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A lead BODIPY-phenylanthracene dyad for application in photodynamic therapy.
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A Lead BODIPY-phenylanthracene photodynamic therapy

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ABSTRACT

Over the past four years we have developed BODIPY dyads capable of triplet state generation from a charge transfer states generated by photoinduced electron transfer. In the current work we identify a lead compound for application in photodynamic therapy. This system is composed of a phenylanthracene electron donor unit and a dimethyl-substituted BODIPY acceptor unit. We have demonstrated that this compound, in submicromolar concentrations, can effectively generate singlet oxygen in polar solvents and induce cytotoxicity in human breast cancer cells (MDA-MB-468) when exposed to light. The photophysical properties of these compounds are chemically tunable and thus open the door not only to a new class of photodynamic therapy photosensitizers but also agents for triplet-triplet annihilation up-conversion applications.

Keywords: BODIPY, Photodynamic Therapy, Triplet State, Singlet Oxygen, Photosensitizer

1. INTRODUCTION

In photodynamic therapy, a therapeutic agent is activated by light of an appropriate wavelength to populate the photosensitizers excited singlet state. The efficiency of subsequent intersystem crossing from the singlet to the triplet state is dependent on the chemical and photophysical properties of the photosensitizer. Energy is then transferred from the triplet state of the type II photosensitizer to ground state molecular oxygen to yield highly reactive singlet oxygen (Figure 1).[1] Singlet oxygen is the major toxic agent that mediates the therapeutic effect by inducing cell death pathways. Therapeutic applications of the photodynamic effect can be achieved if this process is directed to occur in local areas with light or other targeting strategies, resulting in the selective destruction of diseased tissue. Since von Tappeiner and Jesionek treated skin tumors with eosin and light,[2] photodynamic therapy has established itself as a mainstream clinical treatment with success in areas including cancer therapy[3] and dermatology[4] but the search to find novel lead compounds continues due to a variety of drawbacks with current photosensitizers including photosensitivity post-treatment, issues with photosensitizer biocompatibility and less than optimal photosensitizer photophysical properties.

BODIPYs (boron-dipyrromethenes) are primarily chromophores as fluorescent emission is the dominant pathway of decay from the excited state (Figure 1); however, they have entered the photodynamic therapy stage in recent years.[5] Accessing the triplet state is the major focus of BODIPY research from a photodynamic therapy perspective. To achieve this, intersystem crossing is maximized by, among other approaches,[6] the introduction of heavy atoms such as halogens[7] (**1**), conjugation to spin converters[8] or the dimerization of two BODIPY units[9] (**2**). Other advantages of BODIPYs for therapeutic applications include their relatively straightforward synthesis and the facile chemical tuning of their photophysical properties.

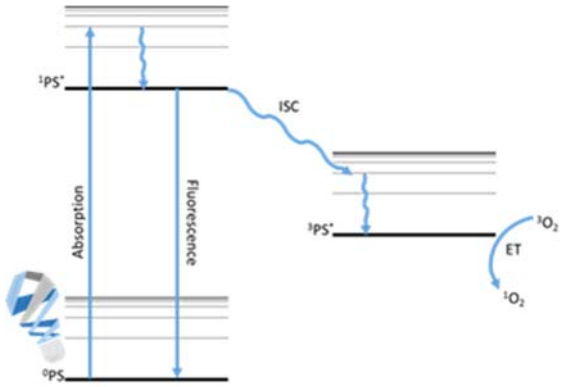


Figure 1. Jablonski diagram representing the photodynamic therapy mechanism.

