Electronic Supplementary Material (ESI) for Analytical Methods.

A mitochondrial-targeted deep-red fluorescent probe for ATP and its application in living cells and zebrafish

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General remarks for experimental

Unless otherwise stated, all materials were obtained from commercial suppliers and used without further purification. All solvents were dried according to the standard methods prior to use. ^{1}HNMR, ^{13}CNMR spectra were measured on a Bruker AV-400 (400 MHz) NMR spectrometer. HRMS spectral data were recorded on a Bruker Daltonics Bio TOF mass spectrometer. UV/Vis and fluorescence spectra were obtained using F7000 (HITACHI). The fluorescence imaging of cells and zebrafish were performed with a Zeiss LSM 880 confocal microscope. All animal experiments have been approved by the Institutional Animal Care and Treatment Committee of Sichuan University in China and were carried out in accordance with the approved guidelines.

UV/Vis and fluorescence spectra analysis

A stock solution of **NIR-A** (10 mM) was prepared in DMSO. All UV/Vis and fluorescence spectra experiments were performed using 10 μM **NIR-A** in PBS buffer solution (10 mM, pH 7.4) at 298K.

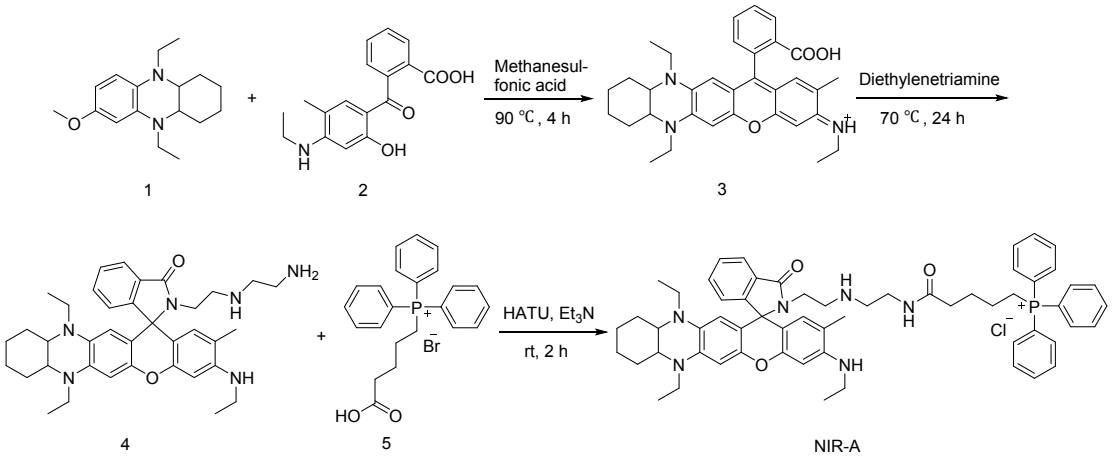
Cell lines

Burkitt's lymphoma cell Ramos used in the study were obtained from the American Type Culture Collection. Cells were propagated in RPIM 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin) in 5% CO_2 at 37℃.

Cell toxicity assay

The cell viability of **NIR-A** treated cells was assessed by CCK-8 assay. Briefly, the exponentially growing Ramos and LO2 cells (2-6×10^3 cells/well) were plated in 96-well plates (100 μL/well) and incubated for 24 h. Then the cells were treated with gradient concentrations of **NIR-A** (10 μM, 20 μM, 40 μM). After treatment for 24 h, respectively, 20 μL CCK-8 was added to each well, and the plates were incubated at 37 ℃ for additional 2-4 h. The color absorbance was recorded at 450 nm using a Bio-rad xMark microplate spectrophotometer. Data shown represents as mean and standard deviation of three independent experiments.

Synthesis



Scheme S1. Synthesis route of NIR-A.

