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Singlet Oxygen Generation by Novel NIR BODIPY Dyes

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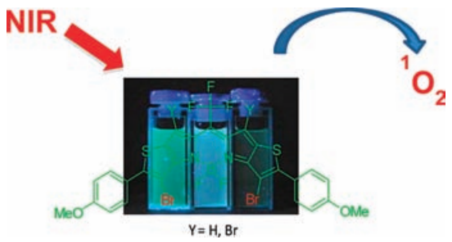
ABSTRACT



Five novel near-infrared BODIPY dyes were prepared for improved singlet oxygen generation using thiophene and bromine. Theoretical, optical, photostable, and singlet oxygen generation characteristics of these dyes were assessed. Predicted excitation energies by TDDFT calculations were in good agreement ($\Delta E \approx 0.06$ eV) with experimental data. All five dyes showed both excitation and emission in the NIR range. In particular, two dyes having sulfur and bromine atoms showed efficient singlet oxygen generation with high photostability.

Singlet oxygen ($^1O_2$) is of prime importance to various applications such as ene reaction, hetero Diels–Alder reaction, [2 + 2] cycloaddition reactions,[1] and photodynamic therapy (PDT). The generation of singlet oxygen in solution requires a photosensitizer (PS) which is converted to the triplet excited state (intersystem crossing, ISC) upon irradiation. The triplet state PS transfers energy to molecular oxygen in a type II process to form singlet oxygen. In a type I process, there is usually superoxide formation by transfer of an electron from the PS to molecular oxygen.[2] Many dyes obtained from natural or synthetic sources with a high intersystem crossing (ISC) have been used for reactions of singlet oxygen and PDT, noninvasive treatments. The heavy atom effect has been a useful chemical approach to improve ISC in several molecules including BODIPY chromophores.[3]

BODIPY (4,4-difluoro-4-bora-3*a*,4*a*-diaza-*s*-indacene), since its first discovery,[4] has received a great deal of attention for various applications[5] such as biological imaging and labeling,[6] sensors,[7] dye-sensitized solar cells (DSSC),[8] light-emitting materials for electroluminescent devices,[9]

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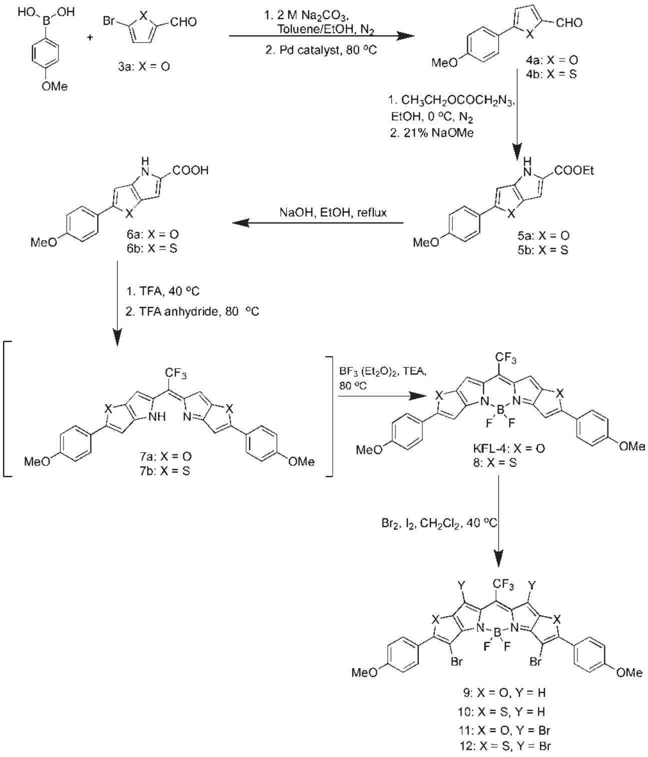
and PDT.[10] Photophysical properties of BODIPY dyes displayed superior characteristics over other dyes such as intense absorption profile, high photostability, high fluorescence quantum yield, high extinction coefficient, and flexible modification for tuning absorption range.[11] In addition, recent versatile synthetic schemes make the modifications of BODIPY feasible for achieving variable photophysical properties including singlet oxygen generation by increasing S1→T1 transitions by attaching heavy atoms to the BODIPY core to enhance spin orbit coupling.[12] Though such heavy-atom-subsituted BODIPY photosensitizers showed improved singlet oxgen generation, the search is still on for NIR absorbing (> 700 nm) BODIPY photosensitizers to produce an enhanced biological effect in deeper tissues.

