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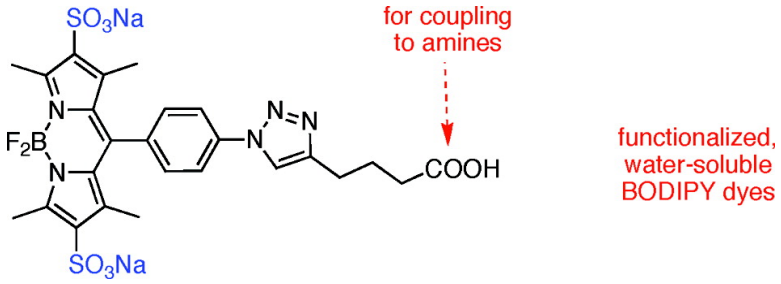

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Syntheses and Spectral Properties of Functionalized, Water-Soluble BODIPY Derivatives

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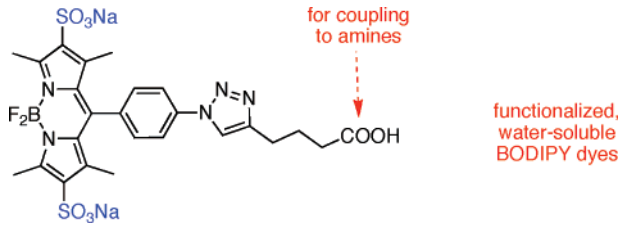

The objective of this work was to form water-soluble 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) derivatives. Sulfonation conditions were developed for several BODIPY dyes to give the monosulfonated products **1a**−**3a** and the disulfonated products **1b**−**3b**. Compounds **1** are functionalized with an aryl iodide for organometallic couplings. Similarly, **2** has an aromatic bromide but also two chlorine atoms that could be replaced via S_NAr reactions. The amine **3** is amenable to couple to biomolecules via acylation reactions. A diazotization/azide reaction sequence was used to convert the amines **3** into azides **4**; the latter may be functionalized via click reactions as illustrated by conversion of **4b** into **5**. Compound **5** was designed to have an acid-functional group to facilitate activation and coupling to amines. Spectral data for these materials indicate they are highly fluorescent probes in aqueous environments.

Introduction

Behind fluorescein, rhodamine, and possibly cyanine derivatives, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene, or BODIPY[1−4] (hereafter abbreviated to BODIPY), dyes are probably the most useful fluorescent probes in biotechnology. This is because they tend to absorb UV radiation efficiently and emit relatively sharp fluorescence peaks at useful wavelengths (typically 520−650 nm) with high quantum yields.

The core of BODIPY dyes is hydrophobic and does not contain any functionality to attach the probes to proteins. Both of these obstacles can be surmounted via synthetic modifications. For instance, there are many BODIPY dyes with carboxylic acid functional groups[5−8] that can be activated and then linked to amino groups on proteins or DNA-derivatives. Further, such carboxylic acids can be activated using sulfonated succinimide reagents;[9] this makes the hydrophobic dyes more water-soluble, enabling them to be dissolved in aqueous media for coupling to various water-soluble biomolecules. Once hydrophobic BODIPY dyes are conjugated to biomolecules, they tend to embed into hydrophobic pockets or even create micellular-like environments via aggregation effects. This is not always disadvantageous; indeed, variations of BODIPY fluorescence with the polarity of their immediate environment can be useful.[10−15]

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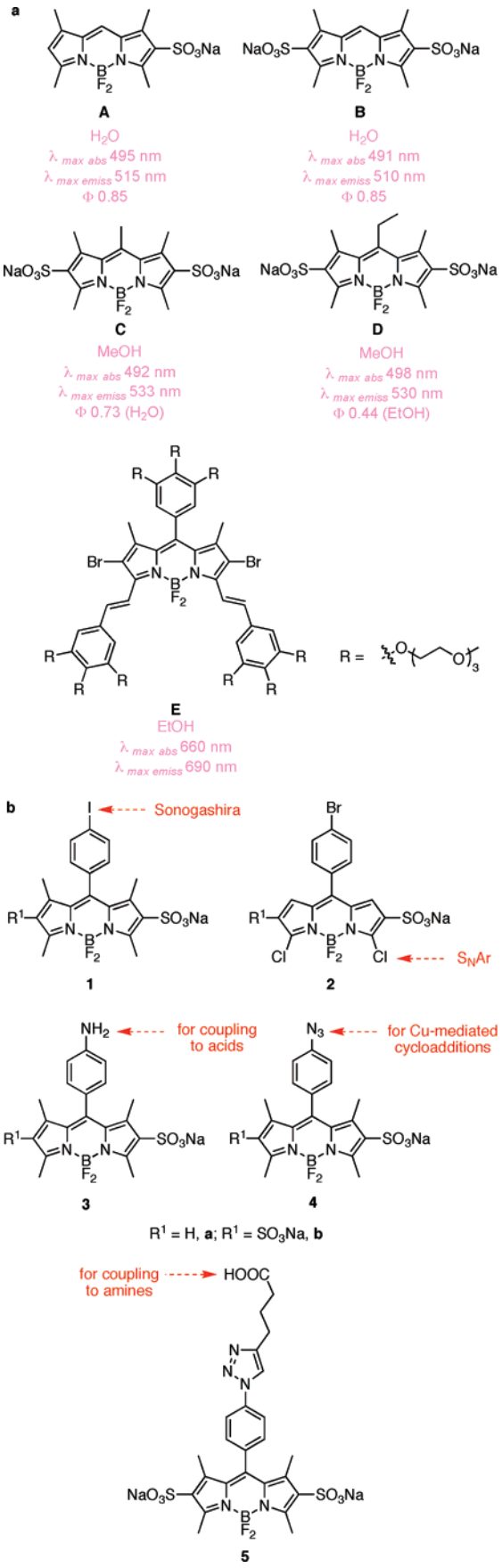

FIGURE 1. (a) Previously known water-soluble BODIPY systems; (b) compounds prepared in this work.

However, in other cases, it is definitely advantageous to have water-soluble BODIPY dyes that can be conjugated easily and that will tend to exist in the aqueous environment that surrounds a biomolecule without perturbing it.

