Supporting Information

An anthracene carboxamide-based fluorescent probe for rapid and sensitive detection of mitochondrial hypochlorite in living cells

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Contents

1. Preparation of reactive oxygen species (ROS) and reactive nitrogen species (RNS)

All the stocking solutions of ROS/RNS were prepared based on the reported literature [1]**.** The stock hydrogen peroxide (H_2O_2), sodium hypochloride (NaClO) and tert-Butyl hydroperoxide (TBHP) slolutions were purchased from Sigma-Aldrich. Superoxide anion (O_2^-) solution was prepared by fully dispersing the potassium dioxide in anhydrous DMSO via ultrasonic treatment. Hydroxyl radicals (·OH) and tert-butoxy radical (tBuO·) were prepared by Fenton reaction, the molar ratio of $FeSO_4$:H_2O_2 and $FeSO_4$:TBHP was 1:10. Peroxyl radicals (ROO·) was generated from 2,2'-azobis(2-amidinopropane)dihydrochloride. Peroxynitrite ($ONOO^-$) solution was prepared by 3-morpholinosydnonimine hydrochloride (SIN-1). NO· were diluted from the commercially available 2,2'-azobis (2-amindinopropane) dihydrochloride and sodium nitroferricyanide(III) dihydrate (SNP) to ultrapure water.

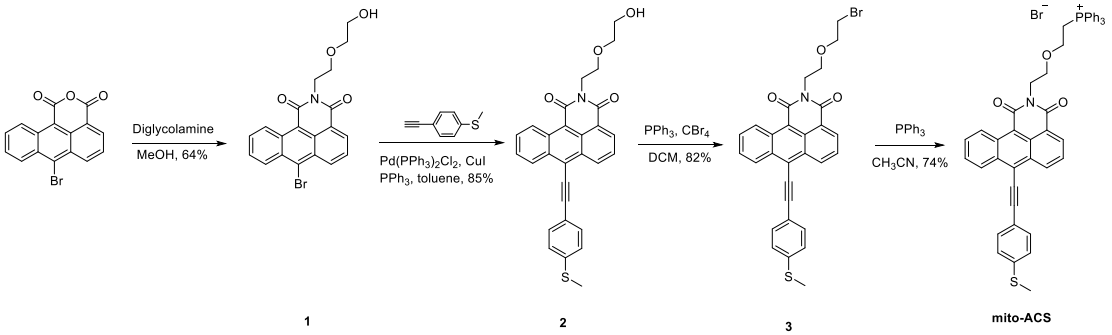
2. Determination of the detection limit

The detection limit was calculated based on the method reported in the previous literature by the equation as follows:

$$\text{Detection limit} = 3\sigma/k \quad [2]$$

Where σ is the standard deviation of blank measurement, k is the slope of the equation between fluorescence intensity and the concentrations of NaOCl. We measured the fluorescence intensity of the probe **mito-ACS** without NaOCl for six times to obtain the standard deviation, and the slope k was obtained according to the linear equation of the fluorescence intensity ratio of F_{452}/F_{638} with the increasing concentration of NaOCl.

3. The synthesis of mito-ACS



The synthesis of compound **1**

6-bromo-anthracene carboxyimide (330 mg, 1 mmol) and 2-(2-Aminoethoxy)ethanol (150 uL, 1.5 mmol) were dissolved in methanol (10 mL) under a nitrogen atmosphere. The reaction mixture was stirred under 60 ^{0}C for 4h. Once the TLC plate showed all the starting material was consumed, reaction mixture was cooled to room temperature and concentrated under vacuum. Then water (50 mL) was added and extracted by dichloromethane (20 mL x 3), organic phase was collected and washed with brine (100

mL), dried over Na_2SO_4 and concentrated via rotary evaporator. The crude product was purified by column chromatography (CH_2Cl_2) to give the product **1** as a yellow solid (64%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.99 (d = 9.1 Hz, 1H), 8.87 (d, *J* = 8.8 Hz, 1H), 8.76 (d, *J* = 7.0 Hz, 1H), 8.65 (d, *J* = 8.8 Hz, 1H), 7.88 - 7.74 (m, 2H), 7.70 (t, *J* = 8 Hz), 4.53 (t, *J* = 5.7 Hz, 2H), 3.92 (t, *J* = 5.7 Hz, 2H), 3.71 (m, 4H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 164.9, 163.4, 135.1, 134.6, 134.0, 134.0, 131.4, 131.4, 129.0, 128.6, 128.4, 128.1, 127.0, 126.8, 122.5, 115.2, 72.3, 68.5, 61.9, 39.9.

