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Amphiphilic Near-IR Emitting 3,5-Bis[2-Pyrrolylethenyl]BODIPY Derivatives: Synthesis, Characterization and Comparison with other (Hetero)Arylethenyl Substituted BODIPYs

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Abstract

A series of 3,5-bis(hetero)arylethynyl-substituted BODIPY derivatives has been prepared in Knoevenagel-type condensation of alkyl-substituted BODIPY with corresponding aldehydes. 2-Pyrrolylethenyl-substituted derivatives feature near-IR emission ($\lambda_{em} > 700$ nm), with high fluorescence quantum yield. Both, emission maxima and fluorescence quantum yields are relatively insensitive to solvent polarity, contrary to corresponding near-IR emitting 4-($N,N$-dimethylaminophenyl)ethenyl derivatives. Alkylation at the $N$-pyrrolic position of ethynyl substituent allows for installation of hydrophilic PEG group and afforded amphiphilic BODIPY derivatives. Overall, 2-pyrrolylethenyl-substituted BODIPY appears to be versatile fluorophores with potential application in near-IR imaging.

Introduction

Near-IR fluorophores are of special interest, due to their potential applications for in vivo and deep-tissue fluorescence imaging.\textsuperscript{1-4} Among various classes of organic fluorophores, BODIPY derivatives are relatively stable, with strong absorption, and high fluorescence quantum yield, and are reasonably straightforward to synthesize.\textsuperscript{5-7} Simple BODIPY derivatives absorb and emit in
the visible region (≈ 500 nm),\textsuperscript{5-6} but there are several strategies to shift absorption and emission towards the deep-red or near-IR spectral windows.\textsuperscript{6} The installation of arylethenyl (styryl) substituents at the 2,5-positions of BODIPY is synthetically straightforward and results in bathochromic shift of absorption and emission to ≈ 650 nm, and up to ≈ 700 nm, if a strongly electron donating \textit{N,N}-dialkylamino substituent is present at the styryl moiety.\textsuperscript{8-12}

For fluorescence deep-tissue imaging it is desirable to utilize fluorophores with wavelength of emission and excitation > 700 nm because of the deeper tissue penetration by near-IR light, and diminished scattering for a longer-wavelength radiation.\textsuperscript{13} Typically, distyryl-BODIPY derivatives absorb and emit ≈ 650 nm, and their absorption and emission shifts bathochromically with increasing electron-donating character of an aryl part of styryl substituent.\textsuperscript{5,6} For example, BODIPY derivatives with 4-\textit{N,N}-dialkylaminophenylethenyl substituents absorb and emit at $\lambda > 700$ nm (e.g. 1, Chart 1).\textsuperscript{8-12} However, absorption and emission for these BODIPY derivatives are solvent- and pH sensitive.\textsuperscript{14} Moreover, electron-rich aminoaryl substituents tend to quench fluorescence through internal charge transfer (ICT).\textsuperscript{14,15} Overall, these characteristics can be problematic for certain \textit{in vivo} imaging applications, when it is desirable that an optical probe behaves in predictable manner in complex, heterogenous biological environment. Therefore, we have been searching for alternative derivatives, which spectral properties are less solvent and pH dependent. Moreover, we were looking for a construct which enables easy installation of hydrophilic (e.g. PEG) groups to create amphiphilic derivatives, suitable for applications in biological media. Distyryl BODIPY derivatives are highly hydrophobic and notoriously undergo aggregation in aqueous media, though several water-soluble derivatives have been reported.\textsuperscript{16-18} Nonetheless, both, hydrophilic and hydrophobic distyryl BODIPY derivatives have been employed as fluorophores for \textit{in vivo} imaging and photosensitizers for photodynamic therapy.\textsuperscript{19-23}
Previously, we have examined a series of amphiphilic energy transfer arrays, which utilized hydrophilic BODIPY derivatives for *in vivo* medicinal imaging.\textsuperscript{24,25} We also examined BODIPY with (2,4,6-trialcoxyaryl)ethenyl substituents (2, Chart 1), which absorbs at 668 nm and functions as an excellent energy donor for bacteriochlorins.\textsuperscript{26} However, this absorption and emission maxima are sub-optimal for *in vivo* application, therefore we envisioned, that 2-pyrrolylethenyl substituents should provide a desirable bathochromic shift, and nitrogen on pyrrole moiety should provide a convenient synthetic handle to attach hydrophilic group.

We also intended to compare the optical properties of 2-pyrrolylethenyl-substituted BODIPY with analogous derivatives comprising furan and thiophene since both the later are also considered electron-donating substituents (see below). While there are several reports on 3,5-bis(2-thienylethenyl),\textsuperscript{27-33} 3,5-bis(2-furylethenyl)\textsuperscript{34} as well as 3,5-bis(3-pyrrolylethenyl)BODIPY (e.g. 3, Chart 1)\textsuperscript{35,36} derivatives, to the best of our knowledge, there was only scarce reports on 3-(2-pyrrolylethenyl)BODIPY,\textsuperscript{37-39} until recently, when during preparation of this manuscript a paper on synthesis of 3,5-di-(2-pyrrolyl)BODIPY (e.g. 4, Chart 1) was published.\textsuperscript{40} Reported derivatives 2 and 3 however, show a negligible fluorescence in polar solvent, which authors attributed to the photoinduced electron transfer from electron-rich dialkyl pyrrole subsituents. Here we present a synthesis and characterization of a series of BIDIPY derivatives with 3,5-bis(heteroarylethenyl) substituents (Scheme 1), including BODIPY with 2-thienylethenyl\textsuperscript{28} (BDP2), 2-furylethenyl (BDP-3) 2-pyrrollylethenyl (BDP4), and N-alkyl-2-pyrrolylethenyl (BDP5 and BDP8, Scheme 2) substituents. For comparison, we examined BODIPYs with 4-N,N-dimethylaminophenylethenyl (BDP-6\textsuperscript{1} Scheme 1) and 4-alcoxyphenylethenyl (BDP7 and BDP9, Schemes 1 and 3). Both BDP6 and pyrolylethenyl-substituted BODIPYs reported here show near-IR (> 700 nm) fluorescence, while the later show much less fluorescence dependence on solvent polarity. Thus, results reported
here also underpin the importance of the proper molecular design and subtle structural and electronic factors for achieving desired optical properties in BODIPY derivatives.

