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Synthesis of paramagnetic BODIPY dyes as new double (spin and fluorescence) sensors

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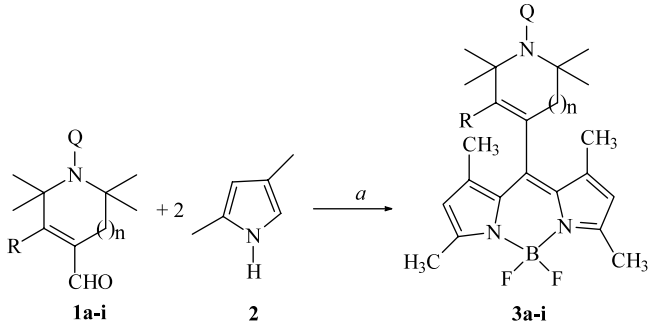
Abstract—Paramagnetic pyrroline and 1,2,3,6-tetrahydropyridine derivatives of BODIPY and their diamagnetic analogs have been synthesized and characterized as novel redox double sensor and cation sensitive reagents.

Fluorescence spectroscopy, fluorescence imaging and fluorescent probes are indispensable tools in numerous fields of modern medicine and science, including analytical chemistry, molecular biology, biophysics, biochemistry, and medical diagnostics.[1]

In the last decade a new class of fluorescent dyes, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) dyes[2] was invented and has found wide application in areas such as biological labeling[3] and the synthesis of molecular devices.[4a] The advantages of these dyes are the tunable emission range (500–700 nm),[5,6] the high molar extinction coefficients, and the insensitivity to pH and solvent polarity. A stable nitroxide covalently linked to a fluorophore results in intramolecular quenching of fluorescence. These donor–acceptor pairs have been used for getting structural information on peptides,[7] redox switches[8] and detection of reactive oxygen species (ROS).[9,10] Several double sensor molecules were synthesized based on nitroxides and naphthalene,[8] fluorescamine,[10] aminophthalimide[11] and dansyl[9] fluorophores. In this paper, we report the first synthesis of paramagnetic BODIPY dyes and their diamagnetic analogs from a series of paramagnetic aldehydes and their diamagnetic forms **1a**–**i**.[12–17]

The acid-sensitive nitroxide moieties of the paramagnetic aldehydes **1a**, **1f**–**h**, fortunately survived the reaction with 2,4-dimethylpyrrole **2** in the presence of a catalytic amount TFA, followed by treatment with DDQ, *i*-Pr_2EtN and boron trifluoride diethyl etherate at ambient temperature in CH_2Cl_2 to give the corresponding BODIPY dyes **3a**–**i**[18] (Scheme 1).

The steady-state fluorescence study of the compounds synthesized in 1,4-dioxane and the more polar acetonitrile (ACN) showed that both emission and excitation maximum slightly shifted hypsochromically in the more polar solvent. The Stokes' shift is about 20–25 nm for compounds with five-membered nitroxides and their derivatives, but a bit smaller for the six-membered derivatives **3h**,**i**. The quantum yield of the diamagnetic derivatives **3b**–**e**, **3i** is about 15–50 fold of the paramagnetic ones (Table 1). The vicinity of donor and acceptor



1a-i **2** **3a-i**

Scheme 1. *Reagents and conditions*: (a) cat. TFA, CH_2Cl_2, N_2, 10 h, rt, then DDQ (1.0 equiv.), 30 min rt, then *i*-Pr_2EtN, $BF_3{\cdot}Et_2O$, 0°C→rt, **3a**: 15%, **3b**: 35%, **3c**: 30%, **3d**: 10%, **3e**: 9%, **3f**: 20%, **3g**: 22%, **3h**: 17%, **3i**: 16%.

