Supporting Information

BODIPY-based photouncaging – green light triggered dual cargo molecule release

Xuemei Chen, Krishnaben V. Sabhaya, Bryan Liu, Gabrielle Bonifacio, Yongqng Yang, Hao Chen, 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, NJ 07102, USA

E-mail: yuanwei.zhang@njit.edu

1. Materials and Methods

Chemicals and Reagents:

All reagents and solvents were obtained from commercial suppliers (e.g., Sigma-Aldrich, TCI, and Fisher Scientific) and used without further purification unless otherwise stated. Analytical grade solvents were used for all reactions and spectral measurements. Thin-layer chromatography was performed using 200 μm aluminum-backed plates purchased from Silicycle, which were visualized under UV light. Flash chromatography was carried out using silica gel from Silicycle (siliaflash P60, 40–63 μm).

Instrumentation:

- NMR spectra: ^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra were recorded on a Bruker 500 MHz ultrashield spectrometer; chemical shifts (δ) are given in parts per million (ppm, δ), relative to tetramethyl silane (TMS) and used CDCl$_3$ solvent.

- Mass spectrometry: Recorded on a high-resolution Orbitrap Q Exactive mass spectrometer (Thermo Scientific, San Jose, CA).

- UV–Vis absorption: Measured on a Tecan infinite M200pro spectrophotometer.

- Fluorescence spectra: Recorded with an Edinburgh Photonics FLS980 spectrometer equipped with a thermoelectric cooled photo multiplier detector (Hamamatsu).
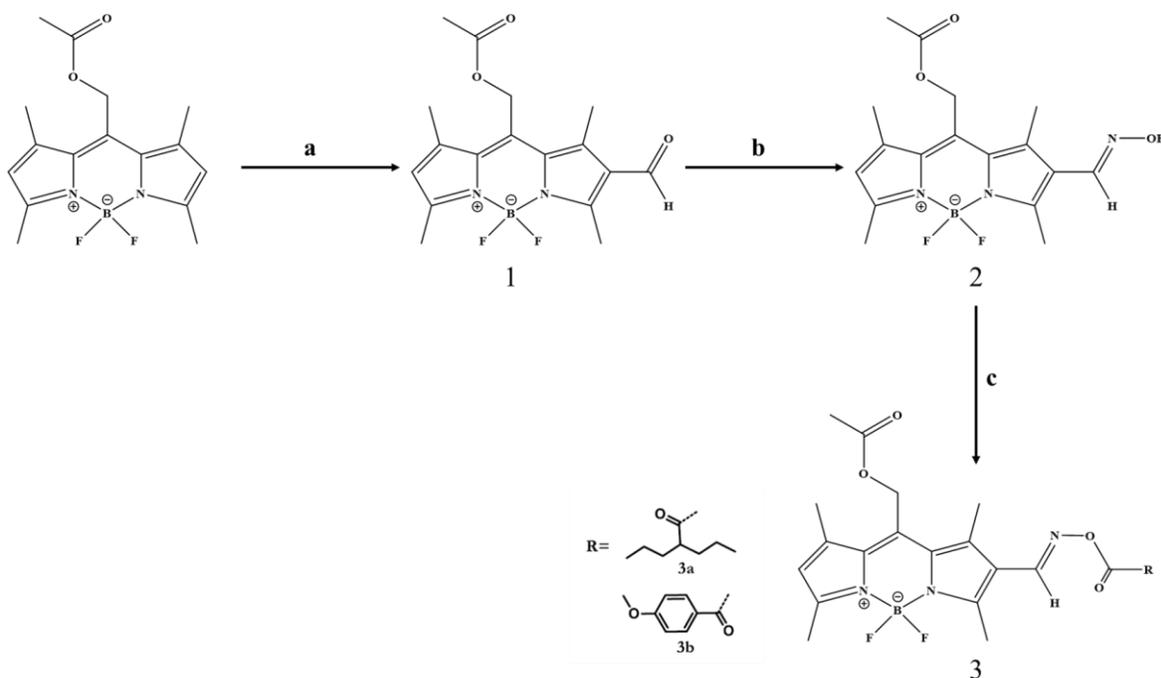
Scheme S1. Reagents: a) DMF /POCl$_3$ /-5 °C; b) NH$_2$OH·HCl /ethanol; c) **3a**: Triethylamine/ DCM/ 2-propylpentanoyl chloride, **3b**: p-methoxy benzoyl chloride/ Triethylamine/ DCM.

2. Synthetic procedure:

Step 1: Preparation of BODIPY–OAc

2,4-Dimethyl pyrrole (1.50 g, 15.7 mmol) was dissolved in dry CH$_2$Cl$_2$ (20 mL) under nitrogen. Acetoxyacetylchloride (1.29 g, 9.6 mmol) was added to the solution dropwise, and the mixture was stirred at room temperature (25°C) for 30 min. The solution was then refluxed at 40 °C for 2 h. The solution was cooled to room temperature, and then N, N-diisopropylethylamine (DIPEA, 4.07 g, 31.5 mmol) was added dropwise. After the mixture was stirred for 1 h, BF$_3$·OEt$_2$ (8.95 g, 63.0 mmol) was added dropwise, and the mixture was stirred overnight.

The solvents were partially evaporated, and the residue was extracted with CH$_2$Cl$_2$ (3 × 50 mL). The organic phase was dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated under reduced pressure. Purification was performed by silica gel column chromatography (hexanes/ethyl acetate 7:5, v/v), affording BDP–OAc as a dark-red solid. ^{1}H NMR (500 MHz, CDCl$_3$) δ (ppm): 6.08 (s,

2H), 5.30 (s, 2H), 2.53 (s, 6H), 2.36 (s, 6H), 2.13 (s, 3H). ^{13}C NMR (500 MHz, CDCl$_3$) δ: 170.69, 156.78, 141.62, 133.46, 132.81, 122.46, 58.01, 20.72, 15.75, 14.82 ppm. HRMS (ESI$^+$): calculated for C$_{16}$H$_{19}$BF$_2$N$_2$O$_2$ [M+H]$^+$ 321.15804, found 321.15865.

Step 2: Formation of BODIPY–OAc Aldehyde (Compound **1**)

In a dry ice bath under a nitrogen atmosphere, POCl$_3$ (4 mL) was added dropwise to DMF (4 mL) with constant stirring to generate the Vilsmeier–Haack reagent. The mixture was maintained in the ice bath for 15 minutes to ensure complete activation. Separately, BODIPY–OAc (previously synthesized) was dissolved in 1, 2-dichloroethane (DCE, 20 mL) and cooled to 0 °C. The freshly prepared Vilsmeier reagent was then added slowly to the BODIPY solution with continuous stirring under nitrogen. The reaction mixture was stirred for 2 hours at ambient temperature (25–30 °C), during which the color gradually deepened, indicating progression of the formylation process. After completion, the reaction was quenched with crushed ice and neutralized with a saturated sodium bicarbonate solution. The organic phase was separated, washed with water and DCM, and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the crude product was purified by silica gel column chromatography using hexane/ethyl acetate (7:5, v/v) as the eluent to afford the purified BODIPY–OAc–CHO as dark orange color. ^{1}H NMR (500 MHz, CDCl$_3$) δ (ppm): 6.26 (s, 1H), 5,34 (s, 2H), 2.76 (s, 3H), 2.63 (s, 3H),2.58 (s, 3H), 2.42 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (500 MHz, CDCl3) δ: 186.09, 170.39, 163.57, 157.15, 145.92, 140.82, 135.67, 130.70, 128.89, 126.36, 125.58, 57.57, 20.62, 16.37, 15.35, 12.96, 12.26 ppm. HRMS (ESI$^+$): calculated for C$_{17}$H$_{19}$BF$_2$N$_2$O$_3$ [M+H]$^+$ 349.15296, found 349.15250.

Step 3: Formation of BODIPY–OAc Oxime Ester Precursor (Compound **2**)

A mixture of hydroxylamine hydrochloride (100 mg, 1.44 mmol) and BODIPY–OAc aldehyde (20 mg, 0.05 mmol) was dissolved in absolute ethanol (20 mL) in a round-bottom flask equipped with a magnetic stirrer and condenser. The reaction mixture was stirred and refluxed at 78 °C for 2 hours under a nitrogen atmosphere. Upon completion, the solvent was removed under reduced pressure, and the residue was diluted with water (20 mL) and extracted with dichloromethane (3 × 15 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, and concentrated to dryness. The resulting crude product was purified by silica gel column

chromatography using dichloromethane/methanol (7:5, v/v) as the eluent to afford BODIPY–OAc oxime ester precursor as a dark-red solid in good yield. ^{1}H NMR (500 MHz, CDCl$_3$) δ (ppm): 8.10 (s, 1H), 6.16 (s, 1H), 5.34 (s, 2H), 2.67 (s, 3H), 2.56 (s, 3H), 2.46 (s, 3H), 2.39 (s, 3H), 2,14 (s, 3H). ^{13}C NMR (100 MHz, CDCl$_3$) δ: 181.65, 170.61, 163.88, 159.55, 155.28, 144.84, 143.41, 138.16, 134.22, 128.25, 123.81, 57.95, 20.73, 18.81, 17.51, 16.13, 15.08, 13.28 ppm. HRMS (ESI$^+$): calculated for: C$_{17}$H$_{20}$BF$_2$N$_3$O$_3$ [M+H]$^+$ 364.16385, found 364.16310.

Step 4: Formation of BODIPY–OAc Oxime Ester (Compound **3a**)

   To a stirred solution of BODIPY–OAc oxime ester precursor (previously synthesized, 1 equiv) in anhydrous dichloromethane (20 mL), a mixture of trimethylamine (50 mL) and DCM (1 mL) was added dropwise under a nitrogen atmosphere at room temperature. After 15 minutes of stirring, a separate solution containing acid chloride (50 mL) and DCM (1 mL) were added slowly to the reaction mixture to initiate esterification. The reaction was maintained under anhydrous conditions and stirred for 4 hours at ambient temperature (25–30 °C). Upon completion, the organic phase was washed with brine. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography using hexane/ethyl acetate (7:5, v/v) as the eluent to yield BODIPY–OAc Oxime Ester **3a** as a red crystalline solid. ^{1}H NMR (500 MHz, CDCl$_3$) δ (ppm): 8.40 (s, 1H), 6.21 (s, 1H), 5.35 (s, 2H), 2.72 (s, 3H), 2.58 (s, 3H), 2.57 (s, 3H), 2.51 (s, 1H), 2.41 (s, 3H), 2.14 (s, 3H), 1.71 (s, 2H), 1.50 (s, 2H), 1,39 (s, 4H), 0.93 (s, 6H). ^{13}C NMR (100 MHz, CDCl$_3$) δ: 174.02, 170.52, 161.34, 155.23, 150.19, 144.64, 139.15, 134.80, 131.71, 130.53, 124.63, 120.11, 57.80, 34.81, 20.83, 20.70, 19.16, 16.31, 15.22, 14.15 ppm. HRMS (ESI$^+$): C$_{25}$H$_{34}$BF$_2$N$_3$O$_4$H$^+$ Calculated Mass: 490.26832, Experimental Mass: 490.26630.

