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Journal
European Journal of Organic Chemistry, 2019(7)

ISSN
1434-193X

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Publication Date
2019-02-21

DOI
10.1002/ejoc.201801758

Peer reviewed

Synthesis, Structure, and Fluorescence Behavior of Profluorescent 8-amino BODIPY Nitroxides

Wiley Schultz-Simonton, Patrick Skelly, Indranil Chakraborty, Pradip Mascharak, and Rebecca Braslau*

Abstract: A series of novel 8-amino BODIPY profluorescent nitroxides and the corresponding *N*-ethoxyamine derivatives were synthesized with varying substitution and molecular geometries. Single crystal X-ray diffraction data for the three 8-amino BODIPY nitroxides were obtained. UV-Vis absorption and emission data for the profluorescent nitroxides and 8-amino BODIPY *N*-ethoxyamine derivatives were examined to assess the utility of these compounds as fluorescent probes.

Introduction

"Profluorescent nitroxides" are fluorescent dyes covalently bonded to a persistent nitroxide radical. Interaction between the radical and the excited state of the fluorophore results in a suppression of the fluorescence emission, occurring both in cases where the nitroxide is covalently bound (intramolecular) and where separate nitroxide and fluorophore molecules have sufficient proximity to form an "encounter complex" (intermolecular). This property, along with the reversible redox properties and unique reactivity of the nitroxide group, allows for design of fluorescent probes which have been utilized to visually monitor a variety of processes which involve redox changes and/or radical intermediates.[1]

The quenching effect observed with profluorescent nitroxides is attributed to an electron exchange process; the unpaired electron provides a "spin-allowed" nonradiative decay mechanism for the fluorophore excited-state.[2] The strength of this fluorescence quenching is influenced by proximity of the fluorophore to the nitroxide radical,[3] and is enhanced in cases where the nitroxide and fluorophore are π conjugated and/or attached by rigid C-C bonds rather than flexible linkages.[4] However, synthetic routes to such compounds can be cumbersome,[5] thus synthesis involving accessible 2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPO) derivatives is appealing for probe design. Relatively little is understood about the precise geometric parameters important in designing profluorescent nitroxides with high quenching efficiencies.[1]

To better understand the molecular and environmental properties that lead to efficient fluorescence quenching, the syntheses of profluorescent 8-amino BODIPY nitroxides **1**, **2**, and **3** and their fluorescent *N*-ethoxyamine analogs **4**, **5**, and **6** was undertaken based on methodology developed by Bañuelos.[7] In general, BODIPY dyes have high photo- and chemical stability, "tunable" excitation and emission wavelengths ($\lambda_{em}/\lambda_{ex}$), large extinction coefficients (ε) and high fluorescence quantum yields (ϕ)[6,8] making them attractive for a wide variety of sensing applications.[5,8,9] Profluorescent nitroxides **1**, **2**, and **3** were selected for their differences in predicted geometries and $\lambda_{em}/\lambda_{ex}$, to explore the effects of variable bond angles and distances on spectroscopic properties. Their usefulness as probes is essentially a function of the ratio of fluorescence quantum yield ϕ between the *N*-ethoxyamine and the corresponding nitroxide: $\phi_{NOEt}/\phi_{NO^{\cdot}}$.

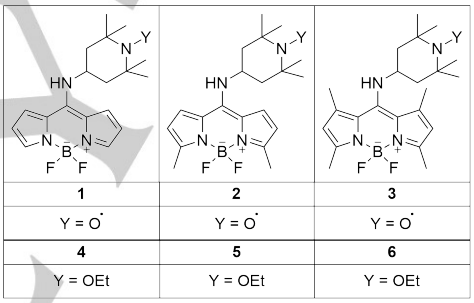
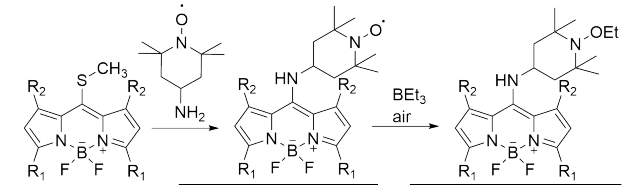


Figure 1. Structure of BODIPY nitroxides **1**, **2**, and **3**, and BODIPY *N*-ethoxyamines **4**, **5**, and **6**

Results and Discussion



	R_1	R_2
7	H	H
8	CH_3	H
9	CH_3	CH_3

	R_1	R_2	% yield
1	H	H	90
2	CH_3	H	51
3	CH_3	CH_3	36

	R_1	R_2	% yield
4	H	H	33
5	CH_3	H	55
6	CH_3	CH_3	55

Scheme 1. Synthesis of profluorescent BODIPY nitroxides **1**, **2**, **3**, and *N*-ethoxyamine derivatives **4**, **5**, and **6**.