Synthesis of compound 3

Compound 1 (274 mg, 1 mmol) and compound 2 (299 mg, 1 mmol) were successively added to methanesulfonic acid (5 mL), the mixture was heated at 90 °C for 4 h. After being cooled, the mixture was poured into ice-water, then perchloric acid (0.5 mL) was added. The resulting precipitate was filtered off and washed with plenty of water. After the sample dried, purification by silica gel using a CH_2Cl_2: EtOH = 50: 1 as the eluent to give compound 3 (261 mg, 51%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, J = 7.7, 1H), 7.83 (dt, J = 27.5, 7.5, 2H), 7.44 (dd, J = 7.6, 4.2, 1H), 7.32 – 7.21 (m, 1H), 6.98 (d, J = 2.2, 1H), 6.92 – 6.82 (m, 2H), 5.92 (d, J = 10.8, 1H), 3.82 (dt, J = 13.8, 6.8, 1H), 3.72 (dq, J = 10.0, 3.4, 1H), 3.58 (dd, J = 14.7, 7.2, 2H), 3.08 (dd, J = 9.3, 5.3, 2H), 2.11 (s, 3H), 2.01 – 1.94 (m, 1H), 1.85 (dt, J = 10.4, 5.6, 1H), 1.64 – 1.52 (m, 3H), 1.45 – 1.23 (m, 10H), 1.05 (d, J = 7.0, 1H), 0.81 (q, J = 7.0, 3H). MS (ESI): calcd for $[C_{33}H_{38}N_3O_3]^+$ 524.2, found 524.3.

Synthesis of compound 4

Diethylenetriamine (10 mL, 90 mmol) and compound 3 (2.1 g, 4 mmol) in ethanol (50 mL). The mixture was stirred at 70 °C for 24 h. After the solvent was removed on a rotary evaporator, the residue was purified by silica gel (CH_2Cl_2: EtOH = 50: 1) to afford compound 4 (560 mg, 24%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 – 7.74 (m, 1H), 7.64 – 7.38 (m, 2H), 7.09 – 6.93 (m, 1H), 6.45 – 6.21 (m, 2H), 6.17 (d, J = 2.6, 1H), 5.43 (d, J = 17.8, 1H), 5.05 (t, J = 5.5, 1H), 3.13 (dt, J = 13.0, 6.5, 11H), 2.91 – 2.79 (m, 2H), 2.69 (t, J = 6.1, 2H), 2.56 (d, J = 6.2, 1H), 2.35 – 2.18 (m, 2H), 1.88 (s, 3H), 1.74 – 1.17 (m, 11H), 1.12 (td, J = 6.9, 4.4, 3H), 0.78 (t, J = 6.9, 2H), 0.56 (t, J = 6.9, 2H). MS (ESI): calcd for $[C_{37}H_{48}N_6O_2]^+$ 608.4, found 609.2.

Synthesis of NIR-A

Compound 4 (608 mg, 1 mmol), compound 5 (330 mg, 0.75 mmol), HATU (380 mg, 1 mmol) and

Et_3N (0.2 ml) were added to 10 mL CH_2Cl_2, the mixture was stirred for 2 h in rt. Then, the mixture was washed with brine twice, the residue was purified by silica gel (CH_2Cl_2: EtOH = 50: 1) to afford compound **NIR-A** (201 mg, 27%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.08 – 7.61 (m, 15H), 7.45 (ddd, J = 5.8, 3.9, 2.2, 2H), 7.10 (dt, J = 5.8, 3.1, 1H), 6.68 (q, J = 6.6, 6.1, 1H), 6.45 – 6.26 (m, 2H), 6.21 (d, J = 3.7, 1H), 5.51 (d, J = 5.8, 1H), 3.57 (s, 1H), 3.41 (s, 1H), 3.36 – 3.08 (m, 10H), 2.95 (s, 1H), 2.82 (s, 1H), 2.67 – 2.57 (m, 2H), 2.35 (dt, J = 11.3, 6.5, 7H), 1.71 (p, J = 7.7, 3H), 1.55 (d, J = 10.8, 2H), 1.44 (d, J = 10.9, 2H), 1.37 – 1.16 (m, 10H), 0.72 (dt, J = 9.5, 7.0, 4H). ^{13}C NMR (100 MHz, DMSO) δ 172.08, 170.79, 167.74, 154.08, 153.99, 151.89, 151.68, 148.06, 144.88, 137.09, 135.39, 135.36, 134.07, 133.97, 133.03, 131.36, 130.77, 130.64, 128.63, 128.08, 124.10, 122.67, 119.38, 118.53, 107.85, 107.47, 105.02, 104.72, 97.49, 96.10, 65.36, 60.21, 56.51, 55.80, 53.49, 48.25, 47.29, 42.21, 37.96, 34.61, 31.19, 29.46, 27.52, 26.51, 26.34, 21.81, 21.22, 20.75, 20.25, 17.42, 14.64, 14.55, 11.90, 10.33. HRMS calcd for $C_{44}H_{61}BrFN_9O_{15}$ $[C_{60}H_{70}N_6O_3P^+]^+$ 953.5242, found 953.5192.