The synthesis of compound **2**

To a mixture of toluene (10 mL), CuI (30 mg, 0.15mmol) and PPh_3 (26 mg, 0.1 mmol), solution of **1** (413 mg, 1 mmol) and 1-Ethynyl-4-(methylthio)benzene (180 mg, 1.2 mmol) were added, and then the mixture was degassed 3 times by evacuating the flask and backfilling of Ar. Then, $PdCl_2$(dppf) (35 mg, 0.05 mmol) was added, and the mixture was stirred at 110 ºC for 12 hours. The reaction mixture was cooled to RT, diluted with dichloromethane (15 mL), and filtered through Celite. The filtrate was extracted with dichloromethane (3 × 20 mL). The combined organic phases were washed with brine (40 mL) and concentrated under reduced pressure. The residue was purified by column chromatography with CH_2Cl_2:MeOH (v/v, 100/1) to afford **2** as a yellow solid (85%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.69 - 9.47 (d, *J*= 6.1, 1H), 8.33 (m, 2H), 8.13 (s, 1H), 7.49 (s, 1H), 7.41 - 7.24 (m, 4H), 7.14 (d, *J* = 6.2 Hz, 2H), 4.30 (m, 2H), 3.80 (m, 2H), 3.66 (m, 4H), 2.47 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 164.47, 163.53, 141.68, 133.28, 132.47, 132.09, 130.82, 128.85, 127.39, 127.30, 127.27, 126.89, 126.77, 125.67, 125.58, 122.09, 118.19, 114.03, 106.43, 85.74, 72.36, 68.52, 61.89, 39.70, 15.13.

The synthesis of compound **3**

Compound **2** (240 mg, 0.5 mmol), triphenylphosphine (200 mg, 0.75 mmol), and carbon tetrabromide (245 mg, 0.75 mmol) were added to 20 mL of dichloromethane. The solution was stirred for 24 h at room temperature. Then, water was added and extracted with dichloromethane (3 × 20 mL). The organic layer was collected and dried over brine (30 mL), anhydrous Na_2SO_4, then, concentrated via rotary evaporator. The crude product was purified by column chromatography CH_2Cl_2:MeOH (v/v, 100/1) and then washed by n-hexane to give the product **3** as a yellow solid (82%).

^{1}H NMR (400 MHz,$CDCl_3$) δ 9.91 (d, *J* = 9.1 Hz, 1H), 8.78 (d, *J* = 9.0 Hz, 1H), 8.67 (d, *J* = 6.9 Hz, 1H), 8.59 (d, *J* = 8.6 Hz, 1H), 7.79 - 7.72 (m, 1H), 7.64 (m, 4H), 7.30 (d, *J* = 8.3 Hz, 2H), 4.50 (t, *J* = 6.1 Hz, 2H), 3.90 (dt, *J* = 8.3, 6.2 Hz, 4H), 3.46 (t, *J* = 6.2 Hz, 2H), 2.57 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 164.68, 163.55, 141.75, 133.67, 133.47, 132.90, 132.61, 132.17, 131.02, 129.39, 127.78, 127.60, 127.16, 127.07, 125.91, 125.77, 122.51, 118.23, 114.62, 106.46, 85.85, 70.63, 68.02, 39.26, 31.95, 15.18.

