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Synthesis of new, BODIPY-based sensors and labels

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Abstract—New, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) dye based thiol-reactive fluorescent label, fluorescent amino acid, and fluoroionophore compounds with 540–560 nm emission are described. Combination of a BODIPY dye with a nitronyl nitroxide or an imino nitroxide or a bifunctional pyrroline nitroxide furnished a nitric oxide, a redox sensitive molecule and a double (spin and fluorescence) label, respectively.
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1. Introduction

Fluorescent probes and sensors have attracted attention because of their high sensitivity and exceptional ease of handling relative to their radioactive counterparts.[1] Mapping of the distribution of different ions within biological and environmental systems is a typical challenge. Binding of the target analyte to a synthetic fluorophore (sensor) can result in either amplification or quenching of the fluorescence.[2,3] These fluorescent probes are either constructed as fluorophore–spacer–receptor or as intrinsic fluorescent probes. In the former case, the receptor and fluorophore are separated by an alkyl chain, whereas in the latter case the receptor is part of a π-electron system.[4,5] The optical properties of intrinsic probes (ICT probes) are determined by a strong solvent-dependent behavior. In fluorophore–spacer–receptor systems only long-range electronic interactions are possible, the most common is photoinduced electron transfer (PET). There are many examples of crown-,[6] cryptand-,[7] podand-,[8] 2,2′-dipyridyl-,[9] and calixarene-based[10] PET sensor molecules as selective for sodium, magnesium, potassium, calcium, and transition metal ions. In these sensors the photoinduced electron transfer quenches the luminescence in the absence of the analyte. Binding the analyte (a metal ion or proton) inhibits the PET and switches on the emission.[11] It has been reported very recently that the combination of ICT and PET switches resulted in a molecular half-subtractor with reconfigurable logic gates.[12] Fluorophores covalently bound to a nitroxide give a unique, redox-sensitive sensor.[13] In these donor–acceptor molecules the paramagnetic nitroxide as an acceptor quenches the fluorescence, however, when nitroxide is reduced to hydroxylamine, the fluorescence increases. This feature of these donor–acceptor pairs was experienced with naphthyl,[13] coumarin,[14] dansyl,[15] aminophthalimide,[16] and BODIPY[17] donors. A sterically hindered amine (e.g., nitroxide precursor) covalently bound to a fluorophore offers the detection of reactive oxygen species (ROS) as formation of the nitroxide quenches the fluorescence.[18]

Another utilization of fluorescence spectroscopy is fluorescent labeling of biomolecules such as proteins, lipids, and DNA. Numerous fluorophores are known as covalent and noncovalent labels. The covalent probes can have a variety of reactive groups, for coupling with amine, sulfhydryl, hydroxyl, or histidine side chains in proteins.[19] In the case of proteins the introduction of a fluorescent, unnatural amino acid by conventional solid phase synthesis[20] or by nonsense suppression technique[21] is also a well-known approach. However, there is still a challenge to synthesize selective, long wavelength emitting probes for modifying biomolecules.[22,23]

Among the aforementioned examples, the BODIPY dyes[24] were used as fluorophores with many advantages, including high extinction coefficient, high quantum yield, narrow emission bandwidth, and therefore, good signal/noise ratio, insensitivity to pH and solvent polarity, and greater chemical and photochemical stabilities in solution and in solid state. Absorption/emission wavelengths can be tuned by the modification of the pyrrole core;[25,26] therefore, visible and infrared regions of the spectrum can be spanned. This property was used in a BODIPY-based, oxidation-sensitive fluorescent lipid peroxidation probe.[27]

In the present account, we wish to describe BODIPY-based labels for modifying proteins and BODIPY-based sensor molecules containing crown ether or nitroxide group to detect metal ions, reducing agents, or nitrogen-monoxide by fluorescence.

Keywords: Fluorescence; Crown ethers; Amino acid; Nitroxides; Double sensors.
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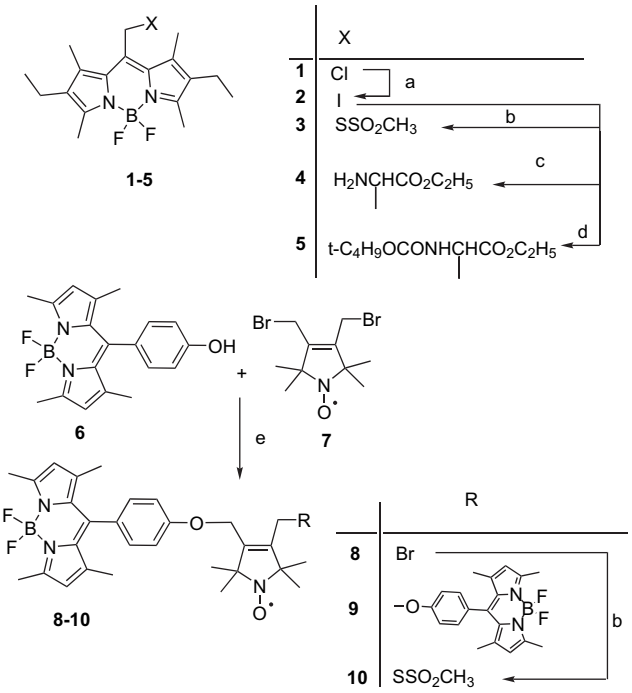
2. Results and discussion

2.1. Synthesis of fluorescent labels

Compound 4,4-difluoro-8-chloromethyl-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (**1**) is readily available from 3-ethyl-2,4-dimethylpyrrole and chloroacetyl chloride[28] and it was quite obvious that this derivative can be functionalized further by nucleophilic substitution. Chloromethyl compound **1** was converted to the more reactive iodomethyl derivative **2** by treatment with NaI in THF. Compound **2** was transformed to methanethiosulfonate derivative (MTS) **3** by a substitution reaction with $NaSSO_2CH_3$ in aq acetone. The resulting compound is a thiol-specific, reversible fluorescent dye with 555 nm emission (Table 1), capable of forming S–S bond with a cysteine SH group, and analogously to the frequently used spin labeling technique[29] compound **3** is a good candidate for site-directed fluorescent labeling of a peptide.[30] The BODIPY dye containing fluorescent D,L-amino acid was obtained by a modified O'Donnell reaction.[31,32] Treatment of carbanion, generated from dibenzylideneglycine ethyl ester with NaHMDS at −78 °C, with compound **2** yielded the fluorescent amino acid ester **4** after acid catalyzed hydrolysis of the imine. The treatment of compound **4** with di-*tert*-butyldicarbonate in THF gave the more stable *N*-Boc protected D,L-amino acid ester **5**. Compounds **3** and **5** are capable of fluorescent modification of the peptide by labeling at the cysteine side-chain or by incorporation of the fluorescent amino acid by solid phase peptide synthesis. Our further idea was the combination of spin and fluorescence labeling, i.e., to synthesize a double (spin and fluorescence) label capable of simultaneous labeling at the same site of a protein. A possible synthetic approach for this was the treatment of phenol containing BODIPY dye **6**[12] with a paramagnetic homobifunctional alkylating nitroxide **7**.[33] Fortunately, this could be accomplished under mild conditions to yield allylic bromide **8** and the two fluorophores containing **9** as a by-product. Compound **8** was then converted to methanethiosulfonate **10** by treatment with $NaSSO_2CH_3$ in aq acetone. The product was an SH-specific double (fluorescent and spin) label with a green emission (508 nm), a reduced quantum yield (Φ=0.22) and 2.5 ns lifetime, which is about half of the regular BODIPY lifetime[34] (Scheme 1).

