Supporting Information for

Naphthylimide-based mitochondrial immobilized probes for polarity-specific imaging of mitochondrial autophagy in cells and mouse cardiac tissue

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Materials and instruments

Unless otherwise stated, all reagents as well as drugs covered in this article were purchased from commercial platforms and used without purification. High-resolution mass spectrometry (HRMS) analyses were measured on a Bruker maxis impact spectrometer; NMR spectra were recorded on a Bruker Advance III HD 600 MHz spectrometer using TMS as an internal standard; absorption spectra were obtained on a Shimadzu UV2600 power spectrometer; fluorescence spectra were recorded with a HITA CHI F7100 fluorescence spectrophotometer; Cell fluorescence spectra were taken with a Nikon Eclipse C1 Plus laser confocal scanning microscope. Fluorescence spectra were recorded on a HITA CHI F7100 fluorescence spectrophotometer; pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; thin layer chromatography was carried out on silica gel plates, and column chromatography was carried out on silica gel (mesh size 200~300).

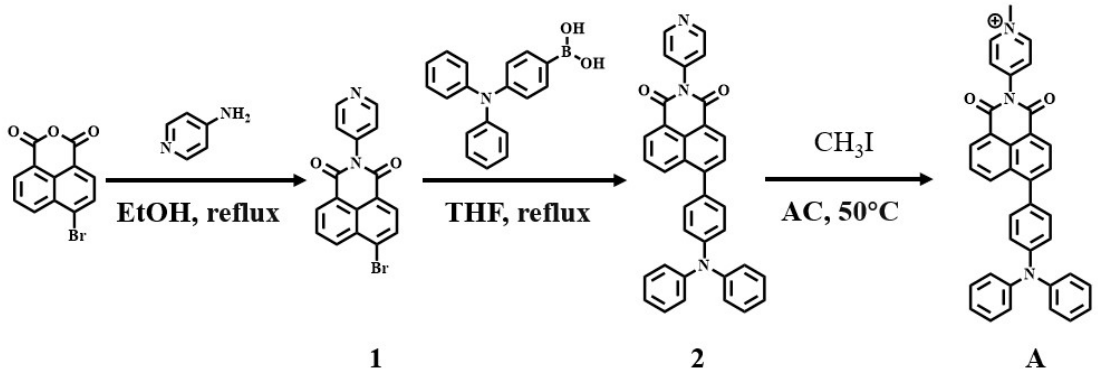
Cell culture

4T1 cells (murine mammary carcinoma cells) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum (FBS) and 1% antibiotic-antimycotic solution (containing 100 μg/mL penicillin and 100 μg/mL streptomycin, Hyclone brand), maintained at 37°C with 5% CO_2 and 95% air. For cytotoxicity assays, 4T1 cells were seeded into 96-well plates containing 100 μL DMEM medium supplemented with 10% FBS and 1% penicillin/streptomycin per well, and incubated overnight under the aforementioned conditions. The following day, wells were supplemented with varying concentrations of **Mito-NT** (final

concentrations: 0, 1, 2, 5, 10, 20 μM) and incubated for a further 24 hours. Following incubation, add 100 μL of DMEM/CCK8 mixture (volume ratio 10:1) to each well and incubate for 1 hour. Finally, measure the absorbance of each well's solution at 492 nm using an enzyme-linked immunosorbent assay reader. Cell viability was calculated from the absorbance values using the following formula:

$$Cell\ survival\ rate\ (\%) = \frac{OD_{492}sample - OD_{492}blank}{OD_{492}control - OD_{492}blank} \times 100\%$$

In this context, the OD_{492} sample value in the formula denotes the OD_{492} reading of the cell well after incubation with CCK8 following the addition of different concentrations of **Mito-NT**. OD_{492} blank (OD value of the blank group) represents the OD_{492} value of wells containing only DMEM/CCK8 mixture (without cells or **Mito-NT**), used to subtract the background absorption of the medium and CCK8 reagent itself; OD_{492} control (OD value of the control group) represents the OD_{492} value of the cell well after incubation with CCK8 following the addition of 0 μM **Mito-NT** (i.e., containing only cells and medium, without drug treatment), corresponding to a 100% cell survival state. To ensure result reliability, each concentration group and control group must include 10 replicate wells. Calculations shall use the average OD_{492} value from each replicate well substituted into the formula.



Scheme S1: Synthesis process of compound A.

Table S1: Polarity parameters of mixed solutions of different proportions of dioxane and EA

Dioxane (%)	EA (%)	E_T (30) (kcal/mol)
0	100	38.1
10	90	37.89
20	80	37.68
30	70	37.47
40	60	37.26
50	50	37.05
60	40	36.84
70	30	36.63
80	20	36.42
90	10	36.21
99	1	36.02

Table S2: Solvation effects of probe **Mito-NT** in different solvents

Solvent	Absorption (nm)	Emission (nm)	E_T (30) (kcal/mol)	Stokes displacement (nm)	Molar absorptivity (L · mol^{-1} · cm^{-1})	Quantum yield (%)
Hexane	424.5	516.0	31.0	91.5	2683	72.58
Toluene	433.5	552.2	33.9	118.7	2411	60.23
Dioxane	430.5	582.2	36.0	151.7	4970	29.98
EA	425.0	626.6	38.1	201.6	3195	10.75
THF	435.5	630.6	37.4	195.1	3789	9.39
DCM	454.0	659.0	41.1	205.0	4052	6.19

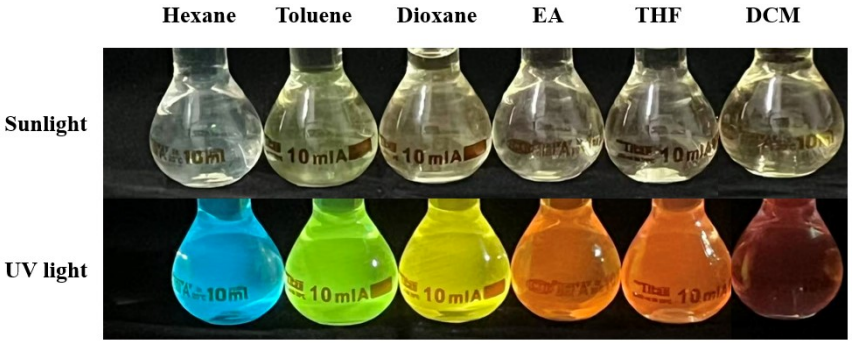


Figure S1: Photographs of the probe **Mito-NT** (10 μM) in different solvents under sunlight and ultraviolet light.

