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Synthesis and Spectroscopic Properties of Rosamines with Cyclic Amine Substituents

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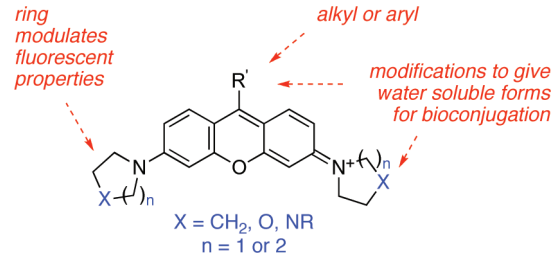

There is a close structural similarity between rosamines **A** and rhodamines **B**, yet a diversity of structures in the rosamine class and their spectral properties have yet to be explored in depth. This manuscript describes a concise, scalable, solution-phase method to obtain rosamines **1**−**5** and **12**−**15**, which include some water-soluble derivatives. In one test case (for **15**) an illustrative protein conjugate was also formed. Throughout these products were isolated and purified, and the syntheses were found to be scalable. Further, the rosamines with these cyclic amine substituents display solvent-dependent fluorescence intensities, and high quantum yields in chlorinated hydrocarbons. In some cases the nature of the cyclic amine substituent was shown to modulate the fluorescence of the parent molecules in pH-dependent ways. The ring size of those amine substituents also correlated with some of their spectroscopic properties. Several water-soluble rosamines were prepared from some of the addition products **1**−**5**, and one of these, **15**, was efficiently conjugated to avidin via an amide linkage. The spectroscopic properties of **15** and **15**-avidin in aqueous media were very similar.

Introduction

From a structural perspective, rosamines **A**[1,2] relate to rhodamines **B** in much the same way that Tokyo Green **C**[3,4] and Pennsylvania Green **D**[5,6] are similar to fluorescein **E**. The recent excitement caused by these fluorescein derivatives[7] can be attributed to the fact that the methyl group on the *meso* aromatic ring attenuates nonradiative decay via free rotation of the aryl group, while lack of a carboxylic acid makes the system impossible to lactonize to much less fluorescent forms at low pH, i.e., it is less acid-sensitive than fluorescein.

There is considerable potential for modulating the chemical and spectroscopic properties of rosamines by varying the *C-meso* substituent, but the scope of such research is limited by synthetic constraints. The first syntheses of rosamines center around condensation reactions of the type shown in Figure 1a.[1,2,8,9] Such approaches tend to involve high temperatures. They can be facilitated via microwave heating,[10] but even in those cases the conversion to product is not very efficient. Other work deals with modification of the 2-carboxylate group of rhodamines (not

(1) Zhang, Y.-Z.; Haugland, R. P. U.S. Patent 5,686,261, 1997.
(2) Mao, F.; Leung, W.-Y.; Haugland, R. P. WO 99/15517, 1999.
(3) Urano, Y.; Kamiya, M.; Kanda, K.; Ueno, T.; Hirose, K.; Nagano, T. *J. Am. Chem. Soc.* **2005**, *127*, 4888–4894.
(4) Martin, M. M.; Lindqvist, L. *J. Lumin.* **1975**, *10*, 381–390.
(5) Mottram, L. F.; Boonyarattanakalin, S.; Kovel, R. E.; Peterson, B. R. *Org. Lett.* **2006**, *8*, 581–584.
(6) Mottram, L. F.; Maddox, E.; Schwab, M.; Beaufils, F.; Peterson, B. R. *Org. Lett.* **2007**, *9*, 3741–3744.
(7) Peterson, B. R. *Chem. Eng. News* **2006**, *84*, 33.
(8) Liu, J.; Diwu, Z.; Leung, W.-Y.; Lu, Y.; Patch, B.; Haugland, R. P. *Tetrahedron Lett.* **2003**, *44*, 4355–4359.
(9) Wang, Z.-Q.; Diwu, Z.; Francisco-Reyes, J.; Yi, G. G. *Chem. Lett.* **2005**, *34*, 404–405.
(10) Jiao, G.-S.; Castro, J. C.; Thoresen, L. H.; Burgess, K. *Org. Lett.* **2003**, *5*, 3675–3677.

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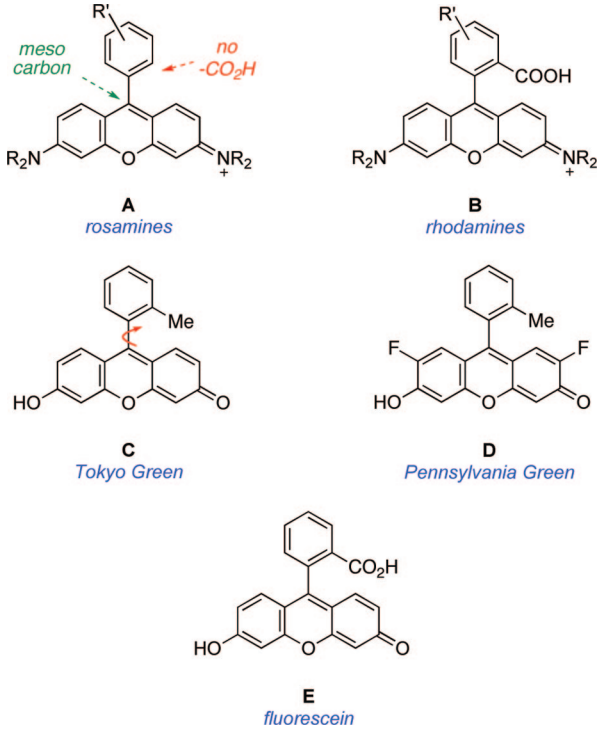


shown),[11,12] but the parent systems are also made via condensation reactions at elevated temperatures. An alternative approach to syntheses of rosamine dyes is to add an organo-lithium or -magnesium compound to the carbonyl group of a xanthone and then protonate to cause loss of water and generate the product.[13] That approach has been applied extensively by Detty and co-workers[14] to make rosamines (and especially S-, Se-, and Te-analogs),[15−17] for their photodynamic and other medicinal properties.[18−22] Similarly, attack of aryl Grignards on solid-supported xanthones **F** was used in a supported parallel synthesis approach to a library of rosamine derivatives **G** (Figure 1b).[23]

For several years, work in our laboratory has focused on a solution-phase approach to the rosamine dye cores **1**−**5** with a variety of *meso*-substituents **a**−**i**. The attributes of this approach are that (i) the xanthone starting materials were obtained via S_NAr reactions of a relatively accessible ditriflate; (ii) the *meso*-substituent can be alkyl or aryl; and (iii) the products were isolated as discrete compounds that could be purified to obtain accurate spectroscopic data, whereas this is difficult via a solid-phase approach. The spectroscopic data obtained on the products show some unexpected dependence on solvent and amine ring size. Further, some of the dyes could be modified to water-soluble forms with functional groups to allow conjugation to proteins.