Chart 1. Examples of previously reported long-wavelength absorbing di(heter)oarylethynyl-substituted BODIPY derivatives: 1, 2, 3, and 4.

Results and Discussion.

Synthesis. BODIPY BDP1 was synthesized following reported procedure. Several different conditions have been reported for the Knoevenagel condensation, leading to installation of arylethenyl substituents at 3 and 5 positions. We found, the moderate to good yields have been obtained when mixture of BDP1 and corresponding aldehyde was treated with piperidine and acetic acid, in the presence of molecular sieves 3Å, in DMF or CH₃CN. Resulting products were easily purified on column chromatography. Under these conditions only minute amount of the corresponding monosubstituted product was detected, however substantial amount of material more polar than product was formed, which was not characterized. Trimethyleneeglycol moiety was subsequently installed on BDP-5 through microwave-assisted 1,3-dipolar cycloaddition reaction with of pre-installed propargyl substituent with corresponding azide under microwave irradiation, to obtain BDP8 and BDP9 in 51% and 71%, respectively (Schemes 1 and 2).
New BODIPY was characterized by $^1$H and $^{13}$C NMR, and HRMS. The corresponding data are consistent with the expected structures. The installation of new arylethenyl moiety is indicated by the presence of two doublets at $\sim 7$-$8$ ppm, with a coupling constants $\sim 16$ Hz, which is consistent with vinyl substituent in trans configuration.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>R</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{CH}_2\text{=CH}$</td>
<td>BDP2</td>
<td>62%</td>
</tr>
<tr>
<td>$\text{CH}_2\text{=S}$</td>
<td>BDP3</td>
<td>21%</td>
</tr>
<tr>
<td>$\text{CH}_2\text{=N}$</td>
<td>BDP4</td>
<td>55%</td>
</tr>
<tr>
<td>$\text{CH}_2\text{=N}$</td>
<td>BDP5</td>
<td>67%</td>
</tr>
<tr>
<td>$\text{CH}_2\text{=N}$</td>
<td>BDP6</td>
<td>45%</td>
</tr>
<tr>
<td>$\text{CH}_2\text{=N}$</td>
<td>BDP7</td>
<td>63%</td>
</tr>
</tbody>
</table>

Scheme 1. Synthesis of diarylethenyl-substituted BODIPY through Knoevenagel-type condensation.
Scheme 2. Synthesis of BDP8.

**Absorption and emission properties.** Absorption and emission properties for all reported BODIPYs were first determined in toluene (Table 1). As expected, absorption spectrum for each dirylethenyl-BODIPY is dominated by a very strong band between 600-700 nm, corresponding to $S_0 \rightarrow S_1$ transition, with a distinctive vibronic band on the blue onset of the manifold, and a weaker band ~ 400 nm, corresponding to $S_0 \rightarrow S_2$ transition. The position of the long-wavelength band is progressively bathochromically shifted with increasing electron-donating ability of aryl part of substituent. In particular, $\lambda_{\text{max}}$ for furyl and thiophene-substituted BDP2 and BDP3 are nearly identical (657 nm and 658 nm), while for pyrrole-substituted BDP3 $\lambda_{\text{max}}$ is shifted to 686 nm. $N$-substitution pyrrole by alkyl group (BDP5, BDP8), causes additional bathochromic shift (695-697 nm), consistently with an electron-donating character of alkyl groups. The most bathochromically shifted derivative is a dimethylaminophenyl-substituted BDP6 (708 nm), that derivative features also elaborated manifold between 300-500 nm.

Emission spectra of new BODIPY derivatives in toluene are similar to these reported for analogous styryl derivatives and consist predominantly 0-0 transition, with much weaker 0-1 transition. Stokes’ shift varies from ~ 10 nm for derivatives absorbing at shorter wavelength (BDP2,3,7 and BDP9), and gradually increases for derivatives absorbing ~700 nm, ranging from 16 nm for BDP4 to 18 nm for BDP5 and to 29 nm for BDP6. Fluorescence quantum yield $\Phi_f$ in toluene is relatively high, ranging from 0.74 for BDP7 to 0.40 for BDP8. Reduction of $\Phi_f$ for long-wavelength emitting derivatives is consistent with an energy-gap rule. $N$-substitution of pyrrole causes minute decrease in $\Phi_f$ (0.46 for BDP4, 0.43 for BDP5 and 0.40 for BDP8. Alkyl groups likely impose conformational flexibility, which enhances internal conversion. It is worth noting,
that thiophene-substituted BDP3 has identical $\Phi_f$ as furan-substituted BDP2. Apparently, thiophene did not substantially enhance $S_1 \rightarrow T_1$ intersystem crossing. Previous reports indicate that thiophene may or may not affect the ISC in BODIPY, depending on manner how thiophene is attached.\textsuperscript{44}

![Absorption and Emission Spectra](image)

Figure 1. Absorption (upper panel) and emission (lower panel) spectra of BDP2 (orange), BDP3 (green), BDP4 (red), BDP5 (purple), BDP6 (blue), BDP7 (black). All spectra are taken in toluene and normalized at the maximum of absorption/emission.