2.2. Synthesis of fluorescent sensors

Fluoroionophores, by changing their optical properties upon complexation with certain ions have great potential for practical applications. It was obvious that compound **2** is a good candidate to modify different ion recognition sites. Alkylation of aza-15-crown-5 or aza-18-crown-6 in acetone in the presence of K_2CO_3 or Na_2CO_3 with compound **2** furnished fluoroionophores **11** or **12**, respectively (Scheme 2). During the study of these ionophores the absorption and emission spectra were recorded in acetonitrile. The ion-free fluoroionophores **11** and **12** gave sharp absorption maxima at 534 nm and emission maxima at 547 and 552 nm, respectively. The spectral response to the addition of ions was measured using the corresponding perchlorate salts and perchloric acid. The absorption spectra of compound **11** shifted to 554 nm upon addition of Ca^{2+} and H^+, and to 556 nm upon addition of Mg^{2+} ions. Addition of Li^+, Na^+, K^+ ions to solution of **11** causes only 1–3 nm red shift with some hypsochromic effect. In the case of compound **12** a small hypsochromic shift and some bathochromic shift occurred, except upon protonation, which resulted in a 19 nm bathochromic shift (Fig. 1). The fluorescence

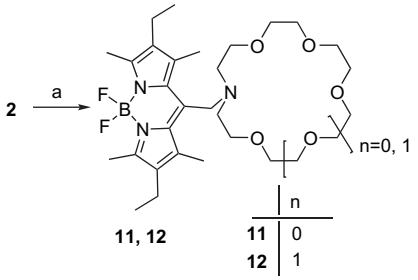


Scheme 1. Reagents and conditions: (a) NaI (2 equiv), THF, reflux, 45 min, 75%; (b) $NaSSO_2CH_3$ (2 equiv), water/acetone, reflux, 30 min, 43–27%; (c) $Ph_2NCH_2CO_2Et$ (1 equiv), THF, NaHMDS (1.1 equiv), −78 °C, 15 min, then **2** (1 equiv), 1 h, then, warm to 0 °C, quench with aq NH_4Cl, EtOH/aq H_2SO_4, rt, 40 min, 38%; (d) Boc_2O (1.0 equiv), THF, 40 °C, 30 min, 76%; (e) **6** (1.5 equiv), acetone, K_2CO_3 (1.5 equiv), 18-crown-6 (0.05 equiv), 10 min, then **7** (1 equiv), reflux, 30 min, 22% for **8** and 30% for **9**.

Table 1. Optical properties of the synthesized new BODIPY derivatives

Compound	λ_{abs} (nm)	ε (L×mol^{-1}×cm^{-1})	λ_{ex} (nm)	λ_{em} (nm)	Φ
3	543	3.37×10^4	543	555	0.80
5	529	3.54×10^4	530	543	0.86
10	498	4.35×10^4	497	508	0.22
11	534	5.87×10^4	530	547	0.001
12	534	7.70×10^4	533	552	0.01
15	499	2.73×10^4	505	518	0.006
20	499	3.43×10^4	510	523	0.005

Dissolved in acetonitrile and referred to fluorescein in 0.1 M NaOH at 496 nm, n=3, accuracy ±10%.



Scheme 2. Reagents and conditions: (a) 1-aza-15-crown-15 for **11**, 1-aza-18-crown-6 for **12** (1 equiv), Na_2CO_3 for **12** or K_2CO_3 for **11** (1.5 equiv), acetone, reflux, 30 min–2 h, 64–77%.

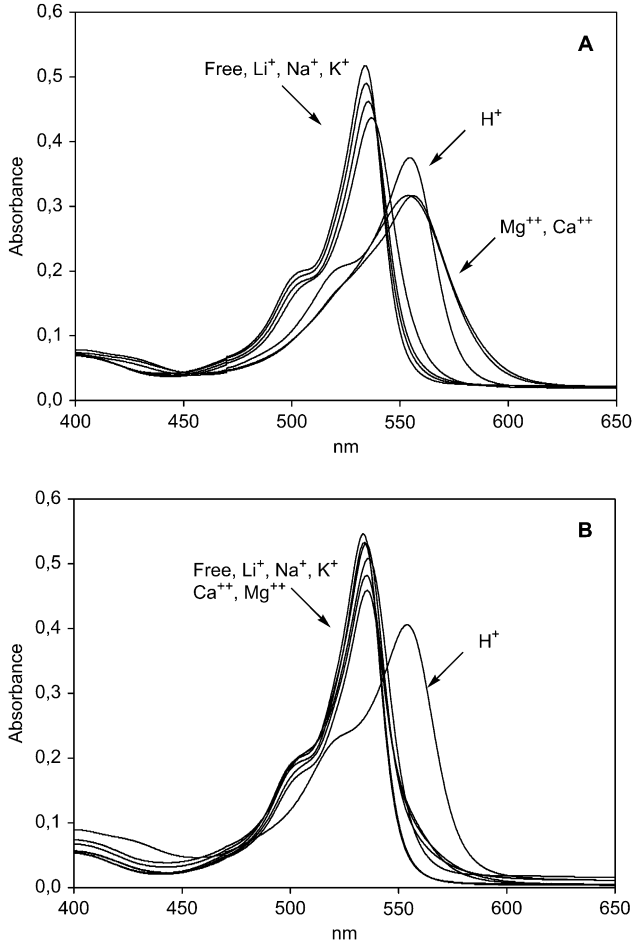


Figure 1. (**A**) Absorption spectra of **11** in acetonitrile and its complexes with 1000 equiv perchlorate salts (1 equiv=1.1 nM); (**B**) absorption spectra of **12** in acetonitrile and its complexes with 1000 equiv perchlorate salts (1 equiv=1.1 μM).

enhancement (quantum yield, Φ/Φ_0) was also studied in acetonitrile solution by the addition of 20 and 10,000 equiv ions (Fig. 2). Upon protonation of compound **11** regardless of the ratio of crown compound/H^+ a 75-fold increase was observed with a red shift to 571 nm. The emission was also red shifted with the addition of a large excess (10,000 equiv) of Ca^{2+} and Mg^{2+} ions with a 17- and 108-fold fluorescence quantum yield increase. This observation, with a fluorescence shift of 1-aza-15-crown-5 when Ca^{2+} is complexed, is in good agreement with Wu et al.'s findings.[35] The addition of Na^+ and Li^+ to an acetonitrile solution of compound **11** caused a 2–3 nm bathochromic shift with 73-fold and 82-fold increase in fluorescence quantum yield when 10,000 equiv ions were added, however only a three-fold fluorescence increase was experienced upon addition of a large excess of K^+ ions. Additions of 20 equiv of ions increased the fluorescence quantum yield of compound **11** to 2–6 times. Compound **12** proved to be more selective, although protonation caused a 17-fold enhancement of fluorescence, but with a red shift to 580 nm. The addition of 20 equiv of K^+ ions caused a six-fold increase, while a large excess of K^+ ions induced a 56-fold enhancement with a small (3 nm) bathochromic shift. Titration of compound **11** with Li^+ and Mg^{2+} indicated the formation of 1:1 complex and the association constants are 3140 and 440 M^{-1}

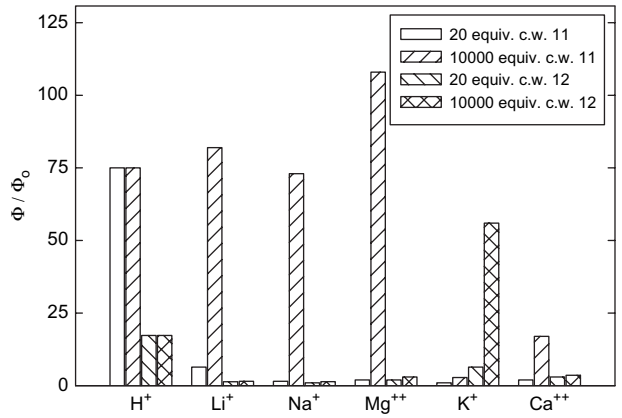


Figure 2. The fluorescence response of aza-crown ethers **11** and **12** to various cations. The first and third bars represent the integrated emissions of compounds **11** and **12** in the presence of 20 equiv of the cations of interest, the second and fourth bars represent the integrated emissions of compounds **11** and **12** in the presence of 10,000 equiv of the cations of interest, respectively (1 equiv=1 μM). The response was normalized with respect to integrated emission of free dye (Φ_0); excitation was provided at 530 nm for **11** and 533 nm for **12**; the emission was integrated from 536 to 700 nm in case of **11** and 539 to 700 nm in case of **12**; the slit width was 3 nm.