Results and Discussion

Synthesis. The ditriflate **6** was easily prepared on a multigram scale without chromatography via a slight modification of a known procedure.[24,25] This reacted with piperidine, morpholine, Boc-piperazine, and pyrrolidine to give the symmetrical products **7**−**10** as indicated in Table 1. Stepwise addition to give an unsymmetrical product was also explored briefly, and this proved to be feasible (reaction 1), presumably because the product of the first addition (a vinylogous amide) is less reactive than the starting material.

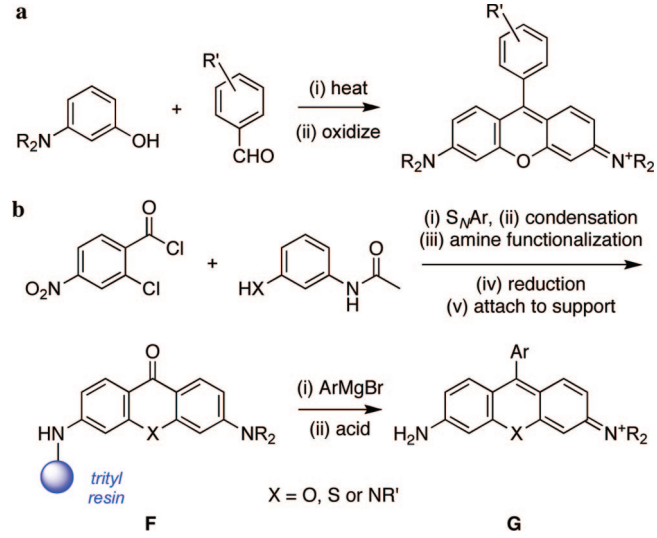
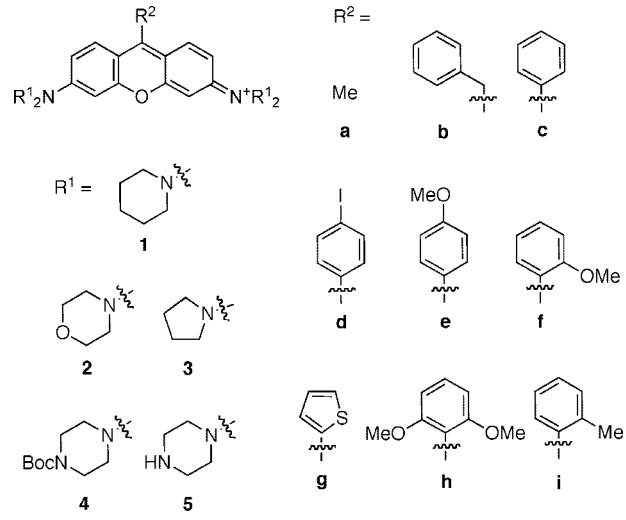
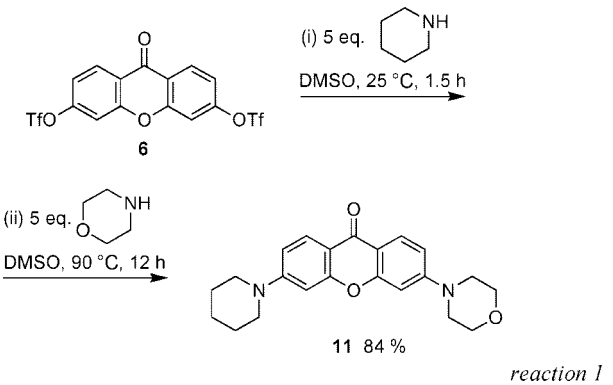


FIGURE 1. Syntheses of rosamine dyes via (a) the conventional condensation approach and (b) nucleophilic additions to xanthones (shown for a solid-supported synthesis).



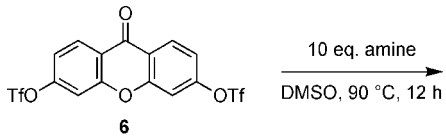


reaction 1

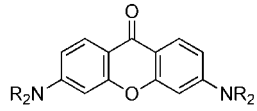
Ketones **7**−**11** are not exceptionally reactive. They are vinylogous ureas, but they combined efficiently with organo-lithium and Grignard reagents to give tertiary alcohols. These adducts were not isolated; instead they were treated with aqueous

(11) Dujols, V.; Ford, F.; Czarnik, A. W. *J. Am. Chem. Soc.* **1997**, *119*, 7386–7387.
(12) Adamczyk, M.; Grote, J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1539–1541.

