Supporting Information

A mitochondria-targeting fluorescent probe for imaging endogenous malondialdehyde in HeLa cells and onion tissues

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Materials and instruments: Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments; Mass spectrometric analyses were measured on a Finnigan MAT 95 XP spectrometer; High resolution mass spectrometric (HRMS) analyses were measured on an Agilent 1100 HPLC/MSD spectrometer; NMR spectra were recorded on an AVANCE III 400 MHz Digital NMR Spectrometer, using TMS as an internal standard; Electronic absorption spectra were obtained on a Shimadzu UV-2700 power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell; The fluorescent images of solution and filter paper strip were excited by a 365 nm lighting of ZF-1 UV analyzer; The fluorescence imaging of cells was performed with a Nikon A1MP confocal microscope; Ultrasonic extraction were carry out on KUDOS ultrasonic cleaner (SK2210HP); Centrifugalization was carried out on a ZONKIA high speed centrifuge (HC-2518); The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

Determination of the fluorescence quantum yield. Fluorescence quantum yield (Φ_f) was determined by using fluorescein ($\Phi_f = 0.95$, in 0.1 M NaOH, excitation at 496 nm) as the fluorescence standard. The quantum yield was calculated using the following 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 solvent used. Subscripts $_S$ and $_X$ refer to the standard and to the unknown,

respectively.

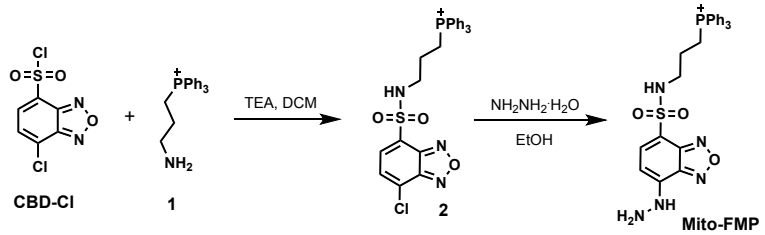
Cells culture. HeLa cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C.

Bioimaging for exogenous MDA in living cells. The cell experiment was divided into two groups. As the control experiment, the first group is that HeLa cells were incubated with **Mito-FMP** (5 μM) for 20 min, then imaged after washing by PBS buffer. In experimental group, cells were pretreated with MDA (0.5 mM) for 30 min, subsequently incubated with **Mito-FMP** (5 μM) for 20 min, and then imaged after washing by PBS buffer. The confocal microscopic imaging uses Nikon A1MP confocal microscope with excitation filter of 405 nm and the collection wavelength range is from 530-580 nm.

Bioimaging for endogenous MDA in living cells. The cell experiment was divided into four groups. The control group were obtained by the same processing method as above. In experimental groups, HeLa cells were pretreated with H_2O_2 (0.5 or 1.0 mM) for 60 min, subsequently incubated with **Mito-FMP** (5 μM) for another 30 min, then imaged after washing by PBS buffer. As negative control experiment, cells were co-pretreated with H_2O_2 (1.0 mM) and ascorbic acid (1.0 mM) for 60 min, subsequently incubated with **Mito-FMP** (5 μM) for another 30 min, then imaged after washing by PBS buffer. The confocal microscopic imaging uses Nikon A1MP confocal microscope with excitation filter of 405 nm and the collection wavelength range is from 530-580 nm.

Bioimaging for endogenous MDA in onion tissues. Taken off one piece of fresh

onion purchased in supermarket, then cut into 0.5-1.0 mm thickness small pieces as the imaging samples. The cell experiment was divided into two groups. In the first group, onion tissues incubated with **Mito-FMP** (10 μM) for 35 min, then imaged after washing by PBS buffer. In the second group, onion tissues were pretreated with L-carnosine (2 mM) for 60 min, subsequently incubated with **Mito-FMP** (10 μM) for 35 min, and then imaged after washing by PBS buffer. The confocal microscopic imaging uses Nikon A1MP confocal microscope with excitation filter of 405 nm and the collection wavelength range is from 530-580 nm.



Scheme S1. The synthetic route of probe **Mito-FMP**.

Synthesis of compound 2. Compound **1** was prepared via previous procedure (*Org. Biomol. Chem.*, 2015, **13**, 6686). The mixture of compound **CBD-Cl** (50 mg, 0.2 mmol) and **1** (80 mg, 0.2 mmol) was dissolved absolutely in dry dichloromethane, followed by addition of one drop of trimethylamine (TEA). Under the protection of nitrogen, the mixture was stirred at room temperature for 1 hour. After complete reaction, the solvent was removed under reduced pressure affording the crude product, which was purified by flash chromatography column using ethyl acetate/petroleum ether (v/v 2:1) to afford faint yellow solid as compound **2** (79 mg, yield 63 %). ^{1}H NMR (400 MHz, CD_3OD), δ (ppm): 1.81-1.87 (m, 2H), 3.15-3.16 (2H), 3.39-3.47 (2H), 7.64-7.66 (d, J = 7.6 Hz, 1H), 7.73-7.75 (7H), 7.77-7.79 (4H), 7.87 (s, 1H), 7.88-7.91 (3H), 8.01-8.03 (d, J = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ (ppm): 20.23, 24.64, 44.02, 119.14, 120.00, 128.03, 129.40, 131.45, 131.59, 131.72, 134.79, 134.89, 135.72, 136.43, 146.64, 150.39. HRMS (ESI) *m/z* calcd for $C_{27}H_{24}ClN_3O_3PS^+$ (M^+): 536.0952. Found 536.0964.

