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Bodipy-based photosensitizers with long alkyl tails at the *meso* position: efficient singlet oxygen generation in Cremophor-EL micelles

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ABSTRACT

Bodipy dyes with *n*-decyloxyphenyl-(**4**, **5**) and pentadecyl-(**8**) *meso* substituents can easily embed themselves into micellar structures formed from Cremophor-EL. In micelles of approximately 20 nm median size, heavy-atom substituted dyes show remarkable photosensitization properties as evidenced by the rate of reaction with an anthracene-based selective singlet oxygen trap in buffered aqueous solutions. Considering the ease of Bodipy derivatization and the advantages of Cremophor-EL carried therapeutic agents, these photosensitizing agents may offer novel targeting opportunities and enhanced chemical and photophysical stability.

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Introduction

Photodynamic therapy is a promising methodology for the treatment of certain cancerous and non-cancerous diseases.[1–8] The treatment protocol involves bringing together three components, namely light, molecular oxygen, and a photosensitizer. In cases where the excited photosensitizer can efficiently undergo intersystem-crossing to the triplet manifold, excitation energy in turn, can be transferred to the ground state (triplet) molecular oxygen generating singlet excited molecular oxygen. Singlet oxygen produced in this way, is the primary cytotoxic agent in photodynamic therapy.[9]

Bodipy-based photosensitizers have received considerable attention as alternative photosensitizers in recent years.[8,10–14] The reasons for this attention are twofold: unlike other photostable dyes such as PDIs[15] and squaraines,[16,17] the absorption peak of these dyes are easily tunable to use the entire visible and even the near IR region of the spectrum,[18] and there are multiple routes to transform these dyes into efficient singlet oxygen generators.[19,20] With these considerations, and due to their strong absorptivity in the visible region, it is clear that Bodipy dyes offer significant potential comparable to porphyrins and phthalocyanines.

One of the most effective methods of drug delivery is using liposomal or micellar systems.[21,22] In principle it becomes straightforward to include various targeting groups in addition to the active agent itself into a micellar or liposomal construct. In this work, we targeted Bodipy derivatives with heavy atom substituents which would facilitate intersystem crossing. In addition, to ensure that these compounds would prefer a micellar structure as opposed to bulk aqueous solution, we incorporated long alkyl chains at the *meso* positions (C-8) of the Bodipy dyes. The micelle forming agent examined was Cremophor EL (Kolliphor EL), a synthetic, non-ionic surfactant made by the reaction of Castor oil (mostly triglyceride) with ethylene oxide, which provides a polyethylene glycol chain.

Results and discussion

The synthetic plan for 8-alkyl and 8-(4′-alkoxy)aryl derivatives differs to some extent (Scheme 1). *p*-Hydroxybenzaldehyde was converted to the corresponding decyl ether by treating it with 1-bromodecane in acetonitrile at reflux. 4-Decyloxybenzaldehyde (**2**) was then first treated with 2,4-dimethylpyrrole and TFA in DCM, followed by oxidation with DDQ. Without isolation, the dipyrrin intermediate was then treated with Et_3N and $BF_3{\cdot}Et_2O$ to yield the green fluorescent Bodipy dye **3**. The 2,6-positions of the Bodipy core were then brominated and iodinated using different

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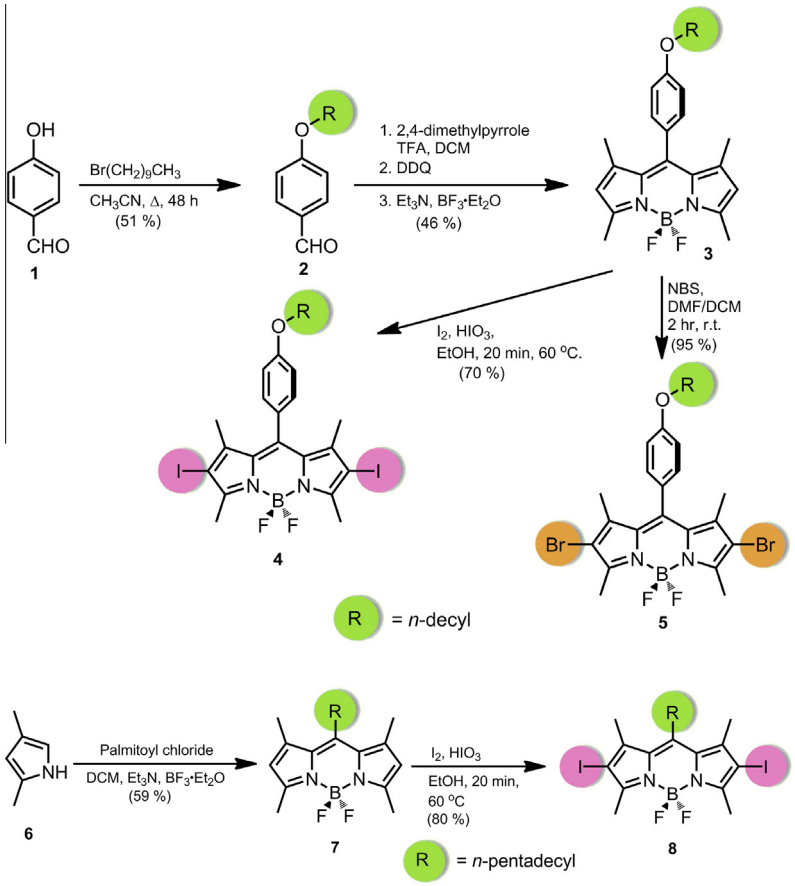

