Electronic Supplementary Material (ESI) for ChemComm.

Photo-Uncaging of BODIPY Oxime Ester for Histone Deacetylases Induced Apoptosis in Tumor Cells

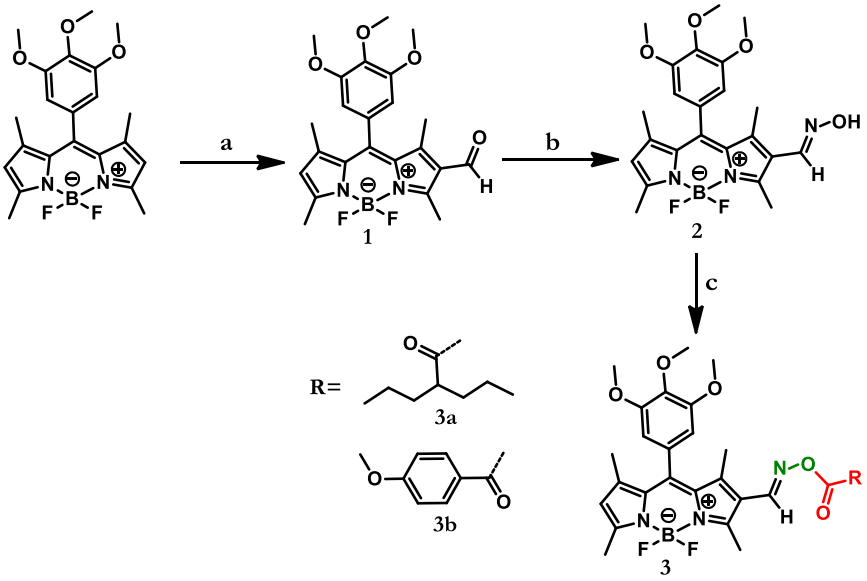
Karthik Sambath, Tinghan Zhao, ZhaoxiongWan, and Yuanwei Zhang*

Department of Chemistry and Environmental Science, College of Science and Liberal Arts, New Jersey Institute of Technology, 323 Martin Luther King Jr. Blvd., Newark, New Jersey 07102, United States

Experimental section

General

All reagents and solvents were purchased from commercial suppliers and used as received without further purification. Compound **2** was synthesized according to their previous reported procedures. ^{1}H and ^{13}C NMR spectra were carried out in $CDCl_3$ solution on a Bruker AVANCE spectrometer (500 MHz). orbitrap mass spectrometry analyses were performed at the Prof. Hao Chen lab, Department of Chemistry and Environmental Science, New jersey institute of technology (NJIT). Flash column chromatography was performed on a CombiFlash® Rf+ automated flash chromatography using RediSep Rf Gold® normal-phase HP silica columns. UV-vis absorption spectra were recorded on a Tecan Infinite M200 PRO plate reader spectrometer in 1 cm path length cuvettes. Fluorescence emission spectra were measured using an Edinburgh FLS980 fluorescence spectrometer. One-photon fluorescence microscopy images were recorded on an Olympus IX-81 DSU microscope equipped with a Hamamatsu EMCCD C9100 digital camera.



Reagents:a) DMF/$POCl_3$/-5 C; b) $NH_2OH.HCl$/ethanol; c) 3a: Triethylamine/ DCM/ 2-propylpentanoyl chloride, 3b: p-methoxy benzoyl chloride/Triethylamine/ DCM.

Synthetic procedure:

Synthesis of BODIPY core: 3,4,5-Trimethoxylbenzaldehyde (1.65 g) and 2,4-dimethylpyrrole (1.6 g) were dissolved in dry CH_2Cl_2 (800 mL) under a nitrogen atmosphere. Nine drops of trifluoroacetic acid (TFA) (about 0.5 mL) were added, and the mixture was stirred at room temperature overnight. After TLC monitoring showed complete disappearance of the aldehyde, a solution of 2, 3-dichloro-5,

6-dicyano-1, 4-benzoquinone (DDQ) (1.9 g) in anhydrous CH_2Cl_2 (200 mL) was added. This mixture was further stirred for 4 h, and N, N-diisopropylethylamine (DIPEA) (16 mL) were added under a nitrogen atmosphere. The solution was stirred at room temperature for 30 min, and BF_3-OE_{t2} (20 mL) was subsequently added. This mixture was stirred for overnight, where upon the complexation was found to be completed by TLC monitoring. The mixture was washed thoroughly with water and brine, dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The crude compound was purified by silica gel column chromatography (eluent: petroleum ether/ethyl acetate: first 10:1 to final 5:1, increasing the polarity of the solvent) to give a shiny green powder as the pure BODIPY dye (600 mg). ^{1}H NMR (500 MHz, $CDCl_3$): δ : 6.53 (s, 2H); 6.00(s, 2H), 3.92 (s, 3H, OCH_3-4), 3.83 (s, 6H, 2 × OCH_3-3, 5), 2.56 (s, 6H), 1.53 (s, 6H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ: 155.6, 154.2, 143.0, 141.3, 138.6, 131.3, 130.1, 121.2, 105.1, 61.3, 56.3, 30.9, 14.2 ppm.

Synthesis of BODIPY Aldehyde (1): A mixture of DMF (6 mL) and $POCl_3$ (6 mL) was stirred at -5 °C for 30 min, to this reaction mixture BODIPY core (150 mg) was dissolved in dichloromethane (5 mL), and the mixture was stirred for 2 h. The crude product was purified using column chromatography (silica gel, hexane/EtOAc 6/4 v/v) to give BODIPY aldehyde (**1**) (142 mg, 89%) as red crystal solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ:10.0 (s, 1H), 6.51 (s, 2H), 6.18 (s, 1H), 3.93 (s, 3H, OCH_3-4), 3.84 (s, 6H, 2 × OCH_3-3, 5), 2.82 (s, 3H), 2.62 (s, 3H), 1.81 (s, 3H), 1.60 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 206.9, 185.8, 161.7, 156.6, 154.5, 147.2, 143.2, 142.8, 139.1, 134.0, 129.1, 123.9, 104.9, 61.4, 56.4, 30.9, 14.7, 11.5 ppm. HRMS-ESI: $C_{23}H_{25}BF_2N_2O_4$, Calculated :443.19482, Found: $[M+H]^+$:443.19475.

Synthesis of BODIPY-oxime parent compound (2): Compound **1** (100 mg) and hydroxylamine hydrochloride (41.4 mg) in absolute ethanol (20.0 mL) were stirred at reflux temperature for 1 h, the residue was purified by column chromatography (hexane/ethyl acetate (7/3). BODIPY-oxime parent compound **2** was obtained as red solid (86.0 mg, 70.0% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.01 (s, 1H), 6.52 (s, 2H), 6.08 (s, 1H), 3.93 (s, 3H, OCH_3-4), 3.83 (s, 6H, 2 × OCH_3-3, 5), 2.70 (s, 3H), 2.58 (s, 3H), 1.62 (s, 3H), 1.55 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ= 158.2, 154.3, 144.8, 142.0, 139.9, 138.9, 130.4, 129.7, 122.4, 121.48, 105.1, 61.4, 56.4, 14.5, 12.3 ppm.

General procedure for synthesis of BODIPY Oxime esters (3a, 3b): A solution of BODIPY oxime (**2**) in dry dichloromethane was cooled to 0 ◦C under a nitrogen atmosphere, triethylamine (0.28 ml) was added followed by acid chloride (1.2eq) in dry dichloromethane and the mixture was allowed to stir for 4 h allowing the temp to slowly rise to 25 °C. The organic phases were then washed with

brine and the residue was purified by column chromatography (hexane/ethyl acetate 7/3 v/v). BODIPY oxime ester was obtained as red solid in good yield.

(3a): ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.32 (s, 1H), 6.51 (s, 2H), 6.11 (s, 1H), 3.91 (s, 3H, OCH_3-4), 3.83 (s, 6H, 2 × OCH_3-3, 5), 2.75 (s, 3H), 2.59 (s, 3H), 2.51-2.46 (m, 1H), 1.72 (s, 3H), 1.69-1.64 (m, 2H), 1.58 (s, 3H), 1.51-1.48 (m, 2H), 1.47-1.36 (m, 2H), 1.25-0.89(t, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 180.6, 173.8, 159.7, 154.4, 150.0, 146.0, 142.4, 140.9, 139.0, 133.0, 130.5, 129.4, 123.1, 119.5, 105.0, 61.4, 60.3, 56.4, 43.9, 34.6, 20.6, 14.6, 13.9 ppm. $C_{31}H_{40}BF_2N_3O_5$ H^+ Calculated Mass: 584.3007, Experimental mass: $[M+H]^+$: 584.3095.

