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Synthesis of boron dipyrromethene fluorescent probes for bioorthogonal labeling

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Abstract

Seven 5-substituted 3-hydrazinyl derivatives of 3a, 4a-diaza-4,4-difluoro-8-phenyl boron dipyrromethene (BODIPY) were prepared for use as bioorthogonal fluorescent labels of aldehydes and ketones. The absorption energies can be tuned to absorb visible light over a large span of wavelengths by changing the nature of the 5-substituent. Optical properties of hydrazones formed with the 5-chloro derivative are affected by the nature of the electrophile such that aliphatic and aromatic hydrazones can be differentiated from each other and from unreacted fluorophore.

Orthogonal labeling of biological molecules involves reactions between functional groups not normally present in a biological molecule or system of interest. Reactions between a modified biomolecule and its chemical partner can be exploited to selectively incorporate a reporter molecule into a chosen site within the biological target.[1,2] For example, reactions between azides and phosphine ligands or alkynes have been successfully exploited to tag proteins, nucleic acid polymers and carbohydrates.[3,4] Aldehyde or ketone moieties can be introduced into many types of biomolecules through chemical means or through genetic manipulation.[5–7] Such functional groups can react with hydrazines or hydroxylamines in aqueous solution to form stable hydrazone or oxime products, respectively. Virtually all commercially available fluorophores that react with aldehydes and ketones, however, possess a hydrazide functional group.[8] Hydrazides are less reactive than corresponding hydrazines and tend to form less stable condensation products.[9] In this work, we have synthesized a series of hydrazine-substituted fluorophores that are suitable for use as bioorthogonal labels of aldehydes and ketones.

Boron dipyrromethene-based fluorescent probes (BODIPY® or BDP) have been widely used to probe biological systems. These fluorophores frequently possess large absorption coefficients, high fluorescence quantum yields and good photostability.[10,11] Emission energies from the visible to the near IR region of the electromagnetic spectrum are accessible through manipulation of the parent molecule. Absorption and emission energies of the BDP are affected by the nature of the substituents on the dipyrromethene and the boron atom. Recently, Rohand et al. showed that molecule **1** is amenable to substitution at the 3- and 5-positions by a variety of nucleophiles[12] and that the photochemical properties of the BDP are highly dependent on the nature of these substituents.[13] We reasoned that putting a hydrazinyl group at this position would lead to a class of probes that could be very useful as labels for suitably functionalized biomolecules. In addition to being more reactive than hydrazide-containing fluorophores, these probes would be expected to undergo changes in absorption and emission properties depending on the electronic nature of the BDP and the electrophile. Such molecules are particularly useful in biological systems such as cells in which background fluorescence from

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Table 1
Preparation of compounds **2**–**8**

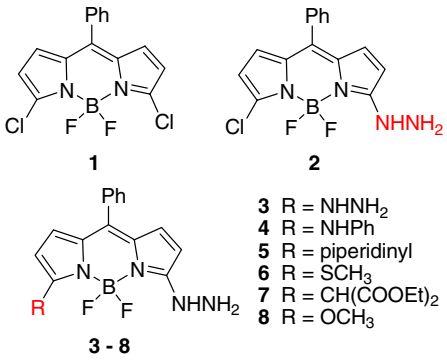
Nucleophile	Temp (°C)	Rxn time (h)	Product	Yield (%)
Hydrazine[a,b]	rt	8.5	**2**	85
Hydrazine[b]	Reflux	5	**3**	44
Aniline[c]	rt	6.5	**4**	87
Piperidine[c]	rt	3	**5**	57
NaSMe[c]	rt	8.5	**6**	64
Diethylmalonate[c]	rt	10	**7**	15
NaOMe[b]	rt	1	**8**	24

[a] The substrate for this reaction was compound **1**; for all other reactions the substrate was compound **2**.
[b] Solvent was methanol.
[c] Solvent was acetonitrile.

unreacted or non-specifically bound reagent can interfere with observation of the covalent product.[14]

Compound **1** was prepared according to the literature procedure.[8] Treatment of **1** with hydrazine hydrate under mild conditions cleanly afforded the monohydrazinyl product **2** in good yield (Table 1). Heating the reaction produced the disubstituted product **3** in moderate yield. Compound **2** could serve as a substrate for the preparation of additional monohydrazinyl derivatives (Scheme 1). Moderate to good yields were achieved with nitrogen and sulfur nucleophiles (**4**, **5**, **6**). A low yield was obtained when diethylmalonate was employed as a nucleophile (**7**). A low yield was also obtained when **2** was treated with sodium methoxide, but this was presumably due to competition between substitution at the 3- and 5-positions, as the major product of the reaction was 3-chloro-5-methoxy-BDP. Attempts were made to improve the yield, including using 3,5-dimethoxy-BDP as the substrate and hydrazine as the nucleophile. In this case, compound **3** was obtained as the major product. Table 1 summarizes the reaction conditions and yields of the hydrazinyl-BDP derivatives.