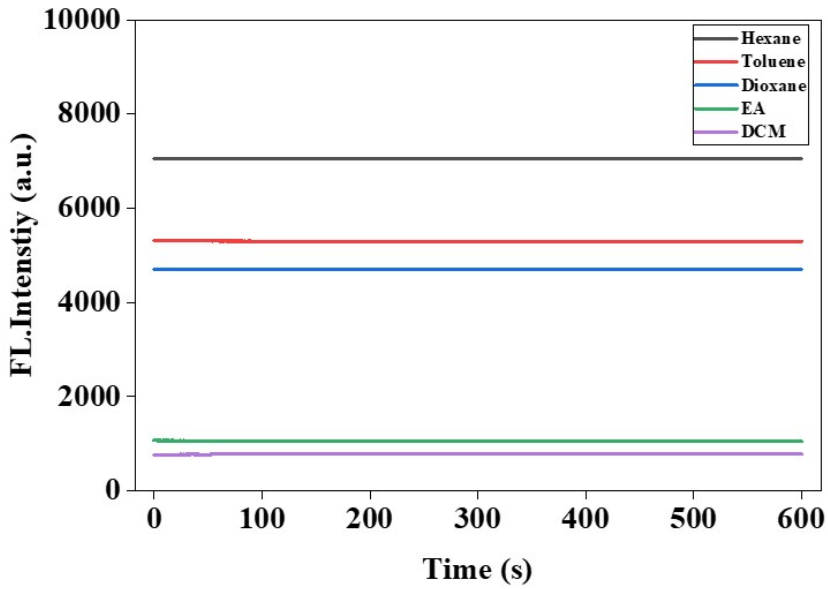


Figure S2: Photostability of the **Mito-NT** probe in different solvents (Hexane, Toluene, Dioxane, EA and DCM).

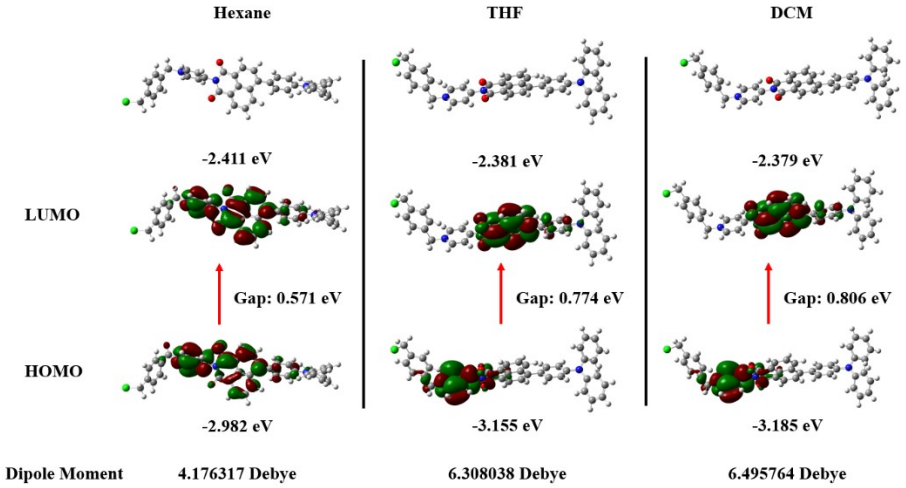


Figure S3: The optimized geometry, orbital energies, and dipole moments of the excited state and frontier molecular orbitals for the probe **Mito-NT**.

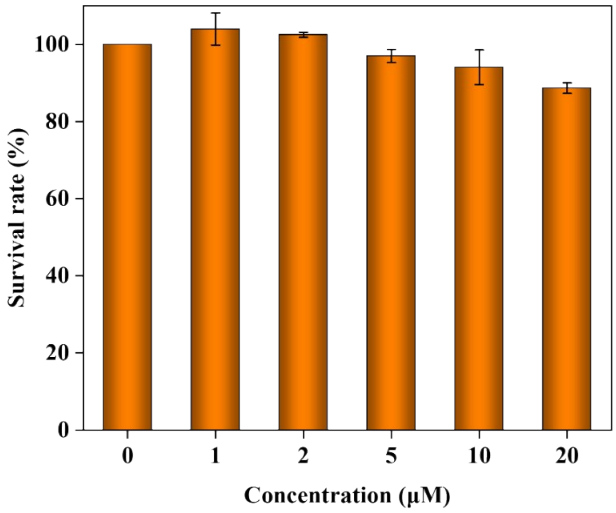


Figure S4: Cytotoxicity assays of **Mito-NT** at different concentrations for 4T1 cells.

