Electronic Supplementary Material (ESI) for Chemical Communications

Thienopyrrole-expanded BODIPY as a Potential NIR Photosensitizer for Photodynamic Therapy

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1. Experimental details

1.1 Methods

MALDI-TOF MS data was measured on a Bruker Daltonics autoflex^{II}. The ^{1}H NMR spectroscopic measurements were made by using a Bruker 500 MHz spectrometer with $CDCl_3$ or CD_2Cl_2 as the solvent. Fluorescence spectral measurements were carried out on a Hitachi F–4600 spectrofluorometer. UV-visible absorption spectra were recorded with a SHIMADZU UV–2550 spectrometer. Luminescence spectra of singlet oxygen sensitized by each photosensitizer solution were recorded using a spectrometer (Jobin Yvon SPEX fluorolog3, HORIBA, Ltd., Kyoto, Japan) equipped with a photomultiplier tube (NIR–PMT R5509−72, Hamamatsu Photonics K.K., Shizuoka, Japan) cooled to 193 K. Flow cytometry analysis was performed with a BD LSRFortessa Cell Analyzer.

1.2 Single crystals and X-Ray structure determination.

Single crystals of **I** and **II** were obtained through the slow diffusion of hexane into a dichloromethane solution. The X-ray crystallographic data for **I** and **II** were carried out at 291K on a Rigaku Saturn CCD spectrometer with graphite monochromatized MoKa radiation (λ = 0.71070 Å). The structure was solved by direct methods and refined on F^2 by full-matrix least-squares using the Crystal Clear and (SHELXS–97) programs.[1] CCDC 893620 & 909470 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; Tel: (+44) 1223–762–911; email: kamila@ccdc.cam.ac.uk).

1.3 Cell culture, MTT assay, confocal fluorescence imaging and flow cytometric analysis.

HeLa cells were maintained following protocols provided by the American Type Tissue Culture Collection. Cells were seeded at a density of 1×10^6 cells mL^{-1} for confocal imaging in RPMI 1640 Medium supplemented with 10% fetal bovine serum (FBS), $NaHCO_3$ (2 g/L), and 1% antibiotics (penicillin/streptomycin, 100 U/ml). Cultures were maintained at 37 °C under a humidified atmosphere containing 5% CO_2.

The cytotoxicity of **I** in cells was investigated both in the presence and absence of light using the MTT assay.[2] HeLa cells (1×10^6 cells/well) were dispersed within replicate 96-well microtiter plates to a total volume of 200 μL $well^{-1}$. Plates were maintained at 37 °C in a 5% CO_2/95% air incubator for 24 h. **I** was diluted to different concentrations of solution with medium and added to each well after the original medium had been removed. Cells were incubated with different concentrations for 36 h. The concentrations of **I** were 0, 1, 2.5, 5, 10, 25, 50 μM and then 100 μL MTT solution (0.5 mg mL^{-1} in PBS) were added to each well. After 4 h, the remaining MTT solution was removed, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. The absorbance was measured at 490 nm in a TRITURUS microplate reader. Cytotoxic studies revealed that **I** is essentially non-cytotoxic in the absence of light but exhibits high photocytotoxicity. The IC_{50} value was calculated to be 7.12 μM.

Confocal fluorescence imaging studies were performed on an LSM 710 confocal laser-scanning microscope (Carl Zeiss Co., Ltd.). Prior to imaging, the medium was removed. Cell imaging was carried out after washing cells with PBS for three times.

To study the cell death induced by **I** upon PDT treatment on Hela cells, a flow cytometric assay of annexin V–FITC and propidium iodide (PI) co-staining was employed.[3] The cells were harvested, rinsed in PBS, resuspended, and determined by flow cytometry.

1.4 Quantification of singlet oxygen generation

The following equation was used to calculate the singlet oxygen quantum yield of the sensitizer with respect to the reference.[4]

$$\phi_\Delta(^1O_2)^{BDP} = \phi_\Delta(^1O_2)^{MB}\frac{S^{BDP}F^{MB}}{S^{MB}F^{BDP}} \qquad \text{Equation S1}$$

where $\Phi_\Delta(^1O_2)$ is the quantum yield of singlet oxygen, superscripts " BODIPY " and " MB " represent **I** and methylene blue, respectively. "*S*" is the slope of a plot of difference in change in absorbance of DPBF (at 411 nm) with the irradiation time, and "F" is the absorption correction factor, which is given by $F = 1 - 10^{-OD}$ (OD at the irradiation wavelength).