(3b): ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.09 (d, 2H), 6.98 (d, 2H), 6.51 (s, 2H), 6.21 (s, 1H),3.95 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3-4), 3.87 (s, 6H, 2 × OCH_3-3, 5), 2.69 (s, 6H), 1.67 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 164.6, 162.3, 160.0, 154.4, 148.7, 145.9, 142.0, 139.0, 137.2, 134.4, 132.8, 129.2, 127.8, 122.7, 122.3, 114.1, 104.9, 61.4, 56.4, 55.6, 29.7, 14.5, 11.4 ppm. $C_{31}H_{32}BF_2N_3O_6$ H^+ Calculated Mass: 592.2430, Experimental mass: $[M-F^-]^+$: 572.92718.

Absorption, Emission spectra and fluorescent quantum yield of compound 3a and 3b: All steady-state absorption, fluorescence emission, of compound **3a** and **3b** were investigated in 10 mm path length quartz cuvettes at room temperature. The steady-state absorption was measured with Tecan Infinite M200 PRO plate reader spectrometer in 1 cm path length cuvetts. Fluorescence emission and excitation spectra were obtained using an Edinburgh Photonics FLS980spectrometer equipped with a thermoelectric cooled photo multiplier detector (Hamamatsu) and a liquid-nitrogen cooled NIR-photomultiplier detector (Hamamatsu). All measurements were carried out with the optical density below 0.12 at the excitation wavelength to avoid reabsorption. The excitation and fluorescence emission spectra were corrected for the spectral sensitivity of Edinburgh Photonics excitation and detection system using factory-measured correction files. Fluorescence lifetimes (τ_F) were determined with the single photon counting technique (TCSPC) and the same fluorescence spectrometer using a pulsed picosecond diode laser (EPL-505) as the excitation source. Molar absorption coefficients (ε) and maximum absorbance wavelengths (λ_{max}) were determine in acetonitrile using Beer's law, from plots of absorbance vs. concentration. Recordings were performed in 10 mm path length quartz cuvettes at room temperature.

a) Fluorescence quantum yields were determined by a reference point method. Fluorescein as standard / exe at 496nm in 0.1 M NaOH (ϕ_f) =0.95 was used as a standard sample to calculate the QY of **3a** and **3b**. The equation as follow.

$$\frac{\phi_S}{\phi_R} = \frac{A_S}{A_R}\,\frac{(Abs)_R}{(Abs)_S}\,\frac{\eta_S^2}{\eta_R^2}$$

Where Φ represents quantum yield, Abs represents absorbance, ***A*** represents area under the fluorescence curve, and η is refractive index of the medium. The subscripts S and R denote the corresponding parameters for the sample and reference, respectively.

Photo-uncaging BODIPY oxime esters (3a, and 3b) and measurement of photochemical quantum yields: Compound **3a** (2 mL of 1×10^{-5} M in MeOH/Water (7/3 v/v) and Compound **3b** (2 mL of 1×10^{-5} M in MeOH/Water (7/3 v/v)) was placed in quartz cuvette (10 mm path). Further the photolysis was followed by biological condition also Compound **3a** (2 mL of 1×10^{-5} M in PBS/DMSO (9.5/0.5 v/v)) was placed in quartz cuvette (10 mm path). The cuvette was placed in front of a light source (Thorlab's M505L3) mounted LED has a nominal wavelength 503 ± 30 nm with a power density of ≈ 80 mW/cm^2, at 1 cm distance and irradiated for the indicated times. The power density was measured by PM100D Compact Power and Engergy Meter (Thorlabs) and the value was confirmed by using photometer IL1400A (International Light). The LED light spectrum was obtained from the vendor, and a wavelength-power density diagram was ploted. After integrating the plot, the total number of photon (light flux) was calculated by sum up the ones at different wavelengths. At regular interval of time point, the cuvette was analysis by UV-Vis spectrophotometer. The photochemical stability was investigated quantitatively by measuring the photochemical decomposition quantum yield, $\Phi_{ph}=N_{ph}/N_{hv}$ (N_{ph} and N_{hv} are the numbers of decomposed molecules and absorbed photons, respectively). The values of Φ_{ph} were determined by an absorption method using a mounted LED for excitation of the compound **3a** and **3b** (excitation wavelength ≈ 503 nm, average beam irradiance ≈ 80 mW/cm^2). According to the well-developed absorption methodology, the values of Φ_{ph} can be determined by the following equation.

$$\mathbf{\Phi_{ph} = (A_t - A_0)N_A/10^3 \times \varepsilon \times I\times[1-10^{(At+A0)/2}]\times t}$$

A_t and A_0 are absorbance maximum at time points t and t_0, respectively. N_A is Avogadro's number, ε is the molar extinction coefficient (M^{-1} cm^{-1}), I is the irradiation intensity (photon cm^{-2} s^{-1}), and t is the irradiation time (sec).

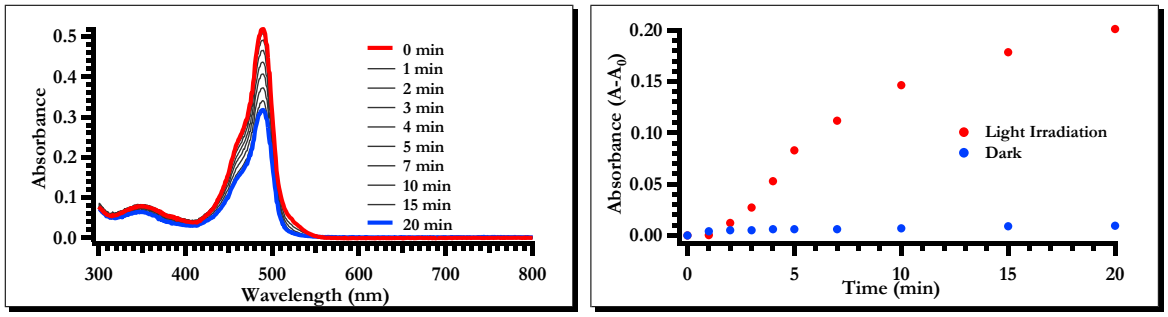


Figure S1: Left panel: UV-Vis spectra of photorelease from compound **3a** in MeOH/Water (7/3 v/v)) Right panel: Absorbance at 505 nm Vs time is plotted, for compound **3a**

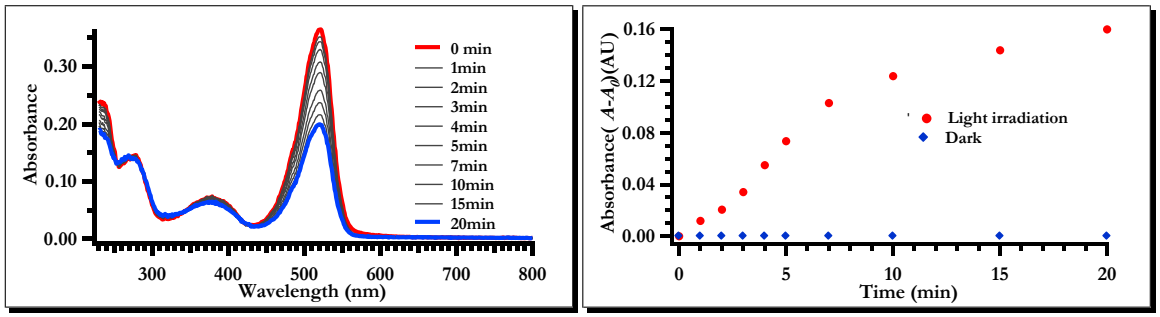


Figure S2: Left panel: UV-Vis spectra of photorelease from compound **3b** in MeOH/Water (7/3 v/v)) Right panel: Absorbance at 505 nm Vs time is plotted, for compound **3b**.

5 mL of Compound **3a** (1×10^{-5} M) in MeOH/Water (7/3 v/v)) solution was placed in quartz cuvette (10 mm path). They were irradiated under mounted LED has a wavelength of 503 ± 30nm with the output power of ≈ 80 mW/cm^2. At regular interval of time, 50 µL of the aliquots was taken and analyzed by LC-MS using mobile phase acetonitrile/water (95/5 v/v), at a flow rate of 0.1 mL/min (detection: UV at wavelength of 500 nm). The reaction was followed until the consumption of the caged oxime ester of the initial area. The peak areas were determined by LC, which indicated gradual increase of peak area with time.