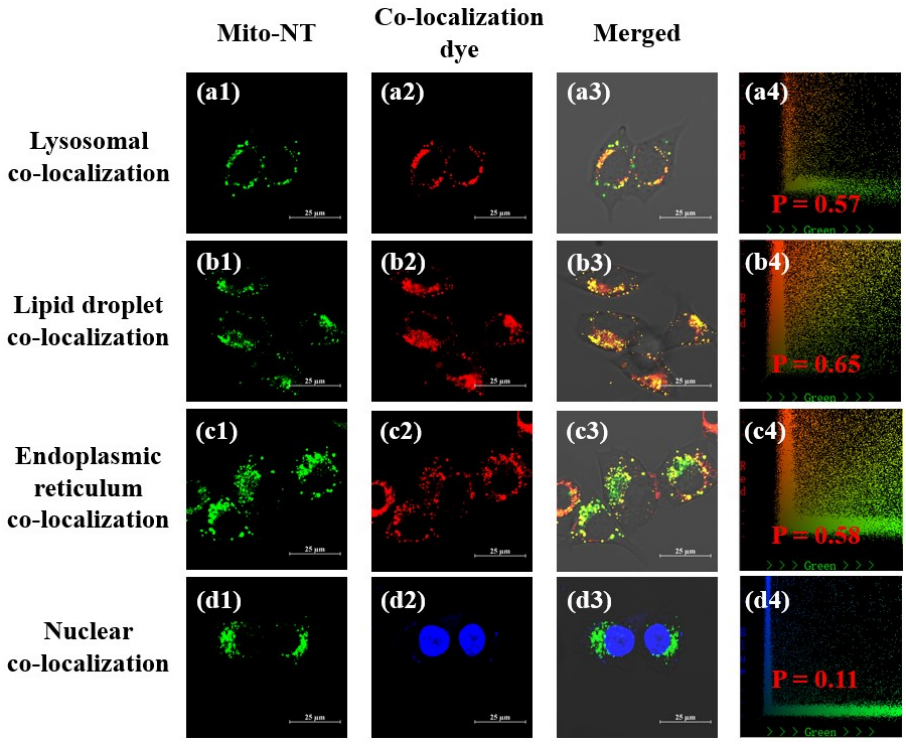


Figure S5: Colocalization imaging of the **Mito-NT** probe (10 μM) with organelle-specific dyes in 4T1 cells. Blue channel: λ_{ex} = 404 nm, λ_{em} = 430–475 nm; Green channel: λ_{ex} = 488 nm, λ_{em} = 500–550 nm. Red channel: λ_{ex} = 561 nm, λ_{em} = 570–616 nm. Scale bars: 25 μm.

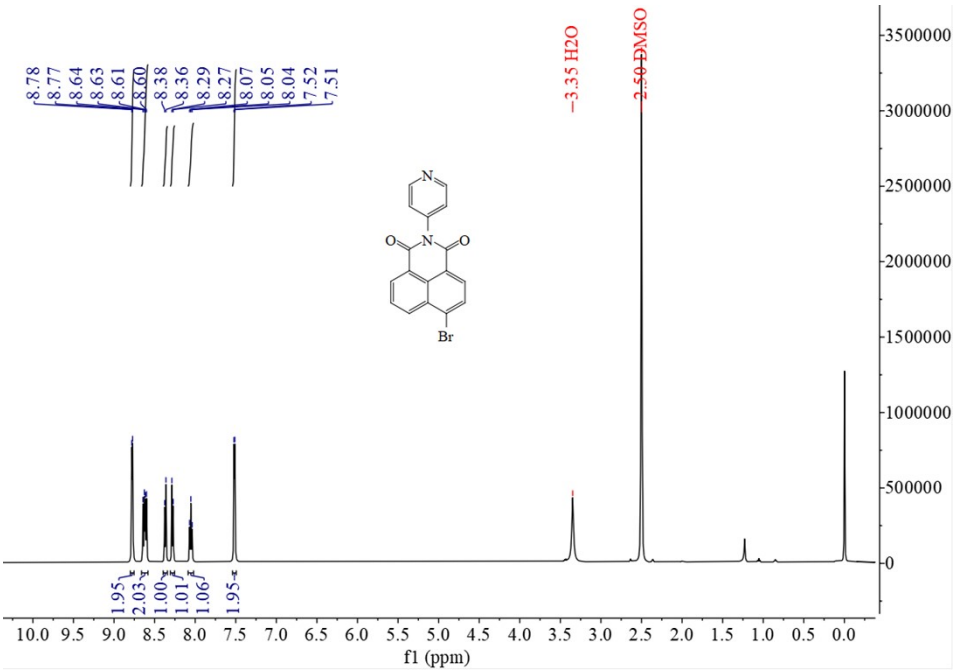


Figure S6. ^{1}H-NMR (DMSO-d_6) spectrum of Compound 1.

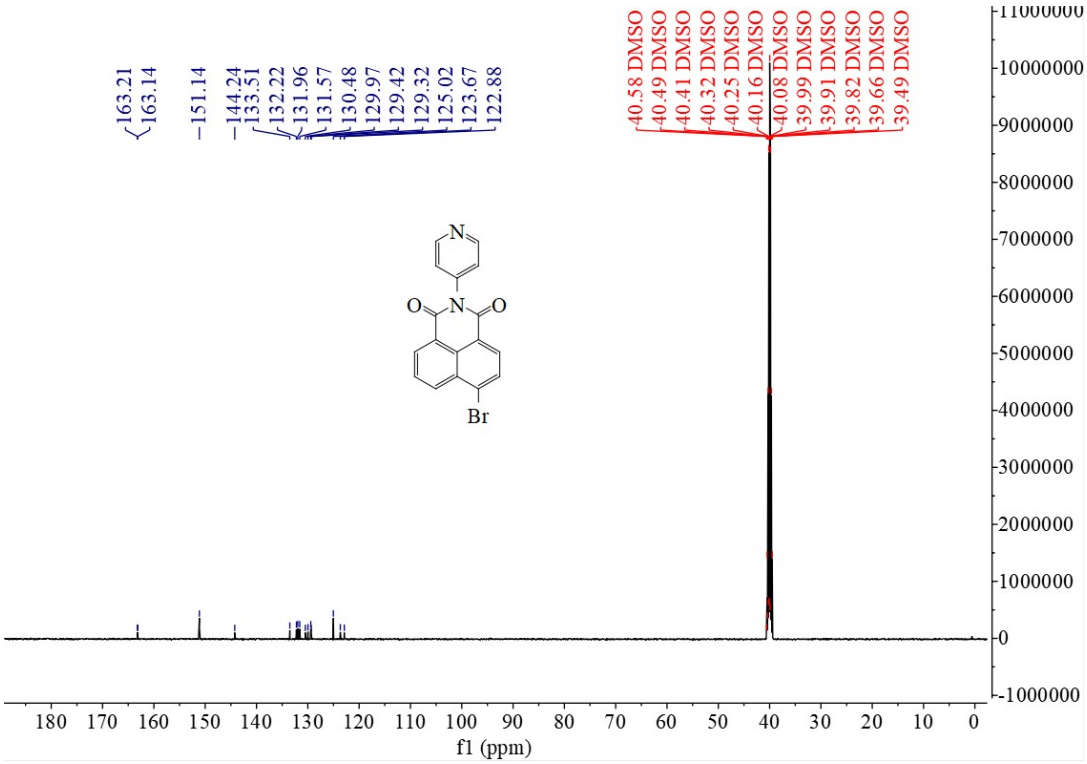


Figure S7. ^{13}C-NMR (DMSO-d_6) spectrum of Compound 1.

