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Water-Soluble BODIPY Derivatives

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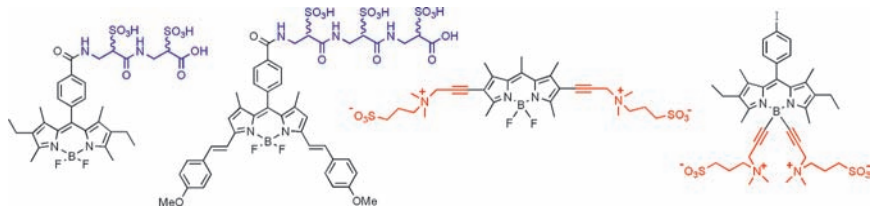
ABSTRACT



New, water-soluble BODIPY dyes have been readily obtained from various BODIPY cores by reactions involving the introduction of novel sulfonated peptide chains by either coupling or substitution to give dimethylpropargylamine derivatives subsequently quaternized by reaction with propanesultone.

Fluorescent organic dyes are widely used as nonradioactive labels in biological analysis[1] or as biomarkers in biomedical applications including imaging diagnosis.[2] Indeed, the attributes of high sensitivity and high spatiotemporal resolution and the availability of simple and rapid analytical systems make fluorescence techniques powerful tools for the interrogation of small sample volumes, small numbers of analyte molecules, and even the study of single components in complex biological systems. However, among the myriad of available synthetic fluorescent molecules exhibiting high extinction coefficients, high quantum yields, narrow emission bands, and photostability,[3] many have limited utility due to poor solubility. For most biological applications both good water solubility and resistance to the formation of nonfluorescent dimer and higher aggregates (especially after conjugation to biological material) are essential. Generally, solubility has been imported by either introducing ionizable hydrophilic groups (carboxylic acid, phosphonic acid, sulfonic acid, and ammonium groups) within the core structure of a fluorescent dye[4] or by grafting the hydrophobic fluorophore to hydrophilic (bio)polymers (carbohydrates, oligonucleotides and polyethylene glycol).[5] Both strategies have been extensively used with benzo[*a*]phenoxazine, xanthene and cyanine dyes, resulting in the development of several families of novel water-soluble fluorescent labels.[6] Some of them are commercially

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(1) Haugland, R. P.; Spence, M. T. Z.; Johnson, I. D.; Basey, A. *The Handbook: A Guide to Fluorescent Probes and Labeling Technologies*, 10th ed.; Molecular Probes: Eugene, OR, 2005.

(2) Ballou, B.; Ernst, L. A.; Waggoner, A. S. *Curr. Med. Chem.* **2005**, *12*, 795–805.

(3) Lavis, L. D.; Raines, R. T. *ACS Chem. Biol.* **2008**, *3*, 142–155.

(4) For recent examples, see: (a) Peneva, K.; Mihov, G.; Nolde, F.; Rocha, S.; Hotta, J.-i.; Braeckmans, K.; Hofkens, J.; Uji-i, H.; Herrmann, A.; Müllen, K. *Angew. Chem., Int. Ed.* **2008**, *47*, 3372–3375. (b) Reddington, M. V. *Bioconjugate Chem.* **2007**, *18*, 2178–2190. (c) Wang, H.; Lu, Z.; Lord, S. J.; Moerner, W. E.; Twieg, R. J. *Tetrahedron Lett.* **2007**, *48*, 3471–3474.

(5) For selected examples, see: (a) Atilgan, S.; Ozdemir, T.; Akkaya, E. U. *Org. Lett.* **2008**, *10*, 4065–4067. (b) Goussu, C.; Vasseur, J.-J.; Bazin, H.; Trinquet, E.; Maurin, F.; Morvan, F. *Bioconjugate Chem.* **2005**, *16*, 465–470. (c) Katritzky, A. R.; Cusido, J.; Narindoshvili, T. *Bioconjugate Chem.* **2008**, *19*, 1471–1475. (d) Reddington, M. V. *J. Chem. Soc., Perkin Trans.* **1998**, *1*, 143–148.