Scheme 1. Synthesis of the targeted photosensitizers **4**, **5**, and **8**.

protocols. The reaction with iodic acid and iodine in ethanol at 60 °C gave diiodo compound **4**, while treatment of **3** with NBS at room temperature resulted in compound **5**.

In a different approach, palmitoyl chloride was reacted with 2,4-dimethylpyrrole followed by installation of the difluoroboron bridge to yield dye **7**. Iodination was performed as for compound **4**, with iodic acid and iodine in ethanol at 60 °C, to give diiodo compound **8**. All new compounds were characterized by ^{1}H, ^{13}C NMR spectroscopy and HRMS. Absorption spectra of the photosensitizers were acquired in DCM, and the major absorption bands in the visible corresponding to S_0–S_1 transition were located around 530 nm (Fig. 1).

Micelles containing Bodipy compounds **4**, **5**, and **8** were prepared following literature protocols.[23–25] The size and the surface charge of the micelles were studied using dynamic light scattering (ESI). The micelles vary in size between 13 and 27 nm. Absorption and emission spectra for the micelle embedded compounds were also acquired in buffered aqueous solutions and were not altered to any significant extent.

The singlet oxygen generation capacity of the photosensitizers in the micelles were studied using a selective singlet oxygen trap 2,2′-(anthracene-9,10-diyl)bis(methylene)dimalonic acid. The absorbance of the trap molecule was adjusted to be approximately 1.0 in an oxygen saturated solution. Micellar photosensitizers at a concentration of 4.0 μM in aqueous buffer solutions were excited in the presence of the trap compound. Initially, a few data points were acquired to eliminate any possibilities of a reaction occurring in the dark. The cuvettes were then exposed to an LED array optimized for 520 nm. The change in the absorption spectra of the anthracene based trap molecule can be clearly seen in Figure 2. It

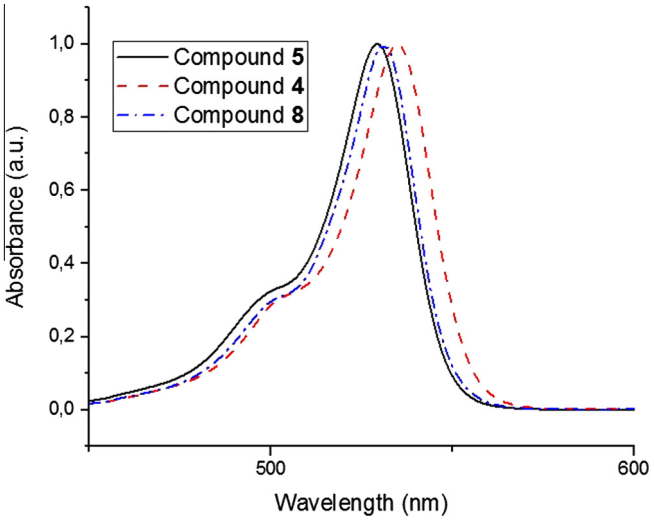

Figure 1. Normalized absorption spectra of photosensitizers **4**, **5**, and **8** in DCM.

is important to note that when kept in the dark, no changes in absorption took place, and also, in the absence of photosensitizers under irradiation with the same source, no change in the absorption took place (ESI).

