## New Fluorogenic Benzothiadiazole and Benzoselenadiazole Reagents to Yield Environment-sensitive Fluorophores via a Reaction with Amines

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We introduce 7-fluoro-4-*N*,*N*-dimethylaminosulfonyl-2,1,3benzothiadiazole and 7-fluoro-4-*N*,*N*-dimethylaminosulfonyl-2,1,3-benzoselenadiazole as new fluorogenic reagents for amines. These reagents are nonfluorescent themselves and can easily react with nonfluorescent amines to produce environmentsensitive fluorophores. Herein, we report the synthesis of the new reagents and the fluorescence properties of the reagents and their amine derivatives as well as the reactivity of the new reagents toward amines.

Environment-sensitive fluorophores change their fluorescence properties (e.g., maximum emission wavelength, fluorescence quantum yield, and fluorescence lifetime) depending on the polarity of the solvent and the degree of hydrogen bonding with the solvent molecules. Originally, environment-sensitive fluorophores were utilized for the evaluation of the microenvironment associated with proteins.<sup>1,2</sup> Recently, environment-sensitive fluorophores have been applied in the development of fluorescent sensors by incorporating the fluorophores into stimulus-responsive macromolecules, such as proteins and synthetic polymers.<sup>3-5</sup> Although different types of environment-sensitive fluorophores are currently available, new compounds with improved properties (e.g., high sensitivity, high photostability, and long emission wavelength) are still in demand. In this letter, we introduce 7fluoro-4-*N*,*N*-dimethylaminosulfonyl-2,1,3-benzothiadiazole (1a) and 7-fluoro-4-N.N-dimethylaminosulfonyl-2,1,3-benzoselenadiazole (2a) (Figure 1) as new fluorogenic reagents for amines: these are nonfluorescent themselves and produce environmentsensitive fluorophores in the presence of amines. The synthesis and photophysical properties of 1a, 2a, and their amine derivatives and the reactivity of 1a and 2a toward amines are discussed.

The reagents 1a and 2a were synthesized from commercially available 2'-fluoroacetanilide using a 6-step reaction (Figure 2). The derivatives of 1a and 2a with *N*,*N*-dimethylamine (1b and 2b) and L-alanine (1c and 2c) were also obtained as model environment-sensitive fluorophores derived from the new reagents.

The absorption and fluorescence spectra of 1a, 1b, 2a, and 2b were obtained in *n*-hexane, ethyl acetate, acetonitrile, methanol, and water. Fluorescence lifetime measurements were also performed on 1b and 2b using the same solvents. Representative absorption and fluorescence spectra of 1a, 1b, 2a, and 2b are provided in Figure 3, and the photophysical properties of 1b and 2b are summarized in Table 1. As illustrated in Figure 3, the reagents 1a and 2a did not exhibit fluorescence in any of the solvents tested. In contrast, the amine derivatives 1b and 2b showed remarkable fluorescence when excited at the maximum absorption wavelength. It should be noted that the fluorescence of 1b and 2b was strongly dependent



Figure 1. Chemical structures of benzothiadiazole derivatives 1a–1d and benzoselenadiazole derivatives 2a–2d.



Figure 2. Synthesis of 1a and 2a. (i) Fuming HNO<sub>3</sub>, 0 °C, 1.5 h; (ii) HCl, MeOH, reflux, 5 h (13% in 2 steps); (iii) Fe, HCl, CH<sub>2</sub>Cl<sub>2</sub>, MeOH, rt, 75 min (66%); (iv) X = S, PhNSO, toluene, reflux, 4.5 h (65%); X = Se, SeO<sub>2</sub>, EtOH, 80 °C, 1 h (97%); (v) ClSO<sub>3</sub>H, 0 °C, 1 h  $\rightarrow$  150 °C, 2 h (X = S, 92%; X = Se, 93%); (vi) Me<sub>2</sub>NH, CH<sub>3</sub>CN, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 70 min  $\rightarrow$  rt, 40 min (X = S, 55%); 0 °C, 15 min  $\rightarrow$  rt, 45 min (X = Se, 45%).



Figure 3. Representative spectra of 1a, 1b, 2a, and 2b. The absorption spectra ( $30 \,\mu$ M) were measured in ethyl acetate (black). The fluorescence spectra ( $1 \,\mu$ M for 1b;  $5 \,\mu$ M for 2b) were measured at the excitation wavelength of  $\lambda_{abs}$  in ethyl acetate (orange), methanol (blue), and water (green).

on the nature of the solvents: whereas efficient fluorescence was observed in an apolar and aprotic solvent, such as *n*-hexane, the fluorescence was dramatically quenched in a polar and protic solvent, such as water. In addition, the maximum emission wavelength shifted bathochromically with increasing solvent polarity. The variation in the fluorescence quantum yields of **1b** and **2b** in different solvents was much larger than that of conventional environment-sensitive fluorophores, such as dan-

**Table 1.** Photophysical properties of **1b** and **2b** at 25 °C: maximum absorption wavelength ( $\lambda_{abs}$ ), maximum emission wavelength ( $\lambda_{em}$ ), fluorescence quantum yield ( $\Phi_f$ ), fluorescence lifetime ( $\tau_f$ ), fluorescence rate constant ( $k_f$ ), and nonradiative rate constant ( $k_{nr}$ )