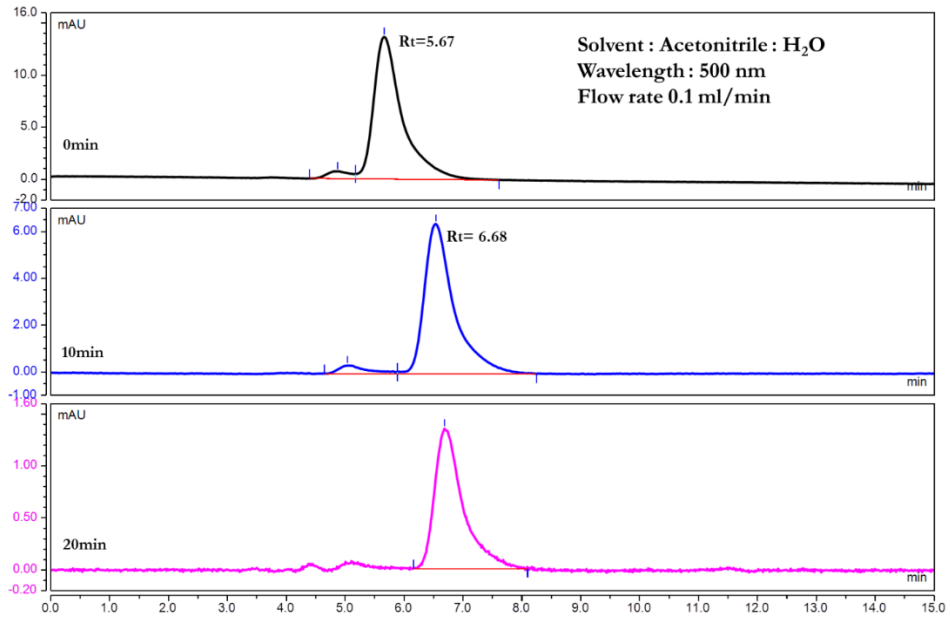
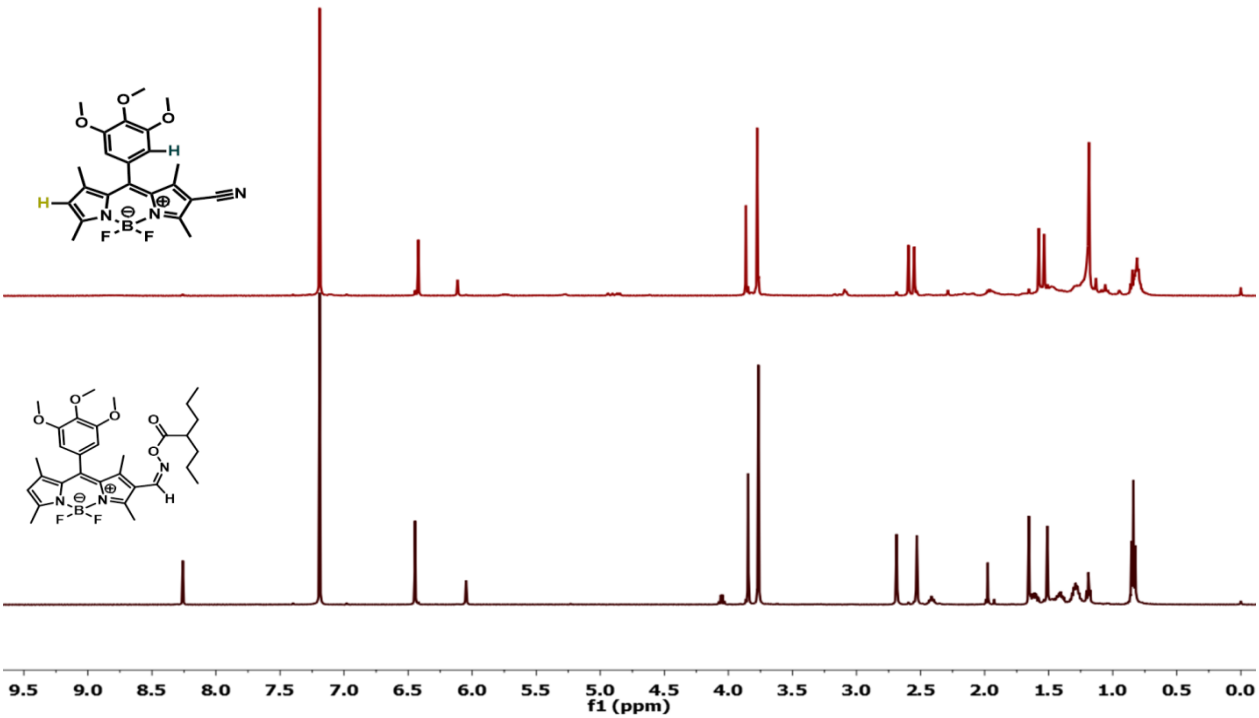


Figure S3: The LC-MS profile of time-dependent photo-uncaging **3a** using green LED (505 nm) irradiation, time point of 0, 10 and 20 min.

We also have followed the course of the photorelease by ^{1}H NMR spectroscopy. A known amount (1 mL) of aqueous suspension of the photolysate was taken at regular intervals of time and was extracted in dichloromethane (DCM), then solvent was evaporated under vacuum and redissolved in $CDCl_3$ and the ^{1}H NMR was recorded. ^{1}H NMR spectra showed a clean photocleavage of the **3a** into corresponding photoproducts.

Preparative photolysis: 5 mg of Compound **3a** in MeOH/Water (7/3 v/v)) solution were irradiated under mounted LED has a wavelength of 503 ± 30 for 10 min. At regular interval of time, the reaction was monitored by TLC. After completion of reaction the photoproduct was isolated using preparative TLC using 15% EtOAc as a mobile phase. The photoproduct of 2-cyano-BODIPY was analyzed by ^{1}H, ^{13}C NMR and mass analysis. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.49 (s, 2H), 6.18 (s, 1H), 3.93 (s, 3H, OCH3-4), 3.87 (s, 6H, 2 × OCH3-3, 5), 2.66-2.62(s, 6H), 1.65-1.61 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 163.37, 154.94, 148.37, 143.01, 139.61, 134.68, 130.76, 129.05, 124.64, 115.32, 114.47, 105.12, 61.79, 56.86, 30.08, 15.57, 15.11. $C_{23}H_{24}BF_2N_3O_3H^+$ Calculated Mass: 440.1951, Experimental mass: 440.1948.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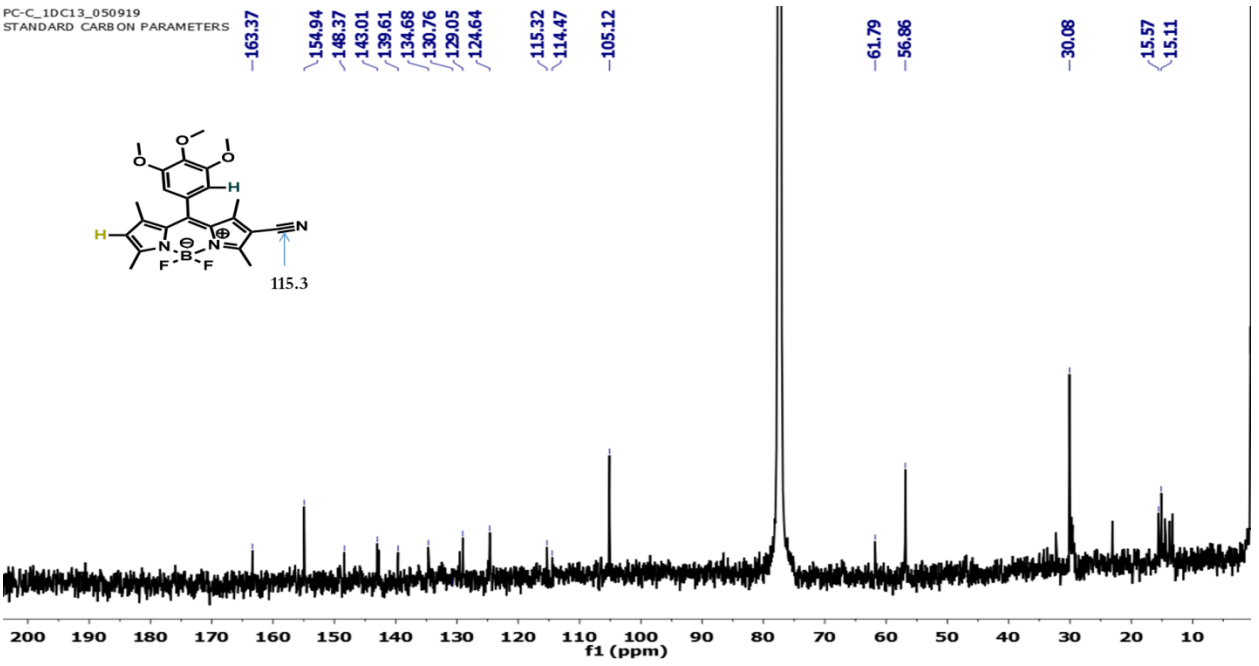
f1 (ppm)