Table 1. Photophysical properties of BODIPY derivatives.\textsuperscript{a}
<table>
<thead>
<tr>
<th>Compound</th>
<th>$\lambda_{\text{abs}}$</th>
<th>$\lambda_{\text{em}}$</th>
<th>$\Phi_f$ (toluene)</th>
<th>$\Phi_f$ (DMF)</th>
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<tr>
<td>BDP2</td>
<td>657 nm</td>
<td>667 nm</td>
<td>0.66</td>
<td>0.46</td>
</tr>
<tr>
<td>BDP3</td>
<td>658 nm</td>
<td>668 nm</td>
<td>0.57</td>
<td>0.46</td>
</tr>
<tr>
<td>BDP4</td>
<td>686 nm</td>
<td>702 nm</td>
<td>0.46</td>
<td>0.31</td>
</tr>
<tr>
<td>BDP5</td>
<td>695 nm</td>
<td>713 nm</td>
<td>0.43</td>
<td>0.34</td>
</tr>
<tr>
<td>BDP6</td>
<td>708 nm</td>
<td>737 nm</td>
<td>0.41</td>
<td>$\sim0.08^c$</td>
</tr>
<tr>
<td>BDP7</td>
<td>648 nm</td>
<td>658 nm</td>
<td>0.74</td>
<td>0.72</td>
</tr>
<tr>
<td>BDP8</td>
<td>697 nm</td>
<td>721 nm</td>
<td>0.40</td>
<td>0.32</td>
</tr>
<tr>
<td>BDP9</td>
<td>644 nm$^b$</td>
<td>656 nm$^b$</td>
<td>$\sim d$</td>
<td>0.57</td>
</tr>
</tbody>
</table>

$^a$Absorption and emission wavelength were determined in toluene, unless noted otherwise. Only the longest wavelength band is reported. Fluorescence quantum yield was determined in air-equilibrated solvents, using tetraphenylporphyrin in air-equilibrated toluene ($\Phi_f = 0.070^{45}$).

$^b$Determined in DMF. $^c$Fluorescence quantum yield could not be accurately determined, due to the limitation in detector sensitivity for $\lambda > 850$ nm. $^d$Insoluble in toluene.

**MOs calculation.** DFT calculations (Table 2, see Supporting Information for more details) show the effect of (hetero)aromatic substituents on HOMO and LUMO energies of the resulting derivatives. Energies of both HOMO and LUMO progressively increase in order of BDP3 < BDP7 < BDP2 < BDP4 < BDP5 < BDP6, compared to benchmark phenylethenyl-BODIPY BDP10. Increase in MOs energies is accompanied by reduction of HOMO-LUMO energy gap, which is congruent with shift of $S_0 - S_1$ absorption band. Overall, both effects, increase in HOMO/LUMO energies and reduction of HOMO-LUMO gap are significantly larger for (2-pyrrolylethenyl)-substituted BODIPY than for corresponding furan or thiophene derivatives. This probably reflects the greater electron-donating ability of pyrrole, compared to thiophene or furan. Electron donating
ability can be quantified, for example, by ionization potential, which is equal to 8.2 eV,⁴⁶,⁴⁷ 7.94 eV,⁴⁷ 8.87 eV,⁴⁸ 8.89 eV,⁴⁹ and 7.14 eV⁵⁰ for pyrrole, N-methylpyrrole, thiophene, furan, and N,N-dimethylpyrrole respectively. Interestingly, 4-methoxyphenylethenyl-substituted BODIPY BDP7 features much lower HOMO/LUMO energies and larger HOMO-LUMO gap, despite having ionization energy (~8.2-8.4 eV)⁵⁰ comparable with that of pyrrole. Apparently, pyrrole is a greater contributor of an electron density through resonance than methoxybenzene (see below).

Table 2. MOs for selected BODIPY derivatives.⁴

<table>
<thead>
<tr>
<th>Compound</th>
<th>HOMO [eV]</th>
<th>LUMO [eV]</th>
<th>LUMO-HOMO [eV]</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDP1</td>
<td>-5.43</td>
<td>-2.44</td>
<td>2.99</td>
</tr>
<tr>
<td>BDP2</td>
<td>-4.71</td>
<td>-2.60</td>
<td>2.11</td>
</tr>
<tr>
<td>BDP3</td>
<td>-4.79</td>
<td>-2.67</td>
<td>2.12</td>
</tr>
<tr>
<td>BDP4</td>
<td>-4.55</td>
<td>-2.49</td>
<td>2.06</td>
</tr>
<tr>
<td>BDP5</td>
<td>-4.54</td>
<td>-2.48</td>
<td>2.06</td>
</tr>
<tr>
<td>BDP6</td>
<td>-4.29</td>
<td>-2.26</td>
<td>2.03</td>
</tr>
<tr>
<td>BDP7</td>
<td>-4.75</td>
<td>-2.58</td>
<td>2.17</td>
</tr>
<tr>
<td>BDP10 ⁵</td>
<td>-4.90</td>
<td>-2.68</td>
<td>2.22</td>
</tr>
</tbody>
</table>

⁴ Calculations were performed in vacuum, employing the DFT B3LYP 6-31G* method. ⁵ BDP-10 is 3,5-diphenylethenyl-BODIPY (see Supporting Information for the structure).

