Supporting Information for

A bright, red-emitting water-soluble BODIPY fluorophore as an alternative to the commercial Mito Tracker Red for high-resolution mitochondrial imaging

Jiang-Lin Wang [a], Lu Zhang [a], Lian-Xun Gao[a], Ji-Lei Chen[a], Te Zhou *[,b], Yi Liu [a,c] and Feng-Lei Jiang *[,a]

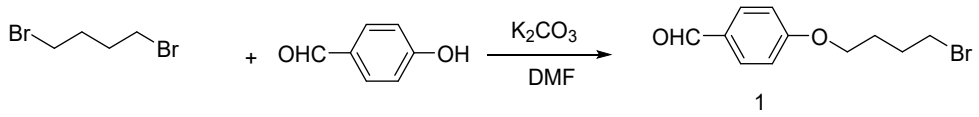
[a] Sauvage Center for Molecular Sciences, College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, P. R. China

[b] The State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) & Key Laboratory of Oral Biomedicine Ministry of Education, School & Hospital of Stomatology, Wuhan University, Wuhan 430079, P. R. China

[c] College of Chemistry and Chemical Engineering, Tiangong University, Tianjin 300387, P. R. China

*Corresponding Author. E-mail: fljiang@whu.edu.cn (F.-L. J.). Tel: +86-27-68756667. isaaczt@whu.edu.cn (T. Z.)

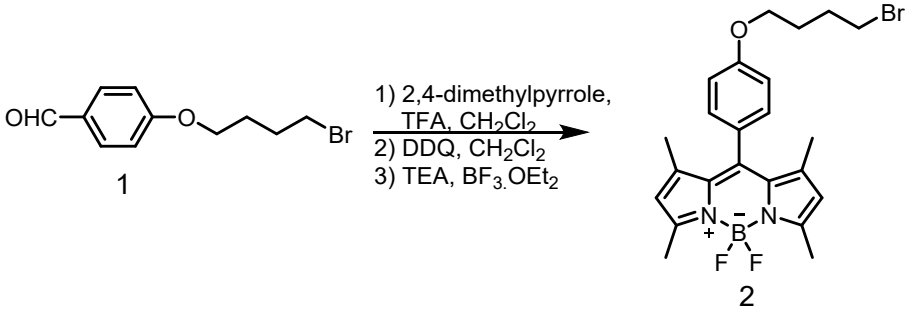
Synthesis of compound **1**



1

K_2CO_3 (27.64 g, 2 mol) was added to a solution of *p*-hydroxybenzaldehyde (24.42 g, 0.20 mol) in DMF. The mixture was stirred continuously for 1 h at room temperature. 1,4-dibromobutane (43.19 g, 0.2 mmol) was dissolved in DMF and added dropwise over a period of 1.0 h. The reaction solution was stirred at room temperature and monitoring by TLC. After the completion of the reaction, the mixture was filtered, then extracted with ethyl acetate and concentrated under reduced pressure. The obtained crude product was purified by silica gel column chromatography, eluted with petroleum ether/ethyl acetate (v / v = 4 : 1) to obtain white waxy solid (yield, 43.71 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1 H), 7.97 – 7.75 (m, 2 H), 7.14 – 6.92 (m, 2 H), 4.09 (t, J = 6.0 Hz, 2 H), 3.51 (d, J = 6.4 Hz, 1 H), 2.13 – 1.92 (m, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.71, 29.31, 33.22, 67.27, 114.73, 129.99, 132.03, 163.91, 190.81 ppm.

Synthesis of compound **2**

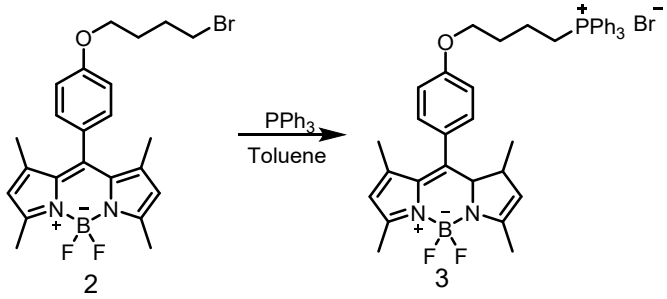


1 2

Catalytic amount of TFA was added to the mixture of compound **1** (1.02 g, 4 mmol) and 2,4-dimethylpyrrole (0.76 g, 8 mmol) in 300 mL of dry dichloromethane under argon atmosphere. Then, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (4.54 g, 40 mmol) was dissolved in dry DCM and added dropwise to the mixture. The reaction solution was continuously stirred for 2.0 h at room temperature and cooled to 0 ℃ by ice water bath. Then, 10 mL of triethylamine (Et_3N) and 10 mL of boron trifluoride diethyl ether (BF_3-OEt_2) were added dropwise over 30 min. After stirring for 12 h, the

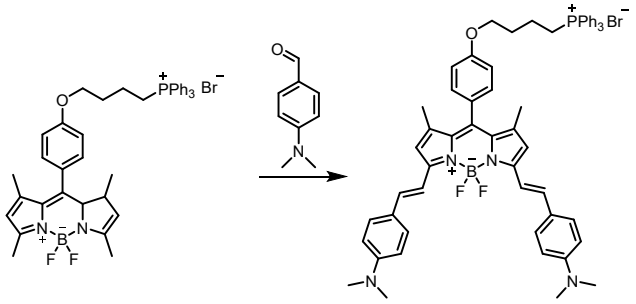
reaction solution was washed successively with saturated $NaHCO_3$ solution and water, then extracted with DCM, dried over anhydrous sodium sulfate. The crude product was purified by column chromatography and the eluent was the mixture of petroleum ether / ethyl acetate (v / v) = 8/1 to give compound **2** (yield, 0.34 g, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 – 7.13 (m, 2 H), 7.05 – 6.93 (m, 2 H), 5.97 (s, 2 H), 4.05 (t, *J* = 6.0 Hz, 2 H), 3.52 (t, *J* = 6.6 Hz, 2 H), 2.55 (s, 6 H), 2.18 – 2.07 (m, 2 H), 2.05 – 1.95 (m, 2H), 1.43 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.58, 14.61, 27.88, 29.49, 33.32, 33.41, 66.97, 115.03, 121.11, 127.14, 129.24, 131.85, 141.82, 143.16, 155.29, 159.41ppm. ESI-MS: m/z= 657.3062.

Synthesis of compound **3**



The reaction mixture of compound **2** (0.47 g, 1.0 mmol) and triphenylphosphine (1.311 g, 5 mmol) were refluxed in 10 mL toluene for 36 h until the orange precipitate occurred. The product would be obtained by vacuum filtration (yield, 0.67 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.79 – 7.91 (m, 15 H), 7.24-7.27 (d, 2 H), 7.04-7.07 (d, 2 H), 6.17 (s, 2 H), 4.10 (s, 2 H), 3.69 (s, 2 H), 2.44 (s, 6 H), 1.96 (s, 2 H), 1.79 (s, 2 H), 1.38 (s, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.65, 14.57, 14.63, 19.44, 21.99, 22.49, 29.56, 46.06, 66.78, 115.09, 118.01, 118.86, 121.10, 127.08, 129.23, 130.41, 130.45, 130.53, 131.84, 133.73, 133.76, 133.83, 134.98, 135.01, 141.83, 143.10, 155.26, 159.34 ppm. ESI-MS: m/z= 717.3813.

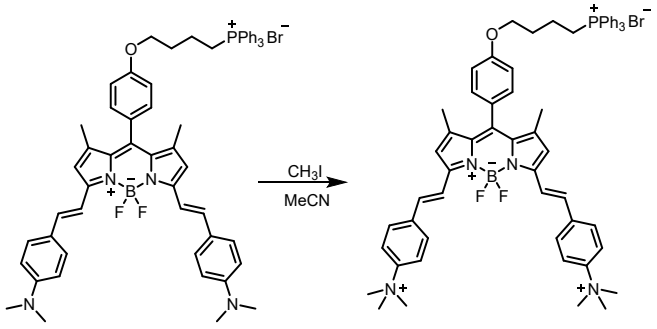
Synthesis of **Mito-BDP 760**



3 Mito-BDP 760

Catalytic amount of piperidine (1.0 mL) was added to the mixture of 4-dimethylaminobenzaldehyde and compound **3**. The reaction was stirred for 12 h. Then, the mixture was cooled to room temperature and extracted with CH_2Cl_2, rinsed with saturated brine, dry over Na_2SO_4, and evaporated the solvent under reduced pressure. The crude product was purified by the column chromatography (CH_2Cl_2 / MeOH = 3: 1) to afford **Mito-BDP 760** (yield, 1.46 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 – 7.82 (m, 6 H), 7.81 – 7.75 (m, 4 H), 7.74 – 7.65 (m, 6 H), 7.59 – 7.47 (m, 6H), 7.21 – 7.10 (m, 4 H), 6.95 – 6.88 (m, 2 H), 6.75 – 6.65 (m, 4 H), 6.57 (s, 2 H), 4.12 (t, J = 5.6 Hz, 2 H), 4.03 – 3.85 (m, 2 H), 3.02 (s, 12 H), 2.28 – 2.14 (m, 2 H), 1.44 (s, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.65, 14.57, 14.63, 19.44, 21.99, 22.49, 29.56, 46.06, 50.66, 52.12, 66.78, 68.49, 115.09, 118.01, 118.86, 121.10, 127.08, 129.23, 130.41, 130.45, 130.53, 131.84, 133.73, 133.76, 133.83, 134.98, 135.01, 141.83, 143.10, 155.26, 159.34 ppm. ESI-MS: m/z= 919.4566.