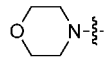
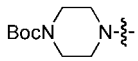
TABLE 1. Amination of the Ditriflyl Xanthone 6



6

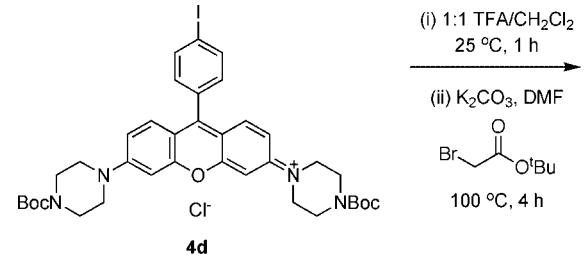


7 - 10

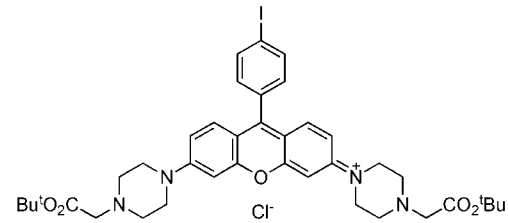
product	NR_2	yield (%)
7		89
8		93
9		60
10		18

acid to give the desired products as indicated in Table 2. All the compounds were isolated in tens to hundreds of milligram amounts via flash chromatography. However, in three cases (**1d**, **1h**, and **4d**), several grams of product were isolated via recrystallization of crude materials obtained on a larger scale. The benzenoid compounds shown in Table 2 have methyl-, iodo-, and one or two methoxy substituents on the *meso*-aryl group. Steric effects associated with *ortho*-substitution did not seem to have any significant adverse effects on the syntheses of any compounds in this series.

None of the compounds shown in Table 2 are water-soluble, but several modifications were tested to demonstrate that water-soluble derivatives are accessible from some of the products. Reaction 2, for instance, illustrates how the piperazine derivative **4d** could be unmasked to give a bisammonium salt. That salt was not isolated, but an aqueous solution of it was strongly fluorescent, and it was neutralized then reacted with *tert*-butyl bromoacetate to give the diester **13**. Treatment of diester **13** with trifluoroacetic acid gave a product that was water-soluble and fluorescent but difficult to isolate. The inference of these experiments is that the intermediate ammonium salt and the diacid derived from it could be prepared via deprotection of their precursors and then used to couple to biomolecules.



4d

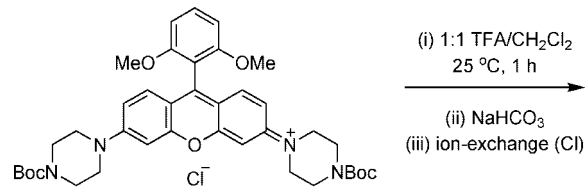


13 76 %

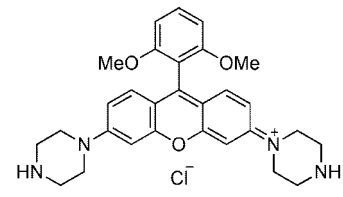
reaction 2

The dimethoxy compound **1h** was also modified to give a water soluble analog. Scheme 1 outlines how this was achieved via a demethylation procedure to give the phenol **14**. That derivative has no functional group for direct attachment to a biomolecule, and in any case it is only slightly water-soluble. Consequently, the two phenol groups were alkylated with *tert*-butyl bromoacetate, and then the esters were deprotected to give the diacid **15**. Diacid **15** is a polar, water-soluble compound that was conveniently isolated by washing away residual starting materials using acetonitrile/ether. This derivative was conjugated to avidin to explore how its spectroscopic properties are modulated by coupling to this protein (see below).

Another modification to give a water-soluble probe is shown in reaction 3. Here the two *tert*-butyloxycarbonyl groups of rosamine **4h** were removed with acid to give, after neutralization and ion exchange, the bisamine **5h**. Compound **5h** dissolves easily in water; its spectroscopic properties in buffers were explored extensively (see below).



4h



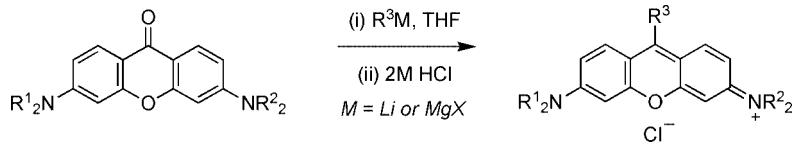
5h 83 %

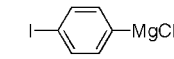
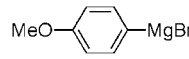
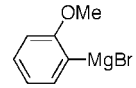
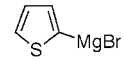
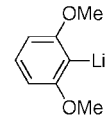
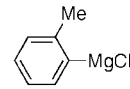
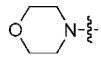
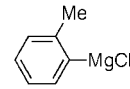
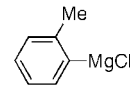
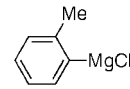
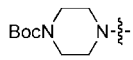
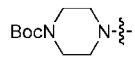
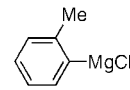
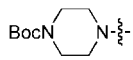
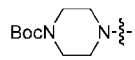
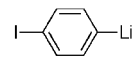
reaction 3

Spectroscopic Properties of Rosamine Derivatives. Table 3 shows UV−vis absorption wavelength maxima, molar extinction

(13) Beach, S. F.; Hepworth, J. D.; Mason, D.; Swarbrick, E. A. *Dyes Pigm.* **1999**, *42*, 71–77.
(14) Gannon, M. K., II.; Detty, M. R. *J. Org. Chem.* **2007**, *72*, 2647–2650.
(15) Brennan, N. K.; Donnelly, D. J.; Detty, M. R. *J. Org. Chem.* **2003**, *68*, 3344–3347.
(16) Del Valle, D. J.; Donnelly, D. J.; Holt, J. J.; Detty, M. R. *Organometallics* **2005**, *24*, 3807–3810.
(17) Holt, J. J.; Calitree, B. D.; Vincek, J.; Gannon, M. K., II.; Detty, M. R. *J. Org. Chem.* **2007**, *72*, 2690–2693.
(18) Holt, J. J.; Gannon, M. K.; Tombline, G.; McCarty, T. A.; Page, P. M.; Bright, F. V.; Detty, M. R. *Bioorg. Med. Chem.* **2006**, *14*, 8635–8643.
(19) Gibson, S. L.; Holt, J. J.; Ye, M.; Donnelly, D. J.; Ohulchanskyy, T. Y.; You, Y.; Detty, M. R. *Bioorg. Med. Chem.* **2005**, *13*, 6394–6403.
(20) Wagner, S. J.; Skripchenko, A.; Donnelly, D. J.; Ramaswamy, K.; Detty, M. R. *Bioorg. Med. Chem.* **2005**, *13*, 5927–5935.
(21) Tombline, G.; Donnelly, D. J.; Holt, J. J.; You, Y.; Ye, M.; Gannon, M. K.; Nygren, C. L.; Detty, M. R. *Biochemistry* **2006**, *45*, 8034–8047.
(22) Tombline, G.; Holt, J. J.; Gannon, M. K.; Donnelly, D. J.; Wetzel, B.; Sawada, G. A.; Raub, T. J.; Detty, M. R. *Biochemistry* **2008**, *47*, 3294–3307.
(23) Ahn, Y.-H.; Lee, J.-S.; Chang, Y.-T. *J. Am. Chem. Soc.* **2007**, *129*, 4534–4535.
(24) Miller, E. W.; Albers, A. E.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 16652–16659.
(25) Wu, L.; Burgess, K. *Org. Lett.* **2008**, *10*, 1779–1782.