Despite the obvious practical value of water-soluble BODIPY dyes, very few have been reported in the open literature. This is even more surprising in view of the fact that BODIPY dyes were first reported in 1969[16] and have been investigated with increasing vigor since then.[3,4] Indeed, the sum total of synthetic procedures to obtain BODIPY dyes includes only the four sulfonated derivatives **A**−**D**[17,18] and several closely related oligo(ethylene glycol)-containing systems, of which **E**[19] is illustrative (Figure 1a). The fact that water-soluble BODIPYs are relatively under-represented in the literature is unsurprising to anyone who has attempted to sulfonate these dyes. Sulfonation reagents tend to be highly reactive, whereas the BODIPY core is relatively fragile. Further, many sulfonated products are not isolated via flash chromatography. Consequently, reaction conditions for sulfonation of BODIPY dyes must be controlled carefully, and some optimization is required to isolate pure materials from these transformations.

This paper describes several procedures for the preparation of sulfonated, water-soluble BODIPY systems (Figure 1b). Mono- (a) and disubstituted (b) tetramethyl-BODIPYs **1** have a 4-iodobenzene substituent at the meso position to enable further functionalization via organometallic cross-coupling reactions. The bromo compounds **2** can be similarly derivatized, but they are also potentially reactive toward nucleophiles in S_N-Ar reactions.[20−22] Compounds **3** are valuable since they can be coupled to active carbonyl groups, the azides **4** are amenable to copper-mediated cycloadditions to alkynes,[23,24] and the disulfonate **5** can be activated and coupled to amino groups on biomolecules. Thus, the end products of this work have potential uses in many different scenarios for labeling biological molecules.

Results and Discussion

The following sections describe the preparation of the unusual BODIPY starting materials, the pivotal sulfonation reactions, and reactions of the sulfonated products to further transform them into useful probes. Finally, the spectral properties of the target molecules are discussed.

BODIPY Starting Materials. The lipophilic starting materials used in this project were generally known compounds,[25−27]

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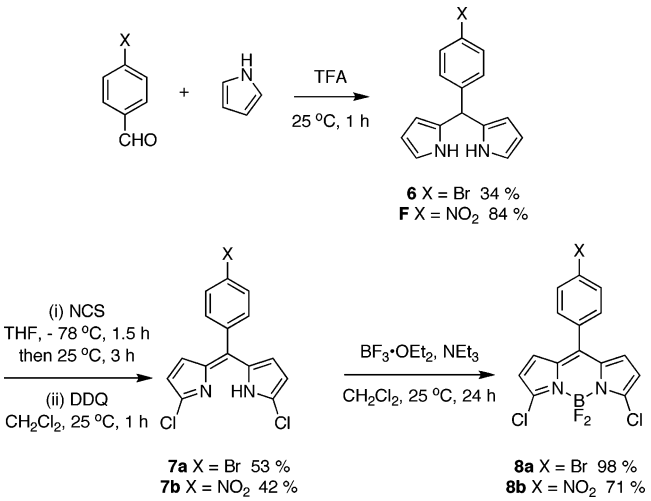
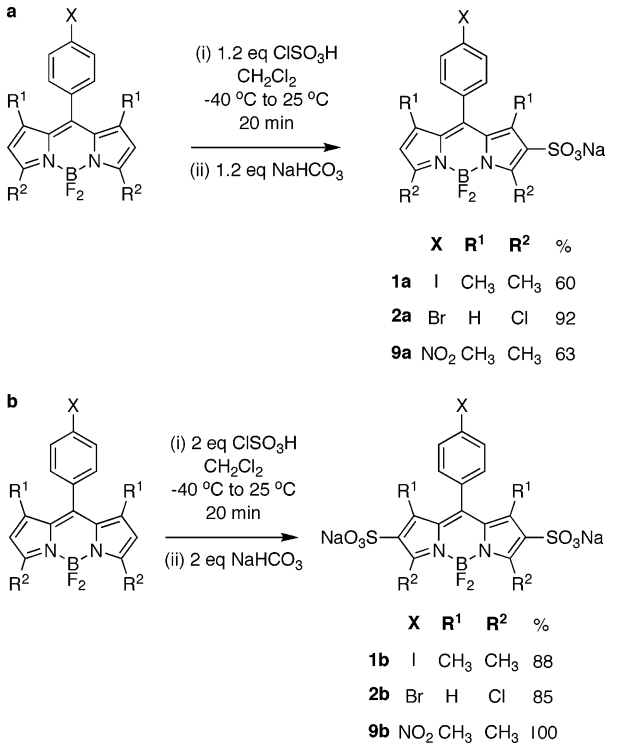
SCHEME 1



SCHEME 2



	X	R^1	R^2	%
1a	I	CH_3	CH_3	60
2a	Br	H	Cl	92
9a	NO_2	CH_3	CH_3	63

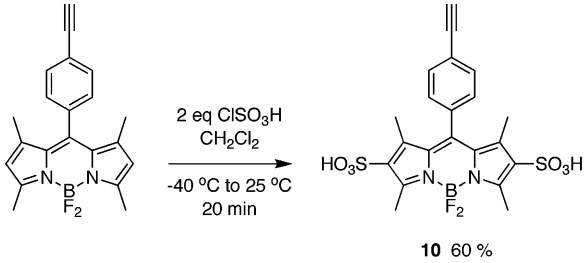
	X	R^1	R^2	%
1b	I	CH_3	CH_3	88
2b	Br	H	Cl	85
9b	NO_2	CH_3	CH_3	100

with the exception of compounds **8** shown in Scheme 1. Condensation of 4-substituted benzaldehydes with pyrrole is a known reaction for formation of the dipyrromethane **F**;[28] this was repeated here, and an analogous procedure was used to prepare the bromoderivative **6**. Chlorination of compounds similar to **6** has been reported by Boens and co-workers.[29] Slight modifications of their procedures enabled us to obtain gram amounts of the dipyrromethenes **7** after a "one-pot" chlorination oxidation sequence and a flash chromatographic separation. Incorporation of the difluoroboron groups was achieved via the standard procedure, though we found that the products **8** could be isolated via recrystallizations rather than column chromatography.

