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Functionalization of the 4,4-difluoro-4-bora-3a, 4a-diaza-*s*-indacene (BODIPY) core

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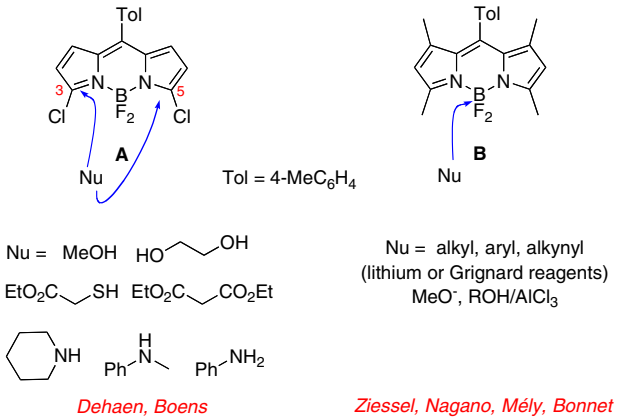
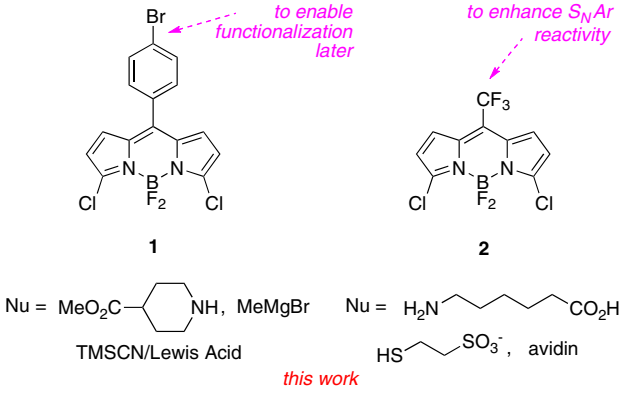
Abstract—The new BODIPY systems **1** and **2** were prepared and then used as substrates to explore S_NAr and *F*-B displacement reactions. Chloride was easily displaced from **1** by a piperidine/ester, methylmagnesium bromide selectively displaced fluoride, and cyanide could attack both sites. System **2** readily added soft nucleophiles to the electrophilic carbon atoms, providing a new method for bioconjugation of BODIPYs to proteins while also introducing a ^{19}F probe.

BODIPY dyes are some of the most widely used fluorescent probes in biotechnology.[1,2] They were first prepared in 1969,[3] but developments relating to their syntheses and functionalization reactions are far from mature. In that regard, two of the most striking recent innovations in the literature are: (i) S_NAr reactions of 3-chloro or 3,5-dichloro systems;[4,5] and (ii) nucleophilic displacement of fluoride atoms from boron.[6–13] These studies, reported from other laboratories, typically feature compounds such as **A** and **B**. Hard nucleophiles were not explored for the S_NAr substrates **A**, cyanide was not studied for either system, and no attempts have been made to use nucleophilic amino acid side-chains on proteins for the functionalization reactions.



Work from our group involving BODIPY dyes has exposed different facets of both reaction types mentioned above. This communication focuses on BODIPYs **1** and **2** to illustrate those new issues. Compound **1** was used to introduce groups functionalized for bioconjugation, and to study cases in which nucleophilic displacement of *F* and S_NAr reactions can compete. Compound **2** was used to illustrate how S_NAr reactions in particular can be used to conjugate dyes to proteins while simultaneously changing the BODIPY core structure, its fluorescent properties, and enhancing its water solubility.



Scheme 1 shows the synthesis developed for the 3,5-dichloroBODIPY **1**. Academically, this follows a procedure similar to that already developed for the similar compound **A**[14] but practically the physical properties of the materials and/or minor variations of the

