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Microwave-Assisted Syntheses of Regioisomerically Pure Bromorhodamine Derivatives

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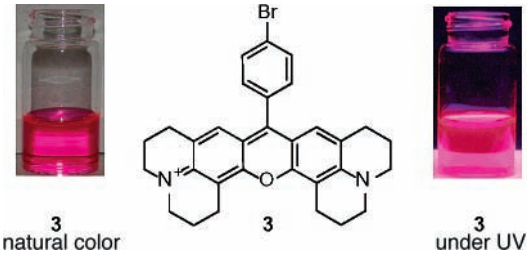
ABSTRACT



Regioisomerically pure bromo-substituted rhodamine derivatives (bromorosamines) were prepared via microwave-accelerated condensation reactions followed by oxidation with chloranil. Reaction optimization was conveniently performed by monitoring UV absorptions attributed to the product.

Regioisomerically pure, bromo-substituted rhodamine derivatives would be desirable starting materials for elaboration of fluorescent dyes via organometallic coupling reactions. We required such compounds for syntheses of superior fluorescent dyes for multiplexing in applications such as high-throughput DNA sequencing.[1,2] However, the well-established condensation route to rhodamines is not ideal for making brominated derivatives because it can give two regioisomeric products that can be hard to separate (Figure 1a). The corresponding compounds without carboxylic acid functionalities, which have been called "rosamines",[3] had not previously been reported with one halogen substituent, but we found them to be interesting because, unlike bromorhodamine syntheses, condensation reactions to form them should give only one regioisomer (Figure 1b). Literature

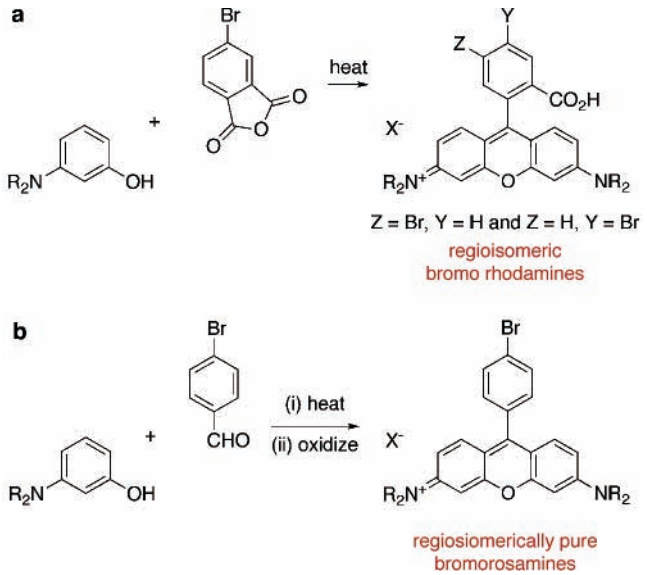


Figure 1. Regioisomerically pure bromorhodamines are hard to obtain via condensations (a), but the corresponding rosamine derivatives should be more accessible (b).

(1) Burgess, K.; Burghart, A.; Chen, J.; Wan, C.-W. *New Chemistry of BODIPY Dyes, and BODIPY Dye Cassettes Featuring Through-Bond Energy Transfer*; SPIE BIOS 2000: San Jose, CA, 2000.

(2) Burghart, A.; Thoresen, L. H.; Chen, J.; Burgess, K.; Bergström, F.; Johansson, L. B.-A. *Chem. Commun.* **2000**, 2203.

(3) Zhang, Y.-Z.; Haugland, R. P. U.S. Patent 5,686,261, 1997. Haugland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*, 6th ed.; Molecular Probes: Eugene, OR, 1996. Mao, F.; Leung, W.-Y.; Haugland, R. P. European Patent WO 99/15517, 1999. Liu, J.; Diwu, Z.; Leung, W.-Y.; Lu, Y.; Patch, B.; Haugland, R. P. *Tetrahedron Lett.* **2003**, *44*, 4355.