Spectra

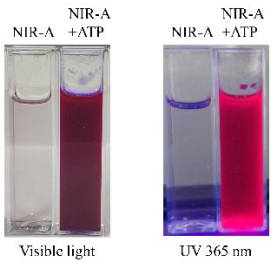


Figure S1. The corresponding color photograph of NIR-A.

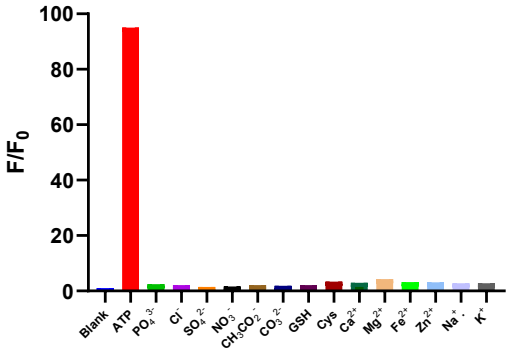


Figure S2. Fluorescence response of NIR-A (10 μM) for 10 mM of ATP or 10 mM of interfering

substances in the PBS buffer solution.

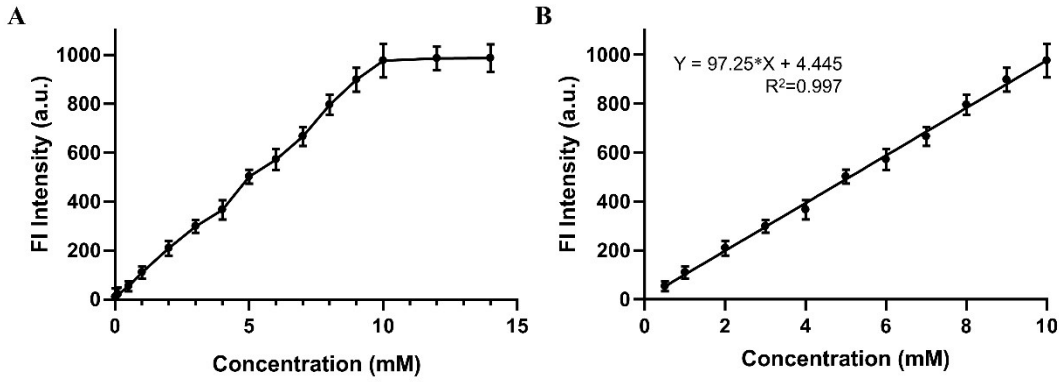


Figure S3. The linear relationship between the fluorescence intensity (663 nm) of NIR-A (10 μM) and the concentration of ATP (0-10 mM).