Synthesis of **Mito-BDP 630**



Mito-BDP 760 Mito-BDP 630

To a solution of **Mito-BDP 760** (40 mg, 0.04 mmol) in MeCN (5 mL) was added

methyl iodide (1.0 mL, 16.06 mmol). The mixture was vigorously stirred at room temperature for 72 h. The reaction was monitored by TLC on a neutral aluminum oxide column (MeCN/H_2O v/v = 80:20) until the starting material point disappeared. Then the solvent was removed by rotary evaporation. The reaction crude was purified by flash neutral aluminum oxide column eluted with MeCN/H_2O (v/v = 90:10) to yield a deep blue solid of **Mito-BDP 630** (yield, 48 mg, 91%). ^{1}H NMR (400 MHz, d_6-DMSO): δ = 8.14 – 8.16 (q, 4 H), 7.85-8.03 (m, 19 H), 7.78-7.82 (d, 2 H), 7.66-7.70 (d, 2 H), 7.42-7.44 (d, 2 H), 7.18-7.20 (d, 2 H), 7.13 (s, 2 H), 4.17-4.23 (m, 2 H), 3.80-3.85 (m, 2 H), 3.75 (s, 18 H), 2.05-2.07 (t, 2 H), 1.87-1.90 (t, 2 H), 1.58 (s, 6 H) ppm. ESI-MS: m/z= 316.5003.

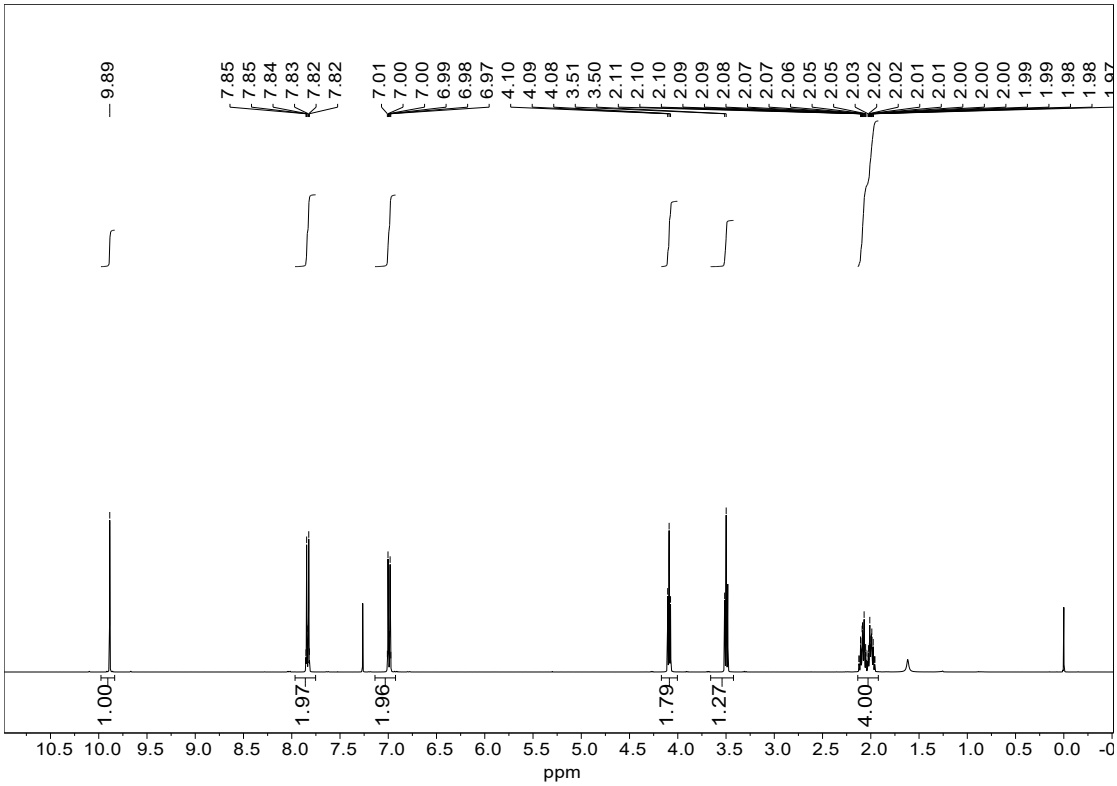


Fig. S1 ^{1}H NMR spectra of compound **1**.

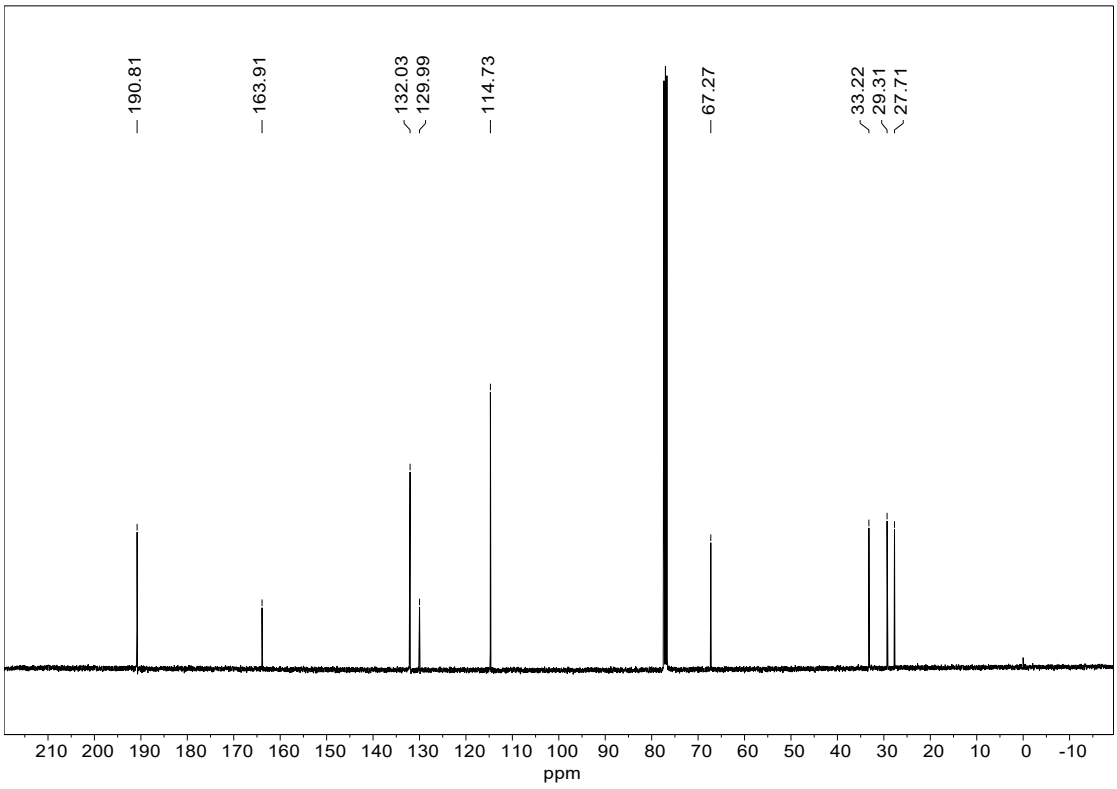


Fig. S2 ^{13}C NMR spectra of compound **1**.