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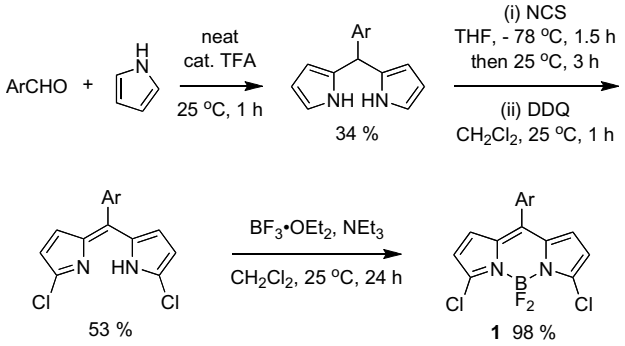
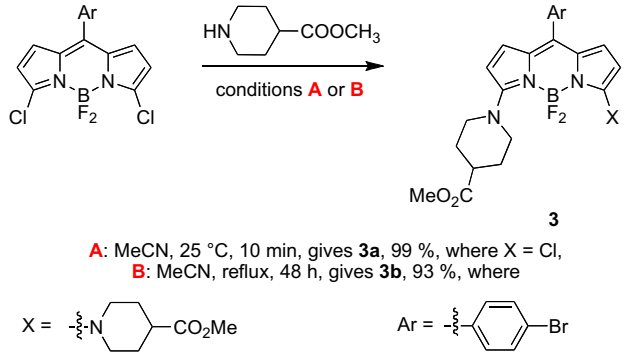


Scheme 1. Synthesis of the 3,5-dichloroBODIPY **1**.



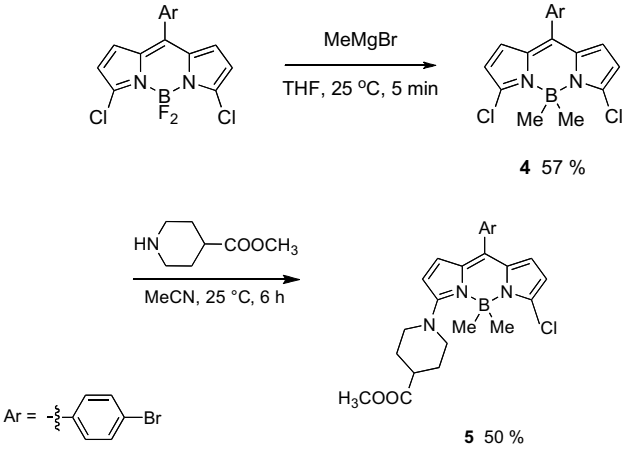
Scheme 2. Mono- and bis-substitution on BODIPY **1**.

procedure made it possible to scale-up the syntheses to 5 g with minimal chromatography.

Displacement of the first of the two chlorines in 3,5-dichloroBODIPY **1** with a piperidine derivative occurs rapidly (Scheme 2). The second chlorine can be displaced using extended reaction times at elevated temperatures. This particular piperidine derivative was of interest because hydrolysis of the methyl ester would unmask two carboxylic acids that could be used for conjugation of this material to biomolecules.

The transformations shown in Scheme 2 were predictable for this substrate in combination with a soft nucleophile. However, reactions of **1** with a harder nucleophile like methylmagnesium bromide could conceivably occur at either of the electrophilic sites. It transpired that displacement of the B-*F* bonds occurred most rapidly, allowing relatively clean production of the *B*-dimethylated compound **4**. This could then be reacted with a piperidine derivative to give the S_NAr product **5**.

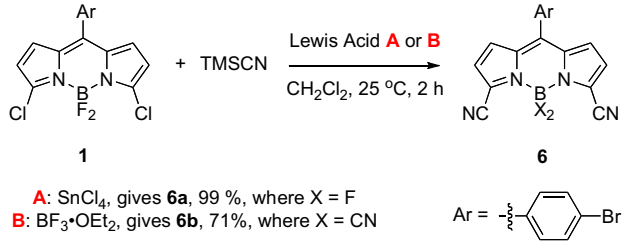
Cyanide is a softer anion than methylmagnesium bromide, but is harder than amines. The reaction of 3,5-dichloroBODIPY **1** with this nucleophile reflects this intermediate character. Displacement was achieved using Lewis acidic activation of trimethylsilyl cyanide



