Electronic Supplementary Information

Potent BODIPY-based photosensitisers for selective mitochondrial dysfunction and effective photodynamic therapy

Edward R. H. Walter,[‡ab] Peter Kam-Keung Leung,[‡cd] Lawrence Cho-Cheung Lee,[bc] Kenneth Kam-Wing Lo*[cd] and Nicholas J. Long*[a]

[a] *Department of Chemistry, Imperial College London, Molecular Sciences Research Hub, London, W12 0BZ, UK.*

[b] *Laboratory for Synthetic Chemistry and Chemical Biology Limited, Units 1503-1511, 15/F, Building 17 W, Hong Kong Science Park, New Territories, Hong Kong, P. R. China.*

[c] *Department of Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, P. R. China.*

[d] *State Key Laboratory of Terahertz and Millimetre Waves, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, P. R. China.*

‡ *Equal contribution from both authors.*

1. Schemes, Figures and Tables

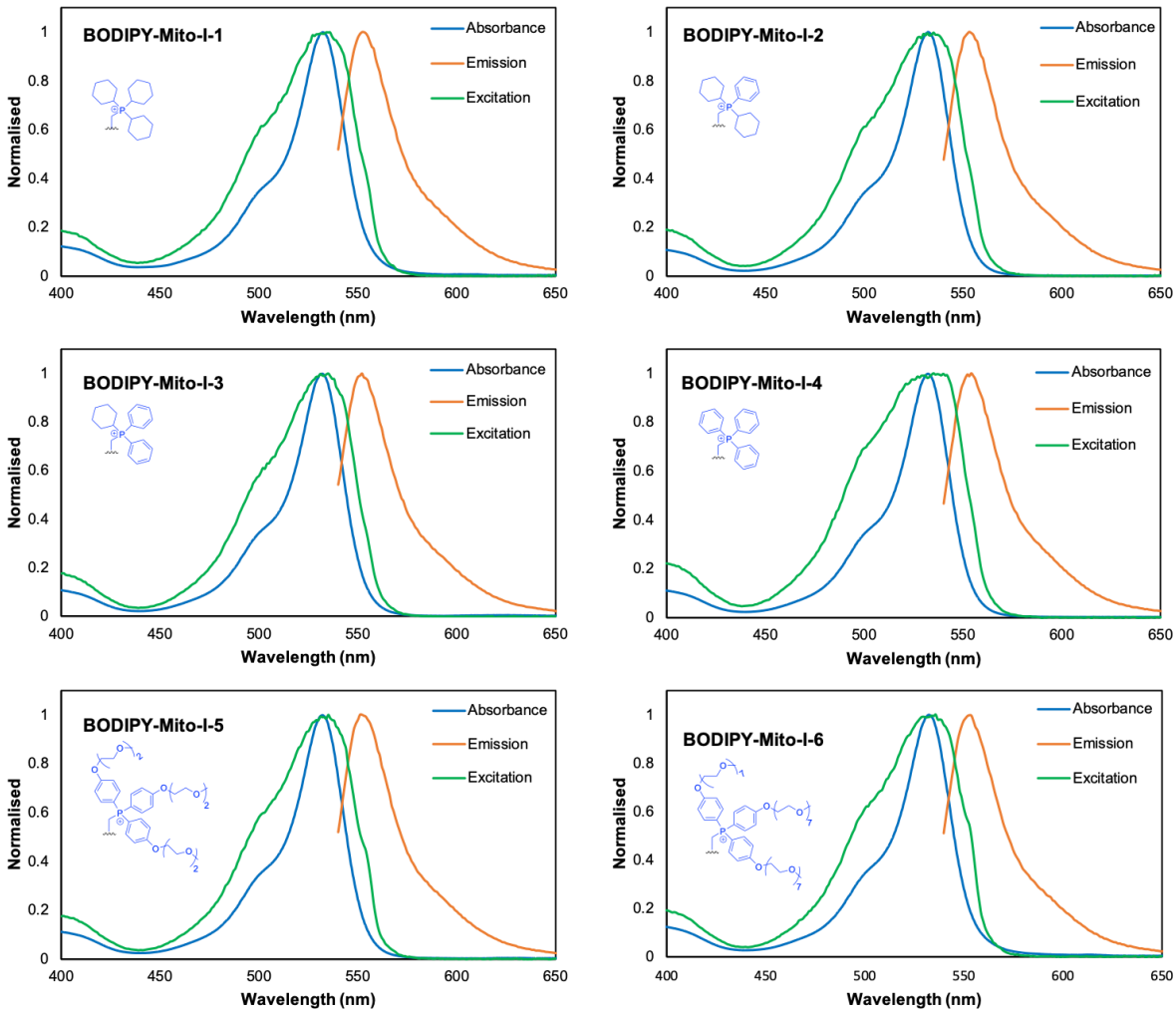


Fig. S1. Normalised absorption (*blue*), emission (*orange*) and excitation (*green*) spectra of **BODIPY-Mito-I-1** to **BODIPY-Mito-I-6** analogues. [**BODIPY**] = 10 μM, CH_3CN, 298 K. λ_{ex} = 532 nm, λ_{em} = 554 nm.

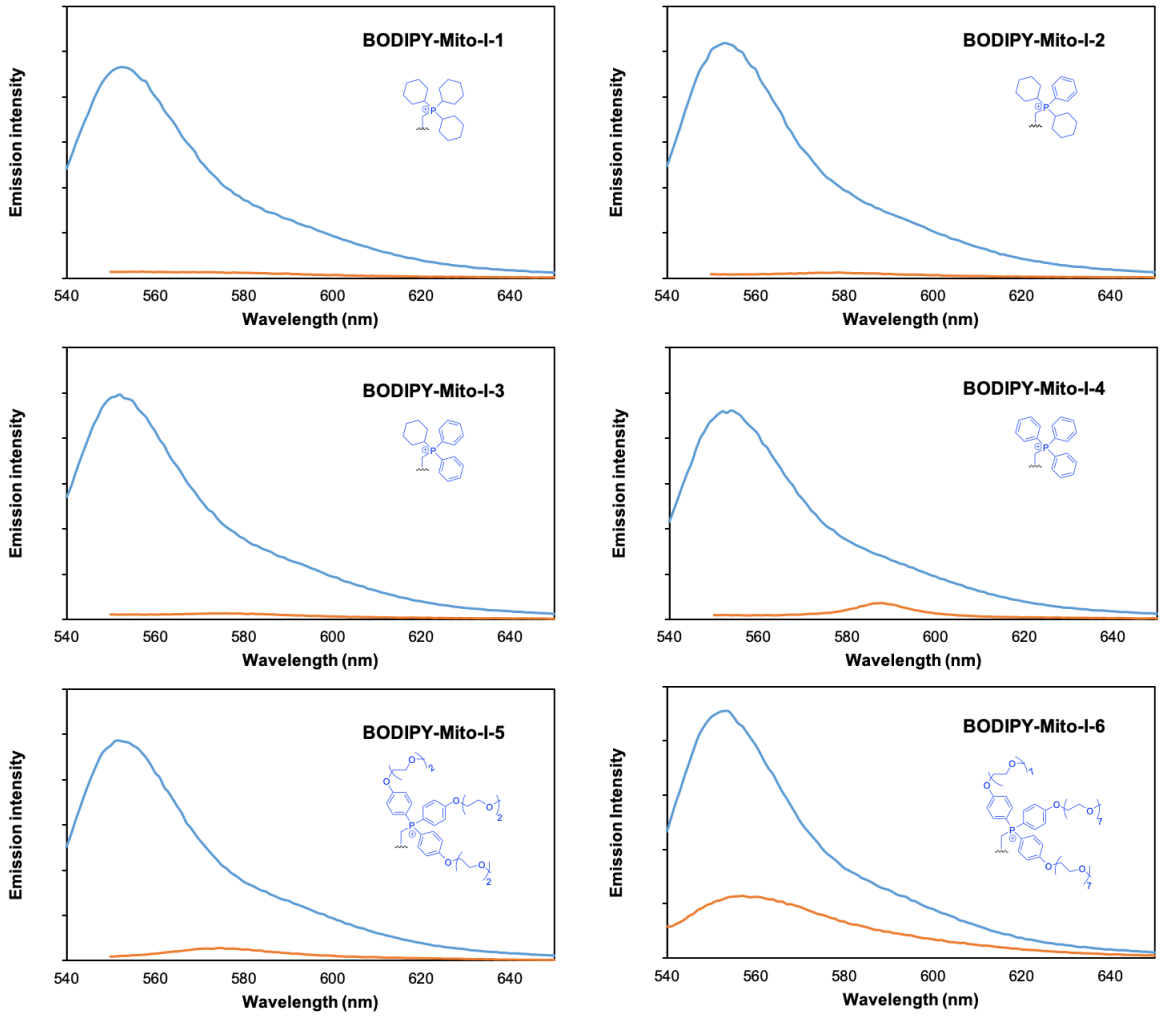


Fig. S2. Emission comparison of **BODIPY-Mito-I-*n*** analogues in CH_3CN (*blue*) and PBS buffer (pH = 7.4) (*orange*). [**BODIPY**] = 10 μM, CH_3CN, 298 K. λ_{ex} = 532 nm (CH_3CN), λ_{ex} = 539 nm (PBS buffer at pH = 7.4).

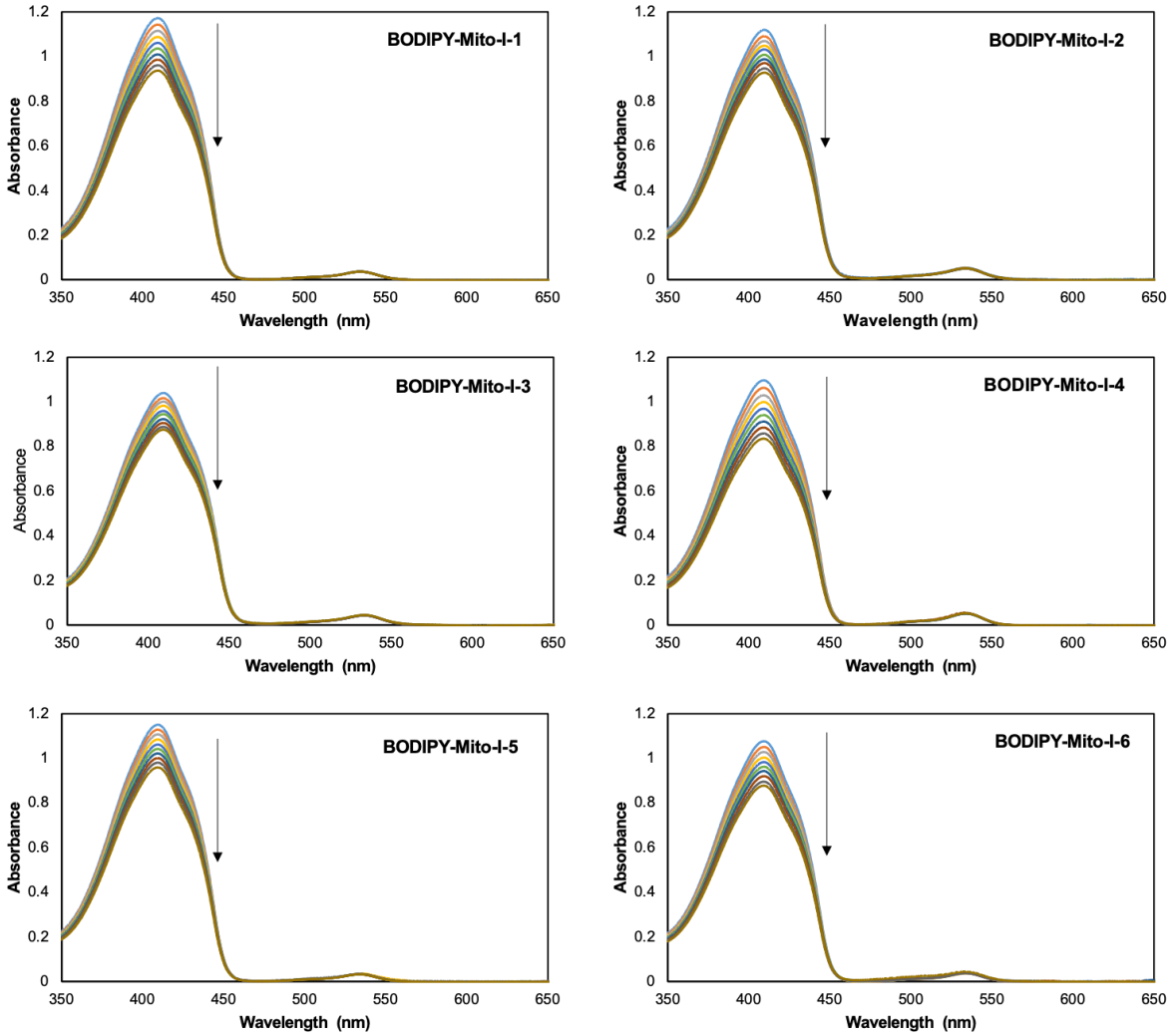


Fig. S3. The decay of absorbance of DPBF at 411 nm in aerated methanol in the presence of **BODIPY-Mito-I-*n*** following irradiation with 525 nm light for 10 s. [**DPBF**] = 50 μM, [**BODIPY**] = 0.5 μM, 298 K.

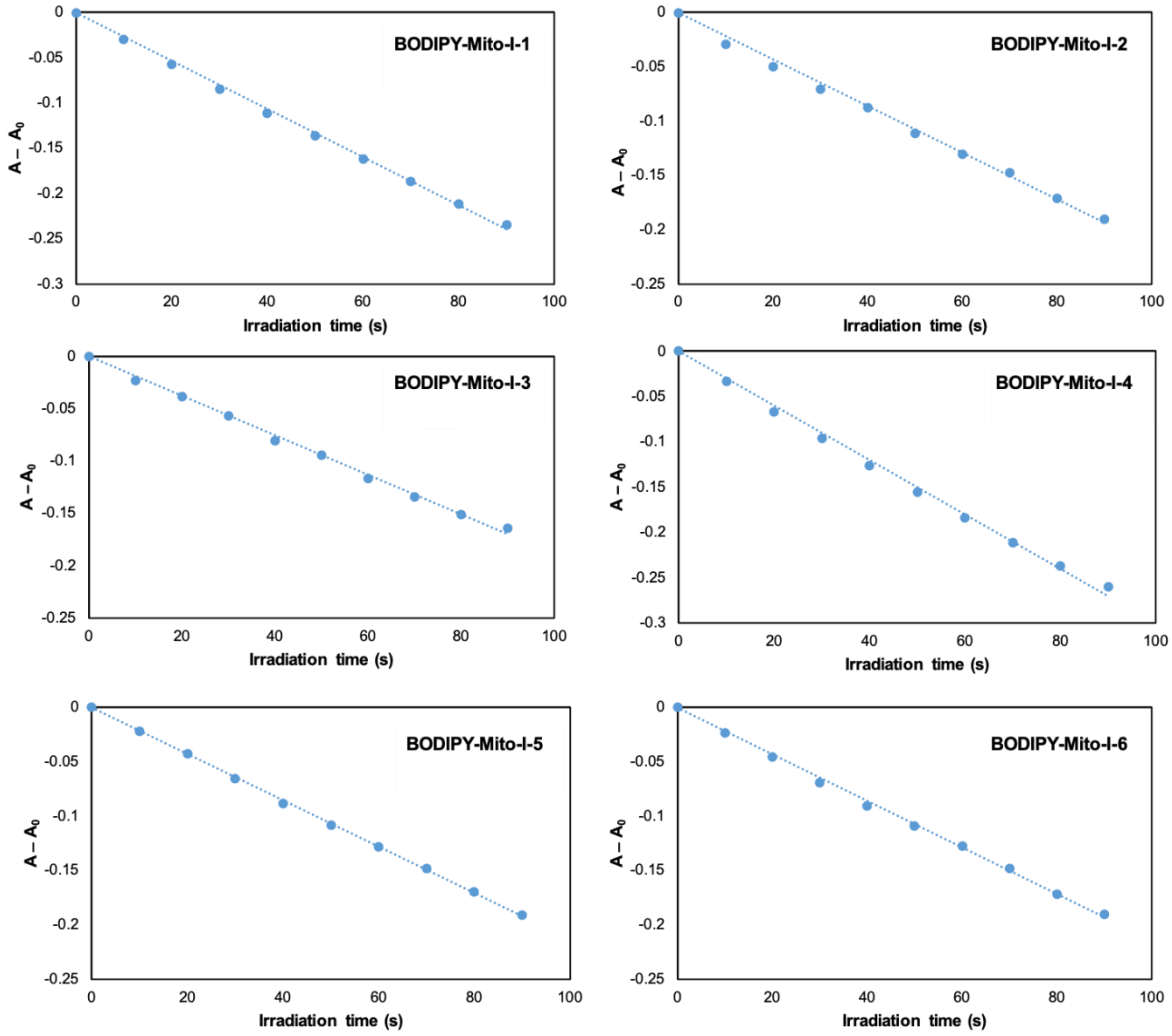


Fig. S4. Rates of decay of absorbance (A – A_0) of DPBF (50 μM) at 411 nm in aerated methanol in the presence of **BODIPY-Mito-I-*n*** following irradiation with 525 nm light.