Rohand et al. showed that the absorption maxima of 3,5-disubstituted-BDP's are strongly influenced by the electronic nature of the substituents.[12] In general, the symmetrically substituted BDP's have absorption maxima that are red shifted relative to the asymmetrically substituted molecules tested. Compounds with a nitrogen or sulfur atom directly bonded to the heterocycle tend to have lowest



Scheme 1. Structures of BDPs **1**–**8**.

Table 2
Absorption and fluorescence emission spectral data of compounds **2**–**10** in dioxane and methanol

Compound	Solvent	λabs_{max} (nm)	λem_{max} (nm)	Φ_F (at 23 °C)
2	Dioxane	478	522	0.004
2	Methanol	463	529	0.002
3	Dioxane	549	567	0.136
3	Methanol	546	564	0.023
4	Dioxane	487	545	0.027
4	Methanol	475	539	0.018
5	Dioxane	486	526	0.016
5	Methanol	473	523	0.010
6	Dioxane	535	563	0.183
6	Methanol	525	559	0.094
7	Dioxane	486	522	0.105
7	Methanol	460	517	0.046
8	Dioxane	500	516	0.034
8	Methanol	495	511	0.012
9	Dioxane	513	543	0.108
9	Methanol	502	539	0.064
10	Dioxane	531	559	0.214
10	Methanol	521	555	0.098

energy absorption maxima. These trends are observed for the 3-hydrazinyl 5-substituted BDP's (Table 2). The BDP with the lowest energy absorption maximum is the symmetrically substituted 3,5-dihydrazinyl-BDP **2**, and the asymmetrically substituted BPD with a sulfur-containing substituent displays the most red shifted absorption maximum of the asymmetrically substituted BDP's.

Absorption spectra of BDP's are normally sharp and narrow.[15] With exception of compounds **3** and **8**, however, the absorption spectra of the hydrazinyl-BDPs in ethanol are broad (Fig. 1). The origin of this phenomenon is unclear, but it seems to be related to conjugation between a nitrogen substituent at the 3-position and the BDP chromophore. For example, there is considerable broadness in the lowest energy absorption band of a 3-(*p*-*N*,*N*-dimethylaminostyryl)-BDP.[16] Also, a 3-amino-BDP derivative displays a broad absorption spectrum in polar solvents.

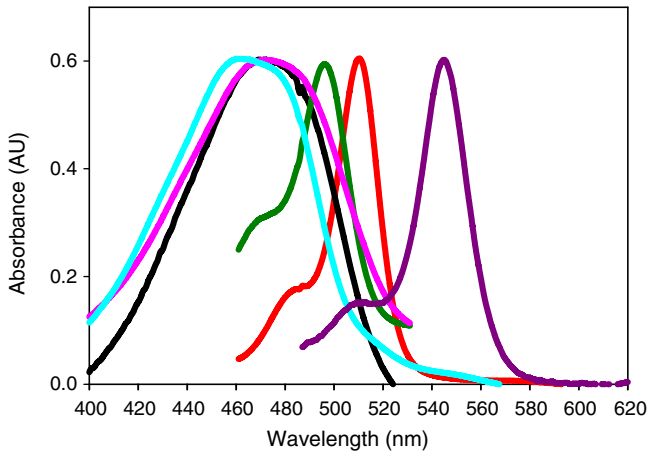
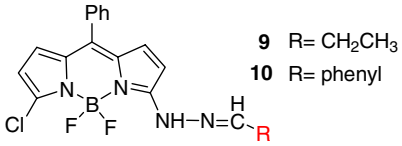


Fig. 1. Normalized absorption spectra of selected compounds in ethanol. From left to right: **7** (cyan), **4** (pink), **2** (black), **8** (dark green), **1** (red), **3** (purple).



Scheme 2. Structures of hydrazones of **2**.

Acylation of the amine sharpens the absorption spectrum and shifts the maximum to longer wavelength.[17]

Fluorescence emission data for compounds **2**–**8** are listed in Table 2. The range of the emission maxima is about 50 nm. All of the hydrazines have rather low fluorescence quantum yields in both methanol and dioxane.

Since the nature of the 3- and 5-substituent affects the optical properties of the BDP, it was expected that hydrazone formation would also affect the absorption and emission spectra of the BDP fluorophore. To test this hypothesis, model compounds an aliphatic hydrazone **9** and an aromatic hydrazone **10** were prepared from compound **2** (Scheme 2).

