European Journal of Chemistry

Journal webpage: www.eurjchem.com

Synthesis and properties of 3-ethynylthiophene containing BODIPY derivatives

Reuben Warshawsky [1,2], Jason Vaal [1] and Priya Hewavitharanage [1,*]

[1] *Department of Chemistry, University of Southern Indiana, Evansville, Indiana 47712, USA*
[2] *School of Medicine, Indiana University, Indianapolis, Indiana 46202, USA*

** Corresponding author at: Department of Chemistry, University of Southern Indiana, Evansville, Indiana 47712, USA. Tel.: +1.812.2285009. Fax: +1.812.4651052. E-mail address: phewavitha@usi.edu (P. Hewavitharanage).*

ARTICLE INFORMATION

DOI: 10.5155/eurjchem.8.4.321-327.1634

Received: 01 August 2017
Received in revised form: 08 September 2017
Accepted: 09 September 2017
Published online: 31 December 2017
Printed: 31 December 2017

KEYWORDS

BODIPY
Thiophene
Stokes shift
Fluorescence
Singlet oxygen
Photodynamic therapy

ABSTRACT

Green, red and far-red emitting Borondipyrromethene (BODIPY) derivatives with 3-ethynylthiophene units at various positions around the BODIPY core were synthesized and their photophysical properties were studied. 3-Ethynylthiophene substitution at the 2,6 positions caused significant increase in Stokes shift while substitution at the 8 and 4,4' positions had no effect. Photooxidation of 1,3-diphenylisobenzofuran (DPBF) in the presence of 3-ethynylthiophene substituted BODIPY derivatives confirmed singlet oxygen generation. 3-Ethynylthiophene substitution at the 2,6 positions is more effective in singlet oxygen generation compared to 4'4 substitutions. Substitution through phenyl group at the meso (8) position gave the lowest rate for singlet oxygen production. All 3-ethynylthiophene containing BODIPY derivatives were highly photo-stable under our experimental conditions.

Cite this: *Eur. J. Chem.* **2017**, *8(4)*, 321-327

1. Introduction

A versatile small fluorescent molecule commonly known as borondipyrromethene has attracted significant attention in the biomedical and electronic fields due to its unique properties [1,2]. BODIPY based molecules are preferred for biological applications due to its small size, high fluorescence quantum yields, sharp absorption and emission peaks and stability [1,2]. They are relatively nontoxic to living cells [3,4] and are widely used as fluorescence probes in molecular labeling and cell imaging [5-7]. Various properties of BODIPY molecules can be fine-tuned by synthetic modifications of the BODIPY core [2,8]. Numerous aromatic and aliphatic substituents have been used to modify the BODIPY core. Substitution at the 2,6 and 3,5 positions has been widely used to extend conjugation [9-12]. Substitution at the meso position and boron center does not cause significant shift in absorption and emission spectra [13]. In general, BODIPY dyes have very small Stokes shifts, often less than 10 nm [14]. A small Stokes shift causes reabsorption of emitted photons or the inner filter effect hence reducing the emission intensity [15]. This is a disadvantage particularly for biomedical applications where small concentrations of fluorophores are used. Resonance energy transfer in a wide variety of BODIPY based donor/acceptor systems has been used to achieve large Stokes shifts [14,15]. However, they are pseudo Stokes shifts that cannot completely eliminate the inner filter effect [16]. Another strategy to enhance Stokes shifts by geometrical relaxation has been reported [17]. Increased geometrical relaxation of excited BODIPY molecules with thienyl substitution at the 2,6 positions caused red shift of the emission, giving large Stokes shifts (96 nm) [17]. Thiophene and its derivatives have been used in modification of the BODIPY core for various applications. Thiophene and oligothiopene substituted BODIPY derivatives have been synthesized for dye sensitized solar cells [18-21]. Substitution of thiophene at the 2,6 and 3,5 positions are used to extend the absorption and emission of BODIPY dyes [22]. Wu *et al.* reported synthesis of BODIPY by attaching oligothiophene moieties at the meso position [23]. However, the effect of thiophene substitution at various positions of the BODIPY core through ethynyl linkage on photophysical properties has not been studied in-depth.

Upon excitation, BODIPY derivatives produce singlet excited states and decay through fluorescence, with high quantum yields. Intersystem crossing (ISC) in BODIPY derivatives is minimal. However, it is possible to enhance ISC via chemical modification of the BODIPY core. It has been shown that incorporation of heavy atoms such as iodine

http://dx.doi.org/10.5155/eurjchem.8.4.321-327.1634

increases ISC by spin-orbit coupling [1,24,25]. Such molecules can act as triplet sensitizers to transfer its energy to the triplet states of other molecules. Energy transfer to molecular oxygen generates singlet oxygen which has several applications including photocatalysis [1] and photodymanic therapy (PDT) [26,27]. Triplet sensitizers are also used in triplet-triplet annihilation based upconversions [28]. BODIPY is a promising candidate for PDT due to its superior properties and non-toxic nature [2]. High ratios of light/dark toxicity in BODIPY derivatives and low quantum yield of photobleaching are additional advantages for biomedical applications [2]. Several types of BODIPY molecules have been synthesized and tested for singlet oxygen generation [2,29]. Impact of heavy atom effect is directly related to where they are positioned on the BODIPY core [2]. It is known that increasing the number of iodine atoms in the BODIPY molecule increases intersystem crossing [2]. However, it increases dark toxicity which limits applications of these molecules in PDT [30]. Incorporation of sulfur containing substituents such as thiophene also promotes ISC in BODIPY [31]. In addition, some thiophene substituted molecules are reported to be permeable to the cell membrane [32]. Hence, thiophene containing BODIPY derivatives are useful candidates for PDT. Efficiency of ISC depends on the nature of the BODIPY-thiophene linkage. Direct attachment of thiophene at the 2,6 positions of BODIPY core via Suzuki coupling does not effectively promote ISC. However, when two thienyl moieties are fused into the BODIPY core, ISC becomes efficient due to large spin orbit coupling and smaller single triplet energy gaps [33]. However, efficiency of singlet oxygen generation when thiophene is attached to different positions of the BODIPY core through ethynyl linkage has not been studied. In this study, we report the synthesis of green, red and far-red emitting BODIPY derivatives with 3-ethynylthiophene at various positions of the BODIPY core. Their photophysical properties and singlet oxygen generation capabilities were studied and compared with a BODIPY compound which contains iodine atoms at the 2,6 positions. We have chosen 3-ethynylthiophene because it provides a route to synthesize multi-functionalized BODIPY molecules as both 2,5 positions of the thiophene moiety are open for further substitution.