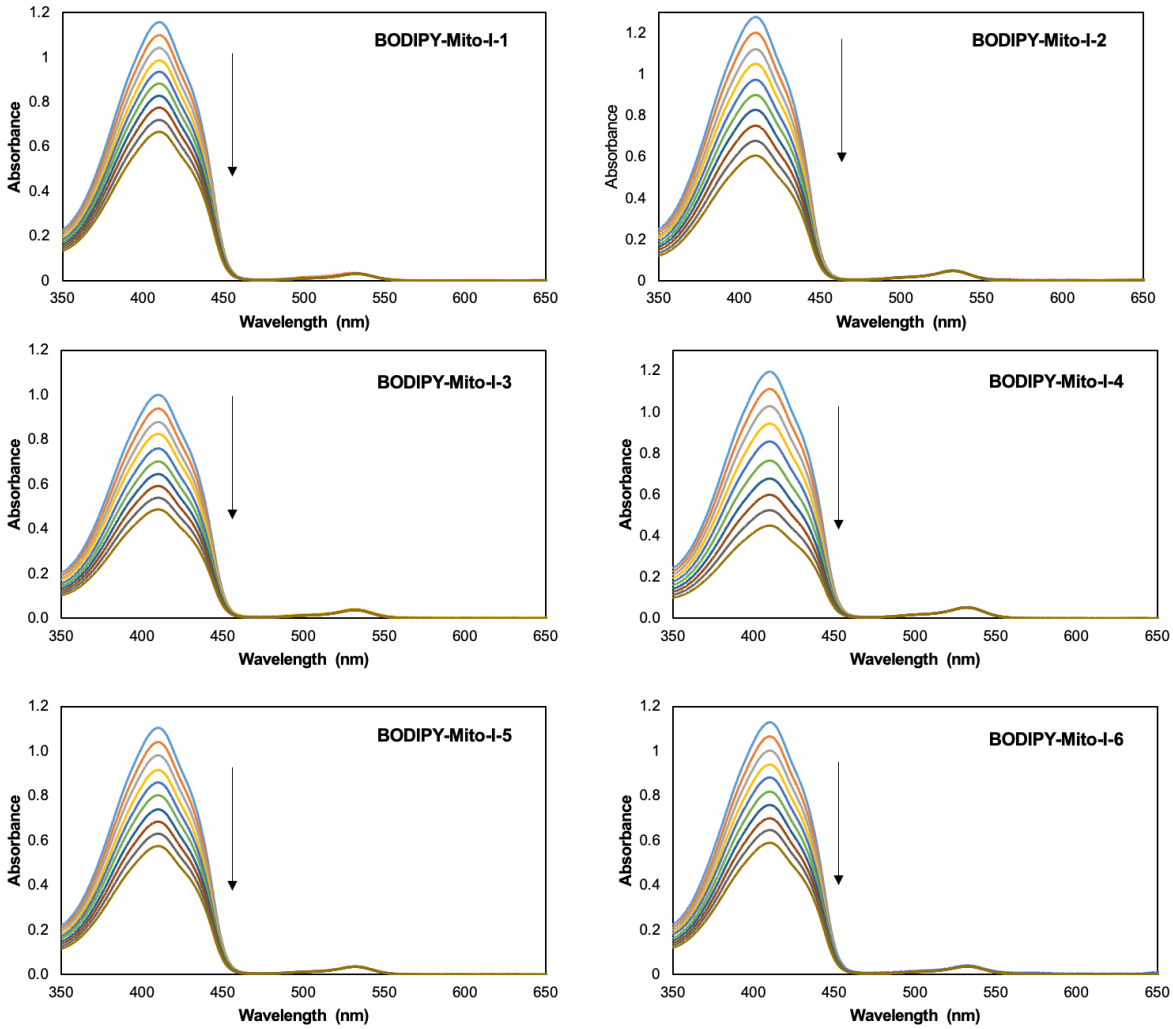


Fig. S5. The decay of absorbance of DPBF at 411 nm in aerated acetonitrile in the presence of **BODIPY-Mito-I-*n*** following irradiation with 525 nm light for 10 s. [**DPBF**] = 50 μM, [**BODIPY**] = 0.5 μM, 298 K.

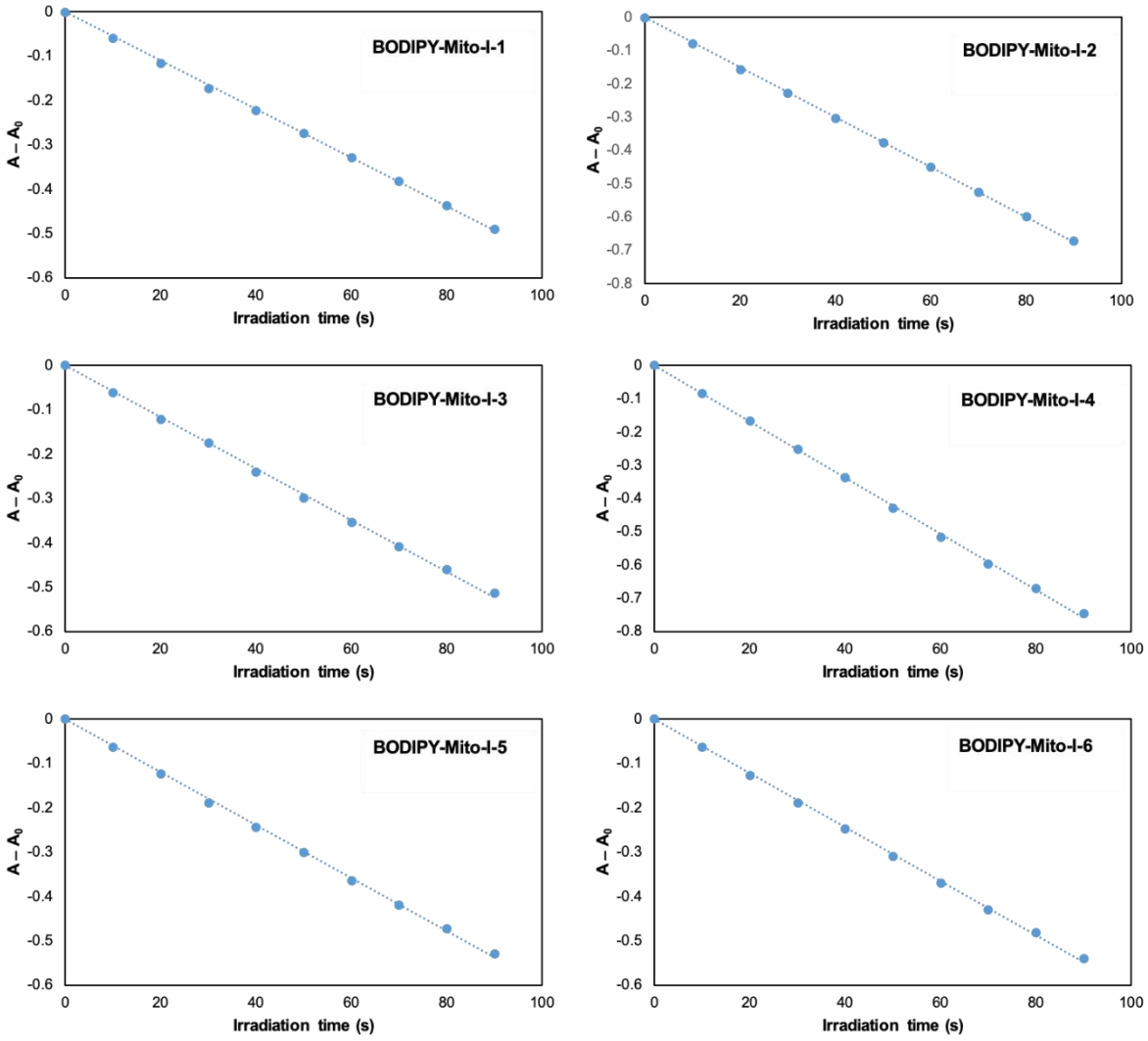


Fig. S6. Rates of decay of absorbance (A – A_0) of DPBF (50 μM) at 411 nm in aerated acetonitrile in the presence of **BODIPY-Mito-I-*n*** following irradiation with 525 nm light.

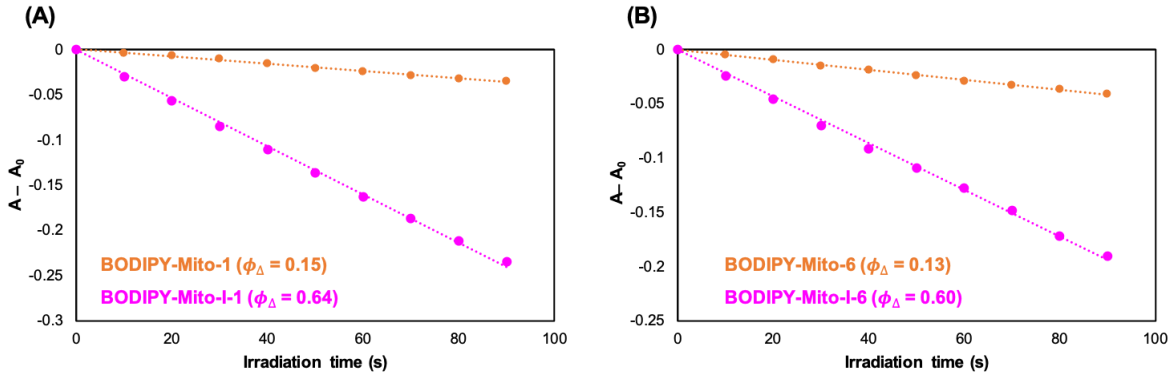


Fig. S7. Rates of decay of absorbance (A – A_0) of DPBF (50 μM) at 411 nm in aerated methanol in the presence of **(A) BODIPY-Mito-1** or **BODIPY-Mito-I-1** and **(B) BODIPY-Mito-6** or **BODIPY-Mito-I-6** following irradiation with 525 nm light.

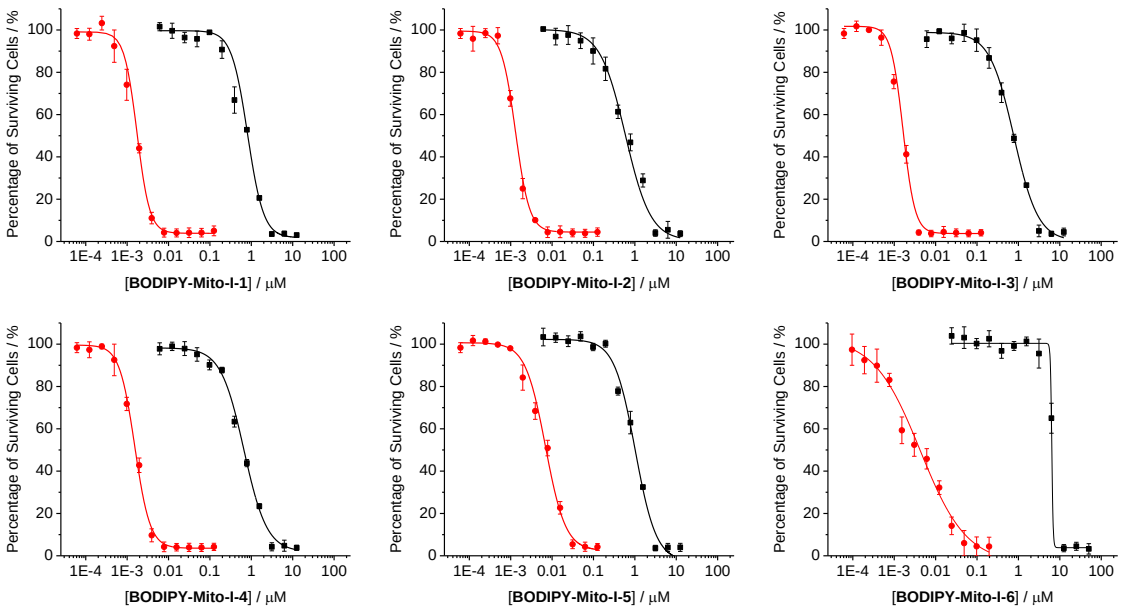


Fig. S8. Percentage of surviving HeLa cells after exposure to **BODIPY-Mito-I-*n*** analogues under dark (*black*) and light irradiation (*red*) conditions, as determined by the MTT assay. The cells were first treated with **BODIPY-Mito-I-*n*** analogues for 24 h, then incubated in the dark or irradiated (525 nm, 10 mW cm^{-2}) for 10 min, and subsequently incubated in fresh medium for 20 h.

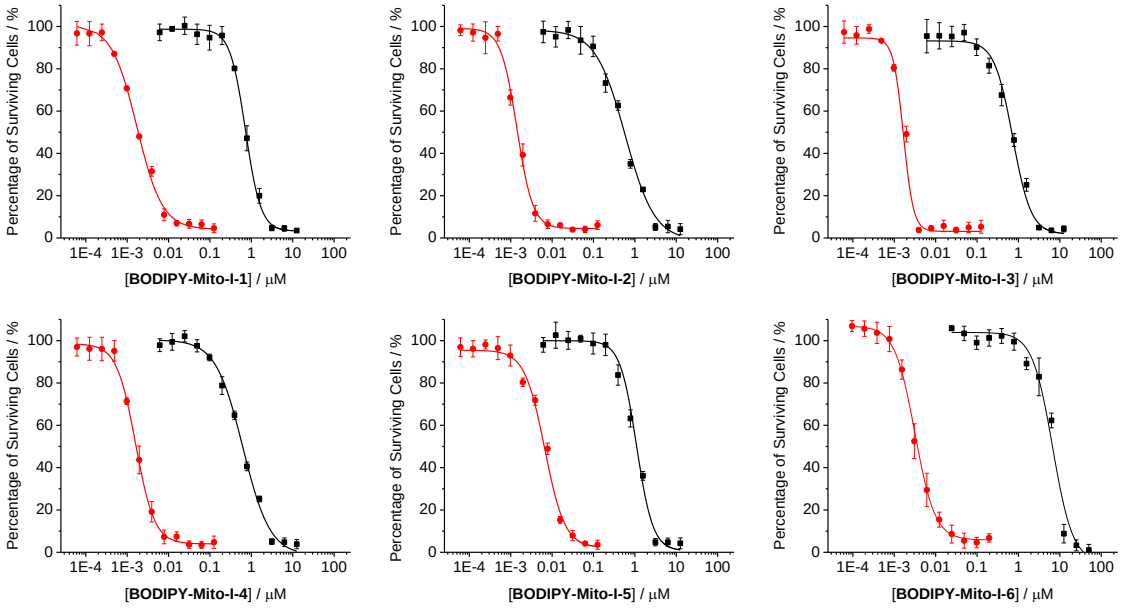


Fig. S9. Percentage of surviving HeLa cells after exposure to **BODIPY-Mito-I-*n*** analogues under dark (*black*) and light irradiation (*red*) conditions, as determined by the NRU assay. The cells were first treated with **BODIPY-Mito-I-*n*** analogues for 24 h, then incubated in the dark or irradiated (525 nm, 10 mW cm^{-2}) for 10 min, and subsequently incubated in fresh medium for 20 h.

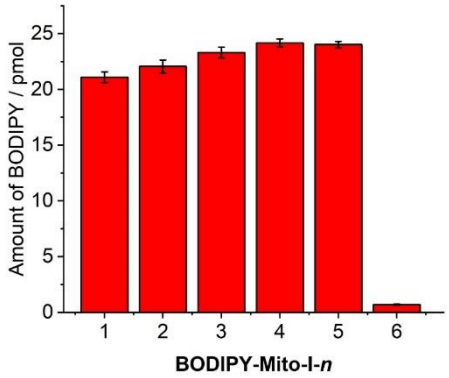


Fig. S10. Amount of BODIPY associated with an average HeLa cell upon incubation with **BODIPY-Mito-I-*n*** analogues (25 μM) at 37 °C for 4 h.

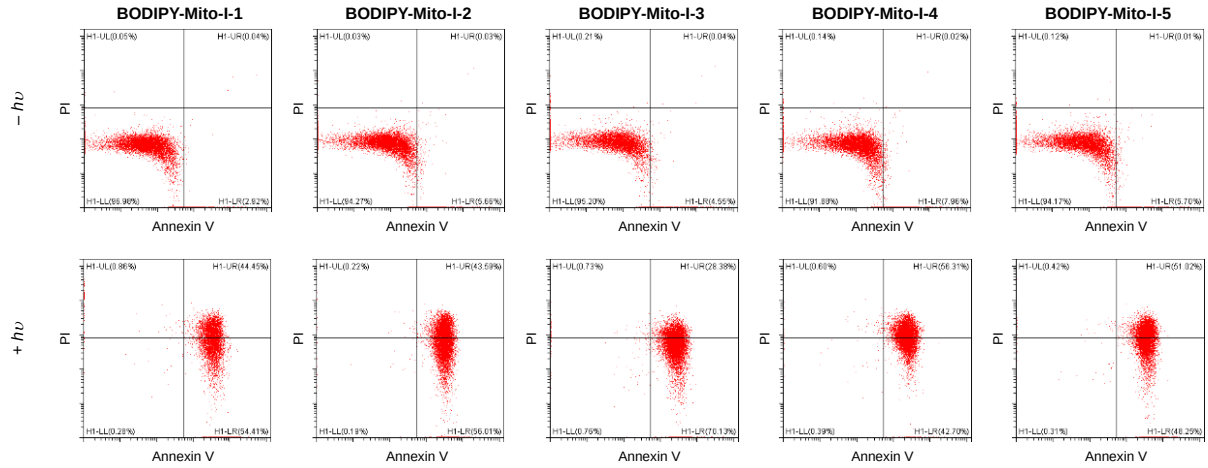


Fig. S11. Flow cytometric analysis of HeLa cells treated with **BODIPY-Mito-I-*n*** analogues (10 nM, 24 h), remained in the dark or irradiated (525 nm, 10 mW cm^{-2}) for 10 min, and then incubated in fresh medium for 20 h. The cells were then stained with Alexa Fluor 647–Annexin V conjugate and PI and analysed by flow cytometry using 488 and 638 nm excitation.

