ORGANIC LETTERS

2010
Vol. 12, No. 14
3219−3221

Design and Investigation of a Series of Rhodamine-Based Fluorescent Probes for Optical Measurements of pH

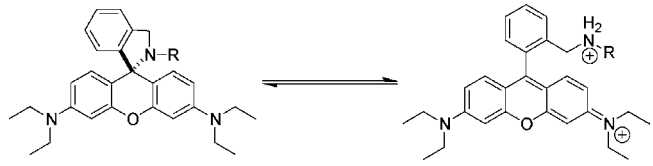
Quinn A. Best, Ruisong Xu, Matthew E. McCarroll, Lichang Wang, and Daniel J. Dyer*

Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, Illinois 62901

ddyer@chem.siu.edu

Received May 24, 2010

ABSTRACT



A series of structurally similar fluorescent probes (1−4), synthesized from rhodamine B, were designed to optically measure pH. Each probe had a unique "off−on" response as the solution went from basic to acidic. Probes 1−3 exhibited a spirocyclic quenching of the pyronin B fluorophore, whereas probe 4 was quenched by PET from the amine moiety.

A chemical sensor capable of measuring pH by optical methods has important implications in analytical and biological chemistry. Fluorescence-based probes have demonstrated a superior ability in the detection of various analytes with high sensitivity and low detection limits. In cellular biology, the pH of specific and localized areas of the cell can be an important indication of cellular events.[1] Such information could potentially be useful in the diagnosis of specific diseases. For example, colorectal cancer,[2] breast cancer,[2b,3] cystic fibrosis,[4] and neurodegenerative disorders[5] have all been linked to abnormal pH regulation, resulting in localized pH values of 4.5−6.0 that are referred to as the acidic window. Currently, a large number of fluorescence-based pH sensors have been reported in the literature and are commercially available.[6] However, many of these probes lack sensitivity or are simply nonresponsive in the so-called "acidic window". Therefore, it would be of great benefit to aid in the diagnosis of these diseases by developing new and more sensitive probes for the measurement of pH.

Many fluorescent probes operate in an "off−on" fashion, where in the "off" state the fluorescence is quenched, while the "on" state the fluorophore is fully fluorescent with a high quantum yield. Among the many fluorophores that potentially could be used as pH sensors, pyronin B has particular advantages in biological imaging because of its high quantum yield ($\Phi = 0.35$), longer excitation and emission wavelength (550−600 nm), spectral insensitivity to solvent polarity, pH, and a high tolerance to photobleaching.[7] With these criteria in mind, we set out to design a mode of regulating the fluorescence of this fluorophore by synthesizing various benzylamine derivatives at the 9-position, which have different interactions with

(1) (a) Chesler, M. *Physiol. Rev* **2003**, *83*, 1183. (b) Izumi, H.; Torigoe, T.; Ishiguchi, H.; Uramoto, H.; Yoshida, Y.; Tanabe, M.; Ise, T.; Murakami, T.; Yoshida, T.; Kohno, K. *Cancer Treat. Rev.* **2003**, *29*, 541. (c) Weylandt, K. H.; Nebrig, M.; Jansen-Rossech, N.; Amey, J. S.; Carmena, D.; Wiedenmann, B.; Higgins, C. F.; Sardini, A. *Mol. Cancer Ther.* **2007**, *6*, 979.

(2) (a) Kellokumpu, S.; Sormunen, R.; Kellokumpu, I. *FEBS Lett.* **1996**, *35*, 2811. (b) Schindler, M.; Grabski, S.; Hoff, E.; Simon, S. M. *Biochemistry* **1996**, *35*, 2811.

(3) Rivinoja, A.; Kokkonen, N.; Kellokumpu, I.; Kellokumpu, S. *J. Cell. Physiol.* **2006**, *208*, 167.

(4) Poschet, J.; Perkett, E.; Deretic, V. *Trends Mol. Med.* **2002**, *8*, 512.

(5) Kogot-Levin, A.; Zeigler, M.; Ornoy, A.; Bach, G. *Pediatr. Res.* **2009**, *65*, 686.

(6) (a) Diwu, Z.; Chen, C. S.; Zhang, C.; Klaubert, D. H.; Haugland, R. P. *Chem. Biol.* **1999**, *6*, 411. (b) Haugland, R. P.; Spence, M. T. Z.; Johnson, I. D. *Handbook of Fluorescent Probes and Research Products*, 9th ed.; Molecular Probes: Eugene, OR, 2002; p 966. (c) Galindo, F.; Burguete, M. I.; Vigara, L.; Luis, S. V.; Kabir, N; Gavrilovic, J.; Russel, D. A. *Angew. Chem., Int. Ed.* **2005**, *44*, 6504. (d) de Silva, A. P.; de Silva, S. S.; Goonesekera, S. S. K.; Gunaratne, H. Q. N.; Lynch, P. L. M.; Nesbitt, K. R.; Patuwathavithana, S. T.; Ramyalal, N. L. D. S. *J. Am. Chem. Soc.* **2007**, *129*, 3050.