Step 4: Formation of BODIPY–OAc Oxime Ester (Compound **3b**)

   ^{1}H NMR (500 MHz, CDCl$_3$) δ (ppm): 8.57(s,1H), 8.07(s, 2H), 6.95(s, 2H), 6.22(s, 1H), 5.37(s, 2H), 3.88(s, 3H), 2.76(s, 3H), 2.62(s, 3H), 2.59(s, 3H), 2.42(s, 3H), 2.15(s, 3H). ^{13}C NMR (500 MHz, CDCl$_3$) δ: 170.79, 170.66, 164.17, 163.81, 154.98, 150.68, 147.98, 144.39, 133.00, 132.50, 131.91, 124.66, 121.67, 114.32, 113.99, 113.91, 57.82, 55.64, 20.73, 16.33, 15.24, 14.34, 13.73 ppm. HRMS (ESI$^+$): C$_{25}$H$_{26}$BF$_2$N$_3$O$_5$H$^+$ Calculated Mass: 498.20063, found 498.19906.

3. Photophysical Properties

(1) 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 cuvettes. Fluorescence emission and excitation spectra were obtained using an Edinburgh Photonics FLS980 spectrometer equipped with a thermoelectric cooled photo multiplier detector (Hamamatsu) and a liquid-nitrogen cooled NIR-photomultiplier detector (Hamamatsu). All measurements were conducted 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.

(2) Fluorescence lifetimes (τ_F) were determined by 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 determined 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. Fluorescence quantum yields were determined by a reference point method. Fluorescein as standard / exe at 510 nm in MeOH (ϕ_f) =0.7 was used as a standard sample to calculate the Φs of **3a** and **3b**. The equation is as follows.

$$\frac{\phi_s}{\phi_R} = \frac{A_s}{A_R} \frac{(Abs)_R}{(Abs)_s} \frac{\eta_s^2}{\eta_R^2}$$

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.

(3) Photochemical properties and stabilities of BODIPY oxime esters (**3a** and **3b**) and measurement of photochemical quantum yields: Photolysis of Compound **3a** (2 mL of 1×10^{-5} M in MeOH/ Water (1/1 v/v) and Compound **3b** (2 mL of 1×10^{-5} M in MeOH/Water (1/1 v/v)) was

placed in quartz cuvette (10 mm path). The cuvette was placed in front of a light source (Thorlabs's M505L3) mounted LED has a nominal wavelength 505 ± 30 nm with a power density of ≈ 10 mW/cm^2, at 1 cm distance and irradiated for the indicated times. The power density was measured by PM100D Compact Power and Energy 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 plotted. After integrating the plot, the total number of photons (light flux) was calculated by summing up the ones at different wavelengths. At regular intervals 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 ≈ 505 nm, average beam irradiance ≈ 10 mW/cm^2). According to the well-developed absorption methodology, the values of Φ_{ph} can be determined by the following equation. $\Phi_{ph} = (A_t - A_0) \, N_A/10^3 \times \varepsilon \times I \times [1-10^{(A_t+A_0)/2}] \times t$ 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).
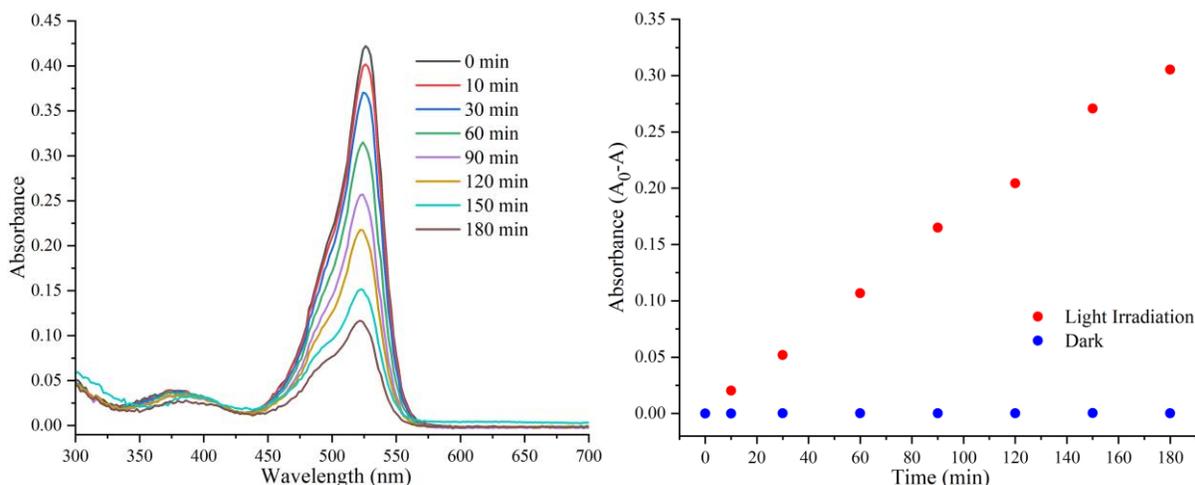
Figure S1: Left panel: UV spectra variation of compound **3a** in MeOH/Water (1/1 v/v) over time upon light exposure Right panel: $\Delta(A_0-A)$ at 505 nm Vs time is plotted, for compound **3a**.
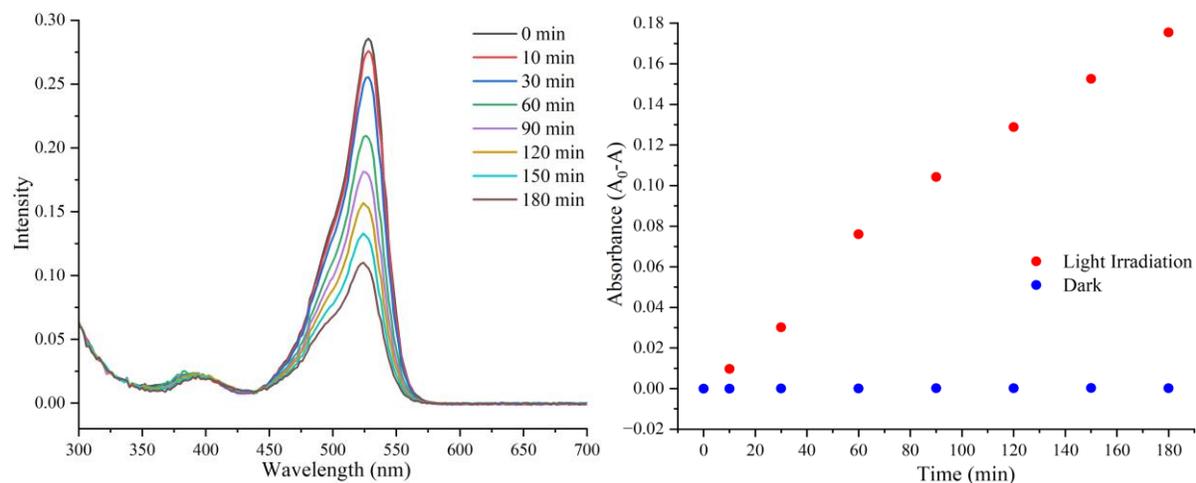
Figure S2: Left panel: UV spectra variation of compound **3b** in MeOH/Water (1/1 v/v) over time upon light exposure Right panel: $\Delta(A_0-A)$ at 505 nm Vs time is plotted, for compound **3b**.
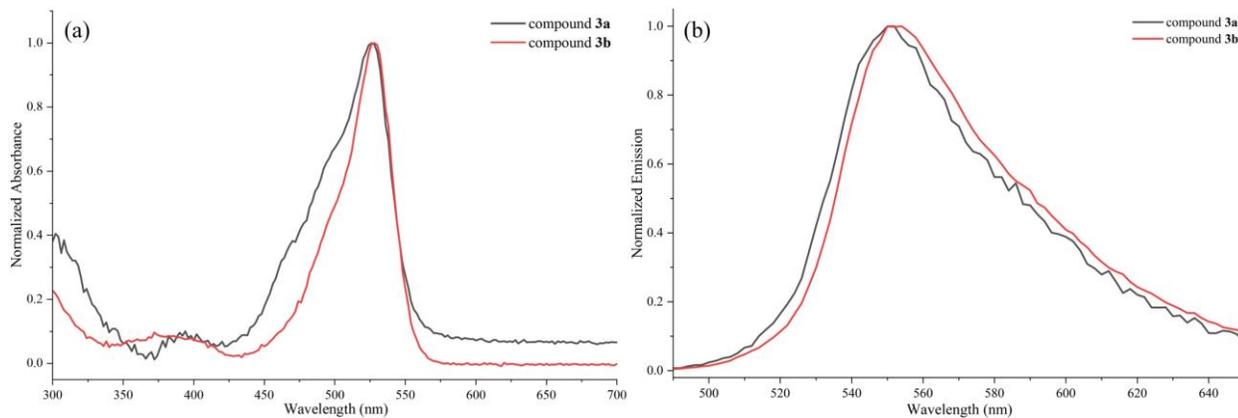
Figure S3: Normalized UV−vis absorption (a) and emission spectra (b, λ_{ex} = 552 nm for **3a** and 556 nm for **3b**) of compound **3a** and **3b** in methanol.
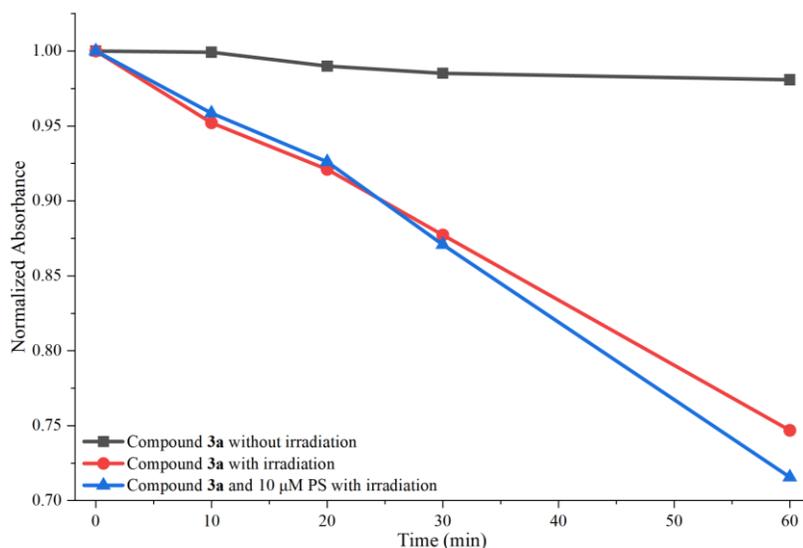
Figure S4: Time course of photolysis for the compound **3a** with different conditions (black curve: dark condition, blue curve: in presence of 10 µM of triplet quencher potassium sorbate (PS), red curve: with irradiation in MeOH/H$_2$O (1/1 v/v). Concentration of compound **3a** is 1×10^{-5} M. compound **3a** was irradiated by 505 nm LED lamp (Thorlab's M505L3, power density: 10 mW/cm^2, 1 cm irradiated distance).