The synthesis of Probe **mito-ACS**

Compound **3** (160 mg, 0.3 mmol) and triphenylphosphine (105 mg, 0.4 mmol) were added to 8 mL of acetonitrile. The solution was refluxed for 3 h, then, after cooled to room temperature, the mixture was concentrated via rotary evaporator. The crude product was purified by column chromatography CH_2Cl_2:MeOH (v/v, 20/1) to give the product **mito-ACS** as red solid (74%).

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.75 (d, *J* = 9.0 Hz, 1H), 8.72 (d, *J* = 8.4 Hz, 1H), 8.52 (d, *J* = 6.6 Hz, 2H), 7.75 (dd, *J* = 12.8, 7.7 Hz, 6H), 7.72 - 7.67 (m, 1H), 7.66 - 7.50 (m, 13H), 7.27 (d, *J* = 8.2 Hz, 2H), 4.18 (m, 2H), 4.12 (t, *J* = 6.0 Hz, 2H), 4.03 (t, *J* = 5.5 Hz, 1H), 3.99 (t, *J* = 5.5 Hz, 1H), 3.51 (t, *J* = 6.0 Hz, 2H), 2.55 (s, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 163.29, 162.16, 141.01, 133.57, 133.55, 133.00, 132.93, 132.75, 132.39, 131.77, 131.48, 131.19, 130.12, 129.07, 128.98, 128.24, 126.80, 126.63, 126.24, 125.82, 124.91, 124.82, 121.22, 118.20, 117.63, 117.05, 113.16, 103.94， 84.75, 67.14, 63.23, 63.17, 37.96, 24.61, 24.26, 14.18.

ESI-MS: m/z calcd for $C_{47}H_{37}NO_3PS^+$ $[M]^+$ 726.2226, found 726.20.

4. Fluorescence spectra of the probe mito-ACS with and without excessive ClO^-

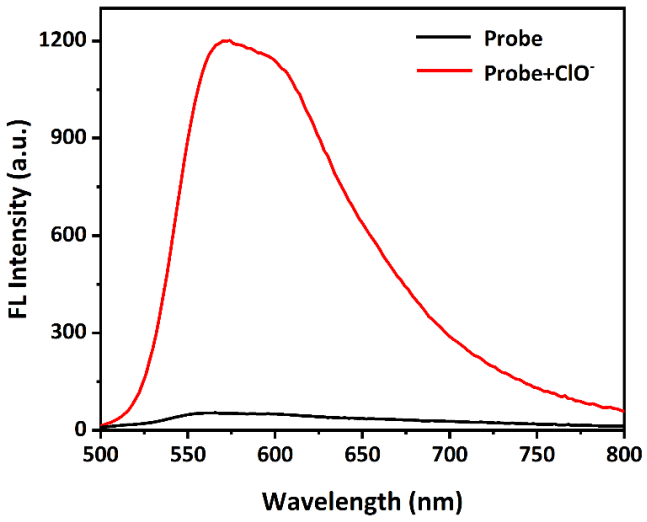


Figure. S1. Fluorescence spectra of the probe **mito-ACS** (10 μM) with and without excessive ClO^- (30 μM) in pure aqueous media, λ_{exc} = 480 nm, slits = 2/2 nm.