Profluorescent nitroxides **1**, **2**, and **3** were obtained in modest to moderate yield by treatment of the corresponding 8-

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Supporting information (Crystallographic details, UV-Vis and fluorescence spectra for compounds **1-6**, ^{1}H and ^{13}C NMR spectra for compounds **1-6**) for this article is given via a link at the end of the document.

methylthio BODIPY with 4-amino TEMPO using methodology developed by Bañuelos et al. (**Scheme 1**).[7,12] Tetramethyl nitroxide **3** was recovered in the lowest yield, presumably due to a more sterically crowded reactive site in precursor **9**. The nitroxides were converted in modest to poor yields to the corresponding *N*-ethoxyamines in unoptimized reactions by treatment with excess triethylborane and slow introduction of air. Particularly low recoveries were achieved for *N*-ethoxyamine **4**, perhaps due to a competing Minisci type reaction at the unsubstituted α position of the BODIPY pyrrole ring.

BODIPY dyes with an 8-amino substituent can be drawn as one of two resonance structures: the "cyanine" and "hemicyanine" forms (**Fig. 2**). For the electron density on the central nitrogen to delocalize significantly into the BODIPY system, the lone pair must adopt a coplanar orientation to the conjugated system. In sterically crowded compounds such as **3**, the nitrogen is precluded from overlap by geometric constraints, whereas in **1** and **2**, the central nitrogen is less sterically constrained and is able to contribute significant cross-conjugated hemicyanine character.

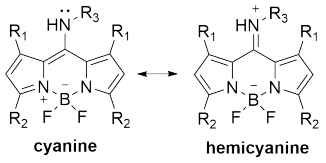

Figure 2. "Hemicyanine" and "cyanine" resonance structures in 8-amino BODIPY dyes.

The molecular structures and selected atomic bond angles and interatomic distances from X-ray data are shown in **Fig. 3**. Consistent with earlier studies on 8-amino BODIPY dyes,[2] sterically unhindered nitroxide **1** has a central nitrogen optimally oriented for π bonding with a small C_3-N_2-C_{10}-C_{11} dihedral angle of 3.1°, and a shortened C_{10}-N_2 bond length indicating a rigid hemicyanine structure. Also consistent with significant hemicyanine character is the presence of six nonequivalent pyrrolic protons in the ^{1}H NMR, and no symmetry in the pyrrolic ^{13}C signals for both **4** and **1**. In contrast, nitroxide **3** adopts a near perpendicular orientation between the BODIPY plane and the TEMPO moiety, and symmetry is observed in both the proton and carbon NMR spectra for the pyrrolic system. Nitroxide **2** adopts a conformation intermediate between **1** and **3** with symmetrical pyrrole signals in the NMR spectra, suggesting unhindered rotation about the C_{11}-N_2 bond in CD_3OD as in **3**. Unlike nitroxide **3**, **2** has significant hemicyanine character as evidenced by the central C-N bond lengths similar to those of **1**, and a significant hypsochromic shift in λ_{ex} and λ_{em} relative to unsubstituted compounds **1** and **4**. The distances between the nitroxide nitrogen atoms N_1 and the BODIPY fluorophore is shortest in **3**, where the TEMPO ring adopts an almost perpendicular orientation to the BODIPY π system, compared to slightly longer central C-N bond lengths in **1** and **2**. No clear correlation between these distances from the crystal structures and fluorescence quenching efficiencies was observed, with the dimethyl compound **2** displaying the largest $\phi_{NOEt}/\phi_{NO^\cdot}$.