(4) The physiological stability of BODIPY oxime esters **3a** and **3b** was evaluated under dark conditions. Each compound was prepared at a concentration of 1.0×10^{-5} M in a MeOH/PBS buffer (pH 7.4, 1:1 v/v) mixture and placed in a quartz cuvette. The samples were incubated at 37 °C in the absence of light. UV – vis absorption spectra were recorded at 30 min intervals, and the absorbance change (A$_0$ - A) at the maximum absorption wavelength (λ_{max} = 526 nm for **3a** and λ_{max} = 528 nm for **3b**) was plotted as a function of time.
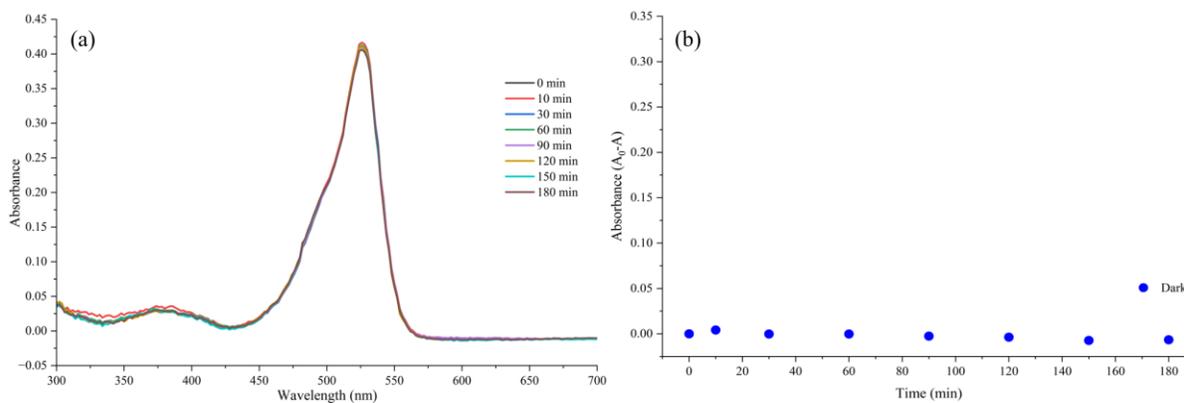
Figure S5: (a) UV spectra variation of compound **3a** in MeOH/PBS (1/1 v/v) over time in absence of light. (b): Δ(A$_0$-A) at 526 nm Vs time is plotted, for compound **3a**.
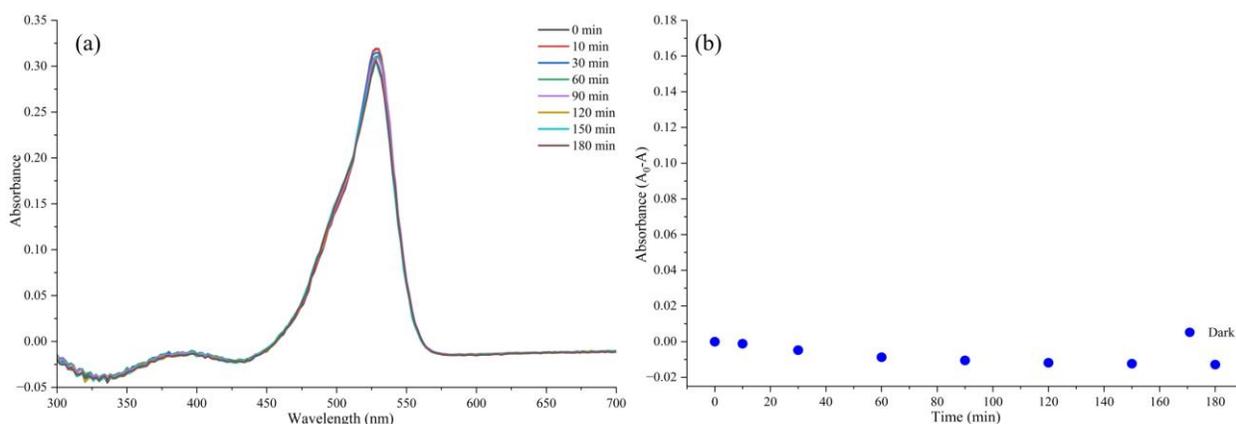
Figure S6: (a) UV spectra variation of compound **3b** in MeOH/PBS (1/1 v/v) over time in absence of light. (b): $\Delta(A_0-A)$ at 528 nm Vs time is plotted, for compound **3b**.

(5) Thermal stability of BODIPY oxime esters **(3a** and **3b)** (1×10^{-5} M in methanol) in a cuvette was merged in a water bath with controlled temperatures, 20, 40, 60 °C, separately. After the compound **3a** and **3b** solution was steady at the set temperature for 30 min, the solution slowly cooled down to room temperature for the UV-Vis measurement. Comparing the absorbance, it showed ignorable changes in varied temperatures.
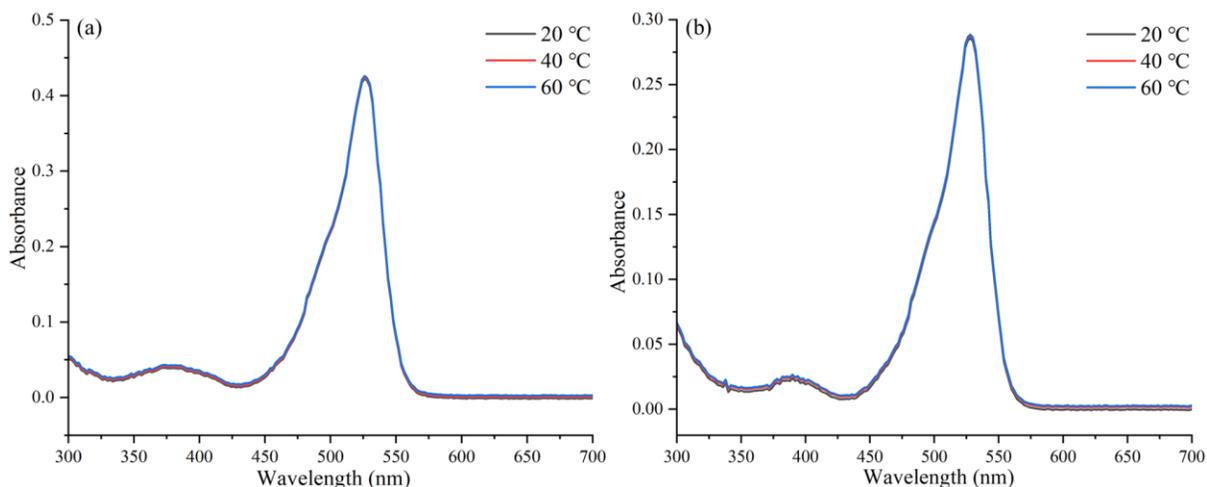
Figure S7: UV-vis absorption spectra of **3a** (a) and **3b** (b) (1×10^{-5} M in MeOH/Water (1/1 v/v)) after being kept at 20, 40, 60 ℃ for 30 min. Spectra were recorded after slowly cooling down the solution to room temperature.

(6) Qualitative analysis of 5 mL of Compound **3a** (1×10^{-5} M) in MeOH/Water (1/1 v/v)) solution was placed in quartz cuvette (10 mm path). They were irradiated under mounted LED has a