Sulfonation of Various BODIPY Derivatives. Scheme 2 shows the key sulfonation reactions featured in this paper. After considerable experimentation, it was discovered that monosulfonations (Scheme 2a) of BODIPYs tend to proceed efficiently using 1.2 equiv of fresh chlorosulfonic acid in CH_2Cl_2. The sulfonating agent in CH_2Cl_2 was added dropwise over a few minutes to a solution of the BODIPY starting material at −40 °C. After the addition was complete, the cooling bath was removed and the reaction was allowed to warm to 25 °C and stirred for 20 min. For syntheses of compounds **1a**, **2a**, and **9a**, the reactions were quenched with $NaHCO_3$(aq), and (after an extraction procedure) the crude products were purified via flash chromatography on silica. A critical observation in this work was the need for the bicarbonate quench; it appears that the protic forms of these sulfonic acids tend to be unstable (though this is not always the case, see below).

Disulfonations of the same starting materials to give products **1b**, **2b**, and **9b** are shown in Scheme 2b. Two equivalents of the chlorosulfonic acid was used to achieve the second sulfonation. Separation in this case is relatively easy because the disulfonic acids precipitate from the dichloromethane solution after 20 min at room temperature. The products were collected by filtration, dissolved in a small amount of aqueous $NaHCO_3$, evaporated to dryness, and then reprecipitated from brine to give essentially pure products. No chromatography is involved, so the procedure is convenient and amenable to scale up.

One exception to the preferred sulfonation conditions was for the alkyne-functionalized BODIPY,[27,30] as shown in reaction 1. Here, the disulfonate **10** precipitated out of the CH_2Cl_2 solution in nearly pure form. This is very fortunate because compound **10** is unstable in aqueous media, undergoing relatively rapid hydrolysis at the alkyne group. For that reason, this is not a particularly useful building block.



(1)

Reaction 2 shows a sulfonation of the relatively electron-poor BODIPY system **8b** with varying equivalents of chlorosulfonic acid. A mixture of mono- (**11a**) and disulfonation (**11b**) products formed if less than 3.5 equiv of the sulfonating agents were used, and neither of these materials precipitated from the solution; it was, however, possible to obtain the yields indicated via flash chromatography. Clean disulfonation was obtained when 3.5 equiv of chlorosulfonic acid was used, and under those conditions, the product **11b** precipitated in a relatively pure form and the sample could be further purified by reprecipitation from brine as described for compounds **1b** and **2b**. The water-

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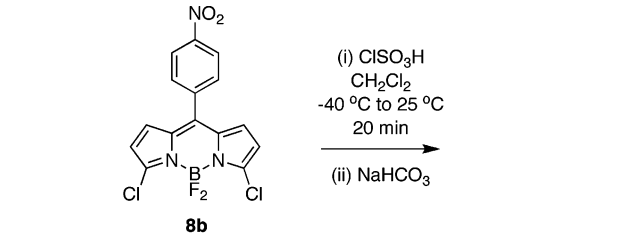
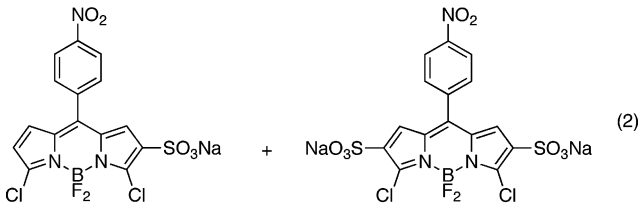
solubility of the monosulfonate **11a** was lower than the similar tetramethyl-BODIPY monosulfonates **1a**, **3a**, **4a**, and **9a**; for instance, it was impossible to obtain a clear ^{13}C NMR spectrum of **11a** in D_2O.





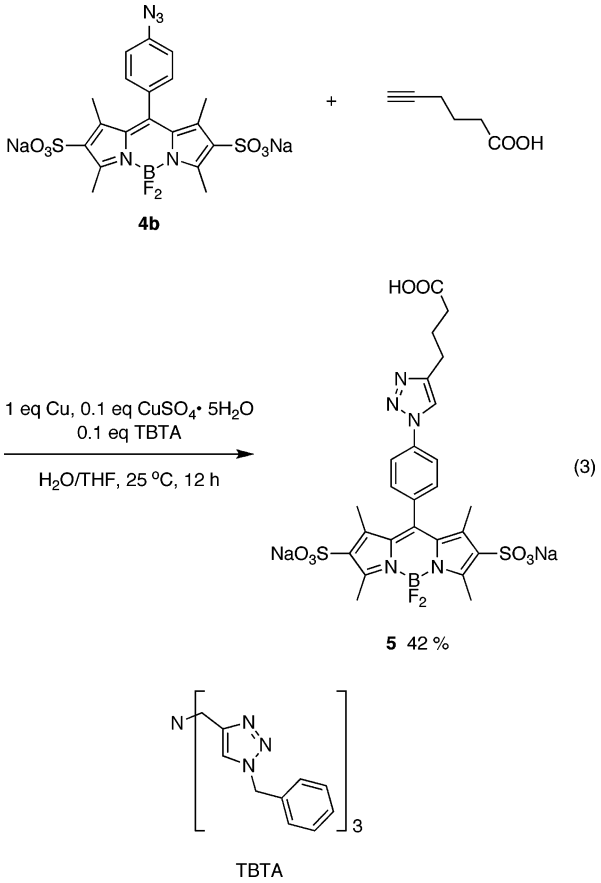
(2)

amount of $ClSO_3H$ (eq)	yield of **11a** (%)	yield of **11b** (%)
1.2	90	0
2.0	68	21
3.0	22	74
3.5	0	97

Derivatization of Some BODIPY Sulfonates. Nitro functionalities on BODIPYs have few direct applications, but this group allows access to more useful derivatives that contain amine or azido "handles". Exploratory work to establish conditions for hydrogenation of the nitro compounds **9** was not encouraging. Almost no reduction was observed under 1 atm of hydrogen in aqueous media. In ethanol, the rates were very slow, and the product amine was contaminated by *N*-ethyl impurities (this is a relatively common occurrence).[31] Reactions featuring $SnCl_2$ in HCl(aq) tended to be complicated, probably by tin complexes that did not elute from silica. Ultimately, hydrazine and catalytic palladium on carbon proved to be effective (Scheme 3).[25] To decrease the risk of an explosion during this reaction, the hydrazine was added dropwise to the substrate and reagents in refluxing ethanol. This procedure has been repeated several times on up to 400 mg of the nitro compound without any mishaps; however, we advocate use of a blast shield and certainly do not recommend that this reaction be performed on a much larger scale. The amino derivatives **3** were isolated via flash chromatography.