Synthesis of compound Mito-FMP. Compound **2** (50 mg, 0.2 mmol) was dissolved absolutely in ethanol, followed by addition of five drops of hydrazine hydrate (80 %). Under the protection of nitrogen, the mixture was stirred at room temperature for 2 hour. After complete reaction, the solvent was removed under reduced pressure affording the crude product, which was purified by flash chromatography column using ethyl acetate/ethanol (v/v 100:1) to afford yellow solid as compound **Mito-FMP** (54 mg, yield 55 %). ^{1}H NMR (400 MHz, CD_3OD), δ (ppm): 1.78-1.86 (m, 2H), 3.14-3.17 (d, *J* = 6.2 Hz, 2H), 3.38-3.45 (2H), 7.73-7.75 (5H), 7.76-7.77 (4H), 7.78 (s, 3H), 7.88-7.89 (1H), 7.90-7.92 (2H), 7.93 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ (ppm): 20.69, 24.38, 43.71, 99.35, 111.29, 119.20, 120.06, 124.90, 131.45, 131.56, 131.68, 133.90, 134.73, 134.82, 136.40, 140.46, 144.48, 144.92, 146.95, 147.59, 150.18. HRMS (ESI) *m/z* calcd for $C_{27}H_{27}ClN_5O_3PS^+$ (M^+): 532.1563. Found 532.1570.

Synthesis of compound SBD-MDA. Compound **Mito-FMP** (30 mg, 0.05 mmol) was dissolved absolutely in ethanol (3 mL), and MDA solution (5 mL, 100 mM) was added and stirred for 1 hour at room temperature. After complete reaction, The mixture was extracted with dichloromethane (3×25 mL). The organic layer was collected, treated with anhydrous Na_2SO_4 and dried under reduced pressure affording the crude product, which was purified by flash chromatography column using dichloromethane/ethanol (v/v 50:1) to afford pale yellow solid as compound **SBD-MDA** (12 mg, yield 38 %). ^{1}H NMR (400 MHz, CD_3OD), δ (ppm): 1.81-1.87 (m, 2H), 3.27-3.30 (t, *J* = 6.6 Hz, 2H), 3.41-3.45 (2H), 6.76-6.78 (t, *J* = 2.2 Hz, 1H), 7.75 (2H), 7.76 (4H), 7.77-7.78 (5H), 7.86-7.87 (1H), 7.88-7.91 (3H), 7.97-7.98 (d, *J* = 1.6 Hz, 1H), 8.14-8.16 (d, *J* = 7.6 Hz, 1H), 8.20-8.22 (d, *J* = 8.0 Hz, 1H), 8.97-8.98 (d, *J* = 2.8 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ (ppm): 20.21, 31.67, 43.71, 111.16, 119.06, 119.93, 124.92, 131.35, 131.49, 131.61, 134.67, 137.77, 135.51, 136.38, 140.64, 145.26, 147.49, 150.12. HRMS (ESI) *m/z* calcd for $C_{30}H_{27}N_5O_3PS^+$ (M^+): 568.1567.

Found 568.1550.

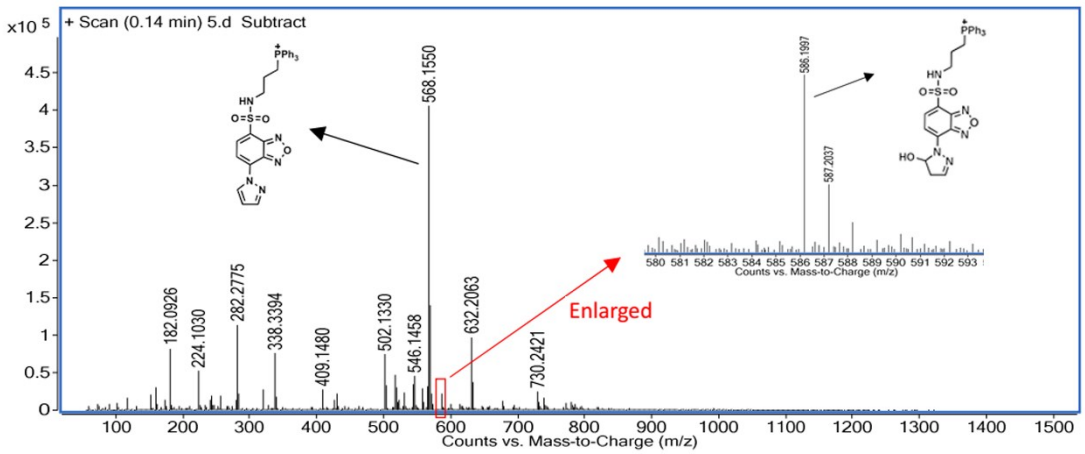


Figure S1. HRMS (ESI) of **Mito-FMP** in the presence of MDA in aqueous solution.