2. Experimental

2.1. Instrumentation

^{1}H NMR (300 or 400 MHz) and ^{13}C NMR (75 or 100 MHz) spectra were recorded at room temperature on JEOL Eclipse nuclear magnetic resonance spectrophotometers. Chemical shifts are reported in parts per million (ppm) in $CDCl_3$, using TMS as the internal reference (0.00). ^{1}H data are reported as follows: multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad). UV-visible spectra were recorded using a Cary 5000 Series UV-VIS-NIR spectrophotometer. Fluorescence spectra were recorded using a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer. Quantum yields were measured using Rhodamine B as the reference (ϕ = 0.65 in ethanol). High resolution mass spectra were obtained at the Department of Chemistry, University of Illinois at Urbana-Champaign.

2.2. Synthesis

All the solvents were freshly distilled under argon before use. Chemicals purchased were used without further purification.

2.2.1. Synthesis of compounds 5,5-difluoro-1,3,7,9-tetramethyl-10-nonyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (1) and 5,5-difluoro-2,8-diiodo-1,3,7,9-tetramethyl-10-nonyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (2)

Synthesis of compounds **1** and **2** is described elsewhere [34] (Scheme 1).

5,5-Difluoro-1,3,7,9-tetramethyl-10-nonyl-5H-4l4, 5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (**1**): ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 6.07 (s, 2H, indacene-H), 2.96 (t, *J* = 8.0 Hz, 2H, CH_2), 2.51 (s, 6H, $2CH_3$), 2.40 (s, 6H, $2CH_3$), 1.62 (br m, 2H, CH_2), 1.48 (br m, 2H, CH_2), 1.27 (br s, 10H, $5CH_2$), 0.87 (t, *J* = 6.6 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 153.8 (2C, indacene-C), 146.8 (1C, indacene-C), 140.4 (2C, indacene-C), 131.5 (2C, indacene-C), 121.6 (2C, indacene-C), 32.0 (2C, $2CH_3$), 30.5 (2C, $2CH_3$), 29.6 (1C, CH_2), 29.5 (1C, CH_2), 29.3 (1C, CH_2), 28.6 (1C, CH_2), 22.7 (2C, $2CH_2$), 16.4 (1C, CH_2), 14.5 (1C, CH_2), 14.1 (1C, CH_3).

5,5-Difluoro-2,8-diiodo-1,3,7,9-tetramethyl-10-nonyl-5H-4l4, 5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (**2**): ^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm): 2.92 (t, *J* = 9.0 Hz, 2H, CH_2), 2.61 (s, 6H, CH_3), 2.48 (s, 6H, CH_3), 1.62 (br m, 2H, CH_2), 1.48 (br m, 2H, CH_2), 1.27 (br s, 10H, $6CH_2$), 0.88 (t, *J* = 6.6 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 155.2 (2C, indacene-C), 146.5, (1C, indacene-C), 142.3 (2C, indacene-C), 131.5 (2C, indacene-C), 86.5 (2C, indacene-C), 31.9 (1C, CH_2), 31.8 (1C, CH_2), 30.4 (1C, CH_2), 29.4 (1C, CH_2), 22.7 (2C, $2CH_2$), 19.0 (1C, CH_2), 16.2 (1C, CH_2), 14.2 (1C, CH_3).

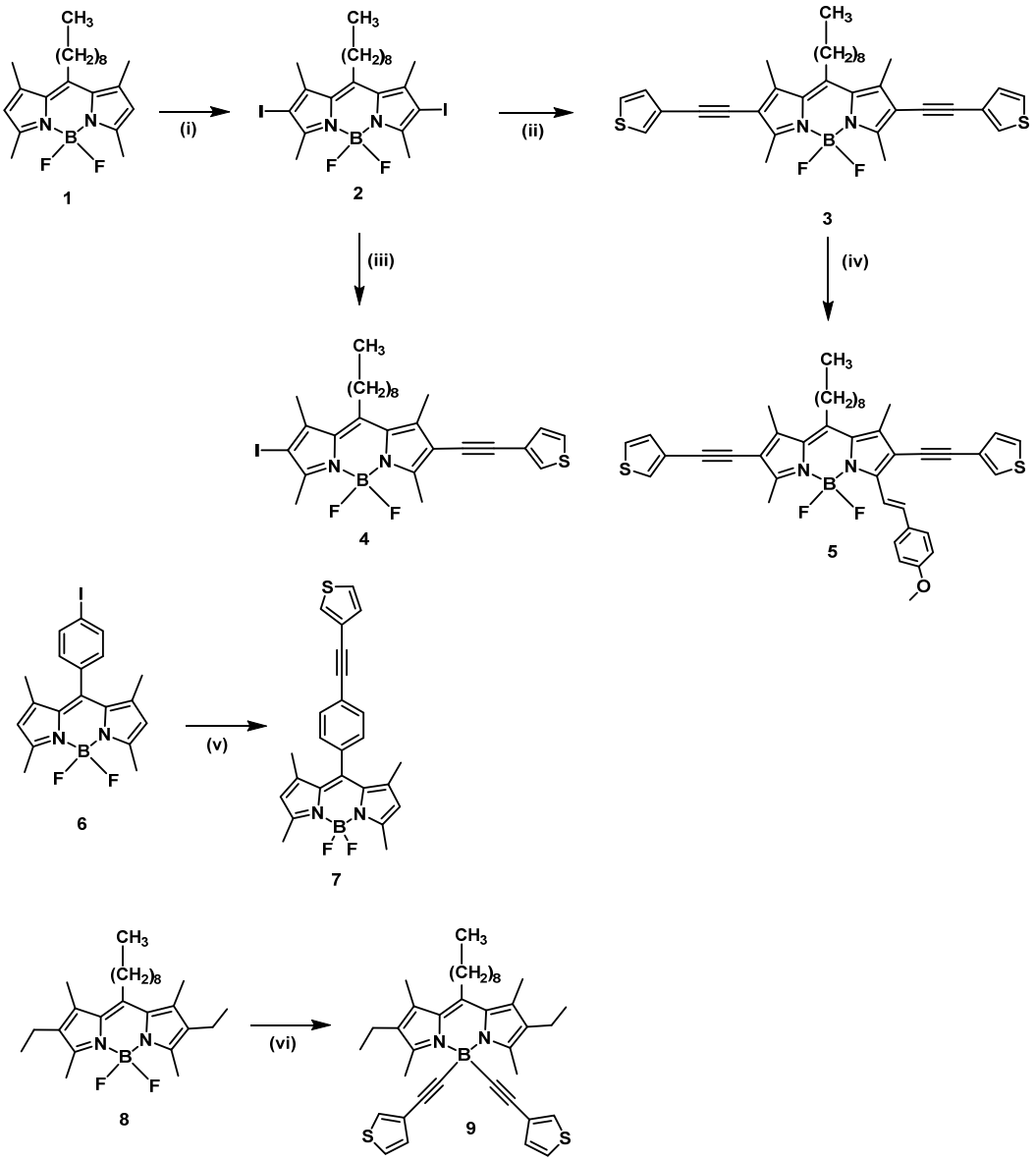
2.2.2. Synthesis of 5,5-difluoro-1,3,7,9-tetramethyl-10-nonyl-2,8-bis(thiophen-3-ylethynyl)-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (3)