The data for each photosensitizer were plotted as the change in absorption of the trap molecule at 382 nm versus irradiation time (Fig. 3). It is clear that all three photosensitizers show significant efficiencies for singlet oxygen generation.

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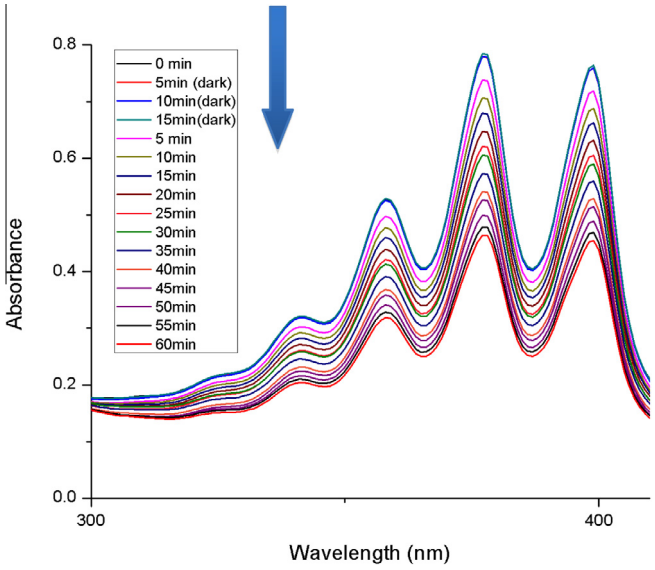

Figure 2. Singlet oxygen mediated bleaching of the trap molecule (2,2′-(anthracene-9,10-diyl)bis(methylene)dimalonic acid) in the presence of Bodipy **5** (4.0 μM) in water. The light source was an LED array with peak emission at 520 nm, fluence rate 2.5 mW/cm^2.

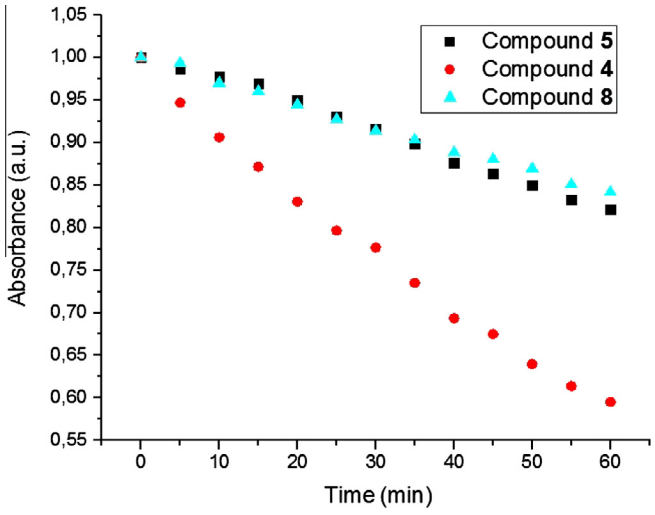

Figure 3. Singlet oxygen mediated bleaching of the trap molecule (2,2′-(anthracene-9,10-diyl)bis(methylene)dimalonic acid) in the presence of photosensitizers **4**, **5**, and **8** (4.0 μM) in PBS buffered water (pH 7.2). Absorbance at 382 nm was plotted as a function of time. The light source was an LED array with peak emission at 520 nm, and a fluence rate of 2.5 mW/cm^2.

Singlet oxygen quantum yields in Cremophor EL micelles in aqueous solutions were calculated (ESI) and vary between 0.14 and 0.46. Interestingly, the bromo derivative, photosensitizer **5**, seems to be the most effective compound in micelle-embedded form. A singlet oxygen quantum yield of almost 50% in a micellar construct is remarkable and compares very well with well-known photosensitizers.[26]

Conclusion

We have reported examples of Bodipy based photosensitizers containing micelle embedding side-chains. We have demonstrated that in aqueous solutions, stable micelles of such compounds can be prepared which act as effective photosensitizers. We are confident that similarly functionalized Bodipy dyes will find practical applications within the context of photodynamic therapy. Our work along that direction is currently in progress.

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Supplementary data

Supplementary data (all experimental methods and procedures, additional spectroscopic and analytical data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.02.033.