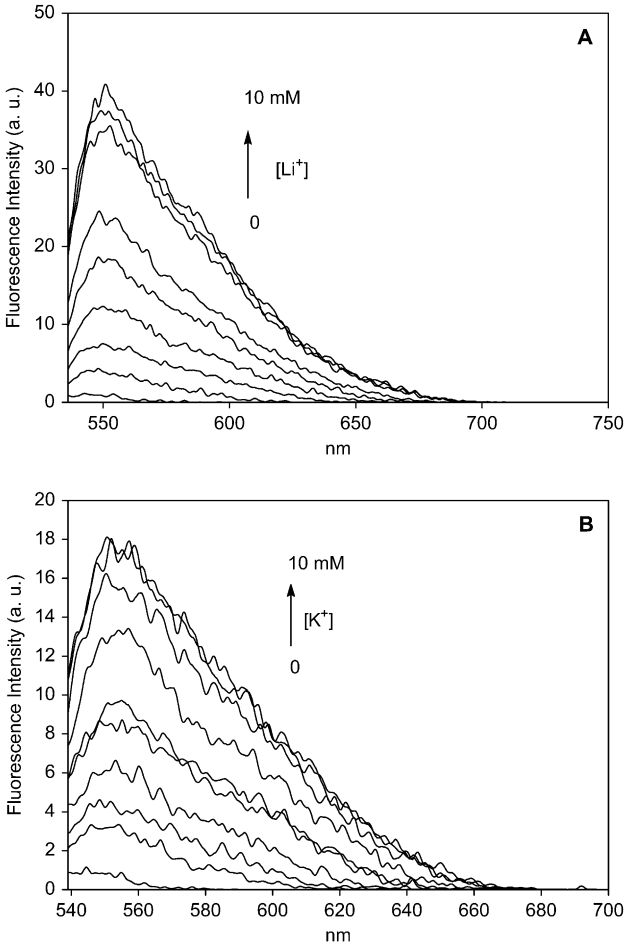
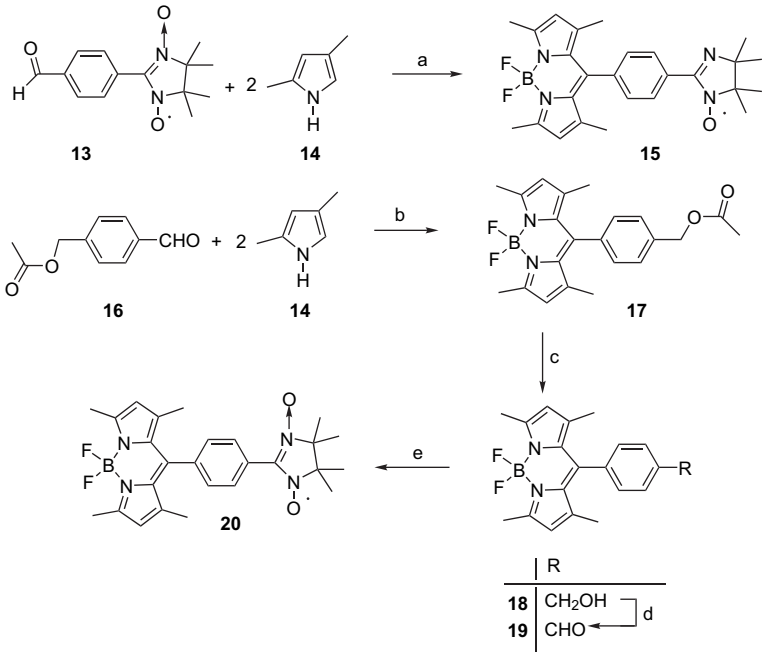


Figure 3. (**A**) Fluorescence emission spectra of compound **11** (1 μM) in acetonitrile with free Li^+ concentrations of 0, 25, 50, 100, 200, 500, 2000, 5000, and 10,000 μM, λ_{ex}=530 nm; (**B**) fluorescence emission spectra of compound **12** (1 μM) in acetonitrile with free K^+ concentrations of 0, 25, 50, 100, 200, 250, 500, 2000, 5000, and 10,000 μM, λ_{ex}=533 nm.

for Li^+ and Mg^{2+}, respectively. Although there is a significant difference between the association constants of **11** and Mg^{2+} versus **11** and Li^+, compound **11** cannot be regarded as a selective fluoroionophore, because Li^+, Na^+, Mg^{2+}, Ca^{2+} all increases the fluorescence. In contrast to compound **11**, compound **12** exhibits good selectivity toward K^+ and the association constant is 6570 M^{-1} in acetonitrile estimated by titration and no other ions caused significant increase in fluorescence quantum yield (except H^+, but this with a red shift) (Fig. 3). An investigation of the sensing mechanism is in progress, however, the changes in spectral band positions are comparatively small, in agreement with a PET signaling mechanism. Binding of a cation alters the redox potential of the aza-crown ether by weakening the nitrogen donor strength thus inhibiting the quenching process and, therefore, increasing the fluorescence. The fluorophore–σ-spacer–receptor arrangement with an electronically decoupled amino nitrogen atom also supports the PET mechanism of sensing rather than an ICT process, as demonstrated earlier by Rurack et al.[36]

From our laboratory, we have demonstrated that nitroxide covalently linked to a BODIPY dye is a good redox sensor reagent. Wang et al. demonstrated that a pyrene fluorophore attached to an imino nitroxide has optical and gate properties, e.g., reduction of nitroxide to *N*-hydroxylamine and protonation of imino nitrogen resulted in fluorescence enhancement.[37] It seemed a real challenge to combine nitronyl nitroxide with the BODIPY-type dye. The reaction of nitronyl nitroxide aldehyde **13**[38] with 2 equiv 2,4-dimethylpyrrole **14** in the presence of TFA in CH_2Cl_2 and after treatment with DDQ, $BF_3\cdot Et_2O$, and *i*-Pr_2EtN and oxidation with PbO_2 gave imino nitroxide **15** instead of the required nitronyl nitroxide. Because the synthetic pathway resulted in the loss of the 3-oxo group, we decided to introduce the nitronyl nitroxide ring in the last step. Reaction of 4-acetoxymethyl benzaldehyde **16**[39] with pyrrole **14** in an acid catalyzed reaction, followed by treatment with DDQ, $BF_3\cdot Et_2O$, and *i*-Pr_2EtN gave compound **17**. Deacetylation of this compound with NaOMe yielded alcohol **18**. Oxidation of compound **18** with activated MnO_2 furnished aldehyde **19**. Treatment of compound **19** with 2,3-bis-hydroxylamine-2,3-dimethylbutane monosulfate salt in the presence of Et_3N in methanol yielded the desired nitronyl nitroxide **20** after oxidation with $NaIO_4$ (Scheme 3).