wavelength of 505 ± 30 nm with the output power of ≈ 10 mW·cm². At regular intervals of time, 100 μL of the aliquots were taken and analyzed by LC-MS using mobile phase acetonitrile/water (95/5 v/v), at a flow rate of 0.2 mL/min. The peak areas were determined by LC, which indicated gradual increase in peak areas with time.
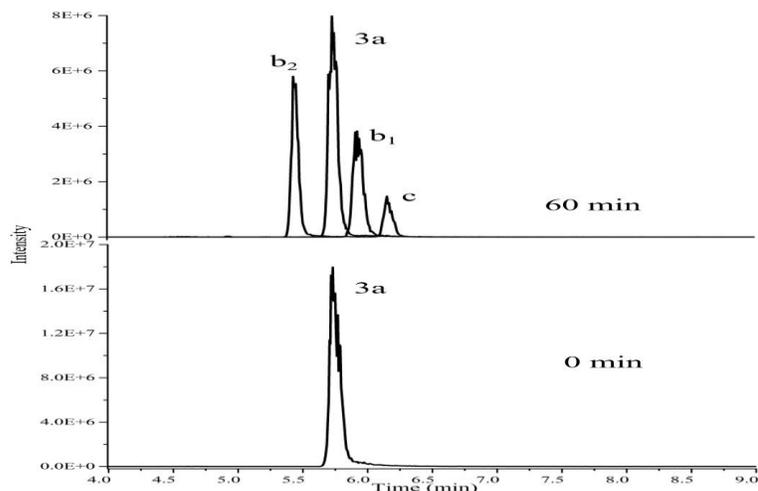
Figure S8: The LC profile of time-dependent compound **3a** using green LED (505 nm) irradiation, time point of 0 and 60 min.
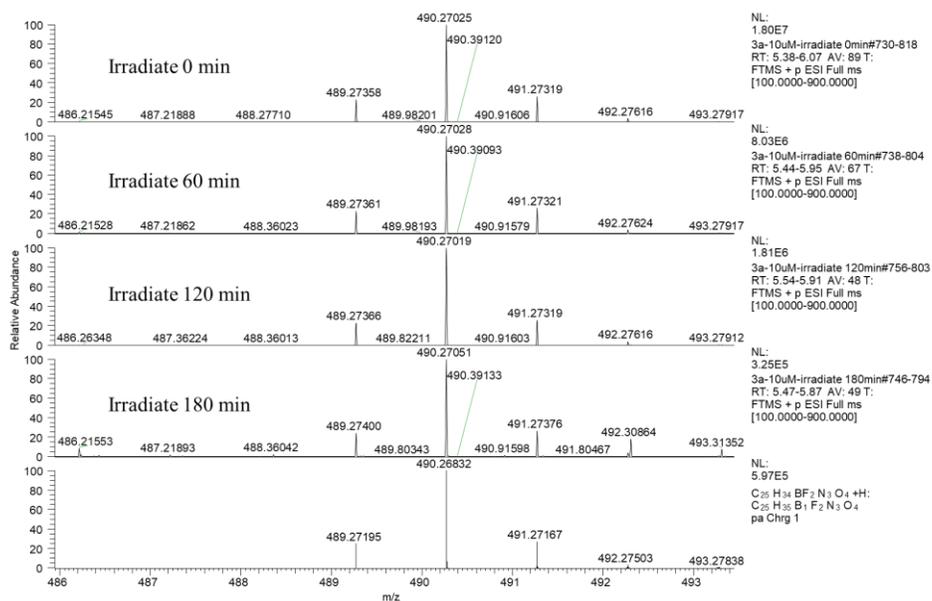
Figure S9: HRMS (ESI+) of Compound **3a** found in MS spectra after varied irradiation times: calculated for $C_{25}H_{34}BF_2N_3O_4$ [M+H]+ 490.26832, found 490.27025.
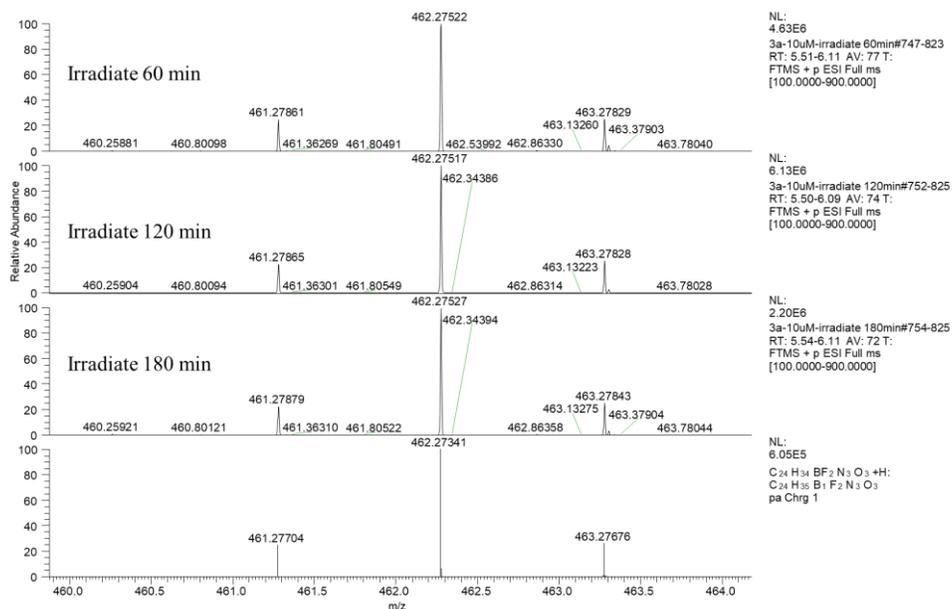
Figure S10: HRMS (ESI+) of intermediate b_1 found in MS spectra after varied irradiation times: calculated for $C_{24}H_{34}BF_2N_3O_3$ [M+H]$^+$ 462.27341, found 462.27522.
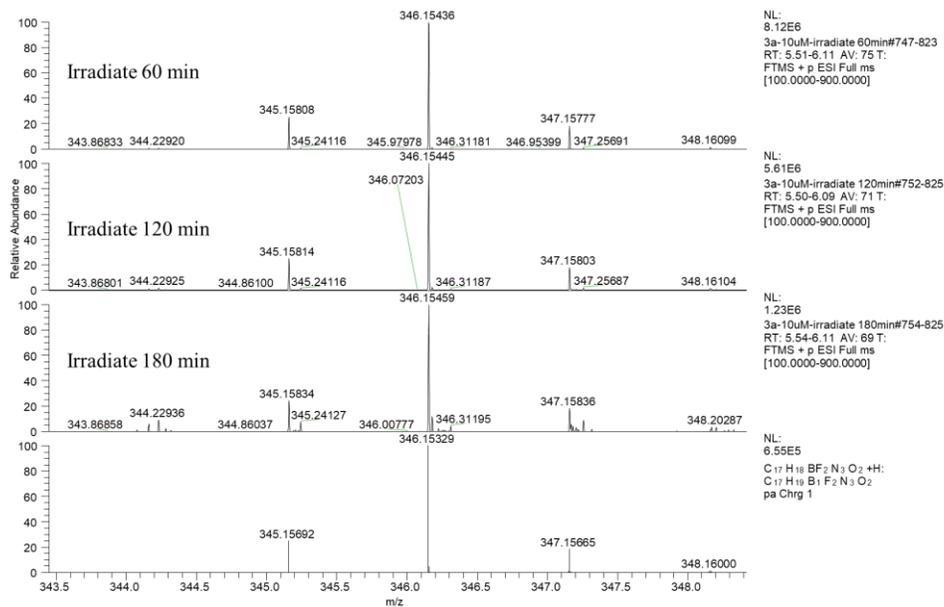
Figure S11: HRMS (ESI+) of intermediate b_2 found in MS spectra after varied irradiation times: calculated for $C_{17}H_{18}BF_2N_3O_2$ [M+H]$^+$ 346.15329, found 346.15436.
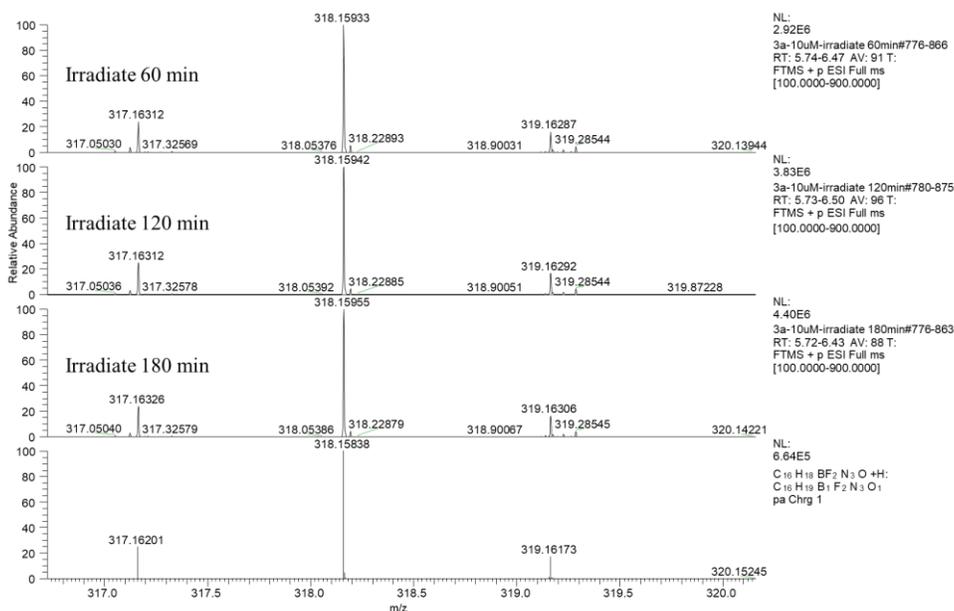
Figure S12: HRMS (ESI+) of product **c** found in MS spectra after varied irradiation times: calculated for $C_{16}H_{18}BF_2N_3O$ [M+H]$^+$ 318.15838, found 318.15933.
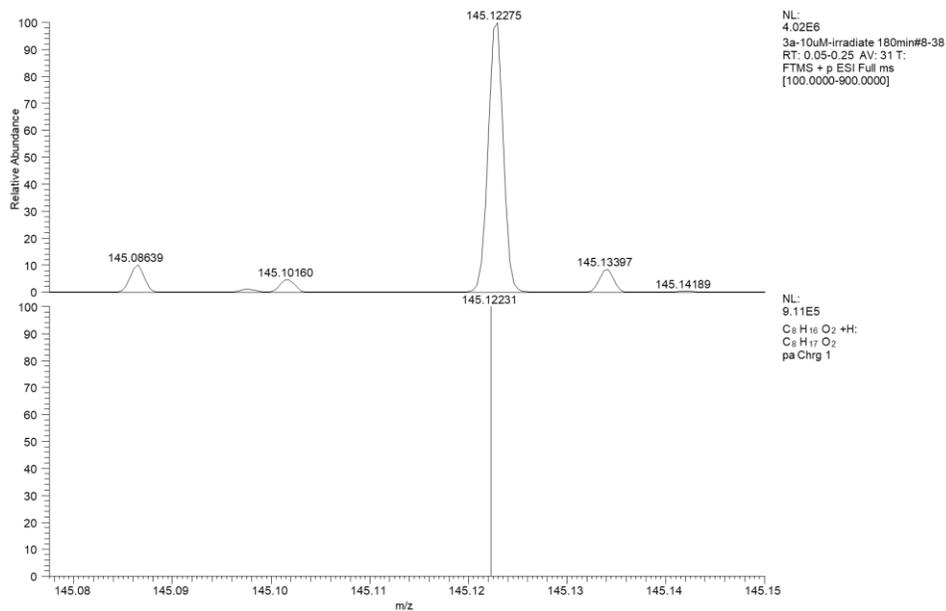
Figure S13: HRMS (ESI+) of valproic acid (VPA) found in MS spectrometry after irradiate **3a** for 60 min under green LED light: calculated for $C_8H_{16}O_2$ [M+H]$^+$ 145.12231, found 145.12275.