10.1021/ol1011967
Published on Web 06/24/2010

the fluorophore. Herein, we describe a new series of rhodamine-based analogues designed for the optical detection of pH.

Starting with commercially available rhodamine B, we synthesized various benzylamine structures (see the Supporting Information for synthesis details) where the amine moiety is primary, secondary, and tertiary (Figure 1). The rhodamine-

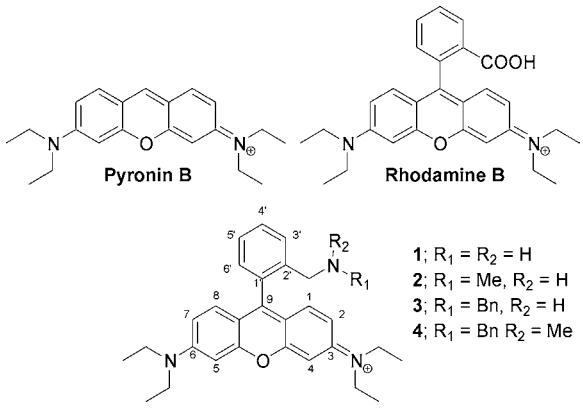


Figure 1. Structures of pyronin B, rhodamine B, and benzylamine moiety substituted pyronin B derivatives **1**−**4**.

based literature is rife with fluorescent probes that are regulated by a spirocyclic structure. These spirocyclic compounds lack absorbance and fluorescence, in the visible spectrum, when engaged in the spirocyclic form but will open upon binding to specific analytes to restore the fluorophore and fluorescence intensity of that compound. Typically, these compounds are in the form of a rhodamine lactone- or lactam-type derivative,[8] which have been mostly applied in the detection of heavy metal cations. However, Nagano et al. recently published work on a probe for hypochlorous acid involving a spiro-thiophene derivative.[7c] We envisioned that our series of structurally related analogues should interact with the pyronin B fluorophore in a similar fashion but differ in their response to pH on the basis of the nucleophilicity and steric hindrance of the amine moiety. Specifically, probes **1**−**3** should take on a spirocyclic structure in the absence of protons at high pH, with **3** maintaining the most spiro-like character. Compound **4**, however, contains a tertiary amine, which will avoid intramolecular cyclization, and should thus maintain its fluorescence. This is analogous to rhodamine derivatives bearing a tertiary amide or an ester functionality at the 2′ position, e.g., rhodamine 6g.

Upon titrating the fluorescence response to pH for probes **1**−**4** (see the Supporting Information for fluorescence spectra), we obtained the following curves shown in Figure 2. The results

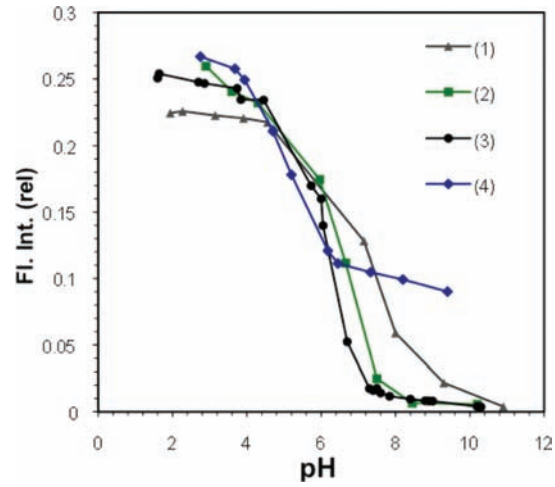


Figure 2. Fluorescence response to pH for compounds **1**−**4** measured using 0.37 μM solutions in 15% EtOH/H_2O (v/v). Excitation wavelength was 550−560 nm. Fluorescence intensities are relative to a 0.37 μM solution of rhodamine B in H_2O.

for probes **1**−**3** were as expected, with each compound showing a completely nonfluorescent form under basic conditions, which gradually increases in fluorescence intensity by 4-fold when exposed to acidic conditions. Probe **1** is the most sensitive to proton concentration, likely due to better solvation and a higher pK_a than the other three. Probe **4**, however, had a very interesting response. The tertiary amine moiety is incapable of undergoing intramolecular cyclization; therefore, it was surprising that this compound had a response to pH. The relative fluorescence intensity remains above 0.1 at all times and triples as the pH changes from ~7 to 4. Of the four analogues used to detect pH, probe **3** in Figure 2 has the most responsive curve in the "acidic window", which is regarded to be of great interest in diagnosis and understanding for some of the aforementioned diseases.