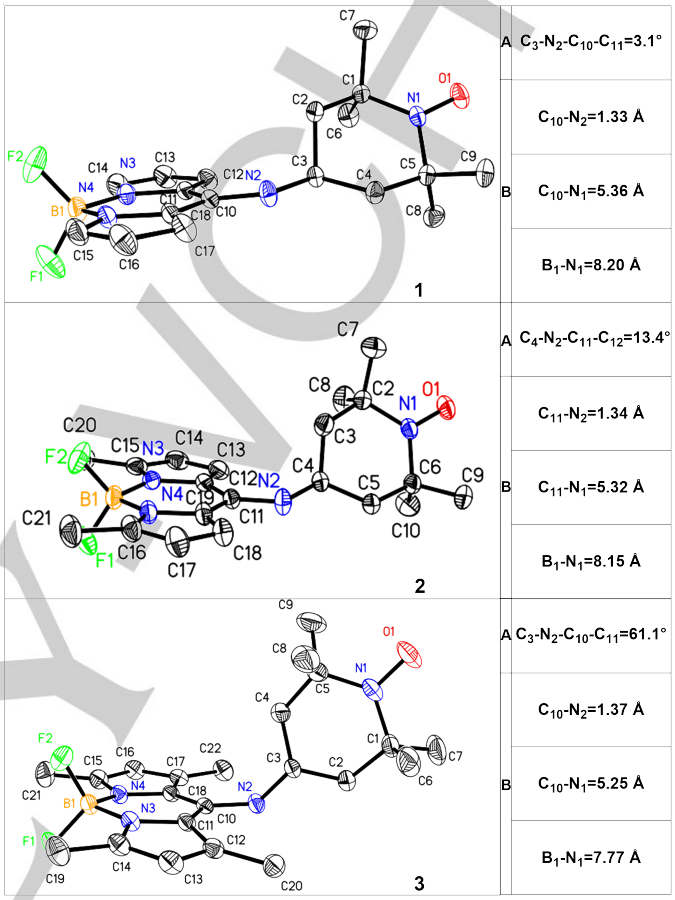

Figure 3. ORTEP structures and selected parameters of nitroxides **1**, **2**, and **3** from single crystal diffraction data with thermal ellipsoids at 50% probability, viewed along the BODIPY ring axis. **A**: Acute dihedral angles (degrees) between TEMPO methine carbon atom C_3/C_4-N_2 and carbons in BODIPY ring **B**: Bond distances from meso carbon of BODIPY C_{10}/C_{11}-N_2 , and interatomic distances between nitroxide center N1 and BODIPY fluorophore atoms C_{10}/C_{11} and B_1.

The λ_{em} / λ_{ex} and other spectroscopic properties of the nitroxides and corresponding ethoxyamines are summarized in **Table 1**. Each dye shows a hypsochromic shift in λ_{ex} going from nonpolar CyH/CH_2Cl_2 to polar MeOH solvent. This effect is most pronounced in dyes **1**, **2**, **4**, and **5** with significant hemicyanine character. The λ_{em} are not significantly affected, which suggests the shift arises from (de)stabilization of the ground state rather than effects on the structures excited states. The Stokes shift decreases in the order **1≈4>2≈5>3≈6**. The fluorescence efficiency ratios $\phi_{NOEt}/\phi_{NO^\cdot}$ between *N*-ethoxyamine and corresponding nitroxide are summarized in **Table 1**, as well as the effective fluorescence efficiency ratios $\phi_{NOEt}/\phi_{NO^\cdot}$ (0.1mM). This value is not a true quantum yield, as the relation between absorption and emission deviates significantly from linearity at this concentration, due to reabsorption and inner filter effects.

However, this value is of interest in considering the utility of the dyes in sensing applications which may require high dye loading. The fluorescence efficiencies of the nitroxides are significantly lower than the *N*-ethoxyamine counterparts, as expected. However, nitroxide **1** displayed a slightly higher fluorescence efficiency than its *N*-ethoxyamine derivative **4** in MeOH solvent. Low fluorescence has been observed for similar 8-amino BODIPYs in polar media, previously attributed to intramolecular charge transfer (ICT) quenching.[2,3,7] However, the solvent dependence on fluorescence quantum yield shown by *N*-ethoxyamine **4** is absent in nitroxide **1**, which shows similar ϕ in MeOH and CyH/CH_2Cl_2. Evidently, in **1**, the quenching effect of the pendant nitroxide outweighs the enhancement in ICT rate afforded by the increase in solvent polarity. *N*-ethoxyamine **5** shows less solvent dependence, and the effect of solvent on ϕ is reversed in nitroxide **2**. The fluorescence of nitroxide **3** and *N*-ethoxyamine **6** appear relatively insensitive to solvent polarity. At higher concentration, differences in the quenching abilities of the dyes are more distinctive; $\phi_{NOEt}/\phi_{NO^\cdot}$ is enhanced at high concentration in most cases, but actually decreases for **2** in CyH/CH_2Cl_2. The $\phi_{NOEt}/\phi_{NO^\cdot}$ (0.1mM) = 130 is very large for nitroxide **3** in CyH/CH_2Cl_2, which suggests the tetramethyl probe may be the most useful of these profluorescent nitroxides as a sensor (which may require high dye concentration). Solid state fluorescence was also observed for **3** and **6**, a property which may have utility in development of visualization techniques using profluorescent nitroxides. The approximately perpendicular orientation of the TEMPO group with respect to the BODIPY fluorophore may disrupt stacking and other intermolecular interactions known to suppress fluorescence, which occur both at high concentration and in the solid.