A diazotization/azide treatment reaction was used to convert the amines **3** into the corresponding azides **4**. Visually, this reaction is interesting. The amine starting materials are weakly green fluorescent; the reaction mixture becomes more fluorescent under acidic conditions, but this fluorescence disappears when sodium nitrite is added, corresponding to formation of the diazo compound. However, addition of azide gives a solution that is more fluorescent than the starting amine.

Reaction 3 shows how the disulfonate **4b** was functionalized via a copper-mediated azide-alkyne cycloaddition reaction. Attempts to perform this reaction in the absence of tris-(benzyltriazolylmethyl)amine (TBTA)[32] gave very little product, but addition of this ligand made the reaction viable. The disulfonate **5** is freely water-soluble and contains an easily accessible carboxylic acid for activation and conjugation to biomolecules.



(3)

Spectroscopic Properties of BODIPY Derivatives. Table 1 summarizes the spectroscopic data collected for the target materials **1**−**5** and some interesting intermediates **9** and **11**. In general, all of these compounds are sufficiently water-soluble to allow their UV and fluorescence properties to be recorded in aqueous media. All of the disulfonates are freely soluble, whereas the monosulfonates will not form relatively concentrated solutions.

All of the compounds shown in Table 1 have absorption maxima in the range 492−518 nm, and their molar absorption coefficients are high ((5.60−14.9) × 10^4 M^{-1} cm^{-1}), as is characteristic of BODIPY dyes in general;[3] they fluoresce in the 507−540 nm range. Throughout, there are small differences between the emission maxima of the mono- and disulfonated forms; in fact, the maxima shift bathochromically between 2 and 4 nm when going from the mono- to disulfonated compound, except for the nitro derivatives where the opposite trend is observed. Longest wavelength fluorescence emission maxima in the series are associated with the dichlorinated compounds **2** and **11**; all of the other probes emit between 507 and 513 nm. Sharp emissions are obtained, as seen in small fwhm (full width at half-maximum height) values (904−1752 cm^{-1}); for comparison, it is informative to consider the series

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SCHEME 3

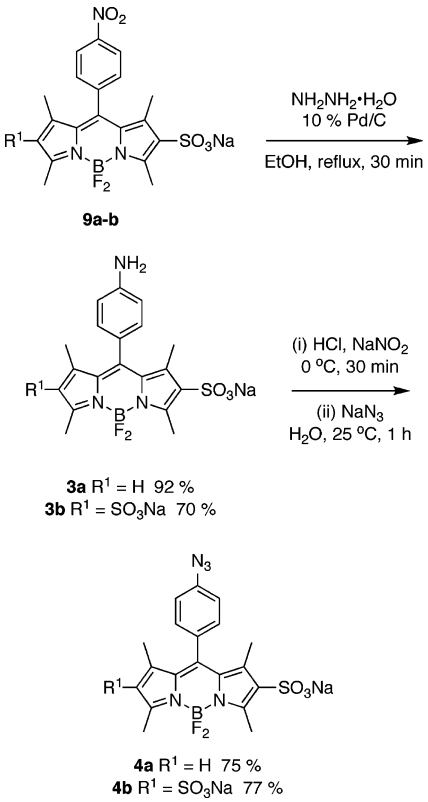

TABLE 1. Spectroscopic Properties of the BODIPY Derivatives

dye	λ_{max}(abs)[a] (nm)	log(ϵ_{max})[a,b]	λ_{max}(em)[a] (nm)	Fwhm[a] (cm^{-1})	Φ[a]
1a[c]	494	5.18 ± 0.01	507	1021	0.47 ± 0.02[d]
1b[c]	498	5.00 ± 0.01	509	994	0.34 ± 0.02[d]
2a[c]	509	4.86 ± 0.01	523	997	0.27 ± 0.01[e]
2b[c]	512	4.89 ± 0.01	524	904	0.41 ± 0.01[e]
3a[f]	492	4.93 ± 0.01	507	1050	0.001[d]
3b[f]	496	5.06 ± 0.01	511	945	0.001[d]
4a[f]	494	4.84 ± 0.01	507	1062	0.34 ± 0.01[d]
4b[f]	498	4.89 ± 0.01	509	1053	0.15 ± 0.001[d]
5[g]	498	4.90 ± 0.01	511	1027	0.49 ± 0.01[d]
9a[c]	497	4.76 ± 0.01	513	1752	0.001[d]
9b[c]	501	4.96 ± 0.01	511	1409	0.002[d]
11a[h]	514	4.82 ± 0.01	540	1440	0.002[e]
11b[h]	518	4.76 ± 0.01	538	1177	0.008[e]

[a] In H_2O. [b] ϵ_{max} were estimated by linear fit of absorbance *A* vs dye concentration c at four dye concentrations (one dye concentration was at zero). [c] Scheme 2. [d] Fluorescein was used as a standard (Φ = 0.92 in 0.1 M $NaOH_{(aq)}$).[40] [e] Rhodamine 6G was used as a standard (Φ = 0.95 in EtOH).[41] [f] Scheme 3. [g] Reaction 3. [h] Reaction 2.

of water-soluble Nile Red derivatives recently reported;[33] these have fwhm values for their fluorescence emission of between 1410 and 1680 cm^{-1}. Figure 2 shows the spectra obtained.