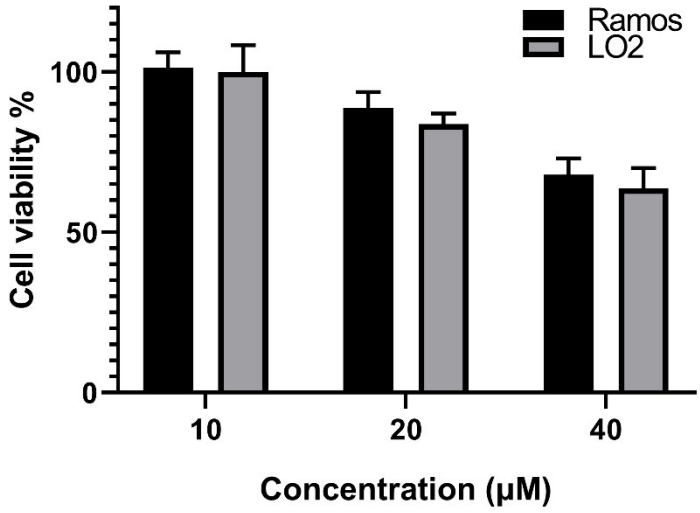


Figure S4. Cytotoxicity assays of NIR-A at different concentrations for Ramos and LO2 cells in 24h. Error bars represent the standard deviations of 3 trials.

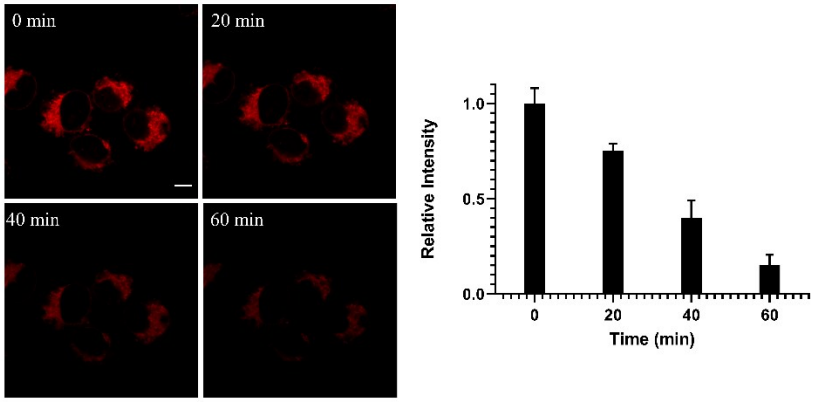


Figure S5. The effects of CCCP (10 μM) on the staining of NIR-A in the mitochondrion of Ramos cells. Scale bar: 10 μm.