1.5 Synthesis section

All reagents were obtained from commercial suppliers and used without further purification unless otherwise indicated. Air and moisture-sensitive reactions were carried out under an argon atmosphere.

Procedure for the preparation of thiophene-fused pyrrole:

(Z)-ethyl 3-(2, 2'-bithiophene-5-yl)-2-azidoacrylate 2: A 250 ml flask was charged with sodium ethylate (2.72 g, 40.0 mmol, 2 eq) and 80 ml of ethanol. A mixture of 2,2'-bithiophene-5-carbaldehyde (3.88 g, 20.0 mmol, 1 eq) and ethyl 2-azidoacetate (5.16 g, 40.0 mmol, 2 eq) in about 10 ml ethanol was then added dropwise over 40 mins at 0 °C under Ar, and the mixture was stirred for 3 h at room temperature. The reaction mixture was quenched with saturated NH_4Cl solution and extracted with diethyl ether, and the organic layer was washed with water, brine and dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure, the residue was purified by column chromatography using silica gel and petroleum ether / ethyl acetate (30/1; v/v) as the eluent to give yellow solids (2.17 g, yield: 35%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (m, *J* = 3.8, 3.1 Hz, 2H), 7.20 (d, *J* = 3.8 Hz, 1H), 7.13 (d, *J* = 3.9 Hz, 1H), 7.10 (s, 1H), 7.07 – 7.03 (m, 1H), 4.36 (q, *J* = 7.1 Hz, 2H), 1.39 (t, *J* = 7.1 Hz, 3H).

Ethyl 2-(thiophene-2-yl)-4H-thieno[3,2-b]pyrrole carboxylate 3: A solution of **2** (1.55 g, 5.1 mmol) in 60 ml of dry toluene was added dropwise into a 250 ml flask charged with 60 ml of dry toluene over an hour. The mixture was then heated to reflux for 1.5 h. The solvent was removed under reduced pressure, the residue was purified by column chromatography using silica gel and petroleum ether / ethyl acetate (4/1; v/v) as the eluent to obtain a brown solid (1.27 g, yield: 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.15 (s, 1H, NH), 7.26 – 7.4 (m, 1H), 7.21 (d, *J* = 2.9 Hz, 1H), 7.09 (s, 1H), 7.04 -7.02 (m, 2H), 4.38 (q, *J* = 7.1 Hz, 2H), 1.39 (t, *J* = 7.1 Hz, 3H).

2-(thiophen-2-yl)-4H-thieno[3,2-b]pyrrole 4: Compound **3** (883 mg, 3.19 mmol, 1 eq) and KOH (717 mg, 12.76 mmol, 4 eq) were dissolved in ethylene glycol and the solution was refluxed under Ar for 2h. The reactant was cooled to about 0°C. The reaction mixture was then quenched with water, extracted with diethyl ether, and dried with anhydrous $MgSO_4$. The solvent was removed under reduced pressure, the residue was purified by column chromatography using silica gel and chloroform as the eluent to obtain a yellow solid (523 mg, yield: 80%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (s, 1H, NH), 7.18 – 7.15 (m, 2H), 7.07 (s, 1H), 7.01 – 6.98 (m, 2H), 6.45 – 6.43 (m, 1H).

The preparation of BODIPY dyes:

BODIPY **II:** Methyl 4-formylbenzoate (82 mg, 0.50 mmol, 1 eq) was added to a solution of **4** (205 mg, 1.00 mmol, 2 eq) in dry dichloromethane (50 ml) in the dark under Ar. Then one drop of trifluoroacetic acid (TFA) was added into the solution under Ar. The mixture was stirred at room temperature for 12 hours. DDQ (118 mg, 0.5 mmol, 1 eq) was added into the mixture, which was stirred for another hour. Triethylamine (TEA) (about 8 ml) and $BF_3{\cdot}Et_2O$ (about 8 ml) were then added, and the solution was stirred for 3 hours at room temperature. The reaction mixture was quenched with water, and filtered. The filtrate was extracted with chloroform and washed with water, and brine. The organic layer was dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure, the residue was chromatographed on silica gel (petroleum ether / ethyl acetate (95/5; v/v)) to give II as a solid with a metallic luster (286 mg, yield: 95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (d, *J* = 8.1 Hz, 2H), 7.68 (d, *J* = 8.1 Hz, 2H), 7.44 - 7.40 (m, 4H), 7.34 (s, 2H), 7.12 – 7.08 (m, 2H), 6.75 (s, 2H), 4.00 (s, 3H); MALDI-MS m/z: calcd for $C_{29}H_{17}BF_2N_2O_2S_4$: 602.526, found: 600.832 $[M–H]^+$, 581.806 $[M–F]^+$. ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.30, 159.15, 151.99, 140.91, 139.82, 138.43, 137.72, 132.52, 131.64, 130.80, 129.56, 128.44, 128.23, 126.74, 118.96, 108.98, 52.42.