Quantum yields for the target compounds **1**, **2**, **4**, and **5** were all reasonably high for fluorescent probes (0.15−0.49). Some BODIPY dyes have quantum yields that are greater than 0.5; the slightly diminished values for **1**, **2**, **4**, and **5** can be attributed to nonradiative decay mechanisms associated with free rotation of the meso substituent.[34] Compounds **3** have a 4-aminobenzene

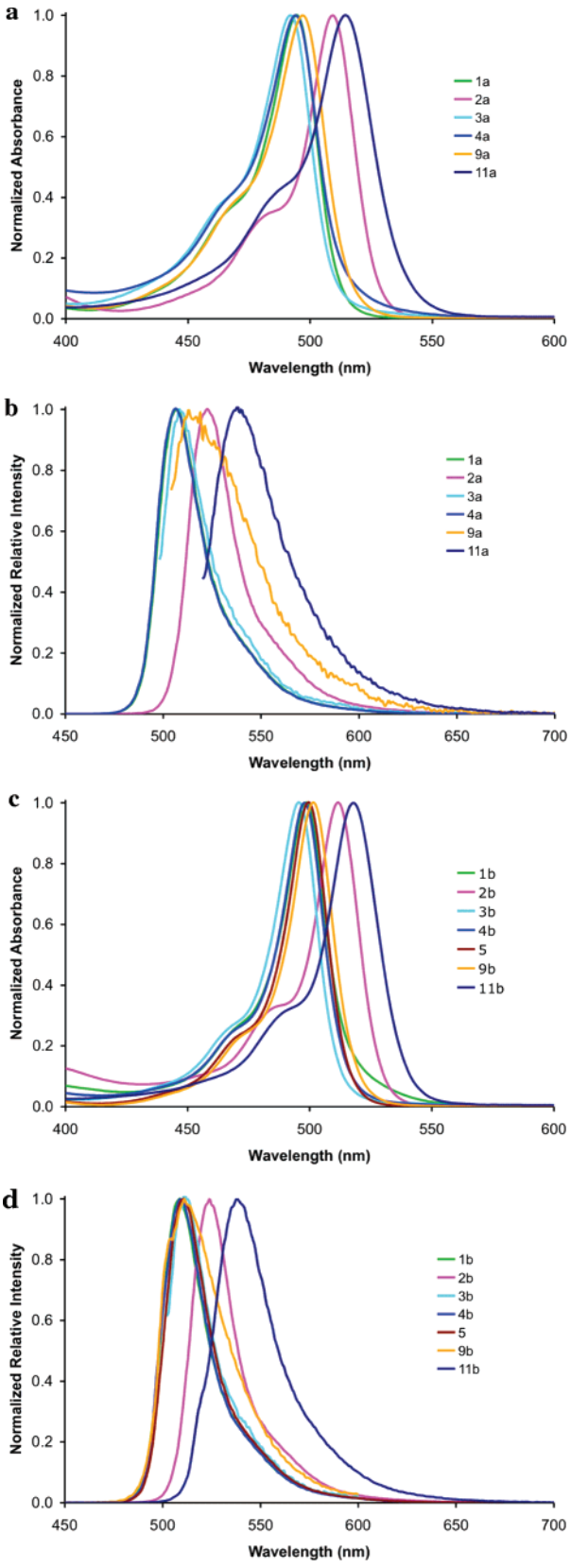

FIGURE 2. (a) UV absorption and (b) fluorescence spectra for the mono-sulfonated BODIPYs. (c) UV absorption and (d) fluorescence spectra for the bis-sulfonated BODIPYs. All of these spectra were recorded in deionized water at concentrations of approximately 10^{-6} M for the UV spectra and 10^{-7}−10^{-6} M for the fluorescence, then normalized.

(33) Jose, J.; Burgess, K. *J. Org. Chem.* **2006**, *71*, 7835−7839.
(34) Yamada, K.; Toyota, T.; Takakura, K.; Ishimaru, M.; Sugawara, T. *New J. Chem.* **2001**, *25*, 667−669.

meso substituent; this electron-rich aromatic ring probably quenches the fluorescence of the BODIPY core via photoinduced electron transfer (PeT) in which the excited-state of the BODIPY fluorophore is reduced by electron transfer from the relatively high-lying HOMO of the electron-rich meso-substituent quencher (i.e., reductive electron transfer). Such effects have been elegantly described as a-PeT by Nagano et al.[35−39] The "a" denotes that the fluorescent chromophore acts as an acceptor. The low quantum yield observed for compounds **3** is *not* a concern if the amine group is transformed into an amide in the bioconjugation process because that will adjust the oxidation potential of the meso substituent, bringing down its HOMO level and restoring the fluorescence.

Compounds **9** and **11** are intermediates rather than target materials. They both have relatively low quantum yields, and these can be rationalized via PeT effects. For these materials, the LUMO of the *meso*-substituent is relatively low-lying due to the influence of the 4-nitro group. This means that the excited state of the BODIPY chromophore can act as an electron donor to the electron-poor meso substituent quencher (i.e., oxidative electron transfer).

Conclusions

Sulfonation reactions of BODIPY derivatives are hard to develop into useful synthetic procedures for two reasons: (i) inappropriate conditions give mixtures of products and (ii) sulfonic acid derivatives of BODIPYs can be hard to purify. The sulfonation reactions shown in Scheme 2 tend to give predominantly one product, and reactions 1 and 2 give essentially binary mixtures that are easily separated by flash chromatography. Conjugation of the target materials to biomolecules could be achieved via amide bond formation to amines or acids or "click" chemistry. Further, some of the dyes presented here can be derivatized via organometallic couplings to the organic halide functionalities and, in the case of the chlorinated derivatives **2** and **11**, via S_NAr reactions.

Experimental Section

Determination of Quantum Yields. Fluorescence quantum yield measurements were performed on a fluorometer and UV/vis instrument. The slit width was 5 nm for both excitation and emission. Relative quantum efficiencies were obtained by comparing the areas under the corrected emission spectrum. The following equation was used to calculate quantum yield.

$$\Phi_x = \Phi_{st}(I_x/I_{st})(A_{st}/A_x)(\eta_x^2/\eta_{st}^2)$$

where Φ_{st} is the reported quantum yield of the standard, I is the integrated emission spectrum, *A* is the absorbance at the excitation wavelength, and η is the refractive index of the solvents used. The subscript x denotes unknown and st denotes standard. Fluorescein ($\Phi = 0.92$ in ethanol) and rhodamine 6G ($\Phi = 0.95$ in ethanol) were used as standards.

