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Synthesis, spectroscopic properties and protein labeling of water soluble 3,5-disubstituted boron dipyrromethenes

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ABSTRACT

Sulfur-containing 3,5-disubstituted boron dipyrromethene (Bodipy®) fluorescent probes with improved water solubility were synthesized. A dicarboxylic acid derivative that can be excited by the 543 nm HeNe laser line is very soluble in aqueous solution and retains high fluorescence quantum yield of the unionizable parent molecule. Conversion of the dicarboxylic acid to the succimidyl or sulfosuccinimidyl diester produces molecules capable of labeling proteins with a bright and stable fluorescence signal.

Boron dipyrromethene fluorophores (Bodipy® or BDP) are widely used as probes in biological systems.[1] These molecules normally have good photostability and high quantum yields,[2,3] but they tend to have low aqueous solubility.[4] The absorption and emission maxima can be tuned to chosen regions of the visible to the near-IR region of the electromagnetic spectrum by varying the substituents on the dipyrromethene chromophore.[5–9] Lowering the energy of the absorption maximum often involves addition on unsaturated or aromatic systems,[10,11] which decreases their aqueous solubility, thereby limiting their utility as biological probes.

Recently efforts have been made to increase the aqueous solubility of BDPs. Burgess' group has shown that addition of a sulfonate group to the 2- and 6-positions increases aqueous solubility without sacrificing quantum yield.[4] They have also made water soluble BDPs for covalent modification of biomolecules and cassettes for energy transfer by appending sulfonic acids to the BDP core and adding carboxylic acid-containing tethers.[12,13] Niu et al. have taken a different approach. They attached hydrophilic molecules containing multiple ionizable groups to various positions on the parent molecule.[14,15] In some cases aqueous concentrations of 100–750 μM could be obtained.

The objective of this work was to make BDP derivatives that fluoresce at long wavelength, are soluble in aqueous solution and can be used for protein labeling. The molecules are based on the 3,5-disubstituted BDP's of Rohand et al.,[9] which are easy to synthesize and whose absorption and emission maxima can be tuned by altering the nature of the functional group.[16,17] The sulfur-substituted compounds possessed the best properties for this type of label: absorption maximum near the HeNe 543 nm line and good quantum yield in both polar and apolar environments.[13,18] Reported here is the synthesis and characterization of water soluble BDPs suitable for biological applications (Fig. 1).

Syntheses of the 3,5-substituted BDPs are presented in Scheme 1. The starting material 3,5-dichloroBDP **1** was prepared according to the literature procedure.[19] Compound **2** was synthesized in reasonable yield by reacting NaSMe with compound **1**. Compound **1** was treated with mercaptoacetic acid to yield a highly fluorescent, water soluble molecule **3** in quantitative yield. This molecule was converted to derivatives suitable for protein labeling through DCC coupling with the appropriate *N*-hydroxysuccinimide. Lower yield was obtained when the *N*-hydroxysulfosuccinimide sodium salt was used as the nucleophile.

All synthesized thio-based BDP compounds were stable enough to handle for the next experiments except compounds **5**–**7**. These compounds must be stored under nitrogen in a desiccator and should also be protected from light until use.

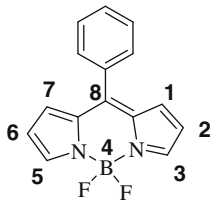

Figure 1. Structure of BDP (4,4-difluoro-8-phenyl-4-bora-3a,4a-diaza-*s*-indacene).

