ORGANIC
LETTERS

2008
Vol. 10, No. 9
1779−1782

Fluorescent Amino- and Thiopyronin Dyes

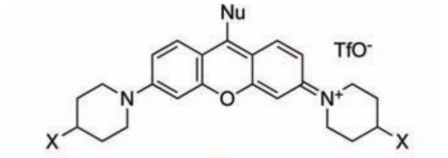
Liangxing Wu and Kevin Burgess*

*Texas A & M University, Chemistry Department, P.O. Box 30012, College Station,
Texas 77842*

burgess@tamu.edu

Received March 7, 2008

ABSTRACT



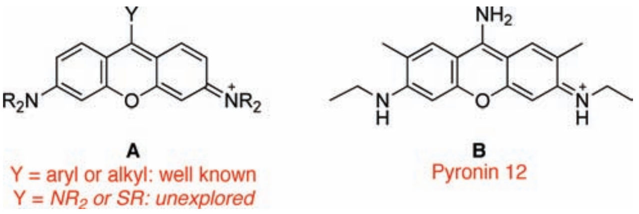
2

X = H or CO_2Me; Nu = NR_2, NHR, or SBn

eg X = H, Nu = SBn; $\lambda_{max\ emiss}$ = 619 nm, Φ 0.79

Highly fluorescent rhodamine/rosamine analogues 2 were prepared from ketone 1.

Fluorescent dyes of structure **A** (Figure 1) where Y = aryl include many compounds of the rhodamine and rosamine class.[1] Compounds **A** where Y = alkyl are known,[2–4] but they are not frequently used. However, there is almost no literature on any substances where Y = NR_2 or SR. In fact, to the best of our knowledge, the only reported compound in this category is “Pyronin 12”, i.e., structure **B**. Dyes that have been christened with a trivial name are often well-known and frequently used. However, Pyronin 12 was mentioned peripherally in a paper on dimerization of dyes in aqueous solution,[5,6] but its synthesis and spectroscopic properties were not reported. Moreover, there do not appear to be any reports of others using this dye, or any like it, since it was first described. This paper outlines syntheses and the spectroscopic properties of a range of dyes **A** where the Y substituent is an amine or a thiol.



A

Y = aryl or alkyl: well known
Y = NR_2 or SR: unexplored

B

Pyronin 12

Figure 1. Rhodamine and rosamine analogues.

The approach used in this work was based on reactions of a ditriflyl xanthone with piperidine derivatives as shown in Figure 2. The starting material for this transformation is a known compound[7] that we prepared via a slight modification of the literature procedure (see the Supporting Information); this modification was scalable to at least 13 g since, unlike the original method, no chromatography was required. Amines related to **1a** and **1b** have been prepared via condensation procedures that involve several steps;[8–13] the reaction outlined below is more direct once the common

---

(1) Haugland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*, 6th ed.; Molecular Probes: Eugene, OR, **1996**.

(2) Tombline, G.; Donnelly, D. J.; Holt, J. J.; You, Y.; Ye, M.; Gannon, M. K.; Nygren, C. L.; Detty, M. R. *Biochemistry* **2006**, *45*, 8034.

(3) Guilhem, J. *Compt. Rend.* **1959**, *248*, 2584.

(4) Babko, A. K.; Chalaya, Z. I. *Zh. Anal. Khim.* **1963**, *18*, 570.

(5) Dare-Doyen, S.; Doizi, D.; Guilbaud, P.; Djedaieni-Pilard, F.; Perly, B.; Millie, P. *J. Phys. Chem. B* **2003**, *107*, 13803.

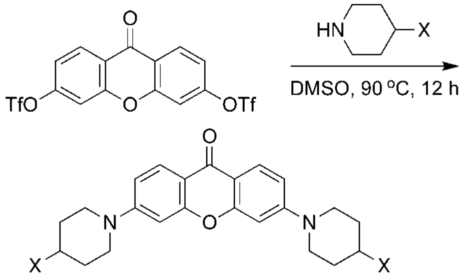
(6) Scala-Valero, C. Thesis. Synthese et caracterisation de nouveaux colorants laser, University of Orleans: Orleans, France, **1997**.

(7) Chang, M. C. Y.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2004**, *126*, 15392.

(8) Beach, S. F.; Hepworth, J. D.; Mason, D.; Swarbrick, E. A. *Dyes Pigm.* **1999**, *42*, 71.

(9) Brennan, N. K.; Donnelly, D. J.; Detty, M. R. *J. Org. Chem.* **2003**, *68*, 3344.