General Procedure for the Preparation of Monosulfonated BODIPYs. A solution of chlorosulfonic acid (1.2 equiv) in CH_2Cl_2 (dry, 2 mL) was added dropwise to a solution of BODIPY (100 mg) in CH_2Cl_2 (dry, 25 mL) over 10 min under N_2 at −40 °C. Then the resulting solution was slowly warmed to room temperature. After 20 min, TLC showed all of the starting material was consumed, and then aqueous $NaHCO_3$ (1.2 equiv, 20 mL) was added to neutralize the solution, and the products were separated from the CH_2Cl_2 into the aqueous layer. The aqueous layer was evaporated to dryness. The residue was dry-loaded onto a silica gel flash column and eluted using 15% MeOH/CH_2Cl_2. All of the products were isolated as orange powders with an approximate $R_f = 0.4$ (20% MeOH/CH_2Cl_2).

Sodium 2-Sulfonate-1,3,5,7-tetramethyl-8-(4′-iodophenyl)-4,4-difluoro-4-bora-3a, 4a-diaza-*s*-indacence (1a). 1,3,5,7-Tetramethyl-8-(4′-iodophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence[26] (100 mg, 0.22 mmol) and chlorosulfonic acid (18 μL, 0.27 mmol) were reacted according to the general procedure giving an orange powder (74 mg, 60%): ^{1}H NMR (500 MHz, CD_3OD) δ 7.95 (d, 2H, $J = 8.3$ Hz), 7.14 (d, 2H, $J = 8.3$ Hz), 6.19 (s, 1H), 2.75 (s, 3H), 2.52 (s, 3H), 1.70 (s, 3H), 1.46 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 159.3, 152.7, 145.7, 142.3, 139.8, 138.8, 134.4, 133.2, 132.7, 130.2, 129.0, 122.8, 94.8, 14.0, 13.6, 13.0, 12.2; MS (ESI) calcd for $C_{19}H_{17}BF_2IN_2O_3S^-$ $(M - Na)^-$ 529.01, found 528.88; IR (thin film) 2922, 1717, 1540, 1312, 1193, 1033, 1006, 678 cm^{-1}.

Sodium 2-Sulfonate-3,5-dichloro-8-(4′-bromophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence (2a). Compound **8a** (100 mg, 0.24 mmol) and chlorosulfonic acid (19.2 μL, 0.29 mmol) were reacted according to the general procedure giving an orange powder (114 mg, 92%): ^{1}H NMR (500 MHz, CD_3OD) δ 7.78 (d, 2H, $J = 8.5$ Hz), 7.51 (d, 2H, $J = 8.5$ Hz), 7.10 (s, 1H), 7.08 (br, 1H), 6.67 (d, 1H, $J = 4.6$ Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 148.9, 145.5, 141.4, 136.3, 135.7, 134.7, 133.5, 133.2, 132.1, 131.6, 130.0, 127.0, 121.7; MS (ESI) calcd for $C_{15}H_7BBrCl_2F_2N_2O_3S^-$ $(M - Na)^-$ 492.88, found 492.76; IR (thin film) 1572, 1379, 1259, 1198, 1119, 1055, 667 cm^{-1}.

Sodium 2-Sulfonate-1,3,5,7-tetramethyl-8-(4′-nitrophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence (9a). 1,3,5,7-Tetramethyl-8-(4′-nitrophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence[25] (100 mg, 0.27 mmol) and chlorosulfonic acid (22 μL, 0.33 mmol) were reacted according to the general procedure giving an orange powder (80 mg, 63%): ^{1}H NMR (500 MHz, CD_3OD) δ 8.46 (d, 2H, $J = 8.8$ Hz), 7.69 (d, 2H, $J = 8.8$ Hz), 6.22 (s, 1H), 2.77 (s, 3H), 2.54 (s, 3H), 1.66 (s, 3H), 1.42 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 161.2, 154.4, 150.0, 146.6, 142.7, 142.1, 140.8, 134.6, 133.4, 131.3, 129.8, 125.7, 124.3, 15.2, 14.9, 14.2, 13.5; MS (ESI) calcd for $C_{19}H_{17}BF_2N_3O_5S^-$ $(M - Na)^-$ 448.09, found 447.98; IR (thin film) 1513, 1343, 1200, 1086, 988, 806 cm^{-1}.

Sodium 2-Sulfonate-3,5-dichloro-8-(4′-nitrophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence (11a). Compound **8b** (100 mg, 0.26 mmol) and chlorosulfonic acid (21 μL, 0.31 mmol) were reacted according to the general procedure giving an orange powder (115 mg, 90%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42 (d, 2H, $J = 8.7$ Hz), 7.93 (d, 2H, $J = 8.8$ Hz), 7.09 (d, 1H, $J = 4.5$ Hz), 6.84 (d, 1H, $J = 4.5$ Hz), 6.82 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 149.6, 145.5, 142.6, 140.1, 138.2, 134.1, 133.4, 132.7, 132.3, 130.8, 130.0, 124.5, 120.8; MS (ESI) calcd for $C_{15}H_7BCl_2F_2N_3O_5S^-$ $(M - Na)^-$ 459.95, found 459.85; IR (thin film) 2982, 1558, 1390, 1348, 1197, 1030, 667 cm^{-1}.

General Procedure for the Preparation of Disulfonated BODIPYs. A solution of chlorosulfonic acid (2 equiv) in dry CH_2Cl_2 was added dropwise to a solution of BODIPY in dry CH_2Cl_2 over 10 min under N_2 at −40 °C. An orange precipitate was formed as the solution mixture warmed slowly to room temperature. The disulfonic acid was isolated by vacuum filtration and treated with water. The aqueous solution was neutralized with $NaHCO_3$ (2 equiv), concentrated to 5−10 mL, and treated with brine.

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The desired product was reprecipitated afterward to afford an orange solid (85−100% yield).