Solvent-dependent absorption and emission properties of diarylethenyl-BODIPY derivatives. 4-(N,N-dimethylamino)phenylethenyl-substituted BODIPYs shows a remarkable dependence of emission maxima and Φᵣ on solvent polarity which is manifested by a significant bathochromic shift of emission band and reduction of Φᵣ when solvent dielectric constants
increases,\textsuperscript{14} This behavior is attributed to significant contribution of internal charge transfer (ICT) state to $S_1$, due to the presence of non-bonding electron pairs on amino nitrogen, which donates electrons via relevant resonance structures (structures III and IV, Scheme 4a).\textsuperscript{14} Corresponding resonance structures can be drown for 2-pyrrolylethenyl substituted BODIPY (Scheme 4b), therefore it can be expected, that BDP5 and BDP8 will exhibit a similar solvent dependence. Moreover, recently a nearly complete fluorescence quenching in DMSO was reported for analogous pyrrolylethenyl-substituted BODIPY derivatives, which was attributed to photoinduced electron transfer, from pyrrole substituents, which efficiently quenches BODIPY excited state.\textsuperscript{40}

![Scheme 4. Possible resonance structures for (a) BDP6\textsuperscript{14} and (b) BDP5 or BDP8.](image)

Significant dependence of $\lambda_{em}$ and $\Phi_f$ on local polarity can be problematic for application of given derivatives in biological imaging, since brightness of the probe would significantly affected by a complex intracellular media. Therefore, here we determined absorption and emission properties of $N$-alkyl-2-pyrrolylethenyl-substituted derivatives in series of solvents of high
dielectric constants, and compared these with the same for \( N,N \)-dimethylaminophenylethenyl-substituted derivative (Figure 2, Table 3).

Absorption spectra of BDP5, BDP6, and BDP8 show comparable, very minor solvent polarity dependence, manifested by a small (< 10 nm) shift of the \( S_0 - S_1 \) absorption band, and even smaller shift for \( S_0 - S_2 \) transition. Interestingly, these spectral shifts do not correlate with solvent dielectric constants (e.g. the largest batochromic shift is observed in PhCN).

Emission spectra in solvents of high dielectric constants are distinct for dimethylaminophenylethenyl-BODIPY BDP6 and \( N \)-alkyl-2-pyrrolylethenyl-BODIPY BDP5 and BDP8. BDP6 exhibits significant broadening and batochromic shift in emission (44 – 75 nm, compared to toluene), when solvent dielectric constant increases (Figure 2b). This observation indicates a huge change in dipole moment in the excited state for BDP6 and it is consistent with substantial contribution of ICT (i.e. structures III and IV) to the excited state.\(^{14}\) On the other hand BDP5 and BDP8 exhibit a small batochromic shift in polar solvent (maximum 21 nm compared to toluene), and without significant broadening. Stokes’ shift increases parallel to increase of dielectric constant of solvent, but this increase is substantially smaller than for BDP6. \( \Phi_f \) for BDP5 and BDP8 are moderately reduced (~1.3-fold) in polar solvents. Larger reduction (1.8-fold compared to that in toluene) is observed in MeOH. This reduction is much smaller than that reported for 4-\( N,N \)-dimethylaminophenylethenyl substituted BODIPY reported previously (e.g. >10-fold reduction of \( \Phi_f \) for monostyryl-substituted BODIPY in DMSO compared to toluene).\(^{14}\) In our case, an accurate determination of \( \Phi_f \) for BDP6 in polar solvents was impossible due to the limitation in detector sensitivity for \( \lambda > 850 \) nm). Our results also contrast with that reported recently for other pyrrolylethenyl-substituted BODIPY, where a negligible emission was observed.
in DMSO, due to the photoinduced electron transfer from pyrrole to photoexcited BODIPY\textsuperscript{40}. Apparently, PET does not operate efficiently for BDP\textsubscript{5} and BDP\textsubscript{8}, even in highly polar DMSO. We are attributing less efficient PET to (a) lower electron-donating ability of pyrrole substituent for BDP\textsubscript{5} and BDP\textsubscript{8}, compared to that in 2 and 3, where more electron rich dialkylpyrrole was used, and (2) lower electron-accepting ability of BODIPY core of BDP\textsubscript{5} and BDP\textsubscript{8}, due to the presence of two methyl groups at 1 and 8 positions\textsuperscript{51}. Overall, these results underpin the importance of an electronic balance, achieved by a substitution pattern, to achieve a high fluorescence quantum yield. We anticipate that further modification of BODIPY structure allows for both greater batochromic shift and high fluorescence quantum yield in pyrolylethenyl substituted BODIPY.

(a)

(b)
Figure 2. Absorption and emission spectra of BDP5 (a), BDP6 (b), and BDP8 (c) in different solvents: toluene (black), MeOH (orange), PhCN (green), DMF (blue), DMSO (red).

Table 3. Absorption and emission data for BODIPY derivatives in solvents of different dielectric constants.