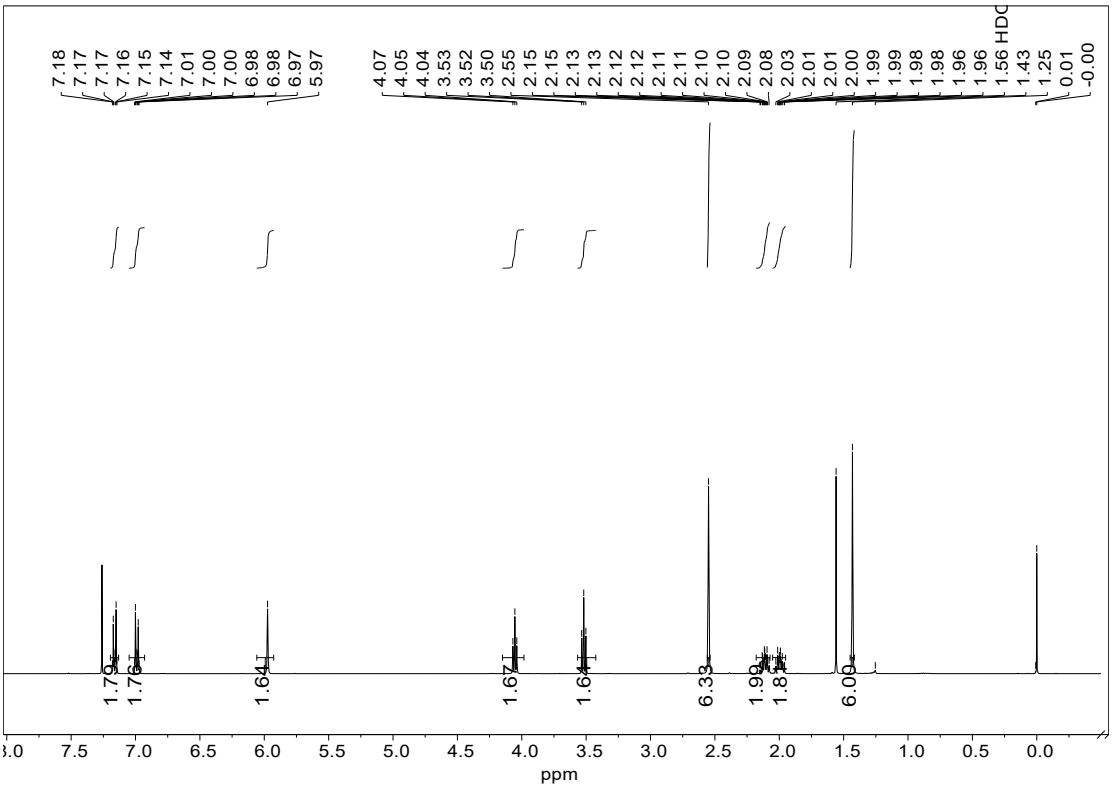


Fig. S3 ^{1}H NMR spectra of compound **2**.

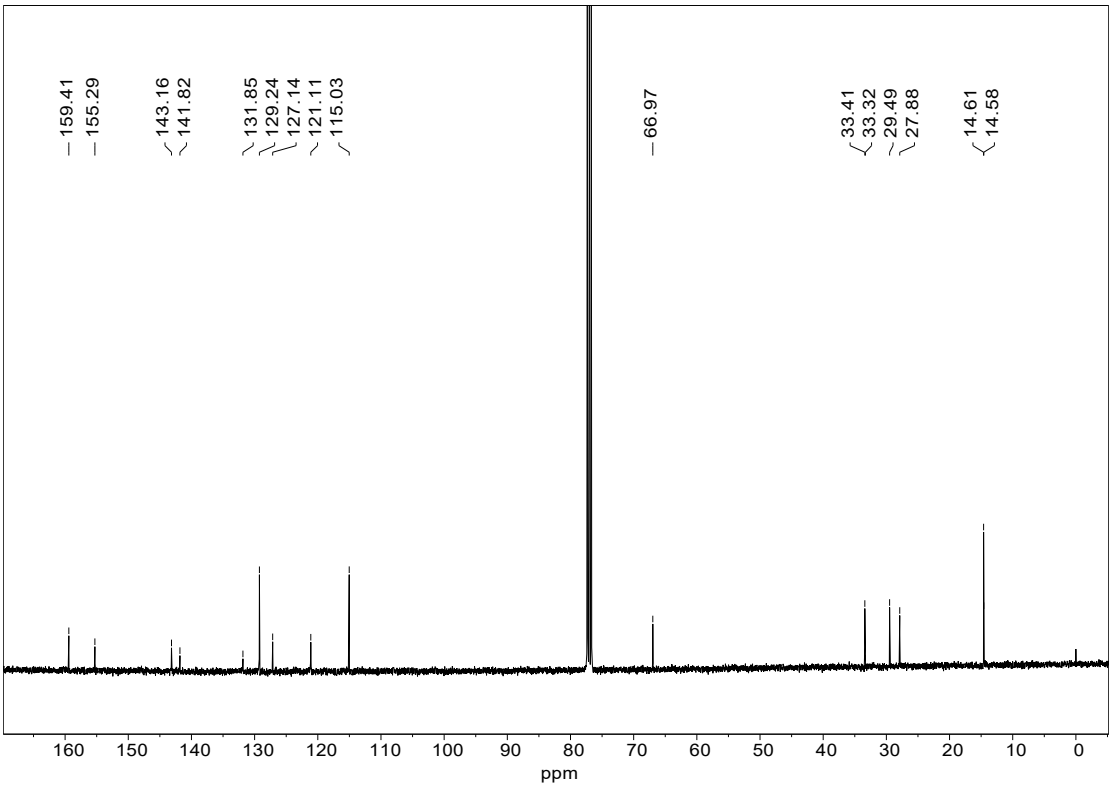


Fig. S4 ^{13}C NMR spectra of compound **2**.

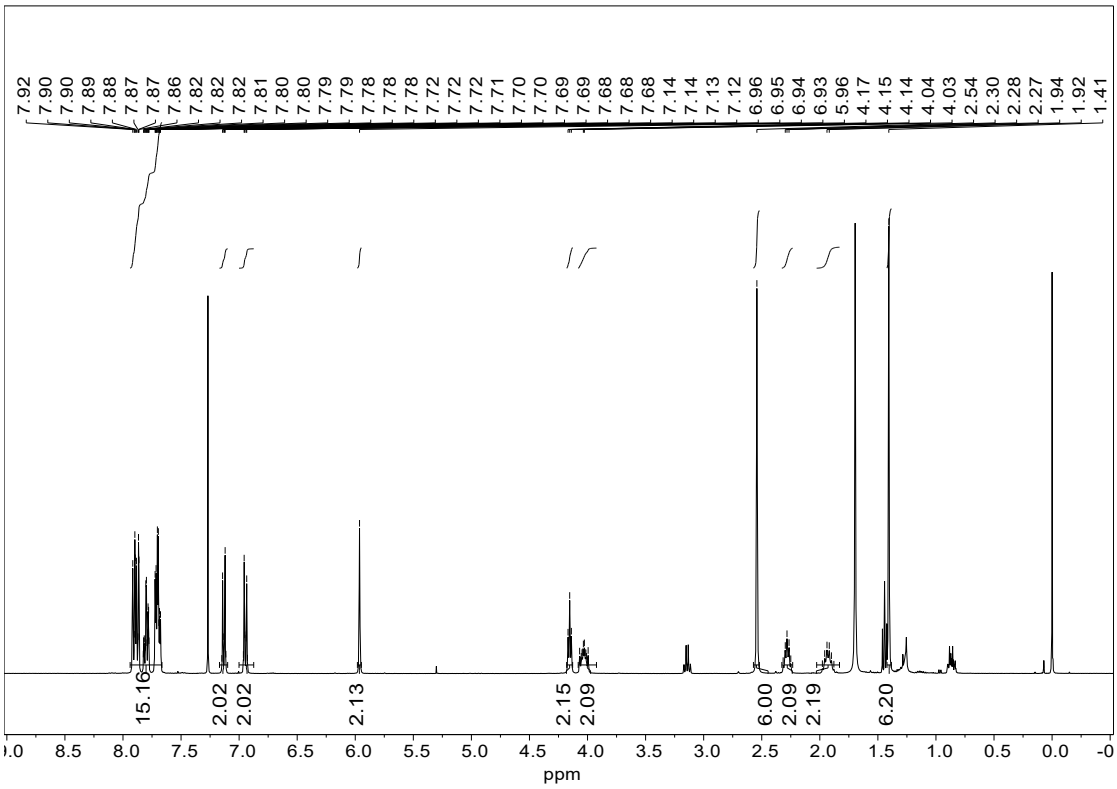


Fig. S5 ^{1}H NMR spectra of compound **3**.

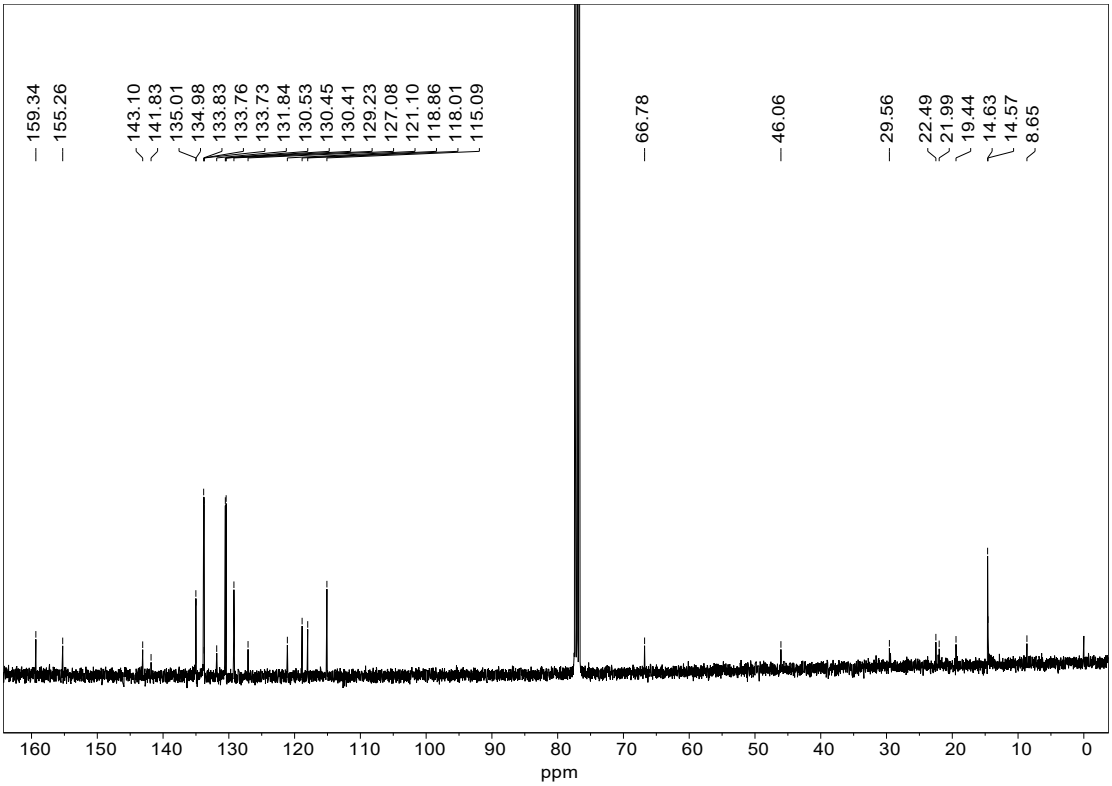


Fig S6 ^{13}C NMR spectra of compound **3**.