Keywords: nitroxides; fluorescence; BODIPY dye; fluorescence quenching.
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Table 1. Steady-state fluorescence data of compounds **3a–i**

Entry	*n*	R	Q	$\lambda_{ex}/\lambda_{em}$ dioxane	$\lambda_{ex}/\lambda_{em}$ ACN	Φ*
3a	0	H	O·	518/542	515/540	0.01
3b	0	H	H	512/538	510/533	0.21
3c	0	H	Ac	520/546	515/540	0.50
3d	0	H	OMe	510/536	507/530	0.49
3e	0	H	OAc	514/536	510/530	0.54
3f	0	Me	O·	517/544	510/535	0.03
3g	0	Br	O·	517/540	515/535	0.02
3h	1	H	O·	512/523	498/509	0.03
3i	1	H	OAc	498/517	497/512	0.45

* In ACN, compared to fluorescein in 0.1 M NaOH, ±10%.

can explain this, also that the double bond of the pyrroline rings is in conjugation with the π-electron system of the fluorophore. The difference in the fluorescence intensity of the paramagnetic and diamagnetic derivatives allows application of these compounds to follow oxidation–reduction processes by two independent methods, EPR and fluorescence spectroscopy (Figs. 1 and 2).

It is interesting to note that the quantum yield of the amino derivative **3b** is about half of the other diamagnetic derivatives. This observation is in good agreement with the finding of Kollmannsberger and co-workers,[4c] although fluorescence quenching is less significant. However, on adding TFA to the acetonitrile solution of **3b** the fluorescence intensity decreased ($\Phi = 0.09$) and a bathochromic shift (9 nm) was observed (Fig. 3). This effect can also be observed by adding glacial acetic acid and *p*-toluenesulfonic acid monohydrate (data not shown) and Zn^{2+} ions. This quenching effect can be explained by protonation/chelation of the pyrroline nitrogen non-bonding electron pair causing oxidative photoinduced electron transfer (PET), probably analogously to the PET observed in the case of pyridine derivatives.[4b]

In conclusion, new paramagnetic and diamagnetic BODIPY derivatives have been synthesized as redox status and proton sensitive fluorescent switches, emitting at the 520–540 nm region. The further elucidation of the quenching mechanism[19] and biological applications of these reagents are in progress.

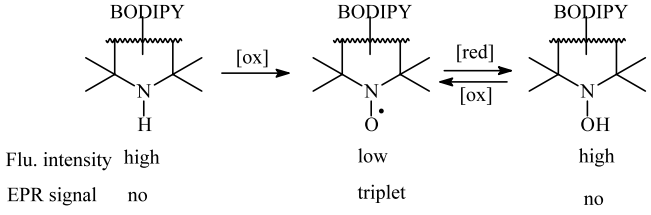

Figure 1. Detection possibilities of the redox state of nitroxides or their precursor attached to BODIPY fluorophore.

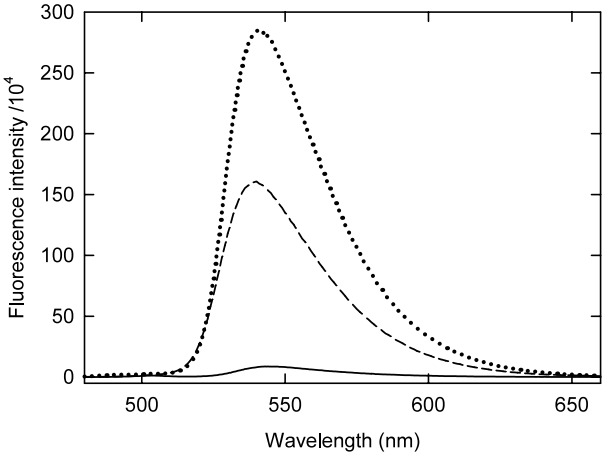

Figure 2. Normalized fluorescence emission spectrum of compounds **3a** (—), **3b** (- - -) and **3e** (· · ·). Excitation was at 510 nm, and emission and excitation slits were set at 5 nm.