2. Experimental Details

General Procedures: All commercially available reagents were used as received from suppliers without further purification. Solvents used were laboratory grade. Anhydrous solvents were obtained from departmental solvent towers and stored over 3 Å molecular sieves. Moisture-sensitive reactions were carried out by Schlenk-line techniques, under an inert atmosphere of nitrogen. Thin-layer chromatography was performed on silica (Merk Art 5554) and visualised under UV irradiation. Automated flash column chromatography was executed using a Biotage Isolera Four unit and KP-SIL silica cartridges (10 g or 25 g). ^{1}H (400 MHz), ^{31}P NMR {^{1}H} (162 MHz) and ^{13}C {^{1}H} (101 MHz) NMR spectra were recorded on a Bruker AV-400 spectrometer, Imperial College London at 298 K. Chemical shifts are reported in parts per million (ppm) and coupling constants in Hertz (Hz). Peak multiplicities are abbreviated as: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, m = multiplet and br = broad. Mass spectrometry analysis

(ESI, accurate mass) was conducted by the Mass Spectrometry Service, Imperial College London, unless stated otherwise.

2.1 Photophysical Characterisation

Sample preparation: Stock solutions of **BODIPY-Mito-I-*n*** analogues were prepared in DMSO (1 mL) with a concentration range of 1 – 3 mM, and stored at -20 °C in the dark. Samples were thawed to room temperature directly before use. All samples were diluted to 10 μM in PBS buffer (pH = 7.4) for the photophysical measurements.

UV-Vis spectroscopy: UV-Visible absorption spectra were measured using an Agilent Technologies Cary 60 Spectrophotometer operating with WinUV software. The sample was held in a quartz cuvette with a path length of 1 cm. Absorption spectra were recorded against a baseline of pure solvent in an optically matched cuvette with a scan rate of 600.0 nm / min and a data interval of 1.0 nm. Extinction coefficients were calculated from the Beer–Lambert law (**Equation 1**).

$$A = \varepsilon c l \qquad \textbf{Equation 1.}$$

where A = the absorbance at a particular wavelength, ε is the extinction coefficient, c is the concentration and l is the path length (width of the quartz cuvette, 1 cm).

Fluorescence spectroscopy: Emission and excitation spectra were acquired on an Agilent Technologies Cary Eclipse Fluorescence Spectrophotometer, in quartz cuvettes with a path length of 1 cm. Emission and excitation spectra were collected with a scan rate of 120.0 nm / min, a delay interval of 1.0 nm and band-passes of 5 nm unless stated otherwise.

Fluorescence quantum yields (Φ_{em}): The fluorescence quantum yields of **BODIPY-Mito-I-*n*** analogues were determined relative to rhodamine 6G (Φ_{488} = 0.94) in ethanol.[1] Solutions of the reference and the sample were prepared and their absorbance was at 488 nm was adjusted to ≤ 0.1. All measurements were recorded under aerated conditions at room temperature in PBS (pH = 7.4). Absorbance and emission spectra were recorded consecutively with identical instrumentation parameters. The fluorescence quantum yields for the **BODIPY-Mito-I-*n*** analogues were calculated from **Equation 2**.

$$\Phi_{\mathrm{a}} = \left(\frac{I_{\mathrm{a}}}{I_{\mathrm{b}}}\right) \times \left(\frac{A_{\mathrm{b}}}{A_{\mathrm{a}}}\right) \times \left(\frac{n_{\mathrm{a}}}{n_{\mathrm{b}}}\right)^2 \times \Phi_{\mathrm{b}} \qquad \textbf{Equation 2.}$$

where Φ is the quantum yield, *I* is the integrated intensity of the emission spectrum, *A* is the absorbance at the excitation wavelength and *n* is the refractive index of the solvent. 'a' refers to the sample and 'b' refers to the standard.

Singlet oxygen quantum yields (Φ_Δ): The singlet oxygen quantum yields were recorded in aerated solutions of methanol and acetonitrile containing **BODIPY-Mito-I-*n*** analogues (0.5 μM) and DPBF (50 μM), in a quartz cuvette with a path length of 1 cm. The absorbance of the **BODIPY-Mito-I-*n*** analogues was adjusted to < 0.1 at 525 nm, before the samples were placed 3.5 cm away from the light source and irradiated with a Nichia NCSG219B-V1 525 nm LED (525 nm, 5450 mW @ 100 % power) operating at 5 % power, and fitted with a collimating lens. The photon flux of the LED operating at 5 % power was reported to be 1.248×10^{16} photons / s^2.

The absorbance of DPBF at 411 nm was monitored following 10 s of irradiation with 525 nm light. Prior to the measurements, the photostability of DPBF was confirmed under identical irradiation conditions. The Φ_Δ of **BODIPY-Mito-I-*n*** analogues in methanol and acetonitrile were determined relative to Rose Bengal in methanol (Φ_Δ = 0.79)[3] and acetonitrile (Φ_Δ = 0.53),[4] respectively, and calculated from **Equation 3**.

$$\Phi_\Delta^{unknown} = \Phi_\Delta^{reference} \times \frac{m^{unknown} \times F^{reference}}{m^{reference} \times F^{unknown}} \qquad \textbf{Equation 3.}$$

where Φ is the quantum yield, *m* is the slope of a linear fit of the change of absorbance ($A - A_o$) of DPBF at 411 nm and *F* is the absorption correlation factor (**Equation 4**).

$$F = 1 - 10^{-AL} \qquad \textbf{Equation 4.}$$

where *A* = absorbance at 525 nm and *L* is the path length of the cuvette.

For comparison, the Φ_Δ of **BODIPY-Mito-1** and **BODIPY-Mito-6** (10 μM) from our previous work[5] were recorded in methanol, in an identical procedure to **BODIPY-Mito-I-1** and **BODIPY-Mito-I-6** analogues highlighted above.

2.2 Biological Characterisation

Cell culture: HeLa cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 1 % penicillin/streptomycin at 37 °C under a 5 % CO_2 atmosphere. They were subcultured every 2 to 3 days.

MTT assays: HeLa cells were seeded in two 96-well flat-bottomed microplates (*ca*. 10000 cells per well) in growth medium (100 μL) and incubated at 37 °C under a 5 % CO_2 atmosphere for 24 h. The growth medium was removed and replaced with the **BODIPY-Mito-I-*n*** compounds at concentrations ranging from 10^{-10} to 10^{-5} M in growth medium/DMSO (99:1, *v* / *v*). Wells containing untreated cells were used as blank controls. The microplate was incubated at 37 °C under a 5 % CO_2 atmosphere for 24 h. After treatment, the medium was removed, the cells were washed with PBS (100 μL) and phenol red-free growth medium (100 μL) was added to each well. One of the microplates was irradiated at λ_{ex} = 525 nm (10 mW cm^{-2}) with an LED cellular photocytotoxicity irradiator (PURI Materials, Shenzhen, China) for 10 min, whereas the other one was kept in the dark. The cells were then replenished with fresh growth medium (100 μL) and further incubated at 37 °C under a 5 % CO_2 atmosphere for 24 h. Then, MTT in PBS (10 μL, 5 mg mL^{-1}) was added to each well. The microplate was incubated at 37 °C under a 5 % CO_2 atmosphere for 3 h. The growth medium was then removed, and DMSO (200 μL) was added to each well. The microplate was further incubated at 37 °C for 15 min. The absorbance of the solutions at 570 nm was measured with an Epoch 2 microplate spectrophotometer (BioTek., Santa Clara, CA). The IC_{50} values of the **BODIPY-Mito-I-*n*** compounds were determined from dose dependence of surviving cells after exposure to the compounds.

Neutral red uptake (NRU) assays: The experimental procedure was similar to that for the MTT assays, except that neutral red in PBS (10 μL, 0.4 mg mL^{-1}) instead of MTT was added to the wells after the treatment. The microplate was incubated at 37 °C under a 5 % CO_2 atmosphere for 3 h. The growth medium was then removed, and a destain solution (EtOH / H_2O / CH_3COOH (glacial), 50 / 49 / 1, *v* / *v* / *v*) (200 μL) was added to each well. The absorbance of the solutions at 540 nm was measured with an Epoch 2 microplate spectrophotometer (BioTek., Santa Clara, CA). The IC_{50} values of the **BODIPY-Mito-I-*n*** compounds were determined from dose dependence of surviving cells after exposure to the compounds.

Cellular uptake: HeLa cells were grown in a 50-mm tissue culture dish and incubated at 37 °C under a 5 % CO_2 atmosphere for 48 h. The culture medium was removed and replaced with the **BODIPY-Mito-I-*n*** compounds (25 μM) in growth medium/DMSO (99:1, *v* / *v*). After incubation at 37 °C under a 5 % CO_2 atmosphere for 4 h, the medium was removed and the cell layer was washed gently with PBS (1 mL × 3). The cell layer was then trypsinised with 0.25 % trypsin–EDTA

(500 μL) and quenched with fresh medium (4.5 mL). The cell number was counted with a Logos Biosystems LUNA-II automated cell counter. The solution was then transferred to centrifuge tubes and centrifuged at 1,500 rpm for 3 min. The cell layer was then washed with PBS (1 mL) and centrifuged again. After removing the PBS, the cells were lysed with DMF. The solution was sonicated for 15 min and centrifuged. The supernatants were separated for UV-Vis spectroscopic measurements to determine the concentration of the **BODIPY-Mito-I-*n*** compounds inside cells. The concentration of the compounds was calculated from their corresponding calibration curves.

Determination of intracellular ROS generation: HeLa cells were seeded on a sterilised coverslip in a 35-mm tissue culture dish and grown at 37 °C under a 5 % CO_2 atmosphere for 48 h. The culture medium was removed and replaced with **BODIPY-Mito-I-6** (25 μM) in growth medium/DMSO (99:1, *v* / *v*). After incubation at 37 °C under a 5 % CO_2 atmosphere for 3 h, the medium was removed and the cell layer was washed gently with PBS (1 mL × 3). The cells were then incubated in phenol red-free growth medium. In the dark condition, the tissue culture dish was kept in the dark for 10 min, while in the light condition, the tissue culture dish was irradiated at λ_{ex} = 525 nm (10 mW cm^{-2}) with an LED cellular photocytotoxicity irradiator (PURI Materials, Shenzhen, China) for 10 min. The medium was removed, washed with PBS (1 mL × 3), and treated with CM-H_2DCFDA (5 μM) at 37 °C under a 5 % CO_2 atmosphere for 30 min. The medium was removed, and the cell layer was washed with PBS (1 mL × 3). The coverslip was mounted to a sterilised glass slide, and then imaging was performed using a Leica TCS SPE confocal microscope with an oil immersion 63× objective and an excitation wavelength at 488 nm.

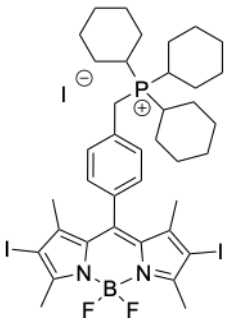
Detection of mitochondria membrane potential: HeLa cells were seeded on a sterilised coverslip in a 35-mm tissue culture dish and grown at 37 °C under a 5 % CO_2 atmosphere for 48 h. The culture medium was removed and replaced with **BODIPY-Mito-I-6** (25 μM) in growth medium/DMSO (99:1, *v* / *v*). After incubation at 37 °C under a 5 % CO_2 atmosphere for 3 h, the medium was removed and the cell layer was washed gently with PBS (1 mL × 3). The cells were then incubated in phenol red-free growth medium. In the dark condition, the tissue culture dish was kept in the dark for 10 min, while in the light condition, the tissue culture dish was irradiated at λ_{ex} = 525 nm (10 mW cm^{-2}) with an LED cellular photocytotoxicity irradiator (PURI Materials, Shenzhen, China) for 10 min. The medium was removed, washed with PBS (1 mL × 3), and treated with Rhodamine 123 (5 μM) at 37 °C under a 5 % CO_2 atmosphere for 30 min. The medium was removed, and the cell layer was washed with PBS (1 mL × 3). The coverslip was mounted to a sterilised glass slide, and then imaging was performed using a Leica TCS SPE confocal microscope with an oil immersion 63× objective and an excitation wavelength at 488 nm.

Annexin V/propidium iodide (PI) assays: HeLa cells in growth medium were seeded in two 6-well flat-bottomed microplates in growth medium and incubated at 37 °C under a 5 % CO_2 atmosphere for 48 h. The growth medium was removed and replaced with the **BODIPY-Mito-I-*n*** compounds (**BODIPY-Mito-I-1 – BODIPY-Mito-I-5**: 10 nM; **BODIPY-Mito-I-**6: 25 nM) in growth medium/DMSO (99:1, *v* / *v*). After incubation for 24 h, the medium was removed and the cells were washed gently with PBS (1 mL × 3). Then phenol red-free growth medium (2 mL) was added to each well. One of the microplates was irradiated at λ_{ex} = 525 nm (10 mW cm^{-2}) with an LED cellular photocytotoxicity irradiator (PURI Materials, Shenzhen, China) for 10 min, whereas the other one was kept in the dark. The cells were then replenished with fresh growth medium (2 mL) and further incubated at 37 °C under a 5 % CO_2 atmosphere for 20 h. After the treatment, the medium was removed and the cells were washed gently with PBS (1 mL × 3). The cell layer was then trypsinised and centrifuged at 1500 rpm for 1 min. The cell pellet was washed with PBS (1 mL) and subjected to centrifugation. The cells were resuspended in an Annexin V binding buffer (100 μL) in the flow cytometer tubes, followed by the addition of Alexa Fluor 647–Annexin V conjugate (5 μL) and PI (2 μL, 100 μg mL^{-1}). The cell suspension was kept in the dark for 15 min. The Annexin V binding buffer (400 μL) was added to the suspension before analysis by flow cytometer (Beckman CytoFLEX). The untreated cultured cells were used as a control group for background correction. The experiments were performed in triplicates and analysed using the CytExpert 2.6 software.

2.3 Compound Synthesis

BODIPY-Mito-*n* analogues were synthesised in accordance with our previous work.[5] Compounds (**1**) and (**2**) were synthesised in accordance with reports in the literature.[6]

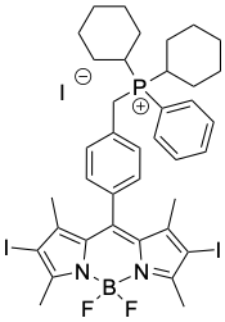
BODIPY-Mito-I-1



Iodine monochloride (1 M in acetic acid, 384 μL) was added to an ice-cold (0 – 5 °C) solution of **BODIPY-Mito-1** (78.9 mg, 0.113 mmol) in CH_2Cl_2 / MeOH (1:1, 18 mL). The reaction was stirred at 0 – 5 °C for 30 min under an inert atmosphere of nitrogen and warmed to room temperature. The bright pink solution was washed with sodium thiosulphate (sat. solution, 10 mL), dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure to form a bright pink residue. Purification by silica gel column chromatography (100 % CH_2Cl_2 to 95 % CH_2Cl_2, 5 % MeOH) and recrystalisation in CH_2Cl_2 / *n*-hexane formed the title compound as a pink solid (78.0 mg, 69 %). ^{1}H NMR (400 MHz, $CDCl_3$) 7.69 (2 H, d, $^3J_{H\text{-}H}$ 7.4), 7.31 (2 H, d, $^3J_{H\text{-}H}$ 7.4), 4.43 (2 H, d, $^2J_{H\text{-}P}$ 14.3), 2.85 – 2.76 (3 H, m), 2.63 (6 H, s), 2.09 – 2.06 (6 H, m), 1.93 – 1.89 (6 H, m), 1.83 – 1.80 (3 H, m), 1.62 – 1.54 (6 H, m), 1.49 – 1.43 (6 H, m), 1.35 (6 H, s), 1.31 – 1.26 (3 H, m); ^{31}P NMR {^{1}H} (162 MHz, $CDCl_3$) 29.6; ^{13}C NMR {^{1}H} (101 MHz, $CDCl_3$) 157.4, 144.7, 139.8, 135.2, 131.9 (d, $^2J_{C\text{-}}$

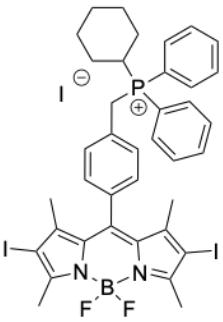
$_P$ 8.1), 131.7 (d, $^3J_{C\text{-}P}$ 4.9), 86.2, 31.2 (d, $^1J_{C\text{-}P}$ 37.4), 27.5, 26.7 (d, $^2J_{C\text{-}P}$ 12.0), 25.6, 23.3 (d, $^1J_{C\text{-}P}$ 41.0), 17.4, 16.2; ESI-LRMS $[C_{38}H_{51}{}^{11}BN_2O_9F_2PI_2]^+$, (+) m/z 869.2, ESI-HRMS calculated for $[C_{38}H_{51}{}^{11}BN_2O_9F_2PI_2]^+$, 869.1941 found, 869.1982.