Another method for acquiring a BODIPY triplet state is photoinduced electron transfer. Molecules that display this phenomenon are dyad systems comprised of an electron donor and acceptor unit. When such molecules are excited by light absorption, an electron is transferred from the donor to the acceptor unit yielding a charge transfer state. This state then undergoes a recombination resulting in a formation of long-lived triplet excited states. In 2017, Filatov *et al.* demonstrated this in heavy atom-free BODIPY-anthracene dyads (**3** and **4**) with triplet state life times of >40 μs. Later, the suggested mechanism of spin-orbit charge transfer induced intersystem crossing was confirmed in these molecules by Wang *et al.*[10] Since then a large library of BODIPY-pyrene, -anthracene and -perylene dyads have been shown to display triplet state generating properties. Additionally, production of the triplet state was shown to be dependent on both solvent polarity and molecular geometry. In polar media conversion to the triplet was predominant due to stabilization of the charge transfer state, while in non-polar media these dyads display strong fluorescence emission from the singlet state .[11]

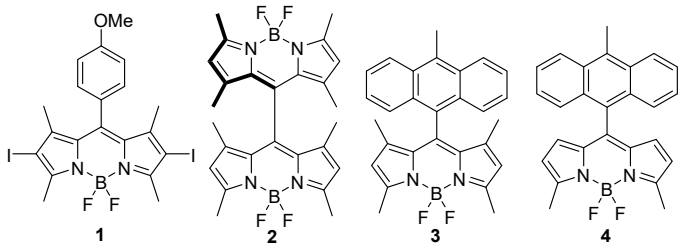
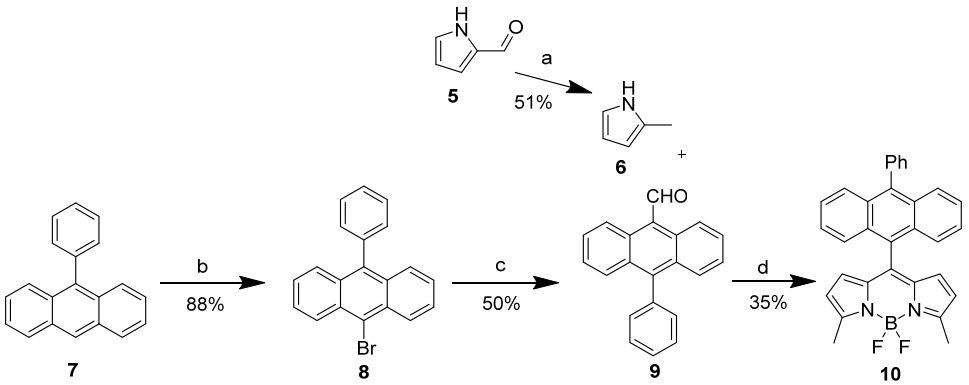


Figure 2. Examples of singlet oxygen generating BODIPY-based systems.

2. SYNTHESIS

2.1 Synthesis of lead BODIPY dyad

The target compound consists of a dimethyl-substituted BODIPY acceptor core and a phenylanthracene donor unit covalently bound at the 8-position. The synthesis of compound **10** was achieved in four steps. Firstly, compound **7** was brominated to yield **8** and then converted to the aldehyde **9** using phenyllithium in accordance with literature procedures.[12] In parallel, the synthesis of 2-methyl-1*H*-pyrrole, compound **6**, was performed following an adapted literature reduction of compound **5**.[13] Compounds **9** together with pyrrole **6**, were used to synthesize the BODIPY over three steps. A condensation reaction between the aldehyde **9** and pyrrole **6** was performed, followed by oxidation of the corresponding dipyrromethane with DDQ. A complexation with boron trifluoride in the presence of a base afforded the 8-substituted BODIPY in an overall yield of 35% (Scheme 1).[11a]