PC-C_1DC13_050919
STANDARD CARBON PARAMETERS
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154.94
148.37
143.01
139.61
134.68
130.76
129.05
124.64
115.32
114.47
105.12
61.79
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30.08
15.57
15.11
115.3
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f1 (ppm)

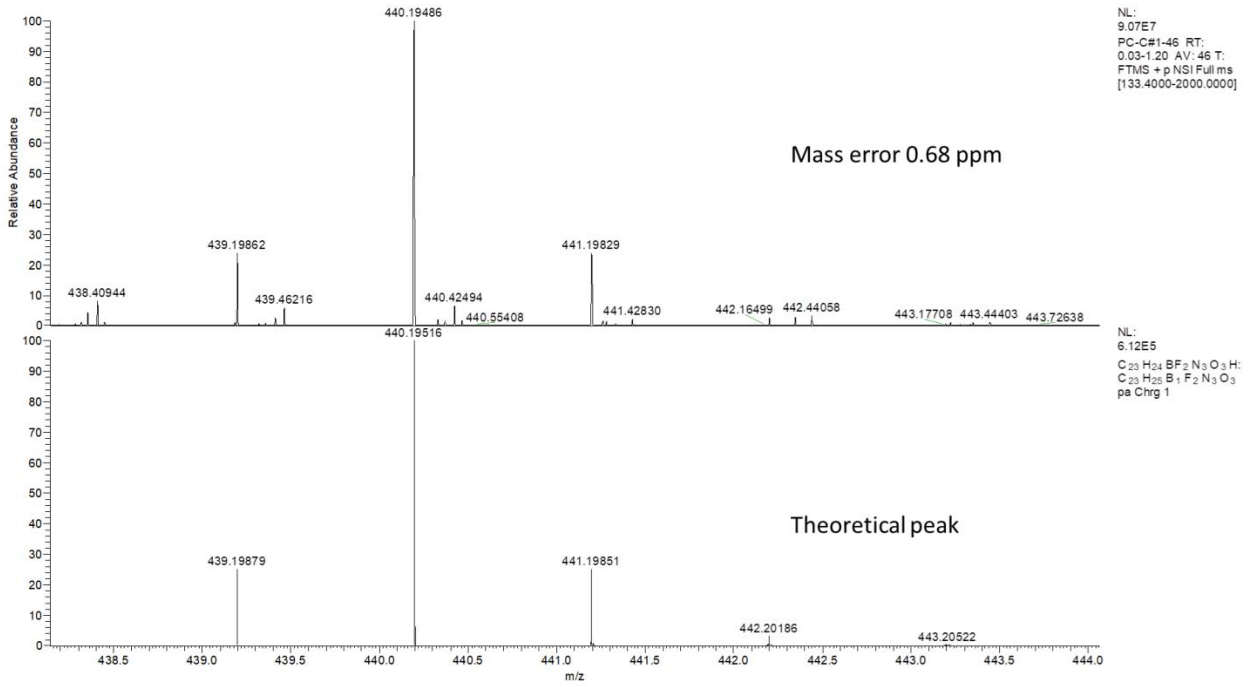


Figure S4. ^{1}H, ^{13}C NMR and Mass spectra of 2-cyano-BODIPY photo-byproduct.

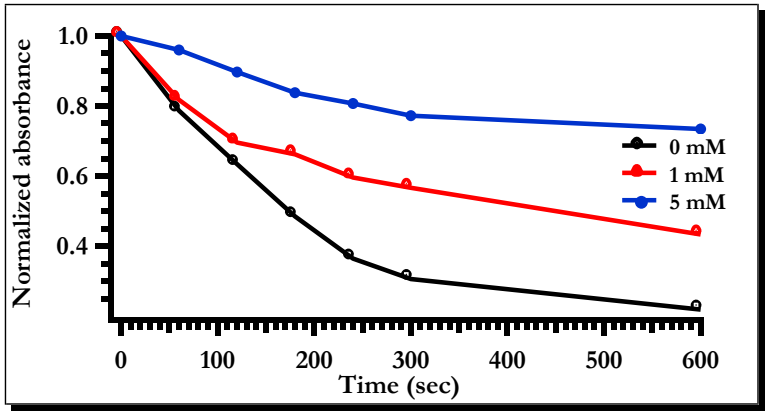


Figure S5. Time course of photolysis for the oxime ester **3a** in presence of different amount of triplet quencher potassium sorbate (PS).

Cell culture: HeLa cell line was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin at 37 °C in a humidified 5% CO_2 incubator.

Cellular Uptake Studies: To investigate the cellular of BDVPA, HeLa cells were employed. All cells were seeded on confocal dish (MatTek) at the density of 4×10^4 cells per dish and incubated for 24

h at 37 ℃. Stock solutions of **3a** dissolved in DMSO were prepared at a nominal concentration of 10^4 μM. The stock solution was diluted to 1 and 10 μM with the DMEM cell medium respectively and freshly placed over cells for 1 h and 4 h incubation period separately. Cells were washed three times with PBS and the live cell imaging solution (Molecular Probes) was added to confocal dishes. Fluorescent images were obtained using an inverted Olympus IX70 microscope coupled with a FITC filter cube (Ex: 482/40; DM: 506; Em: 534/40). Bright field was obtained and **3a** fluorescent images were obtained and issued by Fiji, freely available image processing software.

Cell viability before Photolysis: To assess the cytotoxicity of **3a**, HeLa cells were cultured in the Dulbecco's modified Eagle's medium (DMEM) cell medium supplemented with 10% fetal bovine serum, 1% penicillin, and streptomycin at 37 °C and 5% CO_2. Cells were placed in 96 well plates and incubated until there were no fewer than 5×10^3 cells per well for the experiments. Next, cells were incubated with different concentrations of **3a** (0.1, 0.5, 1, 2.5, 5, 10, 20, 40 μM) for 4 h, where the values within parentheses refer to the nominal concentrations. Then, **3a** was removed and regular DMEM was added for 4 h washed + 24 h group, additional 22 h incubation was performed for both 4 h washed + 24 h and 4 h +24 h groups. After 22 h incubation, 20 μL of the Cell Titer 96 Aqueous One solution reagent (for MTS assay) was added into each well, followed by further incubation for 2 h at 37 °C.

The respective absorbance values were read on a Tecan Infinite M200 PRO plate reader spectrometer at 490 nm. Cell viabilities were calculated on the basis of the following equation

$$Cell\ Viability(\%) = \frac{Abs^{s}{}_{490nm} - Abs^{D}{}_{490nm}}{Abs^{c}{}_{490nm} - Abs^{D2}{}_{490nm}} \times 100\%$$

where $Abs^{s}{}_{490nm}$ is the absorbance of the cells incubated with different concentrations of experimental probe solutions, $Abs^{D}{}_{490nm}$ is the absorbance of cell-free well containing only **3a** at the concentration that was studied, $Abs^{c}{}_{490nm}$ is the absorbance of cells alone incubated in the medium, $Abs^{D2}{}_{490nm}$ is the absorbance of the cell-free well.

Cell viability after Photolysis: To assess the photolysis cytotoxicity of **3a** and the photo-byproducts of 2-cyano-BODIPY, HeLa cells were cultured in the Dulbecco's modified Eagle's medium (DMEM) cell medium supplemented with 10% fetal bovine serum, 1% penicillin, and streptomycin at 37 °C and 5% CO_2. Cells were placed in 96 well plates and incubated until there were no fewer than 5×10^3

cells per well for the experiments. Next, cells were incubated with 20 μM of **3a** for 4 h, except control group which had no treatment by **3a** and with 2-cyano-BODIPY (20 μM) for 4 h, seperately. Then, **3a** was removed and regular DMEM was added for 4 h washed + 24 h group. After that, control, 4h washed + 24 h and 4 h + 24 h groups were placed under the 505 nm LED lamp for various irradiation time (1, 3, 5, 10, 20 min). In addition, the control group incubated with 2-cyano-BODIPY was irradiated and incubated without removing extra cellular 2-cyano-BODIPY. And an additional 22 h incubation was performed after the irradiation. 20 μL of the Cell Titer 96 Aqueous One solution reagent (for MTS assay) was added into each well, followed by further incubation for 2 h at 37 °C.

The respective absorbance values were read on a Tecan Infinite M200 PRO plate reader spectrometer at 490 nm. Cell viabilities were calculated on the basis of above equation.