We have investigated the spectral properties of compounds **15** and **20**. The addition of 2-(*N*,*N*-diethylamino)-diazenolate-2-oxide (diethylamine NONOate), as a solid, water soluble NO source to a solution (0.1 M phosphate buffer solution (PBS)/acetonitrile, 50:1) of nitronyl nitroxide **20** yielded a small (~20%) increase in quantum yield (Fig. 4). In the UV–vis spectrum the $S_0\rightarrow S_1$ band does not change, only bands at 230, 266, and 363 nm disappear, while a new band at 237 nm appears. The significant change is observed only in EPR spectra, the 5 line EPR spectra of nitronyl nitroxide change to the 7 line EPR spectra of an imino nitroxide **15**, as happens with any other nitronyl nitroxide when subjected to nitrogen oxide (Fig. 4).[40] The EPR spectral data are: a_{N1}=4.3 G, a_{N2}=9.2 G, 7 lines and they were identical with the EPR spectra of the directly prepared compound **15**. However, the resulting imino nitroxide **15** has interesting optical properties. Titration of compound **15** in a 0.1 M phosphate buffer with ascorbic acid as a reducing agent, results in about 80% increase in fluorescence quantum yield after adding excess ascorbic acid. However, dissolving compound **15** in TFA/acetonitrile/water (pH=1.5) the fluorescence quantum yield increases five times, without any



Scheme 3. Reagents and conditions: (a) **13** (1 equiv), **14** (2 equiv), CH_2Cl_2, TFA (1.1 equiv), rt, 8 h, then DDQ (1 equiv), 30 min, $BF_3\cdot Et_2O$ (10 equiv) and *i*-Pr_2EtN (15 equiv), rt, 40 min, then the crude product was oxidized with PbO_2 (2 equiv), $CHCl_3$, reflux, 15 min, 10%; (b) **16** (1 equiv), **14** (2 equiv), CH_2Cl_2, TFA (1.1 equiv), rt, 8 h, then DDQ (1 equiv), 40 min, $BF_3\cdot Et_2O$ (10 equiv) and *i*-Pr_2EtN (15 equiv), rt, 30 min, 35%; (c) NaOMe (0.3 equiv), MeOH, rt, 40 min, 59%; (d) MnO_2 (15 equiv), CH_2Cl_2, rt, 2 h, 64%; (e) 2,3-bishydroxylamino-2,3-dimethylbutane monosulfate (1 equiv), MeOH, Et_3N (1.5 equiv), 12 h, rt, then oxidation with aq $NaIO_4$ (10 equiv), rt, 5 min, 38%.

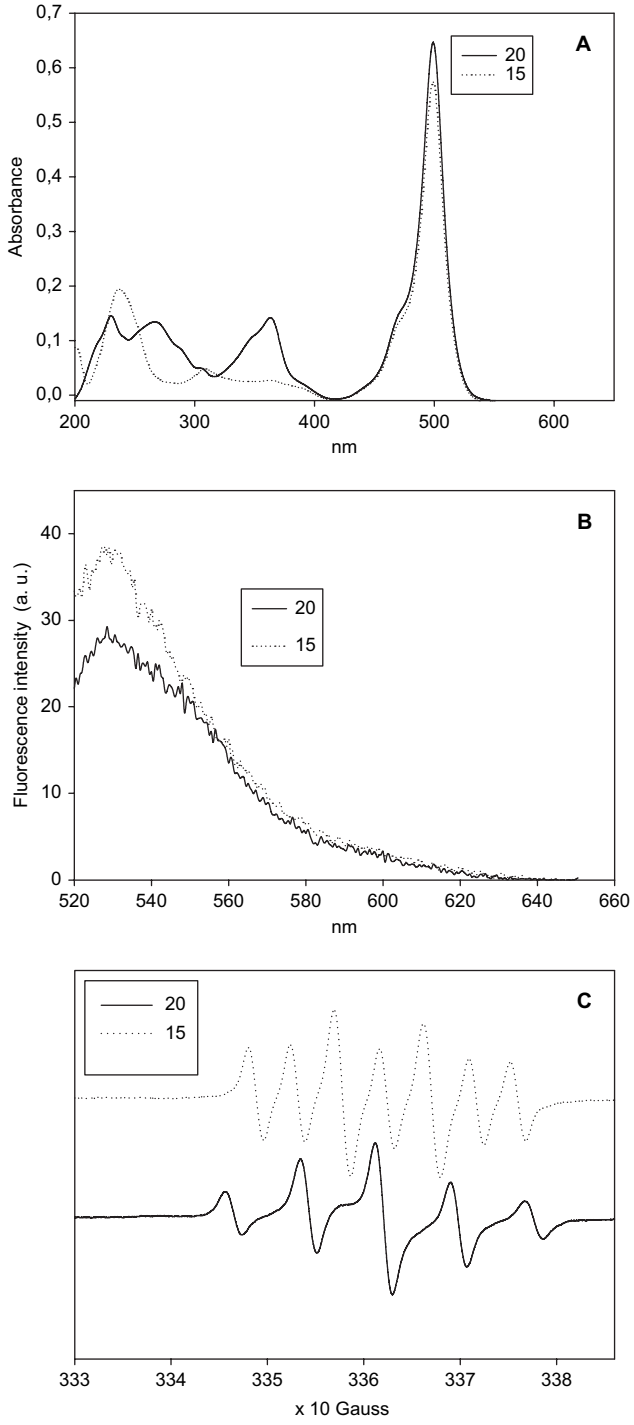


Figure 4. (**A**) Absorption; (**B**) emission spectra of compounds **20** (24 μM) and **15** in phosphate buffer solution (PBS)/acetonitrile 50:1, (pH=7.4). Spectra of **15** were generated by addition of 1 equiv diethylamine NONOate; (**C**) EPR spectra of compounds **20** (427 μM) and **15** in acetonitrile/PBS 1:1 mixture. The spectrum of **15** was generated by addition of 1.07 equiv diethylamine NONOate.

reducing agent. Titration of acidic solution of compound **15** with ascorbic acid resulted in further increases of quantum yield (up to 10 times). This confirms that compound **15** has got a redox center (nitroxide) and a proton sensitive receptor (imidazole nitrogen). Only the reduction of nitroxide and protonation of imidazole nitrogen restore the fluorescence of the BODIPY dye (Fig. 5).

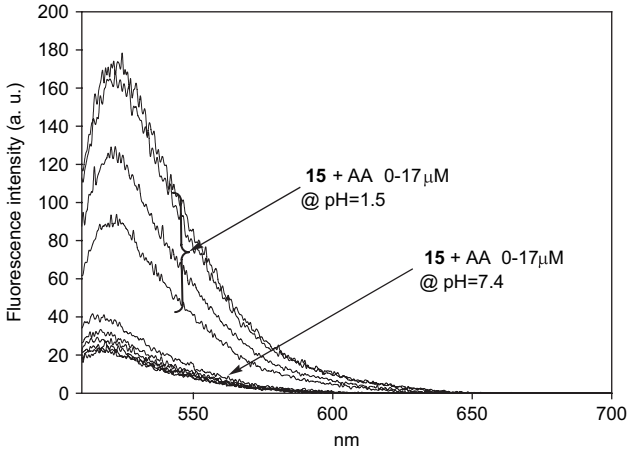


Figure 5. Titration of compound **15** (10 μM) in 0.1 M PBS (pH=7.4)/acetonitrile 50:1 with ascorbic acid (AA) 0, 1.7, 3.4, 6.8, 10.2, 13.6, and 17 μM (final concentration). The upper four plot: titration of compound **15** (10 μM) in water/TFA (pH=1.5)/acetonitrile 50:1 with ascorbic acid (AA) 0, 6.8, 13.6, and 17 μM (final concentration), λ_{ex}: 505 nm.

3. Conclusion

In conclusion, we have reported the synthesis and preliminary study of new BODIPY-based sensor molecules, a thiol-specific fluorescent label, a fluorescent amino acid, and a double (spin and fluorescence) label. Among the fluoroionophores, 1-aza-18-crown-6 in combination with a BODIPY dye is superior to 1-aza-15-crown-5 owing to its larger association constant and potassium-selective spectral change. The imino nitroxide attached BODIPY dye is a H^+- and redox sensitive molecule and a good candidate for Boolean logic gate application. Further work with new labels and sensors is in progress.