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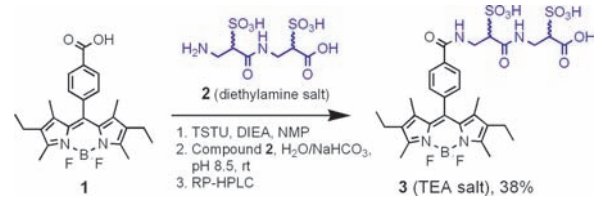
available (e.g., Alexa Fluor,[7] Cy dyes,[8] etc.) and widely used as fluorescent tags in biotechnological applications. Usually, new synthetic routes are required to introduce the desired water-solubilizing groups, which have to be masked during the synthesis to prevent cross reactivity and/or troublesome purifications. It is thus of prime interest to be able to modify postsynthetically the fluorophore skeleton with a flexible and tunable hydrophilic moiety, as we have previously illustrated with cyanine- and rhodamine-based fluorescent species.[9] Surprisingly, little synthetic effort has been devoted to the water-solubilization of 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dyes and few hydrophilic labeling reagents derived from this fluorescent core have been reported.[10] Recently, Burgess et al. explored three different strategies to introduce one or two sulfonate groups onto the hydrophobic BODIPY core, either in the 2- and 6-positions through Heck-type coupling[11] or electrophilic sulfonation with chlorosulfonic acid[12] inspired by the work of Boyer et al.[13] or in the 3-position by S_NAr reaction of a chloro substituent with 2-mercaptosulfonic acid.[14] Only the synthetic approach based on the electrophilic substitution reaction gave water-soluble BODIPY derivatives in good yields without requiring a prior functionalization of the pyrrole moieties (e.g., with chloro substituents). This solubilization strategy could be applied to BODIPY dyes to get the corresponding mono- or disulfonated derivatives, but the water-solubilizing moiety cannot be finely tuned. Thus, it is useful to explore alternative methods, allowing the introduction of a large number of sulfonate groups in a single step by means of an easy-to-handle reagent, especially for an efficient enhancement of water solubility of fluorescent BODIPY cores bearing additional aromatic rings in the meso, 3- and 5-positions (i.e., styryl-BODIPY derivatives).

Herein, we report two straightforward methods to introduce (poly)sulfonated linkers derived from α-sulfo-*β*-alanine or sulfobetaine onto BODIPY scaffolds by postsynthetic derivatization through amide formation, alkyne-coupling, and B-F substitution reactions. The spectral properties of the resulting water-soluble BODIPY derivatives were then evaluated under physiological conditions.

First, we focused on the water solubilization of two different BODIPYs, **1** and **8**, whose spectral properties are close to those of the widely used rhodamine 6G (R6G) and sulfoindocyanine dye Cy 5.0. As their dipyrromethene unit contains a *m*-phenyl substituent carrying a carboxylic acid functional group, a peptide coupling reaction with a hydrophilic linker bearing both an amino and several sulfonate groups was anticipated to be a direct way to introduce water-solubilizing residues. To reach the target, the acids **1** and **8** were prepared in two steps from the corresponding phenyliodo derivatives using first a carboalkoxylation reaction promoted by $[Pd(PPh_3)_2Cl_2]$ with ethanol as the nucleophile, followed by a saponification reaction with KOH in a mixture of methanol and water (for the synthesis details, see Supporting Information). The carboxylic acid **1** was first converted quantitatively into the corresponding *N*-hydroxysuccinimidyl (NHS) ester by treatment with *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU)/diisopropylethylamine (DIEA) in dry *N*-methylpyrrolidone (NMP) (Scheme 1).

Scheme 1. Synthesis of Water-Soluble BODIPY **3**[a]



[a] Compound **3** was obtained as a triethylammonium salt after reverse-phase (RP)-HPLC purification with aqueous triethylammonium bicarbonate (TEAB) buffer as mobile phase.