BODIPY **I:** NBS (118.2 mg, 0.664 mmol, 4 eq) was added to a solution of **II** (100 mg, 0.166 mmol, 1 eq) in THF (40 ml). The mixture was then stirred at room temperature overnight. The reaction was quenched with sodium thiosulfate solution, extracted with chloroform, and washed with water. The organic layer was dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The residue was then recrystallized from $CHCl_3$/hexane (113 mg, yield: 81%). ^{1}H NMR (500 MHz, CD_2Cl_2) δ 8.18 (d, *J* = 7.8, 2H), 7.58 (d, *J* = 7.8, 2H), 7.26 (s, 1H), 7.21–7.23 (m,

3H), 7.12 (s, 2H), 6.61 (s, 1H), 3.97 (s, 3H) MALDI-TOF-MS m/z: calcd for $C_{29}H_{14}BBr_3F_2N_2O_2S_4$: 839.213, found: 838.219 $[M–H]^+$,819.165 $[M–HF]^+$. ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.49, 159.71, 156.21, 151.84, 149.68, 142.71, 139.69, 138.91, 138.70, 136.68, 135.20, 134.58, 132.77, 131.53, 131.50, 131.43, 130.44, 129.52, 127.19, 127.07, 120.06, 116.36, 116.16, 109.41, 108.91, 108.08, 52.47.

2 Supplementary data

2.1

Table S1. Crystal data and structure refinement of compounds **II** and **I**.

	II	**I**
Empirical formula	$C_{59}H_{36}B_2Cl_2F_4N_4O_4S_8$	$C_{30}H_{16}BBr_3Cl_2F_2N_2O_2S_4$
Fw	1289.92	924.13
Crystal size (mm)	0.28 x 0.24 x 0.22 mm	0.28 x 0.24 x 0.22 mm
Crystal system	monoclinic	Triclinic
Space group	C2/c	P–1
Z	4	2
a/Å	33.942(11)	12.003(17)
b/ Å	10.309(3)	12.516(18)
c/ Å	17.344(5)	13.657(11)
a (deg)	90	75.653(2)
ß (deg)	109.456(5)	79.106(3)
γ (deg)	90	63.710(2)
V / $Å^3$	5722(3)	1774.7(4)
r (calc) (Mg/cm^3)	1.497	1.729
Absorption coefficient (mm^{-1})	0.471	3.838
T (K)	291(2)	291(2)
F(000)	2632	904
Max. and min. transmission	0.9034 and 0.8794	0.4856 and 0.4129
T range for data collection (°)	1.27 – 26.00	1.55 – 26.00
Measured reflections (R(int))	16586 / 5622 [R(int) = 0.0343]	14788 / 6965 [R(int) = 0.0657]
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Parameters refined	5622 / 0 / 376	6965 / 0 / 445
R indices (all data)	R1 = 0.0655, wR2 = 0.0981	R1 = 0.0682, wR2 = 0.1575

Goodness-of-fit on F^2	1.034	1.048
Largest diff. peak and hole/(eÅ^{-3})	0.190 and –0.201	0.495 and –0.458

Table S2. Spectroscopic data of **I** and **II** in different solvents at 298 K.

