Supporting Information

BODIPY-vinyl dibromides as highly efficient triplet sensitizers for singlet oxygen generation, photodynamic therapy and Triplet triplet annihilation up conversion

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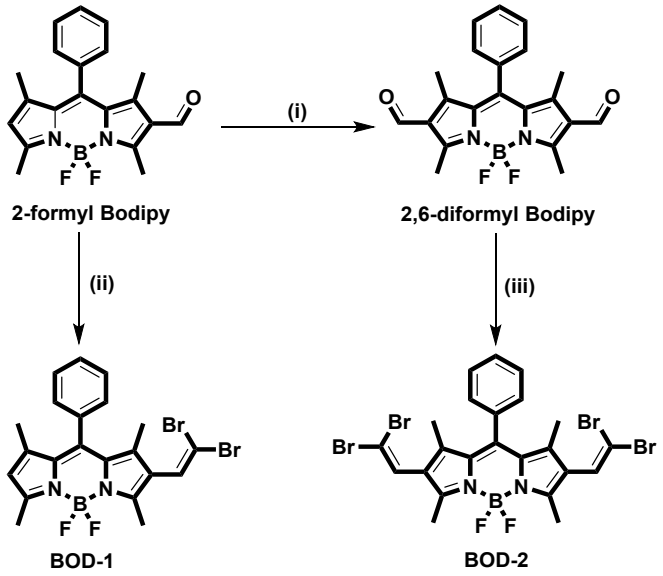
General Methods

All reagents were purchased from commercial suppliers (Aldrich and Merck) and used without any purification. ^{1}H NMR and ^{13}C NMR were measured on a Varian VNMRJ 400 Nuclear Magnetic Resonance Spectrometer. Mass spectrometry analysis was measured on Thermo Fisher Scientific Q-Exactive HRMS. UV absorption spectra and Fluorescence emission spectra were obtained using HORIBA Duetta, the two-in-one Fluorescence and absorbance spectrometer using 10.0 mm path length quartz cuvettes (2.0 mL volume). In singlet oxygen measurements 1,3-Diphenylisobenzofuran was used as a singlet oxygen scavenger and was purchased from Sigma-Aldrich. Fluorescence lifetimes were measured by a time-correlated single photon counting (TCSPC) system using a Edinburgh FLS920 spectrophotometer with excitation wavelength at 480 nm for **BOD-1** and 500 nm for **BOD-2** (pulse width: < 200 ps). The lifetime values were computed by the F900 software. The nanosecond transient absorption spectra and the relative decay curves were measured on LP980 laser flash photolysis spectrometer (Edinburgh Instruments Ltd., UK) with a nanosecond pulsed laser (OpoletteTM 355II+UV nanosecond pulsed laser. OPOTEK, USA), and the signal was digitized on a Tektronix TDS 3012B oscilloscope. The triplet state lifetime values (by monitoring the decay trace of the transients) were obtained with the L900 software. All samples in flash photolysis experiments were deaerated with N_2 for ca. 15 min before measurement. The upconverted fluorescence spectra were recorded with RF-5301PC spectrofluorometer (Shimadzu Ltd., Japan). For fluorescence imaging were recorded with Zeiss Axio Observer.

Synthesis of BOD-1 and BOD-2

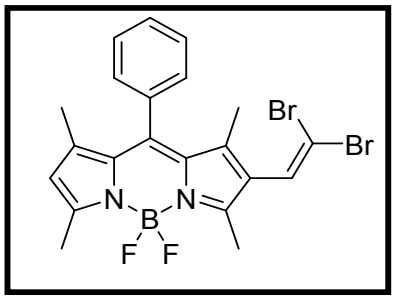
The synthesis pathway for **BOD-1** and **BOD-2** were shown in Scheme 1.

2-formyl Bodipy[1], **2,6-diformyl Bodipy**[2], **BOD-1**[3] and **BOD-2**[3] were synthesized by using literature procedure.



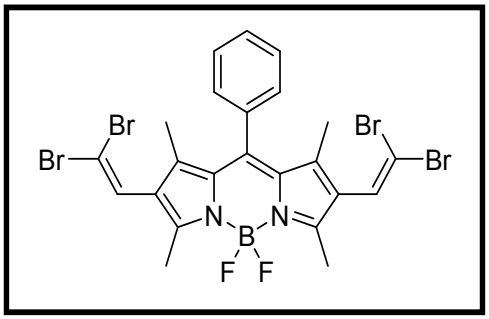
Scheme 1. Synthesis pathway of **BOD-1** and **BOD-2** (i) $POCl_3$, DMF in 1,2-DCE at 60°C. (ii) CBr_4, PPh_3 in DCM at 0°C. (iii) CBr_4, PPh_3 in DCM at 0°C.