Thereafter, acylation of the primary amino group of **2** with the in situ prepared active ester in a mixture of bicarbonate buffer (pH 8.5) and NMP gave the desired water-soluble analogue **3** in a moderate yield (38% after RP-HPLC purification). The use of NMP as organic cosolvent was not sufficient to completely prevent precipitation of the intermediate NHS ester, which slowly reacts with **2** and is prone to hydrolysis to give back the starting BODIPY **1** (which was recovered unmodified in 30% yield). The same postsynthetic sulfonation procedure was applied to the styryl-BODIPY **8**, but its greater hydrophobic character could not be countered by the introduction of only two sulfonate groups. Thus, we explored the chemistry of the tripeptide (α-sulfo-*β*-alanine)$_3$ **7**, which was readily synthesized in two steps from the *N*-Fmoc dipeptide **4** used in the preparation of disulfonated linker **2** (Scheme 2). The diethylammonium salt of this highly hydrophilic linker was isolated in good yield by conventional liquid−liquid extraction, and its structure was confirmed by detailed measurements, including ESI mass spectrometry and NMR analyses.

To avoid complete precipitation of the active ester in the NMP−water mixture (observed in our first attempt for derivatization of **8** with **7**), which would prevent an efficient acylation, the previous synthetic protocol was modified by adding a transesterification step with *N*-hydroxysulfosuccinimide (sulfo-NHS). The resulting sulfo-NHS ester **9** was found to be conveniently soluble in the reaction mixture, and its derivatization with **7** afforded the target water-soluble styryl-BODIPY

(6) For recent examples, see: (a) Boyarskiy, V. P.; Belov, V. N.; Medda, R.; Hein, B.; Bossi, M.; Hell, S. W. *Chem.—Eur. J.* **2008**, *14*, 1784–1792. (b) Ho, N.-H.; Weissleder, R.; Tung, C.-H. *Tetrahedron* **2006**, *62*, 578–585. (c) Jose, J.; Burgess, K. *J. Org. Chem.* **2006**, *71*, 7835–7839.

(7) Berlier, J. E.; Rothe, A.; Buller, G.; Bradford, J.; Gray, D. R.; Filanoski, B. J.; Telford, W. G.; Yue, S.; Liu, J.; Cheung, C.-Y.; Chang, W.; Hirsch, J. D.; Beechem, J. M.; Haugland, R. P.; Haugland, R. P. *J. Histochem. Cytochem.* **2003**, *51*, 1699–1712.

(8) (a) Mujumdar, R. B.; Ernst, L. A.; Mujumdar, S. R.; Lewis, C. J.; Waggoner, A. S. *Bioconjugate Chem.* **1993**, *4*, 105–111. (b) Mujumdar, S. R.; Mujumdar, R. B.; Grant, C. M.; Waggoner, A. S. *Bioconjugate Chem.* **1996**, *7*, 356–362.

(9) Romieu, A.; Brossard, D.; Hamon, M.; Outaabout, H.; Portal, C.; Renard, P.-Y. *Bioconjugate Chem.* **2008**, *19*, 279–289.

(10) Meltola, N. J.; Wahlroos, R.; Soini, A. E. *J. Fluorescence* **2004**, *14*, 635–647.

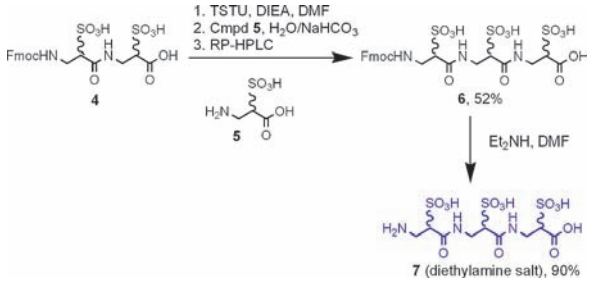
(11) Thivierge, C.; Bandichhor, R.; Burgess, K. *Org. Lett.* **2007**, *9*, 2135–2138.