Table 1. Summary of spectroscopic properties of BODIPY dyes **1**, **2**, **3**, **4**, **5**, and **6** in MeOH and CyH/CH_2Cl_2 solvents.

compound	λ_{ex} (nm) ε (L·mol^{-1}·cm^{-1})	λ_{em} (nm)	ϕ_{rel}*	$\phi_{NOEt}/\phi_{NO^\cdot}$	$\phi_{NOEt}/\phi_{NO^\cdot}$(0.1mM)†
1 (MeOH)	405 (20,000)	469	0.090±0.005	-	-
1 (CyH/CH_2Cl_2)	419 (20,000)	469	0.10±0.01	-	-
2 (MeOH)	426 (30,000)	485	0.097±0.006	-	-
2 (CyH/CH_2Cl_2)	439 (30,000)	488	0.047±.004	-	-
3 (MeOH)	458 (40,000)	492	0.020±0.001	-	-
3 (CyH/CH_2Cl_2)	464 (40,000)	497	0.025±0.002	-	-
4 (MeOH)	402 (20,000)	465	0.031±0.001	0.34	10
4 (CyH/CH_2Cl_2)	415 (20,000)	468	0.69±0.04	6.9	17
5 (MeOH)	424 (30,000)	483	0.58±0.04	6.0	9.9
5 (CyH/CH_2Cl_2)	434 (30,000)	488	0.80±0.07	17	0.97
6 (MeOH)	456 (40,000)	490	0.24±0.01	12	14
6 (CyH/CH_2Cl_2)	459 (40,000)	492	0.21±0.01	8.4	130

* determined relative to Coumarin 153, ϕ=0.53 in EtOH.[18]

† value is not a true quantum yield as absorption/emission behavior is nonlinear at this concentration

The on/off fluorescence performance of the dyes examined here is comparable with previously synthesized tetramethyl substituted BODIPY profluorescent nitroxides where C-C linkages are used.[13a,13b,17] The $\phi_{N\text{-alkoxyamine}}/\phi_{NO^\cdot}$ for meso substituted profluorescent nitroxides **1**-**3** is also on par with that found for isoindoline BODIPY nitroxides with C-C linkages.[5] The performance of these new dyes is significantly better than previously synthesized BODIPY TEMPO conjugates using phenyl "spacers," utilizing alkyne-azide cycloaddition,[14] ester[15] or amide[16] linkages to attach the BODIPY and TEMPO moieties. This methodology for formation of the critical C-N linkages is efficient and allows for shorter distances between the nitroxide radical center and the BODIPY core.

Conclusions

Synthesis and spectroscopic analysis of three novel profluorescent BODIPY nitroxides and their *N*-ethoxyamine analogues was carried out, to assess their fluorescence behavior and usefulness as probes. X-ray diffraction for the three nitroxide probes was analyzed and molecular geometry correlated to fluorescence characteristics. BODIPY **1** exhibits a nearly coplanar relation between the TEMPO moiety and the BODIPY system and asymmetry in the NMR signals, indicating a largely cross-conjugated hemicyanine character. Dye **3** has a nearly perpendicular orientation between the TEMPO and BODIPY moieties, and a hypsochromic shift in $\lambda_{em}/\lambda_{ex}$ that suggests a dominant cyanine resonance contribution. Dye **2** has symmetry in the NMR signals of the BODIPY rings (indicating free rotation about the central C-N bond), but $\lambda_{em}/\lambda_{ex}$ intermediate between **1** and **3**, suggesting this dye has significant hemicyanine resonance contribution as in **1**. The fluorescence of *N*-ethoxyamine **4** shows dramatic sensitivity to solvent polarity that is absent in nitroxide precursor **1**. Dyes **5** and **2** show a fairly large $\phi_{NOEt}/\phi_{NO^\cdot}$, and distinct responses to solvent polarity between nitroxide and *N*-ethoxyamine. No clear trend relating nitroxide-fluorophore distance to $\phi_{NOEt}/\phi_{NO^\cdot}$ was obvious, although these interesting and unexpected results observed may lead to better design of profluorescent nitroxides in the future. The solid state fluorescence and good performance at high concentrations of nitroxide **3** should be valuable in visual sensing applications.