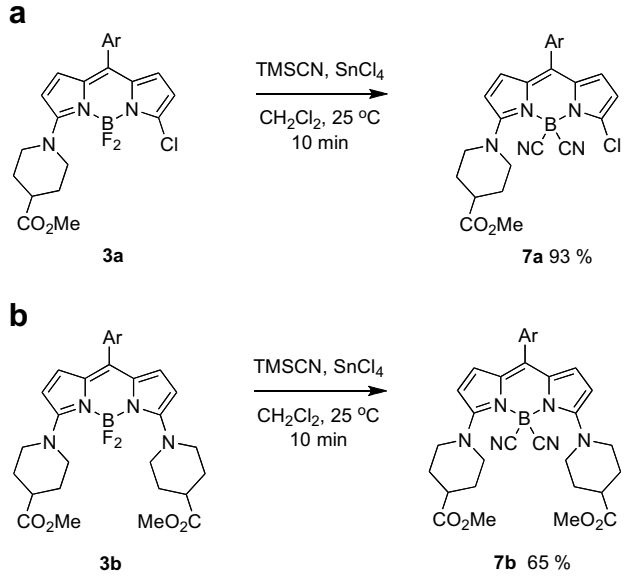
(reactions of solubilized cyanide ions were less satisfactory). The reaction of **1** in the presence of tin tetrachloride occurred selectively at the carbon to give compound **6a**, but boron trifluoride promoted both types of displacement reactions to give the tetracyanide **6b**. Cyanide has been used in S_NAr reactions occurring at the *meso* (or 8-position) of non-halogenated-BODIPY dyes,[15–17] but this is the first time that either mode of reactivity shown in Scheme 3 has been reported.

The Lewis-acid-mediated conditions described above were also applied to the aminated products **3**. This led to the corresponding *B*-dicyanated compounds **7** (Scheme 4).

Table 1 shows photophysical data for molecules **1** and **3**–**7**. Several trends can be observed using the starting material **1** as a basis for comparison. Monoamination and diamination cause red-shifts in the fluorescence, reduced quantum yields, and peak broadening. The Stokes' shift for **3a** is high because the absorption maximum for this material is blue-shifted. The *B*-dimethylated compound **4** is spectroscopically similar to the parent **1** in all respects, and, just like **1** amination to give **5** blue-shifts the absorption maximum. Replacement of the 3,5-dichloro-substituents in **1** with cyanides gave the BF_2 compound **6a** and the $B(CN)_2$-compound **6b**. These changes did not significantly affect the wavelengths for the absorption or fluorescence maxima, but they did increase the quantum yields by a factor of 4–5. Again, monoamination and diamination caused red-shifts in the fluorescence, peak broadening, and reduced quantum yields.

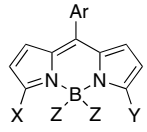


Scheme 3. Syntheses of compound **6** having cyanide substituents.

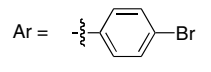


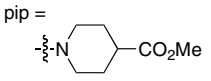
Scheme 4. B-*F* displacements using cyanide anion in: (a) mono-; and (b) diaminated BODIPY dyes.

Table 1. Spectroscopic data for BODIPYs **1** and **3**–**7**



	X	Y	Z
1	Cl	Cl	F
3a	pip	Cl	F
3b	pip	pip	F
4	Cl	Cl	Me
5	pip	pip	Me
6a	CN	CN	F
6b	CN	CN	CN
7a	pip	Cl	CN
7b	pip	pip	CN





Dye	λ_{abs}[a] (nm)	ε[a] (M^{-1} cm^{-1})	λ_{emiss}[a] (nm)	fwhm[a] (nm)	Φ[a]
1	512	77600	523	27	0.15 ± 0.01[b]
3a	482	68400	562	78	0.003[c]
3b	573	39900	614	52	0.01[d]
4	503	134300	516	30	0.13 ± 0.01[c]
5	483	61800	564	84	0.002[c]
6a	514	127400	526	25	0.66 ± 0.01[b]
6b	510	36500	523	25	0.80 ± 0.02[b]
7a	485	33700	560	75	0.006[c]
7b	517	26100	613	61	0.008[b]

[a] In MeOH.
[b] Rhodamine 6G was used as a standard (Φ = 0.94 in EtOH).
[c] Fluorescein was used as a standard (Φ = 0.92 in 0.1 M $NaOH_{aq}$).
[d] Rhodamine 101 was used as a standard (Φ = 1.00 in EtOH).

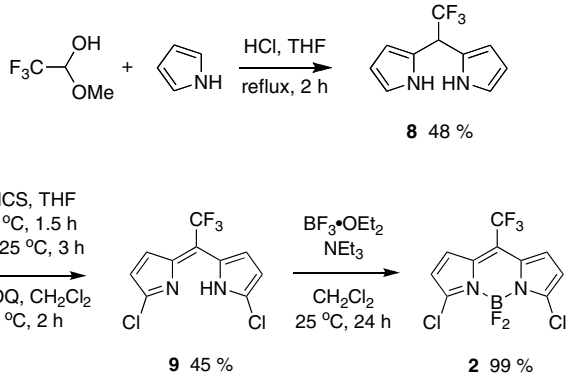
The probes containing piperidine were prepared because the carboxylic acid might be used to conjugate them to biomolecules. However, this was not pursued further since the quantum yields for these derivatives were low. Further, hydrolysis of the esters gave carboxylic acids that, at least in the protonated form, tended to destabilize the structure, perhaps via a pH effect. Consequently, an alternative approach was adopted; this involved derivatives of system **2**.