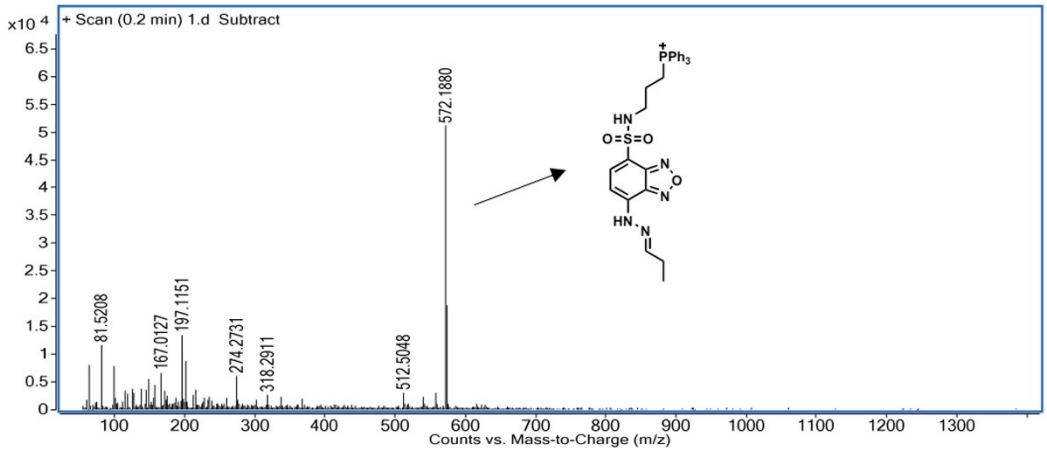


Figure S2. HRMS (ESI) of **Mito-FMP** in the presence of propaldehyde in aqueous solution.

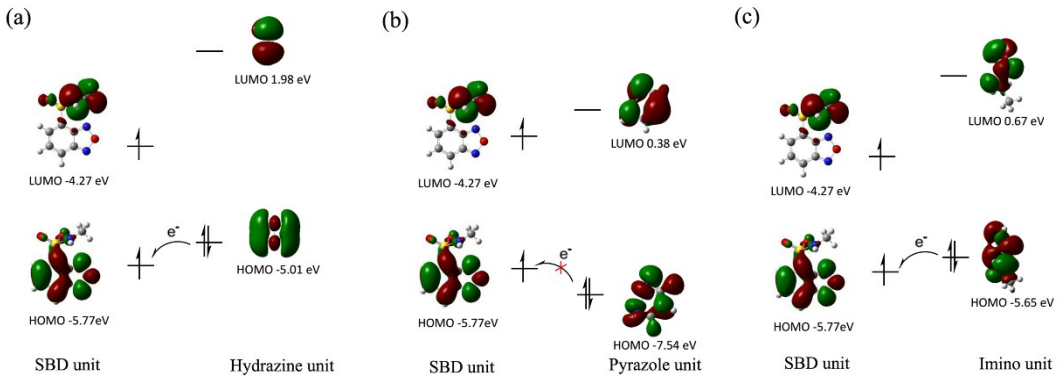


Figure S3. The frontier orbital energies of SBD chromophore and the potential electron donor (hydrazine unit, pyazole unit, and imino unit (a representative condensation product of hydrazine and propaldehyde)) calculated by a suite of Gaussian 09 programs.

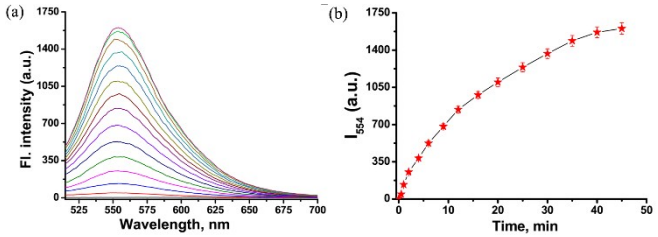


Figure S4. Reaction-time profiles of the probe **Mito-FMP** (10 μM) in the presence of 1.0 mM MDA in PBS buffer (25 mM, pH 7.4, containing 1% ethanol). Excitation: 373 nm.