Comp.	Solvent	$\lambda_{ m abs}$ /nm	$\lambda_{ m em}$ /nm	$arPhi_{ m f}$	$ au_{ m f}$ /ns	$k_{\rm f} / 10^7  {\rm s}^{-1}$	$\frac{k_{\rm nr}}{/10^7  {\rm s}^{-1}}$
1b	<i>n</i> -Hexane	437	520	0.43	20.9	2.0	2.8
	Ethyl acetate	444	570	0.33	19.7	1.7	3.4
	Acetonitrile <sup>a</sup>	445	583	0.20	13.3	1.5	6.0
	Methanol	446	597	0.053	3.9	1.4	24
	Water	448	641	0.0044	0.45	1.0	>200
2b	<i>n</i> -Hexane	476	573	0.19	14.3	1.3	5.7
	Ethyl acetate	479	628	0.077	6.4	1.2	15
	Acetonitrile <sup>a</sup>	481	645	0.046	4.7	1.0	20
	Methanol	481	655	0.013	1.4	0.9	72
	Water	485	688	0.0011	0.20	0.6	>500

<sup>a</sup>Molar absorption coefficients of **1b** and **2b** in acetonitrile are 6900 and  $6600 \text{ M}^{-1} \text{ cm}^{-1}$ , respectively.

sylamine (cf.  $\Phi_{\rm f} = 0.31$  in *n*-hexane and  $\Phi_{\rm f} = 0.05$  in water)<sup>6</sup> and 4-methylamino-7-nitro-2,1,3-benzoxadiazole (cf.  $\Phi_{\rm f} = 0.09$  in *n*-hexane and  $\Phi_{\rm f} = 0.028$  in water).<sup>7</sup>

Because **1b** and **2b** bear an electron-donating dimethylamino group and an electron-withdrawing aromatic ring (i.e., dimethylaminosulfonylbenzothiadiazole or -benzoselenadiazole), the first excited singlet (S<sub>1</sub>) state of **1b** and **2b** exhibits intramolecular charge-transfer characteristics. Thus, the absorption energies required for the transition of **1b** and **2b** from the ground (S<sub>0</sub>) state to the S<sub>1</sub> state are lower than those of **1a** and **2a**, which lack an electron-donating substituent. Similarly, it can be concluded that the bathochromic shift in the maximum emission wavelength of **1b** and **2b** with increasing solvent polarity is caused by the intramolecular charge-transfer character of the S<sub>1</sub> states.<sup>8</sup>

The sensitivity of benzothiadiazole and benzoselenadiazole derivatives toward the surrounding environments was recently discussed in a detailed photophysical and photochemical study carried out using related amine derivatives in ten different solvents.9 The discussion therein is also relevant to the analysis of 1b and 2b. With increasing solvent polarity, the energy gap between the S<sub>0</sub> and S<sub>1</sub> states in **1b** and **2b** decreases, resulting in an increase in the nonradiative relaxation processes according to the energy gap law (cf.  $k_{nr}$  values in Table 1).<sup>10</sup> Whereas the rate of fluorescence ( $k_{\rm f}$  values in Table 1) is fairly solvent independent, the fluorescence quantum yield (which equals  $k_f/(k_f + k_{nr})$ ) is strongly solvent dependent due to the change in the nonradiative rate constant  $(k_{nr})$ . In addition to the increase in the polarity of the solvent, the formation of a hydrogen-bonding network between the fluorophores 1b and 2b and solvent molecules also accelerates the nonradiative relaxation processes from the S<sub>1</sub> state. Higher  $k_{\rm nr}$  values were obtained in protic methanol than in aprotic acetonitrile (Table 1) despite the fact that these solvents have similar polarity (cf. the dielectric constants of methanol and acetonitrile were 32.7 and 37.5, respectively). Thus, both the polarity and hydrogen-bonding ability of the solvent influence the fluorescence properties of 1b and 2b.

It should be noted that the fluorescence properties of **1b** are almost identical to those of a well-established environment-

sensitive fluorophore, 7-*N*,*N*-dimethylamino-4-*N*,*N*-dimethylaminosulfonyl-2,1,3-benzoxadiazole (Figure S1 and Table S1).<sup>13</sup> However, the photostability of **1b** was determined to be approximately 10 times higher than that of the corresponding benzoxadiazole (Figure S2),<sup>13</sup> demonstrating the potential applicability of **1a** for bioimaging.

Finally, the reactivity of fluorinated reagents 1a and 2a with amines was compared with that of the corresponding chlorinated compounds  $1d^{11}$  and  $2d^9$  (Figure 1). L-Alanine was adopted as the model amine, and the photophysical properties of L-alanine derivatives 1c and 2c are provided in Figure S3 and Table S2.<sup>13</sup> In this study, the production of 1c and 2c was monitored using HPLC-UV. The results demonstrate that the fluorinated reagents 1a and 2a (1 mM) were successfully derivatized with excess L-alanine (30 mM) in a mixture of borate buffer (pH 9.2) and acetonitrile (1:1, v/v) at 70 °C after reacting for 10 h (yield: 74– 79%). In contrast, almost no reaction was observed between the chlorinated compounds 1d and 2d and L-alanine under these conditions (<0.35%). The reaction rates of 1a and 1b with L-alanine were estimated to be at least 200 times larger than those of 1d and 2d. The difference in reactivity between fluorinated and chlorinated reagents might be due to the reaction mechanism: the intermediate state of this reaction involves a Meisenheimer complex in which the fluorine atom is less repulsive to nucleophilic alanine than the chlorine atom.<sup>12</sup> Overall, we conclude that the new fluorogenic reagents 1a and 2a are sufficiently reactive toward amines. We are currently working on developing macromolecular fluorescent sensors utilizing these fluorogenic reagents.

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