TABLE 2. Synthesis of Rosamine Derivatives

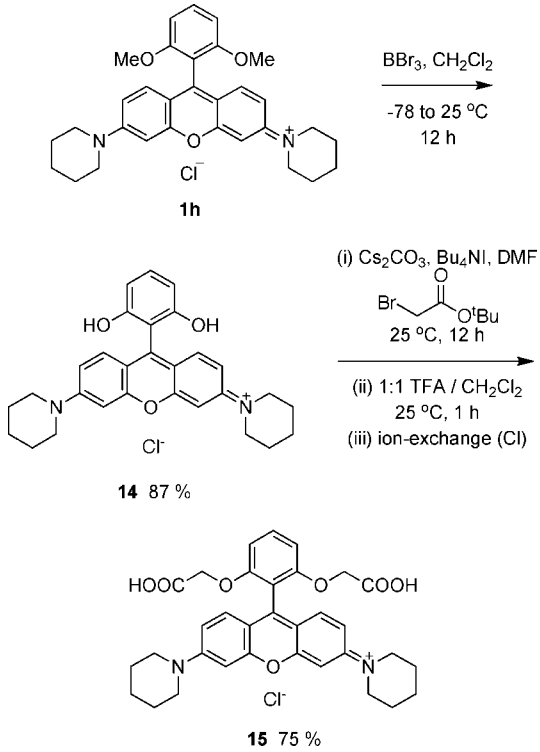


entry	NR^1_2	NR^2_2	R^3M	product	yield (%)
1			CH_3MgBr	**1a**	89
2			$PhCH_2MgCl$	**1b**	70
3			PhMgBr	**1c**	100
4				**1d**	90
5				**1e**	100
6				**1f**	100
7				**1g**	74
8				**1h**	96
9				**1i**	99
10				**2i**	98
11				**3i**	53
12				**12i**	98
13				**4i**	90
14				**4d**	68
15				**4h**	94

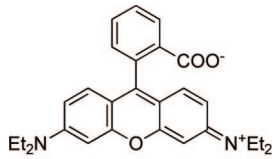
coefficients, fluorescence emission maxima, fluorescence peak broadness (measured by full width at half-maximum height, fwhm), and quantum yields for the target compounds. These data were collected for all the compounds when dichloromethane was used as solvent. However, we observed that the *meso* aliphatic compounds **1a** and **1b** tend to be less stable in polar media.

Relative to the other compounds, the *meso*-methyl derivative **1a** has blue-shifted absorption and fluorescence maxima, but

SCHEME 1. Synthesis of Water-Soluble Rosamine



the extinction coefficient and the quantum yield are comparable with the rosamines having aromatic *meso*-substituents. This indicates that the *meso*-aryl groups are not significantly conjugated to the xanthene core, presumably because steric factors involving these substituents make it energetically unfavorable for the molecules to become planar. Curiously, the benzyl compound **1b** is spectroscopically more similar to the *meso*-phenyl compound **1c** than to the methyl derivative **1a**. The emission peak broadness values in this series range from 31 to 44 nm (CH_2Cl_2); these values are comparable with a typical rhodamine, rhodamine B (36 nm in CH_2Cl_2).



rhodamine B

Several trends emerge from the spectroscopic data recorded for the compounds dissolved in dichloromethane. Compound **1g** is most red-shifted in terms of the wavelengths for absorption maxima and the fluorescence emission maxima. However, the quantum yield for this compound was about a third of that observed for other members in this series; this observation can be attributed to partial fluorescence quenching via electron transfer from the *S*-containing heterocycle.[26] Quantum yields for the Boc-piperazine derivatives were also low relative to other members of the series. The very best quantum yield observed was for the pyrrolidine derivative **3i**. To the best of our knowledge, this work is the first to uncover a relationship between the ring size of the peripheral amines in *rosamines* and their fluorescent quantum yields, but others have commented

TABLE 3. Spectral Properties of Rosamines

	$\lambda_{max\ abs}$ (nm)	ϵ_{max}	λ_{fluor} (nm)	fwhm (nm)[a]	Φ_f[b]
			CH_2Cl_2		
1a	555	101,900	570	35	0.91 ± 0.05
1b	566	95,900	582	34	0.87 ± 0.01
1c	565	106,000	585	37	0.89 ± 0.01
1d	570	115,400	590	38	0.85 ± 0.01
1e	562	116,700	582	35	0.91 ± 0.02
1f	566	118,000	585	37	0.92 ± 0.03
1g	582	107,600	603	41	0.33 ± 0.01[c]
1h	565	87,300	586	36	0.86 ± 0.04
1i	565	123,600	582	34	0.89 ± 0.01
2i	554	106,900	575	37	0.80 ± 0.01
3i	558	127,400	573	31	1.00 ± 0.09
12i	559	111,000	579	36	0.59 ± 0.01
4i	555	120,800	577	40	0.47 ± 0.01
4d	561	77,900	585	42	0.41 ± 0.01
4h	555	109,300	580	40	0.49 ± 0.02
13	564	62,800	586	44	0.007
			EtOH		
1c	564	86,700	588	39	0.30 ± 0.01
1d	568	70,700	592	40	0.28 ± 0.01
1e	561	100,400	582	37	0.31 ± 0.01
1f	564	115,600	586	37	0.36 ± 0.01
1g	581	56,500	606	43	0.10 ± 0.01[c]
1i	564	119,500	584	36	0.28 ± 0.01
2i	556	98,600	582	38	0.16 ± 0.01
3i	558	120,500	576	33	0.95 ± 0.01
12i	560	97,000	582	38	0.14 ± 0.01
4i	556	108,000	580	39	0.19 ± 0.01
14	557	71,000	580	37	0.26 ± 0.01
15	557	84,800	580	38	0.49 ± 0.02
5h	561	81,000	582	45	0.008
			0.1 M phosphate buffer, pH = 7.4		
15	567	76,500	594	42	0.13 ± 0.01
5h	542	71,500	568	41	0.14 ± 0.02