In this study, we report the synthesis and photophysical properties of new NIR BODIPY dyes by making use of the heavy atom effect. The dyes were designed from the BODIPY fluorophore, KFL-4, due to its high molar extinction coefficient and long wavelength absorption maximum at 723 nm.[13] To gain insight into the heavy atom effect, we attached bromine atoms to the modified BODIPY core using a fast and efficient brominating condition employing bromine and trace iodine in an aromatic electrophilic substitution reaction.[14] The oxyen atoms of furan in the BODIPY core were replaced with sulfur atoms (Scheme 1). By introducing the π-electron-rich system, thiophene, it was expected that in addition to extended conjugation of the chromophore the heavy atom would increase the intersystem crossing yield.[15] Furthermore, the attachment of bromine atoms in a di- and tetrasubstituted fashion on the modified BODIPY core composed of both furan and thiophene moieties was to maximally increase ISC and to possibly cause bathochromic shift toward the NIR region of the electromagnetic spectrum.[16]

To confirm the sites of bromination for the dibrominated compounds, **9** and **10** were subjected to a 1D-selective NOESY and ROESY experiment, respectively. In the experiments we were interested in $^1H-^1H$ correlation to confirm the bromination on the furan/thiophene or the pyrrole rings. The experiment works on the principle of determing $^1H-^1H$ proximity of a molecule by through-space nuclear Overhauser effect (NOE). A weak irradiation of the phenyl protons (A) in the precursor of **9** results in an increase in the population of the higher energy level in nearby nonirradiated protons (B) and (C) through space. The excess population after undergoing a T_1 relaxation to a lower energy level gives signal intensities of the nearby protons (B) and (C). The irradiation of (B) gives signal intensities (A) and (D) suggesting a close proximity between the phenyl protons and the furan/thiophene proton (D). In the NOESY/ROESY of KFL-4 and **8**, the interaction of D with B was observed. On the other hand, it was not detected in the NOESY/ROESY of **9** and **10**, which is

Scheme 1. Synthesis of the BODIPY Analogues



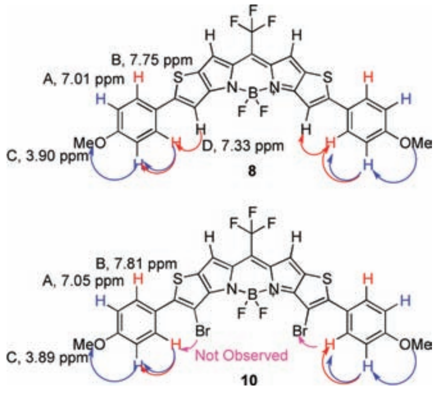


Figure 1. Key NOEs showing the sites of bromination in **10**.

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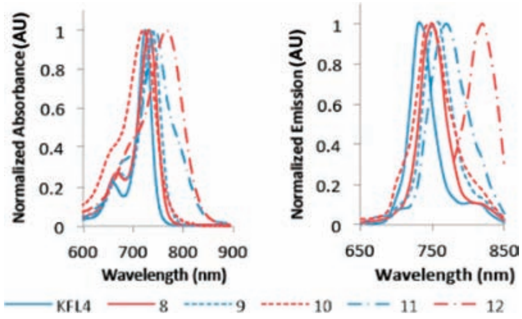

Figure 2. Normalized absorption and fluorescence emission spectra of compounds **8**–**12** in chloroform.

Table 1. Optical Properties of Analogues

dye	solvent	λ_{ab} (nm)	λ_{em} (nm)	ε (M^{-1} cm^{-1})	Φ_F[a]
KFL4	$CHCl_3$	723	738	253 000	0.56
	THF	722	738	229 000	0.56
8	$CHCl_3$	731	754	201 000	0.37
	THF	730	754	179 000	0.39
9	$CHCl_3$	738	756	242 000	0.37
	THF	732	756	203 000	0.28
10	$CHCl_3$	720	754	89 000	0.45
	THF	717	754	64 000	0.17
11	$CHCl_3$	746	765	94 000	0.28
	THF	741	765	139 000	0.11
12	$CHCl_3$	766	820	75 000	0.11
	THF	764	770	51 000	0.04

[a] Reference: **KFL4**.