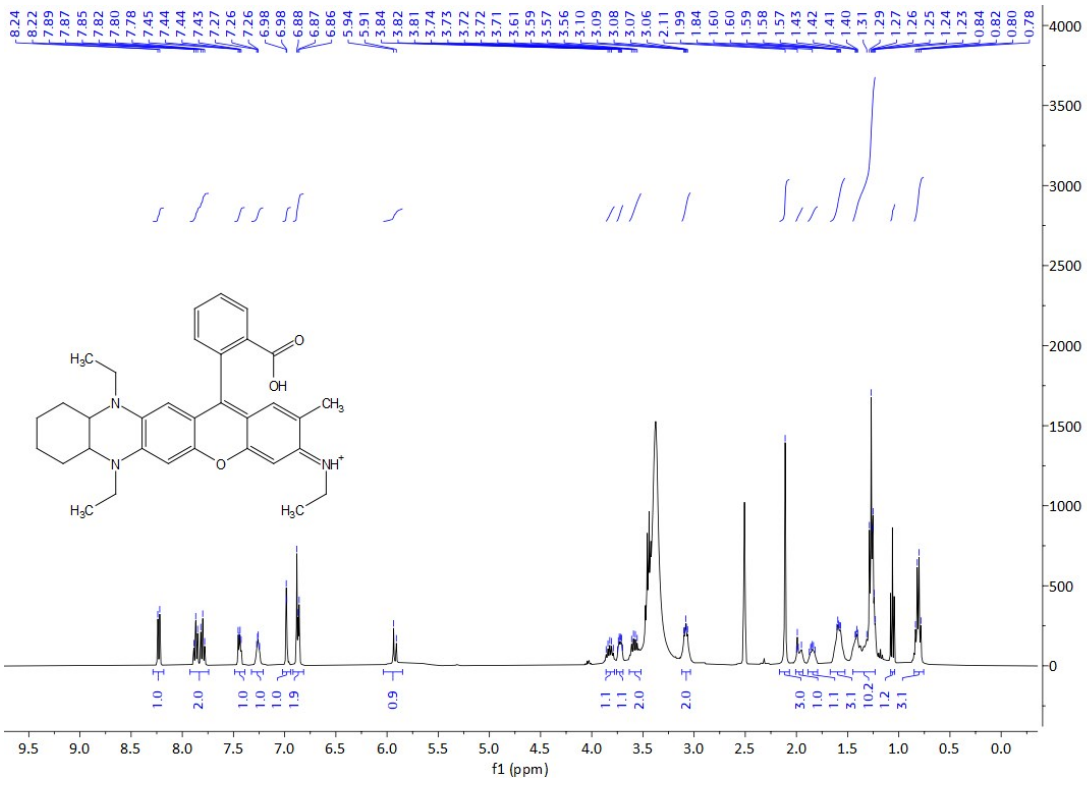


Figure S6. ^{1}H NMR of Compound 3.

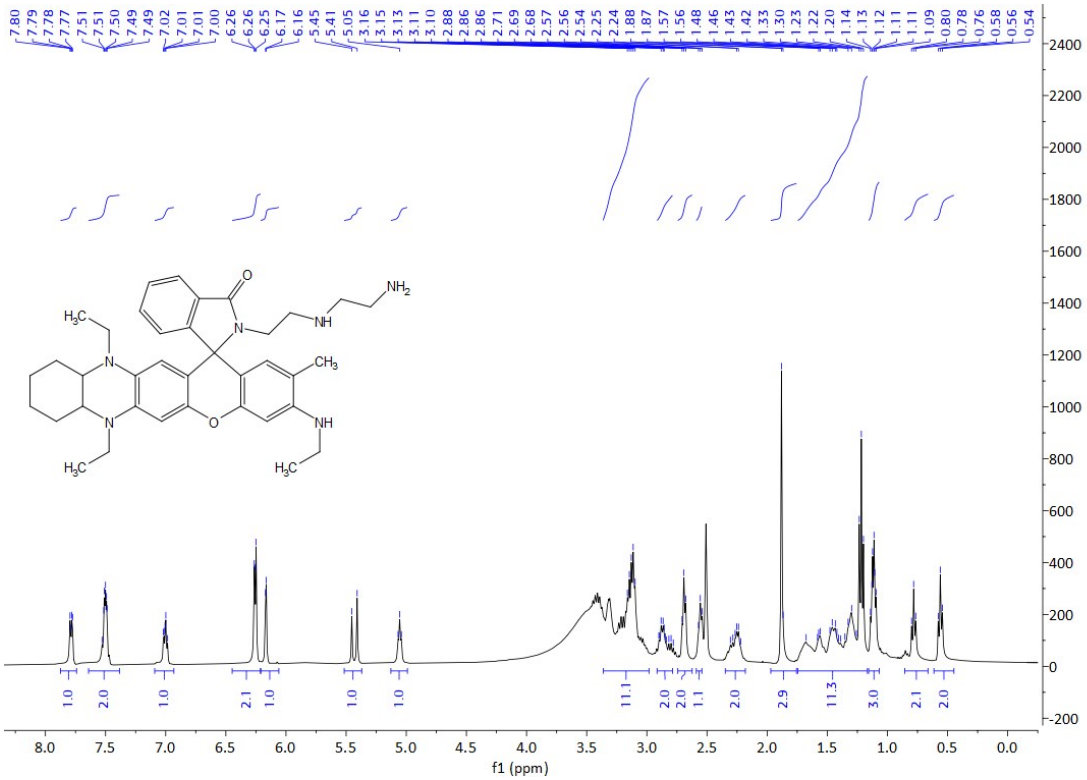


Figure S7. ^{1}H NMR of Compound 4.