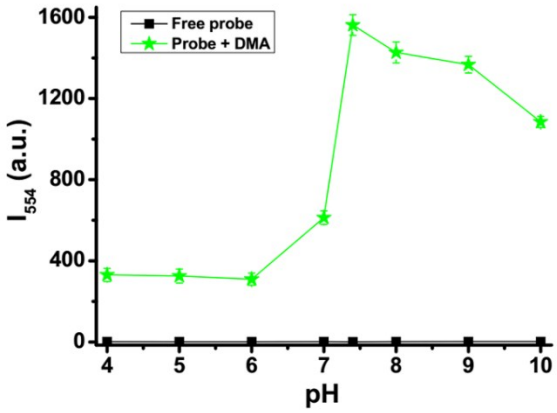


Figure S5. The fluorescence intensity of **Mito-FMP** (10 μM) at 554 nm in the presence or absence of excess MDA in various pH ranging from 4.0 to 10.0 PBS buffer (25 mM, containing 1% ethanol), respectively.

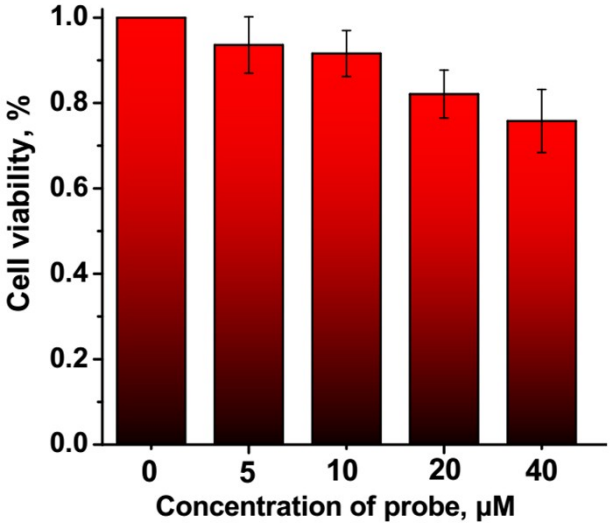


Figure S6. The cell viability of living HeLa cells treated with 5, 10, 20, or 40 μM **Mito-FMP** for 12 hours measured by standard MTT assay.

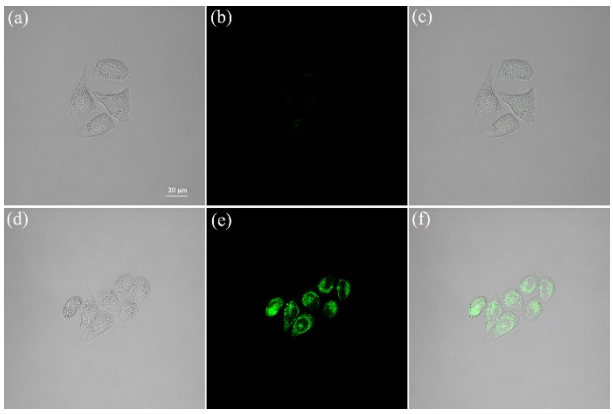


Figure S7. Confocal fluorescence imaging of probe **Mito-FMP** (5 μM) responding to exogenous MDA in living HeLa cells. (a-c) Cells were incubated with **Mito-FMP** for 20 min, then imaged; (d-f) Cells were pretreated with MDA (0.5 mM) for 30 min, subsequently incubated with **Mito-FMP** for 20 min, and then imaged. The fluorescence images were captured from the green channel of 530-580 nm with an excitation at 405 nm. (a, d) bright field images; (b, e) green channel images; (c, f) merged bright field images with green channel images. Scale bar: 20 μm.

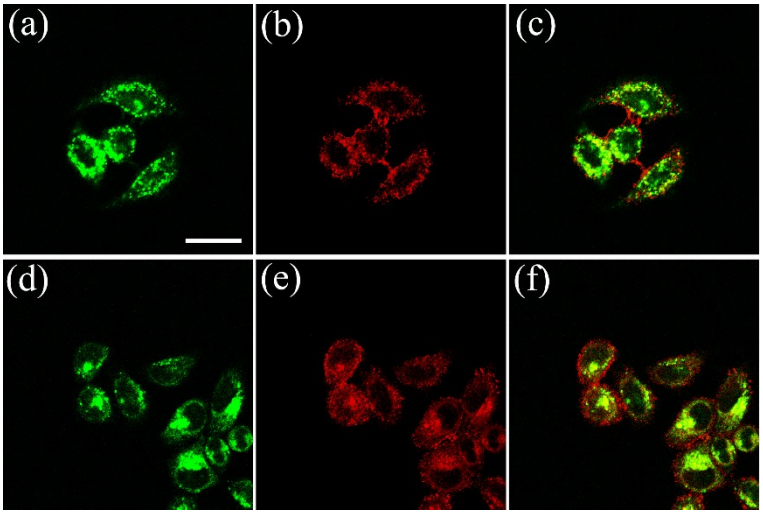


Figure S8. The confocal fluorescence images of living HeLa cells pretreated with MDA (0.5 mM, 30 min) and **Mito-FMP** (5 μM, 20 min), subsequently co-incubated with Lyso-Tracker Red (1 μM, a-c) or for ER-Tracker Red (1 μM, d-f) 10 min. (a, d) Green channel images (λ_{ex}= 405 nm, λ_{em} = 530-580 nm); (b, e) Red channel images (λ_{ex} = 561 nm, λ_{em} = 590-640 nm); (c, f) Merged green and red channel image. Scale bar: 20 μm.