Given that spiro-cyclic structures have been thoroughly investigated in other rhodamine systems, and their existence is proven by X-ray crystallography,[7c,8a,b] we thought an NMR study should be sufficient in distinguishing between a spiro-cyclic and an open structure for our series. Specifically, we examined the ^{13}C and DEPT spectra for insight on our various derivatives.

As shown in Figure 3, probes **1**−**3** all have a quaternary carbon atom in the alkyl region of their ^{13}C spectra. This signal corresponds to the carbon atom at position 9, indicating that probes **1**−**3** exist in a spiro-cyclic structure. Probe **4**, however, has no such quaternary carbon in the alkyl region; therefore, carbon atom 9 must lie in the aromatic region. Further NMR experiments are needed to confirm the exact chemical shift of carbon atom 9 in the aromatic region. This is consistent with ^{13}C spectra of commercial rhodamine ester derivatives.[9]

Further evidence obtained by UV−vis titrations verifies a spirocyclic structure for probes **1**−**3** and proposes a mode

(7) (a) Onganer, Y.; Quitevis, E. L. *J. Phys. Chem.* **1992**, *96*, 165. (b) Boldicke, T.; Struck, F.; Schaper, F.; Tegge, W.; Sobek, H.; Villbrandt, B.; Lankenau, P.; Bocher, M. *Immunol. Methods* **2000**, *240*, 165. (c) Kenmoku, S.; Urano, Y.; Kojima, H.; Nagano, T. *J. Am. Chem. Soc.* **2007**, *129*, 7313. (d) Kobayashi, H.; Ogawa, M.; Alford, R.; Choyke, P. L.; Urano, Y. *Chem. Rev.* **2010**, *110*, 2620.

(8) (a) Wang, X.; Song, M.; Long, Y. *J. Solid State Chem.* **2001**, *156*, 325. (b) Kwon, J. Y.; Jang, Y. J.; Lee, Y. J.; Kim, K. M.; Seo, M. S.; Nam, W.; Yoon, J. *J. Am. Chem. Soc.* **2005**, *127*, 10107. (c) Ko, S. K.; Yang, Y. K.; Tae, J.; Shin, I. *J. Am. Chem. Soc.* **2006**, *128*, 14150. (d) Xiang, Y.; Tong, A. *Org. Lett.* **2006**, *8*, 1549.

(9) Ramos, S. S.; Vilhena, A. F.; Santos, L.; Almeida, P. *Magn. Reson. Chem.* **2000**, *38*, 475.

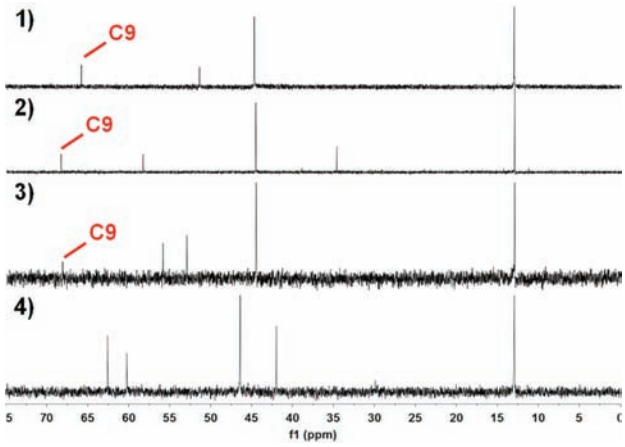


Figure 3. Alkyl region of ^{13}C NMR spectra for compounds **1**−**4**, with the quaternary carbon atom, C9, labeled in **1**−**3**. The chemical shift of C9 was determined by the corresponding DEPT spectra (see the Supporting Information).

of quenching for probe **4**. Probes **1**−**3** undergo dramatic changes in the absorbance spectra (Figure 4) as the pH is