To a Schlenk flask $Pd(PPh_3)_4$ (26.92 mg, 0.032 mmol), CuI (12.17 mg, 0.064 mmol) and compound **2** (100 mg, 0.16 mmol) were added in a glove box and dissolved in freshly distilled THF (15 mL). Then, Et_3N (0.78 mL) was added followed by 3-ethynylthiophene (42.26 mg, 0.4 mmol). The mixture was stirred at room temperature for 24 h, the residue was extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4 and the solvent evaporated. Purification using silica gel column chromatography eluted with dichloromethane:hexane (*v*:*v*, 40:60) yielded compound **3** (Scheme 1).

5,5-Difluoro-1,3,7,9-tetramethyl-10-nonyl-2, 8-bis(thiophen-3-ylethynyl)-5H-4l4,5l4-dipyrrolo[1,2-c:2', 1'-f][1,3,2]diazaborinine (**3**): Color: Dark purple. Yield: 70%. M.p.: 151-153 °C. FT-IR (Neat, ν, cm^{-1}): 3111, 2928, 2851, 2214 (alkyne), 1532, 1201, 1184, 1000, 776. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.43 (dd, *J* = 6.4, 1.6 Hz, 2H, Ar-H), 7.28 (q, *J* = 0.007 Hz, 2H, Ar-H), 7.17 (dd, 6.4 Hz, 1.2 Hz, 2H, Ar-H), 2.65 (s, 6H, $2CH_3$), 2.53 (s, 6H, $2CH_3$), 1.62 (br m, 2H CH_2), 1.49 (br m, 2H CH_2), 1.27 (br m, 12H, $6CH_2$), 0.87 (t, *J* = 6.8 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 156.9 (2C, indacene-C), 147.7 (1C, indacene-C), 141.2 (2C, indacene-C), 131.4 (2C, indacene-C), 129.9 (2C, indacene-C), 128.3 (2C, Ar-C), 125.5 (2C, Ar-C) 122.5 (2C, Ar-C), 116.3 (2C, Ar-C), 91.3 (2C, ethynyl-C), 81.3 (2C, ethynyl-C), 31.9 (1C, CH_2), 30.5 (2C, $2CH_3$), 29.6 (2C, $2CH_3$), 29.3, (1C, CH_2), 28.7, (1C, CH_2), 22.8, (1C, CH_2), 15.3, (1C, CH_2), 14.2, (1C, CH_2), 13.8 (1C, CH_3). UV/Vis (CH_2Cl_2, λ_{max}, nm): 563. HRMS (ESI, *m/z*) calcd. for $C_{34}H_{38}BN_2S_2F_2$ $[M+H]^+$, 587.2538; found 587.3536.

2.2.3. Synthesis of 5,5-difluoro-2-iodo-1,3,7,9-tetramethyl-10-nonyl-8-(thiophen-3-ylethynyl)-5H-4l4,5l4-dipyrrolo [1,2-c:2',1'-f][1,3,2]diazaborinine (4)

The same procedure used to synthesize compound **3** was used to synthesize compound **4** (1:1 molar ratio of compound **2**: 3-ethynylthiophene was used). The reaction mixture was stirred overnight and the compound was purified as described in the synthesis of compound **3** (Scheme 1).



Reagents and conditions: (i) HIO_3, I_2, ethanol (ii) 3-ethynylthiophene (2 mol equiv.), CuI, $Pd(PPh_3)_4$, NEt_3, THF, room temperature, 24 h (iii) 3-ethynylthiophene (1 mol equiv.), CuI, $Pd(PPh_3)_4$, NEt_3 THF, room temperature overnight (iv) 4-methoxybenzaldehyde, $Mg(ClO_4)_2$, piperidine, glacial acetic acid, toluene (v) 3-ethynylthiophene (1 mol equiv.), CuI, $Pd(PPh_3)_4$, NEt_3 THF, room temperature overnight (vi) lithium 3-ethynylthiophene, CH_2Cl_2.