As anticipated, hydrazone formation causes a bathochromic shift in the absorption maximum, which is observable by visual inspection of the solution (Fig. 2). The red shift is larger for the aromatic hydrazone than for the aliphatic hydrazone (53 and 35 nm, respectively, in dioxane). Emission spectra for the hydrazones follow the same pattern (Fig. 3). Formation of the aliphatic hydrazone shifts the emission maximum from 522 nm to 543 nm in dioxane, while formation of the aromatic hydrazone red shifts the emission maximum an additional 16 nm. The difference in the energy of the emitted light is also clearly observed by visual inspection (Fig. 2). Satisfyingly, the red shift in the emission spectrum was also accompanied with a large increase in quantum yield. The emission of compound **2** is feeble in both apolar and polar solvents. Formation of the aliphatic hydrazone **9** increases the emission intensity by 25- to 30-fold in both solvents (Table 2). The quantum yield of the aromatic hydrazone **10** is nearly double that of the aliphatic hydrazone. Therefore, background fluorescence due to unreacted hydrazine is unlikely to interfere with detection of either hydrazone. Covalent labeling of an aromatic aldehyde or ketone, such as a modified phenylalanine or tyrosine, should be distinguishable from products formed with aliphatic carbonyls by judicious choice of excitation and emission wavelengths.

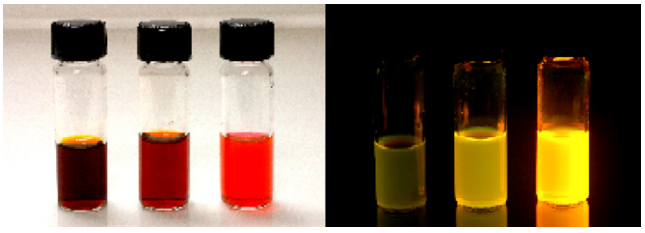


Fig. 2. Solutions of **2**, **9** and **10** in dichloromethane under room light (left) and long wavelength fluorescent light (right).

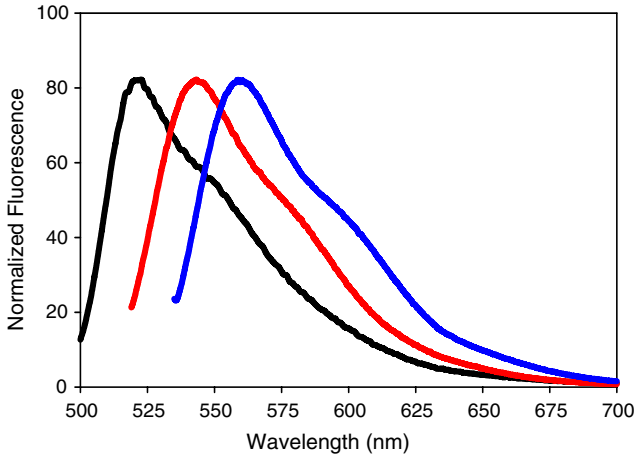


Fig. 3. Normalized fluorescence emission spectra **2**, **9** and **10** in dioxane. From left to right, parent hydrazine **2** (black, $\lambda_{ex} = 478$ nm), aliphatic hydrazone **9** (red, $\lambda_{ex} = 513$ nm), aromatic hydrazone **10** (blue, $\lambda_{ex} = 531$ nm).

Like the asymmetrically substituted hydrazinyl-BDPs, the absorption and emission energies and the quantum yields of the hydrazones **9** and **10** are environmentally sensitive. Therefore, their use as bioorthogonal labels will be most effective when the product occupies a relatively hydrophobic environment. Hydrazinyl-BDPs that are more fluorescent in aqueous solution are likely to form hydrazones suitable for detecting an aldehyde or ketone in aqueous solution. Preparation and characterization of additional hydrazones is currently underway in our laboratory.

Summary: BDP derivatives possessing a 3-hydrazinyl substituent were prepared by nucleophilic substitution reactions beginning with the known compound 3,5-dichloro BDP. The absorption energies of the hydrazinyl-BDP can be tuned to absorb visible light over a large span of wavelengths by changing the nature of the 5-substituent. An aliphatic and an aromatic hydrazone were prepared from one of the hydrazines to test the hypothesis that this approach will allow for selective detection of aliphatic and aromatic carbonyls. Formation of a hydrazone shifts absorption and emission maxima of the probe to longer wavelength; therefore, the fluorescent signal from the covalently bound fluorophore is observable in the presence of unreacted material. The hydrazone formed with an aliphatic aldehyde has different absorption and emission maxima than one formed with an aromatic aldehyde. Thus, a hydrazone formed by reaction with an unnatural amino acid such as a derivatized phenylalanine or tyrosine should yield a distinct fluorescent signal, even if other carbonylated species are present.

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Supplementary data

Experimental procedures and characterization data for **2**–**10**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.12.052.

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