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23. Synthesis of photosensitizer **4**: Compound **3** (90.0 mg, 0.189 mmol) and iodine (200 mg, 0.78 mmol) and ethanol (50 mL) were added to a 250 mL round bottomed flask, and to this solution was added iodic acid (111 mg, 0.63 mmol) in water (2 mL). The reaction mixture was stirred at 60 °C and the progress was monitored by TLC (1:1 $CHCl_3$/Hexanes). Upon completion, saturated aqueous $Na_2S_2O_3$ (50 mL) was added and the product extracted with $CHCl_3$ (3 × 50 mL). The solvent was removed in vacuo and the residue purified by silica gel column chromatography using 1:1 $CHCl_3$/Hexanes as eluant. Red solid (80 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$, 300 K): δ_H = 7.14 (d, *J* = 8.5 Hz, 2H), 7.05 (d, *J* = 8.5 Hz, 2H), 4.04 (t, *J* = 6.5 Hz, 2H), 2.66 (s, 6H), 1.84 (m, 2H), 1.52 (s, 6H), 1.47 (m, 2H), 1.32 (m, 12H), 0.91 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C = 160.2, 156.5, 145.4, 141.8, 129.0, 126.4, 115.4, 85.6, 68.3, 32.0, 29.6, 29.5, 29.4, 29.3, 26.1, 22.7, 17.2, 16.0, 14.2. HRMS (ESI) calcd for $C_{29}H_{37}BF_2I_2N_2O$ $(M-H)^+$ 730.10199, found 730.10249; Δ = 0.68 ppm.
24. Synthesis of photosensitizer **5**: Compound **3** (75 mg, 0.23 mmol) was dissolved in DMF/DCM (1:1, 50 mL). Then, *N*-bromosuccinimide (370 mg, 2.05 mmol) in DCM (25 mL) was added to the reaction mixture dropwise over 15 min. The reaction mixture was stirred at room temperature for 2 h. The progress of the reaction was monitored by TLC. The reaction mixture was extracted with water and DCM and the organic phase dried with Na_2SO_4. The solvent was removed in vacuo and the residue purified by silica gel column chromatography using 3:1 $CHCl_3$/Hexanes as eluant. Yield: 165 mg (95%). ^{1}H NMR (400 MHz, $CDCl_3$, 300 K): δ_H = 7.15 (d, *J* = 8.7 Hz, 2H), 7.03 (d, *J* = 8.7 Hz, 2H), 4.04 (t, *J* = 6.6 Hz, 2H), 2.62 (s, 6H), 1.85 (m, 2H), 1.53 (m, 2H), 1.45 (s, 6H), 1.32 (m, 12H), 0.91 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C = 160.2, 153.7, 142.5, 140.7, 130.9, 129.0, 126.1, 115.4, 111.7, 68.3, 31.9, 29.6, 29.6, 29.4, 29.3, 29.2, 26.1,

22.7, 14.1, 13.9, 13.6. HRMS (ESI) calcd for $C_{29}H_{37}BBr_2F_2N_2O$ $(M-H)^+$ 635.1244, found 635.12963; Δ = 8.23 ppm.

25. Synthesis of photosensitizer **8**: Compound **5** (400 mg, 0.80 mmol) and iodine (500 mg, 1.78 mmol) were added to ethanol (50 mL) in a 250 mL round bottomed flask, and to this solution was added iodic acid (400 mg, 2.27 mmol) in water (3 mL). The reaction mixture was stirred at 60 °C and was monitored by TLC (1:1 $CHCl_3$/Hexanes). Upon completion, aqueous saturated $Na_2S_2O_3$ (50 mL) was added, and the product extracted with $CHCl_3$ (3 × 50 mL). The solvent was removed in vacuo and the residue purified by silica gel column chromatography using 1:1 $CHCl_3$–Hexanes as eluant. Red solid (500 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$, 300 K): δ_H = 3.01 (t, J = 8.4 Hz, 2H), 2.63 (s, 6H), 2.49 (s, 6H), 1.63 (m, 2H), 1.52 (m, 2H), 1.29 (m, 22H), 0.91 (t, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C = 155.2, 146.4, 142.2, 131.4, 86.3, 31.9, 31.7, 30.3, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.4, 29.4, 22.7, 18.9, 16.1, 14.1. HRMS (ESI) calcd for $C_{28}H_{43}BF_2I_2N_2$ $(M-H)^+$ 709.16, found 709.16034; Δ = 0.56 ppm.

26. For a compilation of quantum yields of various photosensitizers, please check the following website: https://www3.nd.edu/~ndrlrcdc/Compilations/QY/IntroQY.htm.