Scheme 1

5, 5-Difluoro-2-iodo-1, 3, 7, 9-tetramethyl-10-nonyl-8-(thiophen-3-ylethynyl)-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (**4**): Color: Dark purple. Yield: 58%. M.p.: 205-207 °C (Dec.). FT-IR (Neat, ν, cm^{-1}): 3115, 2976, 2928, 2861, 2214 (alkyne), 1533, 1183, 1085, 999, 777. ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.43 (dd, *J* = 6.4 Hz, 1.2 Hz, 1H, Ar-H), 7.25 (q, *J* = 0.007 Hz, 1H, Ar-H), 7.12 (dd, 6.4 Hz, 1.2 Hz, 1H, Ar-H), 2.93 (t, *J* = 2.76 Hz, 2H, CH_2), 2.59 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 1.55 (br m, 2H, CH_2), 1.47 (br m, 2H, CH_2), 1.21 (br s, 10H, $5CH_2$), 0.82 (t, *J* = 6.44 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 157.0 (1C, indacene-C), 155.0 (1C, indacene-C), 147.2 (1C, indacene-C), 141.8 (1C, indacene-C), 141.7 (1C, indacene-C), 132.0 (1C, indacene-C), 130.8 (1C, indacene-C), 129.9 (1C, indacene-C), 128.3 (1C, indacene-C), 125.5 (2C-Ar-C), 122.5 (2C-Ar-C), 116.5 (2C-Ar-C), 91.5 (2C-Ar-C), 86.2 (1C, ethynyl-C), 81.2 (1C, ethynyl-C), 31.9, 30.4, 29.7, 29.5, 29.3, 22.7, 18.9, 16.1, 15.4, 14.2, 13.7. UV/Vis (CH_2Cl_2, λ_{max}, nm): 543. HRMS (ESI, *m/z*): calcd. for $C_{28}H_{35}BN_2F_2SI$ $[M+H]^+$, 607.1627; found: 607.1632.

2.2.4. Synthesis of 5,5-difluoro-3-(4-methoxystyryl)-1,7,9-trimethyl-10-nonyl-2,8-bis(thiophen-3-ylethynyl)-5H-5l4, 6l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (5)

Compound **5** was synthesized according to a literature procedure [35]. Compound **3** (100 mg, 0.17mmol) and $Mg(ClO_4)_2$ (22 mg, 0.098 mmol) were dissolved in toluene (2 mL). 4-Methoxybenzaldehyde (46.6 mg, 0.34 mmol) and piperidine (0.299 mL) were added under argon. Then glacial acetic acid (0.11 mL) was added dropwise. Afterword the solution was refluxed in a Dean-Stark apparatus at 120 °C for 1 hour. The crude product was purified using silica gel column

chromatography eluted with dichloromethane:hexane (*v*:*v*, 45:65) to afford the product **5** (Scheme 1).

5, 5-Difluoro-3-(4-methoxystyryl)-1,7,9-trimethyl-10-nonyl-2,8-bis(thiophen-3-ylethynyl)-5H-5l4, 6l4-dipyrrolo[1, 2-c:2', 1'-f][1,3,2]diazaborinine (**5**): Color: Dark blue. Yield: 18%. M.p.: 283-285 °C (Dec). FT-IR (Neat, ν, cm^{-1}): 3117, 2929, 2856, 1725, 1602, 1536, 1512, 1263, 1194, 1014, 801. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.50 (m, 4H, Ar-H) 7.10 (d, *J* = 16 Hz, 1H, C=C-H), 6.84 (d, *J* = 8.8 Hz, 4H, Ar-H), 6.84 (d, *J* = 16 Hz, 1H, C=C-H) 3.77 (s, 3H, Ar-CH_3), 2.88 (t, *J* = 2.76 Hz, 2H, CH_2), 2.64 (s, 3H CH_3), 2.55 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 1.55 (br m, 2H, CH_2), 1.47 (br m, 2H, CH_2), 1.21 (br s, 12H, $6CH_2$), 0.82 (t, *J* = 6.44 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 160.2, 151.4, 152.9, 139.3, 135.1, 133.1, 129.6, 128.9, 117.9, 117.2, 114.2, 55.4, 53.5, 32.1, 31.8, 30.9, 30.3, 29.5, 29.4, 292, 28.4, 22.6, 16.6, 14.1. UV/Vis (CH_2Cl_2, λ_{max}, nm): 615. HRMS (ESI, *m/z*). calcd. for $C_{42}H_{44}BN_2OS_2F_2[M+H]^+$, 705.2956; found 705.2956.

2.2.5. Synthesis of 5,5-difluoro-10-(4-iodophenyl)-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2] diazaborinine (6)

Compound **6** was prepared from condensation of 2,4-dimethylpyrrole with 4-iodobenzoyl chloride in CH_2Cl_2 and then reacted with BF_3OEt_2 [35] (Scheme 1).

5, 5-Difluoro-10-(4-iodophenyl)-1, 3, 7, 9-tetramethyl-5H-4l4, 5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (**6**): ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.81 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.02 (d, *J* = 8.4 Hz, 2H, Ar-H), 5.96 (s, 2H, 2 indecene-CH), 2.52 (s, 6H, 2 CH_3), 1.39 (s, 6H, $2CH_3$).

2.2.6. Synthesis of 5,5-difluoro-1,3,7,9-tetramethyl-10-(4-(thiophen-3-ylethynyl)phenyl)-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (7)

Compound **7** was synthesized from compound **6** using a similar procedure as described for the synthesis of compound **3** (Scheme 1).