[a] Full width at half-maximum height: a measure of the sharpness of the fluorescence peaks. [b] Average of three measurements and rhodamine 101 was used as a standard (Φ = 1.0 in EtOH).[32] [c] Cresyl Violet (Φ = 0.54 in MeOH)[33] was used as a standard.

on similar effects in the context of 4-amino-1,8-napthalimide[27] and 4-aminophthalimide derivatives.[28] The origin of these effects is thought to be that smaller-ring cyclic amine substituents are less inclined to invert at nitrogen atom in the excited state, so this pathway for nonradiative loss is disfavored and the overall quantum yields are higher.[29]

Differences between the piperidine derivatives **1** and the pyrrolidine derivative **3i** are accentuated in a more polar medium (ethanol, second section of Table 3). This is also consistent with previous reports; [27−29] more polar solvents favor intramolecular charge transfer from cyclic amines where the N-atom more closely approximates a planar arrangement as a result of facile interconversion between ring forms. The interconversion is more rapid for piperidine derivatives than the pyrrolidine-based compounds.[27] Overall, the quantum yield data for the compounds in ethanol are, in all the cases measured, less than that in dichloromethane. This is a somewhat unexpected finding since other rosamines are known to be very highly fluorescent even

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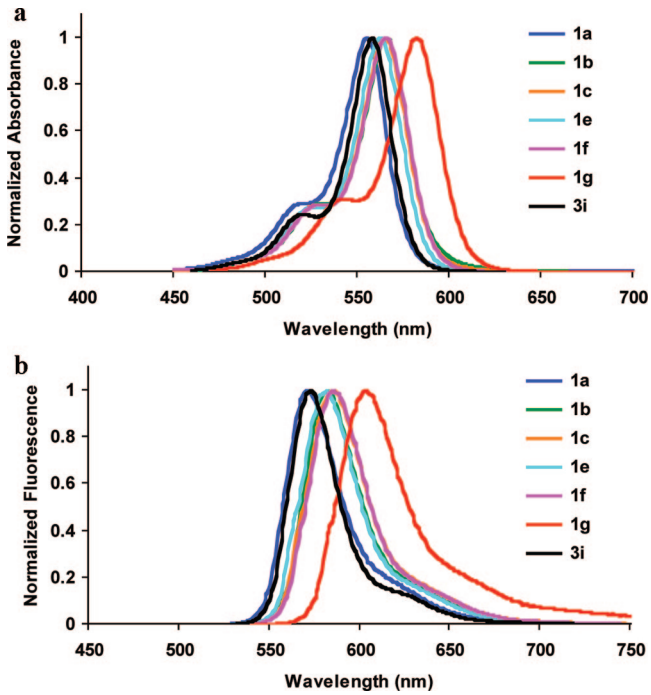

FIGURE 2. (a) UV−vis absorption (10^{-6} M) and (b) fluorescence spectra (10^{-7} M, excited at $\lambda_{max\ abs}$), for representative rosamines in CH_2Cl_2.

in alcohols; for instance, tetramethyl rosamine, has a quantum yield of 0.84 in MeOH.[30,31]

Quantum yields of 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dyes with *meso*-aryl substituents are thought to be increased if the aryl group has an *ortho*-substituent, or if rotation of the aryl is otherwise restricted.[34] Similarly, fluorescein and derivatives with 2-substituted *meso*-aryl groups are far more fluorescent than the corresponding *meso*-phenyl compound.[3,4] However, it is remarkable that in the series outlined in Table 3, unlike the analogous BODIPY dyes, rosamines with *ortho*-substituted *meso*-aryl groups (**1i** {2-Me}, **1f** {2-OMe}, **1h** {2,6-OMe}) have similar quantum yields to the phenyl derivative (**1c**).

The only nonsymmetrical compound (with respect to a plane through the *meso*-substituent) is the piperidine/morpholine derivative **12i**. This has a lower quantum yield than similar symmetrical compounds (i.e., **1i** and **2i**). The water-soluble derivatives **15** and **5h** had quantum yields of approximately 0.13 in 0.1 M phosphate buffer (pH 7.4).

Some illustrative UV−vis absorption and fluorescence emission spectra for molecules of Table 3 are shown in Figure 2. These data show that the *meso*-thiophene derivative **1g** is red-shifted, both in terms of absorption and fluorescence, relative to the other compounds. Absorption and emissions from all the other probes are clustered in a relatively narrow wavelength range, with the *meso*-methyl derivative **1a** at the blue end of the scale.

The piperidine derivative **1i** was chosen as a typical dye to study solvent effects on absorption maxima, fluorescence

TABLE 4. Spectral Properties of Rosamines 1i in Different Solvents

	solvent		spectral properties		
	dielectric constant	dipole moment	$\lambda_{max\ abs}$ (nm)	λ_{fluor} (nm)	Φ_f[a]
$CHCl_3$	4.8	1.04	566	580	0.95 ± 0.02
EtOAc	6.1	1.78	567	586	0.19 ± 0.01
THF	7.5	1.75	567	588	0.32 ± 0.01
CH_2Cl_2	8.9	1.60	565	582	0.89 ± 0.01
acetone	21.0	2.88	564	587	0.15 ± 0.01
EtOH	24.3	1.69	564	584	0.28 ± 0.01
MeOH	33.0	1.70	562	582	0.19 ± 0.01
CH_3CN	36.6	3.92	562	586	0.14 ± 0.01
DMF	38.3	3.82	570	594	0.20 ± 0.01

[a] Rhodamine 101 (Φ = 1.0 in EtOH) was used as a standard.