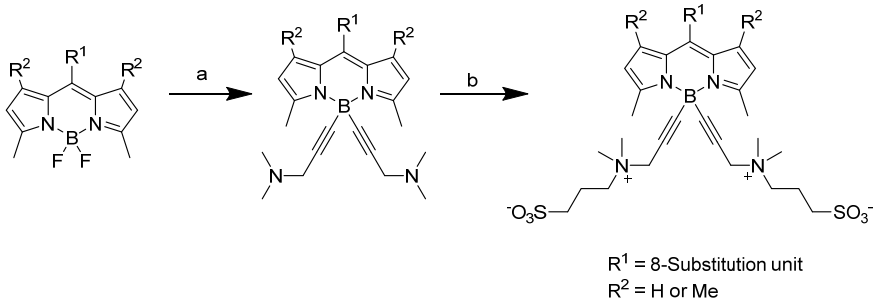


Scheme 1. Synthesis of compound **10**. (a) 1. $LiAlH_4$ (3 eq.), THF, 0 °C, 30 min. 2. 66 °C, 36 h. (b) Br_2 (1 eq.), CH_3COOH, 80 °C, 20 min; (c) 1. PhLi (1 eq.), THF, -78 °C, 1.5 h. 2. DMF, rt, 1 h; (d) 1. TFA (cat.), DCM, rt, 16 h. 2. DDQ (1 eq.), rt, 20 min. 3. DIPEA (10 eq.), $BF_3 \cdot Et_2O$ (10 eq.), rt, 1.5 h.

Compound **10** was one example from a family of BODIPY–anthracene, -pyrene and -perylene dyads shown to produce triplet states from a charge transfer states generated by photoinduced electron transfer. A library of such molecules was subsequently synthesized following the same general method as outlined above, in which **9**, 4-(10-phenylanthracen-9-yl)benzaldehyde, 4-(pyrene-1-yl)benzaldehyde and commercial aldehydes 10-methylanthracene-9-carboxaldehyde, 9-carboxaldehyde, and 1-pyrenecarboxaldehyde, were condensed with the corresponding pyrroles.[11]

2.2 Synthesis of water-soluble derivative

To investigate the *in vitro* cytotoxicity of this new class of photosensitizers eight compounds were identified as efficient singlet oxygen generators and brought forward for further study. Water-solubilizing groups were introduced using a two-step procedure to make the compounds suitable for *in vitro* studies. Firstly, a fluorine substitution to introduce *N,N*-dimethylaminopropyne-1 moieties was performed. A subsequent quaternization of the dimethylamino group with 1,3-propanesultone yielded the corresponding sulfobetaine derivatives. The water-soluble compounds containing zwitterionic fragments were precipitated from the non-polar solvent, to afford compounds **11-18** (Scheme 2, Figure 3). The series of water-soluble dyads was synthesized with substitution yields between 50–74% and quaternization yields between 45–94%. Specifically, for our lead compound **15** the substitution yield was 61% and the quaternization reaction yield was 55%.[14]



Scheme 2. General synthesis of water-soluble dyads. (a) 1. 3-Dimethylamino-1-propyne (4.5 eq.), THF, rt, 30 min. 2. *n*-BuLi (4 eq.), THF, rt, 2 h; (b) 1,3-propanesultone (6 eq.), EtOAc, 80 °C, 2 h.