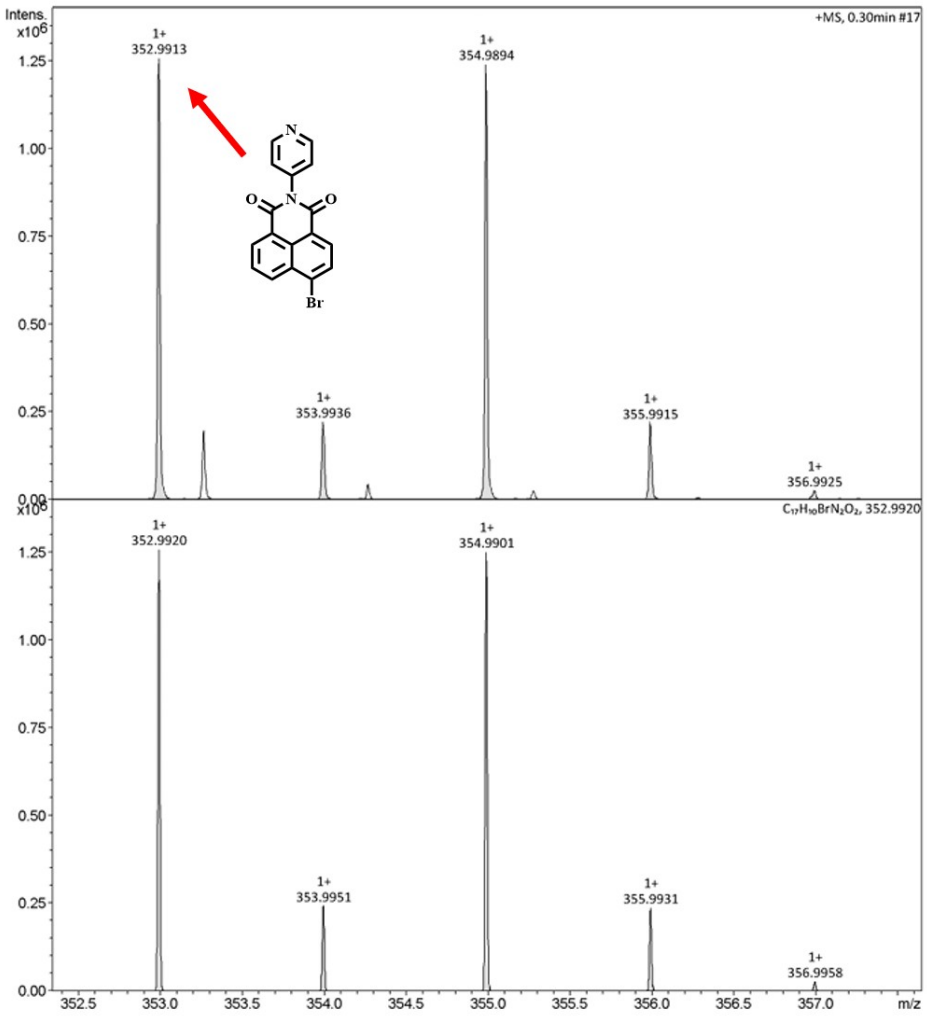


Figure S8. HRMS (ESI) spectrum of Compound 1.

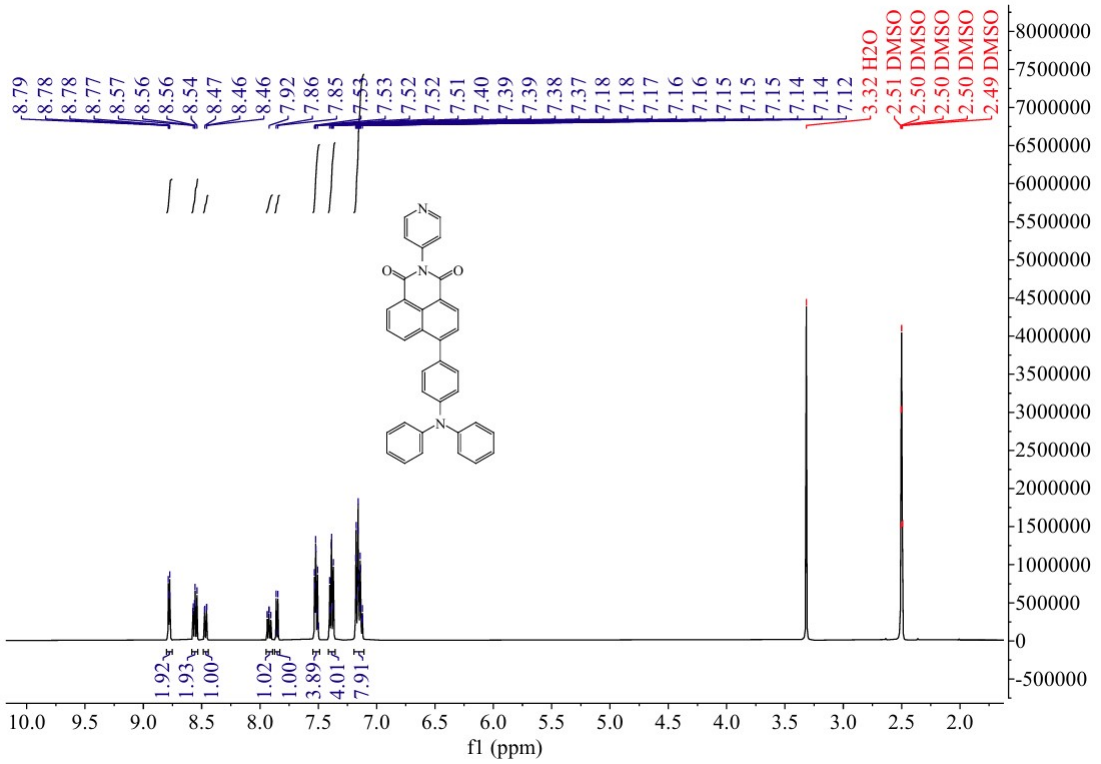


Figure S9. ^{1}H-NMR (DMSO-d_6) spectrum of Compound 2.