Experimental Section

All reagents and solvents were used as received unless otherwise noted. Dry CH_2Cl_2 was obtained by refluxing over CaH_2 and storage over 4Å molecular sieves. Toluene, CH_2Cl_2, CH_3CN, MeOH, and THF were supplied by Fisher. Diethyl ether, $BF_3{\cdot}OMe_2$, $BF_3{\cdot}OEt_2$, CaH_2, cyclohexane and thiophosgene (85%) were supplied by Acros Organics. Ascorbic acid, 1M BEt_3 in hexanes, and 1M BEt_3 in THF were supplied by Sigma Aldrich. 4Å Molecular sieves and pyrrole were supplied by Alfa Aesar. Methyl Iodide was supplied by Spectrum. 2-Methylpyrrole was

supplied by BePharm Limited. 2,4-Dimethylpyrrole was supplied by TCI. TLC analysis was performed on 0.20 mm silica gel 60 plates with UV_{254}, supplied by Macherey-Nagel. Deuterated solvents CD_3OD, C_6D_6, and $CDCl_3$ supplied by Cambridge Isotope Laboratories.

Preparation of 4-amino-2,2,6,6 tetramethylpiperidin-1-oxyl (4-amino TEMPO) was carried out by the method of Zagatto et al.[10] BODIPYs **7**, **8**, and **9** were synthesized according to the procedure of Biellmann.[11]

8-*N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY-amino-TEMPO 1): Following a modified literature procedure,[7] to a dry round bottom flask equipped with a stir bar was charged 50 mg (0.21 mmol, 1.0 eq.) of 8-methylthio BODIPY **7**, dissolved in 10 mL of freshly distilled CH_2Cl_2 and stirred for 10 minutes before adding 60 mg (0.35 mmol, 1.7 eq.) of 4-amino-TEMPO. The mixture was capped and allowed to stir for 22 h, after which TLC analysis showed consumption of **7**. The mixture was evaporated in vacuo and the residue purified by flash chromatography on SiO_2 (gradient: CH_2Cl_2 to CH_2Cl_2/MeOH 96:4) to give 70 mg (90% yield) of **1** as an orange powder, mp = 256-257° C. R_f = 0.21 (99:1 CH_2Cl_2/MeOH). ^{1}H NMR (500 MHz, CD_3OD, ascorbic acid added in situ) 7.55 (s, 1H), 7.41 (s, 1H), 7.35 (s, 1H), 7.32 (s, 1H) , 6.57 (s, 1H), 6.38 (s, 1H), 4.57 (tt, 1H, J= 12.0 Hz, 3.5 Hz), 2.11 (m, 2H), 1.90 (t, 2H, J= 12.0 Hz), 1.33 (s, 6H), 1.26 (s, 6H). ^{13}C NMR, HSQC (125 MHz, CD_3OD, ascorbic acid added in situ) 149.8 (4°), 135.0 (CH), 132.6 (CH), 126.9 (4°), 123.3 (CH), 123.2 (4°), 117.9 (CH), 115.2 (CH), 114.2 (CH), 60.8 (4°), 49.9 (CH), 44.5 (CH_2), 32.3 (CH_3), 20.4 (CH_3). IR (cm^{-1}): 3452 (s), 1712 (br, s), 1458 (s), 1378 (m). HRMS (ESI-Orbitrap) m/z: $(MH+H)^+$ calcd for $(C_{18}H_{26}BF_2N_4O)^+$; 363.2169, found 363.2143.