5. ESI-MS data of mito-ACS and the reaction mixture of mito-ACS with NaOCl

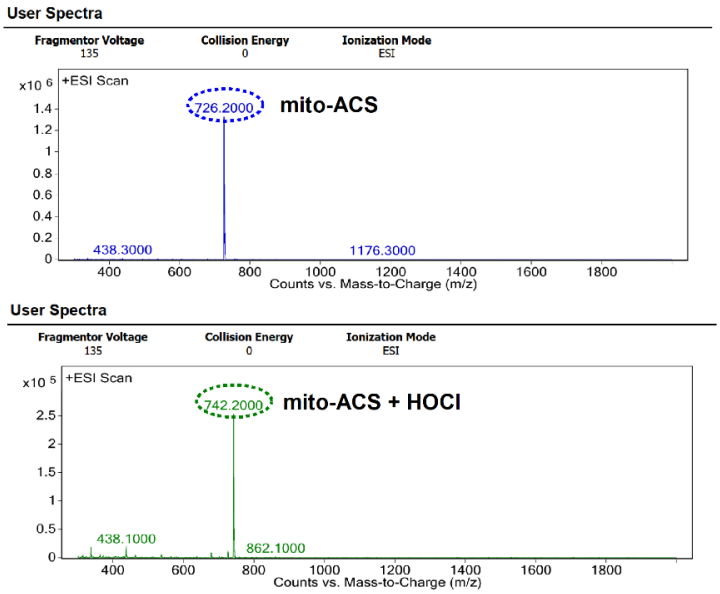


Figure. S2. MS-ESI spectra of **mito-ACS** and the reaction mixture of **mito-ACS** with NaOCl.

6. Photostability of probe mito-ACS and the oxidation product toward NaOCl.

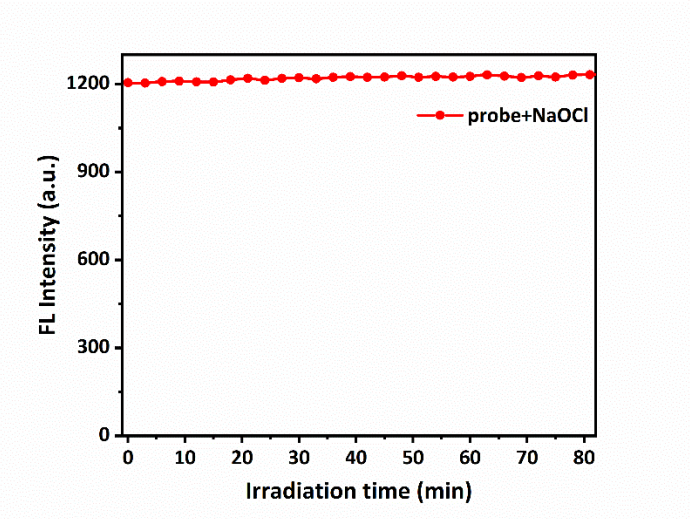


Figure. S3. Time-dependent fluorescence intensity changes of **mito-ACS** (10 μM) under the irradiation by a 450w lamp (pure aqueous media, pH = 7.4), λ_{ex} = 480 nm, slits = 2/2 nm.

7. pH influence of the probe mito-ACS toward NaOCl.

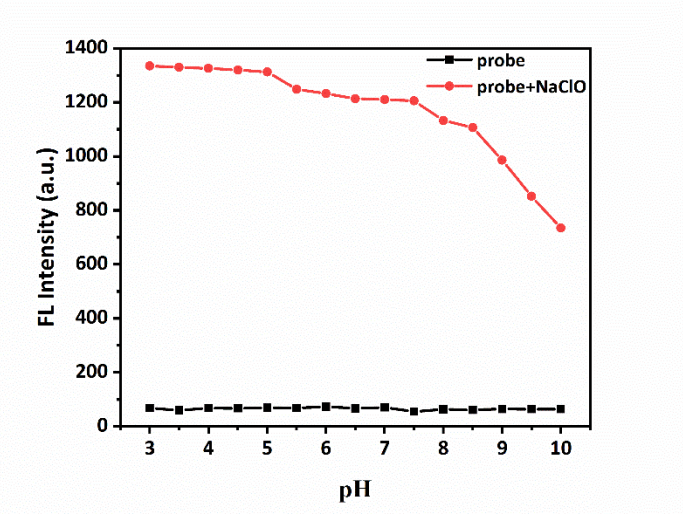


Figure. S4. Fluorescence response of **mito-ACS** (10 μM) in the absence and presence of NaOCl (30 μM) at different pH solutions. All data were recorded in different pH buffer solutions. λ_{exc} = 480 nm, slits = 2/2 nm.