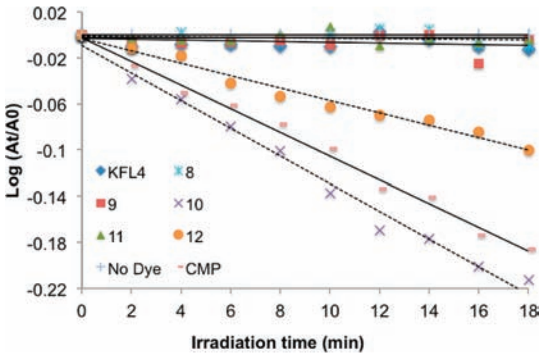

Figure 3. Time-dependent decrease of absorbance at 410 nm by oxidation of DPBF (90 × 10^{-6} M) with a dye (5 × 10^{-6} M) under broad light (400–850 nm at 0.5 mW/cm^2).

the indicative of bromine substitution on the furan or thiophene rather than the pyrrole ring (Figure 1 and spectra in the Supporting Information).

The novel dyes showed excellent optical properties in a solution of solvents of different polarities, $CHCl_3$ and THF (Table 1). The optical properties of **8**–**12** in chloroform display an intense green color and sharp absorption and emission spectra (Figure 2). The incorporation of the thiophene, **8**, caused a bathocromic shift of 8 nm relative to KFL4 while maintaining a high molar extinction coefficient of 201 000 M^{-1} cm^{-1}. Further bathochromic shifting of ~20 nm (KFL4 → **11**) and ~30 nm (**8** → **12**) was observed when four bromine atoms were attached to the modified core containing furan and thiophene moieties respectively. The absorption maxima of **10** was inconsistent with the observed trends as it displayed a hypsochromic shift of 11 nm from its parent **8**. The variation was well captured by the TDDFT calculations (Figure S3) although causes of this variation are not clear. All the analogues showed strong emission spectra in the NIR region ranging from 738 to 820 nm with moderate quantum yields of fluorescence. The low fluorescence quantum yields of the tetrabrominated analogues could be attributed in part to the effective triplet population of the dyes.

A comparative study of singlet oxygen generation in THF solutions was performed to assess the ability of **8**–**12** to generate singlet oxygen. The solutions were irradiated with broad band light, 400–850 nm, at 0.5 mW/cm^2. Singlet oxygen generation was estimated experimentally by 1,3-diphenylisobenzofuran (DPBF), a well-known singlet oxygen indicator.[17] The decrease of the absorbance band at 410 nm was monitored, caused by the oxidation of DPBF with reactive oxygen species such as singlet oxygen. The experiments were performed at initial concentrations of 5 × 10^{-6} M of PS and 90 × 10^{-6} M of DPBF over a period of 18 min. 5-(4-Methoxyphenyl)-10,15,20-tetraphenyl-21,23-dithiaporphyrin) (CMP) was used as a reference PS. In previous experiments, dithiaporphyrins showed high singlet oxygen quantum yield, $\Phi(^1O_2)$ = 0.7–0.8.[18] While compounds **8**, **9**, **11** did not show any significant oxidation of DPBF, compounds **10** and **12** showed fast oxidation of DPBF (Figure 3). A 1.2-fold relative rate of oxygenation of DPBF by **10** was recorded, and a 0.5-fold relative rate for **12** was compared to that of CMP. The slower oxidation of DPBF by **12** might be due to its lower molar extinction coefficient and poor solubility of **12**.