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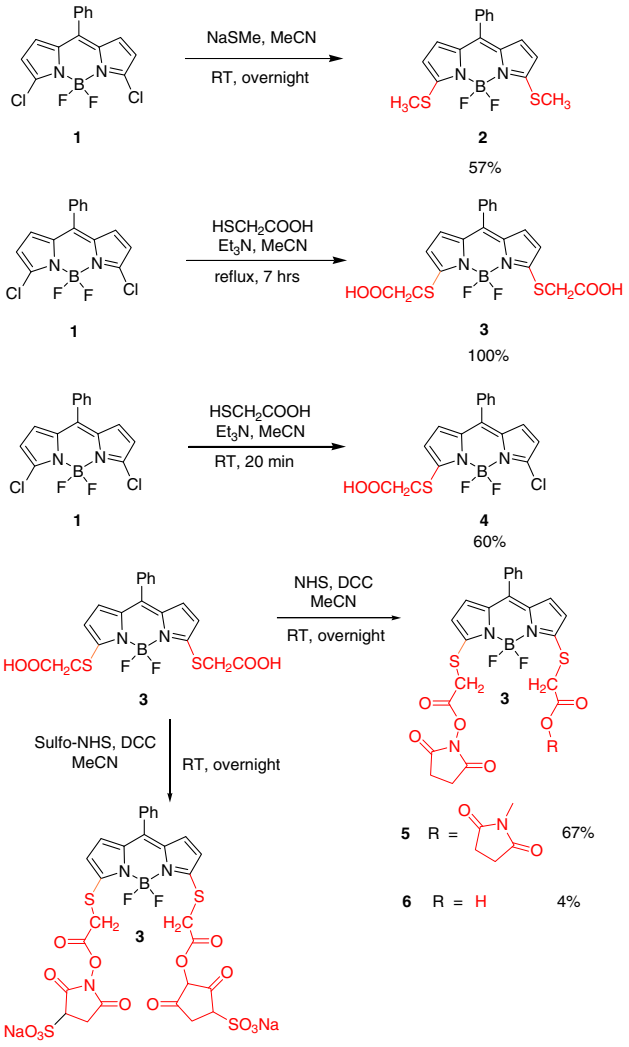

Scheme 1. Synthesis of BDP's **2**–**7**.

The absorbance and fluorescence emission spectra of these thio-BDP derivatives were measured in two solvents (methanol and dioxane). Absorption spectra of compounds **2–6** in dioxane are shown in Figure 2. Note that the absorption maximum for the asymmetrically substituted compound **4** is at higher energy than the dithio-BDPs (**2**, **3**, **5–7**), which is typical for 3,5-disubstituted BDPs.[20–24]

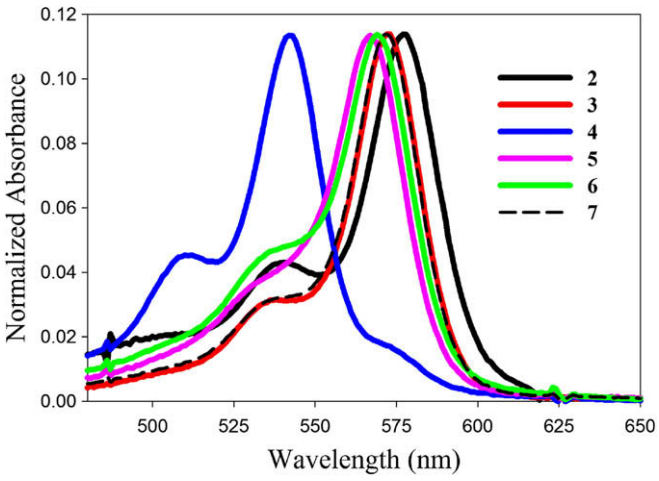

Figure 2. Absorption spectra of compounds **2**–**7** in dioxane.

Absorption and fluorescence data for the novel BDPs are summarized in Table 1.

The emission maxima and quantum yields of the dithio-substituted molecules (**2**, **3**, **5–7**) are similar to one another and greater than the same parameters for the asymmetrically substituted compound **4**. This trend is in agreement with other 3,5-disubstituted BDPs. The quantum yields of the succinimidyl esters are consistently higher than those of the other thio-BDPs, but the reason for the difference is not known.

The quantum yield for compound **3** was also measured in water and in 10 mM phosphate buffer (pH 7.0). Compound **3** was slightly more fluorescent in aqueous solution than in methanol (ϕ = 0.34 and 0.36 in water and phosphate buffer, respectively).

All of the dithio-BDPs emit bright orange fluorescence (Fig. 3).

Figure 4 illustrates the effect of the carboxylic acid on the solubility of the BDP. Since compound **3** is essentially a derivative of **2**, these two molecules are compared. As would be expected from the previous photophysical studies, the hydrophilic carboxylic acid does not significantly change the spectral properties of the dyes (Table 1), but it increases the molecule's solubility. The solubility of compound **2** is about 1 μM in 10 mM phosphate buffer at pH 7, while the carboxylic acid **3** is freely soluble at 40 μM in the same buffer.