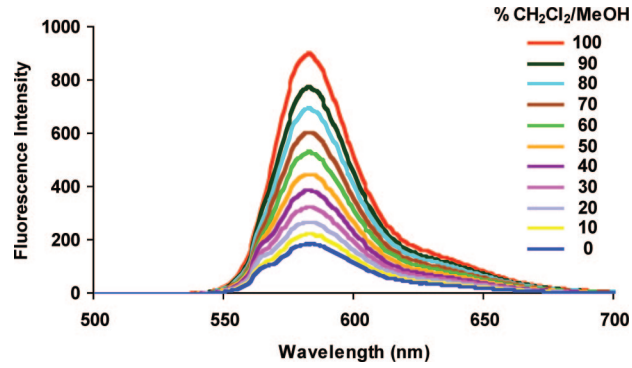

FIGURE 3. Fluorescence intensity (excited at 562 nm) of **1i** at 1.4×10^{-7} M in a CH_2Cl_2/MeOH mixed solvent system. The shoulder observed at around 560 nm is an artifact caused by the excitation source, which was necessarily close in wavelength.

emission maxima, and quantum yields (Table 4). It emerged that the fluorescence efficiency of the rosamines is quite dependent on the solvent used. Two of those with small dipole moments (CH_2Cl_2 and $CHCl_3$, the compounds are not soluble in aliphatic hydrocarbons) gave optimal fluorescence quantum yields. No significant differences were observed in the UV−vis absorption maxima for these compounds. Similarly, the Stokes shifts observed are all within the range 14−24 nm.

Data presented in Table 4 indicate the intensity of fluorescence emission from **1i** can be used as a probe for solvent polarity. This assertion was confirmed via a series of measurements in a dichloromethane/methanol mixed solvent system (Figure 3). The intensity of the fluorescence of this compound (at 1.4×10^{-7} M) increased nearly linearly with the proportion of dichloromethane (see Supporting Information).

The fact that the molecules were most fluorescent in two less polar solvents could be due to aggregation in polar media. This possibility was tested by measuring fluorescence wavelength maxima for **1i** as a function of concentration in ethanol (a representative polar solvent; Figure 4). In fact, the wavelength for emission maxima did not change significantly over a concentration range of 5×10^{-9} to 5×10^{-6} M. This implies aggregation is *not* significant at those concentrations. Experiments with the nonionic detergent Triton-X 100 gave no appreciable change in the fluorescence spectra of the dye in this concentration range (see Supporting Information), further supporting the assertion that **1i** does not aggregate. At a relatively high concentration of the dye, 2×10^{-5} M, there was a 7 nm red shift in the emission maximum for the fluorescence spectrum, indicating aggregation may only be a problem under such conditions.

(30) http://probes.invitrogen.com. In Molecular Probes; Invitrogen Corporation: Carlsbad, CA, 2006.
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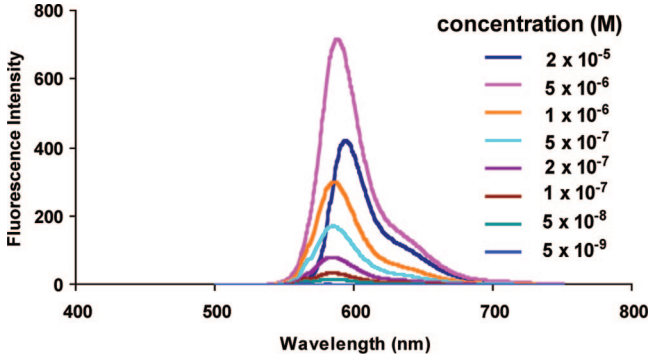

FIGURE 4. Aggregation study of **1i** in EtOH (excited at 564 nm).

Predictably, the quantum yields of the piperazine derivatives **4**, **5h**, and **13** are somewhat dependent on the nitrogen atoms at the periphery of the molecule. Derivatives for which this nitrogen is protected with a *tert*-butyloxycarbonyl groups have moderate quantum yields. However, the free secondary amine of compound **5h**, for example, almost completely quenches the fluorescence unless it is protonated; this is because photoinduced electron transfer (PET) mechanisms may be possible for the amine.[26] Similarly, compound **13** in the free base form is almost completely nonfluorescent in dichloromethane, but solutions of this dye become strongly fluorescent upon protonation. The photophysical properties of rosamine **5h** are pH-dependent. The absorption and fluorescence maxima of dye **5h** slightly red-shifted with increasing pH from 2 to 12 (see Supporting Information for details). Figure 5a shows how the fluorescence of **5h** drops dramatically when the pH is raised above ~6, while Figure 5b demonstrates that these fluorescence changes are reversible.

Finally, the water-soluble probe **15** was conjugated with avidin via activation of the dye-carboxylic acids (*N*-hydroxysuccinimide and *N*,*N*′-diisopropylcarbodiimide in DMF) and then addition of the activated probe to the protein in 0.1 M $NaHCO_3$ at pH 8.3. The dye:protein ratio was calculated to be 0.8 by UV−vis when 3 equiv of the dye was used; this corresponds to 27% labeling efficiency. When 5 equiv of dye was used then a dye:protein ratio of 1.4 (28% labeling efficiency) was observed. The latter sample was used to obtain the spectral data shown in Figure 6. The UV−vis absorption and fluorescence emission maxima of **15** on avidin were observed to be within a few nanometers of the dye in phosphate buffer. The quantum yield of the protein conjugate was 0.06 in phosphate buffer compared with 0.13 for the free dye **15**.