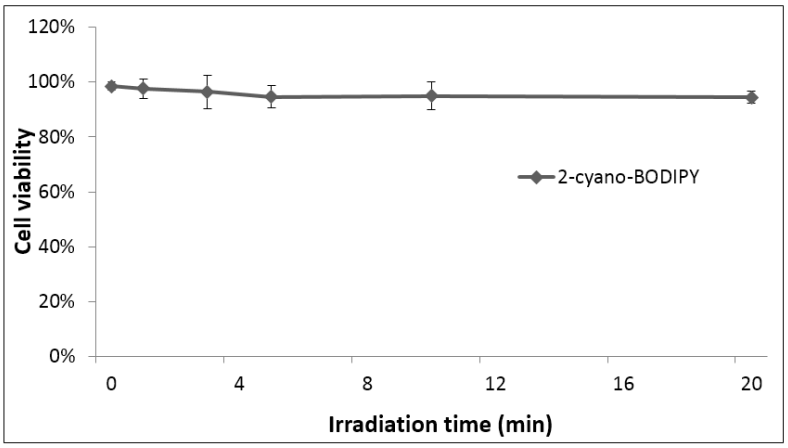
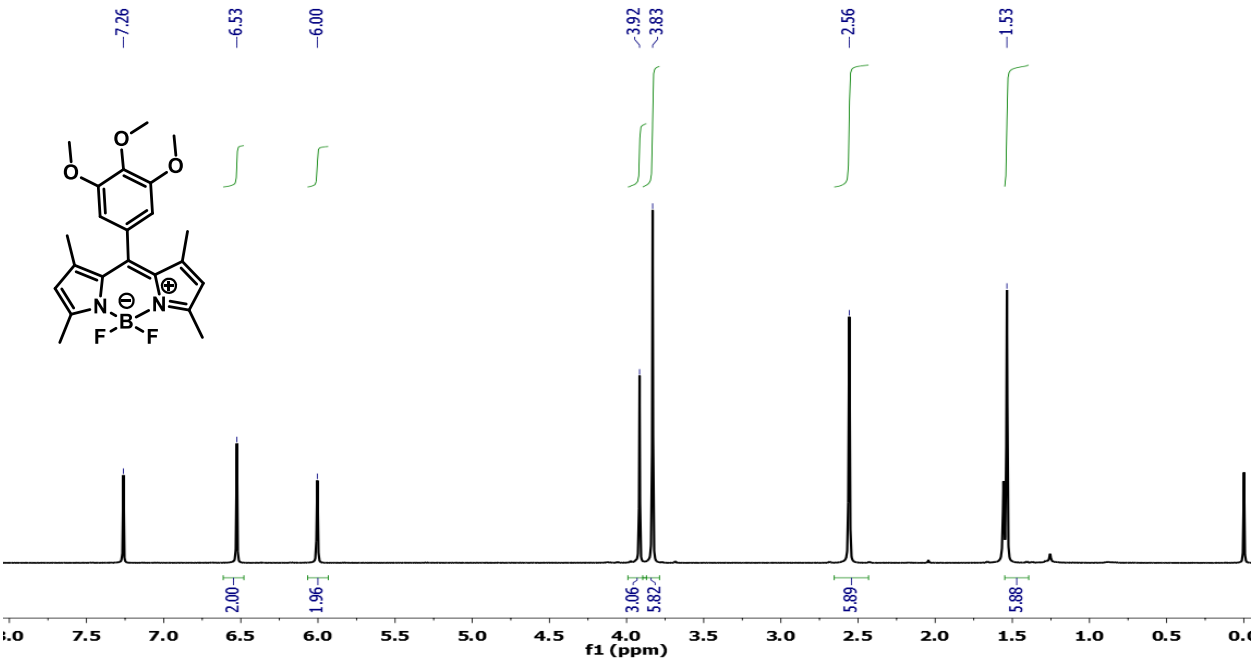
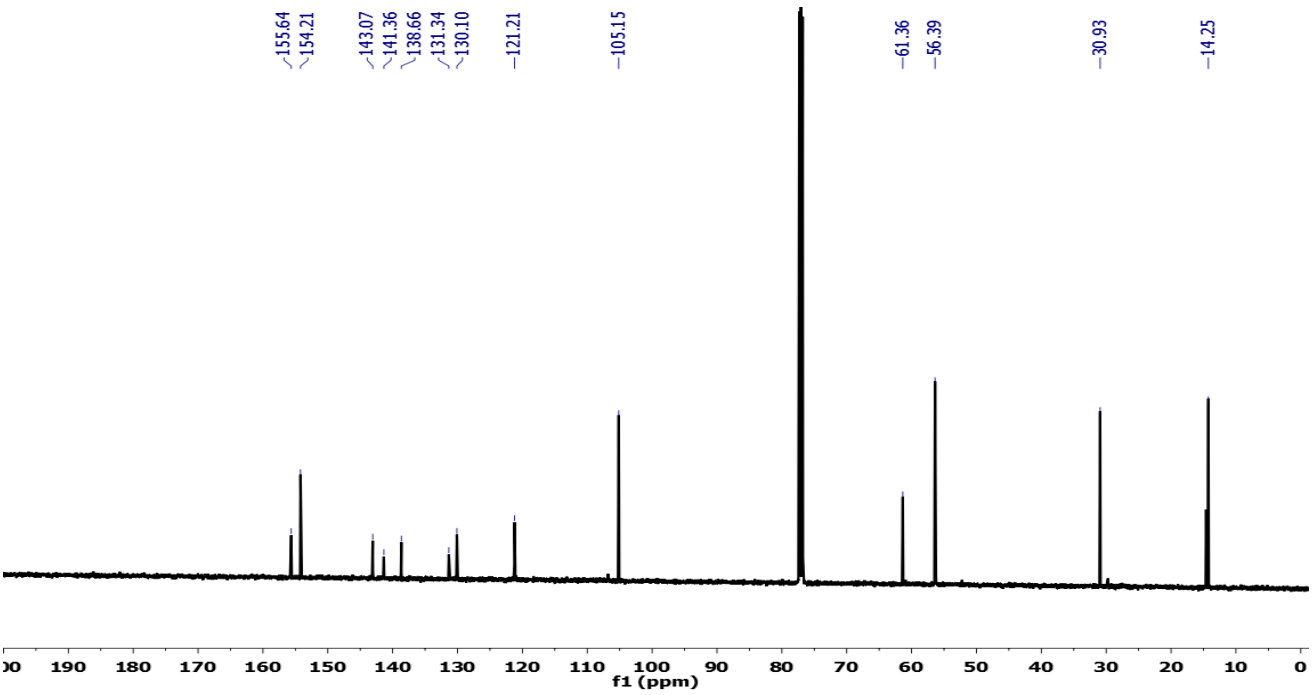


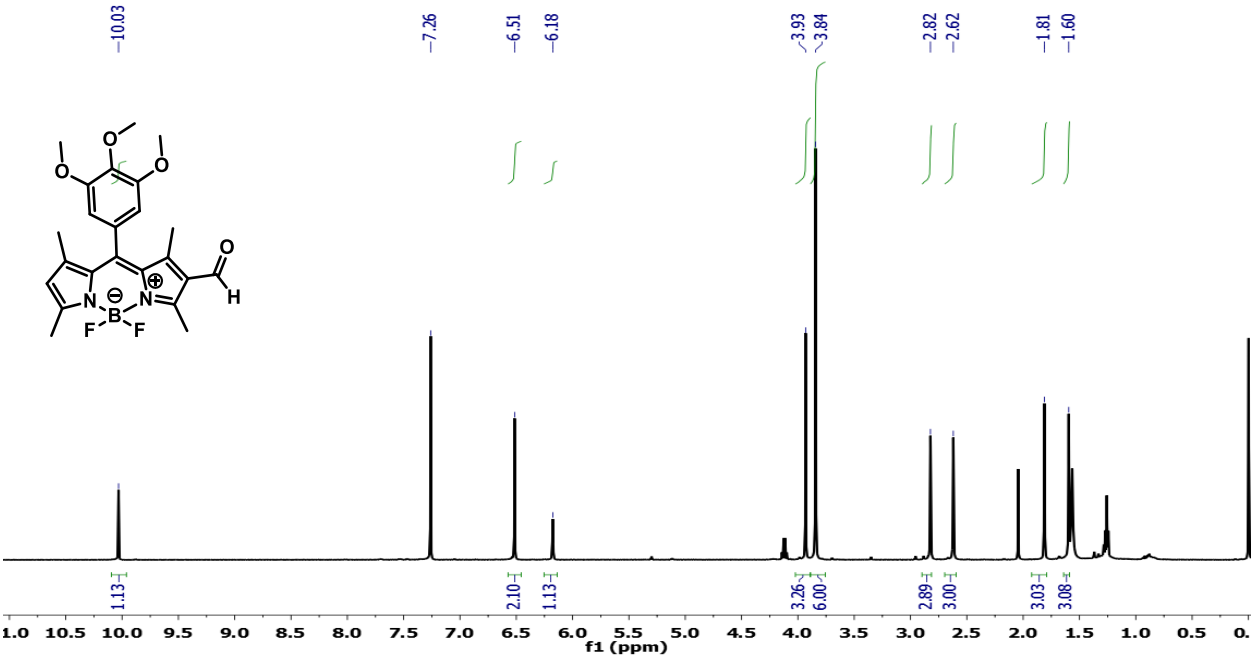
Figure S6. LED (503±30 nm, ~80 mW/cm^2) light irradiation time dependent cell viability of HeLa cells after treatment with 20 μM of 2-cyano-BODIPY. HeLa cells with no treatment was classified as 100%. (N=6).