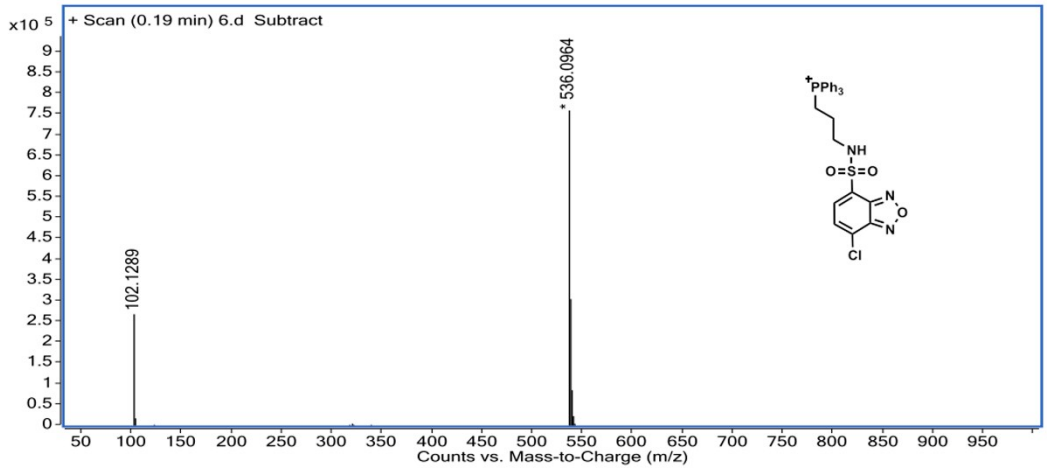


Figure S9. HRMS (ESI) of compound **2**. *m/z* calcd for $C_{27}H_{24}ClN_3O_3PS^+$ (M^+): 536.0952. Found 536.0964.

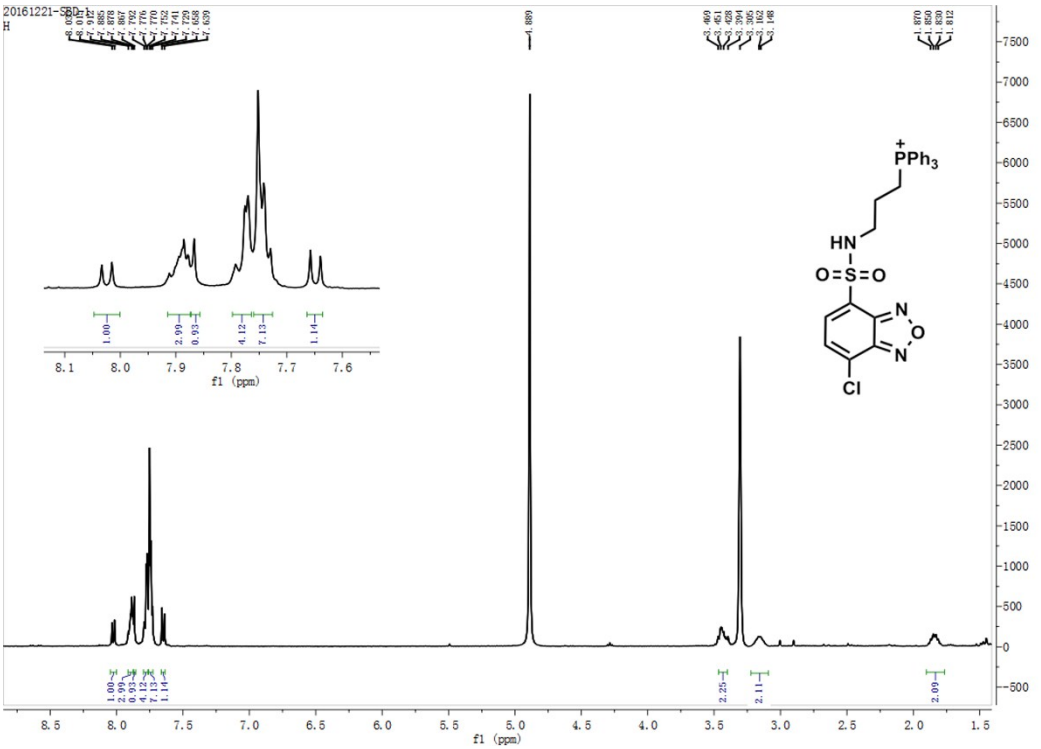


Figure S10. ^{1}H NMR of compound **2** in CD_3OD.