BODIPY-Mito-I-2



Iodine monochloride (1 M in acetic acid, 336.0 μL) was added to an ice-cold (0 – 5 °C) solution of **BODIPY-Mito-2** (68.2 mg, 0.10 mmol) in CH_2Cl_2 / MeOH (1:1, 20 mL). The reaction was stirred at 0 – 5 °C for 30 min under an inert atmosphere of nitrogen and warmed to room temperature. The bright pink solution was washed with sodium thiosulphate (sat. solution, 10 mL), dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure to form a bright pink residue. Purification by silica gel column chromatography (100 % CH_2Cl_2 to 95 % CH_2Cl_2, 5 % MeOH) and recrystalisation in CH_2Cl_2 / *n*-hexane formed the title compound as a pink solid (59.3 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) 7.98 (2 H, t, $^3J_{H\text{-}H}$ 7.7), 7.83 – 7.73 (3 H, m), 7.49 (2 H, d, $^3J_{H\text{-}H}$ 7.8), 7.17 (2 H, d, $^3J_{H\text{-}H}$ 7.8), 4.87 (2 H, d, $^2J_{H\text{-}P}$ 14.6), 3.31 – 3.22 (2 H, m), 2.61 (6 H, s), 2.20 – 2.04 (4 H, m), 1.91 – 1.77 (6 H, m), 1.58 – 1.41 (6 H, m), 1.30 (6 H, s), 1.28 – 1.25 (2 H, m), 1.18 – 1.08 (2 H, m); ^{31}P NMR {^{1}H} (162 MHz, $CDCl_3$) 29.8; ^{13}C NMR {^{1}H} (101 MHz, $CDCl_3$) 157.3, 144.7, 139.8, 135.1, 134.5, 133.5 (d, $^3J_{C\text{-}P}$ 7.1), 131.8 (d, $^3J_{C\text{-}P}$ 5.1), 131.2 (d, $^3J_{C\text{-}P}$ 8.2), 131.2, 130.6 (d, $^2J_{C\text{-}P}$ 11.1), 129.1, 115.0 (d, $^1J_{C\text{-}P}$ 73.2), 86.2, 31.1 (d, $^1J_{C\text{-}P}$ 42.1), 26.7 (d, $^2J_{C\text{-}P}$ 14.31), 26.4 (d, $^3J_{C\text{-}P}$ 5.2), 26.3 (d, $^3J_{C\text{-}P}$ 5.3), 24.6 ($^1J_{C\text{-}P}$ 42.8), 17.4, 16.2; ESI-LRMS $[C_{38}H_{45}{}^{11}BN_2O_9F_2PI_2]^+$, (+) m/z 863.1, ESI-HRMS calculated for $[C_{38}H_{45}{}^{11}BN_2O_9F_2PI_2]^+$, 863.1471 found, 863.1498.

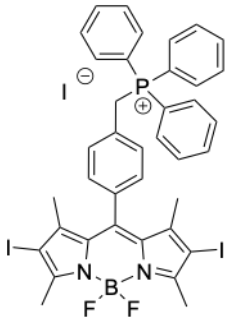
BODIPY-Mito-I-3



Iodine monochloride (1 M in acetic acid, 268.0 μL) was added to an ice-cold (0 – 5 °C) solution of **BODIPY-Mito-3** (54.2 mg, 0.08 mmol) in CH_2Cl_2 / MeOH (1:1, 12 mL). The reaction was stirred at 0 – 5 °C for 30 min under an inert atmosphere of nitrogen and warmed to room temperature. The bright pink solution was washed with sodium thiosulphate (sat. solution, 10 mL), dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure to form a bright pink residue. Purification by silica gel column chromatography (100 % CH_2Cl_2 to 95 % CH_2Cl_2, 5 % MeOH) and recrystalisation in CH_2Cl_2 / *n*-hexane formed the title compound as a pink solid (47.0 mg, 60 %). ^{1}H NMR (400 MHz, $CDCl_3$) 7.98 – 7.93 (4 H, m), 7.84 – 7.80 (2 H, m), 7.72 – 7.67 (4 H, m), 7.22 (2 H, d, $^3J_{H\text{-}H}$ 7.7), 7.96 (2 H, d, $^3J_{H\text{-}H}$ 7.7), 5.27 (2 H, d, $^2J_{H\text{-}P}$ 14.1), 4.40 – 4.32 (1 H, m), 2.59 (6 H, s), 2.26 – 2.24 (2 H, m), 1.72 – 1.66 (4 H, m), 1.16 (6 H, s), 1.07 – 0.96 (4 H, m); ^{31}P NMR {^{1}H} (162 MHz, $CDCl_3$) 32.6; ^{13}C NMR {^{1}H} (101 MHz, $CDCl_3$) 157.2, 144.8, 140.1, 135.1, 134.8 (d, $^2J_{C\text{-}P}$ 8.7), 134.6 (d, $^3J_{C\text{-}P}$ 3.6), 132.0 (d, $^3J_{C\text{-}P}$ 5.4),

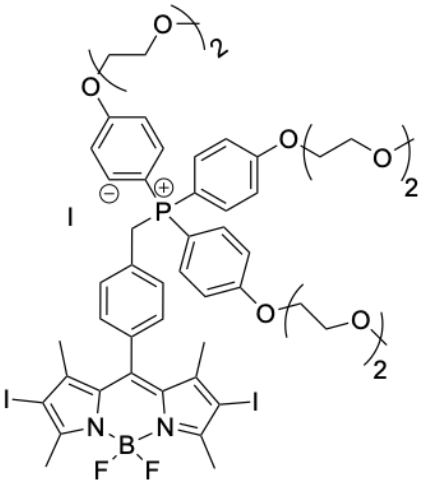
131.1, 130.3 (d, $^{2}J_{C\text{-}P}$ 11.8), 130.3, 128.4, 114.6 (d, $^{1}J_{C\text{-}P}$ 79.8), 86.0, 31.93 (d, $^{1}J_{C\text{-}P}$ 44.1) , 27.3 (d, $^{1}J_{C\text{-}P}$ 44.9), 25.8, 25.5, 25.4, 17.4, 16.2; ESI-LRMS $[C_{38}H_{39}{}^{11}BN_2O_9F_2PI_2]^+$, (+) m/z 857.1, ESI-HRMS calculated for $[C_{38}H_{39}{}^{11}BN_2O_9F_2PI_2]^+$, 857.1002 found, 857.0998.

BODIPY-Mito-I-4



Iodine monochloride (1 M in acetic acid, 150.0 μL) was added to an ice-cold (0 – 5 °C) solution of **BODIPY-Mito-4** (30.0 mg, 0.044 mmol) in CH_2Cl_2 / MeOH (1:1, 6 mL). The reaction was stirred at 0 – 5 °C for 30 min under an inert atmosphere of nitrogen and warmed to room temperature. The bright pink solution was washed with sodium thiosulphate (sat. solution, 10 mL), dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure to form a bright pink residue. Purification by silica gel column chromatography (100 % CH_2Cl_2 to 95 % CH_2Cl_2, 5 % MeOH) and recrystalisation in CH_2Cl_2 / *n*-hexane formed the title compound as a pink solid (30.1 mg, 73 %). ^{1}H NMR (400 MHz, $CDCl_3$) 7.87 – 7.82 (9 H, m), 7.71 – 7.66 (6 H, m), 7.40 (2 H, d, $^{3}J_{H\text{-}H}$ 7.7), 7.09 (2 H, d, $^{3}J_{H\text{-}H}$ 7.7), 5.52 (2 H, d, $^{3}J_{H\text{-}P}$ 14.9), 2.61 (6 H, s), 1.28 (6 H, s); ^{31}P NMR {^{1}H} (162 MHz, $CDCl_3$) 22.5; ^{13}C NMR {^{1}H} (101 MHz, $CDCl_3$) 157.3, 144.8, 140.0, 135.5, 135.2, 134.5 (d, $^{3}J_{C\text{-}P}$ 9.9), 132.8 (d, $^{3}J_{C\text{-}P}$ 5.4), 131.1, 130.5 (d, $^{2}J_{C\text{-}P}$ 12.6), 129.4 (d, $^{2}J_{C\text{-}P}$ 8.4), 128.7, 117.6 (d, $^{1}J_{C\text{-}P}$ 86.4), 86.1, 30.9 (d, $^{1}J_{C\text{-}P}$ 46.7), 17.4, 16.2; ESI-LRMS $[C_{38}H_{33}{}^{11}BN_2O_9F_2PI_2]^+$, (+) m/z 851.1, ESI-HRMS calculated for $[C_{38}H_{33}{}^{11}BN_2O_9F_2PI_2]^+$, 851.0532 found, 851.0526.

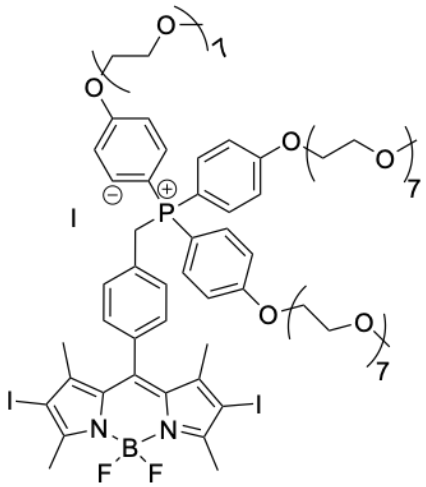
BODIPY-Mito-I-5



Iodine monochloride (1 M in acetic acid, 420.0 μL) was added to an ice-cold (0 – 5 °C) solution of **BODIPY-Mito-5** (128.3 mg, 0.124 mmol) in CH_2Cl_2 / MeOH (1:1, 20 mL). The reaction was stirred at 0 – 5 °C for 30 min under an inert atmosphere of nitrogen and warmed to room temperature. The bright pink solution was washed with sodium thiosulphate (sat. solution, 10 mL), dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure to form a bright pink residue. Purification by silica gel column chromatography (100 % CH_2Cl_2 to 95 % CH_2Cl_2, 5 % MeOH) and recrystalisation in CH_2Cl_2 / *n*-hexane formed the title compound as a pink solid (102.1 mg, 62 %). ^{1}H NMR (400 MHz, $CDCl_3$) 7.67 – 7.61 (6 H, m), 7.36 – 7.34 (2 H, m), 7.15 – 7.10 (8 H, m), 5.20 – 5.16 (2 H, br m), 4.25 – 4.23 (6 H, m), 3.89 – 3.88 (6 H, m), 3.72 – 3.70 (6 H, m), 3.86 – 3.56 (6 H, m), 3.37 (9 H, br s), 2.61 (6 H, s), 1.30 (6 H, s); ^{31}P NMR {^{1}H} (162 MHz, $CDCl_3$) 20.3; ^{13}C NMR {^{1}H} (101 MHz, $CDCl_3$) 164.2, 157.2, 144.8, 140.2, 136.2 (d, $^{3}J_{C\text{-}P}$ 11.4), 135.0, 132.7 (d, $^{4}J_{C\text{-}P}$ 5.1), 131.2, 129.9, 128.5, 116.6 (d, $^{2}J_{C\text{-}P}$ 13.7), 108.3 (d, $^{1}J_{C\text{-}P}$ 94.9), 86.1, 72.0, 70.9, 69.4, 68.2, 59.2, 32.0 (d, $^{1}J_{C\text{-}P}$

49.3), 17.4, 16.2; ESI-LRMS $[C_{53}H_{63}{}^{11}BN_2O_9F_2PI_2]^+$, (+) m/z 1205.2, ESI-HRMS calculated for $[C_{53}H_{63}{}^{11}BN_2O_9F_2PI_2]^+$, 1205.2422 found, 1205.2474.

BODIPY-Mito-I-6



Iodine monochloride (1 M in acetic acid, 75.0 μL) was added to an ice-cold (0 – 5 °C) solution of **BODIPY-Mito-6** (35.4 mg, 0.021 mmol) in CH_2Cl_2 / MeOH (1:1, 4 mL). The reaction was stirred at 0 – 5 °C for 1 h under an inert atmosphere of nitrogen and warmed to room temperature. An additional aliquot of iodine monochloride (1 M in acetic acid, 40.0 μL) was added and the solution was stirred at 0 – 5 °C for 30 min. The bright pink solution was washed with sodium thiosulphate (sat. solution, 10 mL), dried over Na_2SO_4 and filtered. The solvent was removed under reduced pressure to form a bright pink residue. Purification by silica gel column chromatography (100 % CH_2Cl_2 to 95 % CH_2Cl_2, 5 % MeOH) and recrystalisation in CH_2Cl_2 / *n*-hexane formed the title compound as a pink solid (31.8 mg, 76 %). ^{1}H NMR (400 MHz, $CDCl_3$) 7.66 – 7.61 (6 H, m), 7.35 – 7.32 (2 H, m), 7.14 – 7.08 (8 H, m), 5.17 (2 H, d, $^2J_{H\text{-}P}$ 14.8), 4.22 – 4.20 (6 H, m), 3.89 – 3.86 (6 H, m), 3.72 – 3.60 (66 H, m), 3.53 – 3.51 (6 H, m), 3.35 (9 H, s), 2.60 (6 H, s), 1.29 (6 H, s); ^{31}P NMR {^{1}H} (162 MHz, $CDCl_3$) 20.2; ^{13}C NMR {^{1}H} (101 MHz, $CDCl_3$) 164.1 (d, $^4J_{C\text{-}P}$ 3.0), 157.2, 144.8, 140.1, 136.2 ($^3J_{C\text{-}P}$ 11.2), 134.9, 132.7, 131.1, 129.9, 128.5, 116.6 ($^2J_{C\text{-}P}$ 13.6), 108.2 (d, $^1J_{C\text{-}P}$ 94.0), 86.0, 72.0, 70.7, 70.6 – 70.51,* 69.3, 68.2, 59.1, 32.0 (d, $^1J_{C\text{-}P}$ 49.4), 17.4, 16.1; ESI-LRMS $[C_{83}H_{123}{}^{11}BN_2O_{24}F_2PI_2]^+$, (+) m/z 1865.6, ESI-HRMS calculated for $[C_{83}H_{123}{}^{11}BN_2O_{24}F_2PI_2]^+$, 1865.6354 found, 1865.6328.

*multiple carbon environments due to PEG.

2.3.1 NMR Spectra

BODIPY-Mito-I-1

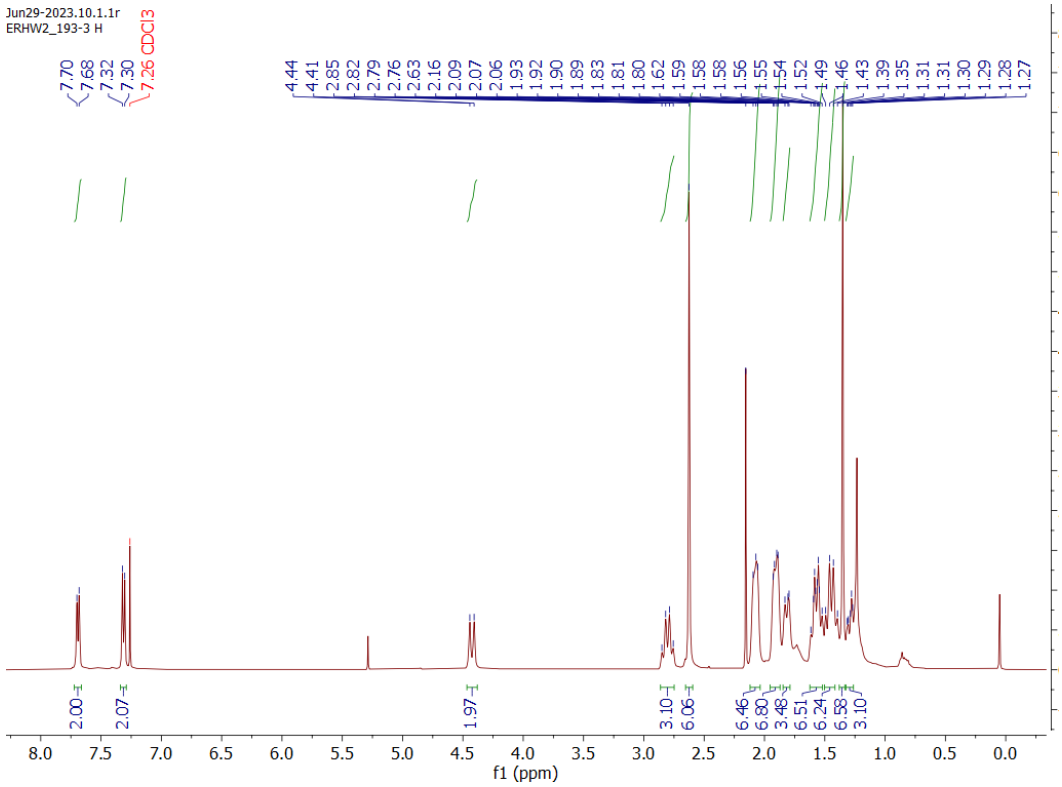


Fig. S12. The ^{1}H NMR spectrum of **BODIPY-Mito-I-1** in $CDCl_3$, 298 K.