<table>
<thead>
<tr>
<th>Solvent (dielectric constant)</th>
<th>$\lambda_{\text{abs}}$</th>
<th>$\lambda_{\text{em}}$</th>
<th>Stokes’ shift (cm$^{-1}$)</th>
<th>$\Phi_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BDP5</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toluene ($\varepsilon = 2.38$)</td>
<td>695 nm</td>
<td>713 nm</td>
<td>363</td>
<td>0.43</td>
</tr>
<tr>
<td>PhCN ($\varepsilon = 26.00$)</td>
<td>699 nm</td>
<td>729 nm</td>
<td>589</td>
<td>0.36</td>
</tr>
<tr>
<td>MeOH ($\varepsilon = 33.00$)</td>
<td>684 nm</td>
<td>716 nm</td>
<td>653</td>
<td>0.24</td>
</tr>
<tr>
<td>DMF ($\varepsilon = 36.71$).</td>
<td>694 nm</td>
<td>722 nm</td>
<td>748</td>
<td>0.34</td>
</tr>
<tr>
<td>DMSO ($\varepsilon = 47.24$)</td>
<td>699 nm</td>
<td>732 nm</td>
<td>645</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>BDP6</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Toluene</td>
<td>708 nm</td>
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<td>556</td>
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</tr>
<tr>
<td>PhCN</td>
<td>732 nm</td>
<td>781 nm</td>
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<tr>
<td>MeOH$^a$</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>DMF</td>
<td>714 nm</td>
<td>793 nm</td>
<td>1395</td>
<td>$\sim 0.08^{b)}$</td>
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<tr>
<td>DMSO</td>
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<td>813 nm</td>
<td>1531</td>
<td>$\sim 0.07^{b)}$</td>
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<tr>
<td><strong>BDP8</strong></td>
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<tr>
<td>Solvent</td>
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<td>λ_{max}</td>
<td>λ_{ vib}</td>
<td>Φ</td>
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<tr>
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<td>736 nm</td>
<td>597</td>
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<tr>
<td>DMSO</td>
<td>705 nm</td>
<td>741 nm</td>
<td>689</td>
<td>0.31</td>
</tr>
</tbody>
</table>

\(^a\) BDP6 is not soluble in MeOH. \(^b\) Fluorescence quantum yield cannot be accurately determined, due to the limitation in sensitivity of detector at wavelength > 850 nm.

**Optical properties in micelles.** Optical properties of PEG-derivatized BDP8 were investigated in series of aqueous micelles formulated from surfactants Pluronics F127, Tween 20, and Triton X100. Absorption spectra of BDP8 at concentration of ~10 µM are presented in Figure 3 and Table 4. Wavelengths of absorption maxima in micelles vary only slightly, compared to that observed in toluene (Table 4) A moderate broadening (~50% compared to toluene) of S\(_0\) → S\(_1\) band is observed in Triton X100 and Tween 20, suggesting a partial aggregation of BODIPY in these media. In Pluronic P127 significant lowering of the ratio of the intensities of 0-0 to 0-1 vibronic bands as well as ratio of intensities of S\(_0\) → S\(_1\) to S\(_0\) → S\(_2\) absorption bands is observed, in addition to broadening of the longest wavelength absorption band. Emission bands in micelles are slightly shifted (~10 mm) compared to that in toluene. The magnitude of a Stokes’ shift suggests, that micelles BODIPY is localized in environment of dielectric constants between that of toluene, and PhCN. Emission band in each micelle is moderately broader (~1.3-fold), compared to that in toluene. However, Φ\(_f\) in micelles is reduced significantly compared to that in toluene, from ~2-fold (in Tween 20 and Triton X100), up to 10-fold (in Pluronic F127).
Figure 3. Normalized absorption and emission spectra of **BDP8** in aqueous micelles and selected organic solvents: toluene (black), MeOH (green), Pluronic F127 (blue), Tween 20 (red), and Triton X100 (orange).

Table 4. Optical properties of **BDP8** in micelles.$^a$

<table>
<thead>
<tr>
<th>Medium</th>
<th>$\lambda_{\text{abs}}$ (FWHM)</th>
<th>$\lambda_{\text{em}}$ (FWHM)</th>
<th>Stokes' shift (cm$^{-1}$)</th>
<th>$\Phi_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>697 nm (42.5 nm)</td>
<td>721 nm (47 nm)</td>
<td>478</td>
<td>0.40</td>
</tr>
<tr>
<td>Pluronic F127</td>
<td>702 nm (104 nm)</td>
<td>731 nm (64 nm)</td>
<td>565</td>
<td>0.03</td>
</tr>
<tr>
<td>Tween 20</td>
<td>701 nm (61 nm)</td>
<td>731 nm (60 nm)</td>
<td>585</td>
<td>0.21</td>
</tr>
<tr>
<td>Triton X100</td>
<td>704 nm (55)</td>
<td>730 nm (61 nm)</td>
<td>506</td>
<td>0.23</td>
</tr>
</tbody>
</table>

$^a$ Measurements performed in 3 mM Triton X-100, 0.15 mM of Pluronic F127, and 0.1% (v/v) of Tween-20 all in PBS (pH = 7.4). $\Phi_f$ (±10%) was determined in air-equilibrated solvents, using tetraphenylporphyrin in toluene as a standard ($\Phi$ = 0.070$^{45}$) and corrected for refractive index $n$ of
the medium. For all measurements done in aqueous surfactant \( n = 1.45 \) was used,\textsuperscript{52} as an average between that for pure hydrocarbons (1.49) and pure water (1.39).

**Conclusion**

Substitution of BODIPY at 3,5-positions with 2-pyrrolylethenyl substitutents causes a significant bathochromic shift of absorption and emission bands, pushing them in near-IR window. This shift in absorption/emission is much larger than in case of analogous derivatives bearing furan or thiophene substituents. N-Pyrrolic positions can be easily derivatized through a click chemistry and allow for introducing hydrophilic substituents and producing amphiphilic derivatives. Novel near-IR emitting BODIPY derivatives show limited dependence of emission properties of microenvironment and show good fluorescence quantum yield in polar and non-polar environment as well as in aqueous micelles, thus they appear to be excellent candidates for neat-IR fluorophores for biological applications. The results of initial *in vivo* testing of BODIPYs described here are described elsewhere.\textsuperscript{53} The research on incorporation of BODIPY derivatives described here into energy transfer arrays to develop fluorophores for multicolor imaging are currently ongoing in our laboratory.