Synthesis of BOD-1



In the first part of the reaction, to a carbontetrabromide (0.34 mmol, 2 equiv.) in 0.35 ml dry DCM, **2-formyl BODIPY** (0.17 mmol, 1 equiv.) in 0.35 ml DCM was added dropwise at 0°C very slowly. Then, triphenylphosphine (0.68 mmol, 4 equiv.) was dissolved in 0.35 ml dry DCM and added to reaction medium at 0°C. After addition, cooling bath was removed and the reaction was stirred at room temperature for 3 hours. Color of the reaction turned from dark orange to dark purple. After completion of the reaction, extraction was done with dichloromethane (3x 30 ml) and dried over $MgSO_4$. Solvent was evaporated in and the resultant compound purified by column chromatography (30 mg, 35% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.49 (s, 3H), 7.28 (s, 2H), 7.11 (s, 1H), 6.01 (s, 1H), 2.56 (s, 3H), 2.52 (s, 3H), 1.37 (s, 3H), 1.29 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 157.2, 157.0, 152.2, 144.4, 142.1, 139.1, 134.8, 130.7, 129.2, 127.8, 122.0, 94.5, 14.7, 14.5, 13.8, 13,2. Calcd. for $C_{21}H_{19}BBr_2F_2N_2$: 530.98553 $[M+Na]^+$, Found: 530.98779 $[M+Na]^+$.

Synthesis of BOD-2



In the first part of the reaction, to a carbon tetrabromide (0.496 mmol, 4 equiv.) in 0.35 ml dry DCM, **2,6-diformyl BODIPY** (0.124 mmol, 1 equiv.) in 0.35 ml DCM was added dropwise at 0°C very slowly. Then, triphenylphosphine (0.992 mmol, 8 equiv.) was dissolved in 0.35 ml dry DCM and added to reaction medium at 0°C. After addition, cooling bath was removed and the reaction was stirred at room temperature for 3 hours. After the completion of reaction, extraction was done with dichloromethane (3x 30 ml) and dried over $MgSO_4$. Solvent was evaporated in and the resultant compound purified by column chromatography (30 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.54 – 7.50 (m, 3H), 7.32 – 7.27 (m, 2H), 7.11 (s, 2H), 2.53 (s, 6H), 1.29 (s, 6H).^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 154.0, 142.6, 140.4, 134.6, 131.3, 130.3, 129.4, 129.3, 128.5, 127.8, 95.0, 13.9, 13.4. Calcd. for $C_{23}H_{19}BBr_4F_2N_2$: 714,81996 $[M+Na]^+$, Found: 714.82324 $[M+Na]^+$.

Determination of Quantum Yields

Fluorescence quantum yields of **BOD-1** and **BOD-2** were determined by using optically matching solutions of Rhodamine 6G (Φ_F=0.95 in ethanol) as a standard.[4] The quantum yield was calculated according to the equation;

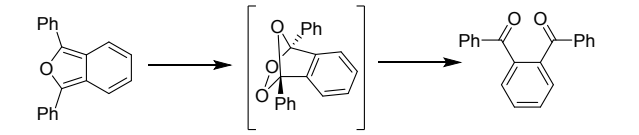
$$\Phi_{F(X)} = \Phi_{F(S)} (A_S F_X / A_X F_S) (n_X / n_S)^2$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and to the unknown, respectively.

Singlet Oxygen Trap Experiments

For singlet oxygen measurements indirect method was performed by using 1,3-diphenylisobenzofuran (**DPBF**) which is a singlet oxygen scavenger in DMSO. It was purchased from a Sigma-Aldrich. In a typical procedure for the detection of singlet oxygen generation by using trap molecules, **BOD-1** (5 μM) and **DPBF** (25 μM) were mixed in O_2 bubbled CH_2Cl_2. Initially several dark measurements were taken followed by irradiation of the mixture at absorption maximum of a sensitizer. Absorbance decay of **DPBF** was monitored suggesting singlet oxygen generation in the presence of light and **BOD-1**. Same procedure was

performed for **BOD-2**. Measurements were performed using 525 nm LED (3.3 mW/cm^2) and samples were irradiated with the light source from a 15 cm distance.



1,3-Diphenylisobenzofuran (DPBF)
λ_{abs}= 414 nm

1,2-dibenzoylbenzene

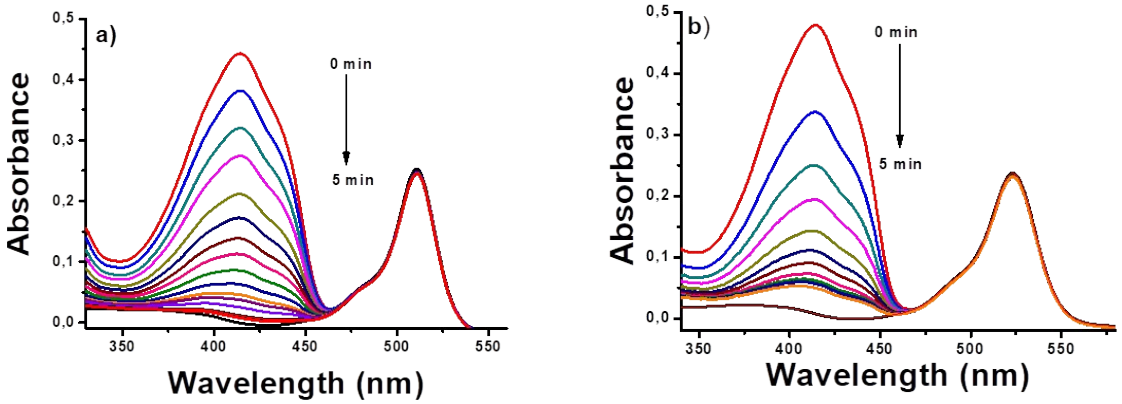


Figure S1. Singlet oxygen mediated bleaching of DPBF (25 µM) in the presence of a) **BOD-1** (5 µM) b) **BOD-2** (5 µM) in CH_2Cl_2.