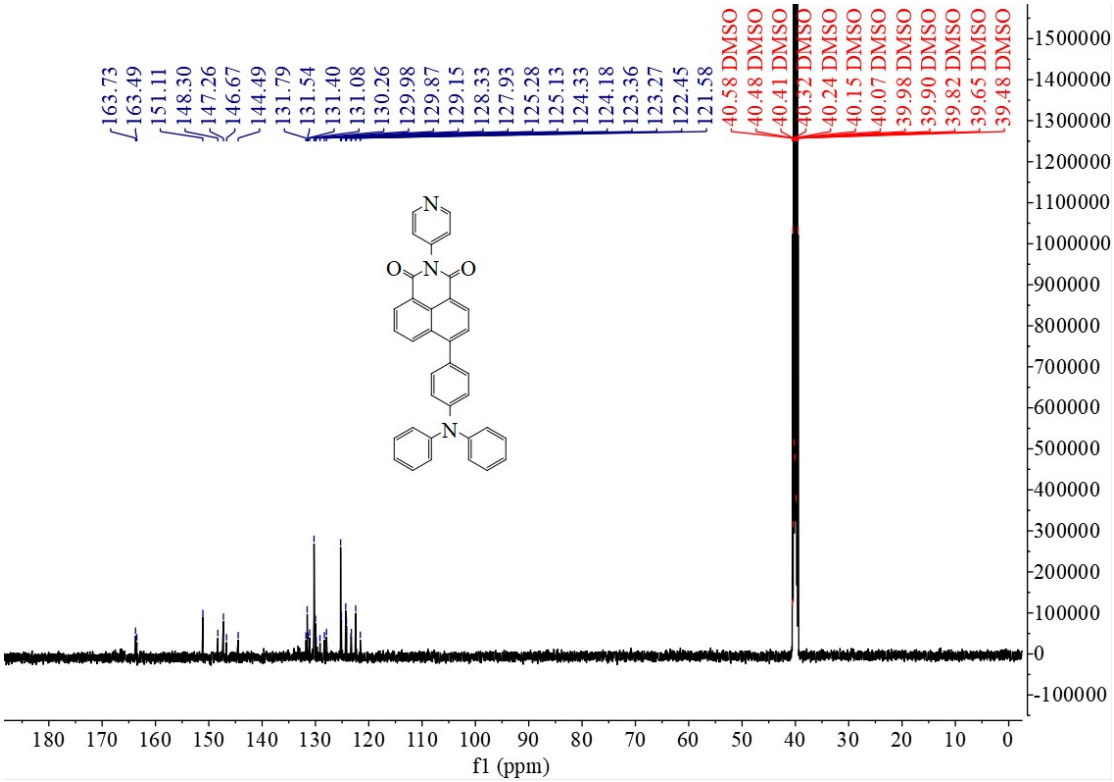


Figure S10. ^{13}C-NMR (DMSO-*d6*) spectrum of Compound 2.