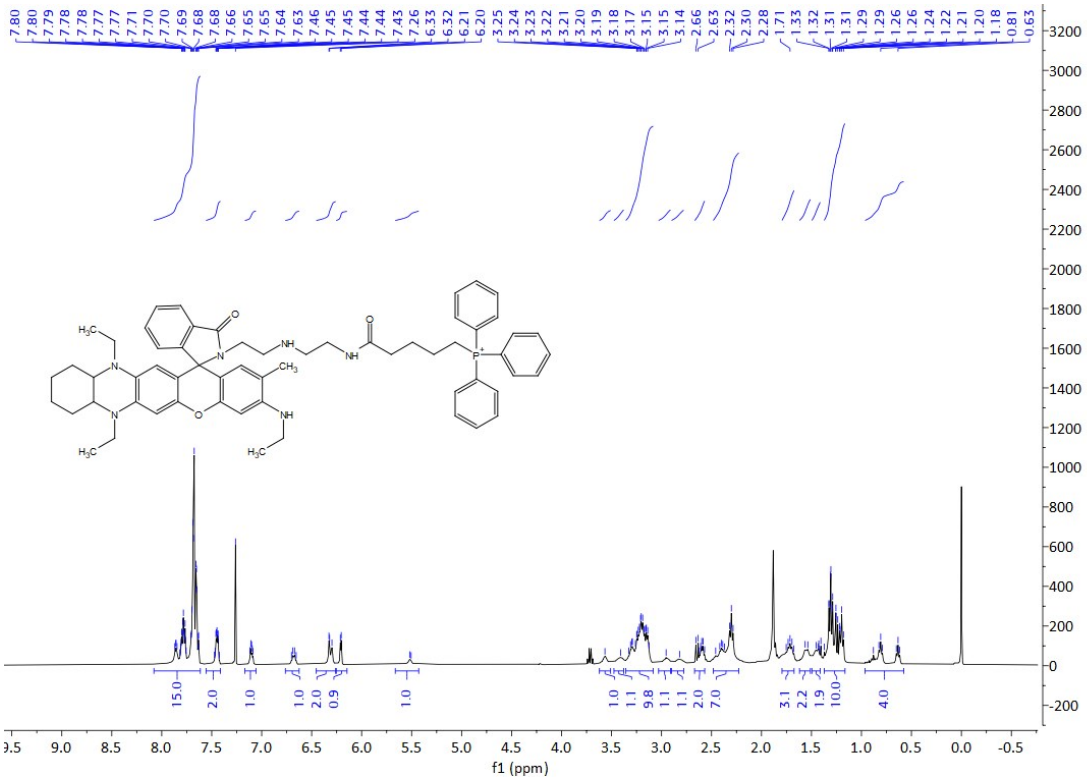


Figure S8. ^{1}H NMR of NIR-A.