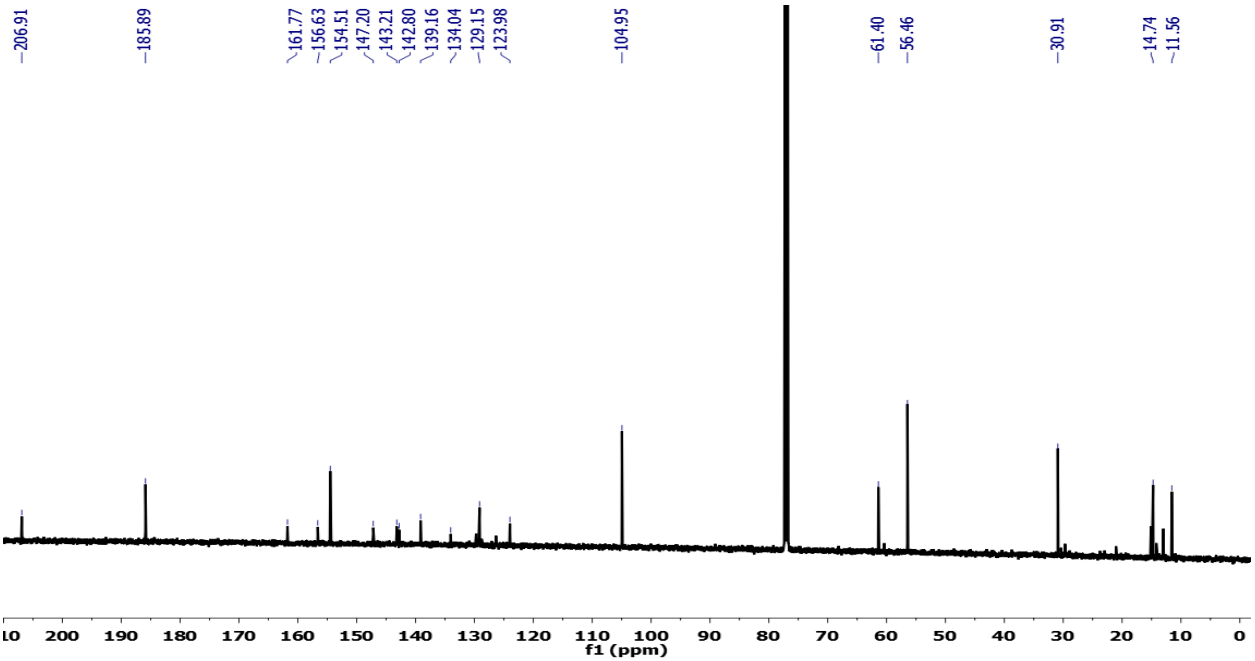
[1] H NMR of BODIPY core compound.



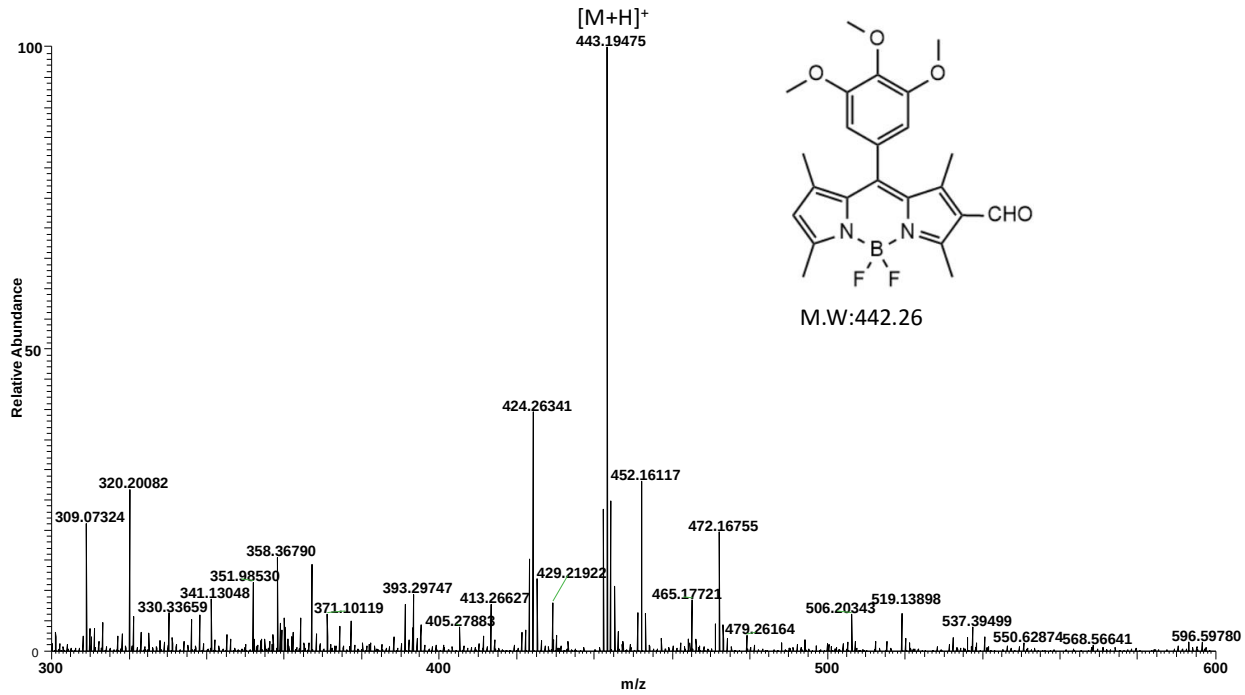
[13] C NMR of BODIPY core compound.



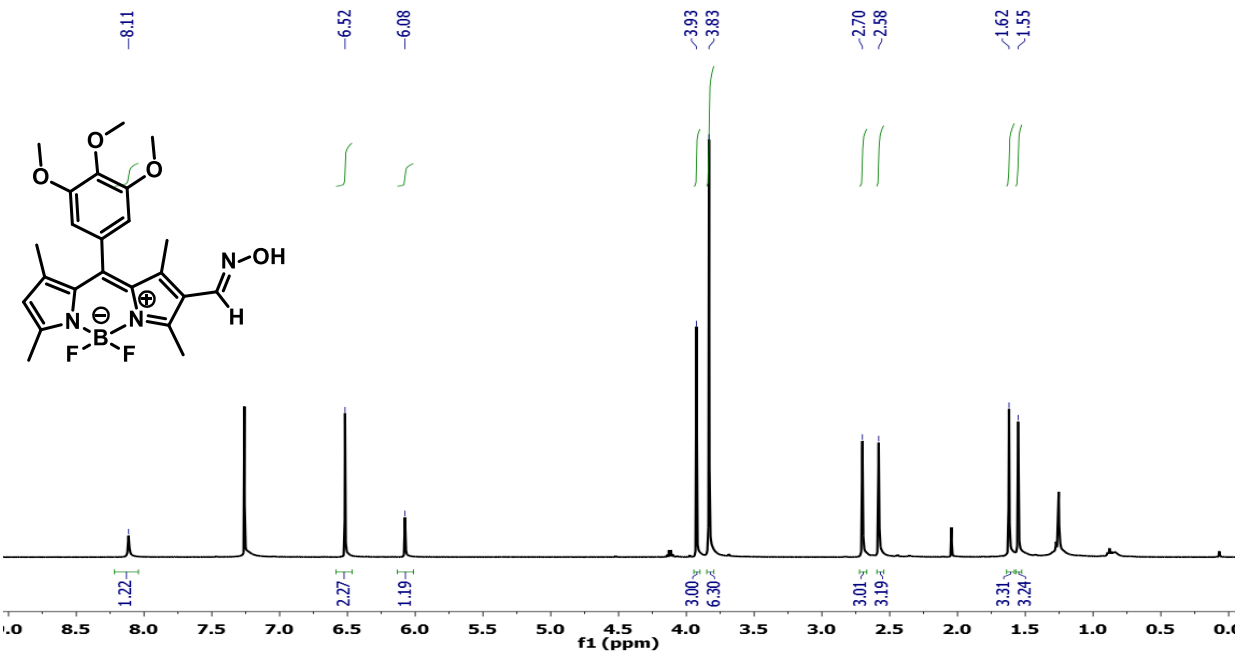
[1] H NMR of aldehyde BODIPY compound **1**.