Singlet Oxygen Quantum Yield Calculations

Relative singlet oxygen quantum yields were calculated by using **2I-BOD** as a reference whose singlet oxygen quantum yield is 0.79 in CH_2Cl_2.[5] Singlet oxygen trap molecule (**DPBF**) and photosensitizers (**BOD-1 and BOD-2**) were placed into cuvette containing oxygen saturated CH_2Cl_2. Solution was kept in dark and mixed by micropipette until absorbance readings were stable. After stabilization, absorbance readings were recorded for 5 minutes by exposing cuvette to light (525 nm) for 60 seconds (from 15 cm distance). Absorbance readings of **DPBF** at its absorbance maxima were recorded against time and recorded graph is given below.

Singlet oxygen quantum yields were calculated according to the equation:

$$\phi\Delta\ (PS) = \phi\Delta(R) \times m(PS)/\ m(R) \times F(R)/\ F(PS) \times PF(R)/\ PF(PS)$$

where PS and R designate photosensitizers and reference dye (**2I-BOD**) respectively. m is the slope of difference in change in absorbance of DPBF at absorbance maxima with the irradiation time. F is the absorption correction factor, which is given as F=1-10-OD, and PF is absorbed photonic flux.

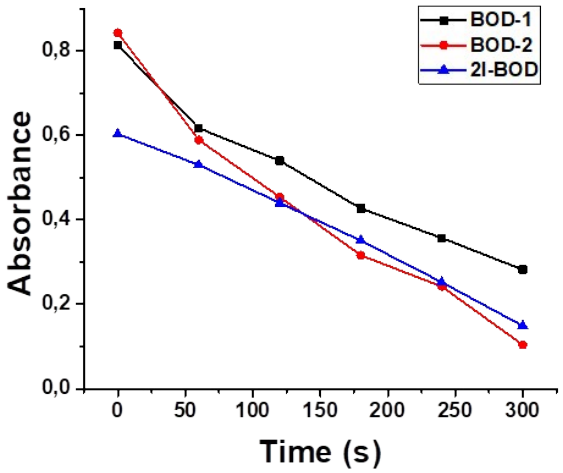


Figure S2. Relative singlet oxygen quantum yield experiment. Absorbance decreases of **DPBF** (25 μM) at absorbance maxima with time in CH_2Cl_2 in the presence of compound **BOD-1** (5 μM) and **BOD-2** (5 μM) as photosensitizers and **2I-BOD** (5 μM) as reference.

TTA Upconversion Calculations

A continuous wave (cw) 510 nm laser was used to excite the sample solutions. The upconverted fluorescence spectra were recorded with RF-5301PC spectrofluorometer (Shimadzu Ltd., Japan). In order to repress the laser scattering, a beam dump was put behind the cuvette. All the samples were deaerated with N_2 for ca. 15 min and kept in N_2 atmosphere during the measurement.

The upconversion quantum yields (Φ_{UC}) were determined with 2,6-diiodo-1,3,5,7-tetramethyl-8-phenyl-BODIPY (**2I-BOD**) as the external standard (Φ_F = 2.7% in acetonitrile). The values were calculated with the equation, where Φ, A, I and η represent the quantum yield, absorbance, integrated photoluminescence intensity and the refractive index of the solvents, respectively. Symbols with 'std' and 'sam' stand the corresponding parameter for the standard and sample.

$$\Phi_{UC} = 2\Phi_{std}\left(\frac{1-10^{-A_{std}}}{1-10^{-A_{sam}}}\right)\left(\frac{I_{sam}}{I_{std}}\right)\left(\frac{\eta_{sam}}{\eta_{std}}\right)^2$$

Cell Studies

Cell Viability

A549 Human Lung Adeno carcinoma cells were seeded in 96-well plates at a density of 1×10^4 cells per well with 100 μL culture and incubated for 24 hours at 37 °C under 5% CO_2. After the overnight incubation, cell medium was removed and replaced with complete medium supplemented with varying concentration of **BOD-1** and **BOD-2**. The experimental group of the cells were illuminated with a yellow light source (525 nm, distance between light source and cells: 15 cm) for 60 min. Then cells were replaced new media and incubated another 24 hours. At the end of incubation periods, medium of the cells were removed and cells were washed by pre-warmed phosphate buffered saline (PBS) to remove any trace of compounds and to prevent color interference during optical density (OD) determination. MTT solution (0.5 mg/mL in PBS) was added into each well and incubated for 4 hours. After the incubation time plates were centrifuged at 1800 rpm for 10 minutes at room temperatures to avoid accidental removal of formazan crystals. Crystals were dissolved with 100 μL DMSO. The absorbance was determined at 540 nm.