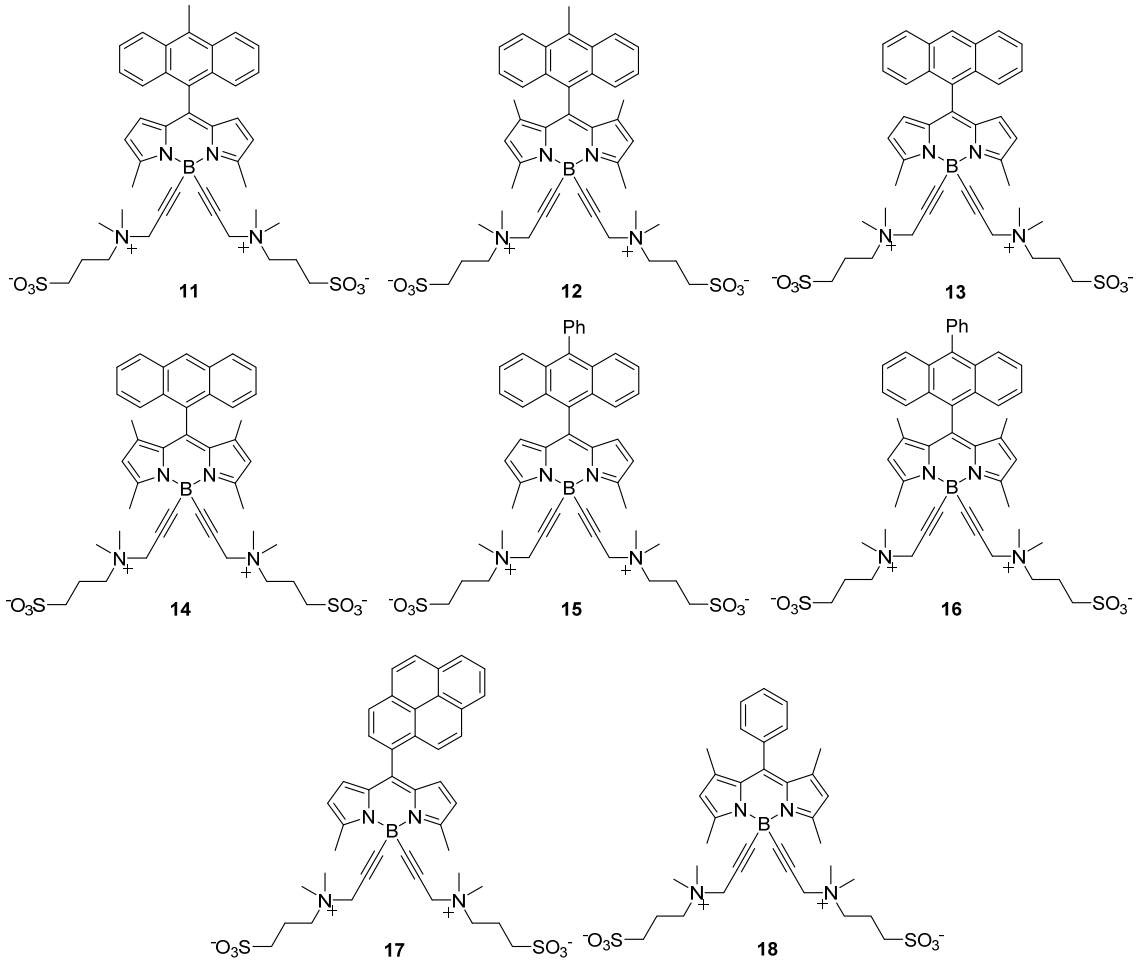


Figure 3. Library of water-soluble anthracene-, pyrene- or phenyl-BODIPYs.

3. APPLICATION IN PHOTODYNAMIC THERAPY

3.1 Optical properties and singlet oxygen quantum yields

Photodynamic therapy relies on the absorption of photons of light to activate the photosensitizer molecule. Thus, it is imperative that efficient photosensitizers have strong absorption properties. Compound **15**, has a characteristic BODIPY absorption profile with a maximum at 514 nm and also absorption below 400 nm corresponding to the anthracene unit. Its emission profile displays stronger fluorescence in ethanol when compared with water (Figure 4).