5, 5-Difluoro-1,3,7,9-tetramethyl-10-(4-(thiophen-3-ylethynyl)phenyl)-5H-4l4, 5l4-dipyrrolo[1, 2-c:2', 1'-f][1,3,2]diazaborinine (**7**): Color: Orange. Yield: 38%. M.p: 268-270 °C (Dec.). FT-IR (Neat, ν, cm^{-1}): 3114, 2975, 2818, 2208 (alkyne), 1536, 1187, 1049, 785. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.66-7.61 (m, 3H, Ar-H), 7.55 (m, 1H, Ar-H), 7.32-7.37 (m, 1H, Ar-H), 7.26 (m, 1H, Ar-H), 7.21 (m, 1H, Ar-H), 5.97 (s, 2H, 2 indacene-H), 2.54 (s, 6H, $2CH_3$), 1.41 (s, 6H, $2CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 155.8 (1C, indacene-C), 143.1 (1C, indacene-C), 140.9 (1C, Ar-C), 135.0 (1C, indacene-C), 132.3 (1C, Ar-C), 132.2 (1C, Ar-C), 132.1 (1C, Ar-C), 132.0 (1C, indacene-C), 131.3 (1C, indacene-C), 129.9 (1C, indacene-C), 129.2 (1C, indacene-C), 128.6 (1C, Ar-C), 128.5 (1C, Ar-C), 128.3 (1C, Ar-C), 125.7 (1C, Ar-C), 124.1 (1C, Ar-C), 121.9 (1C, indacene-C), 121.4 (1C, indacene-C), 88.2 (1C, ethynyl-C), 86.0 (1C, ethynyl-C), 14.6 (4C, CH_3). UV/Vis (CH_2Cl_2, λ_{max}, nm): 503. HRMS (ESI, *m/z*). calcd. for $C_{25}H_{22}BN_2F_2S[M+H]^+$, 431.1557; found 431.1565.

2.2.7. Synthesis of 2,8-diethyl-1,3,7,9-tetramethyl-10-nonyl-5,5-bis(thiophen-3-ylethynyl)-5H-4l4,5l4-dipyrrolo[1,2-c:2', 1'-f][1,3,2]diazaborinine (9)

3-Ethynylthiophene (0.24 mL, 2.25 mmol) was transferred to a Schlenk flask that was previously charged with anhydrous diethyl ether (15 mL). The Schlenk flask was cooled to -78 °C and then *n*-BuLi (1.43 mL, 2.3 mmol) was added. The mixture was stirred at -78 °C for 1 h and at room temperature for 30 min. The mixture was then transferred to a solution of compound **8** (336 mg, 0.78 mmol) in THF (50 mL) at room temperature. The mixture was stirred at room temperature for 30 min., diluted with CH_2Cl_2, and washed with water. After evaporation of solvents the crude mixture was purified using silica gel column chromatography eluted with dichloromethane:hexane (*v*:*v*, 30:70) to yield compound **9** (Scheme 1).

2,8-Diethyl-1,3,7,9-tetramethyl-10-nonyl-5,5-bis(thiophen-3-ylethynyl)-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine (**9**): Color: Orange. Yield: 52%. M.p.: 265-267 °C (Dec.). FT-IR (Neat, ν, cm^{-1}): 3190, 2968, 2899, 2219 (alkyne), 1544, 1262, 1065, 786. ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.26 (m, 2H, Ar-H), 7.16 (m, 2H, Ar-H), 7.04 (m, 2H, Ar-H), 3.03 (m, 2H, CH_2), 2.78 (s, 6H, $2CH_3$), 2.44 (q, *J* = 10 Hz, 4H, $2CH_2$), 2.36 (s, 6H, $2CH_3$), 1.67 (br m, 2H, CH_2), 1.55 (br s, 2H, CH_2), 1.27 (br m, 10H, $5CH_2$), 1.08 (t, *J* = 10 Hz 6H, $2CH_3$), 0.88 (t, *J* = 8.8, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 152.1 (2C, indacene-C), 144.6 (2C, indacene-C), 133.6 (2C, indacene-C), 132.5 (2C, indacene-C), 130.3 (2C, indacene-C), 129.4 (2C-Ar-C), 126.9 (2C-Ar-C), 124.7 (2C-Ar-C), 124.4 (2C, ethynyl-C), 31.9 (1C, CH_2), 30.5 (2C, $2CH_3$), 29.6 (2C, $2CH_3$), 29.3 (1C, $2CH_2$), 28.7 (1C, $2CH_2$), 22.8 (1C, $2CH_2$), 17.6 (1C, CH_2), 15.0 (1C, $2CH_2$), 14.2 (2C, $2CH_3$), 13.9 (2C, $2CH_3$), 13.6 (1C, CH_3). UV/Vis (CH_2Cl_2, λ_{max}, nm): 517. HRMS (ESI, *m/z*). calcd. for for $C_{38}H_{48}BN_2S_2$ $[M+H]^+$, 607.3356; found 607.3352.

2.3. Singlet oxygen generation

Singlet oxygen generation trials were conducted in a quartz cuvette with THF as the solvent. BODIPY dyes were added in a concentration of 5 μM or until the λ_{max} was approximately 0.5 and 1,3-diphenylisobenzofuran (DPBF) was added to give a final concentration of 90 μM. The solution was stirred for 2 minutes in the dark with the cuvette open. The cuvette was then capped and UV-visible spectra were recorded. The samples were irradiated using a 60W halogen bulb for varying intervals of time while stirring, with the UV-Vis spectra being recorded after each interval. This was repeated until the sample was under the light for 22 minutes or until the absorbance of DPBF at 412 nm was stabilized.

3. Results and discussion

3.1. Synthesis

Compounds **1**-**9** were synthesized according to the procedure given in Scheme 1. Iodination of compound **1** was performed using HIO_3 in ethanol to give compound **2** in 76% yield [34]. A small amount of monoiodination product was also isolated. Sonogashira coupling of the BODIPY derivative **2** with 2.5 molar equivalent of 3-ethynylthiophene followed by purification using column chromatography with hexane/CH_2Cl_2 yielded compound **3** as a dark purple solid in 70% yield. Compound **4** was obtained by coupling one molar equivalent of compound **2** and 3-ethynylthiophene using the same procedure. The di-substituted product **3** was also formed in small quantity. The methyl groups on the 3,5 positions are slightly acidic. They undergo base-catalyzed Knoevenagel type condensation with aldehydes. This method has been used in synthesis of NIR BODIPY dyes [36]. Condensation of compound **3** with 4-methoxybenzaldehyde in the presence of piperidine and glacial acetic acid produced the mono-substituted product **5** as a blue solid (18% yield). The di-substituted product was also formed but was not fully characterized due to its low yield. As many unidentified side products formed, the overall product yield was low. Compound **6** was synthesized according to a literature procedure [37]. Sonogashira coupling of compound **6** with 3-ethynylthiophene yielded compound **7** as an orange solid. Reaction of compound **8** [34] with lithium salt of 3-ethynyl thiophene gave compound **9** as an orange solid. Mono-substituted product was not observed under these conditions. All compounds were stable in the air at room temperature when stored in the dark.