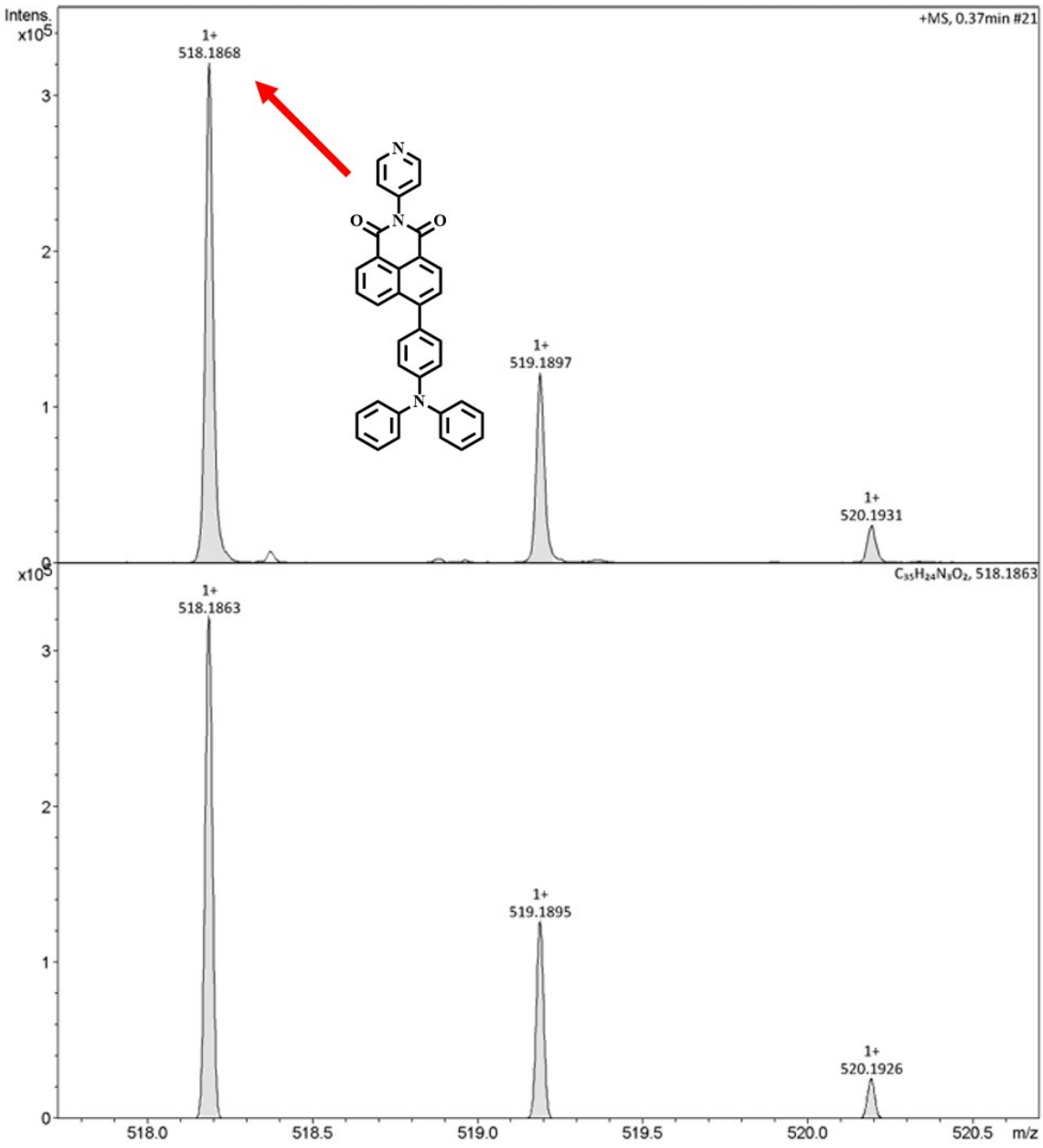


Figure S11. HRMS (ESI) spectrum of 2.

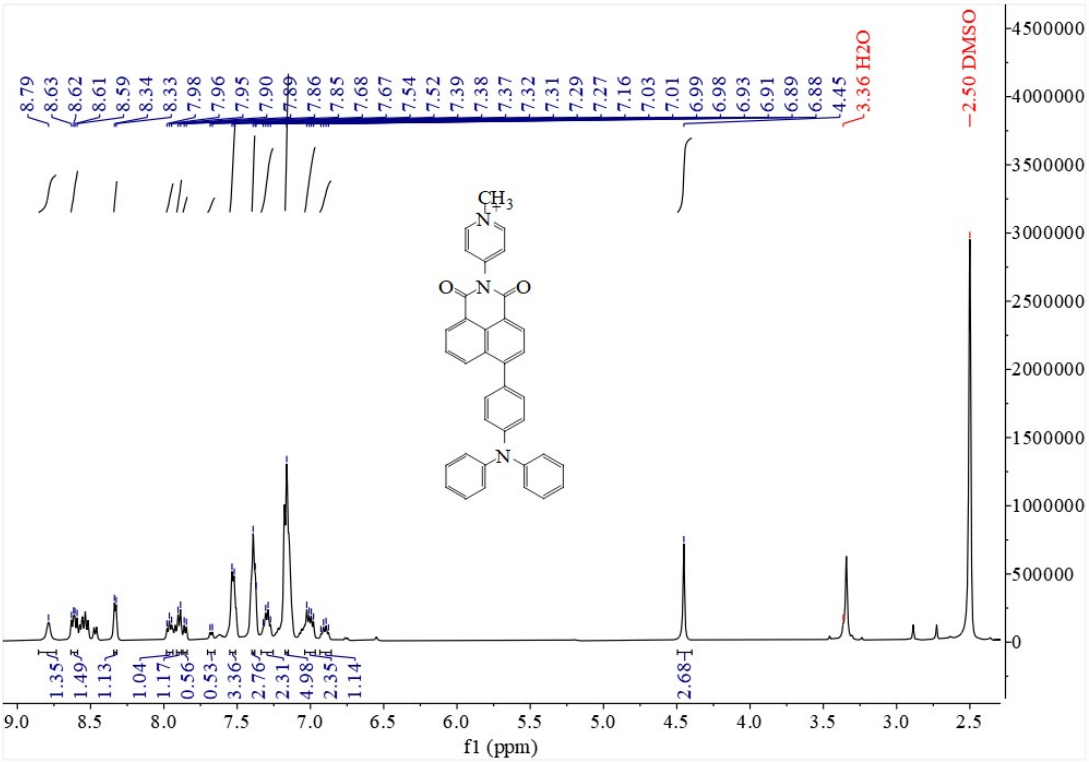


Figure S12. ^{1}H-NMR (DMSO-d_6) spectrum of Compound A.