Conclusions

This paper describes efficient solution-phase syntheses of rosamines with cyclic amine substituents. It is amenable to scale-up and purification of the products. Further, water-soluble derivatives can be prepared via modification of the initial products. The rosamine products are shown to be more fluorescent in dichloromethane and chloroform than in more polar solvents; this is not the case for some commercially available rosamines with acyclic amine substituents. That solvent dependence might be useful in some applications. Further, the data collected here illustrate that the ring size of the cyclic amine substituents has significant effects on the quantum yields; this trend has not been noted previously for rosamine dyes. Finally, the spectroscopic properties of the water-soluble rosamine **15** conjugated to a protein was not significantly different to that of the free dye in aqueous media.

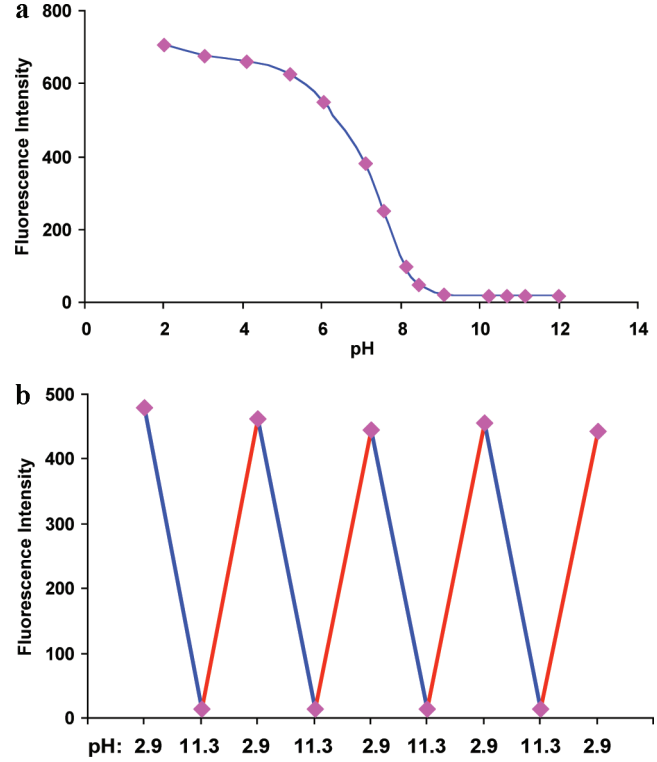

FIGURE 5. Dependence of electronic spectra of **5h** on pH. (a) Fluorescence intensities ($\lambda_{max\ emiss}$, excited at $\lambda_{max\ abs}$, 5.7×10^{-7} M) versus pH in phosphate solutions (50 mM, H_3PO_4 + NaOH); (b) reversibility (excitation at $\lambda_{max\ abs}$ 538 nm and detection at $\lambda_{max\ emiss}$ 565 nm for pH 2.9; excitation at $\lambda_{max\ abs}$ 560nm and detection at $\lambda_{max\ emiss}$ 584 nm for pH 11.3; throughout the dye was used at 5.7×10^{-7} M and the pH was adjusted with 2 M HCl or NaOH).

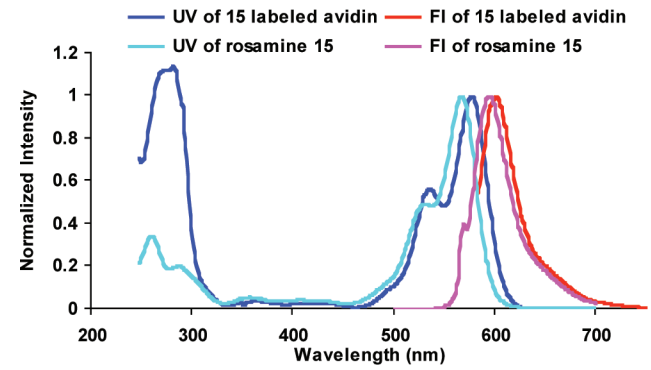

FIGURE 6. UV−vis absorption and fluorescence spectra of rosamines **15** and **15**-labeled avidin in 0.1 M phosphate buffer, pH 7.4.

Experimental Section

General Procedure for Amination of Ditriflyl Xanthone. 3,6-Di-OTf-xanthone **6** (1.0 equiv) was dissolved in DMSO (0.2 M) and the amine (10 equiv) was added. The reaction mixture was heated to 90 °C and stirred for 12 h. After cooling to room temperature, the reaction was quenched with water. The precipitate was collected, washed with saturated aqueous Na_2CO_3 and water to give the crude product, which was recrystallized from EtOAc/hexanes to afford the pure product.

Spectral data for a few representative compounds are listed here. Complete data are given in Supporting Information.

3,6-Dipiperidin-1-yl-xanthen-9-one (7). Yellow solid (3.0 g, 89%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.86 (d, 2H, J = 9.0 Hz), 6.98 (dd, 2H, J = 9.0, 2.3 Hz), 6.74 (d, 2H, J = 2.3 Hz), 3.41 (br, 8H), 1.60 (br, 12H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.0, 157.7, 154.8, 126.8, 111.7, 111.3, 98.8, 47.8, 24.8, 23.9. MS (ESI) m/z calcd for $(M + H)^+$ $C_{23}H_{26}N_2O_2$ 363.21; found 363.21.

3,6-Dimorpholin-4-yl-xanthen-9-one (8). White solid (308 mg, 93%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (d, 2H, J = 9.0 Hz), 6.86 (dd, 2H, J = 9.0, 2.4 Hz), 6.67 (d, 2H, J = 2.4 Hz), 3.86 (t, 8H, J = 4.9 Hz), 3.33 (t, 8H, J = 4.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.1, 158.0, 155.3, 127.7, 114.1, 111.2, 100.0, 66.5, 47.5. MS (ESI) m/z calcd for $(M + H)^+$ $C_{21}H_{23}N_2O_4$ 367.17; found 367.17.