10.1021/ol800526s CCC: $40.75

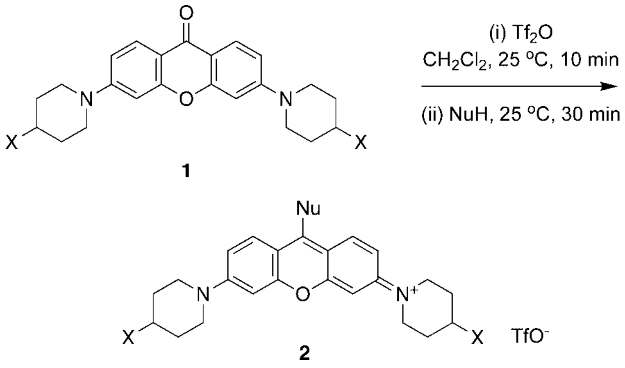


1
a, X = H, 89%
b, X = CO$_2$Me, 51%

Figure 2. S$_N$Ar reactions to prepare diamines **1**.

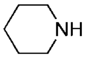
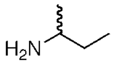
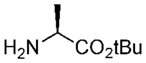
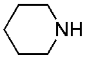
ditriflyl xanthone starting material has been made. The product precipitates from the reaction mixture after addition of water and is then recrystallized. There are, however, restrictions on the amines that can be used; less nucleophilic ones (e.g., HNBu$_2$, HNEt$_2$) tended to give mostly monosubstitution under the conditions studied (DMSO, 90 °C).

Table 1. Synthesis of Novel Pyronins



1

2

entry	X	NuH	yield (%)
2a	H		81
2b	H	Et$_2$NH	61
2c	H		98
2d	H		80
2e	H	BnSH	23
2f	MeO$_2$C		86

The ketone functionality of compounds **1** is less reactive because of the arylamine substituents; it may be considered as a vinylogous urea. Thus, no reaction was observed when piperidine was heated with the ketones under mildly acidic conditions. Conversely, the nucleophilicity of the oxygen atom is increased for the same reason; hence, it can be triflated. These triflates were not isolated, but instead were reacted with amines or benzyl thiol to give the fluorescent compounds **2a**−**f** (Table 1).

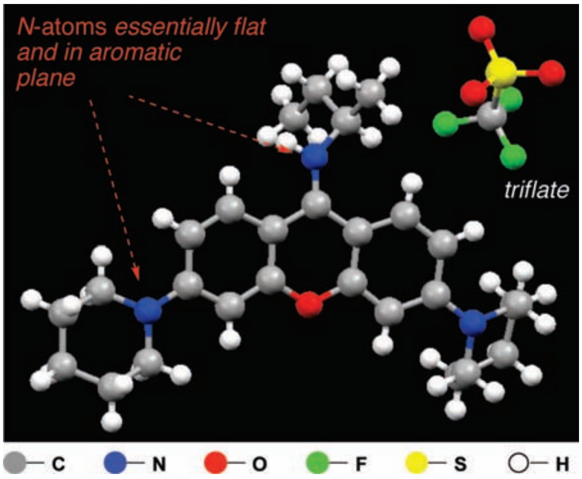


Figure 3. X-ray structure of novel pyronin **2c**.

A single-crystal X-ray structure of compound **2c** was obtained (Figure 3). This provided two pieces of evidence that all three nitrogen atoms are involved in delocalization of the positive charge from the aromatic core. First, all three nitrogens are essentially planar (deviation of the nitrogen away from the plane formed by the three substituents was observed to be 0.062 Å for the central amine and 0.175/0.223 Å for the terminal ones). Second, the deviation of that plane from the one which encapsulated the aromatic segment of the molecule was 9−22°, and the C−N distances for the bonds that connect the amine substituents to the pyronin core were 1.35−1.37 Å (whereas other C − N single bonds in the same molecule were 1.45−1.47 Å). These observations are indicative of lone pair donation from the pyronin amine substituents into the core heterocyclic framework.

Selected spectral properties of the molecules **2** are shown in Table 2. The compounds formed from nitrogen nucleophiles **2a**−**d**, **f** absorb in the range 456−499 nm with high extinction coefficients (51000−62000). They emit in the range 537−562 nm with quite sharp emissions (fwhm = 41−55 nm; cf. the same parameter for rhodamine B in CH$_2$Cl$_2$ is 36 nm, and rhodamines are generally recognized to give sharp fluorescent emissions). Quantum yields are least in the series for the compounds from secondary amines (**2a**, **2b**, and **2f**), and these emit at longer wavelengths. Other compounds in the series that were derived from primary amines (**2c** and **2d**) had markedly higher quantum yields. The *S*-containing compound **2e** absorbs and emits at sig-

---

(10) Detty, M. R.; Prasad, P. N.; Donnelly, D. J.; Ohulchanskyy, T.; Gibson, S. L.; Hilf, R. *Bioorg. Med. Chem.* **2004**, *12*2537.

(11) Del Valle, D. J.; Donnelly, D. J.; Holt, J. J.; Detty, M. R. *Organometallics* **2005**, *24*, 3807.