Dyes	solvent	λ_{abs} [nm]	ε [$M^{-1}cm^{-1}$]/10^5	λ_{em} [nm]	Φ_f [a]
I	THF	688	1.94	707	0.03
	CH_2Cl_2	698	2.30	724	0.04
II	MeCN	673	1.91	708	0.19
	THF	679	2.00	712	0.20
	CH_2Cl_2	684	2.03	716	0.31
	Toluene	687	2.26	718	0.23
	hexane	675	2.09	699	0.39

[a] Methylene blue (MB) in ethanol is used as a reference for these compounds, Φ_f = 0.04.[5]

2.2

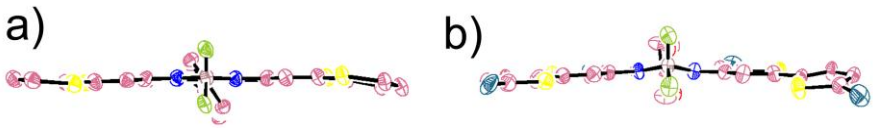

Figure S1. The side ORTEP views of the crystal structures of (a) **II** and (b) **I.**

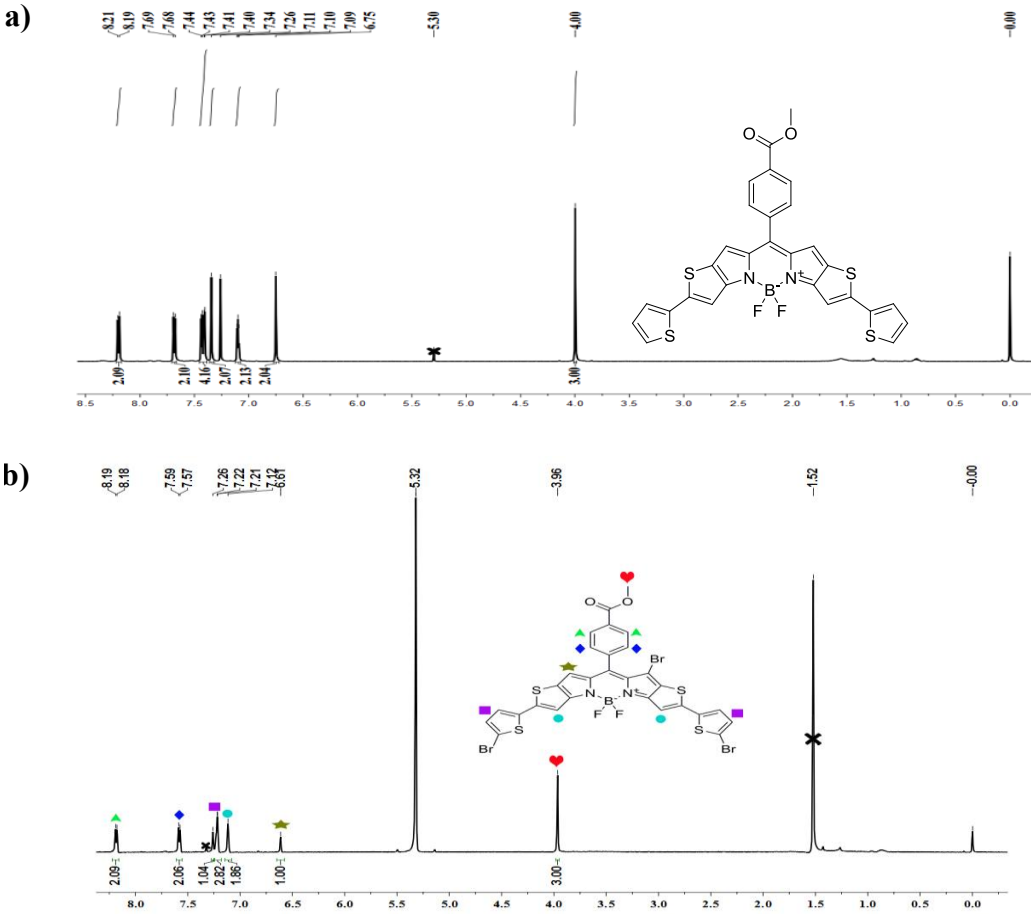

Figure S2. ^{1}H NMR spectra of (a) **II** (in $CDCl_3$, 298 K), (b) **I** (in CD_2Cl_2, 298 K).