The BODIPY dye **2** was targeted because it might have several attributes as a biochemical probe. First, the electron withdrawing trifluoromethyl group would make it more reactive in S_NAr reactions, and we anticipated that these reactions could be used to directly link the probe to proteins. Second, the probe is small. Third, the trifluoromethyl group might be useful as an NMR marker. Finally, the trifluoromethyl substituent makes the carbon that is destined to become the *meso*-carbon more electrophilic, and this facilitates synthesis of the probe. In the event, a convenient synthesis was developed, in which the trifluoromethyl groups stabilized dipyrromethane and dipyrromethene intermediates **8** and **9** so that these were isolable (Scheme 5).[18]

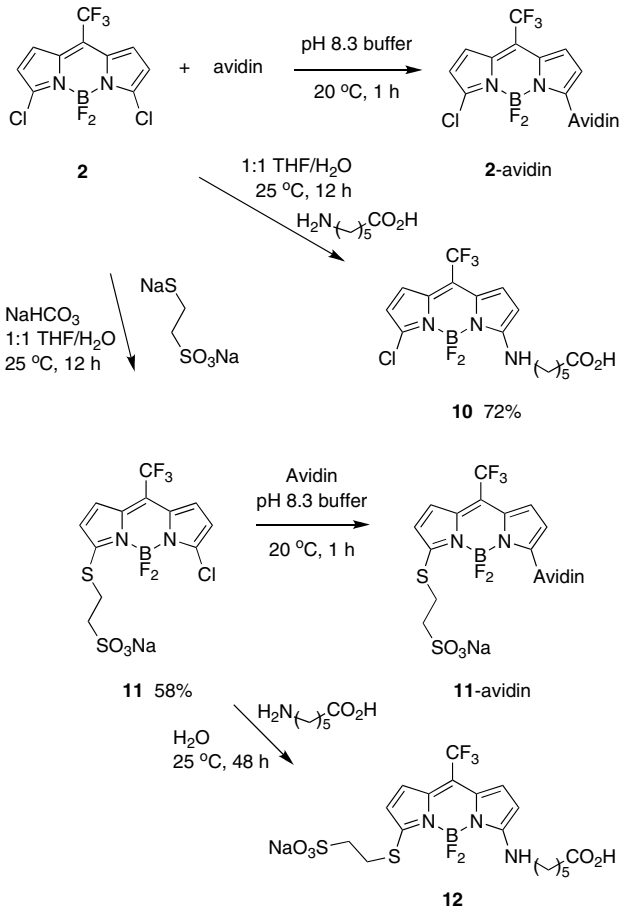
Scheme 6 shows how compound **2** was reacted with avidin (chosen as a model protein). We assume this gave the product of displacement of one chlorine atom since excess of the dye was used, and because of the experiments depicted in Scheme 6 featuring reactions with simpler substrates. Indeed, it was necessary to prepare compounds like **10** and **12** to estimate extinction coefficients for the dyes on proteins, and therefore dye-to-protein ratios.[19] Thus the calculated dye-to-protein ratio was 10:1 for **2**-avidin, and 3:1 for **11**-avidin.

The quantum yields measured for the model compounds **10** and **12** were higher than similar derivatives of **1** (Table 2). This may be attributed to removal of a pathway for non-radiative decay when the freely rotating *meso*-aryl group is replaced by CF_3, and/or change in oxidative potential of the BODIPY core leading to reduced photoinduced electron transfer. Absorbance and fluorescence spectra of **2**- and **11**-avidin are close to the control compounds **10** and **12** (Fig. 1 and Table 2); this supports the assertion that the substitution pattern is predominantly that shown in Scheme 6. Finally, CD spectra recorded for avidin and **2**-avidin showed no significant differences in the 190–320 nm region, indicating the labeling event did not cause a radical change in the protein tertiary structure.