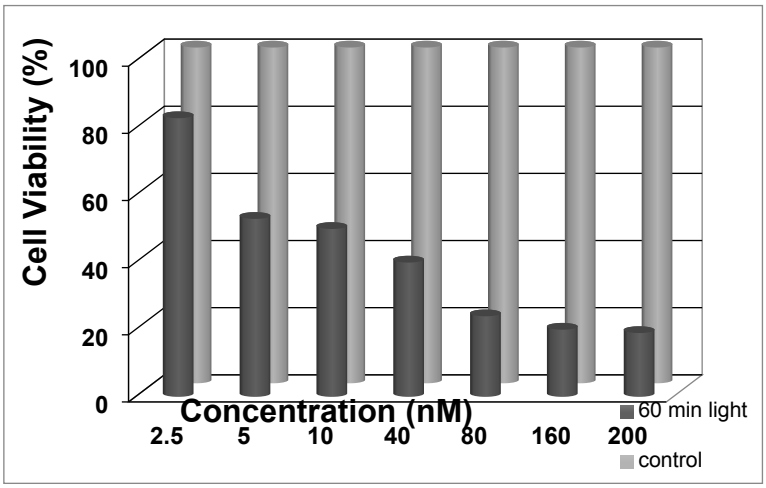


Figure S3. Cell viability of A549 cells after treatment with **BOD-1** at different concentrations. Control group was incubated only with the cell culture medium.

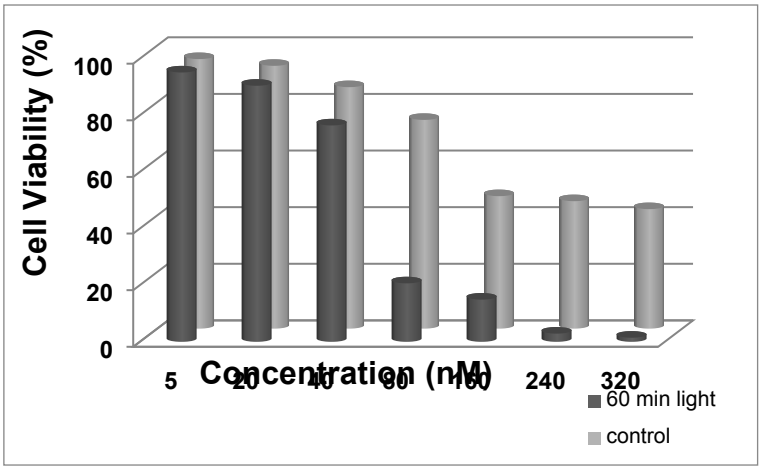


Figure S4. Cell viability of A549 cells after treatment with **BOD-2** at different concentrations. Control group was incubated only with the cell culture medium.

Cell Imaging

A549 Human Lung Adenocarcinoma cell lines were grown in DMEM supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 at 37°C. The cells were plated on 12 mm cover glasses in 6-well plate and allowed to grow for 24h. Before the experiments, the cells were washed with PBS buffer, and then the cells were incubated with **BOD-1** (10 μM) for 30 min at 37°C. Then cells were rinsed with PBS three times and DAPI for 10 min at 37°C. After that cells were washed with PBS three times. Then, the fluorescence images were acquired through a Zeiss Axio fluorescence microscope.

Determination of Photostability of BOD-1 and BOD-2

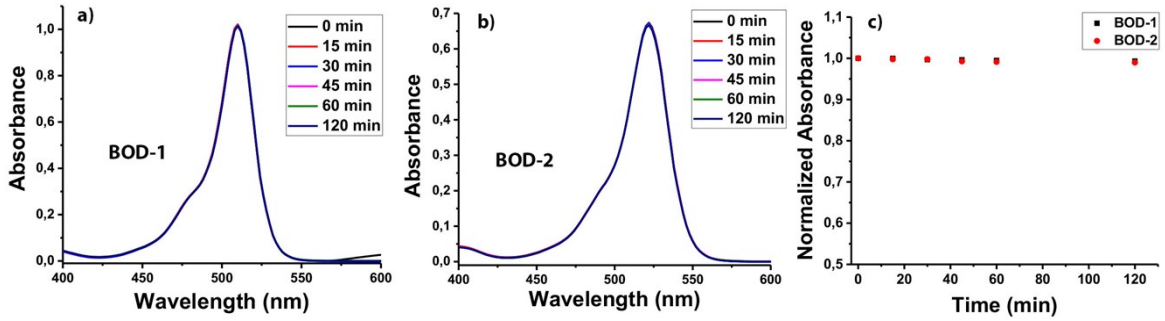
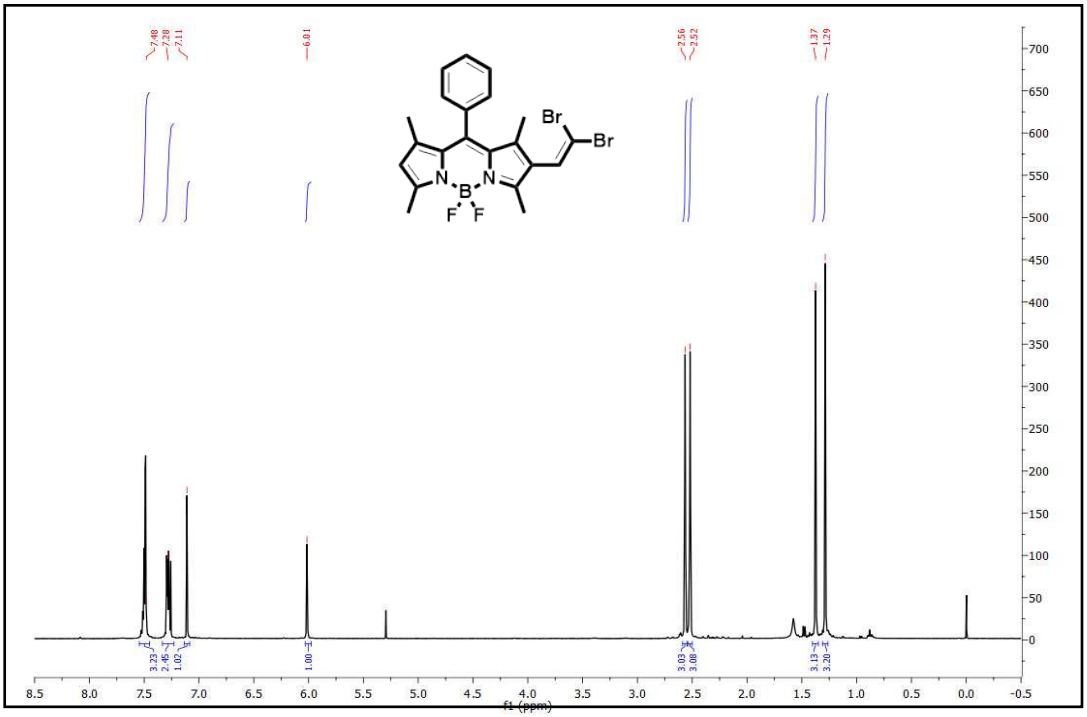