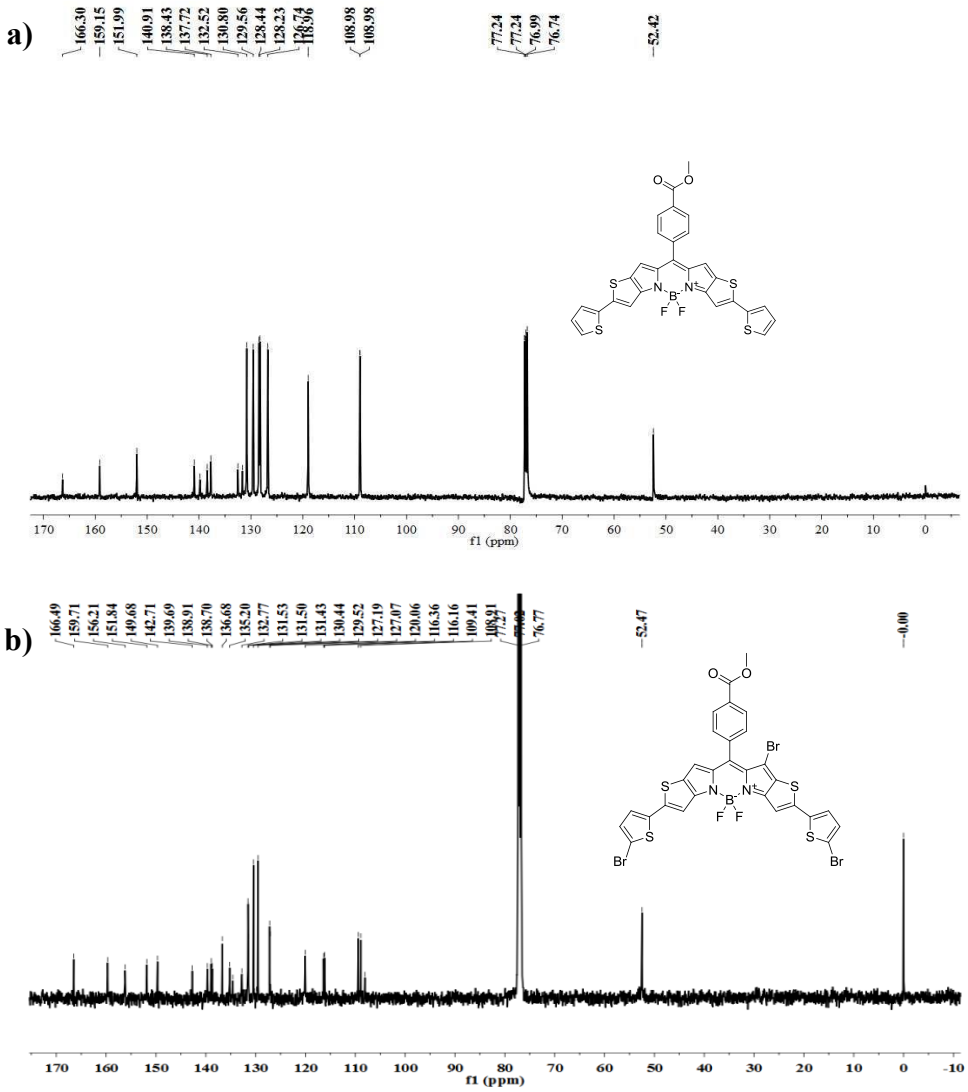

Figure S3. ^{13}C NMR spectra of (a) **II** (in $CDCl_3$, 298 K), (b) **I** (in $CDCl_3$, 298 K).

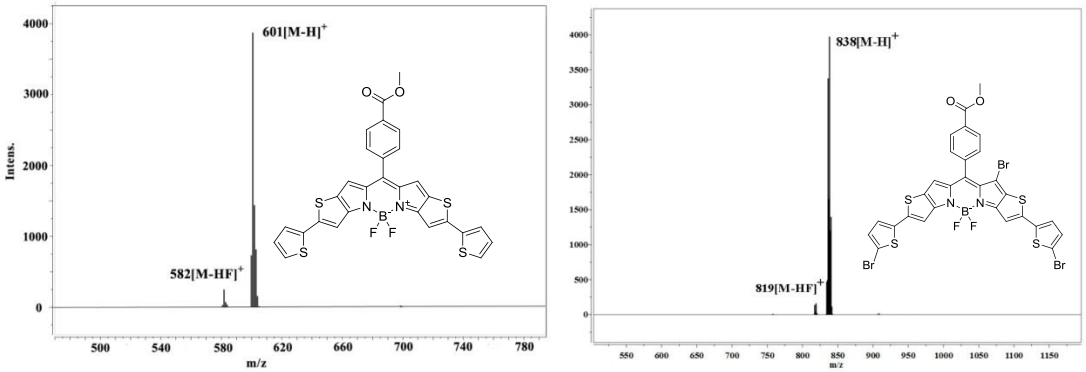

Figure S4. MALDI-TOF MASS spectra of **I** and **II**.