8-*N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-4,4-difluoro-3,5-dimethyl-4-bora-3a,4a-diaza-s-indacene (diMeBODIPY-amino-TEMPO 2): Following a modified literature procedure,[7] to a dry round bottom flask equipped with a stir bar was charged 20 mg (0.075 mmol, 1.0 eq.) of 8-methylthio dimethyl BODIPY **8**, dissolved in 20 mL of freshly distilled CH_2Cl_2 and stirred for 10 minutes before adding 22 mg (0.13 mmol, 1.7 eq.) of 4-amino-TEMPO. The mixture was capped and left stirring for 3 h, after which TLC analysis showed consumption of **8**. The mixture was evaporated in vacuo and the residue purified by flash chromatography on SiO_2 (gradient: hexanes to CH_2Cl_2/hexanes/MeOH 47.5/47.5/5) to give 15 mg (51% yield) of **2** as an orange powder, mp = 239-240° C. R_f = 0.37 (99:1 CH_2Cl_2/MeOH). ^{1}H NMR (500 MHz, CD_3OD, ascorbic acid added in situ) 7.22 (d, J=4.0 Hz, 2H), 6.20 (s, 2H), 4.44 (tt, J= 3.8 Hz, 12.5 Hz, 1H), 2.47 (s, 6H), 2.07 (dd, J= 12.5 Hz, 3.8 Hz, 2H), 1.83 (t, J=12.5 Hz, 2H), 1.30 (s, 6H), 1.25 (s, 6H). ^{13}C NMR (125 MHz, CD_3OD, ascorbic acid added in situ) 147.4 (broad), 126.4, 115.5 (broad), 115.4 (broad), 106.9, 60.3, 44.9, 32.5, 20.4, 14.1. IR (cm^{-1}): 3409 (s), 1710 (br, w), 1482 (s), 1365 (m). HRMS (ESI-Orbitrap) m/z: $(MH+H)^+$ calcd for $(C_{20}H_{30}BF_2N_4O)^+$; 391.2472, found 391.2457.

8-*N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (tetMeBODIPY-amino-TEMPO 3): Following a modified literature procedure,[12] to a round bottom flask equipped with a stir bar was charged 305 mg (1.04 mmol, 1.00 eq.) of 8-methylthio BODIPY **9** dissolved in 4 mL of CH_3CN, and 223 mg (1.30 mmol, 1.25 eq.) of 4-amino-TEMPO was added. The mixture was stirred open to air for 21 h, after which TLC analysis showed consumption of **9**. The mixture was evaporated in vacuo and the residue purified by flash chromatography on SiO_2 (gradient: 98:2 hexanes:EtOAc to 40:60 hexanes:EtOAc) to give 158 mg (36% yield) of **3** as orange crystals, mp = 192-193° C. R_f = 0.57 (99:1 CH_2Cl_2/MeOH). ^{1}H NMR (500 MHz, CD_3OD, ascorbic acid added in situ) 6.09 (s, 2H), 3.91 (tt, J=4 Hz, 12 Hz, 1H), 2.41 (s, 6H) 2.38 (s, 6H), 1.96 (m, 2H), 1.56 (t, J=12 Hz , 2H) 1.14 (s, 6H), 1.07 (s, 6H). ^{13}C NMR (125 MHz, CD_3OD, ascorbic acid added in situ) 149.2, 134.9, 125.8, 119.6, 106.9, 60.3, 56.0, 47.5, 32.7, 20.5, 15.6, 14.2. IR (cm^{-1}): 3431 (s), 1686 (br, s), 1469 (s), 1364 (m). HRMS (ESI-Orbitrap) m/z: $(MH+H)^+$ calcd for $(C_{22}H_{34}BF_2N_4O)^+$; 419.2785, found 419.2760.