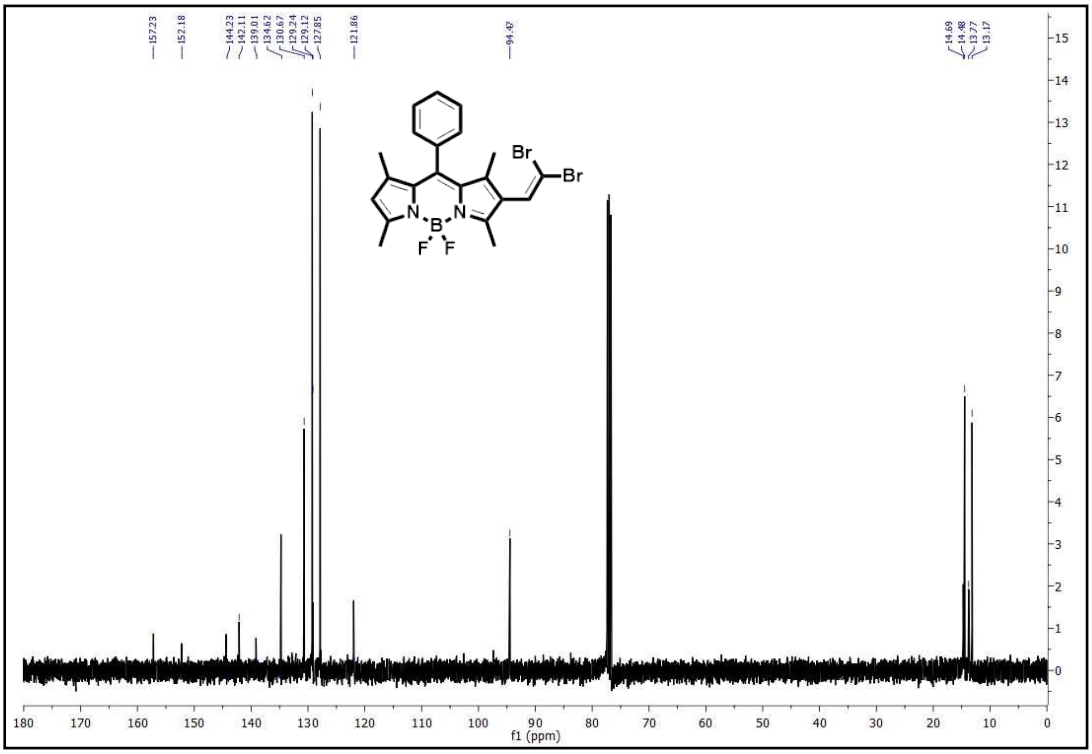
Figure S5. Absorbance spectra of a) BOD-1 and b) BOD-2 during illumination at 530 nm 10mW/cm^2 for 120 min. c) normalized absorbance change of BOD-1 and BOD-2