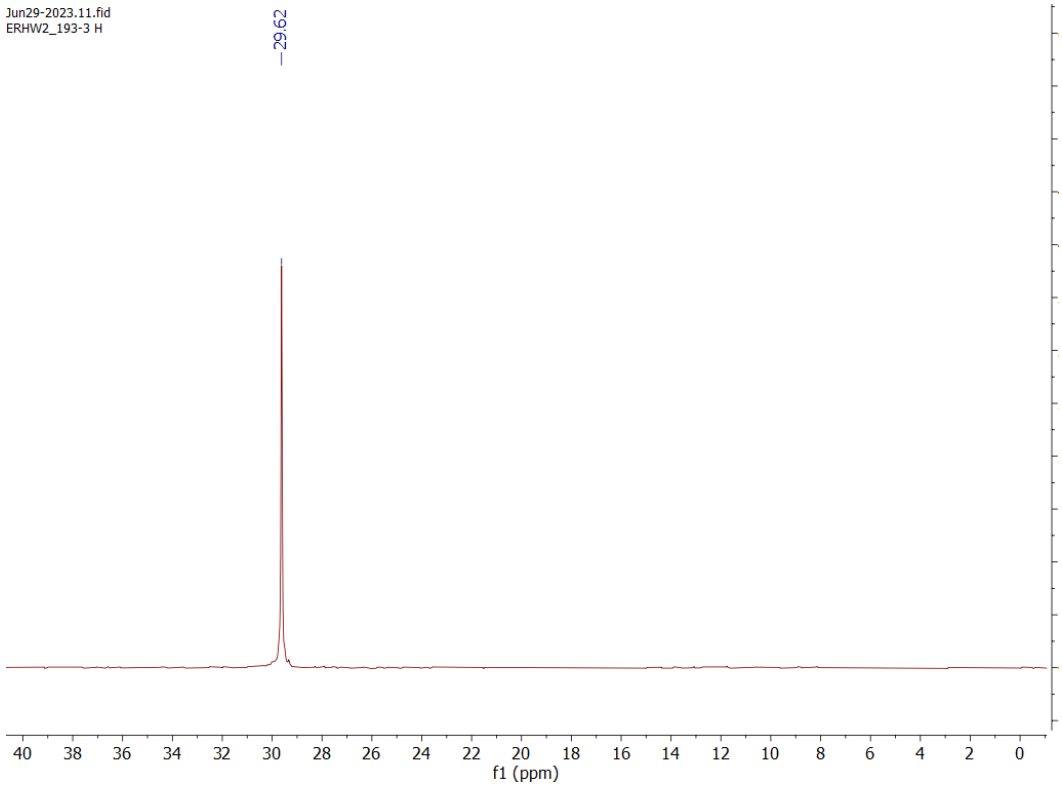


Fig. S13. The ^{31}P NMR spectrum of **BODIPY-Mito-I-1** in $CDCl_3$, 298 K.

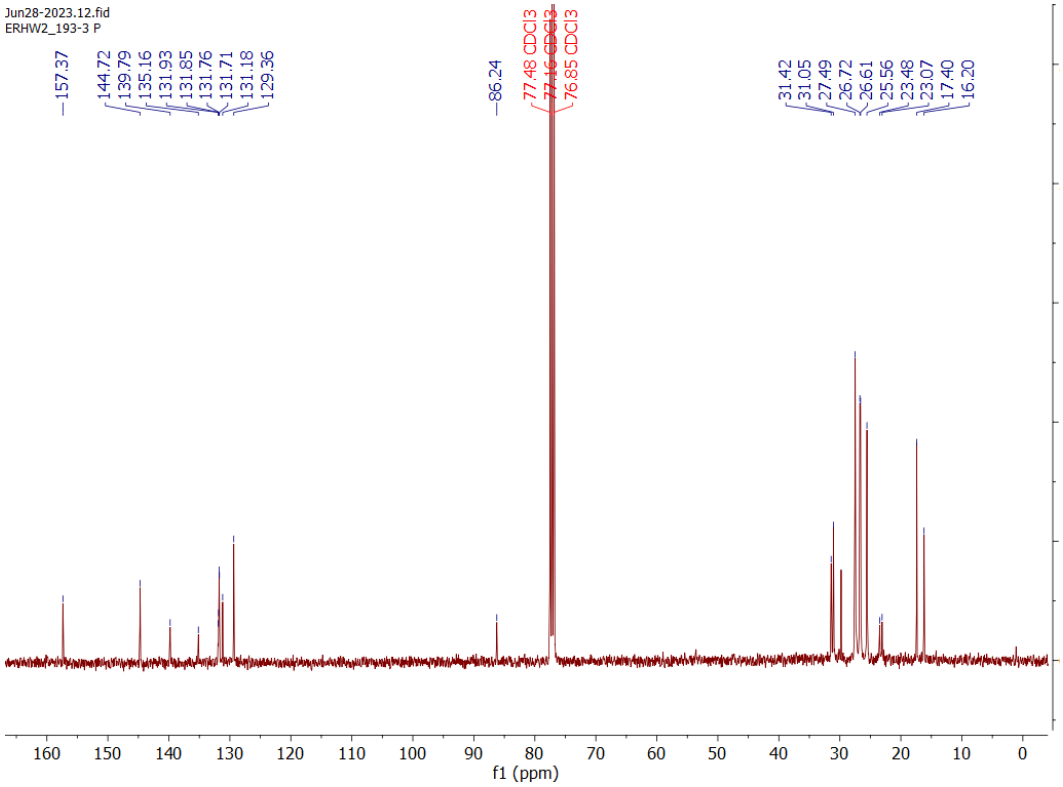


Fig. S14. The ^{13}C NMR spectrum of **BODIPY-Mito-I-1** in $CDCl_3$, 298 K.

BODIPY-Mito-I-2

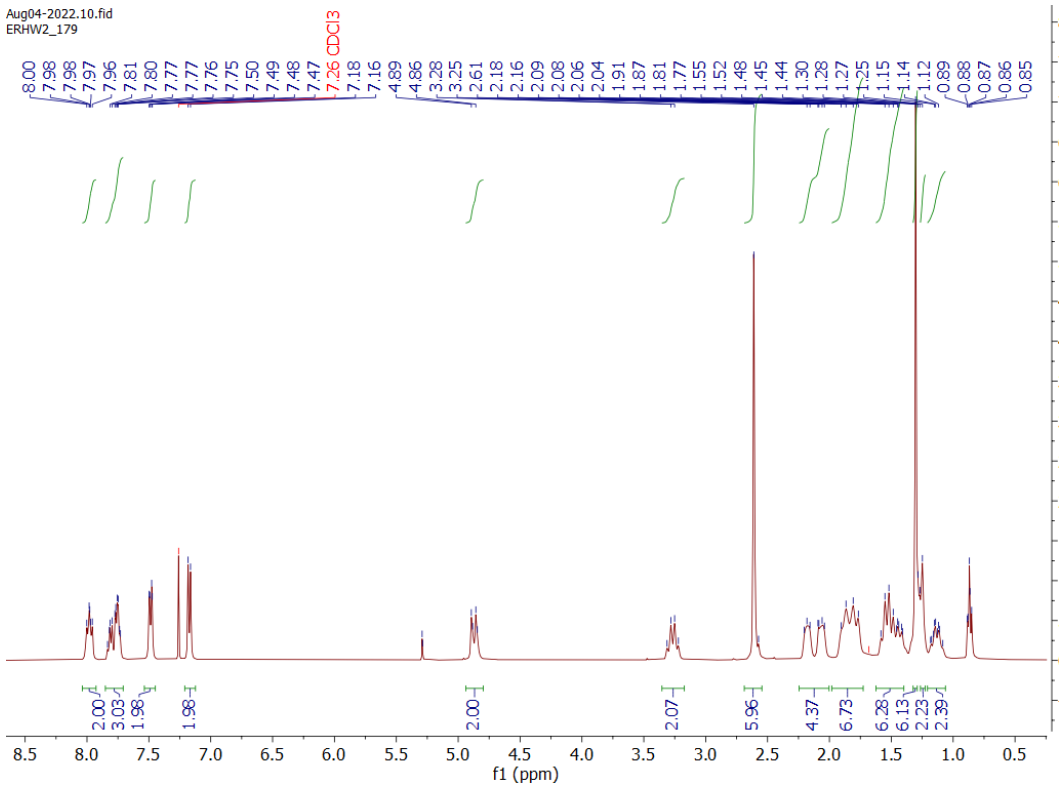


Fig. S15. The ^{1}H NMR spectrum of **BODIPY-Mito-I-2** in $CDCl_3$, 298 K.

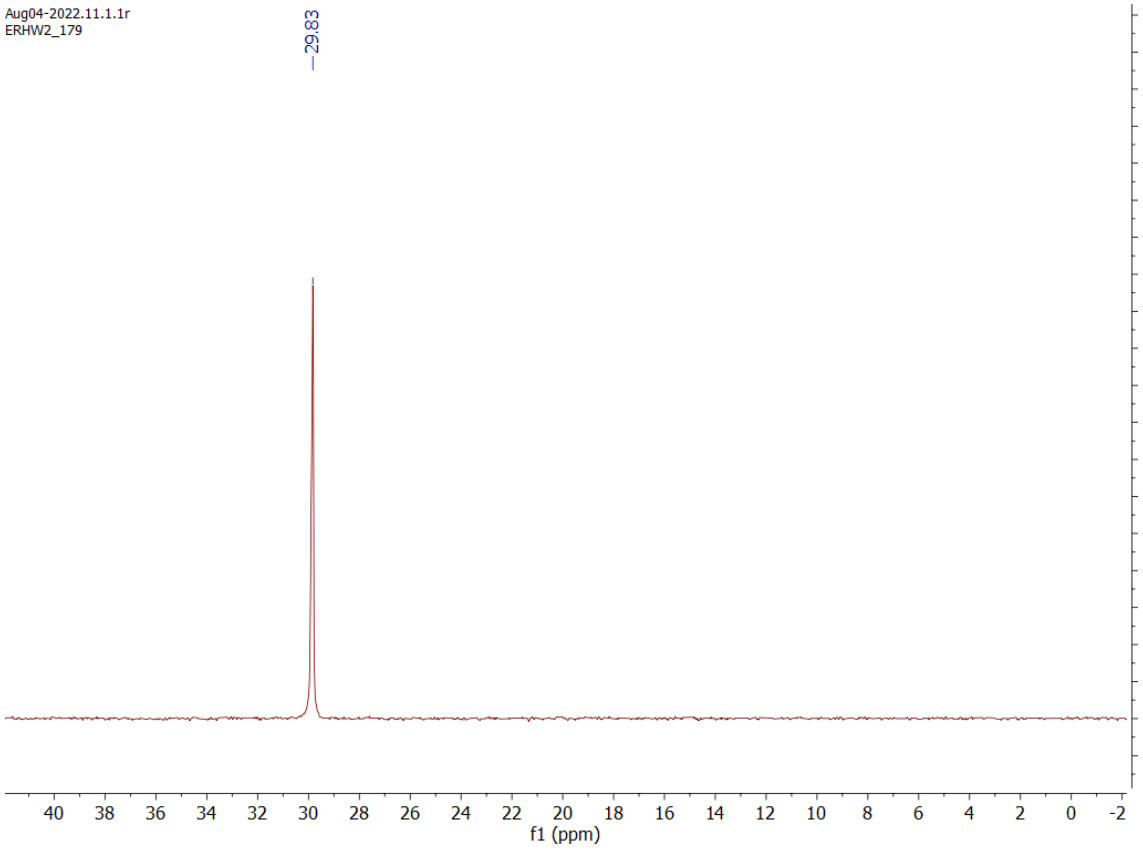


Fig. S16. The ^{31}P NMR spectrum of **BODIPY-Mito-I-2** in $CDCl_3$, 298 K.

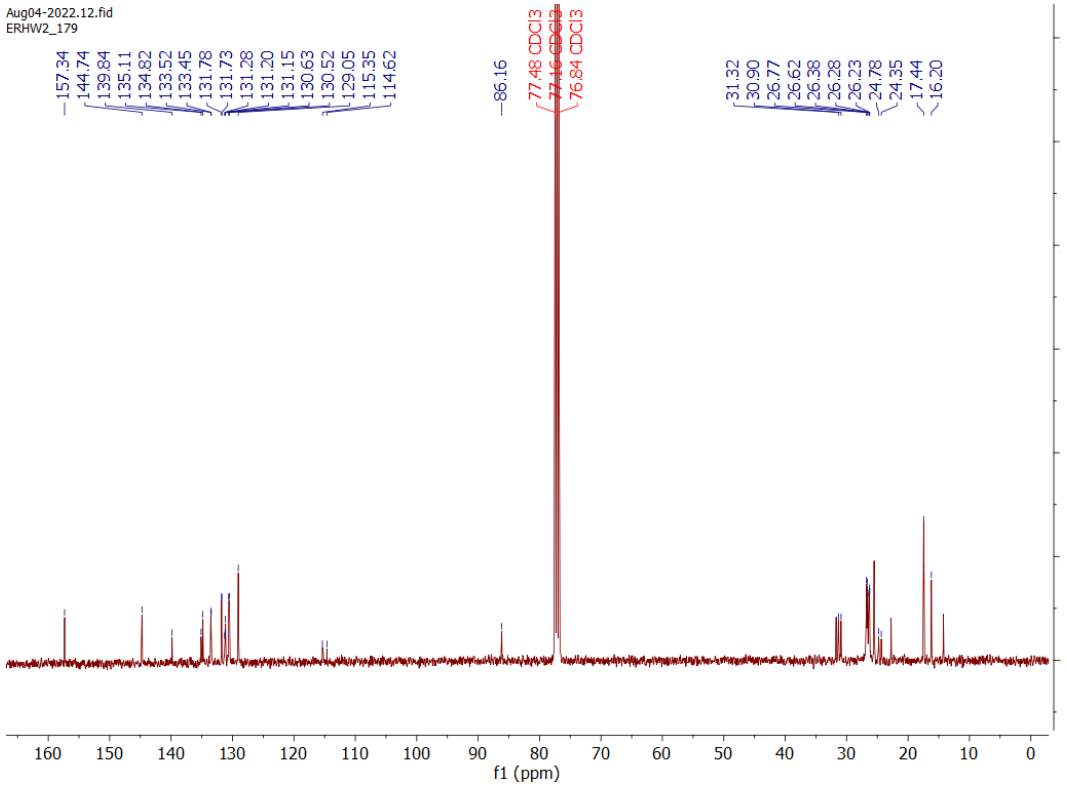


Fig. S17. The ^{13}C NMR spectrum of **BODIPY-Mito-I-2** in $CDCl_3$, 298 K.

BODIPY-Mito-I-3

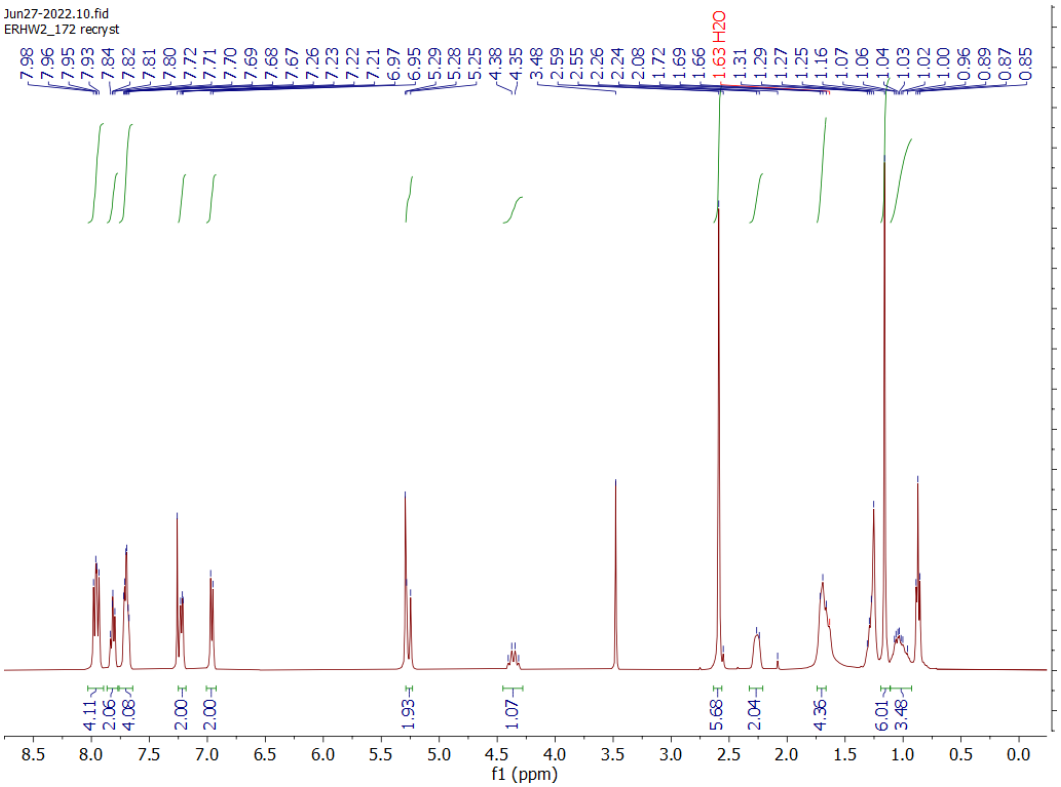


Fig. S18. The ^{1}H NMR spectrum of **BODIPY-Mito-I-3** in $CDCl_3$, 298 K.