(12) Li, L.; Han, J.; Nguyen, B.; Burgess, K. *J. Org. Chem.* **2008**, *73*, 1963–1970.

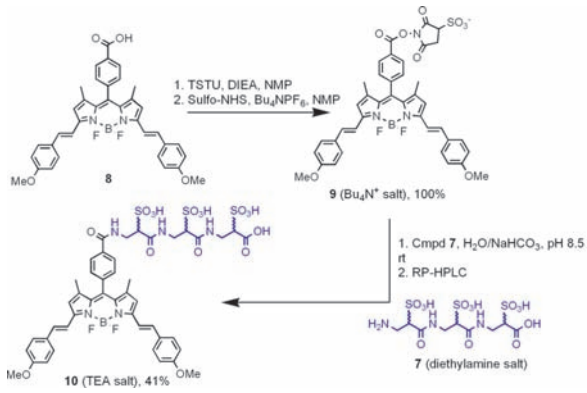
(13) Boyer, J. H.; Haag, A. M.; Sathyamoorthi, G.; Soong, M. L.; Thangaraj, K.; Pavlopoulos, T. G. *Heteroat. Chem.* **1993**, *4*, 39–49.

(14) Li, L.; Nguyen, B.; Burgess, K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3112–3116.

Scheme 2. Synthesis of Trisulfonated Peptidyl Linker **7**



Scheme 3. Synthesis of Water-Soluble Styryl-BODIPY **10**[a]



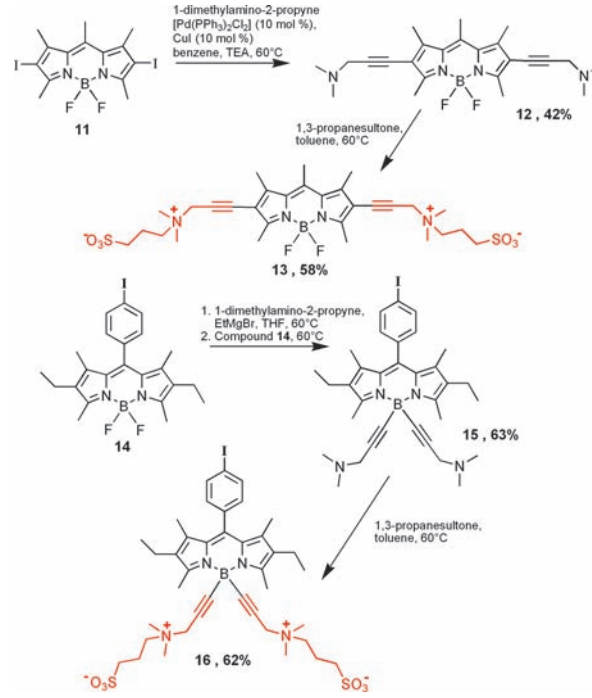
[a] Compound **10** was obtained as a triethylammonium salt when RP-HPLC purification was performed with aqueous TEAB buffer as the mobile phase.

10 (41% after RP-HPLC purification, Scheme 3). All spectroscopic data, especially NMR and mass spectra, were in agreement with the structures assigned for **3** and **10**. As expected, these triethylammonium salts were found to be perfectly soluble in water and related aqueous buffers in the concentration range (1 μM to 10 mM) suitable for biomolecular labeling applications.

To explore further the synthetic opportunities offered by alkynylation protocols for the 2,6-position of a BODIPY framework[15] and by recently developed boron chemistry,[16] we designed and prepared additional water-soluble derivatives using a novel strategy (Scheme 4).