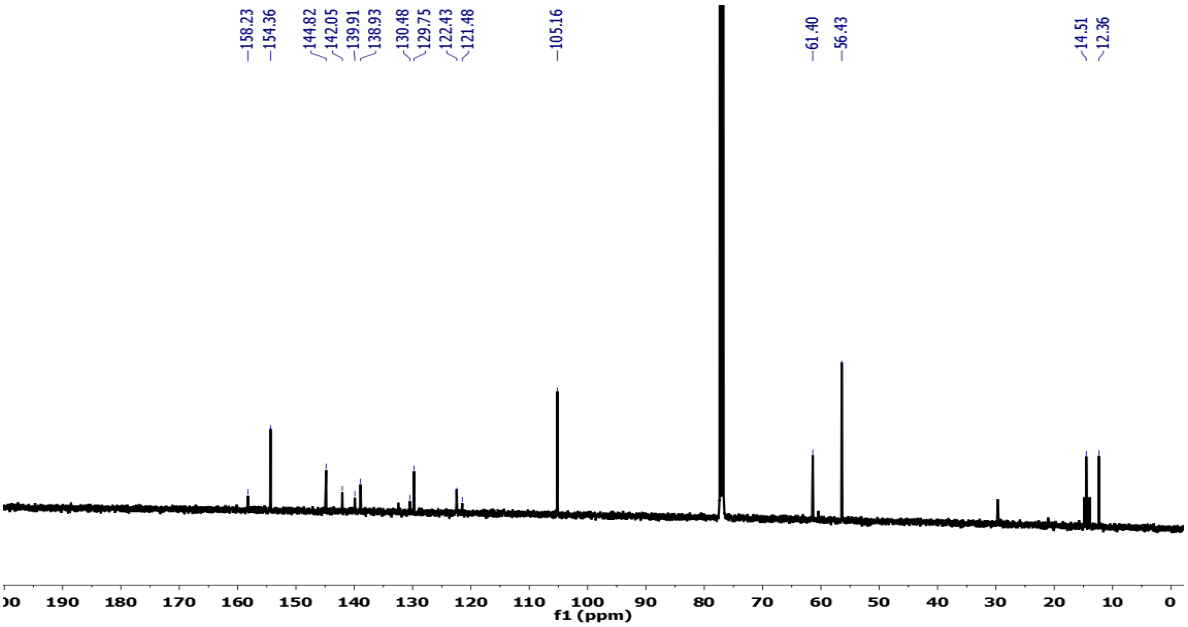
[13] C NMR of aldehyde BODIPY compound **1**.



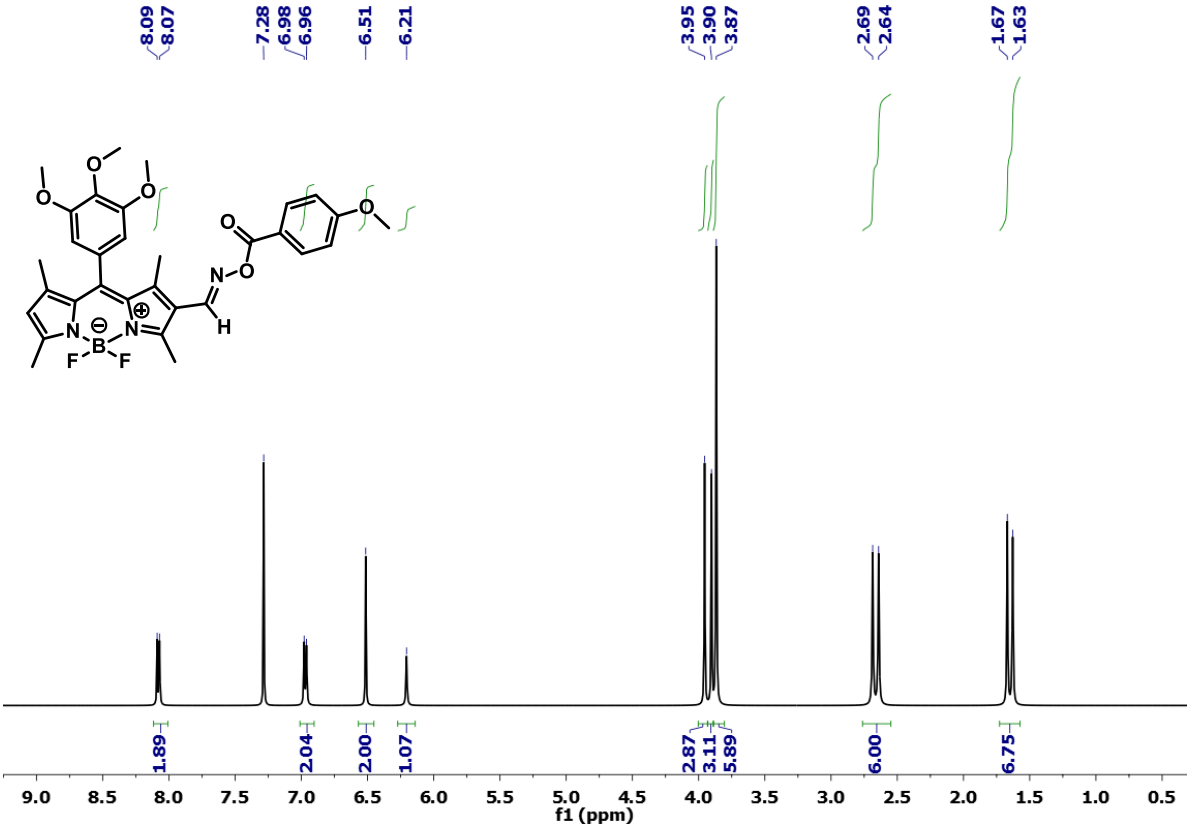
MS spectrum of aldehyde BODIPY compound **1**.



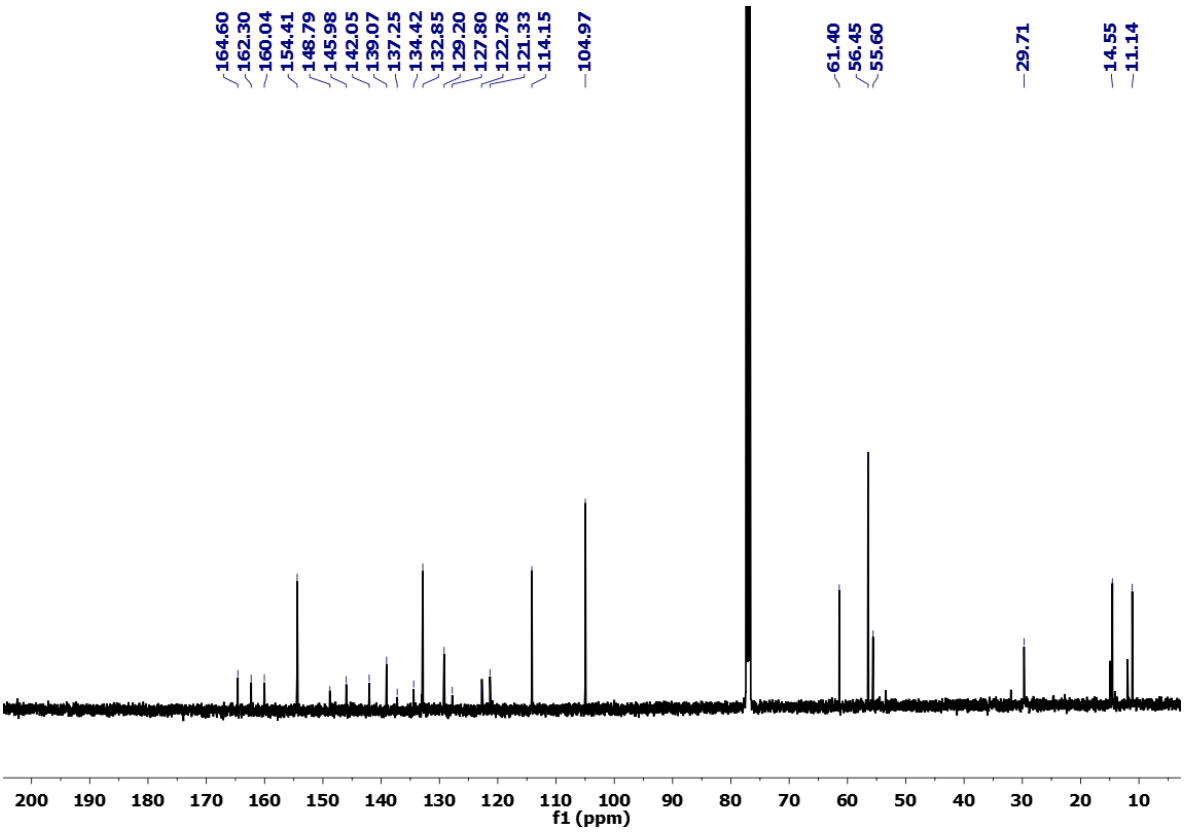
^{1}H NMR of BODIPY compound **2**.