(12) Holt, J. J.; Calitree, B. D.; Vincek, J.; Gannon, M. K., II; Detty, M. R. *J. Org. Chem.* **2007**, *72*, 2690.

(13) Ahn, Y.-H.; Lee, J.-S.; Chang, Y.-T. *J. Am. Chem. Soc.* **2007**, *129*, 4534.

Table 2. Spectral Properties of **2** Measured in CH_2Cl_2

	λ_{max} (nm)	ϵ_{max} ($L{\cdot}mol^{-1}{\cdot}cm^{-1}$)	λ_f (nm)	fwhm[a] (nm)	Φ_f[b]
2a	491	60400	562	55	0.14 ± 0.01
2b	499	51000	558	41	0.11 ± 0.02
2c	456	52400	537	52	0.85 ± 0.01
2d	462	62000	544	52	0.90 ± 0.02
2e	598	82600	619	38	0.79 ± 0.02[c]
2f	482	54900	552	47	0.02 ± 0.01

[a] Full width at half-maximum height: a measurement of the sharpness of the fluorescence spectra. [b] Average of three measurements; fluorescein in 0.1 M NaOH (Φ = 0.92)[14] was used as a standard. [c] Cresyl violet in MeOH (Φ = 0.54)[15] was used as a standard.

nificantly longer wavelengths, and with a sharper output; for these reasons, this is possibly the most interesting dye in the series.

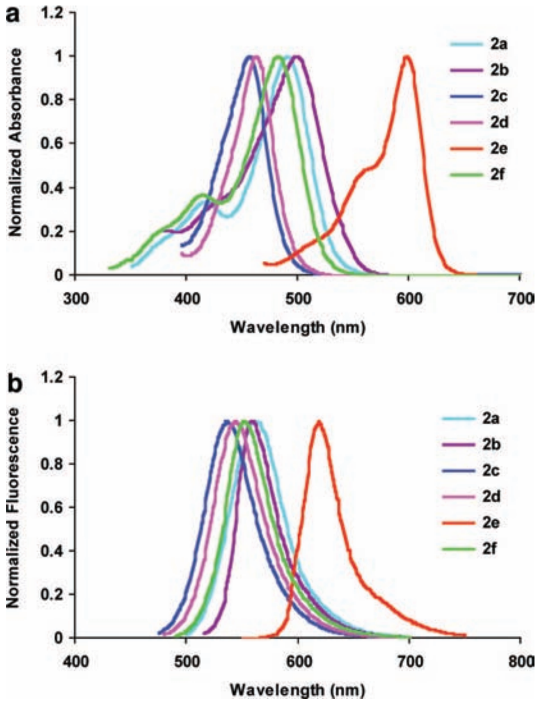


Figure 4. Normalized UV absorbance and fluorescence (excited at $\lambda_{max\ abs}$) of novel pyronins **2** in CH_2Cl_2 (ca. 10^{-6} M for absorbance; 10^{-7} M for fluorescence).