Disodium 2,6-Disulfonate-1,3,5,7-tetramethyl-8-(4′-iodophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence (1b). 1,3,5,7-Tetramethyl-8-(4′-iodophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence[26] (53 mg, 0.118 mmol) and chlorosulfonic acid (16 μL, 0.236 mmol) were reacted according to the general procedure giving an orange powder (68 mg, 88%): ^{1}H NMR (500 MHz, D_2O) δ 7.84 (d, 2H, J = 8.0 Hz), 6.97 (d, 2H, J = 8.0 Hz), 2.57 (s, 6H), 1.49 (s, 6H); ^{13}C NMR (75 MHz, D_2O) δ 155.5, 145.7, 144.0, 139.2, 133.1, 132.7, 130.6, 129.7, 95.7, 13.7, 13.0; MS (ESI) calcd for $C_{19}H_{17}BF_2IN_2O_6S_2^-$ $(M - 2Na + H)^-$ 608.96, found 608.98.

Disodium 2,6-Disulfonate-3,5-dichloro-8-(4′-bromophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence (2b). Compound **8b** (500 mg, 0.12 mmol) and chlorosulfonic acid (160 μL, 0.24 mmol) were reacted by the general procedure giving an orange powder (624 mg, 85%): ^{1}H NMR (500 MHz, D_2O) δ 7.73 (d, 2H, J = 8.4 Hz), 7.45 (d, 2H, J = 8.4 Hz), 7.27 (s, 2H); ^{13}C NMR (75 MHz, D_2O) δ 147.6, 141.9, 133.7, 132.6, 132.3, 131.8, 131.5, 130.0, 126.7; MS (ESI) calcd for $C_{15}H_6BBrCl_2F_2N_2O_6S_2^{2-}$ $(M - 2Na)^{2-}$ 285.91, found 285.84; IR (thin film) 2968, 1572, 1382, 1206, 1033, 650 cm^{-1}.

Disodium 2,6-Disulfonate-1,3,5,7-tetramethyl-8-(4′-nitrophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence (9b). 1,3,5,7-Tetramethyl-8-(4′-nitrophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence[25] (400 mg, 1.08 mmol) and chlorosulfonic acid (144 μL, 2.16 mmol) were reacted according to the general procedure giving an orange powder (630 mg, quant yield): ^{1}H NMR (300 MHz, D_2O) δ 8.49 (d, 2H, J = 8.5 Hz), 7.70 (d, 2H, J = 8.5 Hz), 2.77 (s, 6H), 1.63 (s, 6H); ^{13}C NMR (75 MHz, D_2O) δ 156.1, 148.8, 144.0, 143.6, 140.5, 132.9, 130.2, 129.6, 125.3, 13.8, 13.0; MS (ESI) calcd for $C_{19}H_{16}BF_2N_3O_8S_2^{2-}$ $(M - 2Na)^{2-}$ 263.52 found 263.45; IR (thin film) 1522, 1347, 1190, 1004, 853, 669 cm^{-1}.

1,3,5,7-Tetramethyl-8-(4′-ethynylphenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence 2,6-disulfonic Acid (10). A solution of chlorosulfonic acid (19 μL, 0.276 mmol) in CH_2Cl_2 (2 mL) was added dropwise to a solution of 1,3,5,7-tetramethyl-8-(4′-ethynylphenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence[42] (48 mg, 0.138 mmol) in CH_2Cl_2 (5 mL) over 10 min at −40 °C. An orange precipitate was formed as the solution mixture warmed slowly to room temperature. The disulfonic acid was isolated by vacuum filtration giving the disulfonic acid as an orange powder (42 mg, 60%): ^{1}H NMR (300 MHz, D_2O) δ 7.66 (d, 2H, J = 8.8 Hz), 7.29 (d, 2H, J = 8.5 Hz), 3.48 (s, 1H), 2.63 (s, 6H), 1.54 (s, 6H); MS (ESI) $C_{21}H_{18}BF_2N_2O_6S_2^-$ $(M - H)^-$ 507.07, found 507.08.

Disodium 2,6-Disulfonate-3,5-dichloro-8-(4′-nitrophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence (11b). Compound **8b** (100 mg, 0.26 mmol) and chlorosulfonic acid (61 μL, 0.91 mmol) were reacted according to the general procedure (the only difference is using 3.5 equiv of chlorosulfonic acid and being neutralized with 3.5 equiv of $NaHCO_3$) giving an orange powder (151 mg, 97%): ^{1}H NMR (300 MHz, D_2O) δ 8.30 (d, 2H, J = 7.5 Hz), 7.70 (d, 2H, J = 7.5 Hz), 7.18 (s, 2H); ^{13}C NMR (75 MHz, D_2O) δ 149.4, 145.2, 143.1, 136.8, 134.5, 132.0, 131.3, 124.1 (two carbons may be co-incident in this spectrum); MS (ESI) calcd for $C_{15}H_6BCl_2F_2N_3O_8S_2^{2-}$ $(M - 2Na)^{2-}$ 269.45 found 269.38; IR (thin film) 3113, 1519, 1379, 1348, 1200, 1030, 848, 692, 680, 664 cm^{-1}.

Sodium 2-Sulfonate-1,3,5,7-tetramethyl-8-(4′-aminophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence (3a). A solution of **9a** (35 mg, 0.07 mmol) in EtOH (10 mL) was purged with N_2 for 10 min. Hydrazine monohydrate (0.05 mL) and 10% Pd/C (7.9 mg, 0.1 equiv) were added. The mixture was refluxed under N_2 for 30 min. Then Pd/C was removed under vacuum filtration. After evaporation of the solvent, the residue was dry-loaded onto a silica gel flash column and eluted using 15% MeOH/CH_2Cl_2 to afford an orange solid (30 mg, 92%): R_f = 0.3 (20% MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, CD_3OD) δ 6.97 (d, 2H, J = 8.5 Hz), 6.86 (d, 2H, J = 8.5 Hz), 6.15 (s, 1H), 2.74 (s, 3H), 2.50 (s, 3H), 1.79 (s, 3H), 1.55 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 159.5, 152.7, 150.7, 147.2, 146.5, 141.2, 134.8, 133.7, 131.1, 130.0, 124.2, 123.5, 116.6, 15.2, 14.8, 14.1, 13.4; MS (ESI) calcd for $C_{19}H_{19}BF_2N_3O_3S^-$ $(M - Na)^-$ 418.12 found 418.04; IR (thin film) 3414, 2922, 1608, 1540, 1519, 1196, 1036, 684 cm^{-1}.