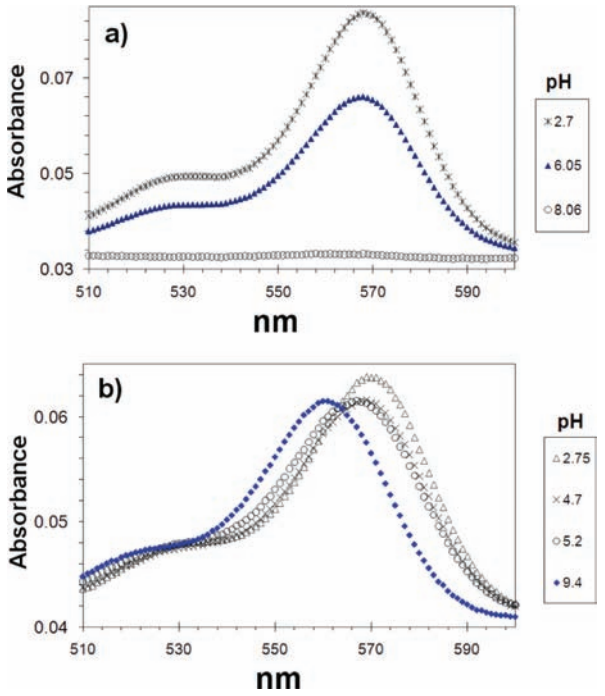
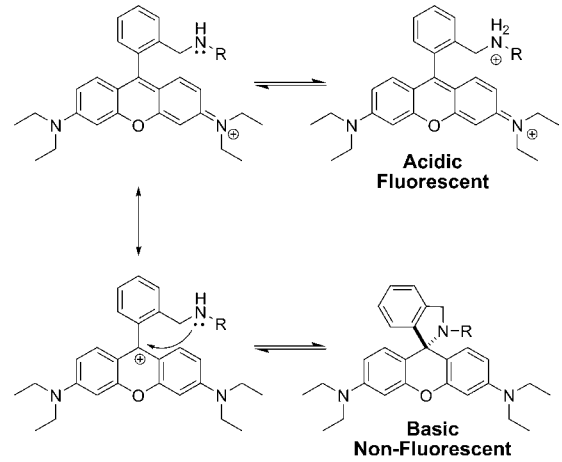


Figure 4. Absorbance spectra for probes **3** (a) and **4** (b) at various values of pH. Measured using 0.37 μM solutions in 15% EtOH/H_2O (v/v).

adjusted from acidic to basic conditions; **1**−**3** lose almost all of their absorbance, while probe **4** remains nearly invariant to changes in pH. This type of behavior where the fluorescence is modulated upon binding to some analyte, while the absorbance remains constant, is indicative of a photoinduced electron transfer mode of quenching.[10] In this proposed PET mechanism, one of the lone pair electrons on the nitrogen moiety is transferred to the excited fluorophore, which allows for a nonradiative relaxation pathway. This mechanism is disrupted when the amine becomes protonated; thus, the fluorescence is fully restored. The efficiency of this quenching process depends on the thermodynamics, allowed by Rehm−Weller formalism[11] and could therefore be "tuned" for future analogues of **4** by the direct attachment of electron-donating or -withdrawing substituents on the amine. Further characterization of the fluorescence properties are ongoing, e.g., fluorescence lifetime experiments.

In summary, a series of rhodamine-base analogues were synthesized from rhodamine B for the fluorescence based detection of pH. Probes **1**−**3** exhibited "off−on" quenching as solutions went from basic to acidic, which was characterized as a spirocyclic structure by UV−vis and NMR. Scheme 1 gives

Scheme 1. Quenching Mechanism for Probes **1**−**3**



a visual summary of the quenching mechanism for probes **1**−**3**. Probe **3** has been proposed to be the most biologically relevant probe, because of its dynamic response in the "acidic window". Probe **4** has interesting photophysical behavior and is related to a PET-type mechanism; further spectroscopic interrogation is underway.

Acknowledgment. We thank Prof. Punit Kohli (Southern Illinois University Carbondale) for use of the UV−vis instrument. This work was supported by the National Institutes of Health (NIGMS-1R15GM080721).

Supporting Information Available: Detailed synthetic procedures and spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

OL1011967

(10) (a) Daffy, L. M.; de Silva, A. P.; Gunaratne, H. Q. N.; Huber, C; Lynch, P. L. M.; Werner, T.; Wolfbeis, O. S. *Chem.—Eur. J.* **1998**, *4*, 1810. (b) Gunnlaugsson, T.; Bichell, B.; Nolan, C. *Tetrahedron Lett.* **2002**, *43*, 4989. (c) Gunnlaugsson, T.; Ali, H. D.; Glynn, M; Kruger, P. E.; Hussey, G. M.; Pfeffer, F. M.; dos Santos, C. M.; Tierney, T. *J. Fluoresc.* **2005**, *15*, 287. (d) Veale, E. B.; Tocci, G. M.; Pfeffer, F. M.; Kruger, P. E.; Gunnlaugsson, T. *Org. Biomol. Chem.* **2009**, *7*, 3447.

(11) Rehm, D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259.