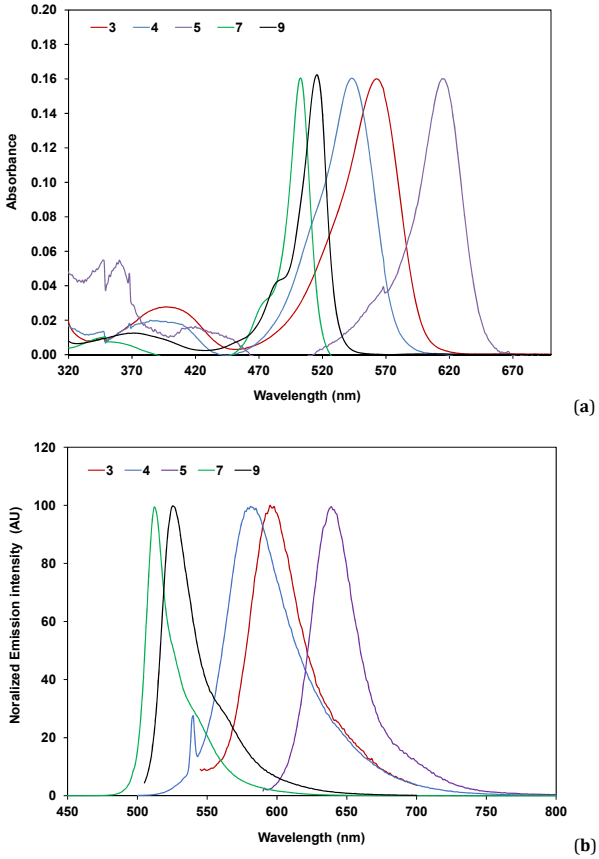


Figure 1. (a) Normalized absorption and (b) emission spectra of compound **3**, **4**, **5**, **7** and **9** in CH_2Cl_2.

3.2. Photophysical properties

The UV-vis absorption and fluorescence experiments of compounds **1**-**9** were carried out in CH_2Cl_2 (1.0×10^{-5} mol/L) using a Cary 5000 Series UV-VIS-NIR Spectrophotometer. Typical BODIPY absorption spectrum has two major absorption bands (Figure 1a). The more prominent, lowest energy absorption band ~500-600 nm, is due to the S_0-S_1 (π-π^*) transition. The weaker absorption band, ~350-430 nm, is due to the S_0-S_2 (π-π^*) transition. Absorption maximum of the BODIPY derivative **3** which has ethynylthiophene units at the 2,6 positions was redshifted by 65 nm in comparison to 2,6 unsubstituted compound **1** (Table 1). It was further redshifted (by 117 nm) with the styryl group at the 3 position in compound **5**. Placing ethynylthiophene at the 4,4' positions has no effect on absorption wavelength as the boron atom is not in conjugation with the BODIPY core (Figure 1a and 2). Attaching ethynylthiophene units through benzene ring at the meso (8) position has no effect on the absorption spectrum (compound **6**, Figure 1a and 2). Sharp emission spectra were observed for all ethynylthiophene substituted compounds (Figure 1b). As expected, emission spectra were red shifted in compounds with 3-ethynylthiophene at the 2,6 positions. Placing one styryl group at the 3 position caused significant red shift (145 nm) in the emission in comparison to unsubstituted compound **1** (Table 1, Figure 1b and 2). Photo-sensitizers that absorb in the deep red and NIR regions penetrates deeper in the tissues and have potential biomedical applications. Similar to absorption spectra, substitution at the meso position and boron center has no significant effect on emission spectra. Geometry of the S_0 state of BODIPY is very similar to that of the ground state S_1 [17]. Thus, BODIPY compounds have small Stokes shifts. Interestingly, Chen *et al.* reported 2,6 thienyl substituted BODIPY molecules with relatively larger Stokes shifts (~70-92 nm). DFT calculations revealed that the dihedral angle between the thienyl and BODIPY moiety in the S1 and S2 was quite different. Hence, large geometry relaxation upon photoexcitation was proposed as the origin of large Stoke shift [17]. When the thiophene ring is connected through a triple bond, the Stokes shift is increased from 6 to 32 nm (Table 1). This is only a 35 nm increase in compound **4**. Absence of larger Stokes shift in ethynylthiophene substituted BODIPY is probably due to smaller geometry relaxation because of restricted rotation of the thiophene ring due to the triple bond.

Table 1. Photophysical properties of compounds **1**-**9** *.

Compound	λ_{max} (nm)	λ_{em} (nm)	Stokes shift	Quantum yield (%)
1	498	504	6	84
2	520	524	4	2
3	563	595	32	34
4	543	584	41	13
5	615	649	34	30
6	504	517	13	57
7	503	512	9	82
8	520	524	4	84
9	517	525	8	86

* Rodamine B was used as reference (ϕ = 65% in ethanol).

3.3. Singlet oxygen generation

The ability of singlet oxygen generation for compounds **3**, **4**, **5**, **7** and **9** was tested in THF solutions. All the solutions were irradiated with a tungsten lamp at 0.5 mW/cm^2.

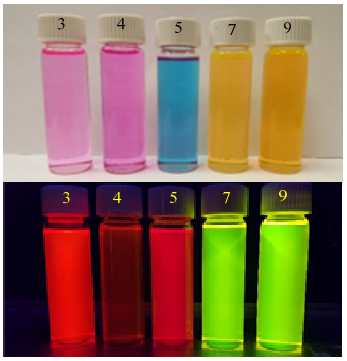


Figure 2. The visual colors of compounds **2**, **3**, **4**, **5**, and **7** under ambient light and when excited at 365 nm using a hand-held UV lamp.

