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Chemosensing in Deep Red: A Squaraine-based Fluorescent Chemosensor for pH.

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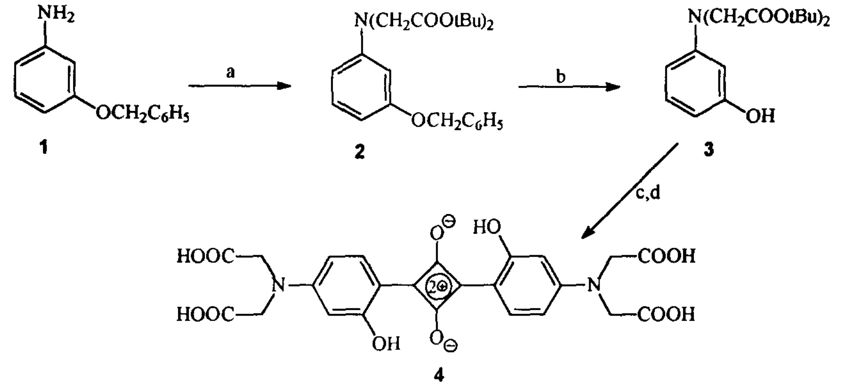
Abstract: We have synthesized and spectrally characterized a squaraine-based fluorescent chemosensor for pH. This chemosensor unlike many others, works in 100 % aqueous solutions, signalling the pH change from 10 to 7 by a 14-fold increase in the emission intensity at 651 nm, when excited at the isosbestic point (614 nm). The average pK_a for the ionizable groups is found to be 8.8. The chemosensor with its molar extinction coefficient > 200,000 and the quantum yield of 0.2, yield an impressive intrinsic brightness value of 40,000.

In recent years, rational design of fluorescent chemosensors have received considerable attention.[1] H^+ concentration, as an important metabolic and cellular parameter, has been a target for various chemosensing schemes.[2] A number of organic compounds respond to changes in pH by a spectral shift in absorption, but only molecules that can transduce the pH signal to a fluorescence emission signal are likely to have the required sensitivity for *in vivo* applications. Substantial charge redistribution accompanies most acid-base equilibria, and that is the origin of different spectral characteristics of the conjugate acid-base pairs. The requirements for a successful fluorescent chemosensor for pH are very straight-forward: starting from the most obvious, water solubility for both acid and base forms; good quantum yields, high extinction coefficients, chemical and photostability, and long wavelength excitation/emission are all desirable. Especially chemosensors which can be excited in the deep-red region of the spectrum (beyond 630 nm) offer compatibility with the solid state lasers, and therefore highly attractive. Other advantages for a long wavelength-chemosensor are also known: since most cells respond very poorly to short wavelength excitation,[3] when an *in vivo* process is to be followed, a long wavelength excitable fluorophore is highly preferred. Secondly, the emission peaks should be located in a region where endogenous background fluorescence (autofluorescence) from cellular pigments are very low. Thirdly, longer wavelength light is much less attenuated in optical fibers. In addition to all these, much reduced Rayleigh and Raman scatterings would yield a very low background signal. Therefore, the search for novel fluorophores and chemosensors in the red-end of the visible spectrum is perfectly justified. Our research group is focussed[4,5] on establishing squaraines as the fluorophore base for the next generation fluorescent chemosensors. Squaraines are a class of compounds with good quantum yields (up to 0.9 in organic solvents, emission peaks in the 640-725 nm range) and very high extinction coefficients (larger than 300,000) in organic solvents, but most of those synthesized so far, are designed for photoreceptor,[6] organic solar cell,[7] optical recording media[8] and non-linear optics[9] applications, and they are known to be water insoluble, in fact poorly soluble in most organic



Scheme 1. (a) $BrCH_2COO$-*t*-Bu, CH_3CN, Proton Sponge; (b) Cyclohexene, EtOH, 10% Pd-C; (c) Squaric acid, *n*-BuOH/$PhCH_3$; (d) TFA

solvents with serious aggregation problems. Their superior fluorescence properties have been exploited only recently as fluorescent dyes[10] and protein labels[11] and as non-specific chemosensors for alkaline and earth-alkaline cations in various organic solvent cocktails.[12] Here, we are reporting the first laser diode excitable chemosensor for pH with a very high "intrinsic brightness"[13] value of 40,000. The chemosensor works in 100 % aqueous solutions with no indication of aggregation. The synthesis is straight-forward, 3-benzyloxyaniline was reacted with t-butylbromoacetate in acetonitrile in the presence of 1,8-bis-(dimethylamino)naphthalene (Proton Sponge). The benzyl group in the resulting product (**2**) was removed by transhydrogenation using cyclohexene and 10% Pd-C in refluxing Ethanol. Removal of the solvents resulted in compound **3** in quantitative yield. This compound, when reacted with squaric acid in Toluene:n-Butanol (50:50) at reflux temperatures, while the water formed was