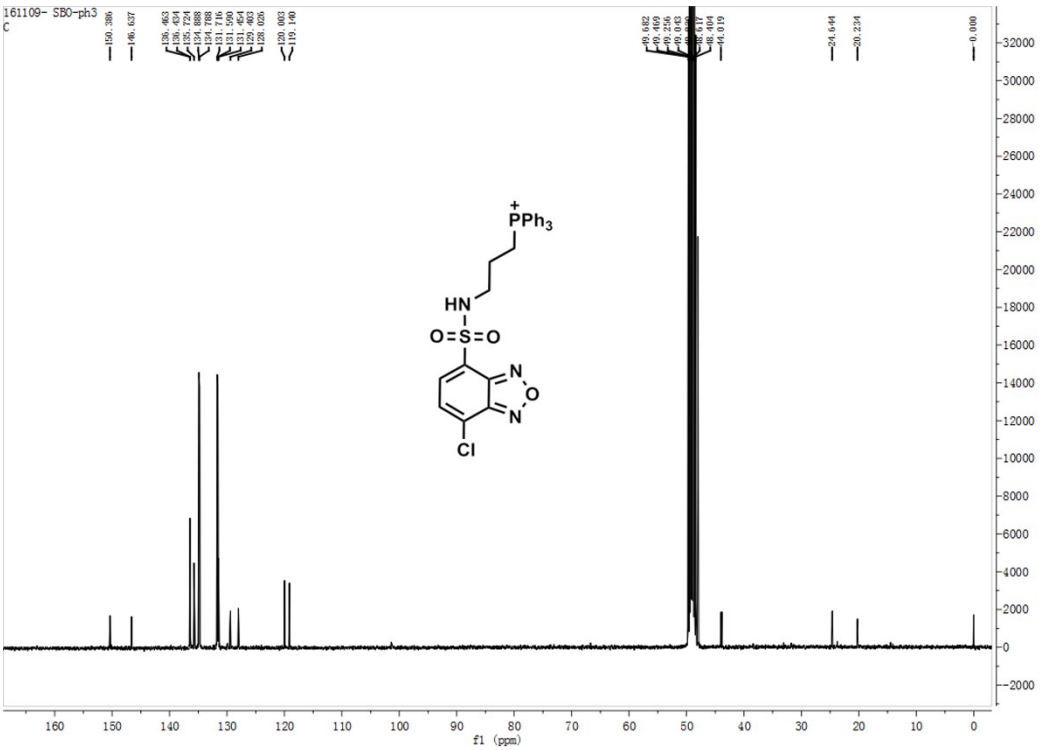


Figure S11. ^{13}C NMR of compound **2** in CD_3OD.

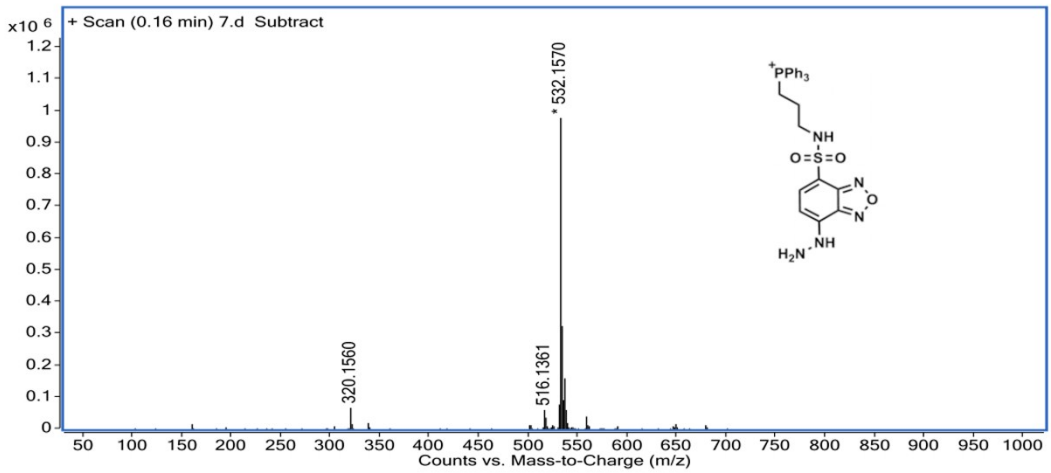


Figure S12. HRMS (ESI) of compound **Mito-FMP**. *m/z* calcd for $C_{27}H_{27}ClN_5O_3PS^+$ (M^+): 532.1563. Found 532.1570.