The idea was to use 1-dimethylamino-2-propyne as the module to carry the nucleophile and to quaternarize the dimethylamino group with 1,3-propanesultone to provide a zwitteranionic fragment (betaine). The efficient solvation of the zwitterionic unit proved to be sufficient to give overall solubility. The use of sultone alkylation is commonly used in medicinal chemistry to convert easily (cyclic) aza-compounds to sulfonate derivatives[17] and has for the first time been applied here to BODIPY dyes. Cross-coupling **11** with excess 1-dimethylamino-2-propyne was promoted by Pd(0) generated in situ from

Scheme 4. Synthesis of Water-Soluble BODIPYs **13** and **16** using Sultone Alkylation



Pd(II) and Cu(I) and yielded compound **12** in 42% yield. Subsequent alkylation with 1,3-propanesultone (in excess) in dry toluene afforded the betaine **13**, which precipitated during the course of the reaction and could be isolated by centrifugation without additional treatment (58% isolated yield). Substitution of the fluoro groups proceeded readily with the Grignard reagent of 1-dimethylamino-2-propyne, providing compound **15** in 63% yield. Again, alkylation with 1,3-propanesultone provided the betaine **16** in 62% isolated yield. Dyes **13** and **16** were soluble in water and related aqueous buffers in the concentration range of 0.3−0.75 mM.

The photophysical properties of the novel water-soluble BODIPY dyes were evaluated under simulated physiological conditions [i.e., phosphate-buffered saline (PBS), pH 7.3] and are collected in Table 1. As would be expected from previous studies, the presence of a hydrophilic linker on the 8-phenyl substituent or sulfobetaine fragments at boron does not affect the spectral properties of the dyes (Figure 1 and Table 1). However, in the case of the 2,6-disubstitution in dye **13**, a bathochromic shift of 9 nm is observed in the emission spectra with respect to dye **16** when measured under the same conditions (Figure 2, top). In the case of dye **16** the absorption spectra in PBS exhibits two strong peaks at 522 and 567 nm. The latter is characteristic of an aggregate and disappears in ethanol (single absorption at 522 nm, Figure 2, top). In PBS or ethanol, a single emission is observed at 532 nm with quantum

(15) (a) Bonardi, L.; Ulrich, G.; Ziessel, R. *Org. Lett.* **2008**, *10*, 2183–2186. (b) Cakmak, Y.; Akkaya, E. U. *Org. Lett.* **2009**, *10*, 85–88.

(16) Goze, C.; Ulrich, G.; Ziessel, R. *J. Org. Chem.* **2007**, *72*, 313–322.

(17) Kong, X.; Migneault, D.; Valade, I.; Wu, X.; Gervais, F. *U.S. Patent 2007/010573*, 2007.

(18) Olmsted, J., III. *J. Phys. Chem.* **1979**, *83*, 2581–2584.

(19) Tleugabulova, D.; Zhang, Z.; Breqnnan, J. D. *J. Phys. Chem. B* **2002**, *106*, 13133–13138.

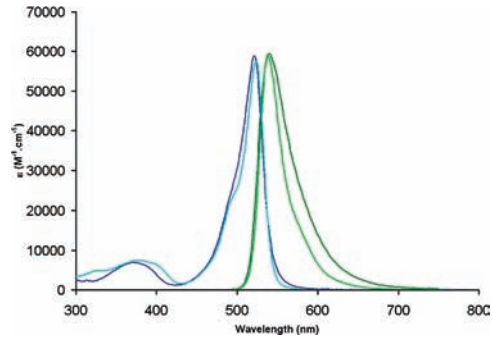


Figure 1. Absorption (light blue) and emission (light green) spectra of **3** at 25 °C in PBS. Absorption (dark blue) and emission (dark green) spectra of **13** at 25 °C in PBS.