NMR Spectra

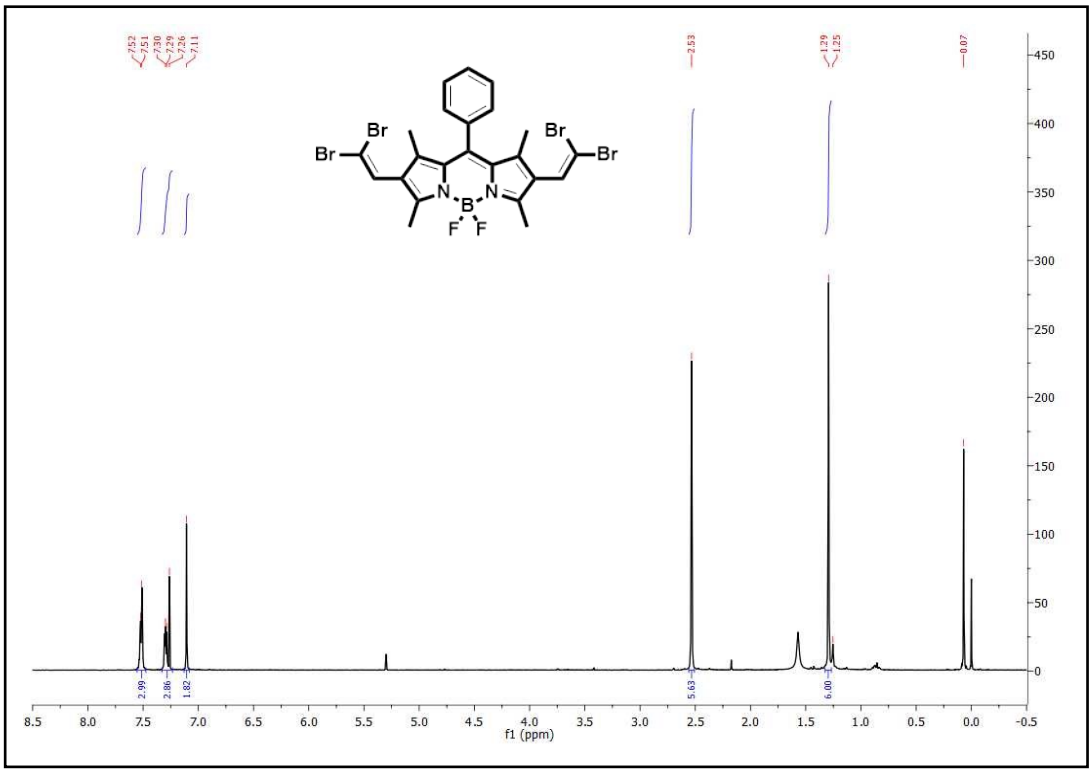
^{1}H-NMR of **BOD-1**



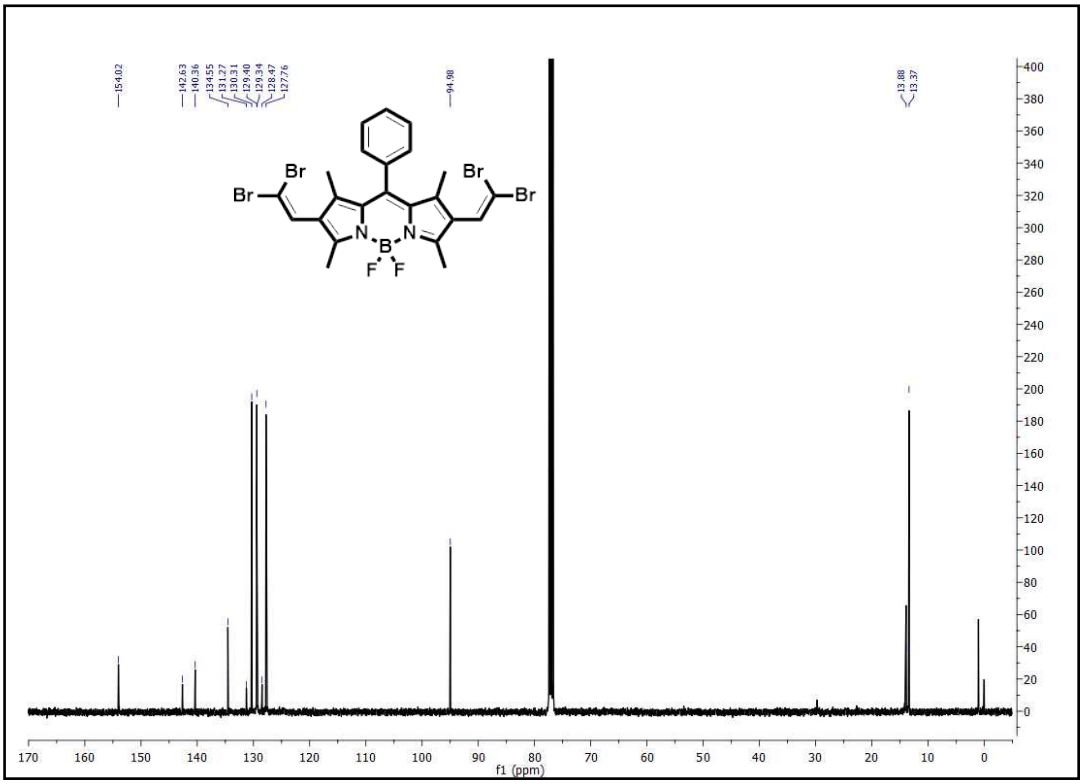
^{13}C-NMR of **BOD-1**



^{1}H-NMR of **BOD-2**

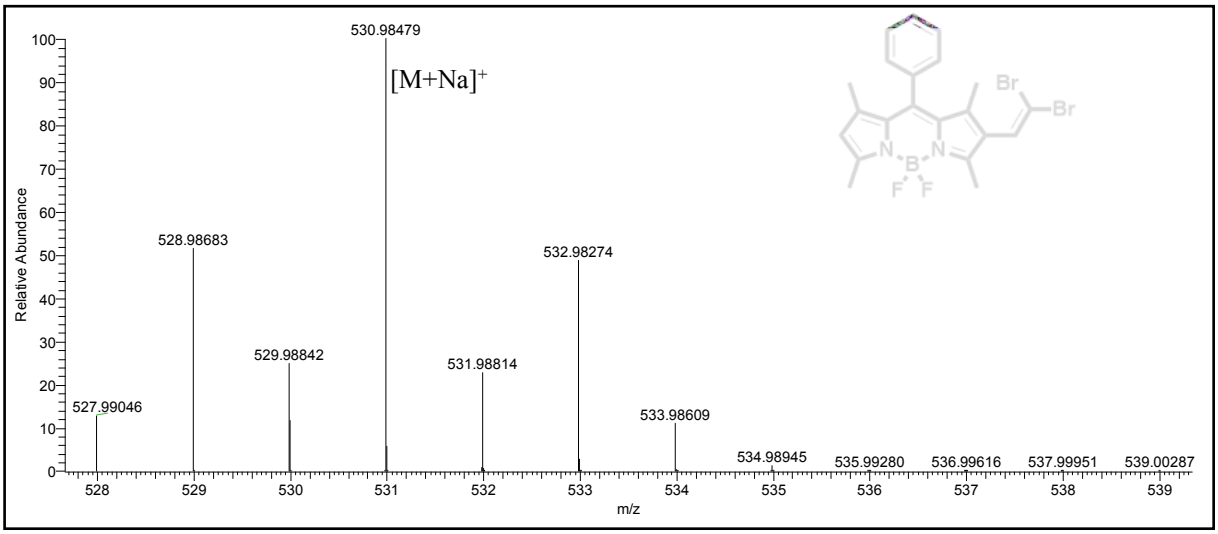