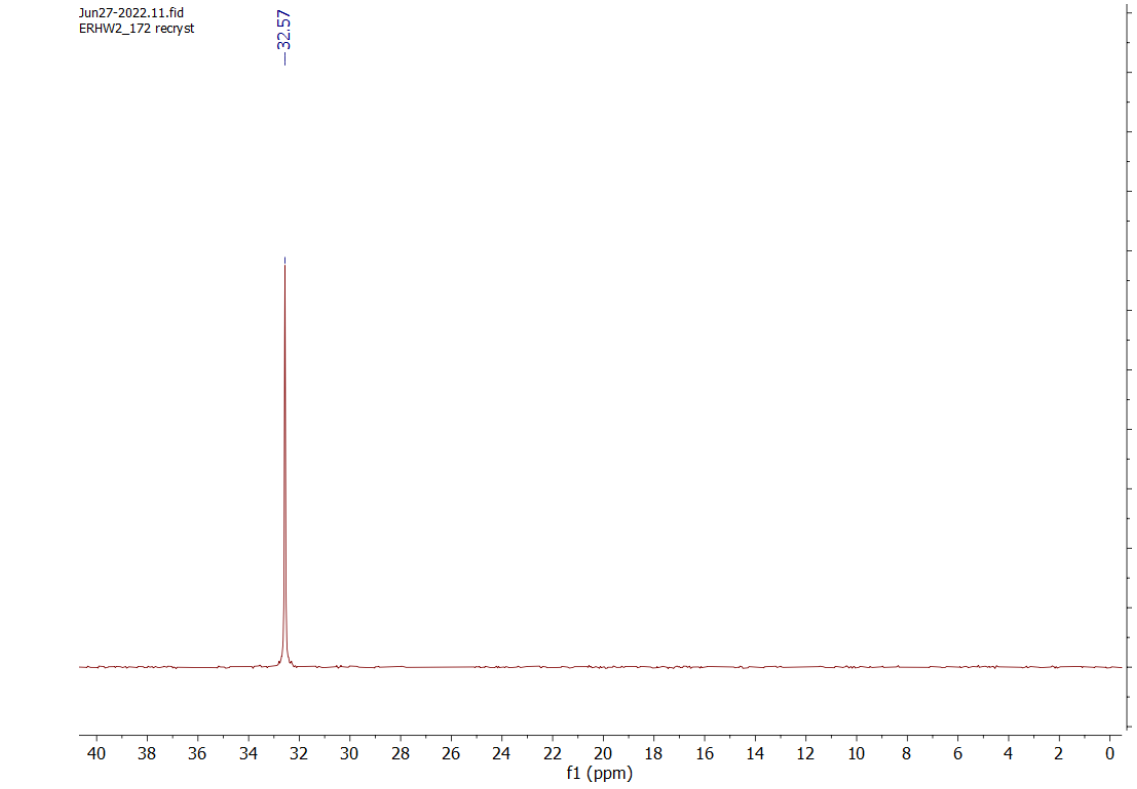


Fig. S19. The ^{31}P NMR spectrum of **BODIPY-Mito-I-3** in $CDCl_3$, 298 K.

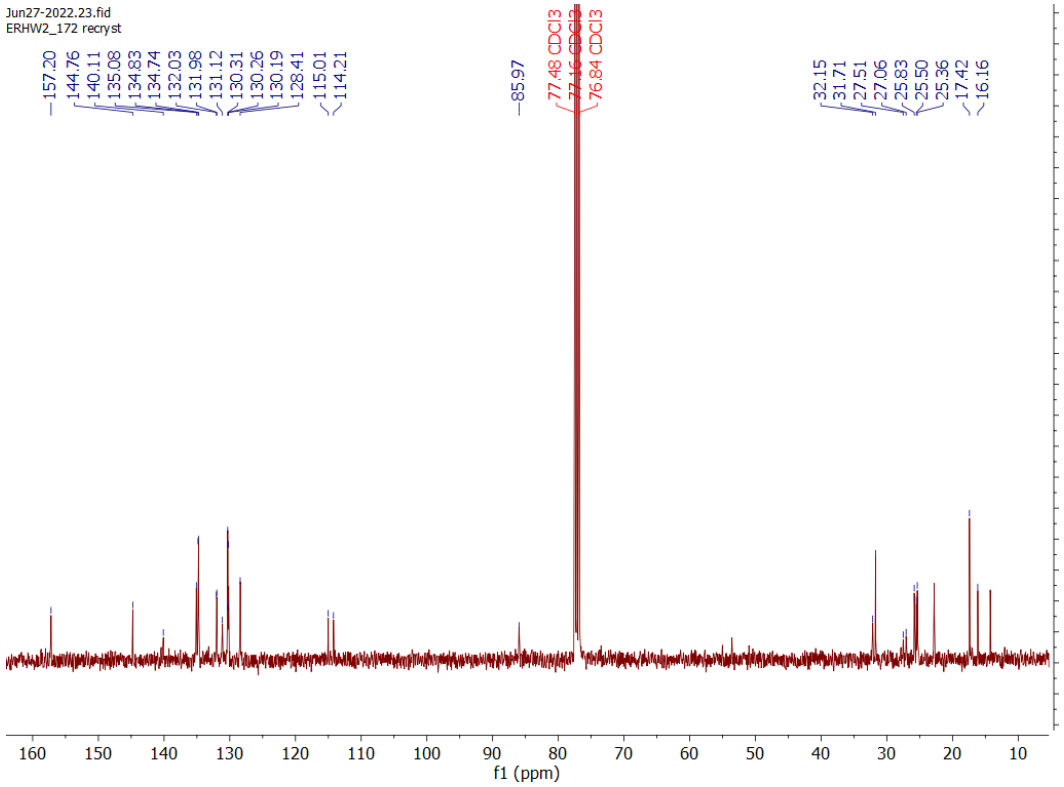


Fig. S20. The ^{13}C NMR spectrum of **BODIPY-Mito-I-3** in $CDCl_3$, 298 K.

BODIPY-Mito-I-4

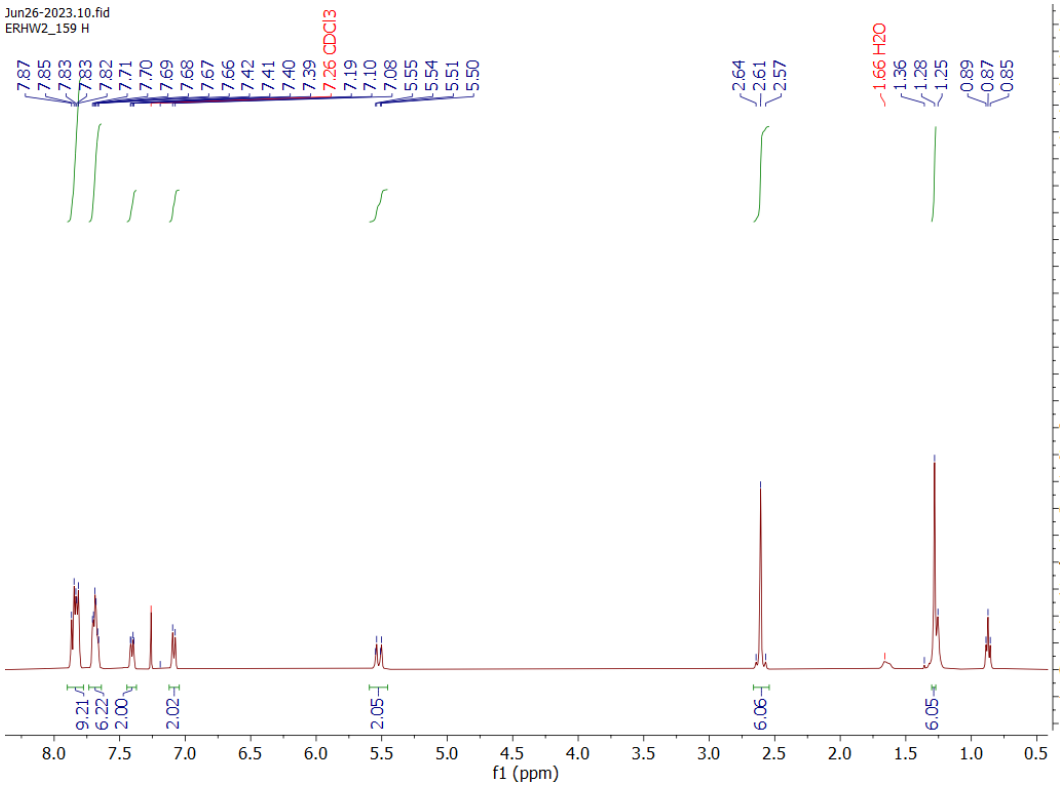


Fig. S21. The ^{1}H NMR spectrum of **BODIPY-Mito-I-4** in $CDCl_3$, 298 K.

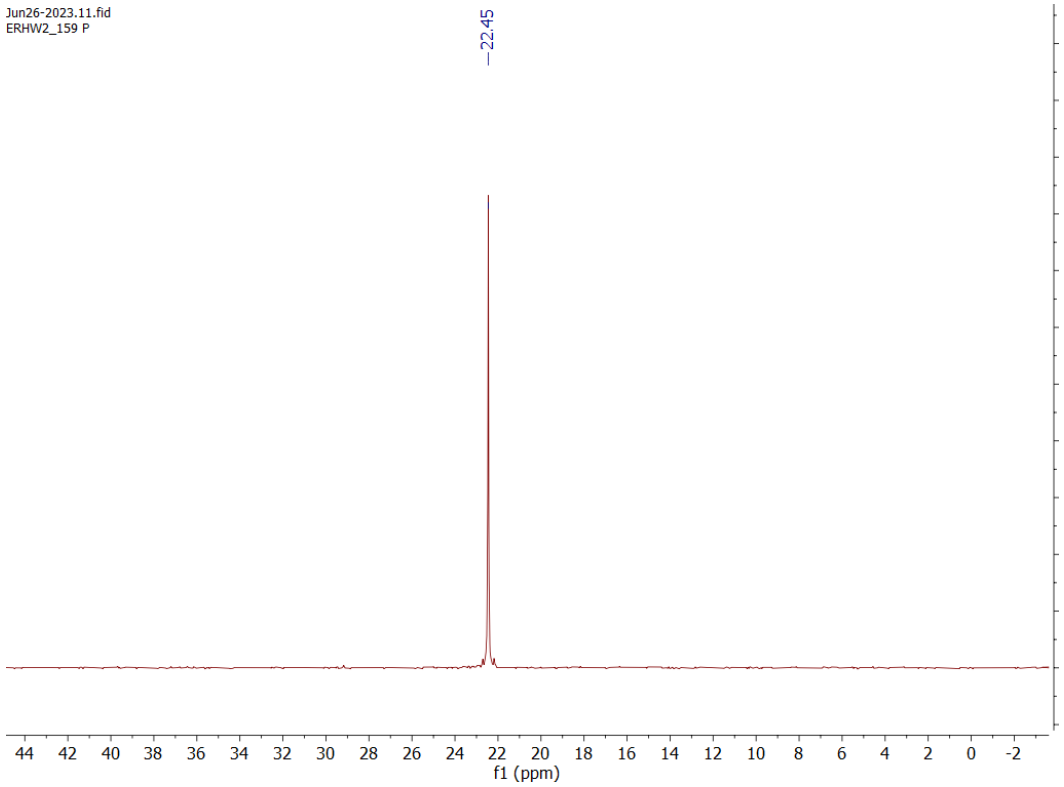


Fig. S22. The ^{31}P NMR spectrum of **BODIPY-Mito-I-4** in $CDCl_3$, 298 K.

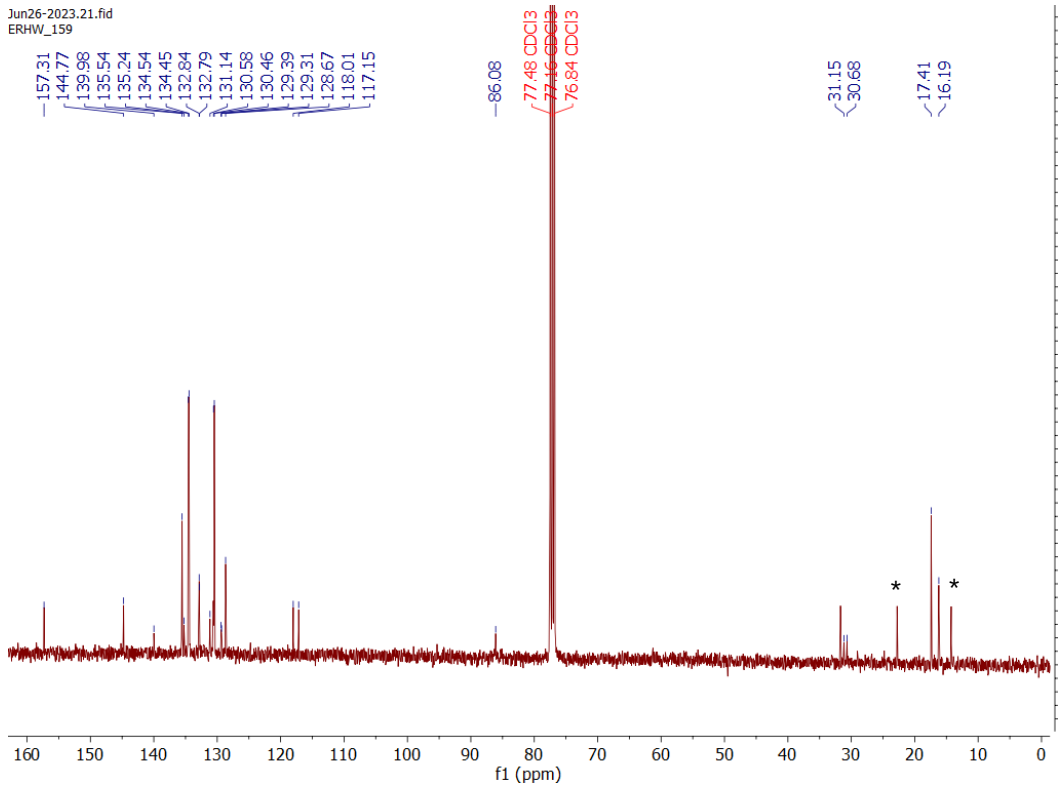


Fig. S23. The ^{13}C NMR spectrum of **BODIPY-Mito-I-4** in $CDCl_3$, 298 K. * Trace amounts of *n*-hexane.

BODIPY-Mito-I-5

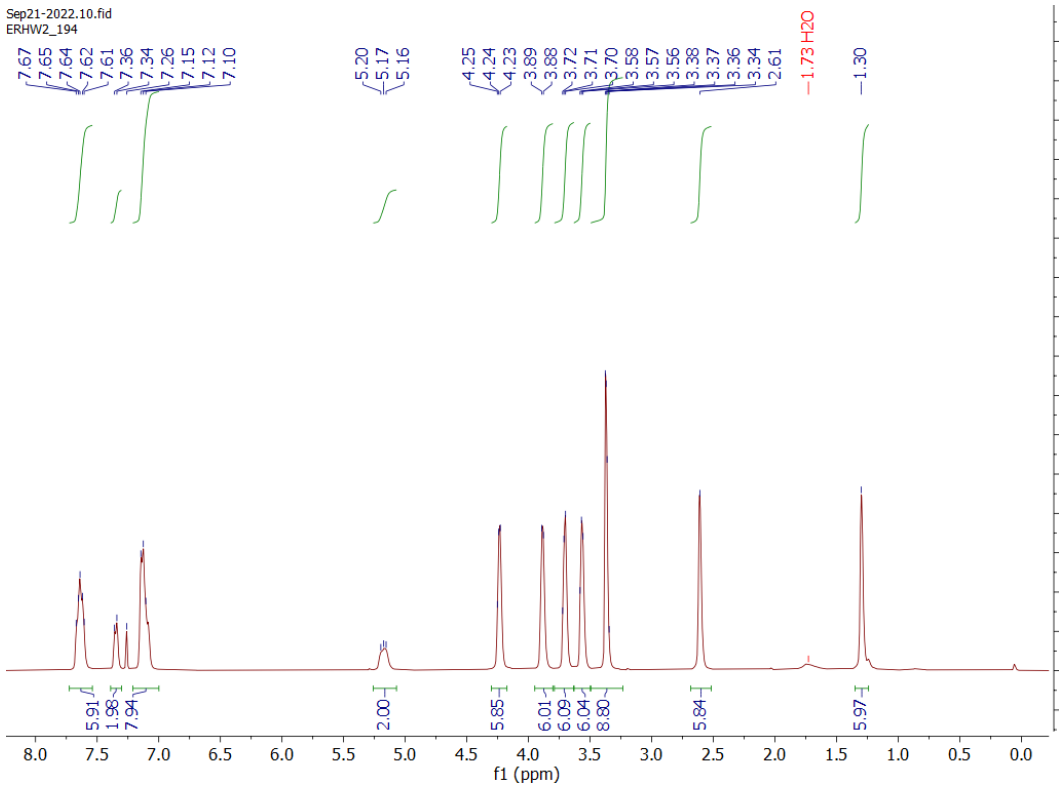


Fig. S24. The ^{1}H NMR spectrum of **BODIPY-Mito-I-5** in $CDCl_3$, 298 K.

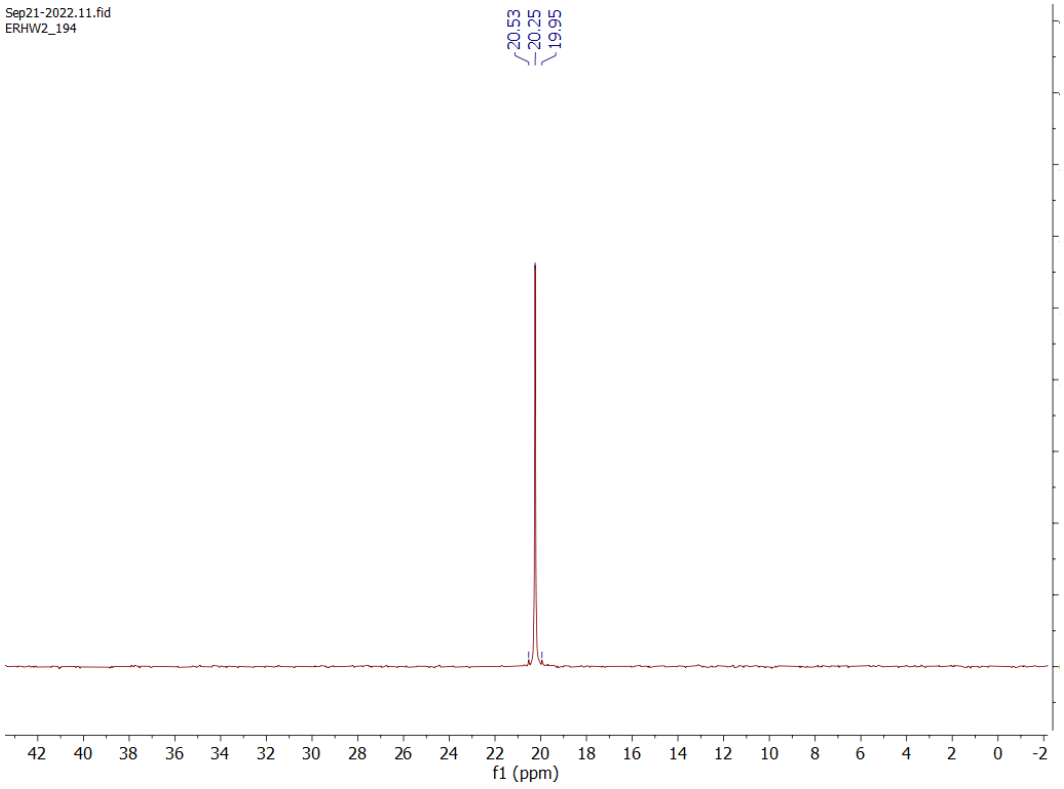


Fig. S25. The ^{31}P NMR spectrum of **BODIPY-Mito-I-5** in $CDCl_3$, 298 K.