Sodium 2-Sulfonate-1,3,5,7-tetramethyl-8-(4′-azidophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence (4a). A solution of **3a** (29 mg, 0.07 mmol) in HCl (2 M, 5 mL) and H_2O (2 mL) was cooled to 0 °C. A solution of $NaNO_2$ (11.3 mg, 0.16mmol) in H_2O (2 mL) was added slowly, and the mixture was kept at 0 °C with stirring for 30 min. Then a solution of NaN_3 (22 mg, 0.33 mmol) in H_2O (2 mL) was added dropwise to the mixture. Stirring was continued at room temperature for 1 h after completion of the addition. The resulting mixture was neutralized with $NaHCO_3$ and evaporated to dryness. The residue was dry-loaded onto a silica gel flash column and eluted using 15% MeOH/CH_2Cl_2 to afford an orange solid (23 mg, 75%): R_f = 0.3 (20% MeOH/CH_2Cl_2); ^{1}H NMR (300 MHz, D_2O) δ 6.92 (d, 2H, J = 7.2 Hz), 6.76 (d, 2H, J = 7.2 Hz), 5.81 (s, 1H), 2.65 (s, 3H), 2.26 (s, 3H), 1.52 (s, 3H), 1.04 (s, 3H); ^{13}C NMR (125 MHz, D_2O) δ 160.0, 152.0, 146.3, 142.7, 141.5, 140.2, 133.0, 131.9, 130.3, 129.3, 129.1, 123.4, 120.1, 14.4, 14.3, 13.5, 12.6; MS (ESI) calcd for $C_{19}H_{17}BF_2N_5O_3S^-$ $(M - Na)^-$ 444.11, found 444.02; IR (thin film) 2128, 2105, 1541, 1304, 1192, 1023, 686 cm^{-1}.

Disodium 2,6-Disulfonate-1,3,5,7-tetramethyl-8-(4′-aminophenyl)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacence (3b). A solution of **9b** (200 mg, 0.35 mmol) in EtOH (10 mL) was purged with N_2 for 10 min. Hydrazine monohydrate (0.2 mL) and 10% Pd/C (37.1 mg, 0.1 equiv) were added. The mixture was refluxed under N_2 for 30 min. Then Pd/C was removed under vacuum filtration. After evaporation of the solvent, the residue was dry-loaded onto a silica gel flash column and eluted using 30% MeOH/CH_2Cl_2 to afford an orange solid (133 mg, 70%): R_f = 0.2 (30% MeOH/CH_2Cl_2); ^{1}H NMR (300 MHz, D_2O) δ 7.02−6.94 (m, 4H), 2.70 (s, 6H), 1.70 (s, 6H); ^{13}C NMR (75 MHz, D_2O) δ 154.7, 148.2, 144.1, 132.3, 131.2, 130.0, 123.9, 117.1, 117.0, 13.0 (2); MS (ESI) $C_{19}H_{19}BF_2N_3Na_2O_6S_2^+$ $(M + H)^+$ 544.0572 found 544.0557; IR (thin film) 3346, 2854, 1608, 1519,1197, 1032, 655 cm^{-1}.

Disodium 2,6-Disulfonate-1,3,5,7-tetramethyl-8-(4′-azidophenyl)-4,4-difluoro-4-bora-3a, 4a-diaza-*s*-indacence (4b). A solution of **3b** (100 mg, 0.18 mmol) in HCl (2 M, 20 mL) and H_2O (5 mL) was cooled to 0 °C. A solution of $NaNO_2$ (32 mg, 0.46 mmol) in H_2O (3 mL) was added slowly, and the mixture was kept at 0 °C with stirring for 30 min. Then a solution of NaN_3 (60 mg, 0.92 mmol) in H_2O (3 mL) was added dropwise to the mixture. Stirring was continued at room temperature for 1 h after completion of the addition. The resulting mixture was neutralized with $NaHCO_3$ and evaporated to dryness. The residue was dry-loaded onto a silica gel flash column and eluted using 30% MeOH/CH_2Cl_2 to afford an orange solid (88 mg, 77%): R_f = 0.2 (30% MeOH/CH_2Cl_2); ^{1}H NMR (300 MHz, D_2O) δ 7.33−7.26 (m, 4H), 2.75 (s, 6H), 1.67 (s, 6H); ^{13}C NMR (75 MHz, D_2O) δ 155.5, 146.2, 143.9, 142.0, 132.7, 130.9, 129.9, 129.5, 120.5, 13.7, 13.0; MS (ESI) calcd for $C_{19}H_{17}BF_2N_5O_6S_2^-$ $(M - 2Na + H)^-$ 523.06, found 523.95; IR (thin film) 2130, 1549, 1295, 1038, 667 cm^{-1}.

Compound 5. Cu (4.5 mg, 0.07 mmol), $CuSO_4 \cdot 5H_2O$ (1.8 mg, 0.007 mmol), and TBTA (3.7 mg, 0.007 mmol) were added to a solution of **4b** (40 mg, 0.07 mmol) and hexynoic acid (15.7 mg, 0.14 mmol) in 1:1 THF/H_2O (5 mL). The reaction mixture was stirred at room temperature for 12 h and evaporated to dryness. The residue was dry-loaded onto a silica gel flash column and eluted

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using 40% MeOH/CH_2Cl_2 to afford an orange solid (20 mg, 42%): $R_f = 0.1$ (30% MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, D_2O) δ 8.30 (s, 1H) 8.00 (d, 2H, $J = 8.3$ Hz), 7.49 (d, 2H, $J = 8.3$ Hz), 2.75 (br, 8H), 2.33 (t, 2H, $J = 7.2$ Hz), 1.97−1.93 (m, 2H), 1.58 (s, 6H); ^{13}C NMR (125 MHz, D_2O) δ 180.0, 155.8, 145.1, 143.8, 137.7, 134.3, 132.9, 130.6, 129.8, 122.5, 122.0, 121.7, 39.7, 24.6, 24.2, 13.8, 13.1; MS (ESI) calcd for $C_{25}H_{24}BF_2N_5O_8S_2^{2-}$ $(M - 2Na)^{2-}$ 317.56, found 317.50.

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Supporting Information Available: Characterization data for compounds **1**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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