8-N-(1-ethoxy-2,2,6,6-tetramethyl-4-piperidinyl)-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY-amino-TEMPOEt 4): To a dry round bottom flask equipped with a stir bar was charged 11 mg (0.030 mmol, 1.0 eq.) of BODIPY-aminoTEMPO **1** dissolved in 2 mL of THF. The mixture was stirred for 20 min before 50 μL (0.05 mmol, 1.7 eq.) of 1M BEt_3 in hexanes was added. After the addition, the mixture was opened to air and stirred for 5 min, after which TLC analysis showed consumption of **1**. The solution was evaporated in vacuo and the residue purified by flash chromatography on SiO_2 (gradient: 95:5 hexanes/EtOAc to 60:40 hexanes/EtOAc) to give 4 mg (33% yield) of **4** as an orange/yellow powder, mp= 210-212° C. R_f= 0.59 (99:1 CH_2Cl_2/MeOH). ^{1}H NMR (500 MHz, CD_3OD) 7.55 (s, 1H), 7.42 (s, 1H), 7.36 (s, 1H), 7.29 (s, 1H), 6.56 (s, 1H), 6.38 (s, 1H), 4.55 (m, 1H), 3.86 (q, 7.5 Hz, 2H), 2.03 (d, J=12.5 Hz, 2H), 1.89 (t, J=12.5 Hz, 2H), 1.33 (s, 6H), 1.28 (s, 6H) 1.15 (t, J=7.5 Hz, 3H). ^{13}C NMR, HSQC (125 MHz, CD_3OD) 149.8 (4°), 134.9 (CH), 132.6 (CH), 126.8 (4°), 123.2 (CH), 123.1 (4°), 117.8 (CH), 115.1 (CH), 114.2 (CH), 73.7 (O-CH_2), 60.9 (4°), 49.9 (CH), 44.9 (CH_2), 33.3 (CH_3), 21.1 (CH_3), 13.8 (CH_3). IR (cm^{-1}): 3397 (s), 1733 (br, s), 1574 (s), 1462 (m). HRMS (ESI-Orbitrap) m/z: $(M+H)^+$ calcd for $(C_{20}H_{30}BF_2N_4O)^+$; 391.2472, found 391.2459.

8-*N*-(1-ethoxyl-2,2,6,6-tetramethyl-4-piperidinyl)-4,4-difluoro-3,5-dimethyl-4-bora-3a,4a-diaza-s-indacene (diMeBODIPY-amino-TEMPOEt 5): To a dry round bottom flask equipped with a stir bar was charged 14 mg (0.036 mmol, 1.0 eq.) of diMeBODIPY-amino-TEMPO **2** dissolved in 2 mL of THF. The mixture was stirred for 10 min before 90 μL (0.090 mmol, 2.5 eq.) of 1M BEt_3 in THF was added. After the addition, the mixture was opened to air via needle tip and left stirring for 25 h, after which TLC analysis showed consumption of **2**. The solution was evaporated in vacuo and the residue purified by flash chromatography on SiO_2 (gradient: 95:5 hexanes/EtOAc to 60:40 hexanes/EtOAc). The product was recrystallized from hexanes to give 8 mg (55% yield) of **5** as orange crystals, mp =

188-191° C. R_f = 0.64 (99:1 CH_2Cl_2/MeOH). ^{1}H NMR (500 MHz, CD_3OD) 7.21 (s, 2H) 6.19 (s, 2H), 4.40 (tt, J= 12.0 Hz, 4.0 Hz, 1H), 3.84 (q, J= 7.0 Hz, 2H), 2.47 (s, 6H), 1.99 (d, J= 12.0 Hz, 2H), 1.81 (t, 2H, J= 12 Hz), 1.30 (s, 6H), 1.26 (s, 6H), 1.14 (t, J= 7.0 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD + a minimal amount of DMSO d_6 was added to increase solubility) 147.4, 123.4 (broad), 118.0 (broad), 116.2 (broad), 115.0 (broad), 73.6, 60.9, 45.1, 33.4, 21.2, 14.6, 14.4, 13.9. IR (cm^{-1}): 3405 (s), 1720 (br, s), 1578 (s), 1417 (s). HRMS (ESI-Orbitrap) m/z: $(M+H)^+$ calcd for $(C_{22}H_{34}BF_2N_4O)^+$; 419.2785, found 419.2772.