(7) Preparative photolysis: 5 mg of Compound **3a** in MeOH/Water (1/1 v/v)) solution were irradiated under mounted LED has a wavelength of 505 ± 30 for 5 h. At regular interval of time,

the reaction was monitored by TLC. The photoproduct **c** was analyzed by ^{1}H, ^{13}C NMR and Mass analysis.
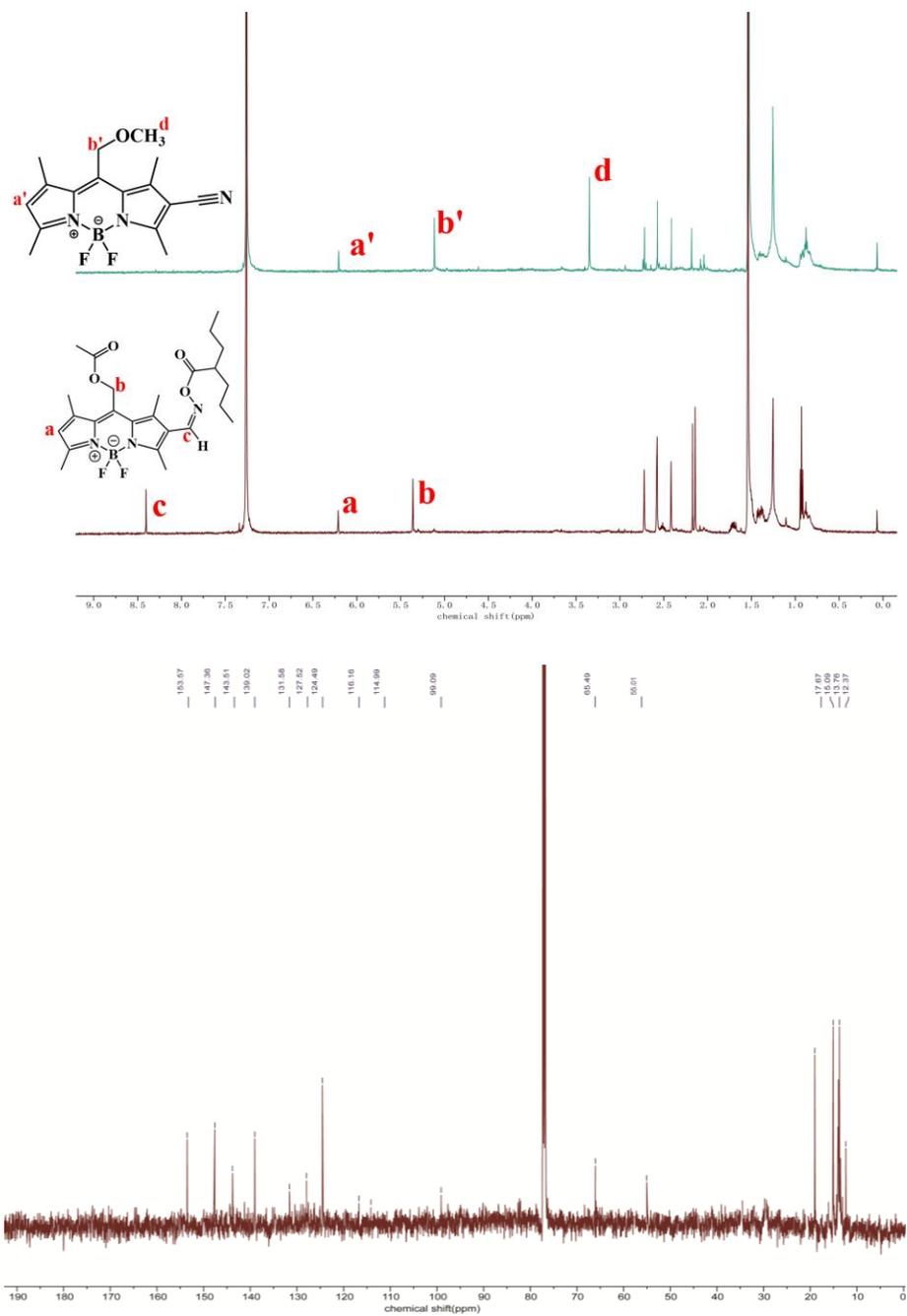
Figure S14: ^{1}H and ^{13}C NMR spectra of product **c**.
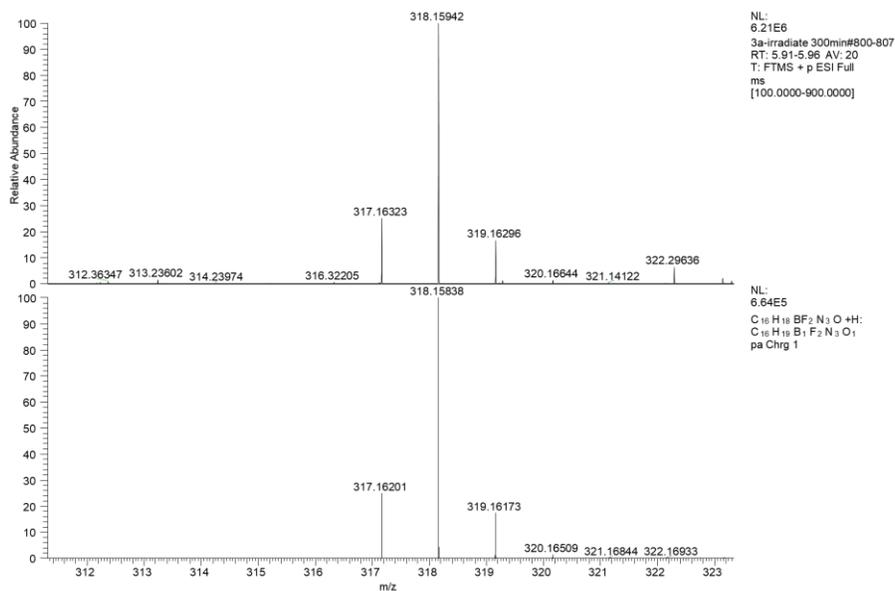
Figure S15: HRMS (ESI+) of product **c** after 5 hours' irradiation: calculated for $C_{16}H_{18}BF_2N_3O$ [M+H]$^+$ 318.15838, found 318.15942.

4. Characterization Data

i. BODIPY–OAc
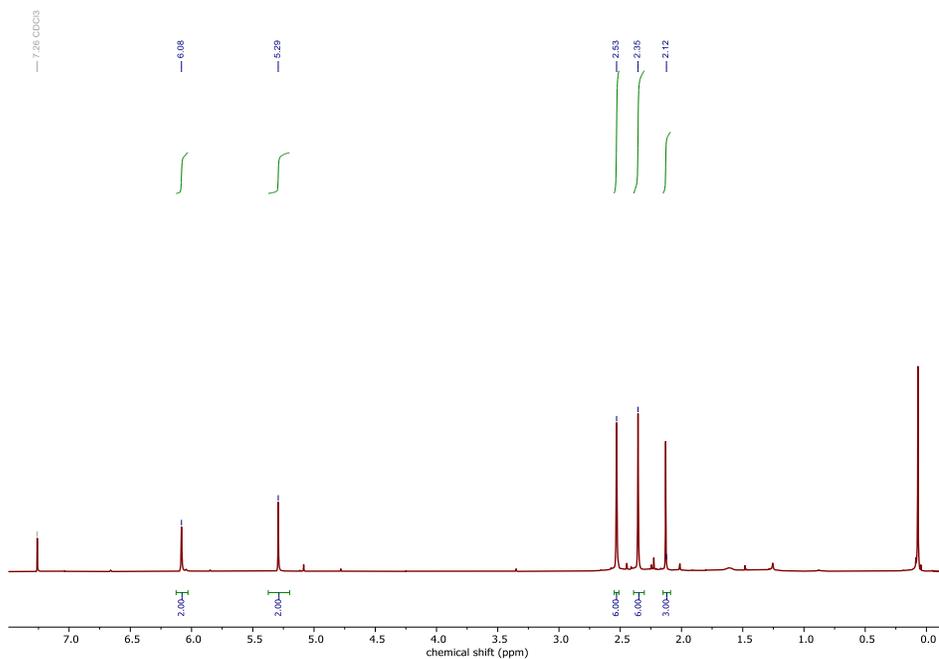
^{1}H NMR of BODIPY–OAc.
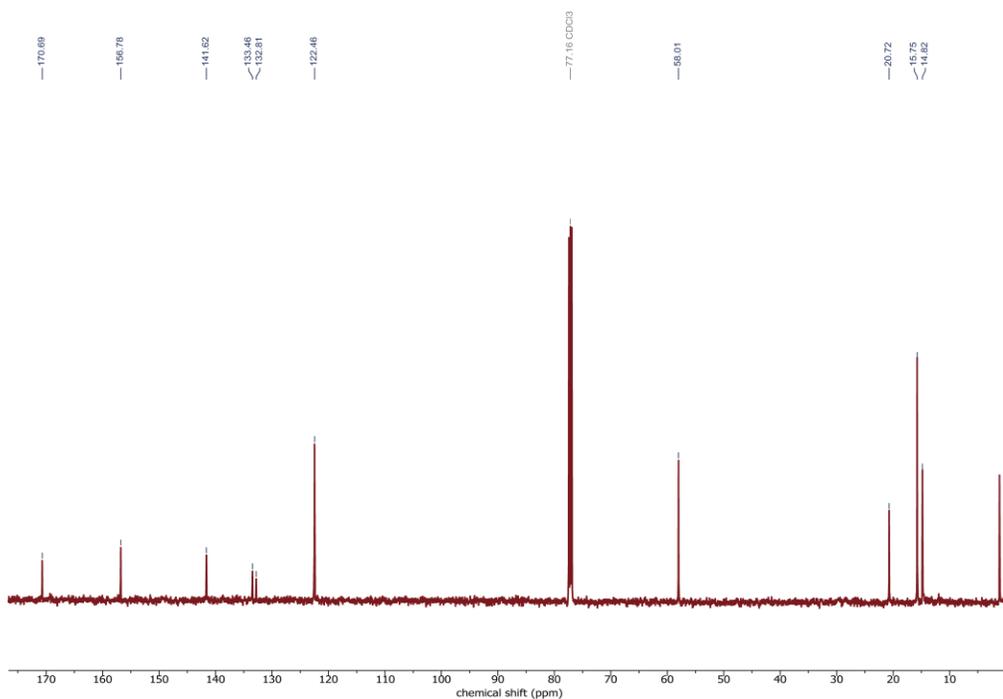
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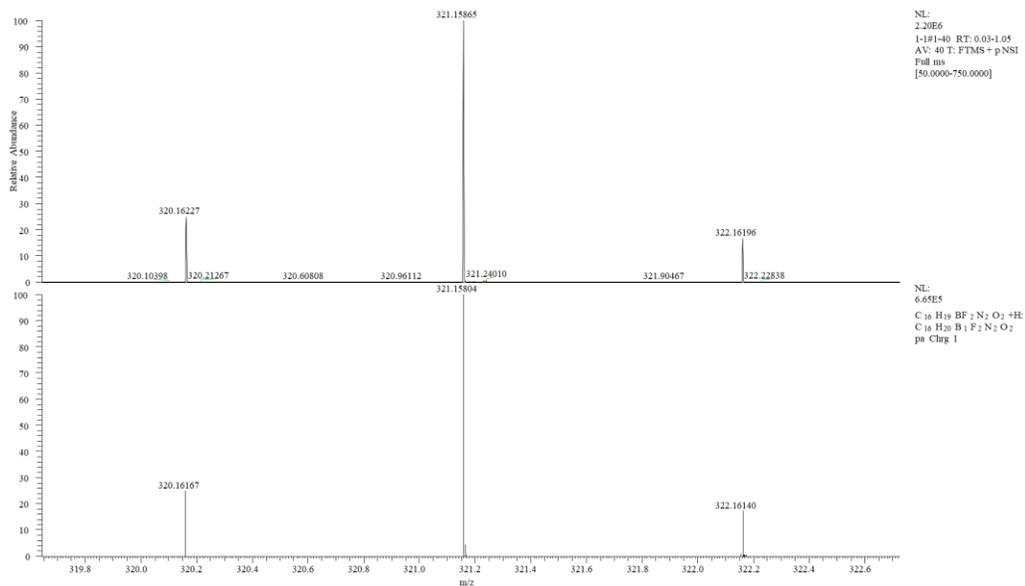
HRMS (ESI$^+$) of BODIPY–OAc: calculated for $C_{16}H_{19}BF_2N_2O_2$ [M+H]$^+$ 321.15804, found 321.15865.