4. Experimental

4.1. General

Melting points were determined with a Boetius micro melting point apparatus and are uncorrected. Elemental analyses (C, H, N, and S) were performed on a Carlo Erba EA 1110 CHNS elemental analyzer. Mass spectra were recorded on an Automass Multi instrument in the EI mode (70 eV, direct inlet) or on a VG TRIO-2 instrument with thermospray technique. ESR spectra were obtained from 10^{-5} M solutions ($CHCl_3$), using a Magnettech MS200 spectrometer. Preparative flash column chromatography was performed on Merck Kieselgel 60 (0.040–0.063 mm). The UV spectra were taken with a Specord 40 (Jena Analytic) in acetonitrile using 1 cm quartz cells and solute concentrations (2–0.5)$\times 10^{-5}$ M. The molar extinction coefficients (ε) at absorption maximum were obtained from slope of absorbance versus concentration using five solutions of different concentrations. Fluorescence spectra of compounds dissolved in acetonitrile were measured with Perkin–Elmer LS50B spectrofluorimeter, with 3 nm slits, with correction of instrumental factors by means of a rhodamine B quantum counter and correction files supplied by the manufacturer. Quantum yields were referred to fluorescein dissolved in 0.1 M NaOH

(Φ'=0.95). The values were calculated from equation $\Phi=(I/I')(A'/A)(n/n')\Phi'$, where I', A', and Φ' are the integrated emission, absorbance (at the excitation wavelength), and quantum yield of the reference sample, respectively. n' is the refractive index of the solvent used for reference sample. I, A, n, and Φ are related to sample with the same definitions applied to reference sample. The fluorescence data of all final compounds are listed in Table 1. Lifetimes were measured with ISS K2 multifrequency phase fluorimeter and referred to glycogen. The association constant (K_a) of complexes were estimated by Eq. 1, which is a linear plot $F_0/(F-F_0)$ versus $1/C$.

$$F_0/(F-F_0)=\left(\frac{\Phi_M\varepsilon_M}{\Phi_C\varepsilon_C-\Phi_M\varepsilon_M}\right)\times\left(\frac{1}{K_aC}+1\right) \quad (1)$$

F_0 denotes the fluorescence intensity of metal free complex at a selected wavelength, F the fluorescence intensity of metal–fluoroionophore complex, C the metal ion concentration, Φ_M and Φ_C are the quantum yields of free and metal–fluoroionophore complex, ε_M and ε_C are the molar extinction coefficients. Qualitative TLC was carried out on commercially prepared plates (20×20×0.02 cm) coated with Merck Kieselgel GF_{254}. ^{1}H NMR spectra of diamagnetic compounds were recorded with Varian Unity Inova 400 WB spectrometer; chemical shifts were referenced to TMS. $NaSSO_2CH_3$,[41] compounds **1**, **6**, **7**, **13**, **16**, and 2,3-bishydroxylamine-2,3-dimethylbutane monosulfate salt[42] were prepared as described earlier and all other reagents and compounds were purchased from Aldrich or Fluka.

4.1.1. 4,4-Difluoro-8-iodomethyl-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (2). A solution of compound **1** (1.76 g, 5.0 mmol) and NaI (1.50 g, 10.0 mmol) was stirred and refluxed in dry THF (20 mL) for 45 min. After cooling, the solution was diluted with Et_2O (20 mL), washed with water (20 mL), and the organic phase was dried ($MgSO_4$), filtered, and evaporated. The residue was purified by flash column chromatography (hexane/Et_2O) to give the title compound as a dark purple solid 1.66 g (75%), mp 145–147 °C, R_f: 0.63 (hexane/Et_2O, 2:1). EA: calcd $C_{18}H_{24}BF_2IN_2$: C, 48.68; H, 5.45; N, 6.31. Found: C, 48.55; H, 5.39; N, 6.20. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=4.69 (s, 2H, CH_2I), 2.51, 2.48 (two s, each 6H, 4×ArCH_3), 2.36 (q, J=6.8 Hz, 4H, 2×CH_2CH$_3$), 1.04 (t, J=6.8 Hz, 6H, 2×CH$_2$CH_3). IR (Nujol) ν: 1605, 1560, 1510 (C=C) cm^{-1}. MS (EI) m/z: 444 (M$^+$, 24), 425 (9), 410 (5), 317 (100).

4.2. General procedure for the synthesis of methanethiosulfonates (3 and 10)

A solution of compound **2** (444 mg, 1.0 mmol) or compound **8** (585 mg, 1.0 mmol) and $NaSSO_2CH_3$ (270 mg, 2.0 mmol) was dissolved in acetone (10 mL) and water (3 mL), and the mixture was heated at reflux until the consumption of the starting halogen compound (~30 min). After cooling, the acetone was evaporated off, water (5 mL) was added, and the residue was partitioned between water and EtOAc (20 mL). The organic phase was separated, dried ($MgSO_4$), filtered, and evaporated, and flash column chromatography (hexane/EtOAc) purification afforded methanethiosulfonates **3** or **10**.

4.2.1. 4,4-Difluoro-8-methanesulfonylmethyl-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (3). Yield 184 mg (43%), red solid, mp 186–189 °C, R_f: 0.22 (hexane/EtOAc, 2:1). EA: calcd for $C_{19}H_{27}BF_2N_2O_2S_2$: C, 53.27; H, 6.35; N, 6.54; S, 14.97. Found: C, 53.10; H, 6.41; N, 6.33; S, 14.88. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=4.70 (s, 2H, CH_2S), 3.37 (s, 3H, SO_2CH_3), 2.51, 2.48 (two s, each 6H, 4×ArCH_3), 2.37 (q, J=7.2 Hz, 4H, 2×CH_2CH$_3$), 1.04 (t, J=7.2 Hz, 6H, 2×CH$_2$CH_3). IR (Nujol) ν: 1600, 1560, 1505 cm^{-1} (C=C). MS (EI) m/z: 428 (M$^+$, 59), 413 (2), 396 (30), 317 (100).

4.2.2. 3-Methanethiosulfonylmethyl-2,2,5,5-tetramethyl-4-[(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene-8-phenyl-4′-oxy)ylmethyl]-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical (10). Yield 166 mg (27%), red solid, mp 148–150 °C, R_f: 0.14 (hexane/EtOAc, 2:1). EA: calcd for $C_{30}H_{37}BF_2N_3O_4S_2$: C, 58.42; H, 6.05; N, 6.82; S, 10.40. Found: C, 58.26; H, 6.13; N, 6.73; S, 10.23. IR (Nujol) ν: 1650, 1560, 1535, 1505 (C=C) cm^{-1}. MS (thermospray) m/z: 617 (M+H)$^+$. EPR (in $CHCl_3$): triplet, a_N=14.6 G.

4.2.3. D,L-2-Amino-3-(4,4-difluoro-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene-8-yl)-propionic acid ethyl ester (4). To a solution of *N*-(diphenylmethylene)glycine ethyl ester (267 mg, 1.0 mmol) in dry THF (10 mL), NaHMDS (1.0 M THF solution) (1.1 mL, 1.1 mmol) was added at −78 °C in one portion and the mixture was stirred for 15 min at this temperature, then compound **2** (444 mg, 1.0 mmol) dissolved in dry THF (10 mL) was added dropwise. After 1 h stirring at −78 °C the mixture was allowed to warm to 0 °C and quenched with satd aq NH_4Cl solution (10 mL). After evaporation of THF, water (10 mL) was added and the aq phase was extracted with $CHCl_3$ (2×20 mL). The organic phase was dried ($MgSO_4$), filtered, and evaporated. The crude residue was dissolved in EtOH (10 mL) and 5% aq H_2SO_4 (5 mL) was added and the mixture was allowed to stand at rt for 40 min. The solution was diluted with water (15 mL) and the solution was concentrated to half in vacuo. The aq phase was washed with EtOAc (2×10 mL) to remove the benzophenone and other by-products, and organic phase was discarded. The aq phase pH was adjusted to 8 by addition of solid K_2CO_3 and extracted with $CHCl_3$ (2×20 mL), then dried ($MgSO_4$), filtered, and evaporated to give 159 mg (38%) of a thick purple oil, R_f: 0.48 ($CHCl_3$/Et_2O, 2:1). EA: calcd for $C_{22}H_{32}BF_2N_3O_2$: C, 63.02; H, 7.69; N, 10.02. Found: C, 62.97; H, 7.55; N, 10.00. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=4.22 (br s, 1H, CH), 3.96 (q, J=7 Hz, 2H, OCH_2), 3.51 (d, J=6 Hz, 2H, ArCH$_2$), 2.48, 2.47 (two s, each 6H, 4×ArCH_3), 2.36 (q, J=7.2 Hz, 4H, 2×CH_2CH$_3$), 1.27 (t, J=7 Hz, 3H, OCH$_2$CH_3), 1.04 (t, J=7.2 Hz, 6H, 2×CH$_2$CH_3). IR (neat) ν: 3400 (NH$_2$), 1740 (C=O), 1615, 1560, 1540, 1500 (C=C) cm^{-1}. MS (EI) m/z: 419 (M$^+$, 11), 418 (4), 318 (45), 182 (80), 41 (100).