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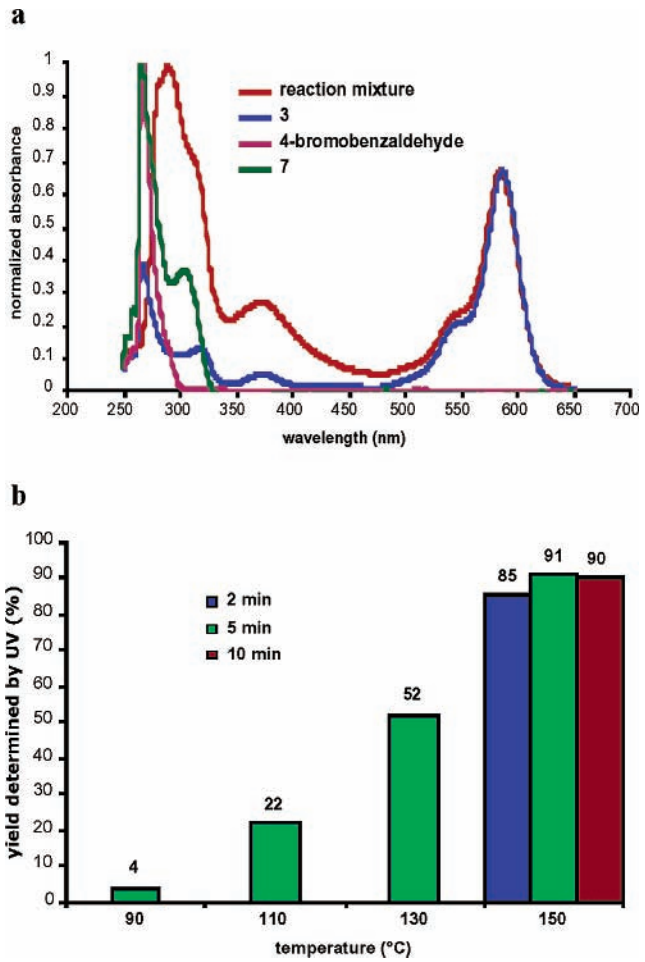

Figure 2. (a) UV spectra of reactants, product, and a typical reaction mixture in the microwave synthesis. (b) Temperature and time optimization for the microwave synthesis of **3**.

routes to rosamines involve extended high-temperature reactions that tend to give complicated mixtures.[3] This Letter reports cleaner, microwave-assisted[4,5] syntheses of rosamines illustrated by preparation of regioisomerically pure 4-bromo derivatives.

Rosamines **1**−**4** were made via the reactions of 4-bromobenzaldehyde with the phenolic amines **5**−**8**. Preparation of **3** was chosen as a model system. Attempts to optimize reaction conditions using NMR were complicated by the extent of impurities formed at the high temperatures involved and by insolubilities of the products in some solvent systems. Consequently, a UV-based method was developed. Standardized concentrations of the reagents were reacted under various conditions, an aliquot of the reaction mixture was then diluted in DMF, allowed to oxidize in the air until the UV showed no change, and then analyzed via UV. Figure 2a illustrates that the starting materials do not absorb significantly above 350 nm, whereas the desired products do. Thus the extent of conversion was obtained via a calibrated UV plot.

(4) Perreux, L.; Loupy, A. *Tetrahedron* **2001**, *57*, 9199.
(5) Lidstrom, P.; Tierney, J.; Wathey, B.; Westman, J. *Tetrahedron* **2001**, *57*, 9225.

Figure 2b shows how this method was used to determine that a good yield of product **3** could be obtained at 150 °C using a 5 min microwave irradiation time. These reactions were performed using 60% sulfuric acid as the medium. Similar experiments using methanesulfonic acid as a solvent gave inferior results (see Supporting Information). Shorter periods of irradiation gave diminished yields, whereas longer ones gave no advantage. Consequently, 10 min of microwave irradiation at 150 °C was set as a standard for comparison with typical thermal syntheses of this system (the reaction time was increased from 5 to 10 min because the scale of the reactions was also increased). Preparations of the other dyes were similarly optimized.

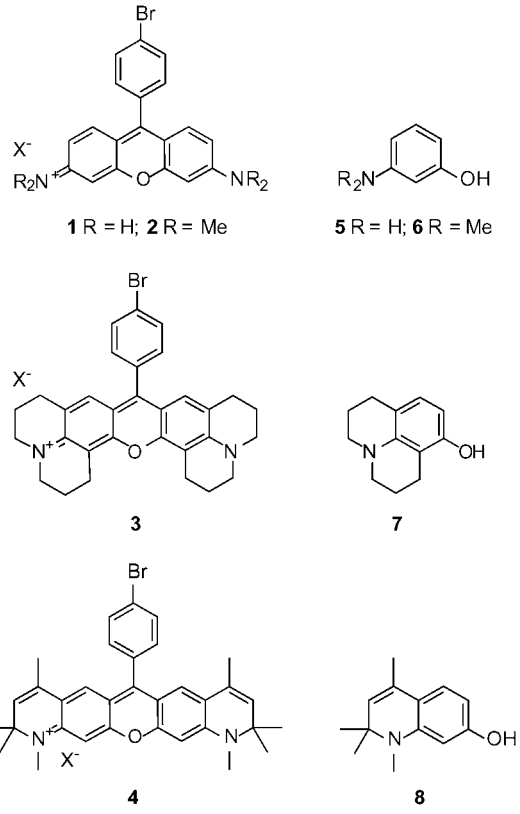

Table 1 compares isolated yields of compounds **1**−**4** under various microwave and thermal conditions. Reaction times