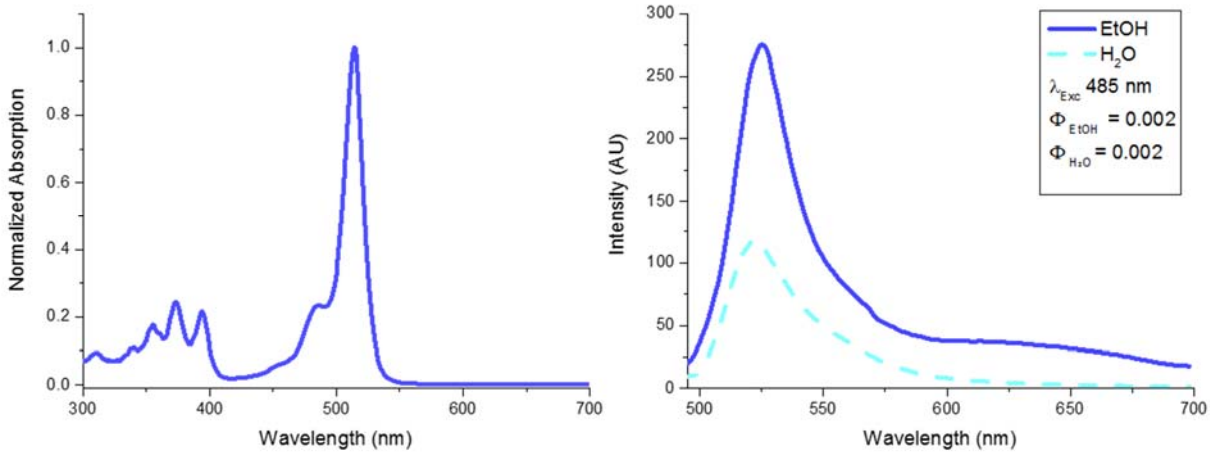


Figure 4. Right: Normalized absorption spectrum for compound **15** ethanol. Left: Emission spectra for **15** in water (dashed) and ethanol (solid). The quantum yields of the compounds were measured relative to the fluorescence of fluorescein in 0.1 M NaOH ($\varphi_f = 0.95$) and at a concentration of 1×10^{-5} M.[15]

The family of water-soluble derivatives **11-18** showed efficient singlet oxygen generation in ethanol (Table 1). Singlet oxygen quantum yields were measured using 1,3-diphenylisobenzofuran as a singlet oxygen trap, with compound **15** having a quantum yield of 48%.[16] Also of note is that compound **18** containing a phenyl group at the 8-position had a very low singlet oxygen quantum yield due to absence of photoinduced electron transfer.

Table 1. Singlet oxygen quantum yields of water-soluble dyads (**11–18**) in ethanol.

Compound	Φ_Δ EtOH	Compound	Φ_Δ EtOH
11	0.38	**15**	0.48
12	0.52	**16**	0.66
13	0.64	**17**	0.03
14	0.39	**18**	0.02

3.2 *In vitro* studies

Compound **15** along with the other water-soluble derivatives were tested on human breast cancer (MDA-MB-468) cells to demonstrate the photodynamic effect *in vitro*. The cells were incubated at various concentrations ($8 \times 10^{-4} - 5 \times 10^{-8}$ M) for 1 h. Following the incubation, they were irradiated with broad-band visible light (400–700 nm, 23.8 mW cm^{-2}). The cells were returned to the incubator for 24 h and an MTT assay was performed to determine cell viability.[17] All compounds displayed cytotoxicity at higher concentrations upon irradiation of light and minimal dark toxicity, except for the control **18** which was not capable of singlet oxygen generation. LD_{50} values of are shown in Table 2.

Table 2. LD_{50} values for compounds **11–18** in human breast cancer (MDA-MB-468) cells

Compound	LD_{50} values	Compound	LD_{50} values
11	8.7×10^{-6}	**15**	2.8×10^{-7}
12	6.1×10^{-6}	**16**	4.2×10^{-6}
13	1.3×10^{-6}	**17**	5.26×10^{-6}
14	1.7×10^{-5}	**18**	8.2×10^{-4}

Target **15**, our lead compound, showed an LD_{50} value of 2.8×10^{-7} M making it the most potent of the compounds explored here with a distinct photodynamic therapeutic response at submicromolar concentration. As shown in Figure 5, at a compound concentration of 5×10^{-7} M only 15% of cells survived following irradiation compared to 89% cell viability under dark conditions. One can also see that at higher concentrations (1×10^{-6}–1×10^{-5} M) phototoxicity was increased while dark toxicity remains minimal.