8-*N*-(1-ethoxy-2,2,6,6-tetramethyl-4-piperidinyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (tetMeBODIPY-amino-TEMPOEt 6): To a dry round bottom flask equipped with a stir bar was charged 29 mg (0.069 mmol, 1.0 eq.) of tetMeBODIPY-amino-TEMPO **3** dissolved in 20 mL of hexanes and stirred for 20 minutes before adding 200 μL (0.20 mmol, 2.9 eq.) of 1M BEt_3 in hexanes. After the addition, the mixture was left stirring for 24 h, then the solution opened to air and a further 200 μL (0.20 mmol, 2.9 eq.) of 1M BEt_3 in hexanes was added and left stirring for 24 h. Another 400 μL (0.40 mmol, 5.8 eq.) of 1M BEt_3 in hexanes was added; the solution darkened and TLC analysis showed consumption of **3**. The mixture was evaporated in vacuo and the residue purified by flash chromatography on SiO_2 (isocratic: 9:1 hexanes/EtOAc) to give 17 mg (55% yield) of **6** as an orange powder, mp = 159-160° C. R_f= 0.20 (1:1 CH_2Cl_2/Hexanes). ^{1}H NMR (500 MHz, CD_3OD) 6.08 (s, 2H), 3.92 (tt, J= 12.0 Hz, 4.0 Hz, 1H) 3.80 (q, 2H, J= 7.0 Hz), 1.93 (d, J= 12.0 Hz, 2H), 1.60 (t, J= 12.0 Hz, 2H), 1.20 (s, 6H), 1.12 (s, 6H), 1.10 (t, 3H, J= 7.0 Hz). ^{13}C NMR, HSQC (125 MHz, CD_3OD) 153.0 (4°), 142.9 (4°), 134.9 (4°), 125.8 (4°), 119.6 (CH), 73.6 (O-CH_2), 61.2 (4°), 55.7 (CH), 47.6 (CH_2), 33.2 (CH_3), 21.2 (CH_3), 15.6 (CH_3), 14.2 (CH_3), 13.7 (CH_3). IR (cm^{-1}): 3414 (s), 1723 (br, s), 1576 (s), 1454 (m). HRMS (ESI-Orbitrap) m/z: $(M+H)^+$ calcd for $(C_{24}H_{38}BF_2N_4O)^+$; 447.3098, found 447.3080.

Single crystals of **1**, **2**, and **3** were obtained by slow evaporation from CH_2Cl_2/hexanes. Data were collected on a Bruker APEX II single-crystal X-ray diffractometer. Structures deposited into the Cambridge Structural Database (CCDC 1863622-1863624). UV-Vis measurements were taken in HPLC grade methanol and spectroscopic grade cyclohexane/dichloromethane (7:3 v/v ratio, to prevent precipitation of the marginally soluble nitroxides) on a Shimadzu UV-2700 UV-Vis Spectrofluorometer. Photoluminescence spectra were obtained on a Horiba FluoroMax-4 Spectrofluorometer, with an excitation and emission slit width of 2.5 nm. UV-Vis and fluorescence emission spectra were taken at room temperature. Extinction coefficients were estimated from an average of no less than three separately weighed stock solutions at 100 μM. Relative fluorescence quantum yields were measured using Coumarin 153 as a standard (0.53 in EtOH).[18] Error in relative quantum yields were calculated by propagation of uncertainty in linear regressions of absorption versus integrated total fluorescence. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker Avance III HD 4 channel 500 MHz Oxford Magnet NMR spectrometer with Automation or a Bruker Avance III HD 800 MHz NMR Spectrometer. FTIR spectra taken in $CHCl_3$ and recorded with a Perkin Elmer Spectrum One spectrometer in NaCl microsolution cells. HRMS was recorded with a Thermo Scientific LTQ-Orbitrap Velos Pro MS.

Acknowledgments

The authors gratefully acknowledge funding provided by the Gura family, and valuable input on the manuscript from Professor Alex Ayzner.

Keywords: Fluorescent Probes • Fluorescence • Radicals

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Entry for the Table of Contents

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FULL PAPER

Profluorescent Nitroxides

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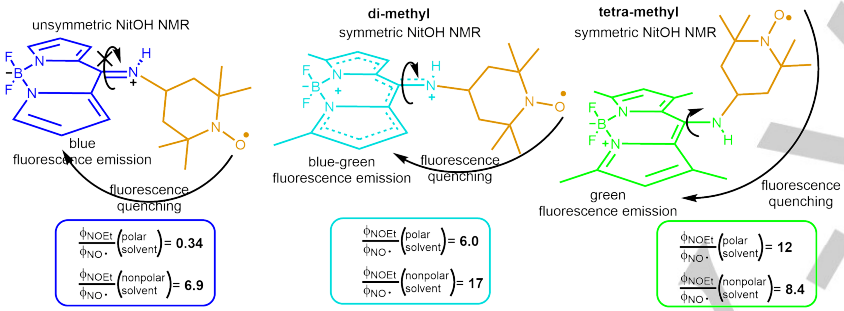
Synthesis, Structure, and Fluorescence Behavior of Profluorescent 8-amino BODIPY Nitroxides



Structure of novel profluorescent BODIPY nitroxides **1**, **2**, and **3**, showing fluorescence efficiency ratios of corresponding *N*-ethoxyamines and nitroxides $\phi_{NOEt}/\phi_{NO\cdot}$ in polar (MeOH) and nonpolar (CyH/CH_2Cl_2) media