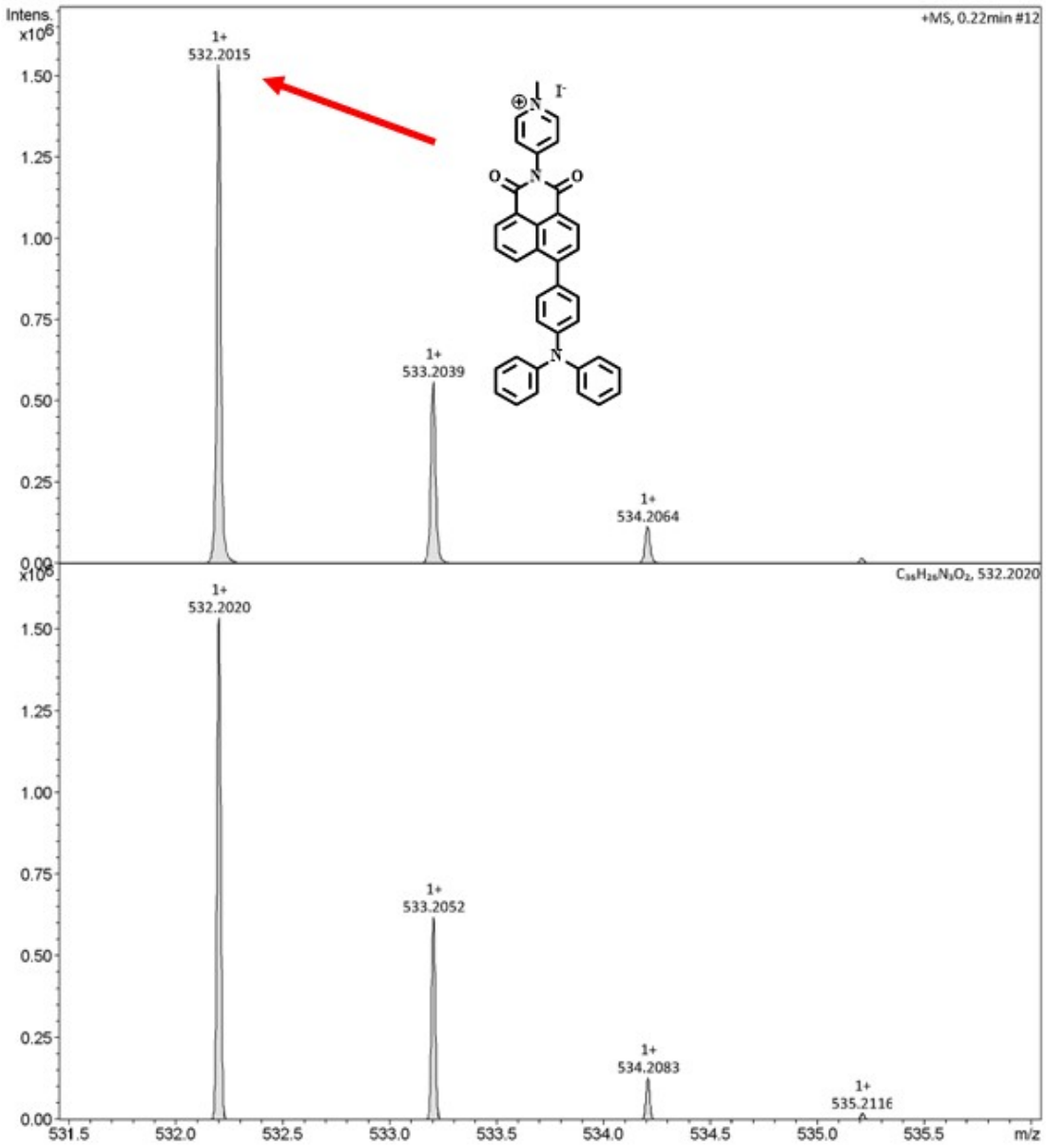


Figure S13. HRMS (ESI) spectrum of Compound A.

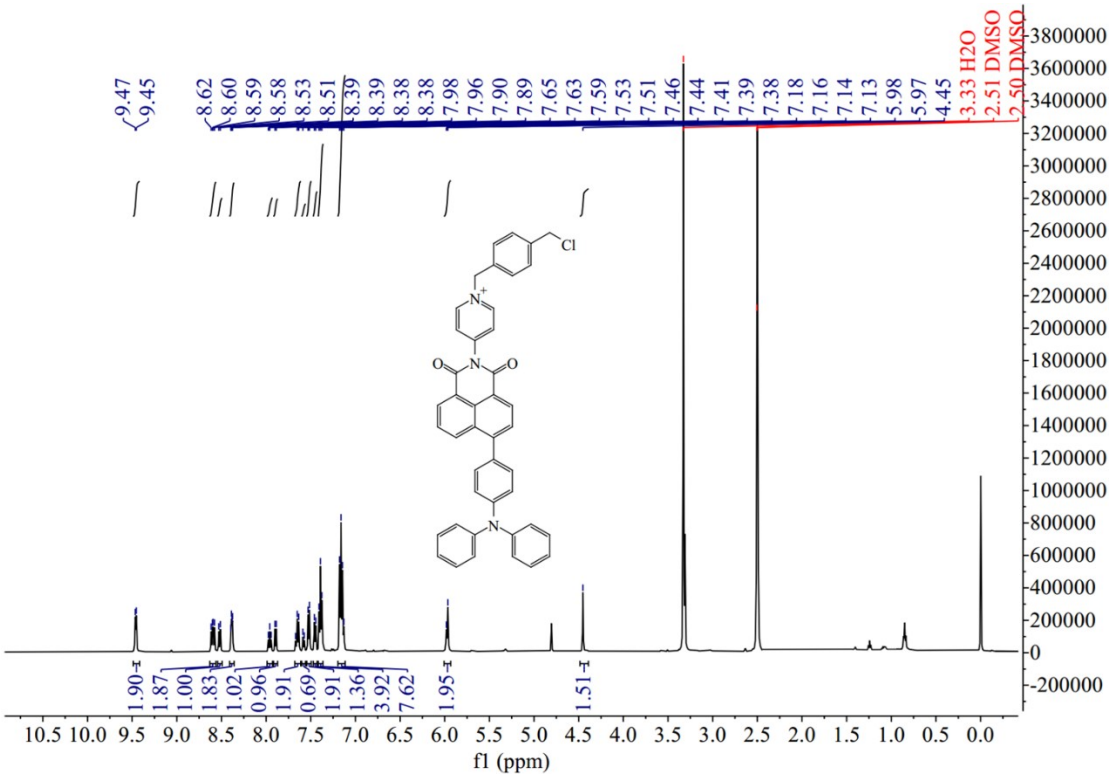


Figure S14. ^{1}H-NMR (DMSO-d_6) spectrum of Compound **Mito-NT**.