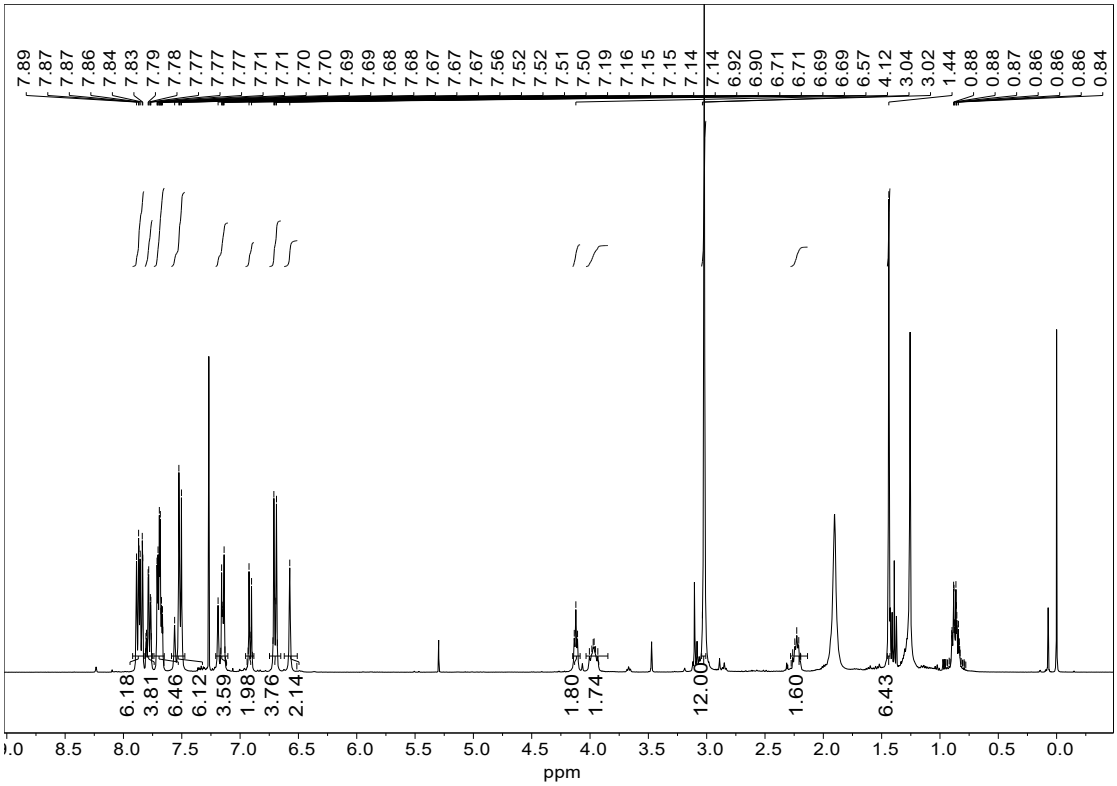


Fig. S7 ^{1}H NMR spectra of compound **Mito-BDP 760**.

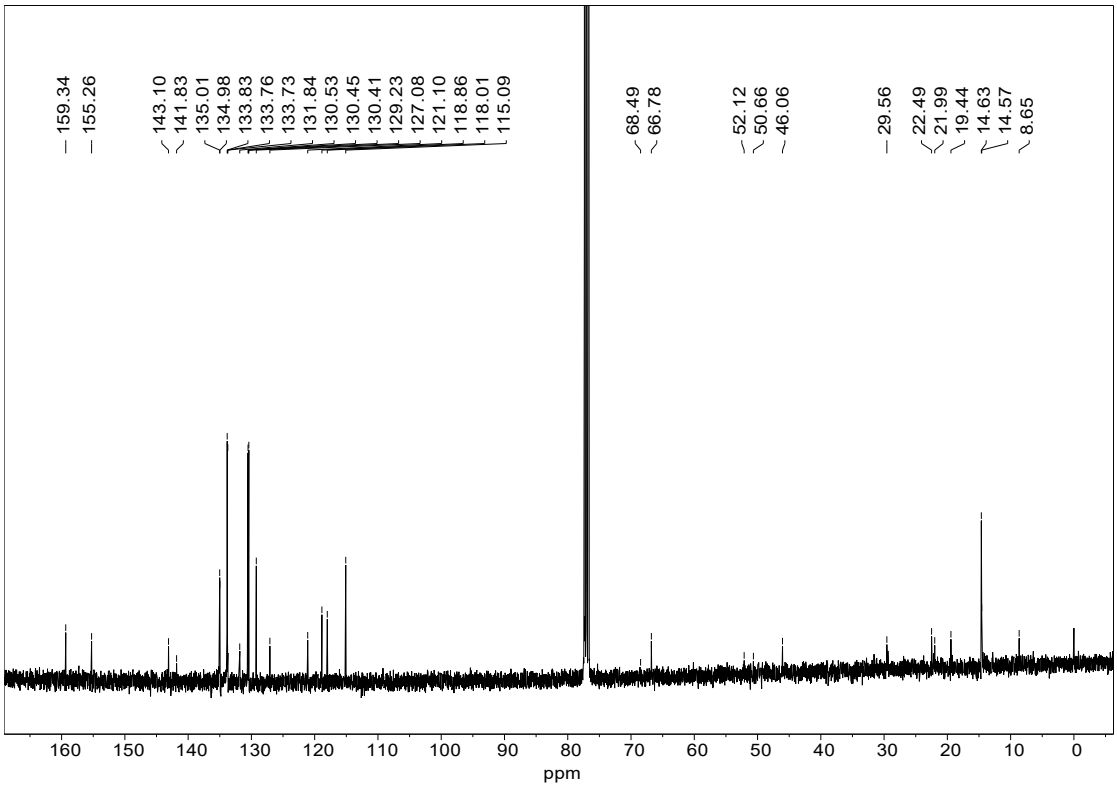


Fig. S8 ^{13}C NMR spectra of compound **Mito-BDP 760**.

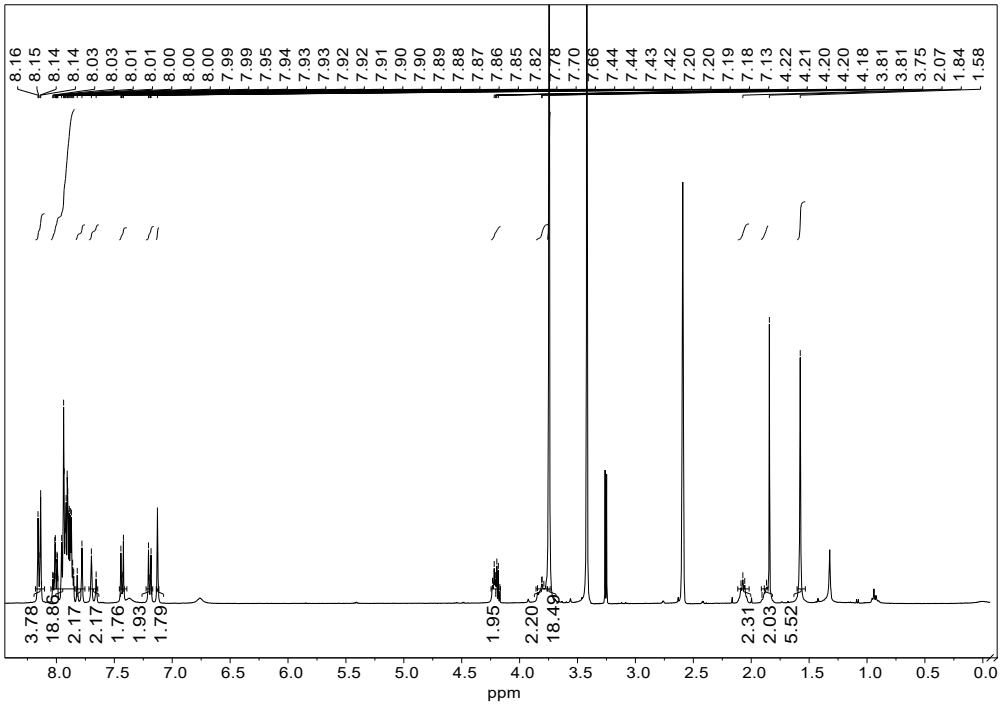


Fig. S9 ^{1}H NMR spectra of compound **Mito-BDP 760**.