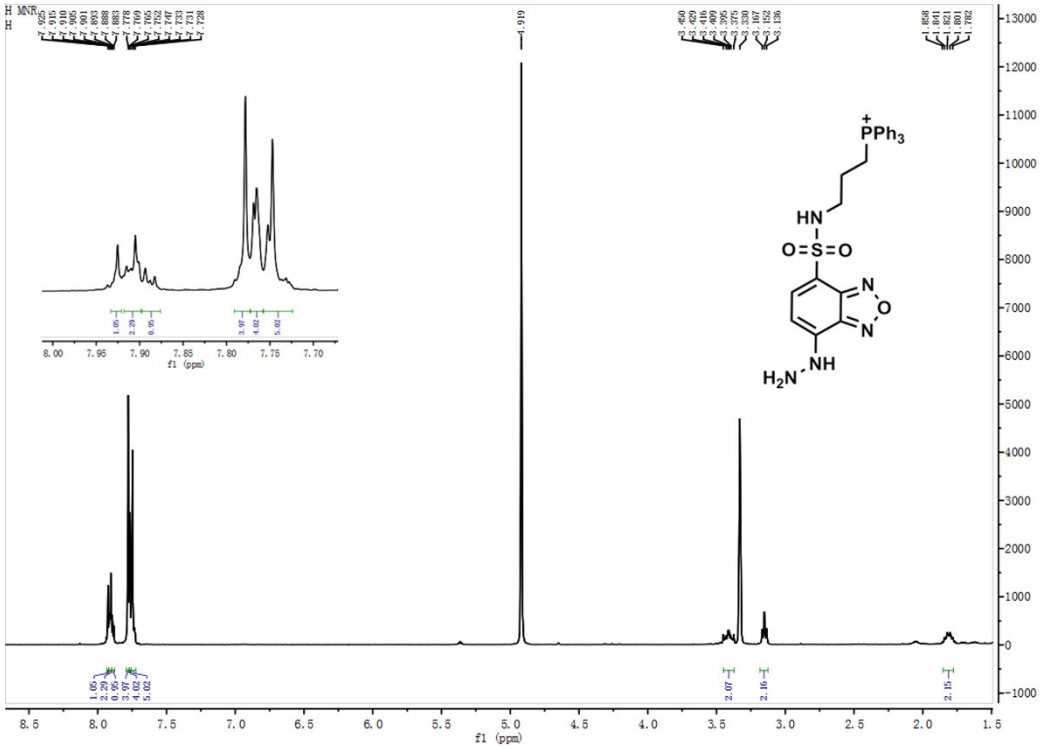


Figure S13. ^{1}H NMR of compound **Mito-FMP** in CD_3OD.

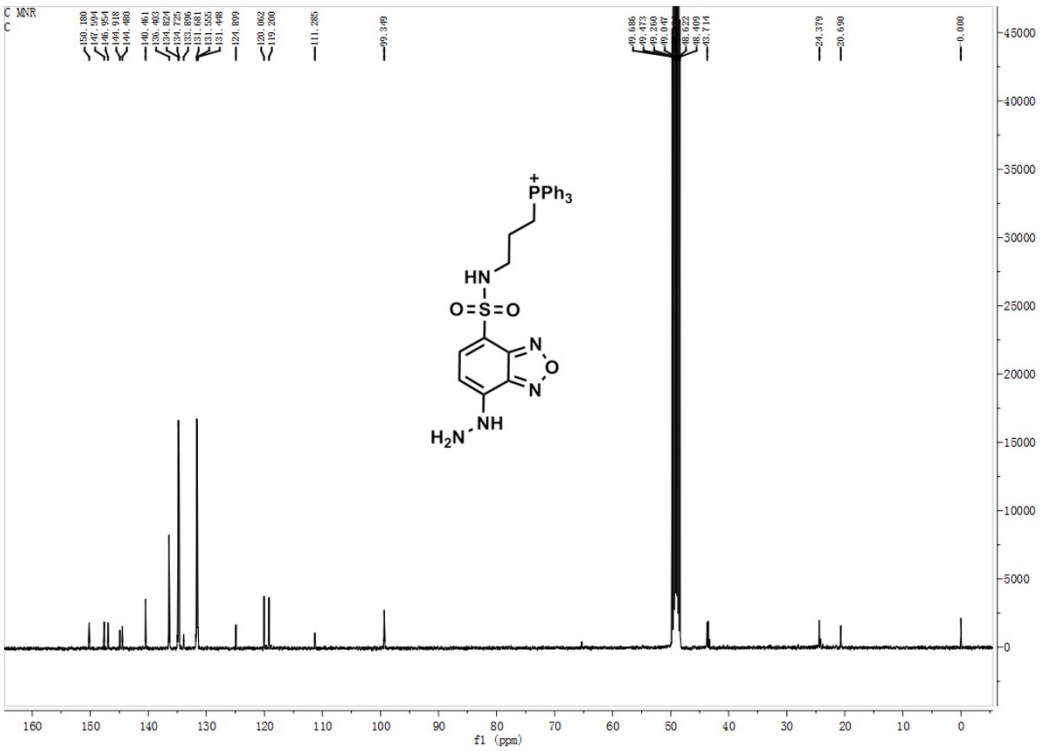


Figure S14. ^{13}C NMR of compound **Mito-FMP** in CD_3OD.