4.2.4. D,L-2-*tert*-Butoxycarbonylamino-3-(4,4-difluoro-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene-8-yl)propionic acid ethyl ester (5). To a solution of compound **4** (155 mg, 0.37 mmol) in THF (10 mL) was added *tert*-butoxycarbonyl anhydride (81 mg, 0.37 mmol)

and the mixture was stirred at 40 °C for 30 min. After cooling, Et_2O (10 mL) was added, the organic phase was washed with brine (10 mL), then the organic phase was separated, dried ($MgSO_4$), filtered, and evaporated, and compound **5** (130 mg, 76%) was obtained after flash column chromatography purification as a red solid, mp 170–172 °C, R_f: 0.55 (hexane/EtOAc, 2:1). EA: calcd for $C_{27}H_{40}BF_2N_3O_4$: C, 62.43; H; 7.76; N, 8.09. Found: C, 62.38; H, 7.69; N, 8.00. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=5.18 (br s, 1H, *H*N), 4.44 (br s, 1H, C*H*), 3.96 (q, J=7 Hz, 2H, OCH_2), 3.51 (d, J=6 Hz, 2H, ArCH_2), 2.48, 2.47 (two s, each 6H, 4×ArCH_3), 2.36 (q, J=7.2 Hz, 4H, 2×CH_2CH$_3$), 1.36 (s, 9H, C(CH_3)$_3$), 1.27 (t, J=7 Hz, 3H, OCH$_2$CH_3). 1.04 (t, J=7.2 Hz, 6H, 2×CH$_2$CH_3). IR (Nujol) ν: 3330 (NH), 1745, 1680 (C=O), 1560, 1540, 1505 (C=C) cm^{-1}. MS (EI) *m/z*: 519 (M^+, 2), 463 (1), 317 (4), 287 (7), 57 (100).

4.2.5. 3-Bromomethyl-2,2,5,5-tetramethyl-4-[(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene-8-phenyl-4′-oxy)-ylmethyl]-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical (8) and 2,2,5,5-tetramethyl-3,4-bis[(4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene-8-phenyl-4′-oxy)-ylmethyl]-2,5-dihydro-1*H*-pyrrol-1-yloxyl radical (9). A solution of compound **6** (510 mg, 1.5 mmol), 18-crown-6 (19 mg), and powdered K_2CO_3 (207 mg, 1.5 mmol) and acetone were stirred at 40 °C for 10 min, then compound **7** (326 mg, 1.0 mmol) was added dropwise, dissolved in acetone (5 mL), and the mixture was stirred and heated at reflux for 30 min. After cooling, the acetone was evaporated off and the residue was dissolved in $CHCl_3$ (20 mL), the organic phase was washed with brine (10 mL), dried ($MgSO_4$), filtered, and evaporated, and the residue was purified by flash column chromatography (hexane/Et_2O followed by hexane/EtOAc). The first two bands are the remains of starting materials **6** and **7**, respectively, and then compound **8** R_f: 0.44 (hexane/EtOAc, 2:1), then compound **9** R_f: 0.21 (hexane/EtOAc, 2:1) eluted. The yield of compound **8** is 128 mg (22%), red solid, mp 195–197 °C. EA: calcd for $C_{29}H_{34}BBrF_2N_3O_2$: C, 59.97; H, 5.87; N, 7.19. Found: C, 59.88; H, 5.78; N, 7.10. MS (EI) *m/z*: 586/584 (M^+, 10/10), 542/540 (25/25), 340 (58), 186 (100). IR (Nujol) ν: 1660, 1625, 1570, 1550, 1510 (C=C) cm^{-1}. Yield of compound **9** is 253 mg (30%), red solid, mp 183–185 °C. EA: calcd for $C_{48}H_{52}B_2F_4N_5O_3$: C, 68.21; H, 6.21; N, 8.29. Found: C, 68.15; H, 6.15; N, 8.25. IR (Nujol) ν: 1650, 1555, 1535, 1505 (C=C) cm^{-1}. MS (thermospray) *m/z*: 845 (M+H)$^+$.

4.3. General procedure for the alkylation of aza-crown ethers (11 and 12)

A solution of compound **2** (444 mg, 1.0 mmol), 1-aza-15-crown-5 (219 mg, 1.0 mmol), and powdered K_2CO_3 (207 mg, 1.5 mmol) or 1-aza-18-crown-6 (263 mg, 1.0 mmol) and Na_2CO_3 (159 mg, 1.5 mmol) was stirred and refluxed in acetone (10 mL) until compound **2** (30 min for **12** and 2 h for **11**) was consumed. After cooling, the inorganic salts were filtered off, washed with $CHCl_3$ (5 mL), and the solvents were evaporated under reduced pressure. The residue was purified by flash column chromatography ($CHCl_3$/Et_2O and $CHCl_3$/MeOH) to yield the title compounds.

4.3.1. 13-(4,4-Difluoro-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene-8-methylenyl)-1,4,7,10-tetraoxa-13-azacyclopentadecane (11). Yield 412 mg (77%), red solid, mp 148–150 °C, R_f: 0.34 ($CHCl_3$/Et_2O, 2:1). EA: calcd for $C_{28}H_{44}BF_2N_3O_4$: C, 62.80; H, 8.28; N, 7.85. Found: C, 62.82; H, 8.30; N, 7.66. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=3.99 (s, 2H, ArCH_2N), 3.61, 3.56 (two s, 16H, 8×CH_2), 2.89 (s, 4H, 2×NCH_2), 2.48, 2.40 (two s, each 6H, 4×ArCH_3), 2.36 (q, J=7.2 Hz, 4H, 2×CH_2CH$_3$), 1.03 (t, J=7.6 Hz, 6H, 2×CH$_2$CH_3). IR (Nujol) ν: 1645, 1540, 1505 (C=C) cm^{-1}.

4.3.2. 16-(4,4-Difluoro-1,3,5,7-pentamethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene-8-methylenyl)-1,4,7,10,13-pentaoxa-16-azacyclooctadecane (12). Yield 370 mg (64%), red solid, mp 84–86 °C, R_f: 0.85 ($CHCl_3$/MeOH, 9:1). EA: calcd for $C_{30}H_{48}BF_2N_3O_5$: C, 62.18; H, 8.35; N, 7.25. Found: C, 62.15; H, 8.31; N, 7.12. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=4.02 (s, 2H, ArCH_2N), 3.62, 3.55 (two s, 20H, 10×CH_2), 2.93 (s, 4H, 2×NCH_2), 2.47, 2.40 (two s, each 6H, 4×ArCH_3), 2.36 (q, J=7.2 Hz, 4H, 2×CH_2CH$_3$), 1.03 (t, J=7.6 Hz, 6H, 2×CH$_2$CH_3). IR (Nujol) ν: 1640, 1550, 1505 (C=C) cm^{-1}.