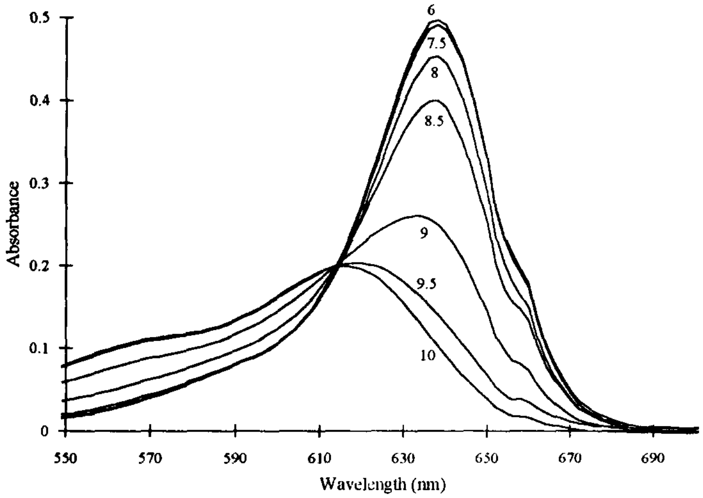


Figure 1. Absorbance spectra of the chemosensor 4 in the aqueous buffers at the indicated pH values.

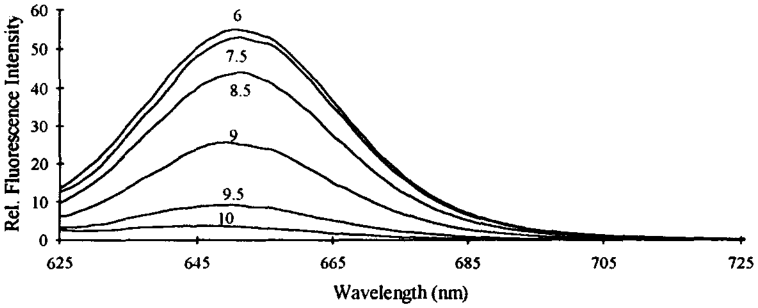


Figure 2. Emission spectra of the chemosensor 4 in the aqueous buffers at the indicated pH values. Excitation was at the isosbestic point (614 nm).

removed azeotropically, formed a dark green solution with an intense red fluorescence. On cooling, the product crystallized out of the solution in the form of green needles. t-Butyl protecting groups were then removed by trifluoroacetic acid treatment to yield the target compound (**4**) in analytically pure state (^{1}H and ^{13}C NMR, EI-Mass, elemental analysis). Overall yield in this 4-step synthesis is 82 %. pH dependent absorption spectra are shown in Figure 1. Acid form has just one sharp peak in the entire visible region (λ_{max} of 635 nm), whereas the base form has a more broad absorption with a peak at 616 nm and a shoulder at 570 nm. isosbestic point is observed at 614 nm. Emission peak for the acid form is at 651 nm, and the base form displays a small blue-shift to 645 nm, with an increase in intensity as the pH is decreased from 10 to neutrality (Figure 2). Phenolic hydroxyl functions yield an average pK_a of 8.8.[14] 2,2'-OH's in squaraine structures are known[15] to form intramolecular hydrogen bonding with squaryl oxygens and hold the entire squaraine structure planar and in full-conjugation. The signal is believed to originate from loss of planarity and conjugation on deprotonation. Since the acid and base forms have two distinct absorbance spectra, we determined 568/635 nm excitation (emission at 651 nm) ratio, it changes approximately 2-fold on a pH change from 10.0 to 7.0. More impressive is the 14-fold increase in the emission intensity at 651 nm when excited at the isosbestic point. By altering substituents on the aromatic rings, the pK_a's of the phenolic groups can be fine-tuned for the desired range. Compound **4** will serve as a prototype for novel squaraine-based chemosensors for pH. Our own work in further refinement of such chemosensors is in progress.

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(14) The average pK_a is calculated using the following equation: $pK_a = pH - \log[(A_{max} - A)/(A - A_{min})]$.

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