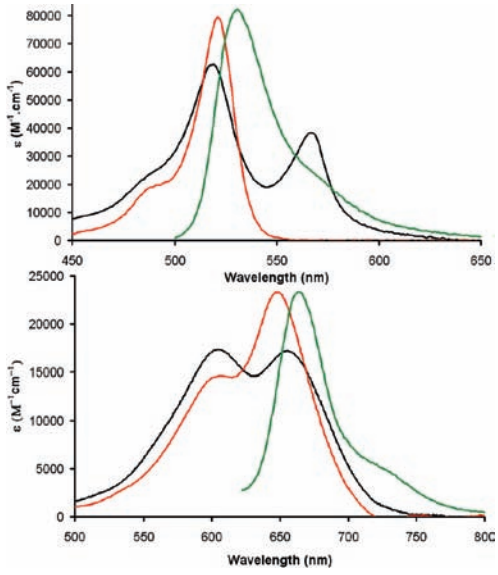


Figure 2. Top: absorption in PBS (black line), absorption in PBS + 10% EtOH (red line), and emission (green line, exc. $\lambda = 522$ nm, in PBS) spectra of **16** at 25 °C. Bottom: absorption in PBS (black line), absorption (red line), and emission (green line, exc. $\lambda = 603$ nm) spectra in PBS + 10% DMSO of **10** at 25 °C.

yields of 61% and 71%, respectively. Note that excitation at 567 nm does not cause any fluorescence of the dye. The excitation spectrum of the emissive species in PBS matches the absorption spectra of **16** in ethanol. This confirms that the emissive species is **16**.

Surprisingly, despite the presence of polysulfonated moieties, compound **10** shows a tendency to aggregate in aqueous solution and quench fluorescence, and the blue shift in the absorption maximum at 609 nm is in keeping with the formation of H-dimers[19] (Figure 2). This aggregation is persistent even at concentrations as low as 1 μM in PBS. The free fluorescent monomer is obtained in organic solvents such as DMSO and in PBS with 10% DMSO or ethanol. Under these conditions, the excitation spectrum matches the absorption spectrum, proving the absence of aggregates.

Table 1. Optical Properties of the BODIPY Derivatives

compound	solvent	$\lambda_{max,abs}$ (nm)	$\lambda_{max,em}$ (nm)	Stokes shift (cm^{-1})	Φ_F[a]
3	PBS	523 307	539	567	0.62[b]
10	PBS	609 656	nonfl	nonfl	nonfl
10	PBS + 10% DMSO	371 648	664	372	0.15
10	PBS + 10% EtOH	368 653	661	185	0.10
10	DMSO	374 649	666	393	0.40
12	CH_2Cl_2	543	565	717	0.54
13	PBS	521	540	675	0.78
15	CH_2Cl_2	521	532	396	0.52
16	PBS	522 567	530 -	289	0.61
16	EtOH	521	532	396	0.71

[a] Determined at 25 °C by using either R6G (for **3**, $\Phi_F = 0.76$ in water, ex $\lambda = 488$ nm) or sulfoindocyanine dye Cy 5.0 (for **10**, $\Phi_F = 0.20$ in PBS, ex $\lambda = 603$ nm) as standards.[8a,18] All Φ_F are corrected for changes in refractive index. [b] QY in DMSO was found to be 0.82. [c] Value for a 5×10^{-6} M solution.

Note that the water-soluble BODIPY dyes **3** and **16** were designed to have, after postsynthetic derivatization, an acid functional group suitable for conjugation to biomolecules through amide bond formation, and their good quantum yields in aqueous environments are compatible with their use as fluorescent biological labels.

In summary, the work highlights two original, tunable synthetic methods to postsynthetically convert hydrophobic BODIPY cores into corresponding water-soluble derivatives. Both approaches provide hydrophilic fluorophores with a handle (i.e., carboxylic acid or iodo functional group) for their conjugation to various biological materials. Interestingly, the mild conditions (i.e., NMP−water, pH 8.5, rt) of the amine/acid coupling reaction involved in the "acylation approach" are fully compatible with the presence of additional sophisticated functional groups. Thus, this method in combination with standard electrophilic sulfonation or B-F displacement reaction has a strong potential to enhance the water solubility without harming the optical properties of these multicomponent dyes. Current work is directed toward introducing additional sulfonate groups at the boron atom or on the styryl-anisole moieties of compound **10** to disrupt its detrimental aggregation tendency in physiological conditions.

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Supporting Information Available: Synthetic procedures and analytical data reported herein. This material is available free of charge via the Internet at http://pubs.acs.org.

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