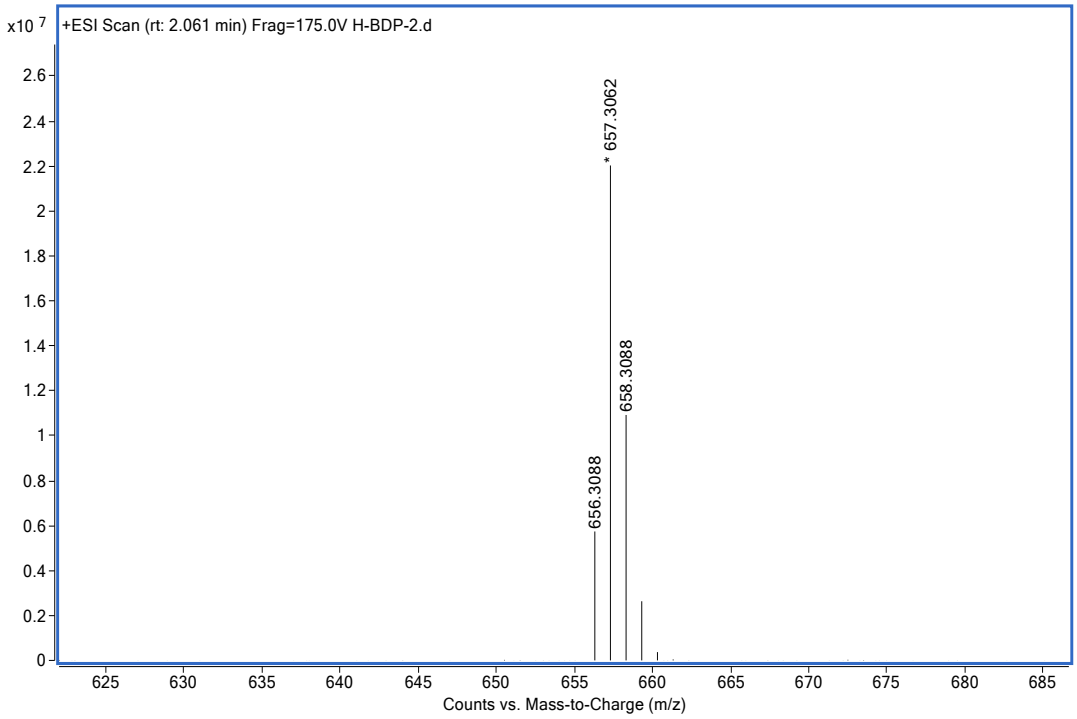


Fig. S10 HR-MS spectra of compound **2**.

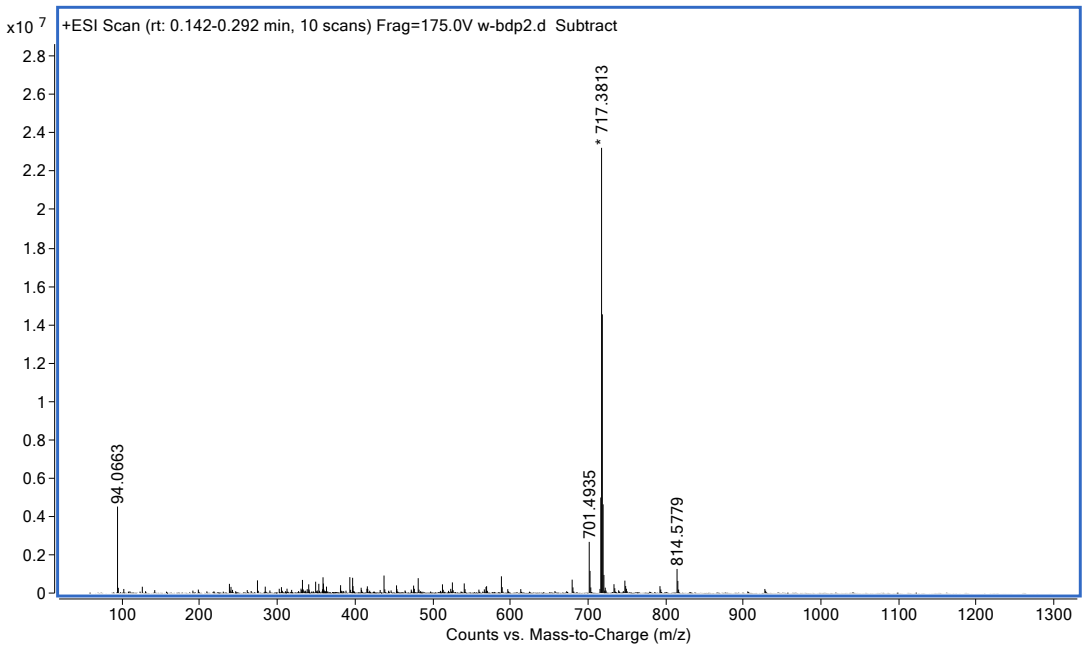


Fig. S11 HR-MS spectra of compound **3**.

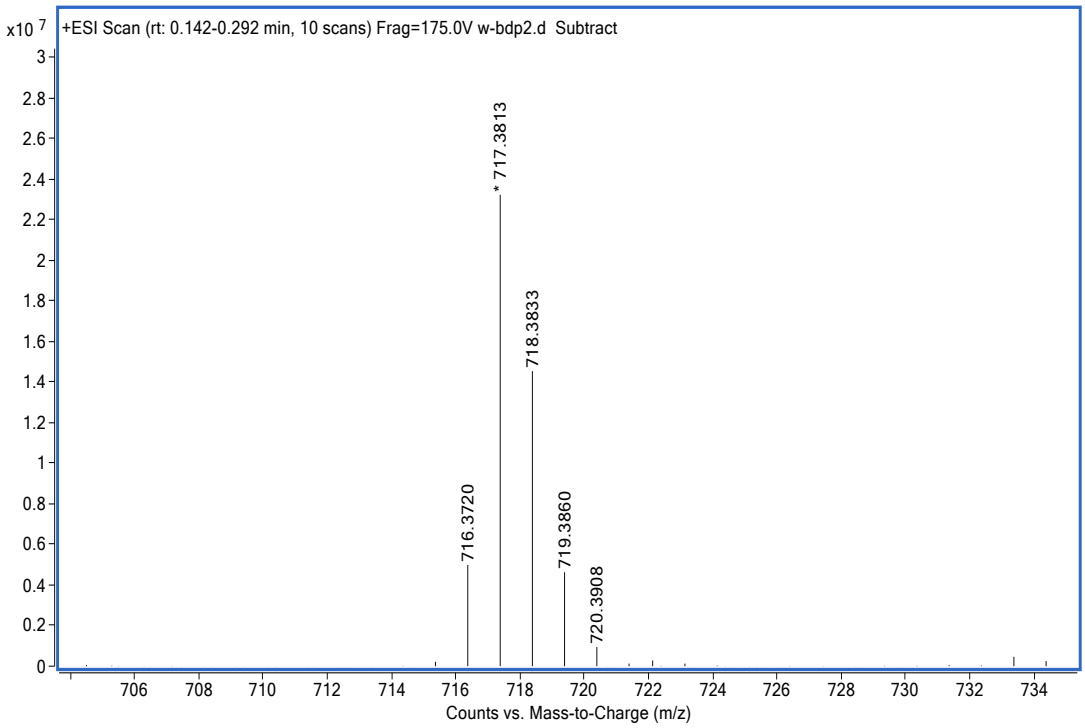


Fig. S12 Mass-spectrometric analysis for isotope of compound **3**.

