simple synthesis method outlined here, should facilitate work directed towards the realization of this goal. Our work to that end is in progress.

Acknowledgment

We gratefully acknowledge support from Scientific and Technical Research Council of Turkey (TUBITAK, Project No.: TBAG-1420) and Middle East Technical University Research Funds (ODTU-AFP, Project No.: AFP-95-01-03-06).

References and Notes:

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- Characterization of the chemosensor: ^1H NMR (CDCl_3) δ 3.48-3.73 (m, 48 H, azamacrocycle CH_2 's), 5.86 (s, 4 H, Ar-H), 10.84 (s, 4H, OH); ^{13}C NMR (CDCl_3) δ 57.12, 73.88, 75.69, 75.85, 75.96, 99.13, 107.82, 163.87, 166.05, 167.70, 186.63 (one of the peaks obscured by CDCl_3); EI Mass spectrum, m/e 818 ($\text{M}-2\text{H}^+$), 411 ($\text{Azacrown-Ar-CH=C=O}^+$). Anal. Calcd for $\text{C}_{40}\text{H}_{56}\text{N}_2\text{O}_{16} \cdot 1/4 \text{H}_2\text{O}$: C, 58.21; H, 6.89; N, 3.39. Found: C, 57.86; H, 6.50; N, 3.47.
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(Received in UK 17 April 1997; accepted 9 May 1997)