ii.   BODIPY–OAc aldehyde (Compound **1**)
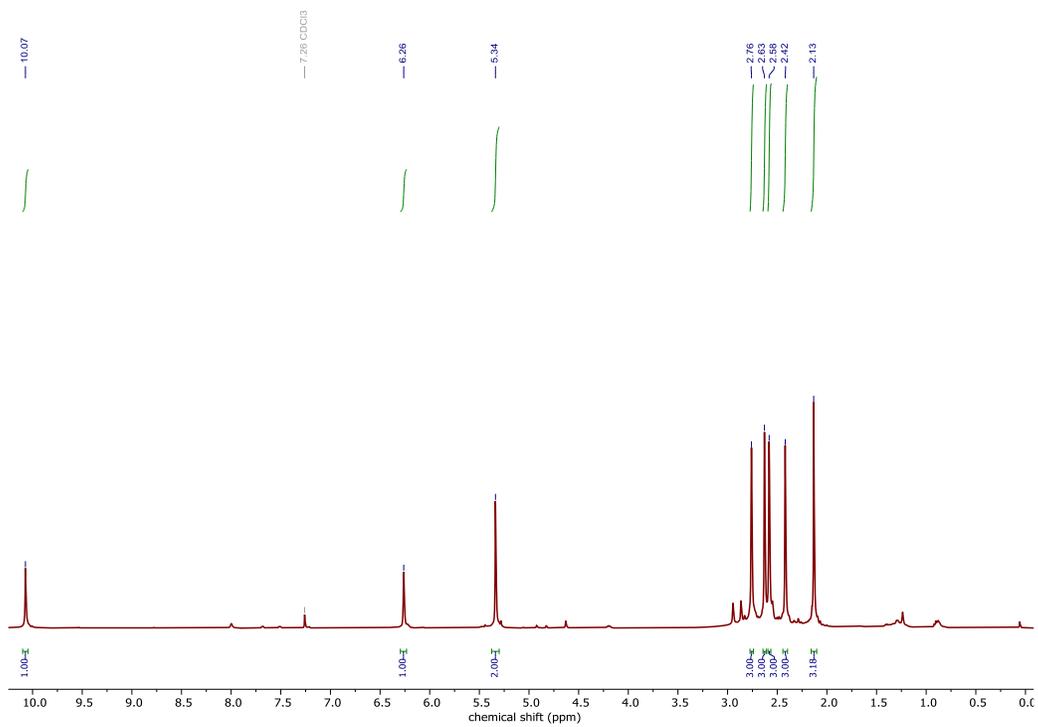
^{1}H NMR of BODIPY–OAc aldehyde (Compound **1**).
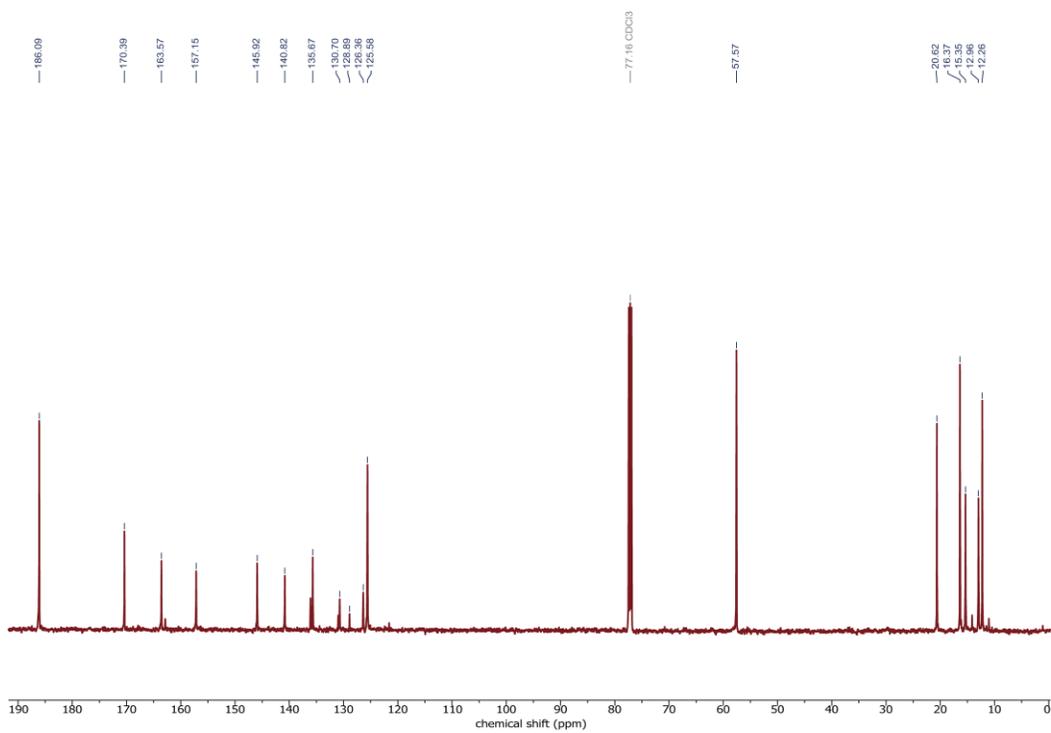
^{13}C NMR of BODIPY–OAc aldehyde (Compound **1**).
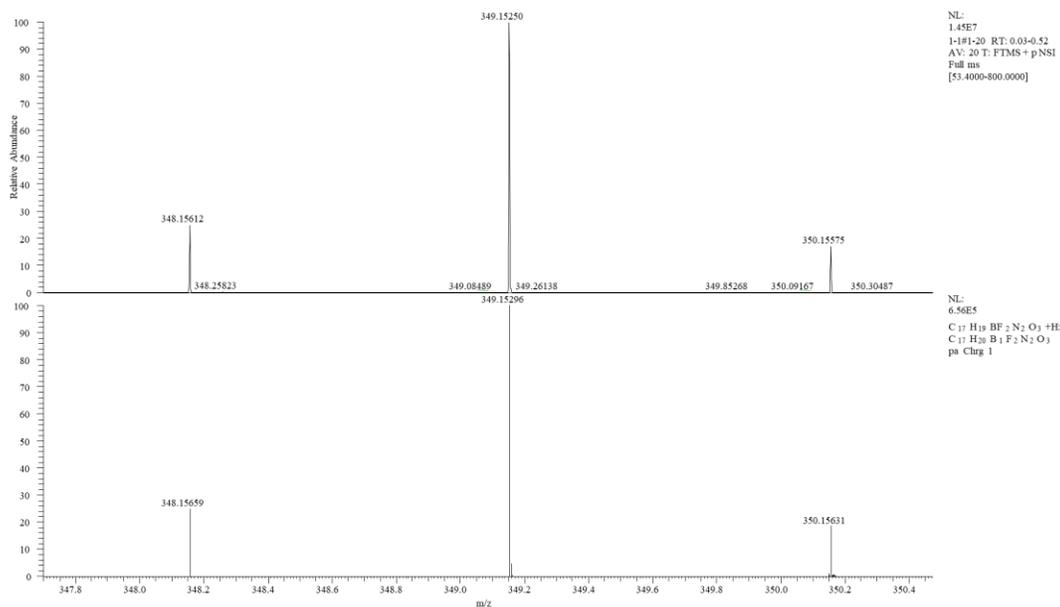
HRMS (ESI⁺) of BODIPY–OAc aldehyde (Compound **1**): calculated for $C_{17}H_{19}BF_2N_2O_3$ [M+H]⁺ 349.15296, found 349.15250.