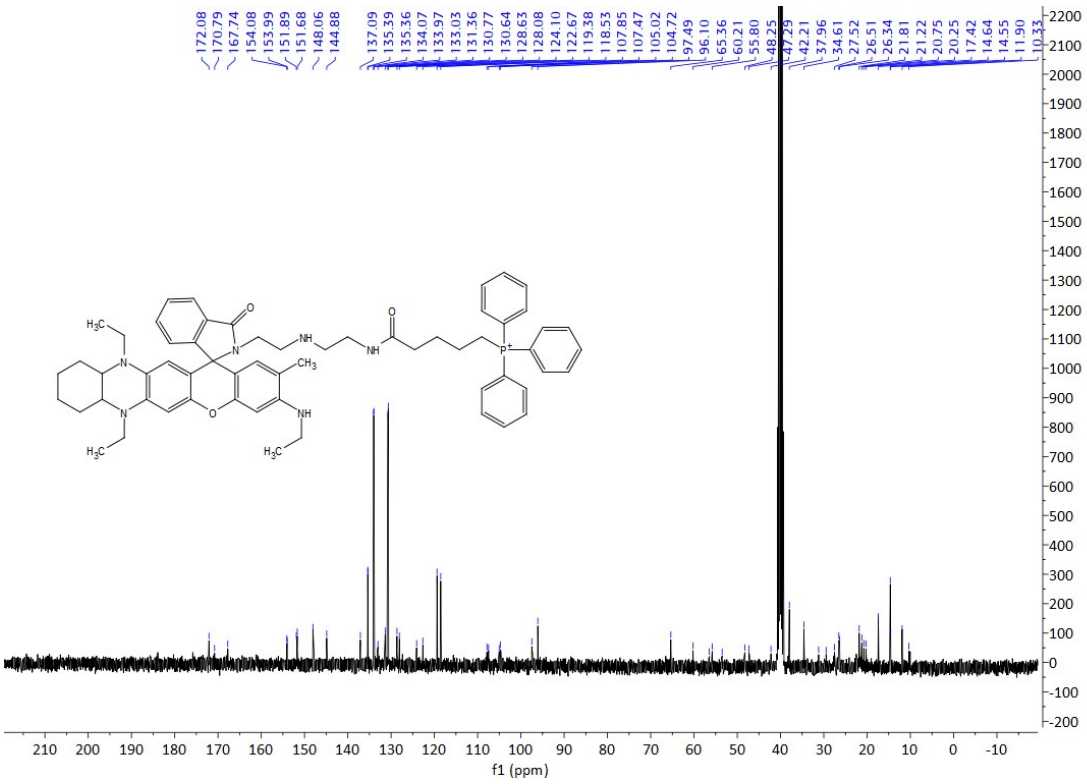


Figure S9. ^{13}C NMR of NIR-A.

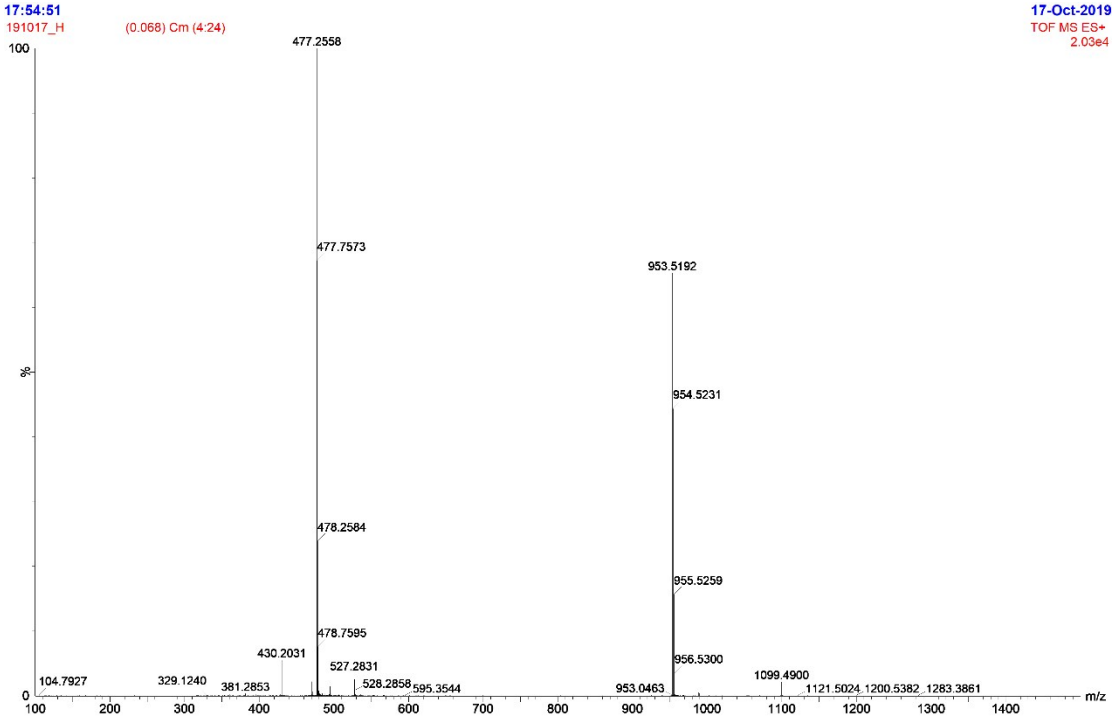


Figure S10. Mass spectra of NIR-A.