**Experimental Section**

*General. Synthesis.* All reagents, solvents, etc. not prepared in house were purchased through either Sigma-Aldrich or Fisher Scientific and used without further purification. All solvents used are ACS grade, unless noted otherwise.

*Microwave Reactions* Microwave reactions were performed in CEM Discover CEM, Mathew, NC) microwave instrument. All reactions were performed in 80 mL CEM microwave vessel, with continuous monitoring of pressure and temperature. Temperature was monitored using
built-in IR sensor. Microwave reactions involves three stages: (1) “Run time” - reaction mixture was irradiated with 150 W until it reaches designated temperature (30-120 sec); (2) “Hold time” – reaction mixture was maintained at designated temperature for set time, (3) “Cooling time” – reaction mixture was kept in closed reaction vials until reaches 50 °C (approximately 5-10 min).

Characterization All NMR spectra were acquired on either 400 MHz NMR or 500 MHz NMR. All HRMS data acquired on FT-ICR MS.

Spectroscopic Studies. Fluorescence measurements were performed with a sample absorbance of approximately 0.1. All measurements were performed in HPLC grade solvents.

DFT calculations were performed, and results were visualized using Spartan 10 for Windows (Wavefunction Inc, Irvine, CA).

Known compounds 1,54 and BDP141 were synthesized following the reported procedure.

BDP2. A mixture of BDP1 (150 mg, 0.392 mmol), furfural (377 mg, 3.92 mmol), and molecular sieves (3Å, ~1.0 g) in acetonitrile (15 mL) was treated with acetic acid (15 μL) and piperidine (30 μL). The resulting mixture was protected from light and refluxed for 18 hours. The reaction mixture was diluted with ethyl acetate and washed (water and brine), dried (Na₂SO₄), and concentrated. Column chromatography [silica, hexanes/CH₂Cl₂ (1:10), first band (blue)] provided a green-gold solid. (130 mg, 62%). ¹H NMR (400 MHz, acetone-δ₆): δ 1.44 (s, 6H), 3.94 (s, 3H), 6.59 (dd, J = 1.8 Hz, 3.4 Hz, 2H), 6.67 (d, J = 3.4 Hz, 2H), 6.84 (s, 2H), 7.31 (d, J = 16.3 Hz, 2H), 7.59 (d, J = 16.5 Hz, 2H), 7.65 (d, J = 8.5 Hz, 2H), 7.73 (m, 2H), 8.22 (d, J = 8.5 Hz, 2H); ¹³C NMR (126 MHz, CDCl₃): δ 15.1, 52.6, 57.5, 60.8, 112.5, 112.7, 117.7, 117.7 118.3, 119.9, 123.5, 128.4, 129.3, 129.3, 130.6, 144.4, 153.3, 181.6; HRMS (ESI-TOF) m/z [M+Cs]⁺ Calcd for C₃₁H₂₅BF₂N₂O₄, 671.0930; Found: 671.0928.
**BDP3.** BDP3 was reported previously\(^2\) and here it was prepared following a slightly modified procedure. A mixture of BDP1 (30 mg, 0.078 mmol), 2-thiophenecarboxaldehyde (88.3 mg, 0.784 mmol), and molecular sieves (3 Å, ~1.0 g) in acetonitrile (5 mL) was treated with acetic acid (3 μL) and piperidine (6 μL). The resulting mixture was refluxed, protected from light, for 18 hours. The reaction mixture was diluted with ethyl acetate, washed (water and brine), dried (Na\(_2\)SO\(_4\)), and concentrated. Column chromatography [silica, hexanes/ethyl acetate (5:1), first band (blue)] provided a dark blue-purple film (9.6 mg, 21%). Characterization data (\(^1\)H NMR, HRMS) are consistent with the one reported previously.\(^2\)^\(^1\)H NMR (400 MHz, acetone-\(d_6\)): \(\delta\) 1.51 (s, 6H), 4.00 (s, 3H), 6.93 (s, 2H), 7.19 (dd, \(J = 3.6\) Hz, 5.1 Hz, 2H), 7.38 (d, \(J = 3.6\) Hz, 2H), 7.55 (d, \(J = 16.6\) Hz, 2H), 7.63 (d, \(J = 5.7\) Hz, 2H), 7.71 (d, \(J = 8.5\) Hz, 2H), 7.77 (d, \(J = 16.1\) Hz, 2H), 8.28 (d, \(J = 8.5\) Hz, 2H); HRMS (ESI-TOF) \(m/z\) [M+Cs]\(^+\) Calcd for C\(_{31}\)H\(_{25}\)BF\(_2\)N\(_2\)O\(_2\)S\(_2\), 703.0473, Found 703.0477.