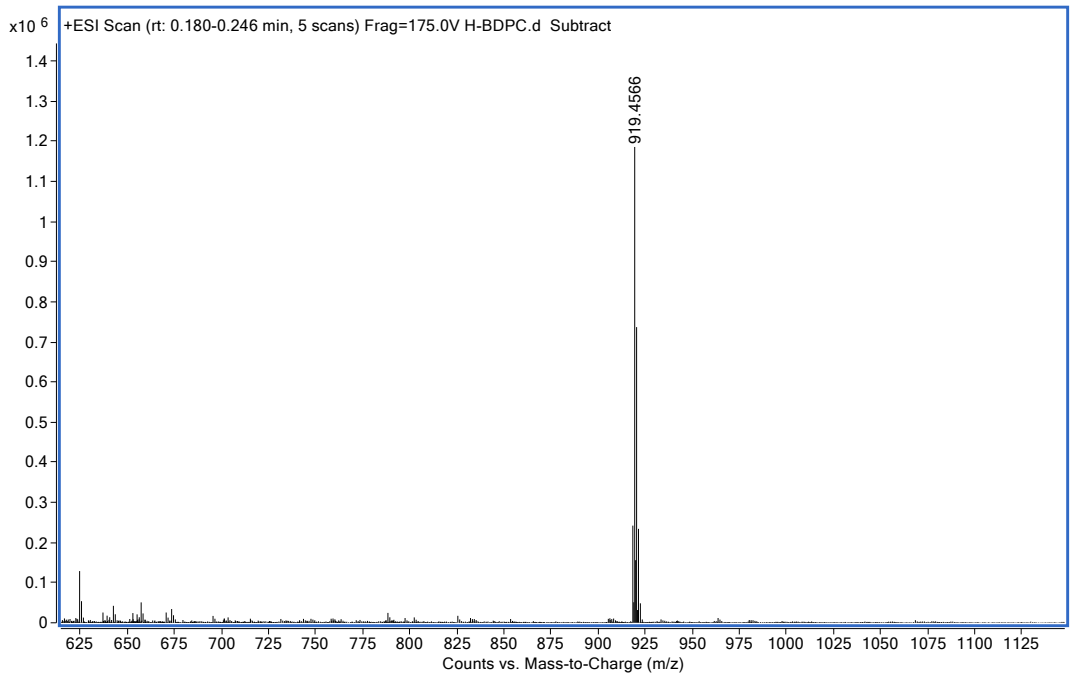


Fig. S13 HR-MS spectra of **Mito-BDP 760**.

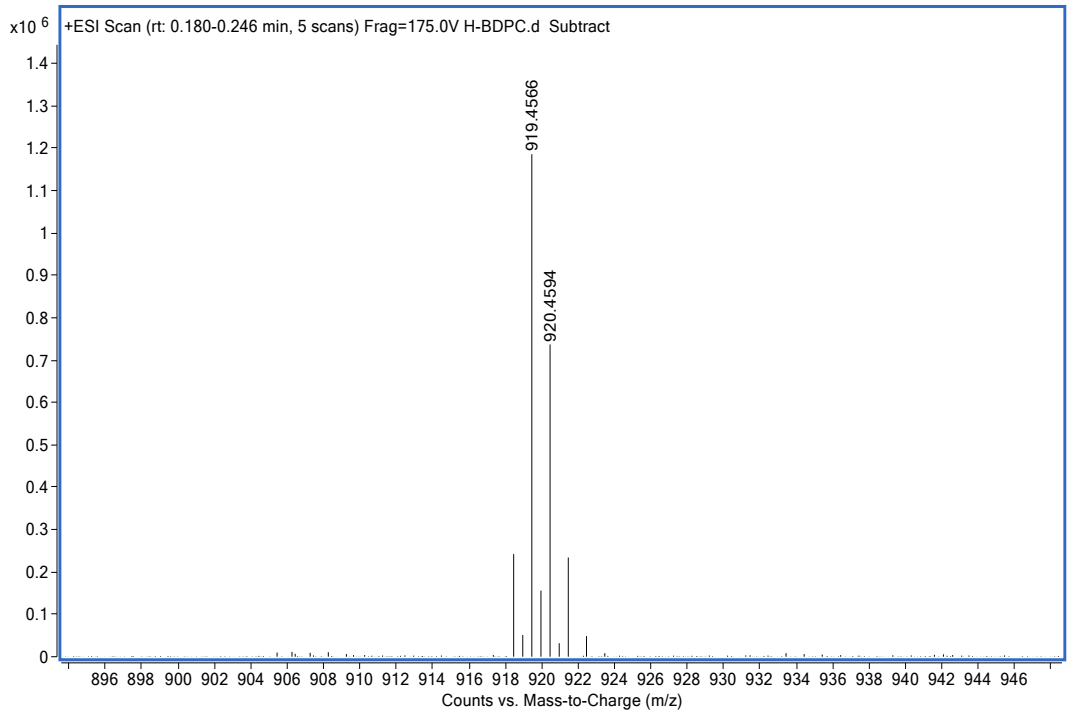


Fig. S14 Mass-spectrometric analysis for isotope of **Mito-BDP 760**.

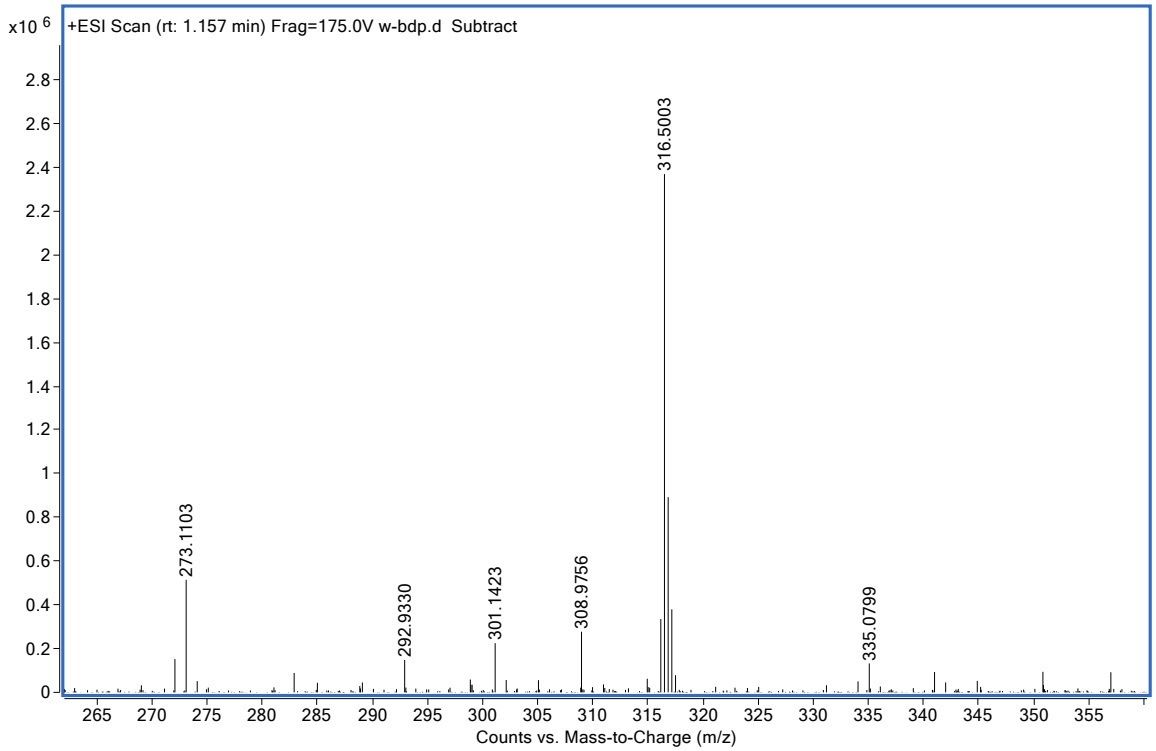


Fig. S15 HR-MS spectra of **Mito-BDP 630**.

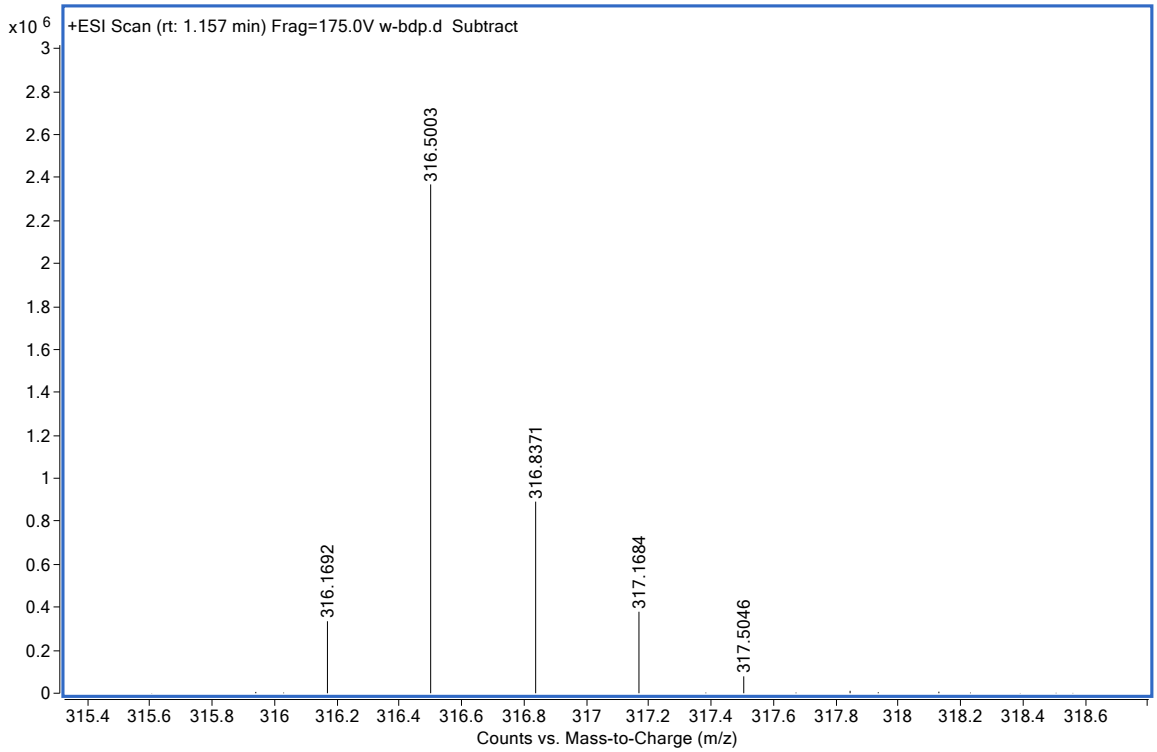


Fig. S16 Mass-spectrometric analysis for isotope of **Mito-BDP 630**.