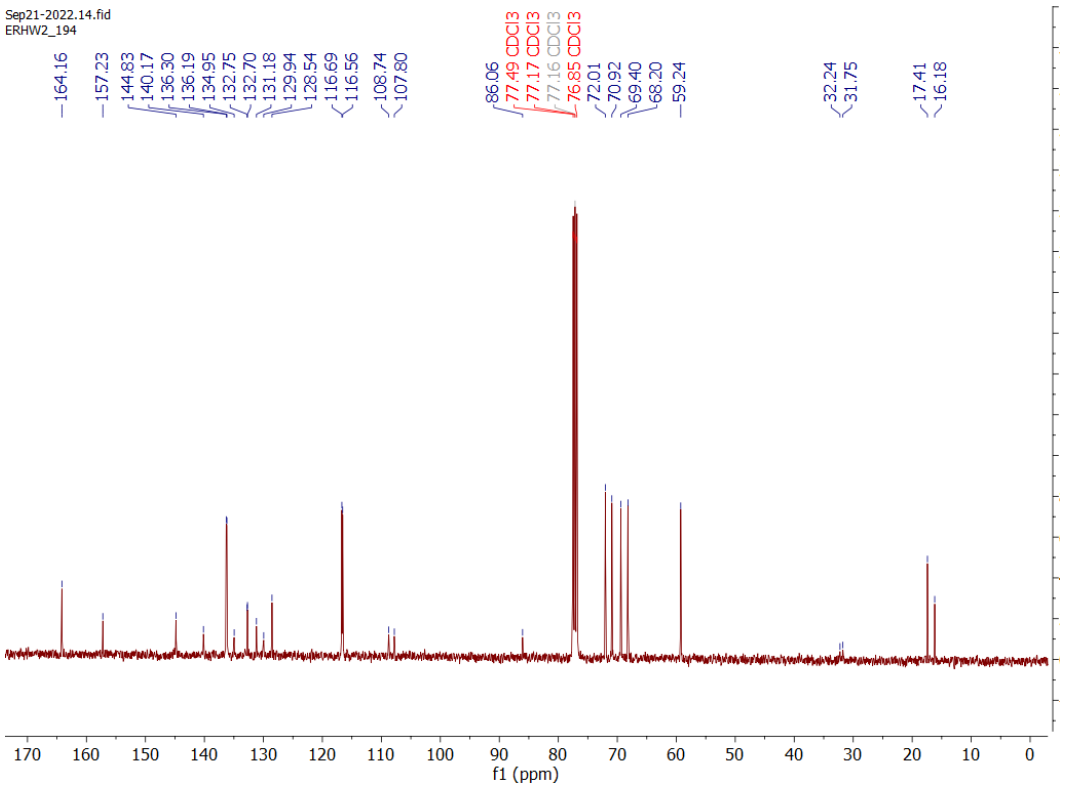


Fig. S26. The ^{13}C NMR spectrum of **BODIPY-Mito-I-5** in $CDCl_3$, 298 K.

BODIPY-Mito-I-6

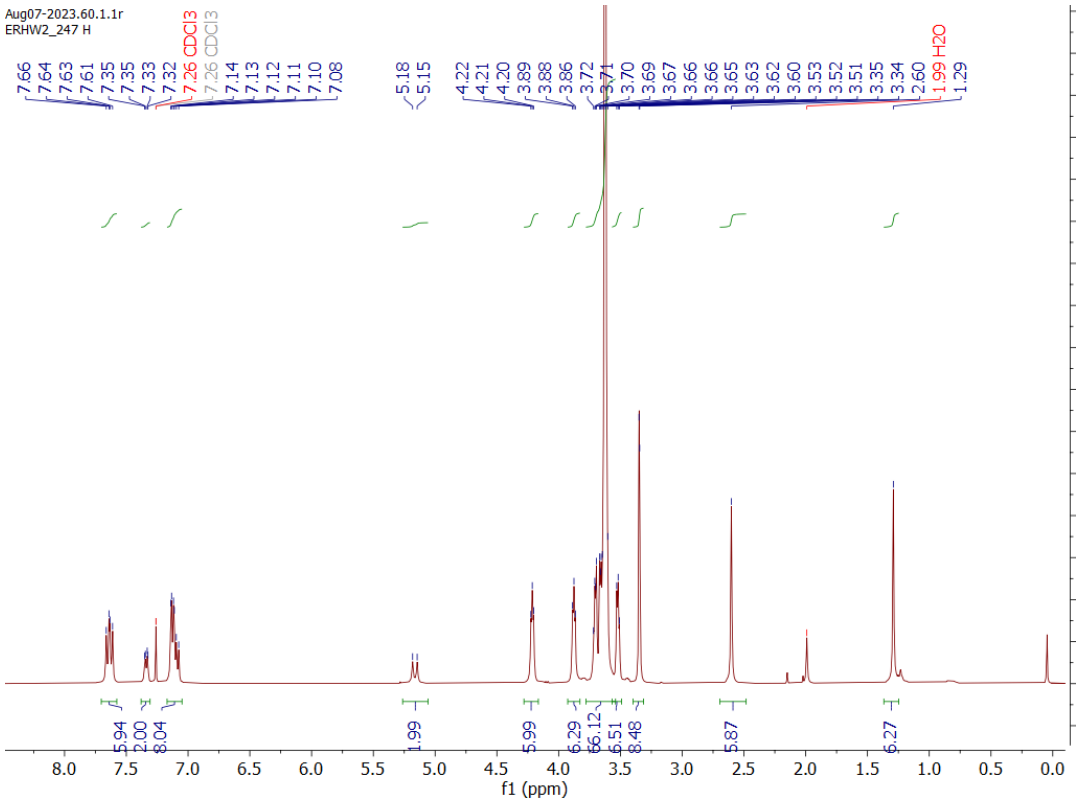


Fig. S27. The ^{1}H NMR spectrum of **BODIPY-Mito-I-6** in $CDCl_3$, 298 K.

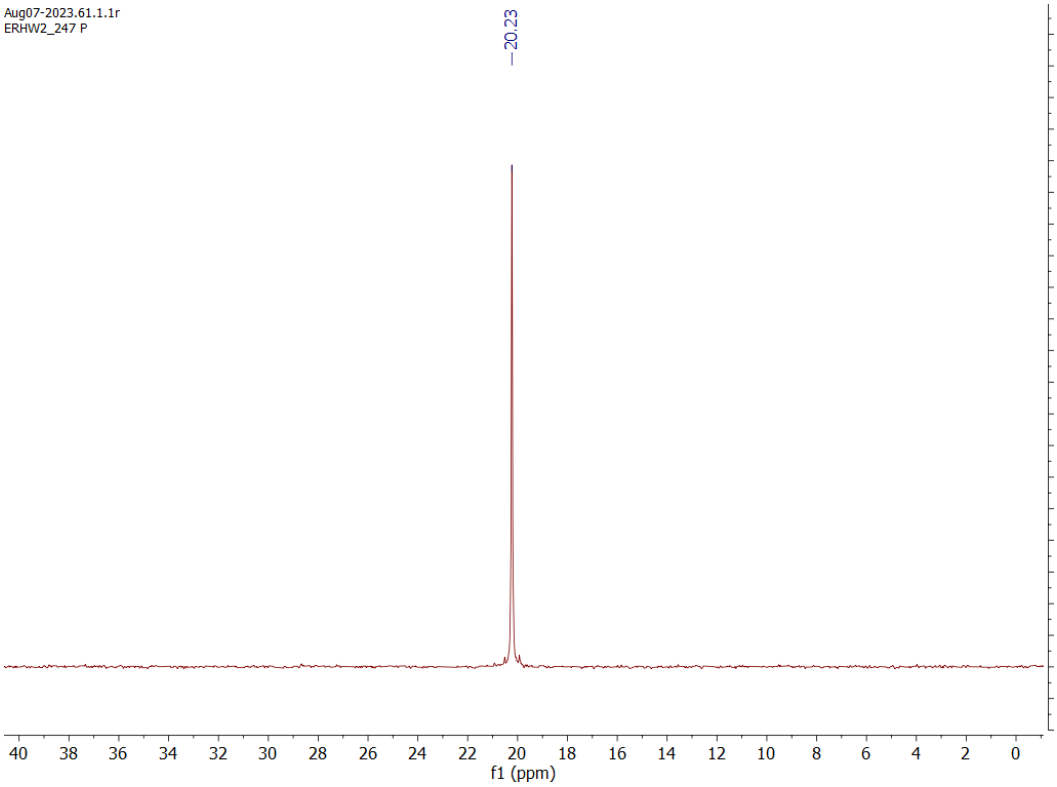


Fig. S28. The ^{31}P NMR spectrum of **BODIPY-Mito-I-6** in $CDCl_3$, 298 K.

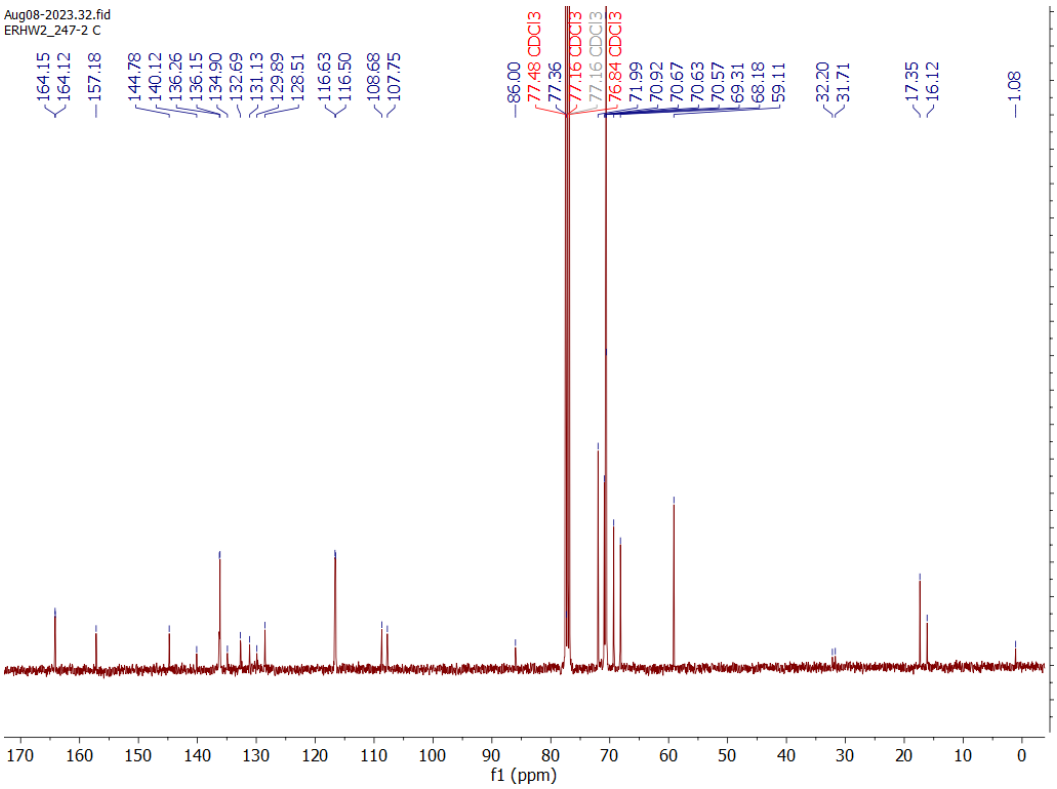
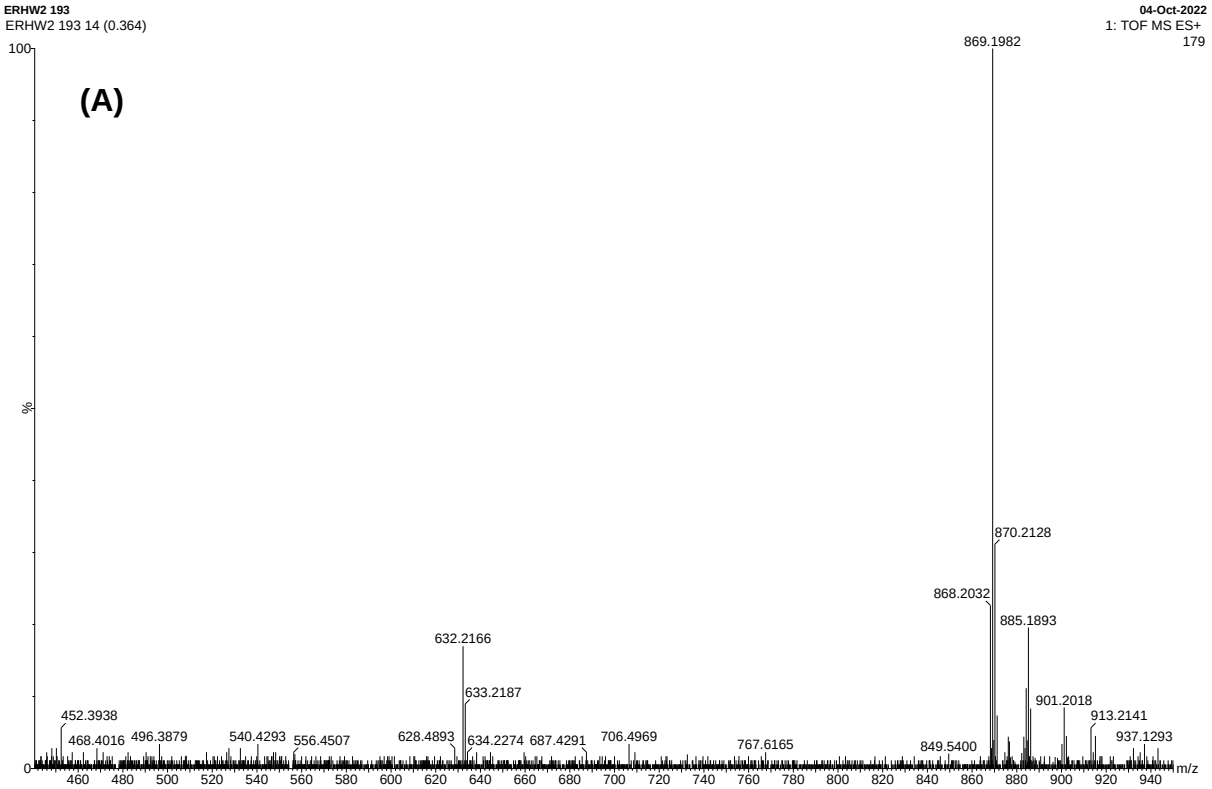


Fig. S29. The ^{13}C NMR spectrum of **BODIPY-Mito-I-6** in $CDCl_3$, 298 K.

2.3.2 Mass Spectrometry Data

BODIPY-Mito-I-1



(B)

Monoisotopic Mass, Even Electron Ions

60 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

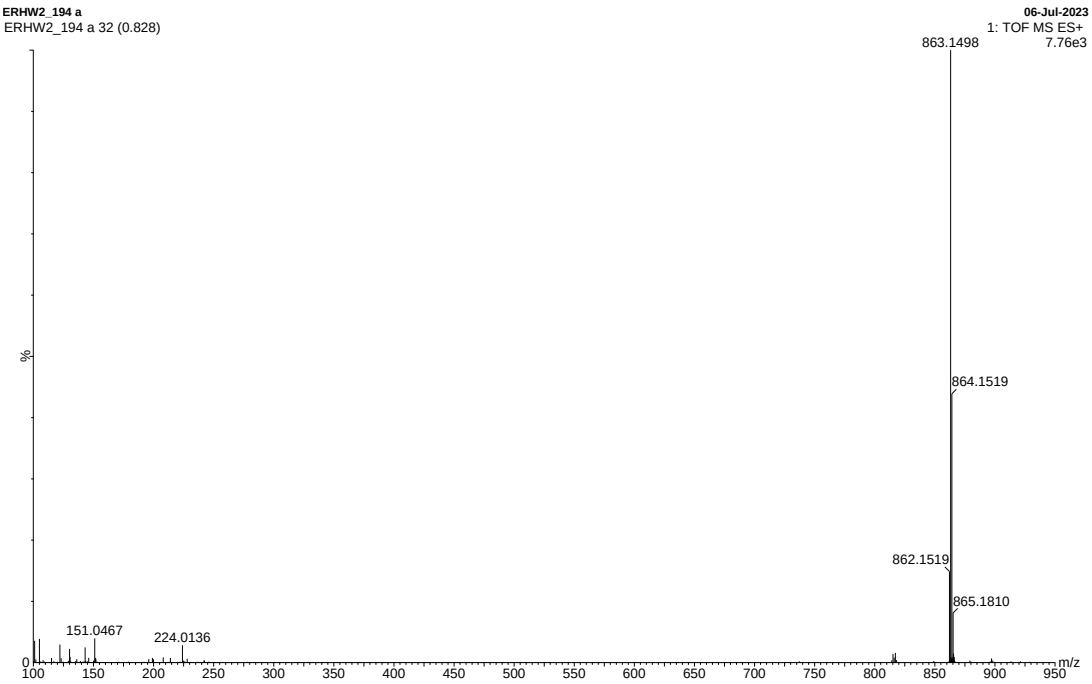
C: 38-38 H: 0-80 N: 1-11 P: 0-2 F: 2-2 B: 1-1 I: 1-2

Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
869.1982	869.1941	4.1	4.7	13.5	33.5	0.0	C38 H51 N2 P F2 B I2

Fig. S30. (A) The ESI-MS of **BODIPY-Mito-I-1**, and **(B)** the calculated and found HRMS data.