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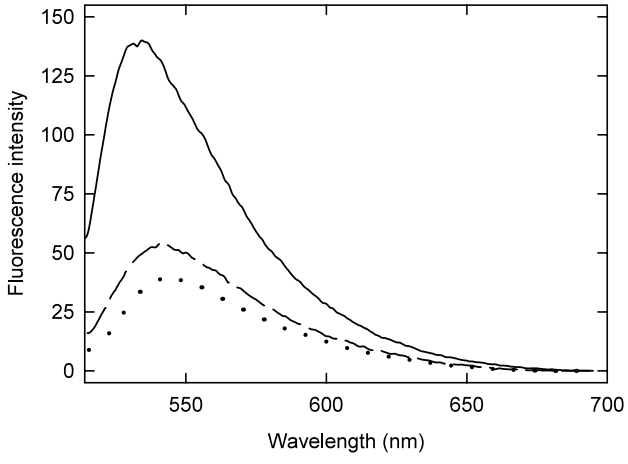

Figure 3. Emission spectra of compound **3b** 2.1 μM (—), **3b** 2.0 μM+Zn^{2+} 160 μM (- - -) and **3b** 2.1 μM+TFA 0.013 M (· · ·) in ACN, excitation was at 510 nm, and emission and excitation slits were set at 3 nm.

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18. Experimental procedure: To a deoxygenated solution of the aldehyde **1a**–**i** (1.0 mmol) and 2,4-dimethylpyrrole in CH_2Cl_2 two drops of TFA (1.05 mmol in the case of **3b**) were added and the mixture was stirred overnight under N_2 at rt. The red solution was treated with DDQ (227 mg, 1.0 mmol), stirred for 30 min then *i*-Pr_2EtN (2.0 mL) and $BF_3{\cdot}Et_2O$ (2 mL) were added at 0°C and the mixture was stirred at rt for further 40 min. After washing with satd aq. $NaHCO_3$, the organic phase was separated, dried ($MgSO_4$), filtered and concentrated. In the case of the paramagnetic compounds, activated MnO_2 (79 mg, 1.0 mmol) was added and O_2 was bubbled through for 30 min. The residue was purified by flash column chromatography collecting the first red/purple fraction affording BODIPY dyes, yield: 9–35%. Spectroscopic data of selected compounds **3a**: mp 249–251°C. Anal. calcd for $C_{21}H_{27}BF_2N_3O$: C, 65.30; H, 7.05; N, 10.88, found: C, 65.29; H, 6.95; N, 11.01. MS (EI) m/z: 386 (M^+, 68), 372 (50), 356 (100), 341 (53). **3b**: mp 225–228°C. Anal. calcd for $C_{21}H_{28}BF_2N_3$: C, 67.94; H, 7.60; N, 11.32, found: C, 67.98; H, 7.61; N, 11.36. ^{1}H NMR (DMSO-d_6) (400 MHz) 6.18 (s, 2H), 5.89 (s, 1H), 2.38 (s, 6H), 2.26 (s, 6H), 1.28 (s, 6H), 1.10 (s, 6H). MS (EI) m/z: 371 (M^+, 28), 356 (42), 314 (100), 299 (76). **3e**: mp 272–274°C. Anal. calcd for $C_{23}H_{30}BF_2N_3O_2$: C, 64.35; H, 7.04; N, 9.79 found: C, 64.41; H, 7.22; N, 9.88. MS (EI) m/z: 429 (M^+, 48), 387 (11), 372 (100), 355 (47). **3h**: mp 209–210°C. Anal. calcd for $C_{22}H_{29}BF_2N_3O$: C, 66.01; H, 7.30; N, 10.50, found: C, 66.07; H, 7.48; N, 10.50. MS (EI) m/z: 400 (M^+, 26), 386 (100), 370 (52), 355 (40). **3i**: mp 272–275°C. Anal. calcd for $C_{24}H_{32}BF_2N_3O_2$: C, 65.02; H, 7.28; N, 9.48, found: C, 65.11; H, 7.22; N, 9.49. ^{1}H NMR ($CDCl_3$) (400 MHz) 6.03 (s, 1H), 6.02 (s, 1H), 5.53 (s, 1H), 2.64 (d, 1H, $J_{AB}=18.4$ Hz), 2.50 (s, 6H), 2.42 (s, 3H), 2.35 (s, 3H), 2.30 (d, 1H, $J_{AB}=18.4$ Hz), 2.11 (s, 3H), 1.37 (d, 6H), 1.20 (s, 6H). The CH_2 behaves as an AB spin system, because of slow nitrogen inversion in 1,2,3,6-tetrahydropyridine ring on NMR time scale. MS (EI) m/z: 443 (M^+, 79), 401 (13), 386 (100), 370 (64).
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