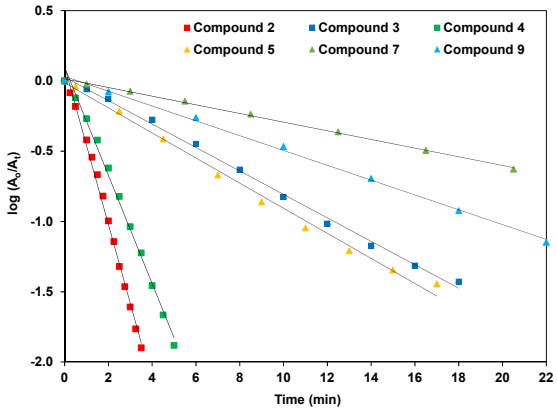


Figure 3. Time-dependent decrease of absorbance at 412 nm by oxidation of DPBF (90×10^{-6} M) with a dye (5×10^{-6} M) in THF under 60 W halogen lamp at 0.5 mW/cm^2.

1,3-Diphenylisobenzofuran (DPBF) was used as the singlet oxygen indicator [38]. DPBF reacts rapidly with singlet oxygen to form *o*-dibenzoylbenzene. Decrease of the absorbance at 412 nm due to oxidation of DPBF by singlet oxygen was monitored. Compound **2** which has two iodine atoms at the 2,6 position was used as the reference since iodine containing compounds are known to sensitize singlet oxygen generation. Rapid DPBF oxidation in compound **2** is consistent with the well-known fact that iodine increases singlet oxygen generation due to the heavy atom effect [39,40]. Compound **4** which has one 3-ethynylthiophene unit and one iodine showed only slight decrease in oxidation rate in comparison to compound **2** (Figure 3). When both iodine atoms were replaced by 3-ethynylthiophene units on the 2,6 positions (**3**), a significant decrease in oxidation rate compared to compound **2** was observed. Compound **5** with one styryl group showed slightly higher DPBF oxidation rate in comparison to compound **3**. Attaching 3-ethynylthiophene units on the 4,4' positions in compound **9** was not effective for singlet oxygen generation (Figure 3) because ethynylthiophene units at 4,4' positions are not in conjugation with the BODIPY core. Compound **7** which has phenylethynylthiophene at the meso position showed the slowest oxidation rate of DPBF. Similar effect has been reported for compound **6** with iodo-phenyl substitution at the meso position. The absence of direct attachment of iodine atoms to the BODIPY core and twisted arrangement of the phenyl group relative to the BODIPY core has been used to explain the low quantum yield of ISC in compound **6** [2]. Absorbance of all BODIPY compounds ~500-700 nm region remained unchanged under our experimental conditions indicating high resistance to photo-bleaching (550 nm region for compound 3, Figure 4). According to our preliminary studies, all 3-ethynyl containing compounds are permeable to cell membrane.

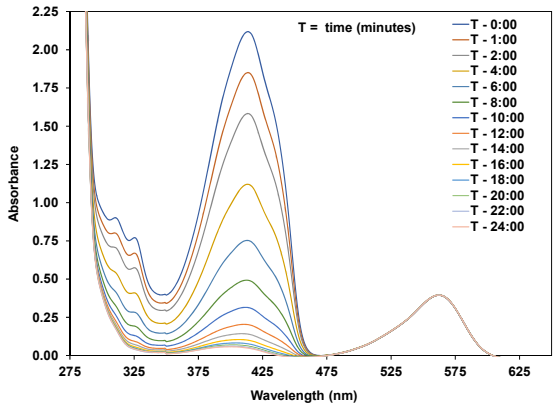


Figure 4. Singlet oxygen generation experiment in THF solution. Decrease in absorption spectrum of DPBF at 412 nm in the presence of compound **3** (λ_{max} = 563 nm) (5×10^{-6} M).

4. Conclusion

In conclusion, we have synthesized new BODIPY derivatives with 3-ethynylthiophene units at various positions of the BODIPY core and studied their photophysical properties. All these derivatives were resistant to photo-bleaching under our

experimental conditions. Styryl substituted compound **5** showed a strong far-red emission. 3-ethynylthiophene units at the 2,6 positions were less effective in increasing Stokes shifts in comparison to the 2,6 thienyl substituted BODIPY. The ability of these compounds to generate singlet oxygen was studied. The effectiveness of 3-ethynylthiophene containing BODIPY derivatives in singlet oxygen generation depends on the position around the BODIPY core. Placing 3-ethynylthiophene units on the 2,6 positions is more effective compared to the 4,4' and the meso substitutions. Styryl substitution at the 3 position has no significant effect on singlet oxygen generation. Cellular localization studies of these dyes are underway.

Acknowledgment

This work was supported by the University of Southern Indiana Endeavor Award for Research & Creativity (Project no: 17786-01000) and by the Lilly Summer Student Research Fellowship (Project no: 25075-01820).