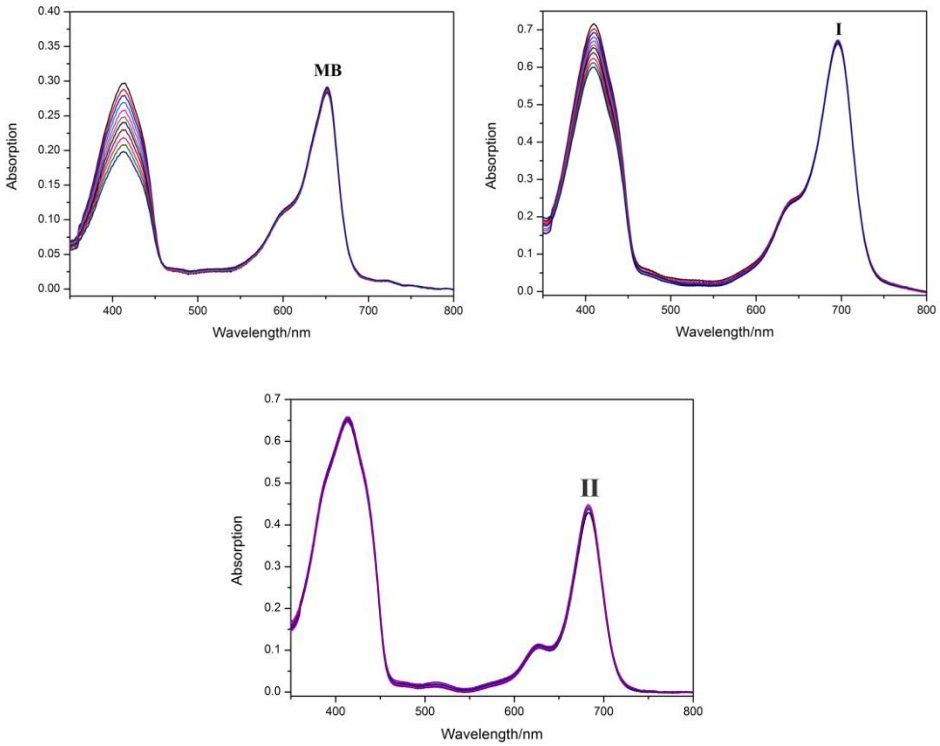

Figure S5. Changes in the absorption spectra of DPBF upon irradiation (λ_{irr} = 635nm) with 10s interval in the presence of **I** and **II**. The absorbance of **I**, **II** and the methylene blue (MB) standard at the irradiation wavelength was adjusted to 0.1 – 0.3.

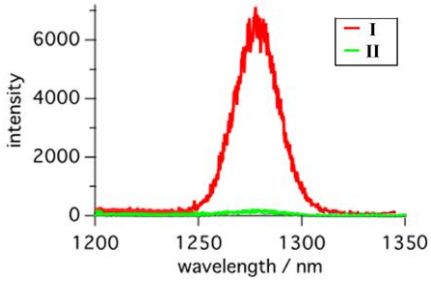

Figure S6. The $^1O_2$ phosphorescence spectra of **I** (red), **II** (green) in $CDCl_3$ (λ_{irr} = 635 nm).

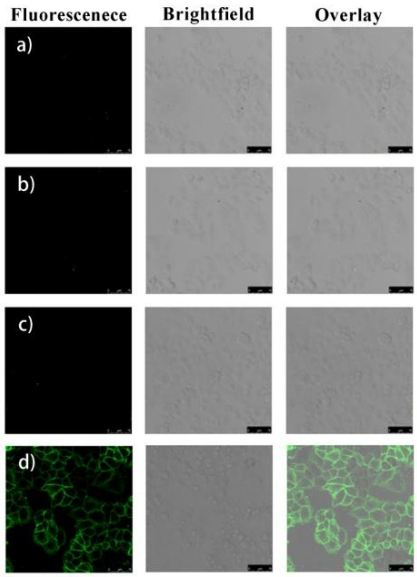

Figure S7. Confocal fluorescence images in HeLa cells (λ_{ex} = 488 nm, λ_{em} = 510–530 nm). (a) cells were untreated (control); (b) untreated cells irradiated by a 635 nm laser beam with a power density of 400 mW/cm^2 for 100 s; (c) **I** (10 μM) treated cells in the dark; (d) **I** (10 μM) treated cells irradiated by a 635 nm laser beam with a power density of 400 mW/cm^2 for 100 s.