^{13}C-NMR of **BOD-2**



HRMS Spectrum of **BOD-1 and BOD-2**

Theoretical BOD-1:



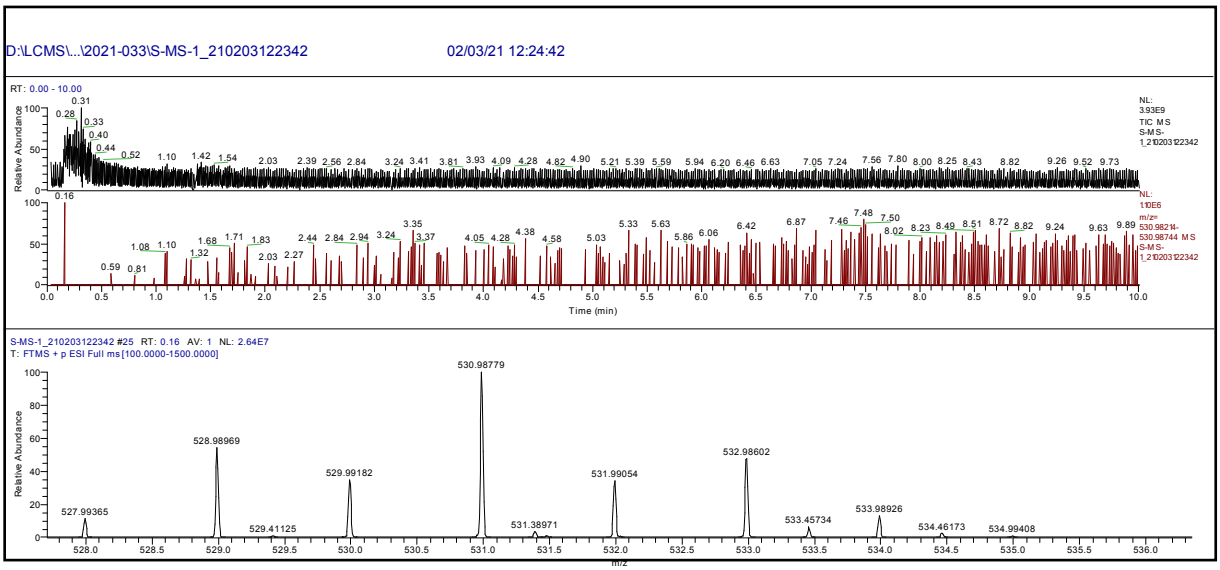
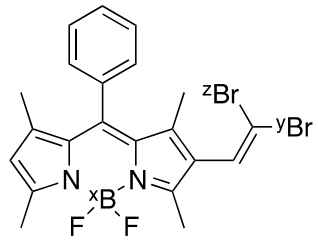
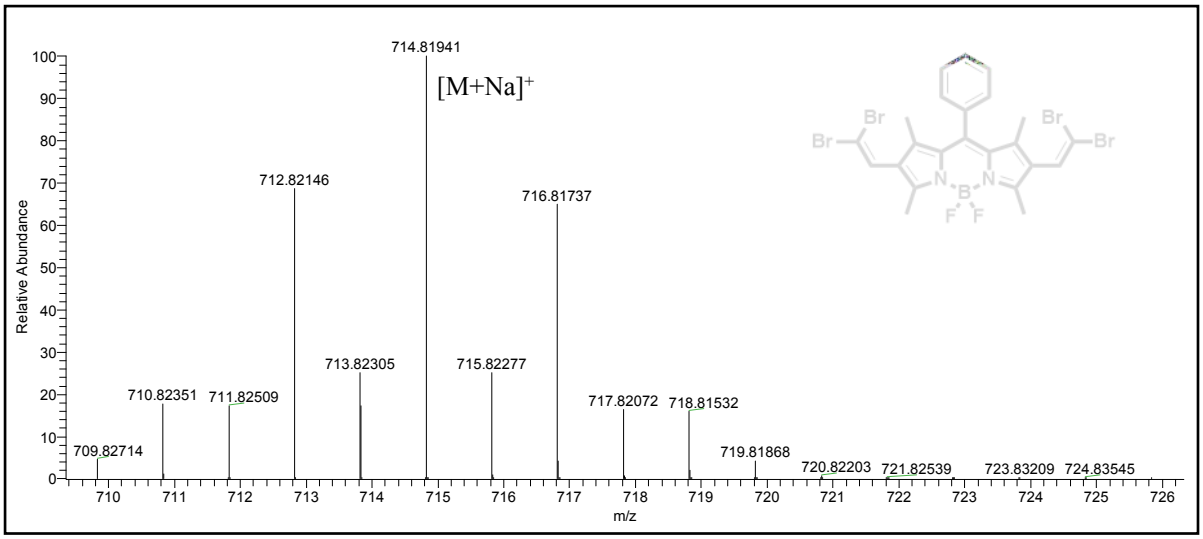