In summary, the work described in this paper highlights numerous ways in which the BODIPY core may be modified via nucleophilic substitution reactions. In the particular case of S_NAr reactions on compound **2** provides a route to label proteins that breaks with well-used



Scheme 5. Synthesis of CF_3-dichloroBODIPY **2**.



Scheme 6. Syntheses of dye avidin conjugates and model compounds.

Table 2. Photophysics studies on compounds **2**, **10**, **11**, and **12**

Dye	λ_{abs}[a] (nm)	ε[a] (M^{-1} cm^{-1})	λ_{emiss}[a] (nm)	Φ[a]
2[b]	548	89000	554	1.00 ± 0.02[c]
2-Avidin	481	—	546	—
10	469	17100	542	0.74 ± 0.02[d]
11	569	57000	584	0.95 ± 0.02[e]
12	477	41800	584	0.70. ± 0.01[d]
11-Avidin	492	—	592	—

[a] In pH 7.4 buffer (0.1 N lithium phosphate).
[b] In CH_2Cl_2.
[c] Rhodamine B was used as a standard (Φ = 0.73 in EtOH).
[d] Fluorescein was used as a standard (Φ = 0.92 in 0.1 M $NaOH_{aq}$).
[e] Rhodamine 101 was used as a standard (Φ = 1.00 in EtOH).

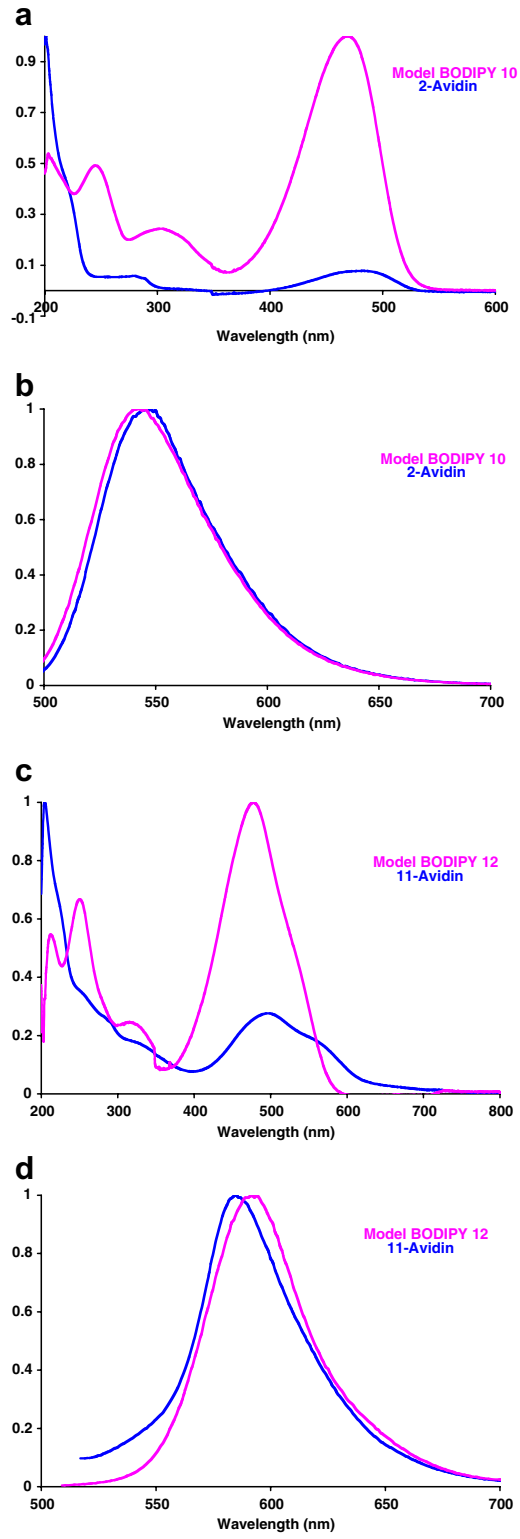


Figure 1. (a) Absorption and (b) emission spectra for **2**-avidin and its model compound **10**; and (c) absorption and (d) emission for compound **11**-avidin and its model compound **12**. Throughout, spectra were recorded in 0.1 M lithium phosphate buffer, pH 7.4, at 10^{-6} M conc in protein or 10^{-5} M dye.

approaches involving activation of pendant carboxylic acid functionalities on the dye.

Acknowledgments

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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.2007.10.103.

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