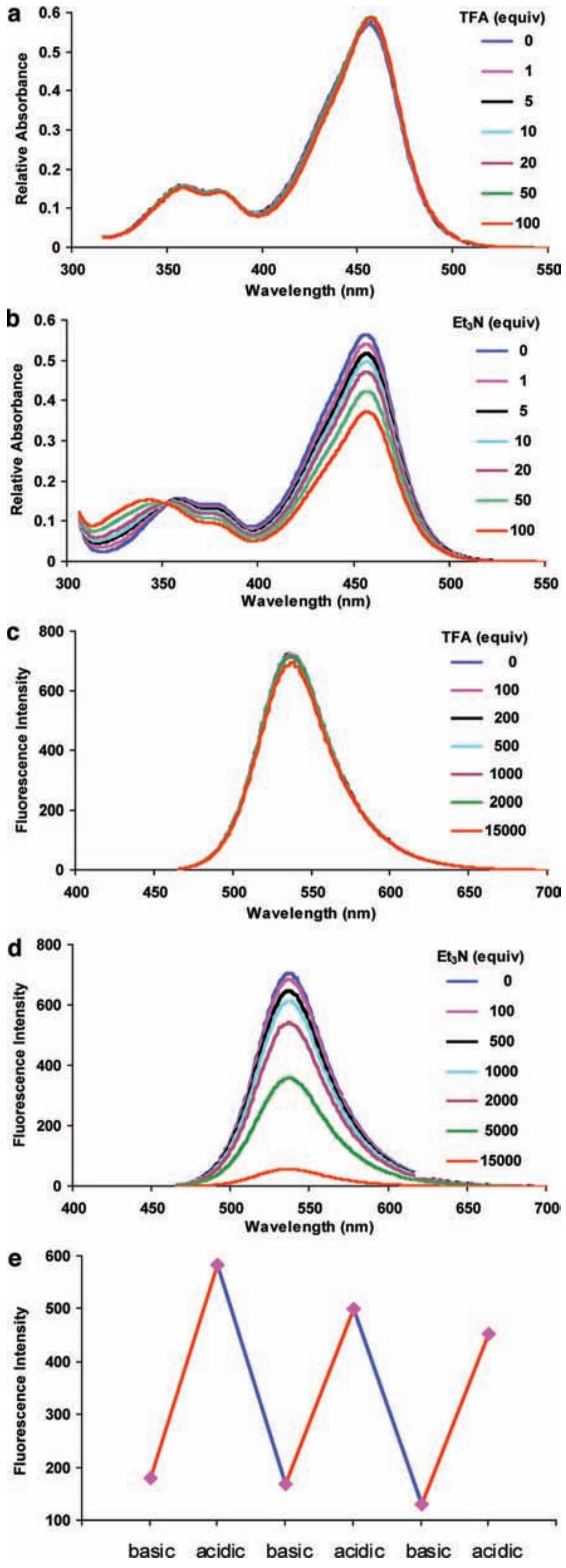


Figure 5. pH dependence of UV absorbance for **2c** under (a) acidic and (b) basic conditions (CH_2Cl_2, 1.1 × 10^{-5} M). Fluorescence pH dependence of **2c** under (c) acidic and (d) basic conditions (CH_2Cl_2, 2.5 × 10^{-7} M, excited at 456 nm). (e) Fluorescence intensity of **2c** under alternating basic (Et_3N) and acidic (TFA) conditions (CH_2Cl_2, 2.5 × 10^{-7} M, excited at 456 nm).

Normalized spectra that correspond to the data in Table 2 are shown in Figure 4. Compounds **2c** and **2d** absorb UV and fluoresce at shorter wavelengths than the others. Conversely, the absorption and fluorescence of compound **2e** is further to the red of the others in the series.

Compounds **2a**, **2c**, and **2e** were selected for pH dependence studies; data for **2c** are shown here. The UV absorption

---

(14) Weber, G.; Teale, F. W. J. *Trans. Faraday Soc.* **1958**, *54*, 640.
(15) Magde, D.; Brannon, J. H. *J. Phys. Chem.* **1979**, *83*, 696.

of this compound was uneffected by addition of TFA but was decreased by addition of NEt_3 (Figure 5a,b). The fluorescent emission characteristics of this compound followed the same trend (Figure 5c,d). Figure 5e shows that the impact of NEt_3 on the fluorescence of this compound is essentially reversible. Dye **2a**, which has a tertiary amine *meso*-substituent, showed a different pH dependence (see the Supporting Information) possibly because it does not have a relatively acidic proton. Thiol derivative **2e** showed no UV or fluorescence change on addition of TFA, but addition of NEt_3 caused decomposition (see the Supporting Information).

The spectral data summarized above are consistent with reversible deprotonation of the central amine under mildly basic conditions. That proton loss gives a less conjugated and nonfluorescent imine derivative (Figure 6).

In summary, a direct route to novel heteroatom analogs of rhodamine and rosamine systems is described here. Spectral properties of the compounds are highly dependent on the nature of the nucleophile that is used to add to the central carbon atom. In that regard, the *S*-containing compound **2e** is of particular interest to the community seeking fluorophores that emit above the 600 nm mark.

Acknowledgment. We thank Dr. J. Reibenspies at TAMU X-ray Diffraction Laboratory for the X-ray crystallographic study, and the TAMU/LBMS-Applications Laboratory directed by Dr. Shane Tichy for assistance with mass spectrometry. Support for this work was provided by The National Institutes of Health (GM72041) and by The Robert A. Welch Foundation.

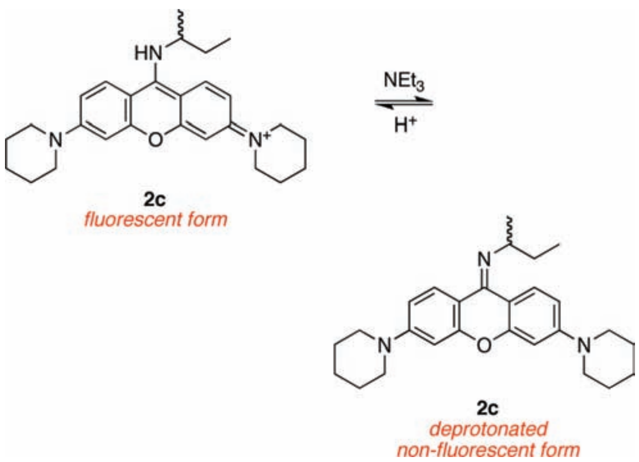


Figure 6. Likely acid/base equilibrium for **2c**.

Supporting Information Available: Experimental procedures and characterization data for the new compounds and crystallographic data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

OL800526S