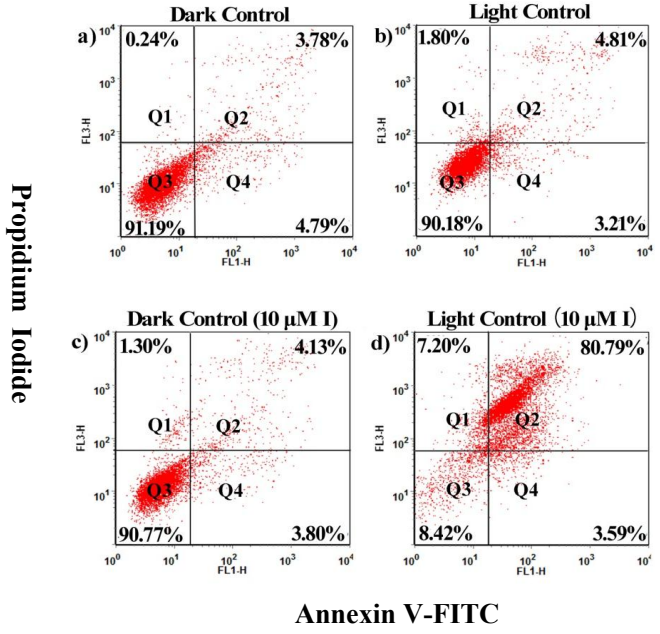

Figure S8. A flow cytometric analysis of the cell death induced by **I** upon PDT treatment of Hela cells. The lower left quadrant (Q3) of each panel shows the viable cells. The higher right quadrants (Q2) represent the apoptotic cells. (a) untreated cells in the dark; (b) untreated cells irradiated by a 635 nm laser beam with a power density of 400 mW/cm^2 for 100 s; (c) **I** (10 μM) treated cells in the dark; (d) **I** (10 μM) treated cells irradiated by a 635 nm laser beam with a power density of 400 mW/cm^2 for 100 s.

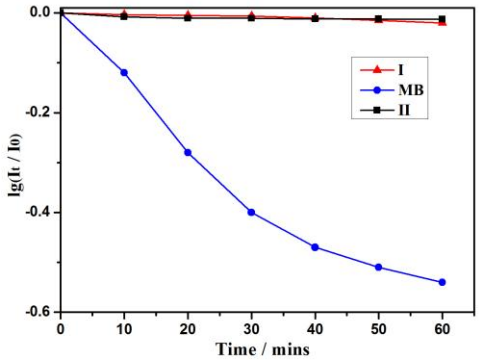

Figure S9. Photostability of MB (blue) and BODIPY-**I** (red), **II** (black) in CH_2Cl_2 using a laser beam (635 nm, 400 mW/cm^2) over a period of 60 min.

References:

(1) Gaussian 03, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, Jr., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts , R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A.; Gaussian, Inc., Wallingford CT, **2004**.

(2) (a) Achilefu, S.; Bloch, S.; Markiewicz, M. A.; Zhong, T.; Ye, Y.; Dorshow, R. B.; Chance, B.; Liang, K. *PNAS*, **2005**, 102 7976. (b) Lovell, J. F.; Chan, M. W.; Qi, Q.; Chen, J.; Zheng, G. *J. Am. Chem. Soc.*, **2011**, 133, 18580.

(3) Vermes, I.; Haanen, C.; Reutelingsperger, C. *J. Immunol, Methods*. **2000**, 243, 167.

(4) (a) M. Mirenda,; Strassert, C. A.; Dicelio, L. E.; San Roman, E. *ACS Appl. Mater. Interfaces* **2010**, 2, 1556. (b) Thomas, A. P.; Saneesh Babu, P. S.; Nair, S. A.; Ramakrishnan, S.; Ramaiah, D.; Chandrashekar, T. K.; Srinivasan, A.; Pillai, M. R. *J. Med. Chem.* **2012**, 55, 5110. (c) Adarsh, N.; Avirah, R. R.; Ramaiah, D. *Org. Lett.* **2010**, 12, 5720.

(5) Olmsted III, J. *J. Phys. Chem.* **1979**, 83, 2581.