BODIPY-Mito-I-2

(A)

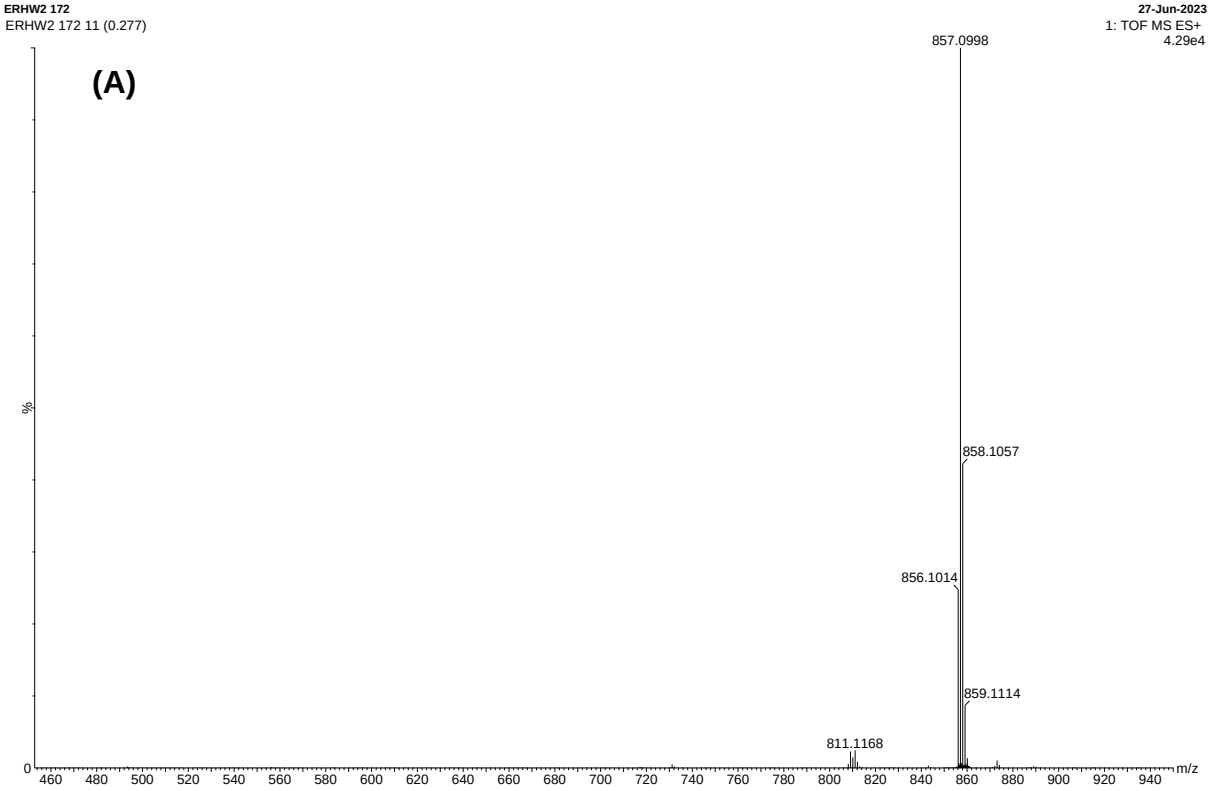


(B)

Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
863.1498	863.1471	2.7	3.1	16.5	100.2	0.0	C38 H45 N2 B F2 I2 P

Fig. S31. (A) The ESI-MS of **BODIPY-Mito-I-2**, and **(B)** the calculated and found HRMS data.

BODIPY-Mito-I-3



(B)

Monoisotopic Mass, Even Electron Ions

43 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

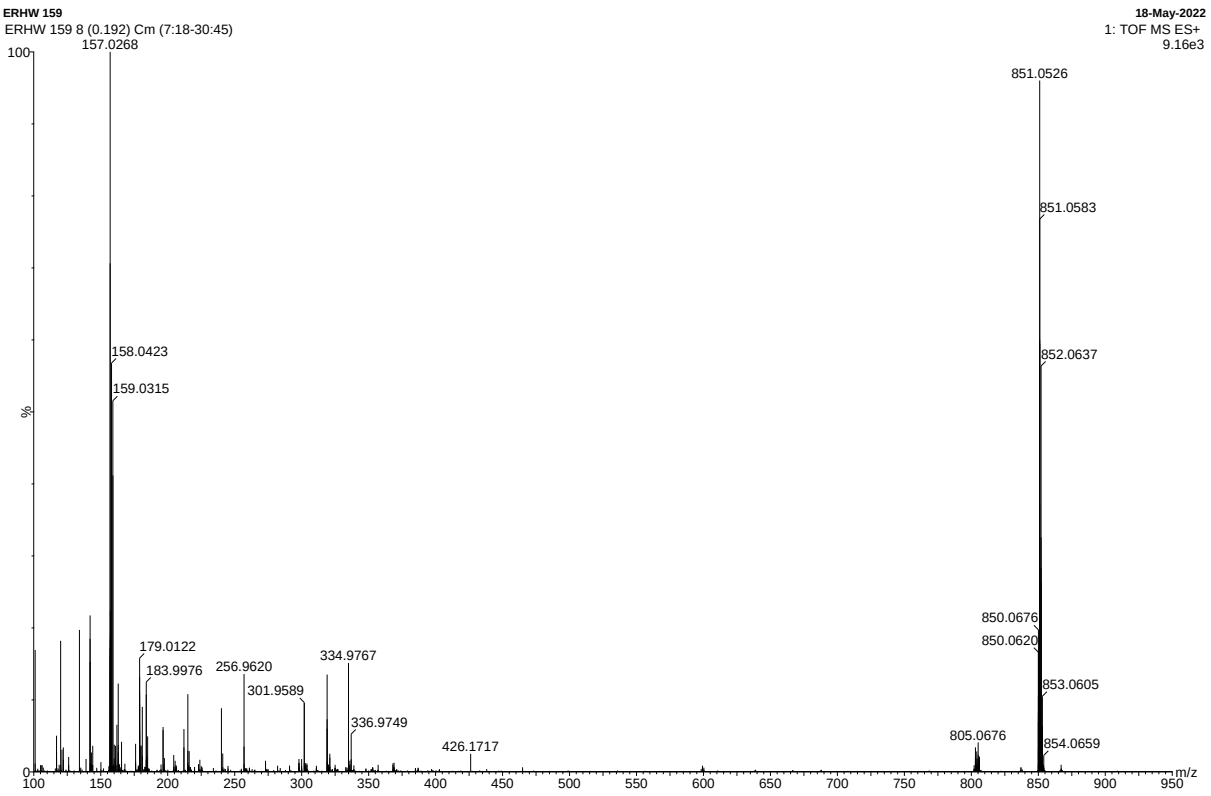
C: 38-38 H: 0-45 N: 0-5 P: 1-1 F: 2-2 I: 1-2 B: 1-4

Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
857.0998	857.1002	-0.4	-0.5	19.5	162.9	0.0	C38 H39 N2 P F2 I2 B

Fig. S32. (A) The ESI-MS of **BODIPY-Mito-I-3**, and **(B)** the calculated and found HRMS data.

BODIPY-Mito-I-4

(A)



(B)

Monoisotopic Mass, Even Electron Ions

43 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

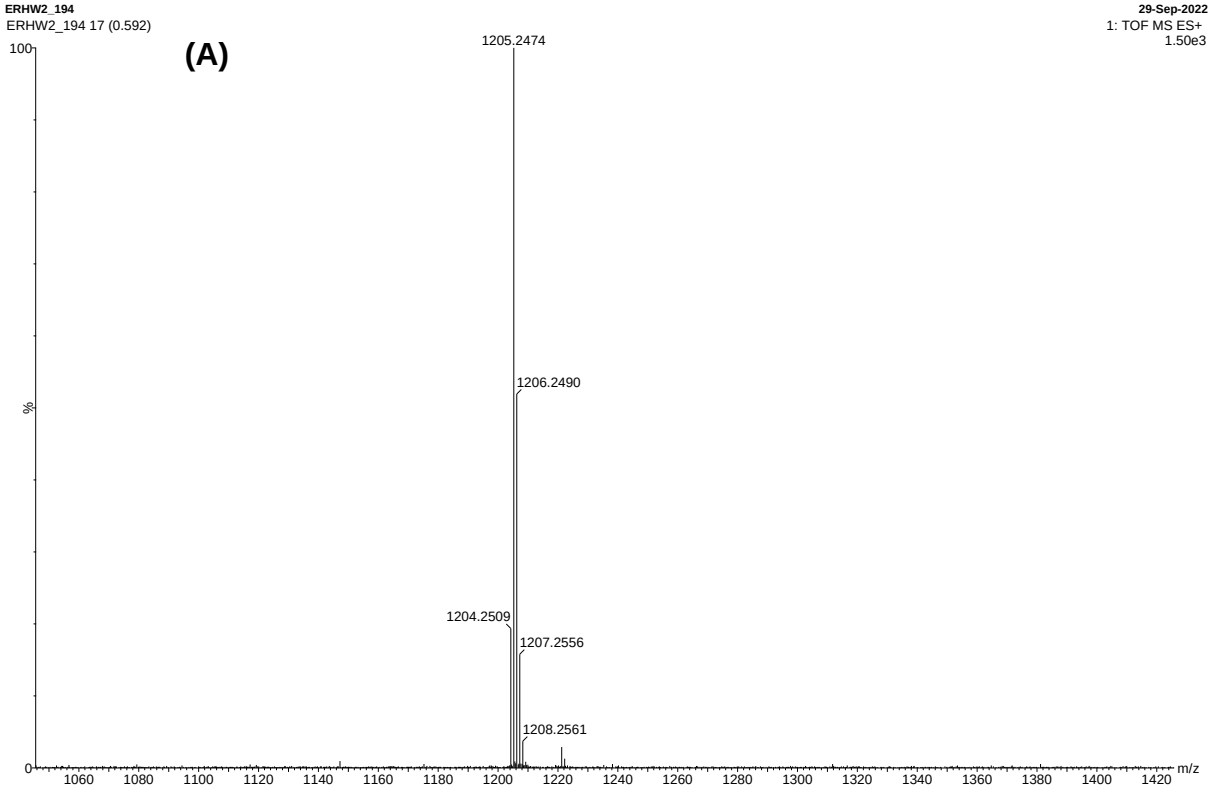
Elements Used:

C: 38-38 H: 0-45 N: 0-5 P: 1-1 F: 2-2 I: 1-2 B: 1-4

Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
857.0998	857.1002	-0.4	-0.5	19.5	162.9	0.0	C38 H39 N2 P F2 I2 B

Fig. S33. (A) The ESI-MS of **BODIPY-Mito-I-4**, and **(B)** the calculated and found HRMS data.

BODIPY-Mito-I-5



(B)

Monoisotopic Mass, Even Electron Ions

72 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:

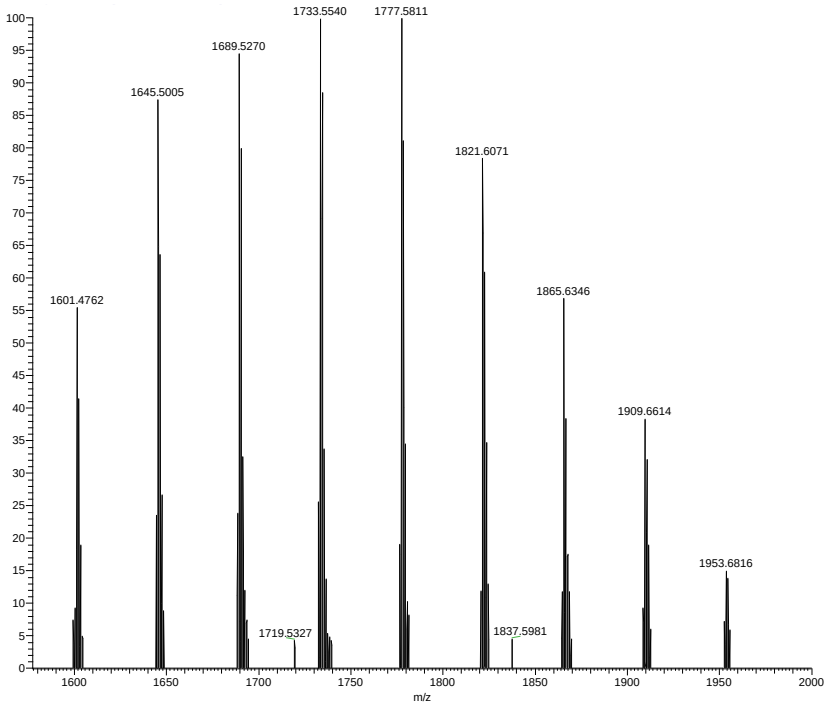
C: 53-53 H: 0-80 B: 1-1 N: 1-2 O: 1-10 F: 2-2 P: 0-1 I: 1-2

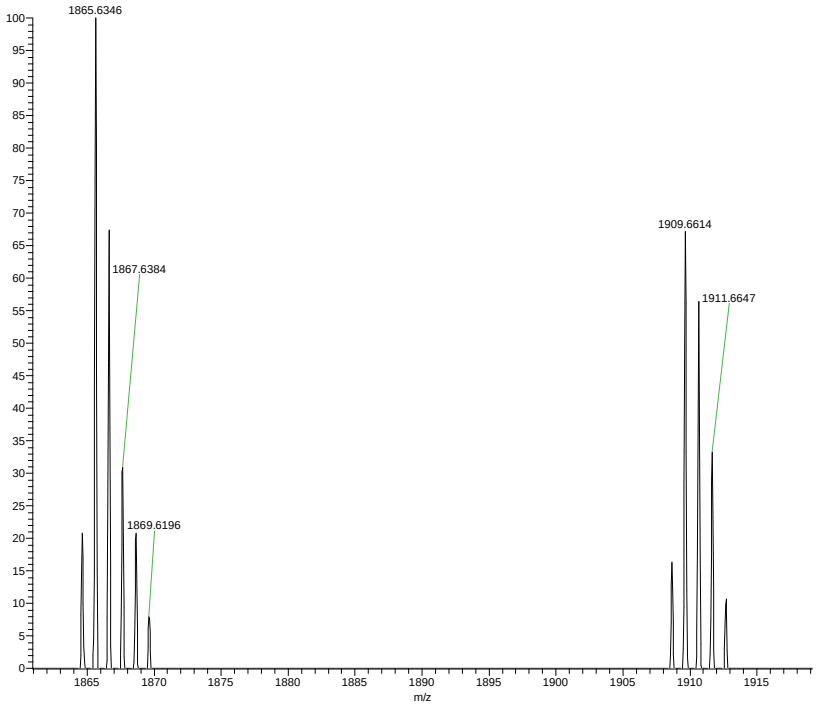
Minimum:				-1.5			
Maximum:		5.0	10.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
1205.2474	1205.2422	5.2	4.3	22.5	44.5	0.0	C53 H63 B N2 O9 F2 P I2

Fig. S34. (A) The ESI-MS of **BODIPY-Mito-I-5**, and **(B)** the calculated and found HRMS data.

BODIPY-Mito-I-6

(A)





(B)

Theoretical mass for C83H123BF2I2N2O24P+: 1865.6361
Found mass: 1865.6346
Error (ppm): -0.8

Fig. S35. (A) The ESI-MS of **BODIPY-Mito-I-6**, and **(B)** the calculated and found HRMS data.

References

1 M. Fischer and J. Georges, *Chem. Phys. Lett.*, 1996, **260**, 115–118.

2 A. Gonzalez, M. Odaybat, M. Le, J. L. Greenfield, A. J. P. White, X. Li, M. J. Fuchter and G. G. D. Han, *J. Am. Chem. Soc.*, 2022, **144**, 19430–19436.

3 R. W. Redmond and J. N. Gamlin, *Photochem. Photobiol.*, 1999, **70**, 391–475.

4 N. Epelde-Elezcano, V. Martínez-Martínez, E. Peña-Cabrera, C. F. A. Gómez-Durán, I. L. Arbeloa and S. Lacombe, *RSC Adv.*, 2016, **6**, 41991–41998.

5 E. R. H. Walter, L. C.-C. Lee, P. K.-K. Leung, K. K.-W. Lo and N. J. Long, *Chem. Sci.*, 2024, **15**, 4846–4852.

6 B. Yuan, H. Wu, H. Wang, B. Tang, J. F. Xu and X. Zhang, *Angew. Chem. Int. Ed.*, 2021, **60**, 706–710.