Table 1. Isolated Yields of the Dyes under Microwave and Thermal Conditions[a]

dye	microwave[b]		thermal	
	temp, time (°C, min)	yield (%)	temp, time (°C, h)	yield (%)
1	150, 20	27	160, 24	8
2	90, 30	41	160, 22	12
3	90, 30	38	90, 18	35
	150, 10	73		
4	150, 10[c]	53	160, 24[c]	5

[a] In 60% H_2SO_4 unless otherwise indicated. [b] After microwave, 2 equiv of chloranil was added to the reaction mixtures to ensure complete oxidation, and then the products were isolated via flash chromatography. [c] Neat: no solvent or acid.

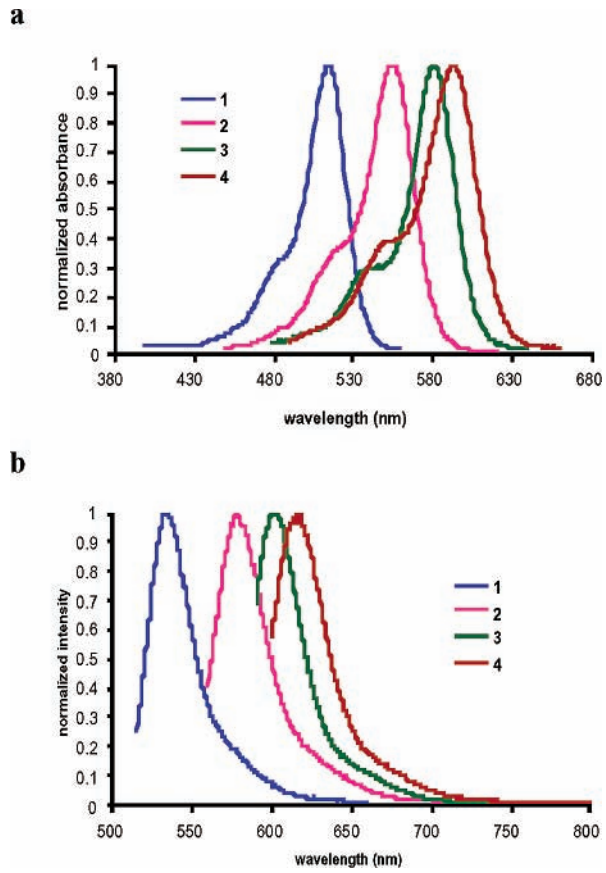

Figure 3. (a) UV spectra and (b) fluorescence emission spectra of dyes **1**−**4** in EtOH.

in the microwave reactions were significantly shorter; thus, the transformations could be performed more conveniently in the microwave apparatus, and, perhaps more importantly, the process of optimizing the reaction conditions was more facile. In each case, chloranil was added after the reaction period, and then the mixtures were stirred for at least 10 min at 25 °C to ensure oxidation of the intermediate condensation product.

Compounds **1**−**4** are highly colored in solution (dilute EtOH solutions are yellow, pink, magenta, and purple, respectively). In fact, they are so strongly colored that it is difficult to recrystallize them, simply because it is almost impossible to see when the solid dissolves and when crystals form. Consequently, there is some ambiguity about the counterion for these salts, but we believe they are obtained from the syntheses as hydroxide salts.

Figure 3 shows the UV absorption and fluorescence emission spectra of **1**−**4**. Their fluorescence maxima span the range 532−616 nm in EtOH. Consequently, these molecular fragments can be used as acceptors for energy transfer cassettes to enable fluorescence detection in multiplexing via well-resolved emission maxima.

In conclusion, the high temperatures required for formation of rhodamine derivatives are easily obtained via microwave heating. Microwave-assisted syntheses of compounds **1**−**4** were efficiently optimized and rapidly repeated as a direct result. There is a high probability that microwave heating also could be applied to make similar systems such as fluoresceins and other xanthene-based dyes. In this work, use of 4-bromobenzaldehyde to give the rosamine compounds rather than 3-bromophthalic anhydride to give rhodamines circumvents the issue of regioisomer formation.

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Supporting Information Available: Temperature and time optimization for microwave synthesis of compound **3**, experimental details for the preparation of compounds **1**−**4**, tabulated spectroscopic data for compounds **1**−**4**, and pictures of the compounds in solution. This material is available free of charge via the Internet at http://pubs.acs.org.

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