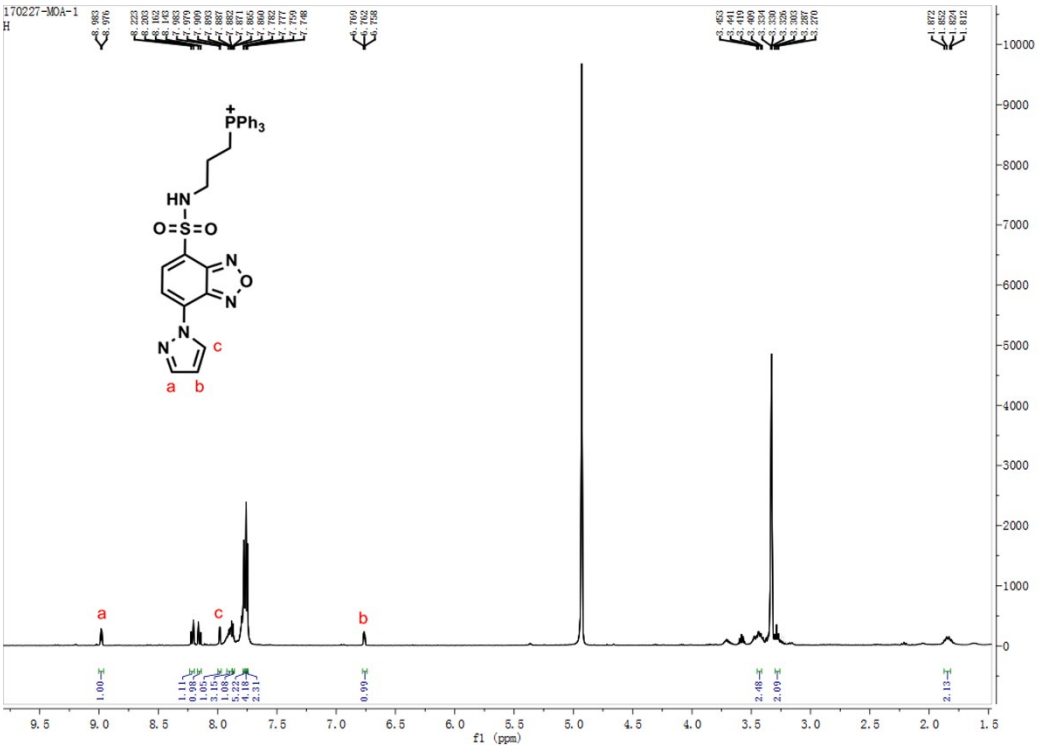


Figure S15. ^{1}H NMR of compound **SBD-MDA** in CD_3OD.

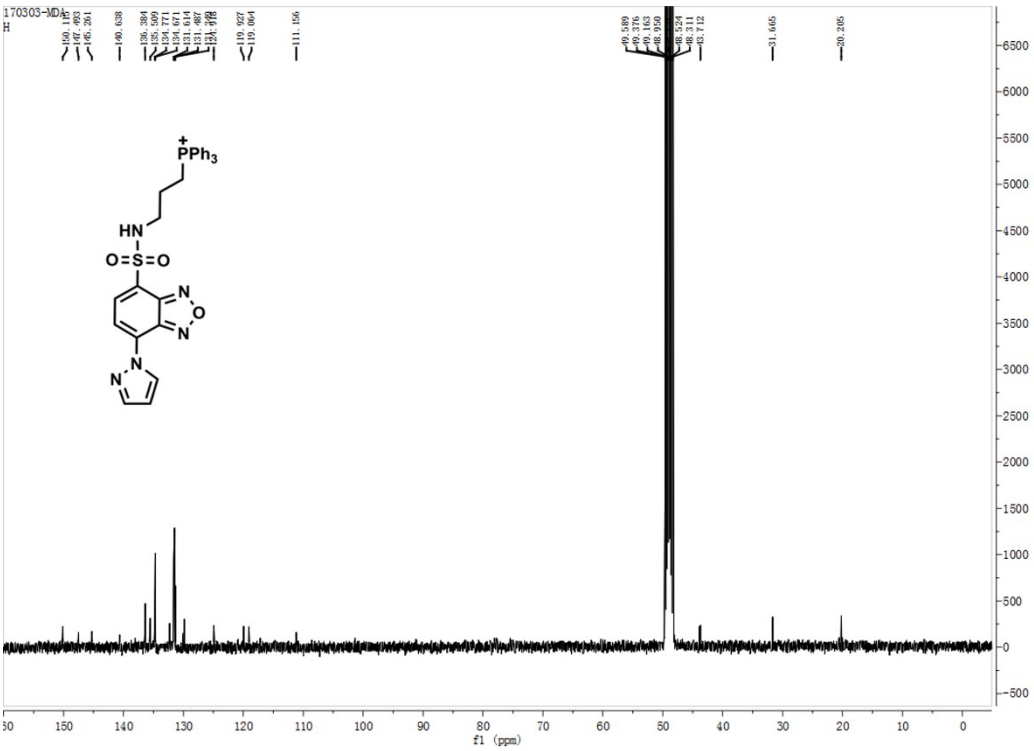


Figure S16. ^{13}C NMR of compound **SBD-MDA** in CD_3OD.