iii. BODIPY-OAc Oxime Ester Precursor (Compound **2**)
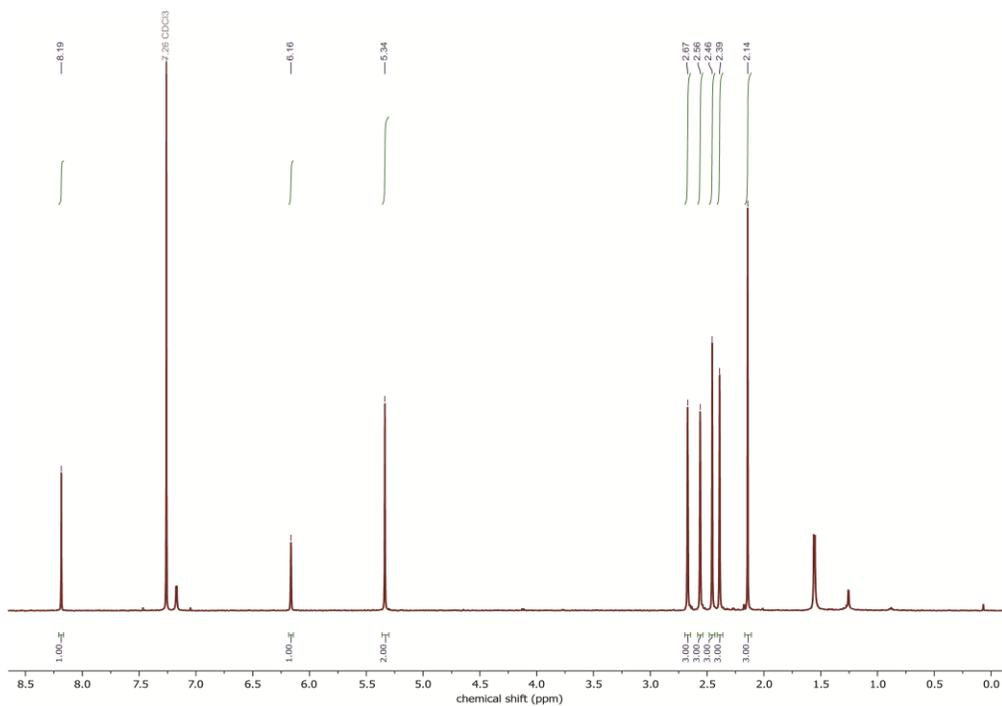
¹H NMR of BODIPY-OAc Oxime Ester Precursor (Compound **2**).
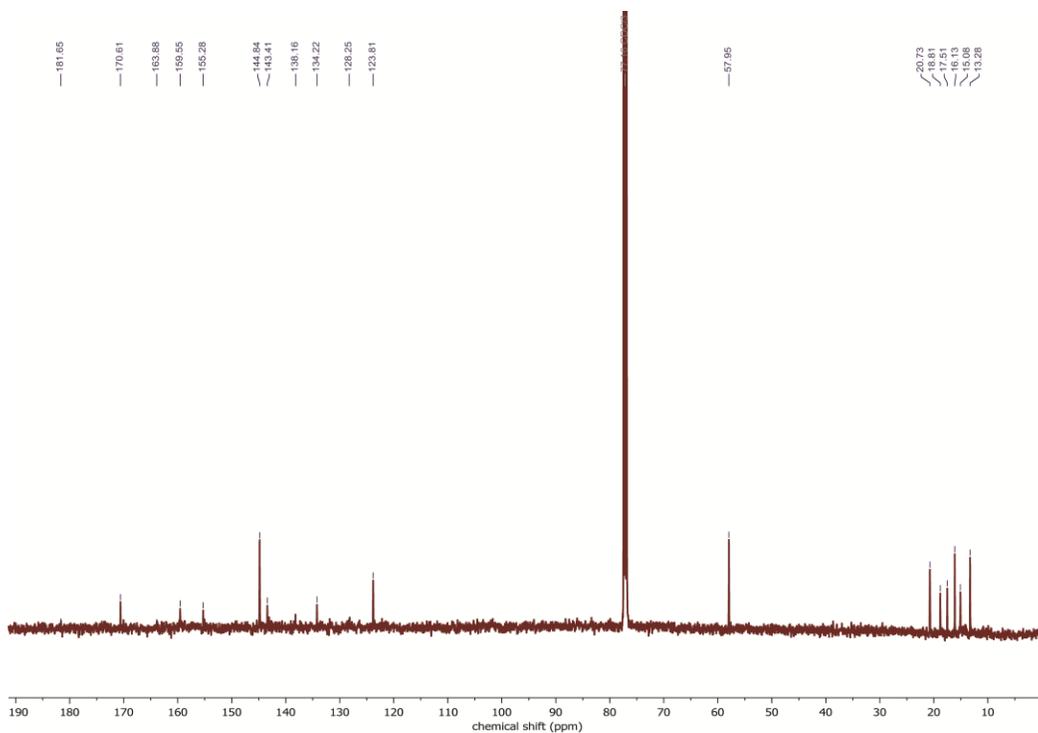
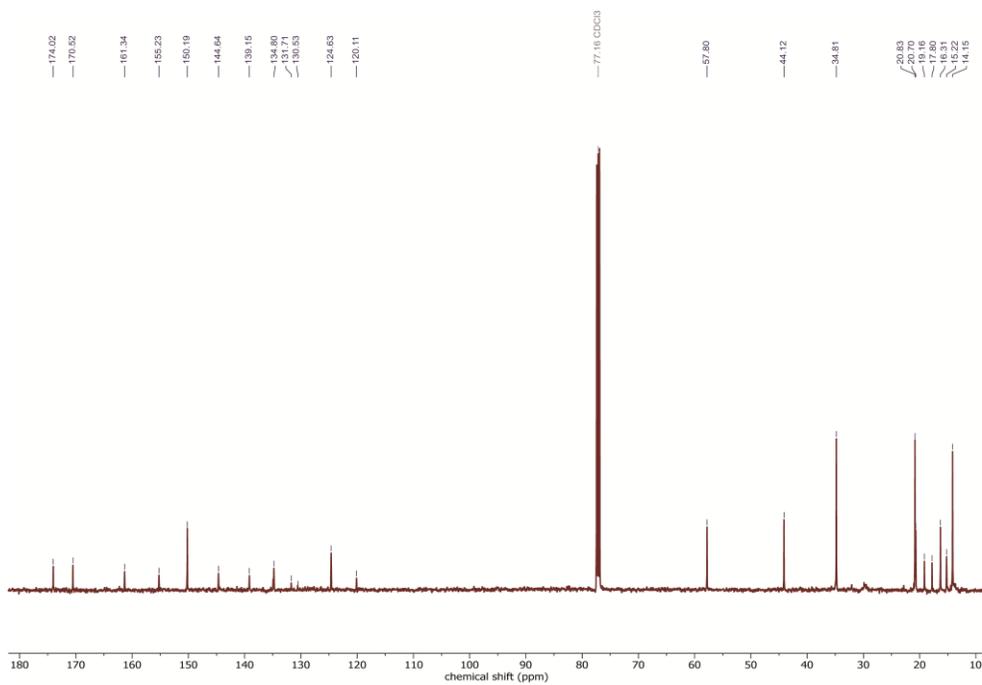^{13}C NMR BODIPY-OAc Oxime Ester Precursor (Compound **2**).
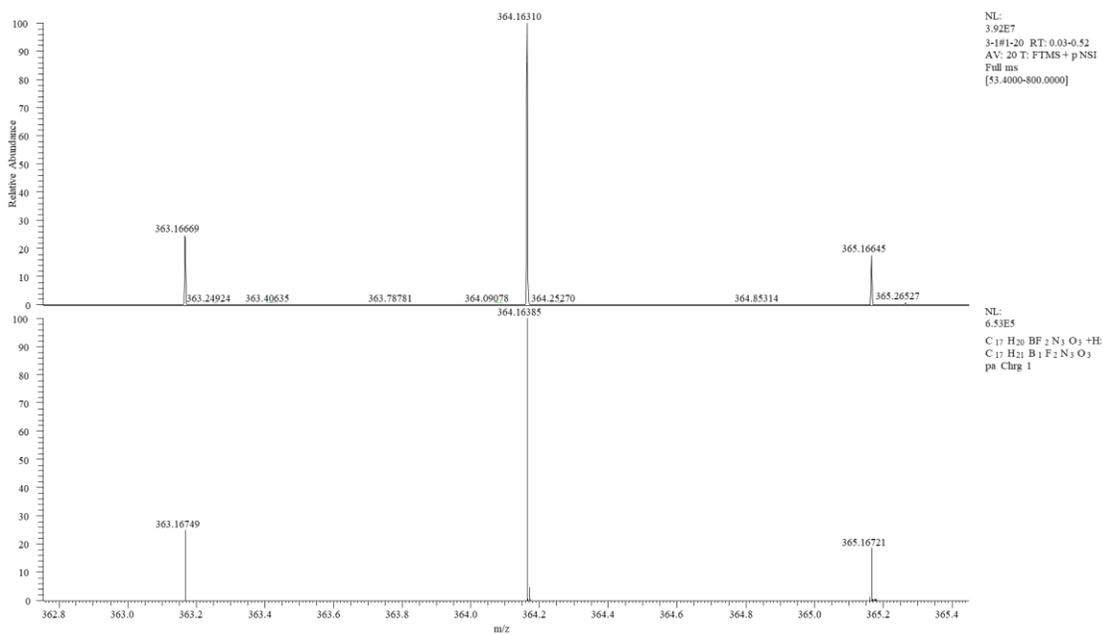
HRMS (ESI$^+$) BODIPY-OAc Oxime Ester Precursor (Compound **2**): calculated for: $C_{17}H_{20}BF_2N_3O_3$ [M+H]$^+$ 364.16385, found 364.16310.
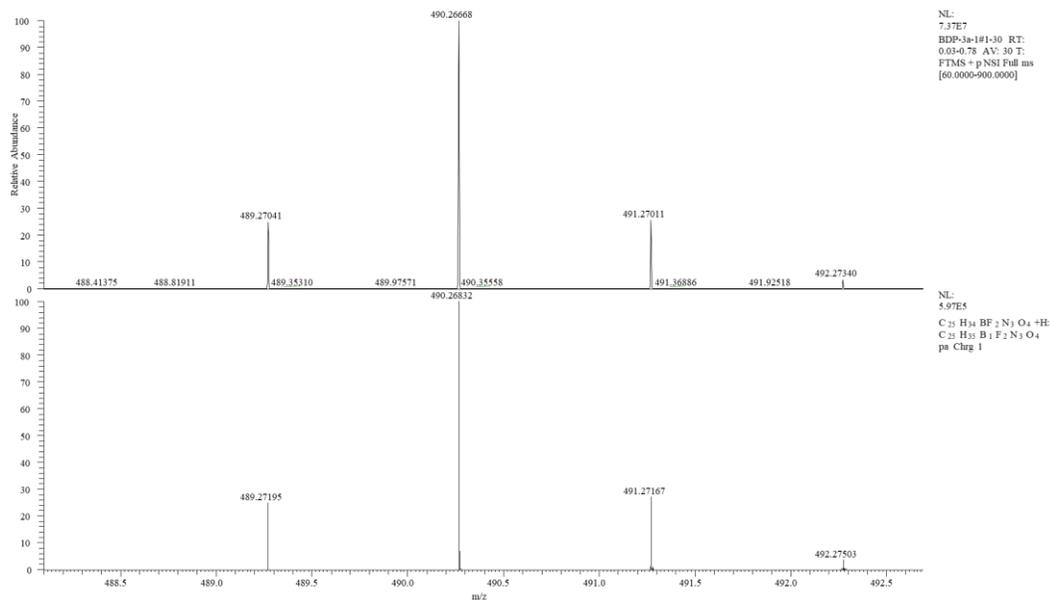
iv.   BODIPY–OAc Oxime Ester (Compound **3a**)
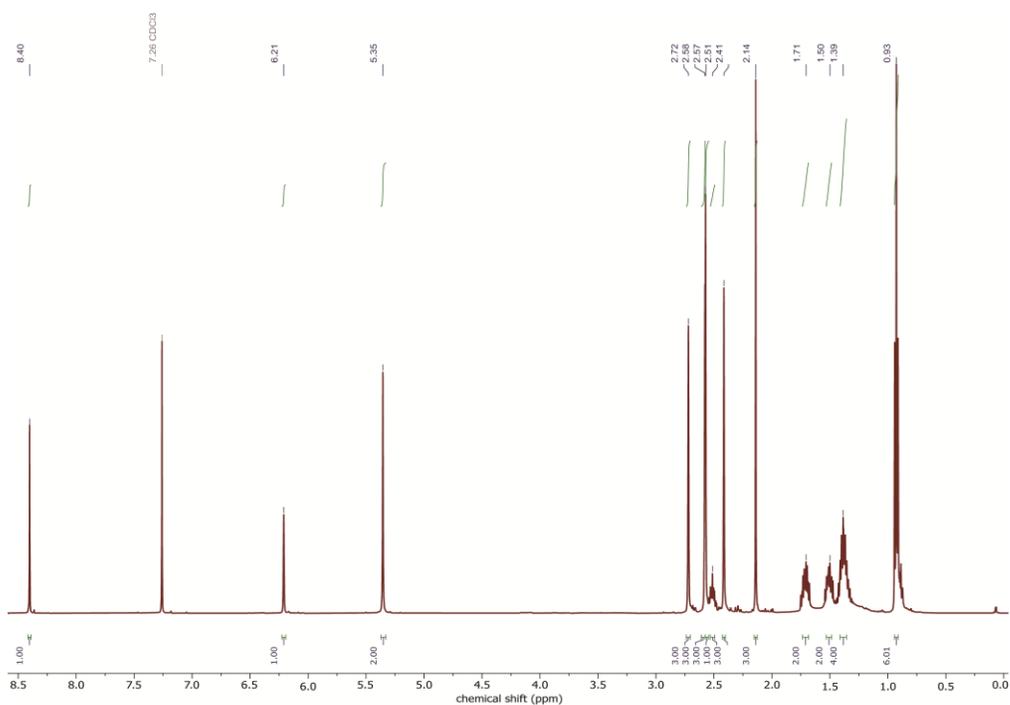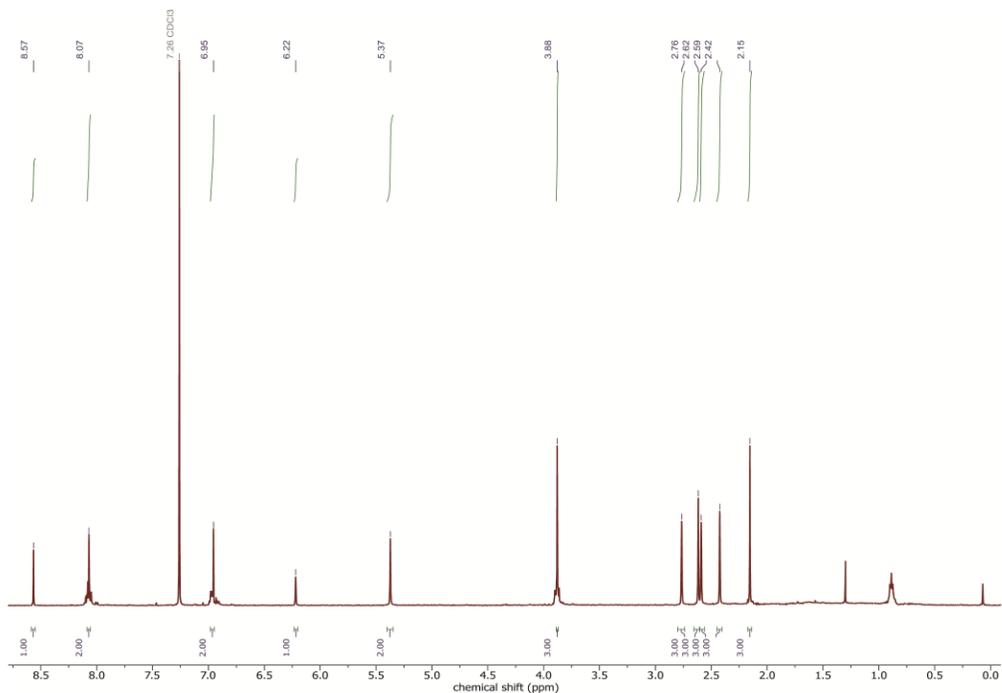