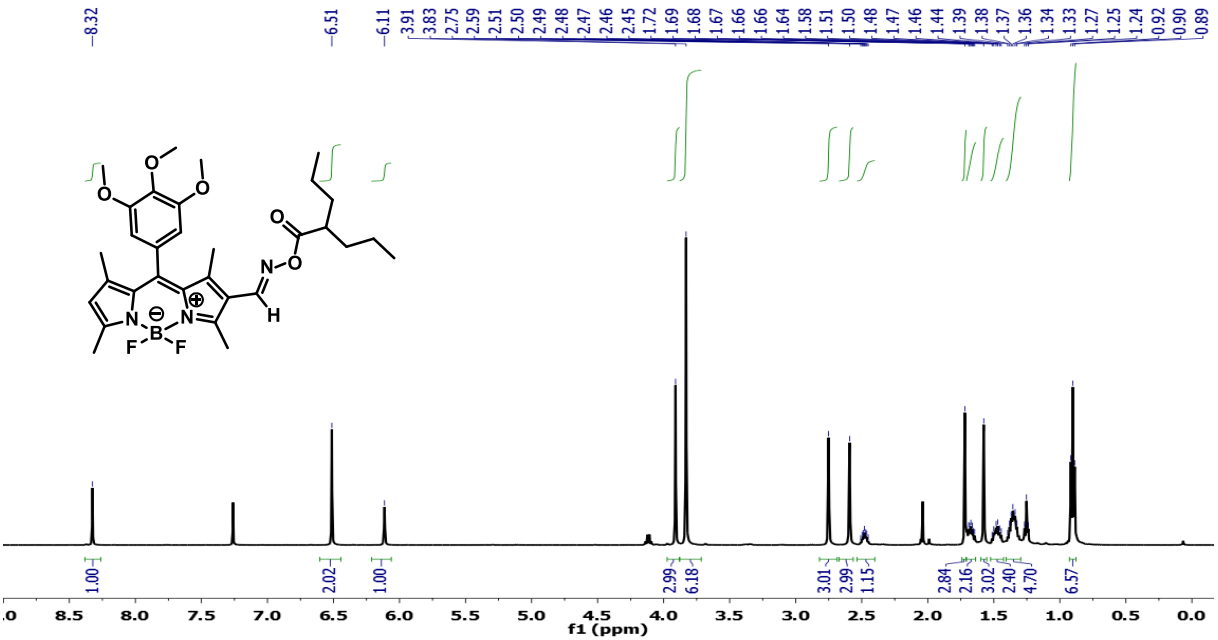
^{13}C NMR of BODIPY compound **2**.



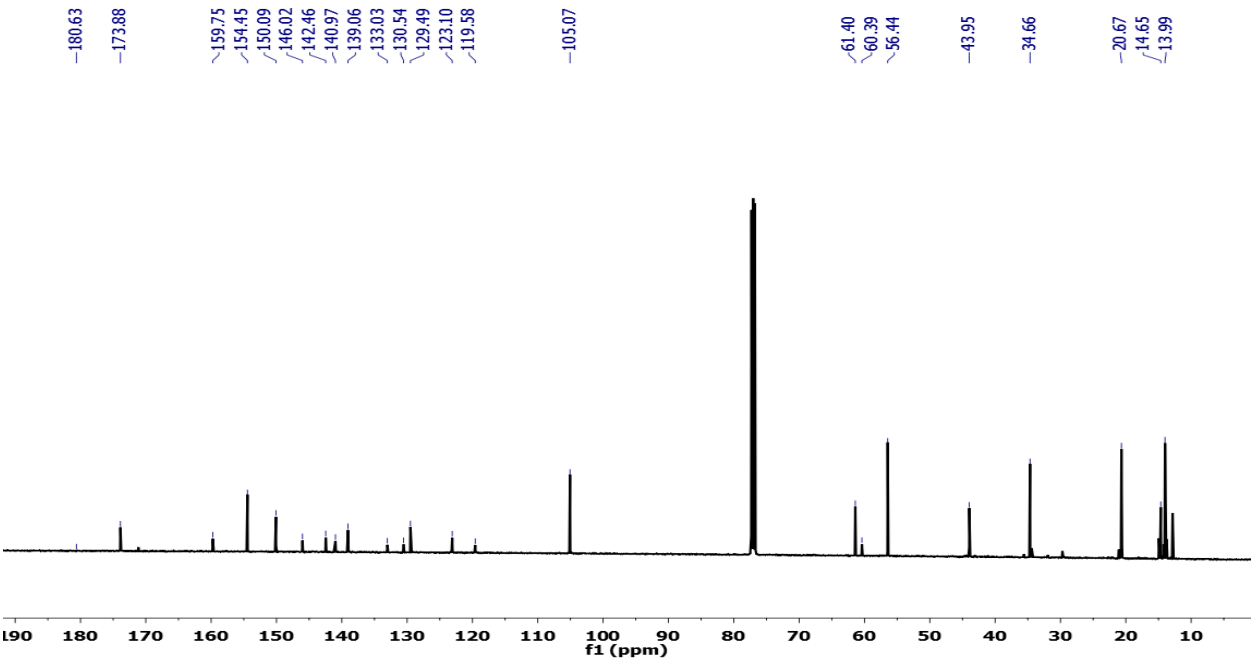
^{1}H NMR of BODIPY compound **3b**.



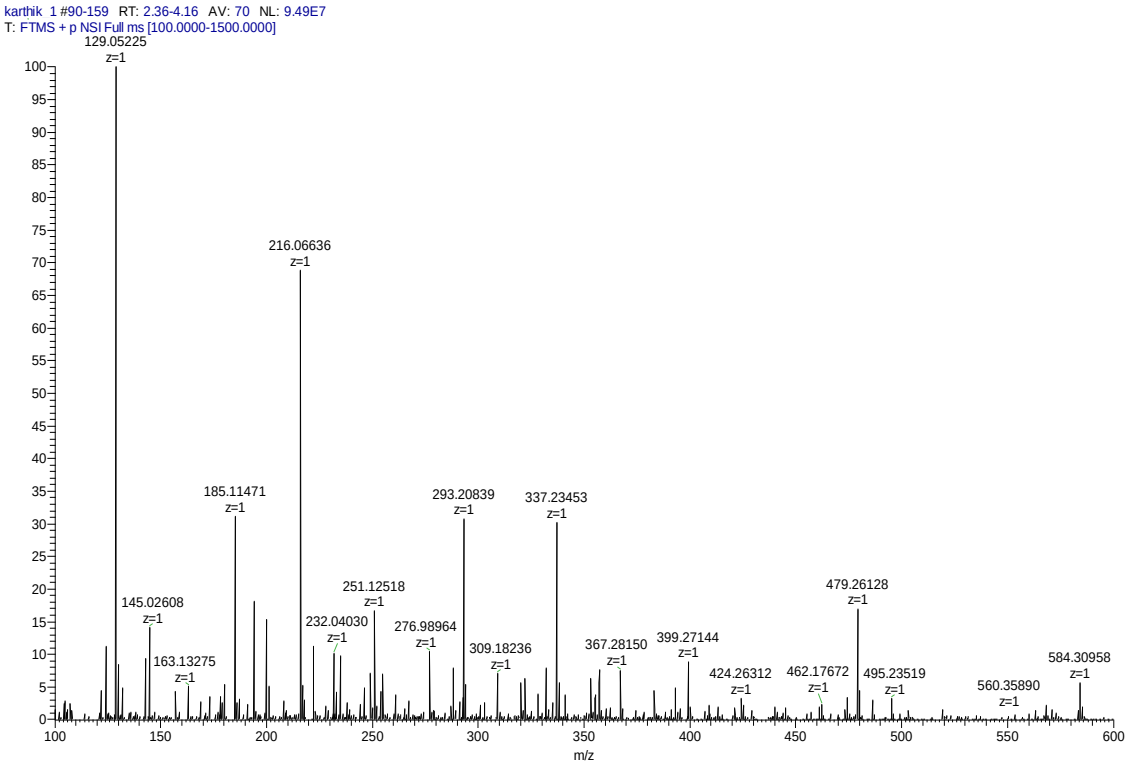
^{13}C NMR of BODIPY compound **3b**.



^{1}H NMR of BODIPY compound **3a**.



^{13}C NMR of BODIPY compound **3a**.



MS spectrum of BODIPY compound **3a**.