References

[1]. Ulrich, G.; Ziessel, R.; Harriman, A. *Angew. Chem. Int. Ed.* **2008**, *47*, 1184-1201.
[2]. Loudet, A.; Burgess, K. *Chem. Rev.* **2007**, *107*, 4891-4932.
[3]. Chang, T. C.; Kuo, C. T.; Chiang, C. C.; Cheng, J. Y.; Yan, C. S.; Peck, K. *Phys. Chem*. **1999**, *1*, 3783-3787.
[4]. Giebler, K.; Griesser, H.; Göhringer, D.; Sabirov, T.; Richert, C. *Eur. J. Org. Chem.* **2010**, *19*, 3611-3620.
[5]. Goncalves, M. S. T. *Chem. Rev*. **2009**, *109*, 190-212.
[6]. Rostron, J. P.; Ulrich, G.; Retailleau, P.; Hamman, A.; Ziessel, R. *New J. Chem*. **2005**, *29*, 1241-1244.
[7]. Khatchadourian, A.; Krumova, K.; Boridy, S.; Ngo, A. T. *Biochemistry*. **2009**, *48*, 5658-5668.
[8]. Jiao, L.; Yu, C.; Uppal, T.; Liu, M.; Li, Y.; Zhou, Y.; Hao, E.; Hu, X.; Vicente, G. H. *Org. Biomol. Chem.* **2010**, *8*, 2517-2519.
[9]. Kang, H. C.; Haugland, R. P. U. S. Patent 5, 433, 896, July 18, 1995.
[10]. Rihn, S.; Retailleau, P.; Bugsaliewicz, N.; De Nicola, A.; Ziessel, R. *Tetrahedron Lett.* **2009**, *50*, 7008-7013.
[11]. Sobenina, L. N.; Vasil'tsov, A. M.; Petrova, O. V.; Petrushenko, K. B.; Ushakov, I. A.; Clavier, G.; Meallet-Renault, R.; Mikhaleva, A. I.; Trofimov, B. A. *Org. Lett.* **2011**, *13*, 2524-2527.
[12]. Poirel, A.; Nicola, A. D.; Ziesse, R. *Org. Lett.* **2012**, *14*, 5696-5699.
[13]. Hewavitharanage, P.; Nzeata, P.; Wiggins, J. *Eur. J. Chem.* **2012**, *3*, 13-16.
[14]. Goze, C.; Ulrich, G.; Ziessel, R. *J. Org. Chem.* **2007**, *72*, 313-322.
[15]. Goze, C.; Ulrich, G.; Ziessel, R. *Org. Lett*. **2006**, *8*, 4445-4448.
[16]. Harriman, A.; Mallon, L. J.; Elliot, K. J.; Haefele, A.; Ulrich, G.; Ziessel, R. *J. Am. Chem. Soc.* **2009**, *131*, 13375-13386.
[17]. Chen, Y.; Zhao, J.; Guo, H.; Xie, L. *J. Org. Chem.* **2012**, *77*, 2192-2206.
[18]. Kaneza, N.; Zhang, J.; Haiying, L.; Archana, P. S.; Shan, Z.; Vasiliu, M.; Polansky, S. H.; Dixon, D. A.; Adams, R. E.; Schmehl, R. H. *J. Phys. Chem. C*. **2016**, *120*, 9068-9080.
[19]. Mirloup, A.; Leclerc, N.; Rihn, S.; Bura, T.; Bechara, R.; Hebraud, A.; Leveque, P.; Heiser, T.; Ziessel, R. *New J. Chem.* **2014**, *38*, 3644-3653.
[20]. Shimizu, S.; Iino, T.; Saeki, A.; Seki, S.; Kobayashi, N. *Chem. Eur. J.* **2015**, *21*, 2893-2904.
[21]. Cortizo-Lacalle, D.; Howells, C. T.; Pandey, U. K.; Cameron, J.; Findlay, N. J.; Inigo, A. R.; Tuttle, T.; Skabara, P. J.; Samuel, I. D. W. *Beilstein J. Org. Chem*. **2014**, *10*, 2683-2695.
[22]. Poirel, A.; De Nicola, A.; Ziessel, R. *Org. Lett.* **2012**, *14*, 5696-5699.
[23]. Wu, Y.; Klaubert, D. H.; Kang, H. C.; Zhang, Y. Z. U. S. Patent 6 005 113, 1999.
[24]. Yogo, T.; Urano, Y.; Ishitsuka, Y.; Maniwa, F.; Nagano, T. *J. Am. Chem. Soc.* **2005**, *127*, 12162-12163.
[25]. Caishun, Z.; Jianzhang, Z.; Shuo, W. , Zilong, W.; Wanhua, W.; Jie, M.; Song, G.; Ling, H. *J. Am. Chem. Soc.* **2013**, *135*, 10566-10578.
[26]. He, H.; Lo, P. C.; Yeung, S. L.; Fong, W. P.; Ng, D. K. P. *J. Med. Chem.* **2011**, *54*, 3097-3102.
[27]. He, H.; Lo, P. C.; Yeung, S. L.; Fong, W. P.; Ng, D. K. P. *Chem. Commun.* **2011**, *47*, 4748-4750.
[28]. Wu, W; Guo, H; Wu, W.; Ji, S.; Zhao, J. *J. Org. Chem.* **2011**, *76*, 7056-7064.
[29]. Awuah, S. G.; Polreis, J.; Biradar, v.; You, Y. *Org. Lett.* **2011**, *15*, 3884-3887.
[30]. Topel, S. D.; Cin, G. T.; Akkaya, E. U. *Chem. Commun*. **2014**, *50*, 8896-8899.
[31]. Umezawa, K.; Matsui, A.; Nakamura, Y.; Citterio, D.; Suzuki, K. *Chem. Eur. J.* **2009**, *15*, 1096-1106.
[32]. Jiang, N.; Fan, J.; Liu, T.; Cao, J.; Qiao, B.; Wang, J.; Gao, P.; Peng, X. *Chem. Commun.* **2013**, *49*, 10620-10622.
[33]. Ji, S.; Ge, J.; Escudero, D.; Wang, Z.; Zhao, J.; Jacquemin, D. *J. Org. Chem.* **2015**, *80*, 5958-5963.
[34]. Vincent, M.; Beabout, E.; Bennett, R. Hewavitharanage, P. *Tetrahedron Lett.* **2013**, *54*: 2050-2054.
[35]. Niu, S.; Ulrich, G.; Retailleau, P.; Ziessel, R. *Org. Lett*. **2011**, *13*, 4996-4999.
[36]. Singh-Rachford, T. N.; Haefele, A.; Ziessel, R.; Castellano, F. N. *J. Am. Chem. Soc*. **2008**, *130*, 16164-16165.
[37]. Coskun, A.; Yilmaz, M. D.; Akkaya, E. U. *Org. Lett.* **2007**, *9*, 607-609.
[38]. Lissi, E. A.; Encinas, M. V.; Lemp, E.; Rubio, M. A. *Chem. Rev.* **1993**, *93*, 699-723.
[39]. Adarsh, N.; Avirah, R. R.; Ramaiah, D. *Org. Lett.* **2010**, 12, 5720-5723.
[40]. Kim, S.; Ohulchanskyy, T. Y.; Baev, A.; Prasad, P. N. *J. Mater. Chem.* **2009**, *19*, 3181-3188.