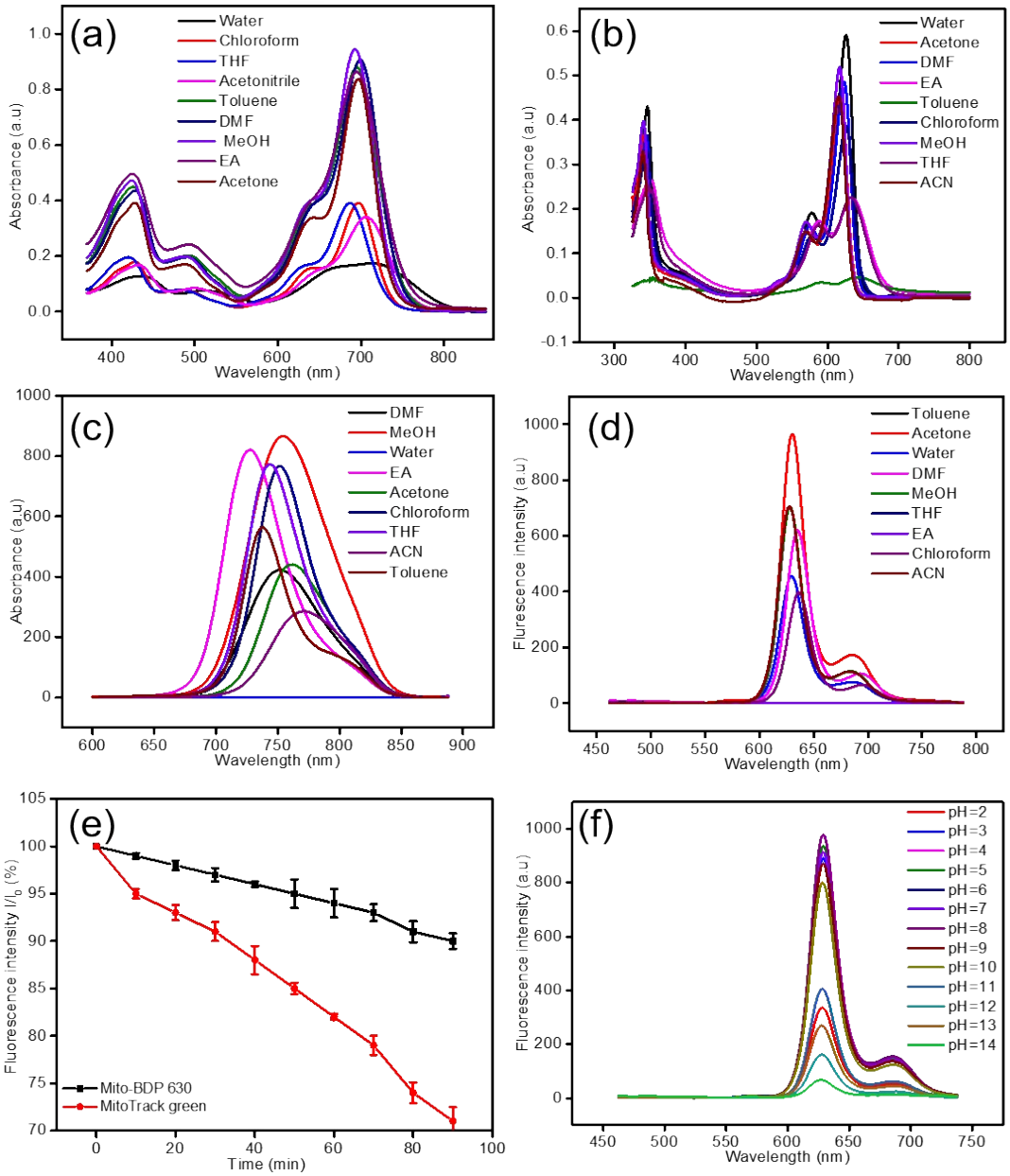


Fig. S17 (a) Absorption spectra of **Mito-BDP 760** (5.0 μM) in different solvents. (b) Absorption spectra of **Mito-BDP 630** (5.0 μM) in different solvent. (c) Fluorescence emission spectra of **Mito-BDP 760** (5.0 μM) in different solvent. (λ_{ex}= 485 nm). (d) Fluorescence emission spectra of **Mito-BDP 630** (5.0 μM) in different solvent (λ_{ex}= 385 nm). (e) Photostability of **Mito-BDP 630** and MTG (2.5 μM in DMSO). (d) Fluorescence emission spectra of **Mito-BDP 630** (5.0 μM) at different pH (λ_{ex}= 385 nm).

Table S1 Optical parameters of BODIPY derivatives.

	Solvent	λ_{abs}（nm）	ε（M^{-1} cm^{-1}）	λ_{em}（nm）	τ(ns)	Φ
Mito-BDP	DMSO	469	78000	509	4.41	0.75
	H_2O	-	-	-	6.05	0.08
Mito-BDP630	DMSO	626	118136	640	4.11	0.73
	H_2O	617	61302	630	4.30	0.43
Mito-BDP760	DMSO	705	170548	769	4.14	0.32
	H_2O	717	34854	-	-	0.03
Mito Tracker Red	DMSO	579	-	599	3.23	0.87
	H_2O	575	-	-	3.72	0.38

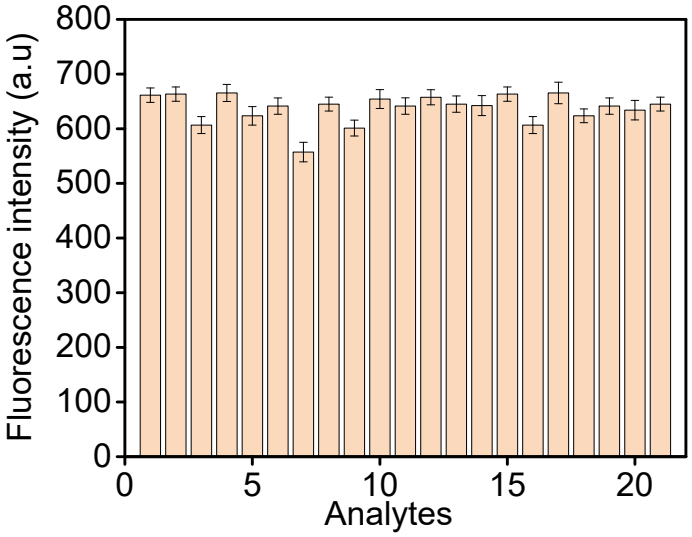


Fig. S18 The fluorescence intensities of **Mito-BDP 630** (2.5 μM) in the presence of various analytes (100 mM except for specific labels) in PBS solution (10 mM, pH = 7.4), 1–21: blank, sulfur species (HSO_3^- , HSO_4^- , $S_2O_3^{2-}$, HS^-); halogen anion (F^-, Cl^-, Br^-, I^-); metal cation (K^+, Ca^{2+}, Cu^{2+}, Cr^{3+}, Na^+, Mg^{2+}, Fe^{3+}) and oxygen species (NO_3^-, NO_2^-, HO•, H_2O_2, ClO^-).

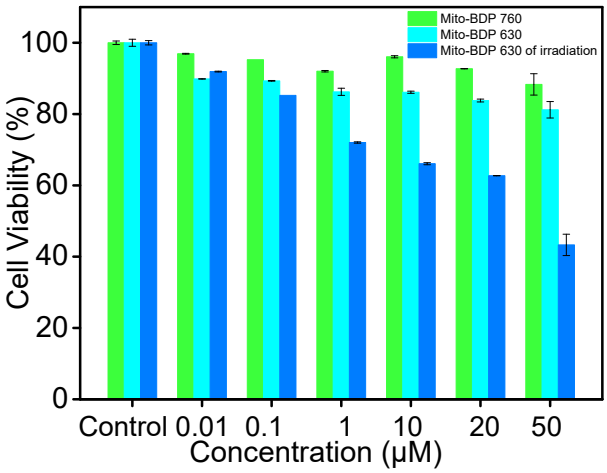


Fig. S19 MTT assays of **Mito-BDP 630** and **Mito-BDP 760**.