^{1}H NMR of BODIPY–OAc Oxime Ester (Compound **3a**).

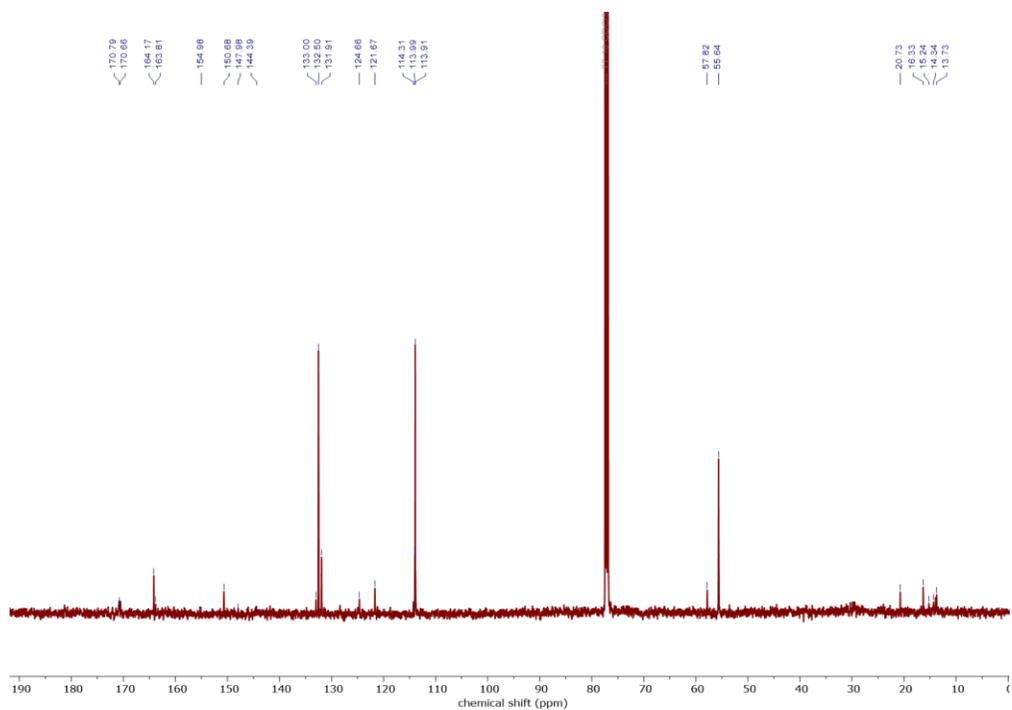
^{13}C NMR BODIPY–OAc Oxime Ester (Compound **3a**).

HRMS (ESI⁺) BODIPY–OAc Oxime Ester (Compound **3a**): calculated for $C_{25}H_{34}BF_2N_3O_4$ [M+H]⁺ 490.26832, found 490.26630.
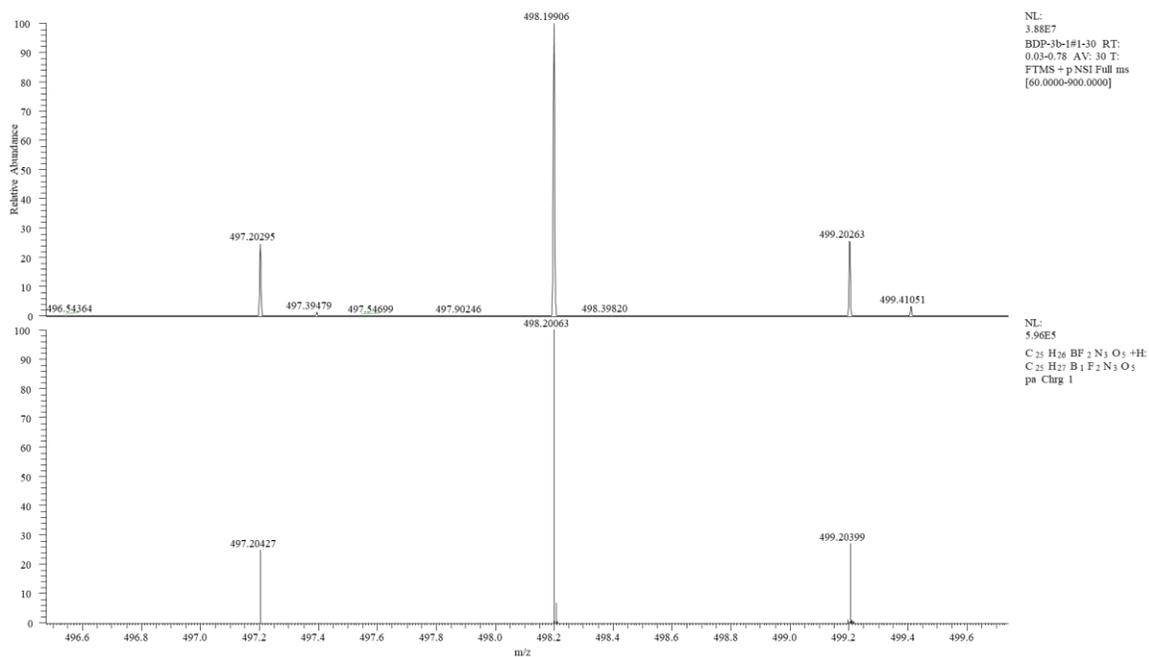
v.   BODIPY–OAc Oxime Ester (Compound **3b**)

¹H NMR of BODIPY–OAc Oxime Ester (Compound **3b**).

^{13}C NMR BODIPY–OAc Oxime Ester (Compound **3b**).

HRMS (ESI$^+$) BODIPY–OAc Oxime Ester (Compound **3b**): calculated for $C_{25}H_{26}BF_2N_3O_5$ [M+H]$^+$ 498.20063, found 498.19906.