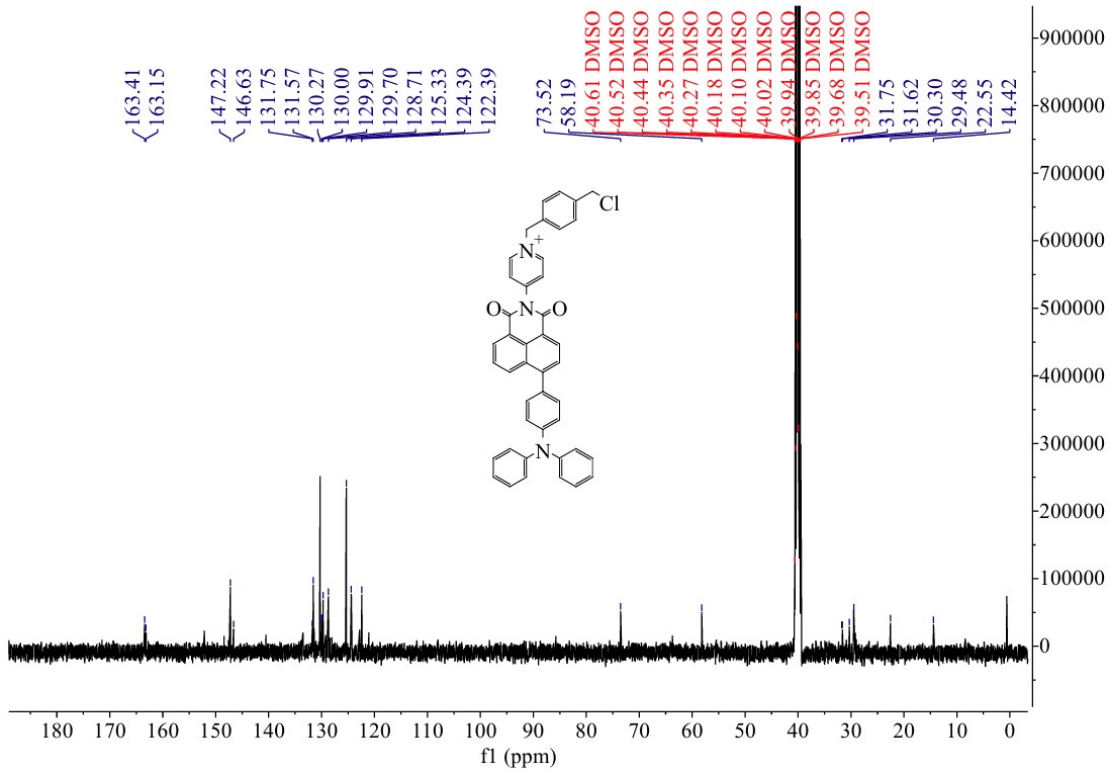


Figure S15. ^{13}C-NMR (DMSO-*d6*) spectrum of Compound **Mito-NT**.

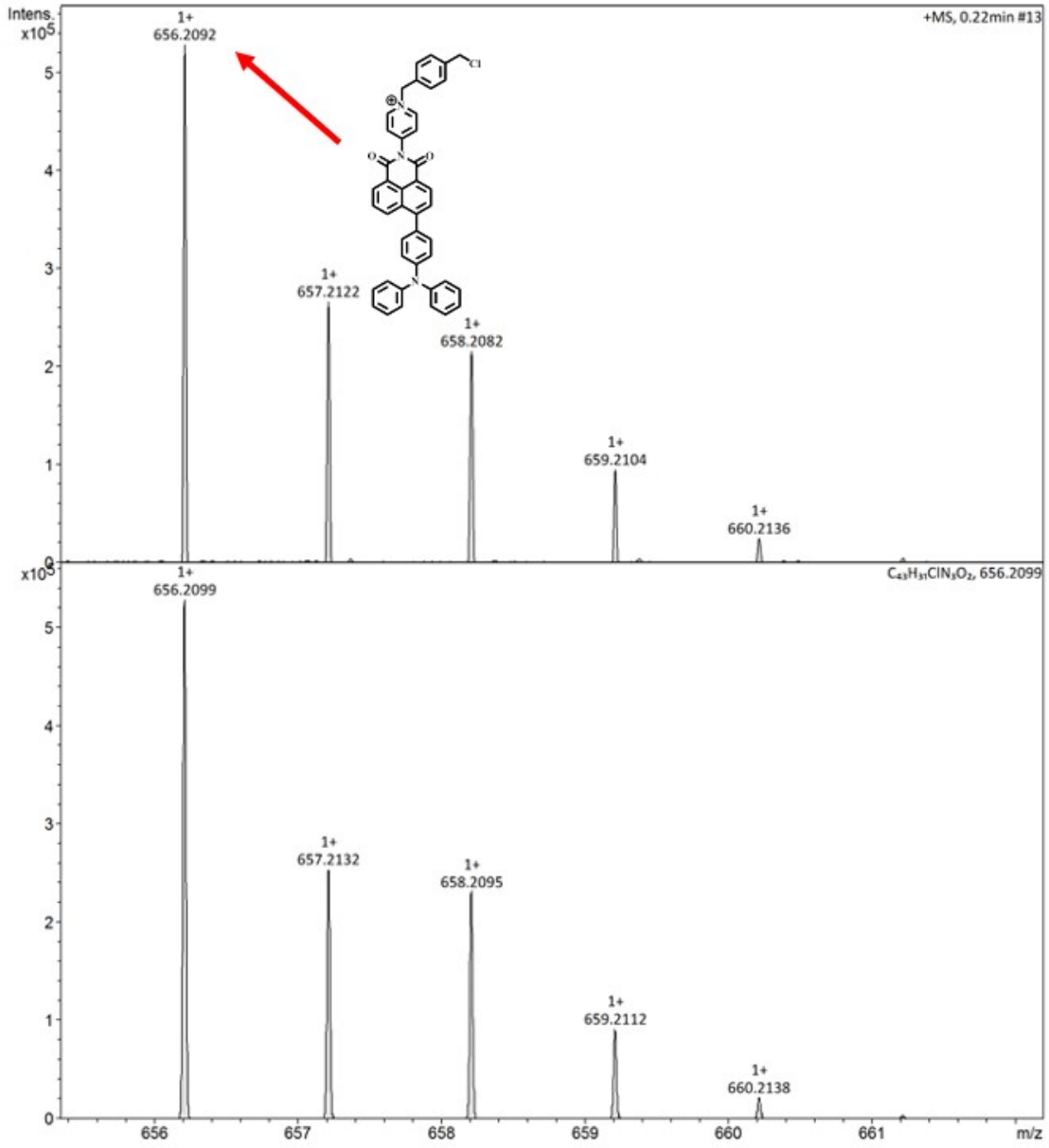


Figure S16. HRMS (ESI) spectrum of **Mito-NT**.