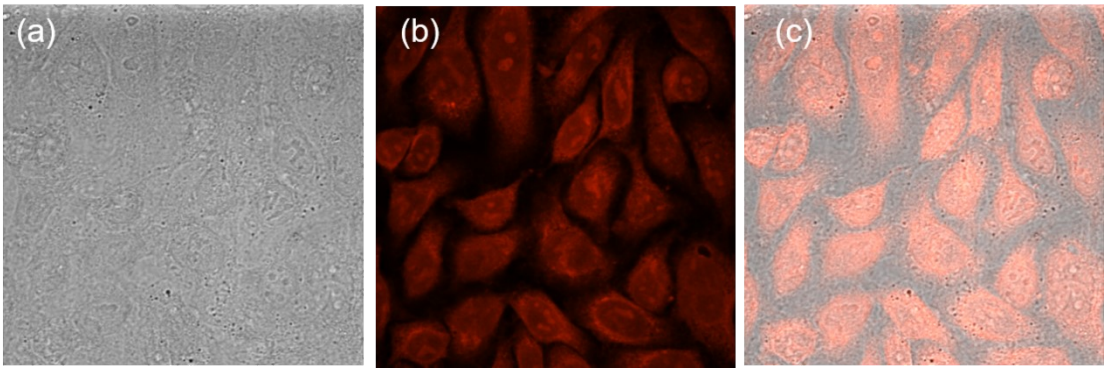


Fig. S20 Confocal microscopy images of HeLa Cells incubated with **Mito-BDP 630**. (a) Bright field. (b) +CCCP (20.0 μM) (red channel: excitation at 561 nm). (c) Merged image. Scale bar: 20.0 μm.

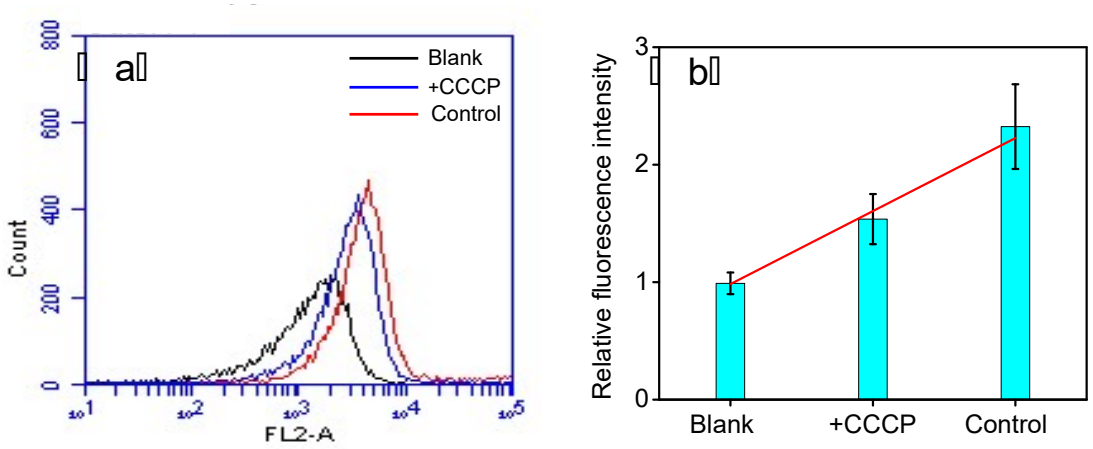


Fig. S21 (a) Flow cytometric analyses of HeLa cells incubated with 2.5 μM **Mito-BDP 630** in the absence (Control) and presence of 20.0 μM CCCP. (b) Corresponding histogram.

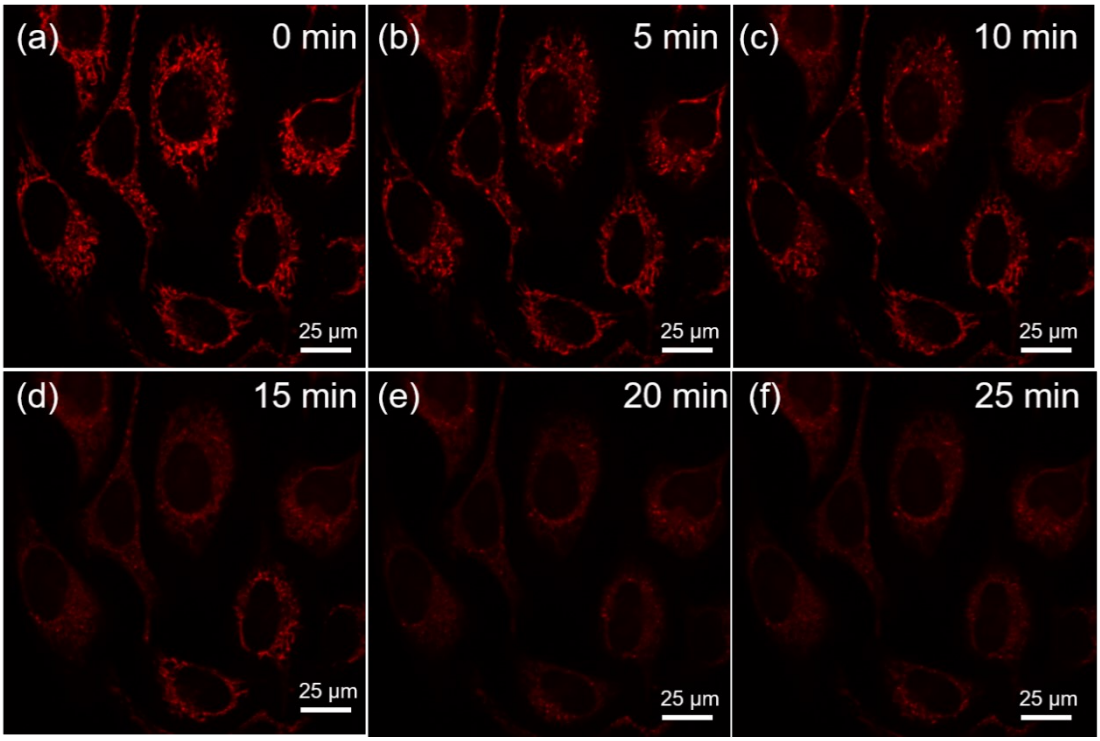


Fig. S22 The photostability of Mito Tracker Red in living HeLa cells. The HeLa cells were incubated with Mito Tracker Red and irradiated by a confocal laser for 25 min. (a-f) 0 - 25 min.

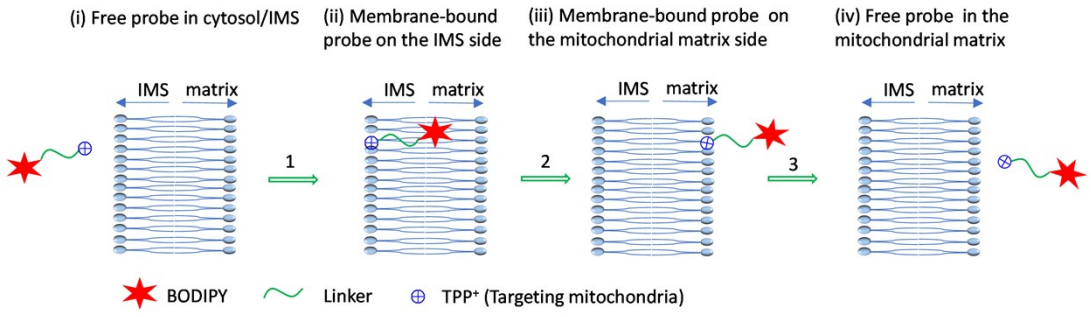


Fig. S23 The process of **Mito-BDP 630** passing through the mitochondrial membrane.

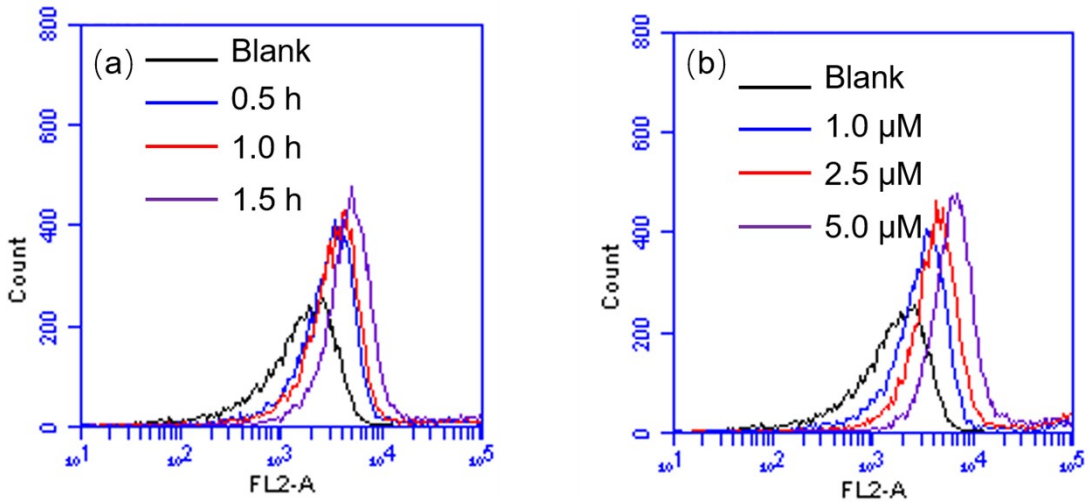


Fig. S24 (a) Flow cytometric analyses of HeLa cells incubated with 2.5 μM **Mito-BDP 630** at different times. (b) Flow cytometric analyses of HeLa cells incubated with different concentrations of **Mito-BDP 630**.