4.3.3. 2-[(4,4-Difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene-8-phenyl)-4-yl]-4,4,5,5-tetramethyl-4,5-dihydro-1*H*-imidazol-1-yloxyl radical (15). To a deoxygenated solution of aldehyde **13** (1.0 mmol, 261 mg) and 2,4-dimethylpyrrole (2.0 mmol, 190 mg) in CH_2Cl_2 (200 mL), TFA (125 mg, 1.1 mmol) was 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, 11.6 mmol) and $BF_3 \cdot Et_2O$ (1.25 mL, 10.0 mmol) 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. The residue was dissolved in $CHCl_3$ (10 mL), PbO_2 (478 mg, 2.0 mmol) was added, and the mixture was stirred and refluxed for 15 min. The PbO_2 was filtered off, the solvent was evaporated under reduced pressure, and the residue was purified by flash column chromatography (hexane/EtOAc), collecting the first red/purple fraction afforded the imino nitroxide linked to the BODIPY dye, 46 mg (10%), orange-red solid, mp 195 °C (decomposes on heating), R_f: 0.57 (hexane/EtOAc, 2:1). EA: calcd for $C_{26}H_{30}BF_2N_4O$: C, 67.40; H, 6.53; N, 12.09. Found: C, 67.51; H, 6.48; N, 12.00. IR (Nujol) ν: 1560, 1540, 1505 (C=C) cm^{-1}. MS (EI) *m/z*: 463: (M^+, 1), 391 (1), 349 (5), 41 (100). EPR (in $CHCl_3$): 7 lines, a_{N1}=4.3 G, a_{N2}=9.0 G and (in acetonitrile/PBS 1:1) 7 lines, a_{N1}=4.3 G, a_{N2}=9.2 G.

4.3.4. 4,4-Difluoro-8-(4-acetoxymethylphenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (17). To a deoxygenated solution of aldehyde **16** (1.78g, 10.0 mmol) and 2,4-dimethylpyrrole (20.0 mmol, 1.90 g) in CH_2Cl_2 (500 mL), TFA (114 mg, 1.0 mmol) was added and the mixture was stirred overnight under N_2 at rt. The red solution was treated with DDQ (2.27 g, 10.0 mmol), stirred for 30 min, then *i*-Pr_2EtN (25.7 mL, 0.15 mol) and $BF_3 \cdot Et_2O$ (12.5 mL, 0.1 mol) 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. The residue was purified by flash column chromatography (hexane/EtOAc), collecting the first brownish fraction afforded the acetoxy derivative **17**, 1.38 g (35%), brownish-red solid, mp 86–88 °C (decomposes on heating), R_f: 0.60 (hexane/EtOAc, 2:1). EA: calcd for $C_{22}H_{23}BF_2N_2O_2$: C, 66.69; H, 5.85; N, 7.07. Found: C, 66.70; H, 5.77; N, 7.01. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.45 (d, J=7.6 Hz, 2H, Ar*H*), 7.25 (d, J=7.6 Hz, 2H, Ar*H*), 5.96 (s, 2H, pyrrole *H*), 5.09 (s, 2H, CH_2O), 2.54 (s, 6H, 2×CH_3), 2.13 (s, 3H, COCH_3), 1.36 (s, 6H, 2×CH_3). IR (Nujol) ν: 1735 (C=O), 1630, 1560, 1540, 1510 (C=C) cm^{-1}. MS (EI) *m*/*z*: 396 (M$^+$, 18), 350 (6), 256 (74), 162 (82), 43 (100).

4.3.5. 4,4-Difluoro-8-(4-hydroxymethylphenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (18). To a solution of ester **17** (792 mg, 2.0 mmol) in MeOH (10 mL), NaOMe solution (0.6 mmol, freshly made from 14 mg Na metal and 5 mL MeOH) was added and the mixture was allowed to stand at rt for 40 min. Then the solution was diluted with water (10 mL) and extracted with $CHCl_3$ (2×15 mL). The combined organic phase was dried ($MgSO_4$), filtered, evaporated, and the residue was purified by flash column chromatography ($CHCl_3$/Et_2O) to yield the alcohol **18** as a dark red solid with a greenish shine, 417 mg (59%), mp 194–195 °C, R_f: 0.73 ($CHCl_3$/Et_2O, 2:1). EA: calcd for $C_{20}H_{21}BF_2N_2O$: C, 67.82; H, 5.98; N, 7.91. Found: C, 67.81; H, 5.88; N, 7.83. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=7.47 (d, J=7.2 Hz, 2H, Ar*H*), 7.26 (d, J=7.6 Hz, 2H, Ar*H*), 5.96 (s, 2H, pyrrole *H*), 4.79 (s, 2H, CH_2O), 2.54 (s, 6H, 2×CH_3), 1.36 (s, 6H, 2×CH_3). IR (Nujol) ν: 3300 (OH), 1645, 1560, 1545, 1505 (C=C) cm^{-1}. MS (EI) *m*/*z*: 354: (M$^+$, 100), 334 (52), 287 (36), 91 (75), 77 (67).

4.3.6. 4,4-Difluoro-8-(4-formylphenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (19). To a solution of alcohol **18** (400 mg, 1.13 mmol) in CH_2Cl_2 (10 mL), activated MnO_2 (1.46 g, 17.0 mmol) was added and the mixture was stirred at rt until the consumption of alcohol was complete (2 h, as monitored by TLC). The mixture was filtered through Celite, the filtrate was evaporated, and then the solid residue was further purified by flash column chromatography (hexane/EtOAc) to yield the aldehyde as a red powder, 254 mg (64%), mp 174–176 °C, R_f: 0.66 (hexane/EtOAc, 2:1). EA: calcd for $C_{20}H_{19}BF_2N_2O$: C, 68.21; H, 5.44; N, 7.95. Found: C, 68.19; H, 5.35; N, 7.89. ^{1}H NMR ($CDCl_3$, 400 MHz): δ=10.10 (s, 1H, C*H*O), 8.02 (d, J=8 Hz, 2H, Ar*H*), 7.49 (d, J=8 Hz, 2H, Ar*H*), 5.98 (s, 2H, pyrrole *H*), 2.59 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.34 (s, 3H, CH_3). IR (Nujol) ν: 1700 (C=O), 1655, 1560, 1540, 1505 (C=C) cm^{-1}. MS (EI) *m*/*z*: 352: (M$^+$, 100), 332 (47), 287 (16), 136 (53).

4.3.7. 2-[(4,4-Difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene-8-phenyl)-4-yl]-4,4,5,5-tetramethyl-4,5-dihydro-1*H*-imidazol-3-oxo-1-yloxyl radical (20). To a deoxygenated solution in MeOH (10 mL) of 2,3-bis-hydroxylamino-2,3-dimethylbutane monosulfate (1.0 mmol, 246 mg) and Et_3N (150 mg, 1.5 mmol), aldehyde **19** (352 mg, 1.0 mmol) was added and stirred overnight under N_2. After evaporation of the solvent, the residue was partitioned between $CHCl_3$ (15 mL) and water (20 mL) containing $NaIO_4$ (2.13g, 10.0 mmol), and the mixture was vigorously shaken for 5 min. The organic phase was then separated, dried ($MgSO_4$), filtered, and evaporated, and after flash chromatography (hexane/EtOAc) we got the title compound as dark brown crystals with a greenish shine, 182 mg (38%), mp 280 °C, decomp., R_f: 0.18 (hexane/EtOAc, 2:1). EA: calcd for $C_{26}H_{30}BF_2N_4O_2$: C, 65.15; H, 6.31; N, 11.69. Found: C, 65.12; H, 6.22; N, 11.52. MS (EI) *m*/*z*: 479 (M$^+$, 1), 463 (3), 391 (5), 349 (21), 217 (67), 84 (100). IR (Nujol) ν: 1555, 1535, 1505 (C=C) cm^{-1}. EPR (in $CHCl_3$): 5 lines, a_N=7.4 G.