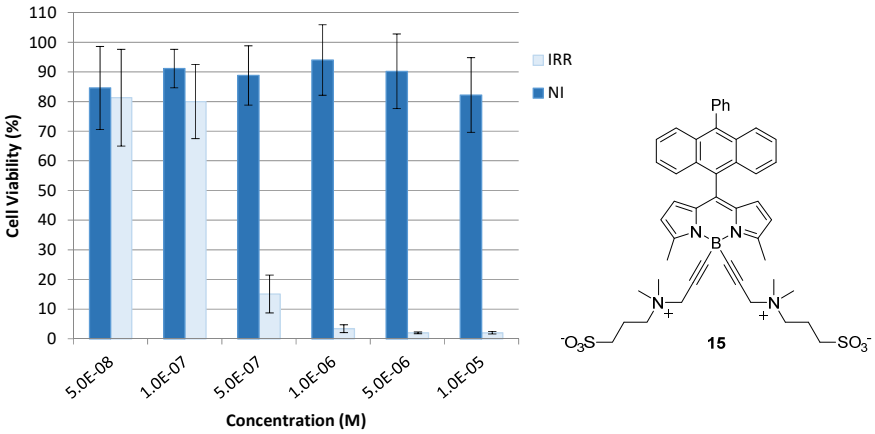


Figure 5. MDA-MB-468 cell viabilities after 1 h of incubation with **15**, with and without light irradiation (400–700 nm, 20 J cm^{-2}) at various concentrations (1×10^{-5}—5×10^{-8} M).

4. OUTLOOK

In summary, a library of water-soluble heavy atom-free BODIPYs have been prepared and studied, and a lead compound has been identified. It has been shown that **15** produces singlet oxygen *in vitro* due to triplet states formation *via* photoinduced electron transfer process. The efficiency of singlet oxygen generation is sufficient to induce cytotoxicity at a concentration of 5×10^{-7} M. The next step in this project will be to expand our understanding in an *in vivo* model.

This family of BODIPY dyads has also generated interest outside of the photodynamic therapy community; particularly in the field of triplet-triplet annihilation photon up-conversion (TTA-UC), which has found application in solar energy and bioimaging.[18] As well as confirming the spin-orbit charge transfer intersystem crossing mechanism using time-resolved electron paramagnetic resonance spectroscopy, Wang *et al*. also observed TTA-based delayed fluorescence in the BODIPY-anthracene dyads.[10b] This was followed by an investigation into the effect of the vertical dipole moment orientation on triplet state generation, in which they found that attaching the anthracene unit at the 8-position of the BODIPY core resulted in triplet state yields of up to 96% in comparison to the dyads with the anthracene unit attached at the 2-position of the BODIPY core which had triplet state yields of <31%. In this study, they also observed a TTA-UC quantum yield of 15.8% for the studied BODIPY-anthracene dyad, using perylene as the triplet acceptor at 510 nm excitation.[19] Recently, the anti-Strokes shift was increased by direct excitation of the charge transfer absorption band of the triplet resulting in improved TTA-UC efficiency in a BODIPY-perylene dyad.[20] In a final example, Kiseleva *et al.* showed that a BODIPY-anthracene dyad could be used as both the sensitizer and emitter molecule in a TTA-UC system by exploiting their polarity dependence. As previously discussed, these dyad systems display efficient ISC in polar media and in non-polar media the emission pathway dominates. In the first systems under investigation, the BODIPY acted as a sensitizer when combined with perylene in polar dichloromethane. When the BODIPY was excited at 525 nm blue emission of perylene was observed. Alternatively, the BODIPY acted as an emitter in a system with {5,10,15,20-tetrakis(4-fluorophenyl)tetrabenzoporphyrinato}palladium(II) in non-polar toluene. The BODIPY emitted green light after excitation of the tetrapyrrole at 638 nm.[21]

From the examples outlined above the future direction of this class of compounds is envisioned to be diverse and as we gain a better understanding of their nuances, they are expected to find additional practical applications in photomedicine, material sciences and bioimaging.

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