For a number of second generation PSs, in particular bacteriochlorin PSs, photobleaching is one of the major problems in PDT.[19] Photostabilities of the new BODIPY compounds were evaluated using more vigorous irradiation conditions: 400–800 nm at 100 mW/cm^2 using a xenon lamp source. The photobleaching kinetics of compounds **8**–**12**, mTHPBC, and mTHPC (clinically approved PDT agent), used for comparative purposes, was obtained experimentally by observing the absorption maxima of the compounds in their respective oxygen saturated

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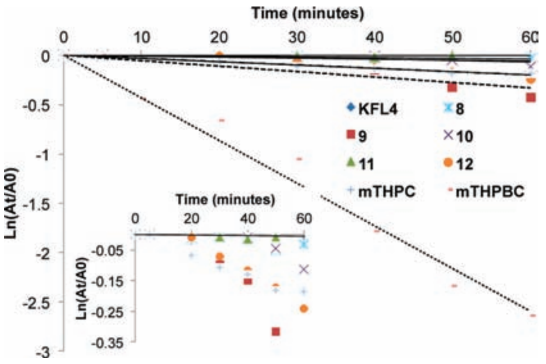

Figure 4. Photobleaching kinetics of mTHPC and mTHPBC (in MeOH) and compounds **8**–**12** (in THF) using a xenon arc lamp (400–800 nm, 100 mW/cm^2) over a period of 60 min.

solutions for 1 h. The first-order kinetics for the compounds that generated singlet oxygen was compared with mTHPBC and mTHPC (Figure 4). The first-order rate constant k (min^{-1}) in the DPBF solution with dye **10** was −0.001, and that of **12** was comparable with that of mTHPC at −0.0033. Dye **9** showed bleaching with a k value of −0.0055 over the period with the others remaining highly resistant to photobleaching. It is important to note that under the irradiation conditions mTHPBC photobleached much more rapidly than all the BODIPY analogues.

To gain insight into the excited state in predicting the absorption behavior of these compounds, theoretical characterization by density functional theory (DFT) was used.[20] Geometry optimizations of these compounds were performed using the 6-311 G* basis set while the electronic excitations corresponding to the absorption spectra were calculated using time-dependent-DFT (TDDFT) and the PBEPBE correlation functional in vacuo as expressed by Gaussian 09.[21] The TD-DFT calculations predicted strong absorption maxima of compounds **8**–**12** in the NIR region with relatively small deviations from the experimental data (ΔE = 0.03–0.15 eV)[22] (Table 2). The observed trend seen in calculated excitation energies in vacuo was consistent with the experimental data obtained from absorption spectra (Figure S3, Supporting Information). The solvent effect might have increased the spectral shifts, resulting in the differences observed.

Table 2. TDDFT-Calculated Excitation Energies for the Lowest Transition (eV, nm), Oscillator Strengths (f), and Experimental Absorption Maxima (exp)

dye	state	excitation	E (eV, nm)	f	exp (eV, nm)
KFL4	S1	H → L (103%)	1.87 (662)	1.66	1.72 (723)
8	S1	H → L (81%)	1.72 (718)	0.47	1.69 (731)
9	S1	H → L (96%)	1.73 (717)	1.28	1.68 (738)
10	S1	H → L (22%)	1.78 (697)	0.54	1.72 (720)
11	S1	H → L (97%)	1.71 (723)	1.23	1.66 (746)
12	S3	H2 → L (79%)	1.64 (757)	0.40	1.61 (766)

In conclusion, we developed new BODIPY analogues that absorb (720–766 nm) and emit (738–820 nm) in the NIR region of the electromagnetic spectrum. Compounds **10** and **12** displayed good singlet oxygen generation comparative to CMP and a relatively high resistance to photobleaching, showing promise of use as NIR photosensitizers in PDT. In particular, compound **10** showed high brightness (BT = $\varepsilon \times \Phi_f$), 40 050 and 10 880 M^{-1} cm^{-1} in $CHCl_3$ and THF, respectively, in addition to efficient generation of singlet oxygen. Such chromophores could be useful for dual purposes: imaging and PDT. Relatively consistent predictions of absorption bands by DFT calculation support it as a good tool in the design of new BODIPY analogues with tuned absorption bands. Work is underway on the synthesis and characterization of more water-soluble derivatives to understand their biological effects and potential PDT applications.

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Supporting Information Available. Available experimental details and characterization data of new compounds were reported. This material is available free of charge via the Internet at http://pubs.acs.org.

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