**BDP4.** A mixture of BDP1 (150 mg, 0.392 mmol), pyrrole-2-carboxaldehyde (373 mg, 3.92 mmol), and molecular sieves (3Å, ~1.0 g) in acetonitrile (15 mL) was treated with acetic acid (15 μL) and piperidine (30 μL). The resulting mixture was refluxed for 18 hours, protected from light. The reaction mixture was diluted with ethyl acetate, washed (water and brine), dried (Na\(_2\)SO\(_4\)), and concentrated. Column chromatography [silica, CH\(_2\)Cl\(_2\), second band (green)] provided a dark green solid. The semi-pure product was dissolved in the minimum amount of CH\(_2\)Cl\(_2\) and small portions of cold hexanes were added to remove residual impurities. The resulting dark-red solid was collected (115 mg, 55% yield). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 1.45 (s, 6H) 4.00 (s, 3H), 6.14 (m, 2H), 6.29 (s, 2H), 6.54 (s, 2H), 6.57 (m, 2H), 6.99 (d, \(J = 16.3\) Hz, 2H), 7.46 (d, \(J = 16.4\) Hz, 2H), 7.52 (d, \(J = 8.4\) Hz, 2H), 8.22 (d, \(J = 8.4\) Hz, 2H), 9.54 (s, 2H); \(^13\)C NMR (101 MHz, CDCl\(_3\)): \(\delta\) 14.9, 52.5, 100.0, 110.3, 112.1, 114.5, 117.5, 122.5, 126.8, 129.4, 130.3,
BDP5. A mixture of BDP1 (580 mg, 1.52 mmol), 1-(prop-2-yn-1-yl)-1H-pyrrole-2-carboxaldehyde (2.02 g, 15.2 mmol), and molecular sieves (3Å, ~1.0 g) in acetonitrile (46 mL) was treated with acetic acid (58 μL) and piperidine (116 μL). The resulting mixture was refluxed for 18 hours, protected by light. Upon reaction completion, the mixture was concentrated. Column chromatography [silica, hexanes/CH₂Cl₂ (1:2), second band (green)] provided a dark green solid. The semi-pure product was washed with hexanes to remove excess aldehyde. Product is a dark green solid (612.6 mg, 67% yield). ¹H NMR (400 MHz, CDCl₃): δ 1.41 (s, 6H), 2.47 (t, J = 2.5 Hz, 2H), 3.98 (s, 3H), 4.78 (d, J = 2.5 Hz, 4H), 6.26 (m, 2H), 6.57 (s, 2H), 6.83 (dd, J = 1.1 Hz, 3.9 Hz, 2H), 6.89 (dd, J = 1.6 Hz, 2.6 Hz, 2H), 7.19 (d, J = 16.0 Hz, 2H), 7.46 (m, 4H), 8.18 (d, J = 8.5 Hz, 2H); ¹³C NMR (101 MHz, CDCl₃): δ 14.9, 36.9, 52.5, 74.9, 77.8, 110.2, 111.8, 116.4, 117.6, 123.4, 124.8, 129.3, 130.3, 130.7, 131.5, 132.9, 135.2, 140.5, 141.0, 152.8, 166.7; HRMS (ESI-TOF) m/z [M] Calcd for C₃₁H₂₇BF₂N₄O₂, 536.2195, Found; 536.2191.

BDP6. A mixture of BDP1 (80 mg, 0.209 mmol), p-N,N-dimethylaminobenzaldehyde (312 mg, 2.09 mmol), and molecular sieves (4 Å, ~1.0 g) in acetonitrile (8 mL) was treated with acetic acid (96 μL) and piperidine (248 μL). The reaction was refluxed for two days, protected from light. The resulting mixture was dissolved in ethyl acetate, washed (water and brine), dried (Na₂SO₄), and concentrated. Column chromatography [silica, hexanes/CH₂Cl₂ (1:5), third band (green)] provided a dark red solid (60.2 mg, 45% yield). ¹H NMR (400 MHz, CDCl₃): δ 1.39 (s, 6H), 3.03 (s, 12H), 3.97 (s, 3H), 6.59 (s, 2H), 6.71 (d, J = 8.9 Hz, 4H), 7.19 (d, J = 16.1 Hz, 2H), 7.45 (d, J = 8.5 Hz, 2H), 7.53 (m, 6H), 8.16 (d, J = 8.5 Hz, 2H); ¹³C NMR (101 MHz, CDCl₃): δ 14.9, 40.4, 52.4, 112.2, 114.9, 117.5, 125.1, 129.3, 129.4, 129.4, 130.2, 130.6, 130.6, 136.8, 140.6, 140.8,
BDP7. A mixture of BDP1 (0.668 g, 1.75 mmol), p-propargyloxybenzaldehyde (0.617 g, 3.85 mmol), and molecular sieves (3Å ~1g) in anhydrous MeCN (70 mL) was treated dropwise with AcOH (2.1 mL, 0.25 mmol) and piperidine (3.5 mL, 0.25 mmol) and refluxed under N2. After 21 hours, the reaction mixture was concentrated to dryness. Column chromatography [silica, hexanes/CH₂Cl₂ (1:1)→(2:3)→(1:2)→(0:1)] yielded a blue-gold solid, with a minor impurity visible on TLC, located just beneath product. This impurity was removed by dissolving product in CH₂Cl₂ and precipitating through addition of hexanes. Precipitate was filtered and dried, yielding a blue-gold solid (732 mg, 63%). ¹H NMR (CDCl₃, 400 MHz): δ 1.42 (s, 6H), 2.56 (t, J = 2.3 Hz, 2H), 3.98 (s, 3H), 4.74 (s, 4H), 6.62 (s, 2H), 7.02 (d, J = 8.8 Hz, 4H), 7.22 (d, J = 16.1 Hz, 2H), 7.46 (d, J = 8.1 Hz, 2H), 7.59-7.64 (m, 6H), 8.19 (d, J = 8.1 Hz, 2H); ¹³C NMR (CDCl₃, 126 MHz): δ 15.1, 52.8, 56.2, 76.2, 78.6, 115.8, 118.0, 118.2, 129.3, 129.4, 130.6, 131.1, 133.1, 136.3, 137.1, 140.5, 141.9, 153.4, 158.7, 166.9; HRMS (ESI-TOF) m/z [M+H]+ Calcd for C₄₁H₃₄BF₂N₂O₄ 666.2503; Found 666.2501.