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References and notes

1. Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Kluwer Academic/Plenum: New York, NY, 1999.
2. Valeur, B. *Molecular Fluorescence*; Wiley-VCH: Weinheim, 2002.
3. de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. P.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515–1566.
4. Kollmannsberger, M.; Rurack, K.; Resch-Genger, U.; Daub, J. *J. Phys. Chem. A* **1998**, *102*, 10211–10220.
5. Rurack, K.; Resch-Genger, U. *Chem. Soc. Rev.* **2002**, *31*, 116–127.
6. (a) Bourson, J.; Poget, J.; Valeur, B. *J. Phys. Chem.* **1993**, *97*, 4552–4557; (b) Yamada, K.; Nomura, Y.; Citterio, D.; Iwasawa, N.; Suzuki, K. *J. Am. Chem. Soc.* **2005**, *127*, 6956–6957; (c) Baruah, M.; Qin, W.; Vallée, R. A. L.; Beljonne, D.; Rohand, T.; Dehaen, W.; Boens, N. *Org. Lett.* **2005**, *7*, 4377–4380.
7. de Silva, A. P.; Gunaratne, H.; Sandanayake, K. *Tetrahedron Lett.* **1990**, *31*, 5193–5196.
8. Arunkhumar, E.; Ajayaghosh, A. *Chem. Commun.* **2005**, 599–601.
9. (a) Turfan, B.; Akkaya, E. U. *Org. Lett.* **2002**, *4*, 2857–2859; (b) Wu, Y.; Peng, X.; Gou, B.; Fan, J.; Zhang, Z.; Wang, J.; Cui, A.; Gao, J. *Org. Biomol. Chem.* **2005**, *3*, 1387–1392; (c) Ulrich, G.; Ziessel, R. *Synlett* **2004**, 439–444; (d) Ziessel, R.; Bonardi, L.; Retilleau, P.; Ulrich, G. *J. Org. Chem.* **2006**, *71*, 3093–3102.
10. Kim, S. K.; Lee, S. H.; Lee, J. Y.; Lee, J. Y.; Bartsch, R. A.; Kim, J. S. *J. Am. Chem. Soc.* **2004**, *126*, 16499–16506.
11. Callan, J. F.; de Silva, P. A.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551–8588.
12. Coskun, A.; Deniz, E.; Akkaya, E. U. *Org. Lett.* **2005**, *7*, 5187–5189.
13. Green, S. A.; Simpson, D. J.; Zhou, G.; Ho, P. S.; Blough, N. V. *J. Am. Chem. Soc.* **1990**, *112*, 7337–7346.

14. Bognár, B.; Ősz, E.; Hideg, K.; Kálai, T. *J. Heterocycl. Chem.* **2006**, *43*, 81–86.
15. Bilski, P.; Hideg, K.; Kálai, T.; Bilska, M. A.; Chignell, C. F. *Free Radical Biol. Med.* **2003**, *34*, 489–495.
16. Hankovszky, H. O.; Kálai, T.; Hideg, É.; Jekő, J.; Hideg, K. *Synth. Commun.* **2001**, *31*, 975–986.
17. Kálai, T.; Hideg, É.; Jekő, J.; Hideg, K. *Tetrahedron Lett.* **2003**, *44*, 8497–8499.
18. Hideg, É.; Kálai, T.; Hideg, K.; Vass, I. *Biochemistry* **1998**, *37*, 11405–11411.
19. Hermanson, G. T. *Bioconjugate Techniques*; Academic: Boca Raton, FL, 1996.
20. van Regenmortel, M. H. V.; Briand, J. P.; Muller, S.; Plaué, S. *Synthetic Polypeptides as Antigens*; Elsevier: Amsterdam, 1988.
21. England, P. M. *Biochemistry* **2004**, *43*, 11623–11629.
22. Emmerson, J. P.; Archer, S.; El-Hamouly, W.; Mansour, A.; Akil, H.; Medzihradsky, F. *Biochem. Pharmacol.* **1997**, *54*, 1315–1322.
23. Ulrich, G.; Goze, C.; Guardigli, M.; Roda, A.; Ziessel, R. *Angew. Chem., Int. Ed.* **2005**, *44*, 3694–3698.
24. Haugland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*, 10th ed.; Molecular Probes: Eugene, OR, 2005.
25. Thoresen, L. H.; Kim, H.; Welch, M. B.; Burghart, A.; Burgess, K. *Synlett* **1998**, 1276–1278.
26. Rurack, K.; Kollmannsberger, M.; Daub, J. *New J. Chem.* **2001**, *25*, 289–292.
27. Drummen, G. P.; van Liebergen, L. C. M.; den Kamp, J. A. O. P.; Post, J. A. *Free Radical Biol. Med.* **2002**, *33*, 472–490.
28. Guerri-Amat, F.; Liras, M.; Carrascoso, M. L.; Sastre, R. *Photochem. Photobiol.* **2003**, *77*, 577–584.
29. Berliner, L. J.; Grunwald, J.; Hankovszky, H. O.; Hideg, K. *Anal. Biochem.* **1982**, *119*, 450–455.
30. Hubbell, W. L.; Altenbach, C.; Hubbell, C. M.; Khorana, H. G. *Adv. Protein Chem.* **2003**, *63*, 243–290.
31. Kotha, S.; Kuki, A. *Tetrahedron Lett.* **1992**, *33*, 1565–1568.
32. (a) Balog, M.; Kálai, T.; Jekő, J.; Berente, Z.; Steinhoff, H.-J.; Engelhard, M.; Hideg, K. *Tetrahedron Lett.* **2003**, *44*, 9213–9217; (b) Balog, M.; Kálai, T.; Jekő, J.; Steinhoff, H.-J.; Engelhard, M.; Hideg, K. *Synlett* **2004**, 2591–2593; (c) Becker, C. F. W.; Lausecker, K.; Balog, N.; Kálai, T.; Hideg, K.; Steinhoff, H.-J.; Engelhard, M. *Magn. Reson. Chem.* **2005**, *43*, S34–S39.
33. Kálai, T.; Balog, M.; Jekő, J.; Hideg, K. *Synthesis* **1999**, 973–980.
34. Karolin, J.; Johansson, L. B.-A.; Strandberg, L.; Ny, T. *J. Am. Chem. Soc.* **1994**, *116*, 7801–7806.
35. Wu, K.-C.; Lin, Y.-S.; Yeh, Y.-S.; Chen, C.-Y.; Ahmed, M. O.; Chou, P.-T.; Hon, Y.-S. *Tetrahedron* **2004**, *60*, 11861–11868.
36. Rurack, K.; Danel, A.; Rozkiewitz, K.; Grabka, D.; Spieles, M.; Rettig, W. *Org. Lett.* **2002**, *4*, 4647–4650.
37. Wang, H. M.; Zhang, D. Q.; Guo, X. F.; Zhu, L. Y.; Shuai, Z. G.; Zhu, D. B. *Chem. Commun.* **2004**, 670–671.
38. Ullman, E. F.; Osiecki, J. H.; Boocock, D. G.; Darcy, R. *J. Am. Chem. Soc.* **1972**, *94*, 7049–7059.
39. Gennari, C.; Ceccarelli, S.; Piarulli, U.; Aboutayab, K.; Donghi, M.; Paterson, I. *Tetrahedron* **1998**, *54*, 14999–15016.
40. Kalyanaraman, J. J. B.; Hyde, J. S. *Biochem. Biophys. Res. Commun.* **1993**, *192*, 926–934.
41. Mintel, R.; Westley, J. *J. Biol. Chem.* **1966**, *241*, 3381–3385.
42. Lamchen, M.; Mittag, T. W. *J. Chem. Soc. C* **1966**, 2300–2303.