The ability of the succinimidyl ester to covalently modify a model protein was assessed. Purified tubulin[25] in 0.1 M $NaHCO_3$ buffer, pH 8.3, was incubated with varying concentrations of com-

Table 1
Spectral properties of BDP derivatives **2**–**7**

Compound	Solvent	λabs_{max} (nm)	λem_{max} (nm)	Φ_F[a] (at 23 °C)	log (ε_{max})
2	Dioxane	577	591	0.30	—
	Methanol	571	585	0.34	4.04 ± 0.01
3	Dioxane	573	585	0.35	—
	Methanol	573	587	0.29	4.17 ± 0.01
4	Dioxane	543	555	0.16	—
	Methanol	540	556	0.11	4.60 ± 0.01
5	Dioxane	567	579	0.55	—
	Methanol	562	578	0.46	3.86 ± 0.01
6	Dioxane	569	582	0.42	—
	Methanol	564	579	0.41	3.85 ± 0.01
7	Dioxane	572	585	0.43	
	Methanol	566	578	0.30	4.00 ± 0.01

[a] Quinine sulfate was used as a standard (ϕ = 0.57 in 0.1 M H_2SO_4). Relative errors in quantum yields are 10–15% of the reported value.

Figure 3. Solutions of **3** in methanol under room light (left) and long wavelength fluorescent light (right).

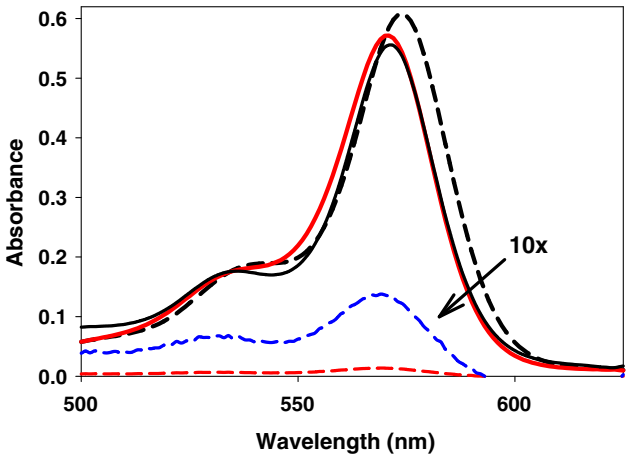

Figure 4. Absorption spectra of **2** and **3** in methanol and pH 7 buffer. Samples were prepared in the following manner: An aliquot of a stock solution in methanol was removed and the solvent was evaporated. Methanol or 10 mM phosphate buffer, pH 7.0, was added to the dried sample to yield a final concentration of 40 μM, and the absorption spectra of the solutions were measured. Solid lines: compound **3** in methanol (black) or phosphate buffer (red). Dashed lines: compound **2** in methanol (black) or phosphate buffer (red). The blue dashed line is the absorption spectrum of **2** in phosphate buffer increased by a factor of 10.

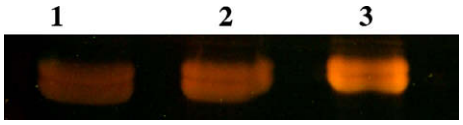

Figure 5. SDS–PAGE of bovine brain tubulin labeled with compound **5** and visualized using long wavelength UV light from a hand held device. Lane 1—10:1 dye:protein; Lane 2—20:1 dye:protein; Lane 3—40:1 dye:protein. The upper band is α-tubulin and the lower band is β-tubulin.

pound **5** using standard lysine labeling conditions.[26] Figure 5 shows a concentration dependent increase in fluorescence of both the α- and β-subunits of tubulin. Even though there are two reactive groups per fluorophore, no evidence of protein crosslinking was detected in the gel; that is, no bands of higher molecular mass were detected by either protein staining or fluorescence visualization (data not shown). It was therefore unnecessary to use the compound with a single chemically reactive succidimidyl ester (**6**) for protein labeling. The fluorescent signal appears to be stable: the photograph in Figure 5 was taken after the gels were stored overnight in acetic acid:methanol:water destaining solution.

In summary, a 3,5-disubstituted BDP dye with strong absorbance near 543 nm was derivatized to extend the biological applications of molecules in this series. The dicarboxylic acid **3** retains the desirable spectral properties of the thioether **2** while possessing high aqueous solubility. Compound **3** can also be converted to a disuccidimidyl ester that can be used for general protein labeling. The reactions to prepare compounds **3**, **5**, and **7** are straightforward and can be accomplished with very little specialized equipment. The synthetic ease by which these molecules can be prepared from the starting material should make these molecules accessible to many laboratories that normally lack synthetic capabilities.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.080.

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