3,6-Bis(4-Boc-piperazin-1-yl)-xanthen-9-one (9). White solid (680 mg, 60%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (d, 2H, J = 9.0 Hz), 6.86 (dd, 2H, J = 9.0, 2.3 Hz), 6.66 (d, 2H, J = 2.3 Hz), 3.59 (t, 8H, J = 5.2 Hz), 3.37 (t, 8H, J = 5.2 Hz), 1.47 (s, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0, 158.1, 155.0, 154.6, 127.8, 114.0, 111.7, 100.3, 80.2, 47.3(2C), 28.4. MS (ESI) m/z calcd for $(M + H)^+$ $C_{31}H_{41}N_4O_6$ 565.30; found 565.29.

3,6-Dipyrrolidin-1-yl-xanthen-9-one (10). Yellow solid (90 mg, 18%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (d, 2H, J = 8.9 Hz), 6.50 (dd, 2H, J = 8.9, 2.2 Hz), 6.28 (d, 2H, J = 2.2 Hz), 3.34 (t, 8H, J = 6.6 Hz), 2.01 (t, 8H, J = 6.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0, 158.1, 151.6, 127.6, 111.5, 109.1, 96.5, 47.6, 25.4. MS (ESI) m/z calcd for $(M + H)^+$ $C_{21}H_{23}N_2O_2$ 335.18; found 335.18.

General Procedure for the Synthesis of Rosamine Derivatives. A Grignard reagent or lithium reagent (1.0 mmol) was added dropwise over 1 min to the solution of 3,6-diamino-xanthen-9-one (0.2 mmol) in 5 mL of THF at 0 °C. After stirring for 12 h at room temperature, the reaction mixture was quenched by addition of 2 mL of 2 M aqueous HCl, stirred for 10 min, and then diluted with 20 mL of CH_2Cl_2. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash chromatography (5% to 10% MeOH/CH_2Cl_2) to give the pure product.

Rosamine 1a. Purple solid (71 mg, 89%). R_f = 0.28 (10% MeOH/CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (d, 2H, J = 9.6 Hz), 7.22 (dd, 2H, J = 9.6, 2.5 Hz), 6.77 (d, 2H, J = 2.5 Hz), 3.66 (br, 8H), 2.87 (s, 3H), 1.72 (br, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.6, 156.4, 156.3, 130.1, 114.8, 114.0, 96.8, 48.8, 25.8, 24.1, 14.8; IR (thin film) 1643, 1597, 1486, 1401, 1235, 1200 cm^{-1}; HRMS (ESI) m/z calcd for $(M - Cl)^+$ $C_{24}H_{29}N_2O$ 361.2280; found 361.2287.

Rosamine 2i. Green solid (94 mg, 98%). R_f = 0.26 (10% MeOH/CH_2Cl_2). ^{1}H NMR (500 MHz, CD_3OD) δ 7.59−7.46 (m, 3H), 7.30−7.23 (m, 7H), 3.87−3.85 (m, 8H), 3.79−3.77 (m, 8H), 2.06 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 160.2, 159.9, 159.1, 137.2, 133.0, 132.8, 131.9, 131.4, 130.1, 127.3, 116.3, 115.8, 98.6, 67.4, 48.5, 19.6; IR (thin film) 1646, 1590, 1481, 1415, 1383, 1235, 1190 cm^{-1}; HRMS (ESI) m/z calcd for $(M - Cl)^+$ $C_{28}H_{29}N_2O_3$ 441.2178; found 441.2184.

Rosamine 3i. Green solid (47 mg, 53%). R_f = 0.28 (10% MeOH/CH_2Cl_2). ^{1}H NMR (500 MHz, CD_3OD) δ 7.58−7.45 (m, 3H), 7.26 (d, 1H, J = 7.5 Hz), 7.16 (d, 2H, J = 9.5 Hz), 6.95 (dd, 2H, J = 9.5, 2.2 Hz), 6.84 (d, 2H, J = 2.2 Hz), 3.63 (br, 8H), 2.15 (br, 8H), 2.06 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 159.3, 159.1, 156.4, 137.2, 133.4, 132.4, 131.9, 131.2, 130.1, 127.3, 116.6, 114.7, 97.9, 50.1, 26.3, 19.6. IR (thin film) 1648, 1596, 1413, 1378, 1344, 1189 cm^{-1}; HRMS (ESI) m/z calcd for $(M - Cl)^+$ $C_{28}H_{29}N_2O$ 409.2280; found 409.2277.

Rosamine 4d. Green solid (107 mg, 68%). R_f = 0.31 (10% MeOH/CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (d, 2H, J = 8.3 Hz), 7.37 (d, 2H, J = 9.5 Hz), 7.19−7.15 (m, 4H), 7.11 (d, 2H, J = 8.3 Hz), 3.80 (br, 8H), 3.67−3.65 (m, 8H), 1.45 (s, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.2, 156.9, 156.8, 154.4, 138.2, 131.8, 131.0, 130.8, 115.4, 114.0, 98.3, 97.3, 80.7, 47.2(2C), 28.3; IR (thin film) 1694, 1644, 1592, 1479, 1415, 1387, 1226, 1161 cm^{-1}; HRMS (ESI) m/z calcd for $(M - Cl)^+$ $C_{37}H_{44}N_4O_5$ 751.2356; found 751.2342.

Rosamine 12i. Green solid (93 mg, 98%). R_f = 0.30 (10% MeOH/CH_2Cl_2). ^{1}H NMR (500 MHz, CD_3OD) δ 7.58−7.45 (m, 3H), 7.29−7.19 (m, 7H), 3.86−3.81 (m, 8H), 3.74−3.72 (m, 4H), 2.07 (s, 3H), 1.85−1.75 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 160.3, 159.5, 159.3, 158.7, 158.6, 137.3, 133.1, 133.0, 132.5, 131.9, 131.3, 130.1, 127.3, 116.8, 115.7, 115.6, 115.3, 98.7, 98.1, 67.4, 50.2, 48.4, 27.3, 25.3, 19.6; IR (thin film) 1646, 1590, 1480, 1415, 1387, 1236, 1189 cm^{-1}; HRMS (ESI) m/z calcd for $(M - Cl)^+$ $C_{29}H_{31}N_2O_2$ 439.2386; found 439.2384.

Acknowledgment. We thank 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.

Supporting Information Available: Complete experimental details and characterization data for all the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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