BDP8. A mixture of BDP5 (613 mg, 1.00 mmol), azide 1 (1.87 g, 8.00 mmol), CuSO₄·5H₂O (335 mg, 1.34 mmol), and L-ascorbic acid (266 mg, 1.34 mmol) in acetone/water ((5:1), 6 mL). Was placed in a microwave tube and irradiated at 65°C for one hour, as described in General Experimental Section. The resulting mixture was diluted with ethyl acetate, washed (water and brine), dried (Na₂SO₄) and concentrated. Column chromatography [ethyl acetate/CH₃OH (10:1), third band (green, red fluorescent)] provided a dark green sticky solid (544 mg, 51% yield). ¹H NMR (400 MHz, CDCl₃): δ 1.37 (s, 6H), 3.33 (s, 6H), 3.54 (m, 27H), 3.80 (m, 4H), 3.96 (s, 3H), 4.46 (m, 4H), 5.33 (s, 4H), 6.25 (dd, J = 2.7 Hz, 3.7 Hz, 2H), 6.53 (s, 2H), 6.83
(dd, $J = 1.4$ Hz, 3.9 Hz, 2H), 6.88 (dd, $J = 1.6$ Hz, 2.6 Hz, 2H), 7.19 (d, $J = 16.1$ Hz, 2H), 7.38 (d, $J = 16.0$ Hz, 2H), 7.42 (d, $J = 8.5$ Hz, 2H), 7.48 (s, 2H), 8.16 (d, $J = 8.45$ Hz, 2H); $^{13}$C NMR (101 MHz, CDCl$_3$): $\delta$ 15.0, 43.4, 50.7, 52.7, 59.3, 69.6, 70.8, 72.2, 110.6, 111.7, 116.2, 118.0, 123.6, 124.1, 125.6, 129.5, 130.5, 131.0, 131.6, 133.1, 135.2, 140.7, 141.2, 145.1, 152.9, 166.9.; HRMS (ESI-TOF) $m/z$ [M+H]$^+$ Calcd for C$_{55}$H$_{69}$BF$_2$N$_{10}$O$_{10}$, 1079.5341, Found; 1079.5329.

**BDP9.** A suspension of BDP7 (150.0 mg, 0.225 mmol), azide $\mathbf{1}$ (136.5 mg, 0.585 mmol), CuSO$_4$·5H$_2$O (28.1 mg, 0.113 mmol), L ascorbic acid sodium salt (22.4 mg, 0.113 mmol) in acetone/H$_2$O (5:1, 48 mL) was placed in 80 mL microwave vessel The contents of the vessel were then subjected to two microwave irradiation cycles of, 30-minute hold time, power of 150W at 65 ºC. TLC indicated reaction was incomplete, thus another batch of $\mathbf{1}$ (136.5 mg, 0.585 mmol) was added, and a third microwave exposure was performed. After third exposure reaction was still incomplete, another batch of $\mathbf{1}$ (136 mg, 0.585 mmol) CuSO$_4$·5H$_2$O (28.1 mg, 0.113 mmol) and sodium ascorbate 22.4 mg (0.113 mmol) were added, and the contents were exposed to two further irradiation cycles for a total of five cycles (2.5 hours). Once the reaction was determined to be complete (TLC), the reaction was diluted with CH$_2$Cl$_2$, washed (water and brine), dried (Na$_2$SO$_4$) and concentrated. Column chromatography [silica, CH$_2$Cl$_2$/MeOH (1:0)→(40:1)→(30:1)→(20:1)] yielded a dark blue oil, that solidified upon prolonged drying under high vacuum. $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 1.39 (s, 6H), 3.33 (s, 6H), 3.48-3.52 (m, 4H), 3.58-3.62 (m, 20H), 3.87 (t, $J = 5.0$ Hz, 4H), 3.96 (s, 3H), 4.55 (t, $J = 5.0$ Hz, 4H), 7.02 (d, $J = 8.8$Hz, 4H), 7.20 (d, $J = 16.2$ Hz, 2H), 7.43 (d, $J =8.4$ Hz, 2H), 7.53-7.62 (m, 6H), 7.86 (s, 2H), 8.17 (d, $J = 8.5$ Hz, 2H); $^{13}$C NMR (CDCl$_3$, 126 MHz): $\delta$ 15.1, 50.6, 52.7, 59.3, 62.3, 69.7, 70.7, 70.76, 70.82, 70.83, 72.2, 115.5, 117.6, 118.1, 124.5, 129.2, 129.4, 130.2, 130.5, 131.0, 133.0, 136.3, 136.9, 140.4, 141.8, 143.9,
153.3, 159.4, 166.8; HRMS (ESI-TOF) m/z [M+Na]⁺ Calcd for C₅₉H₇₁BF₂N₈O₁₂Na 1155.5154; Found 1155.5170.

**Supporting Information Available.** Structure of model compound **BDP10**, details of calculations, and copies of ¹H and ¹³C spectra for new compounds. This material is available free of charge via the Internet at [http://pubs.acs.org](http://pubs.acs.org)

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**References**


Data taken from NIST:

https://webbook.nist.gov/cgi/cbook.cgi?ID=C100663&Mask=20