Table: Mass spectrum peak assignments and isotopic patterns of **BOD-1**

BOD-1; $C_{21}H_{19}BBr_2F_2N_2$	m/z (Theoretical)	m/z (Found)	isotopes [^{x}B,^{y}Br,^{z}Br]
	527.99046	527.99365	[^{10}B^{79}Br^{79}Br]
	528.98683	528.98969	[^{11}B^{79}Br^{79}Br]
	529.98842	529.99182	[^{10}B^{79}Br^{81}Br]
	530.98479	530.98779	[^{11}B^{79}Br^{81}Br]
	531.98814	531.99054	[^{10}B^{81}Br^{81}Br]
	532.99274	532.98602	[^{11}B^{81}Br^{81}Br]
	533.98609	533.98926	[^{10}B^{81}Br^{81}Br]

Theoretical BOD-2:



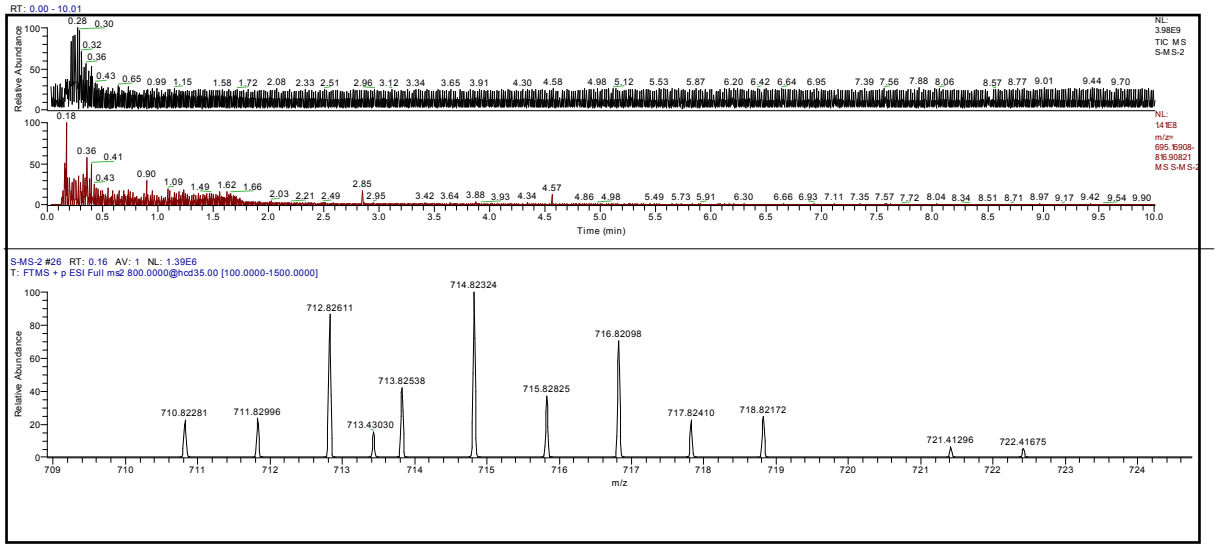
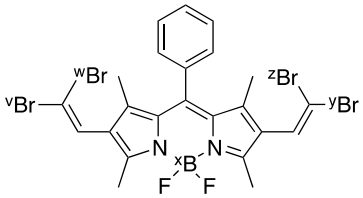


Table: Mass spectrum peak assignments and isotopic patterns of **BOD-2**

BOD-2; $C_{21}H_{19}BBr_2F_2N_2$	m/z (Theoretical)	m/z (Found)	isotopes [^{x}B,^{y}Br,^{z}Br, ^{v}Br,^{w}Br]
	710.82351	710.82281	[^{11}B^{79}Br^{79}Br^{79}Br^{79}B]
	711.82509	711.82996	[^{10}B^{81}Br^{79}Br^{79}Br^{79}B]
	712.82146	712.82611	[^{11}B^{81}Br^{79}Br^{79}Br^{79}B]
	713.82395	713.82538	[^{10}B^{81}Br^{81}Br^{79}Br^{79}B]
	714.81941	714.82324	[^{11}B^{81}Br^{81}Br^{79}Br^{79}B]
	715.82277	715.82825	[^{10}B^{81}Br^{81}Br^{81}Br^{79}B]
	716.81737	716.82093	[^{11}B^{81}Br^{81}Br^{81}Br^{79}B]
	717.82072	717.82410	[^{10}B^{81}Br^{81}Br^{81}Br^{81}B]
	718.81532	718.82172	[^{11}B^{81}Br^{81}Br^{81}Br^{81}B]

References

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