7. Cytotoxicity assays

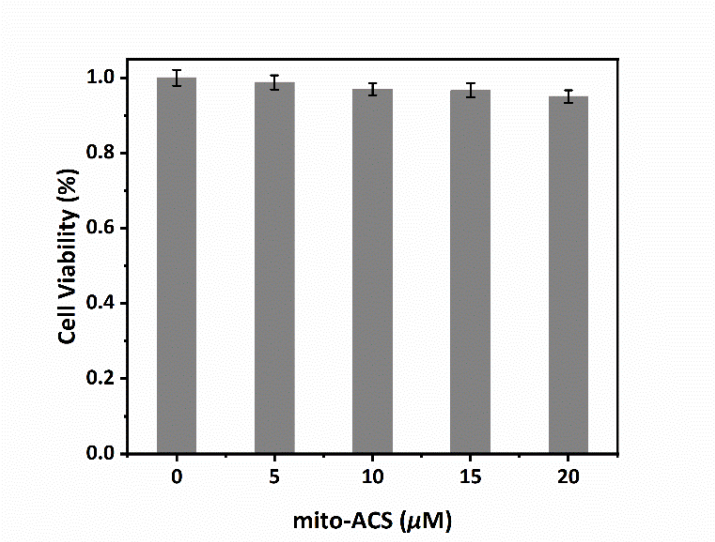


Figure. S5. Cell viability of HeLa cells treated with different concentrations of **mito-ACS** (0, 5, 10, 15, 20 μM) for 24 h.

8. NMR spectra

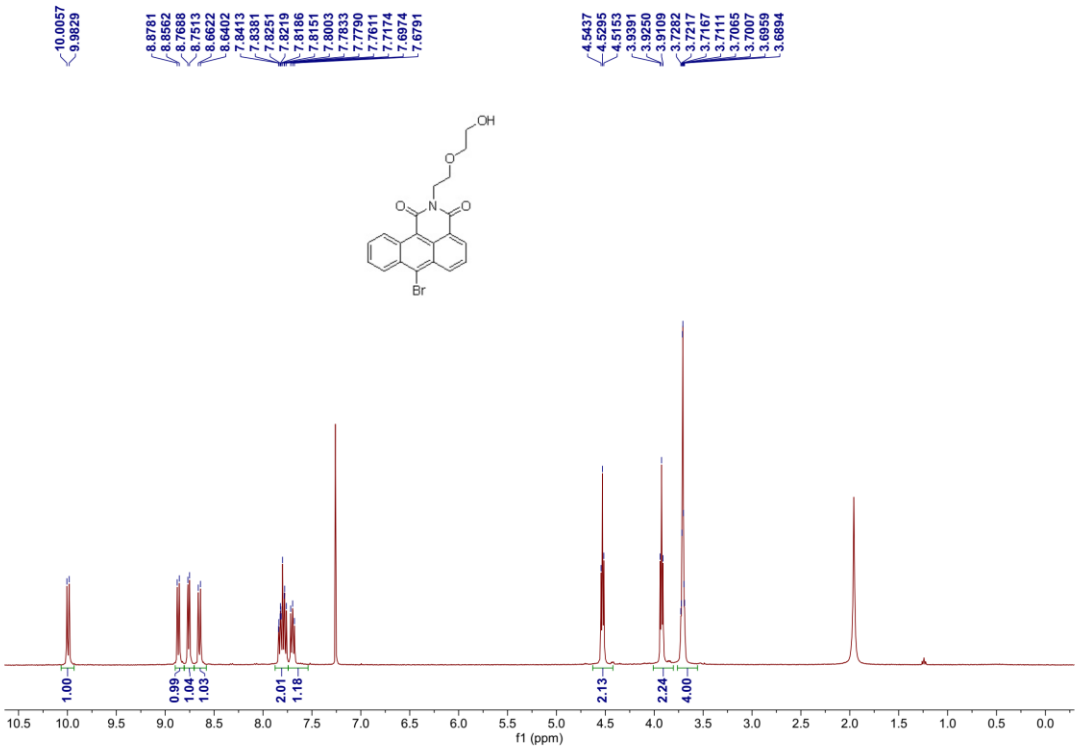


Figure. S6. ^{1}H NMR (400 MHz) spectrum of **1** in $CDCl_3$.

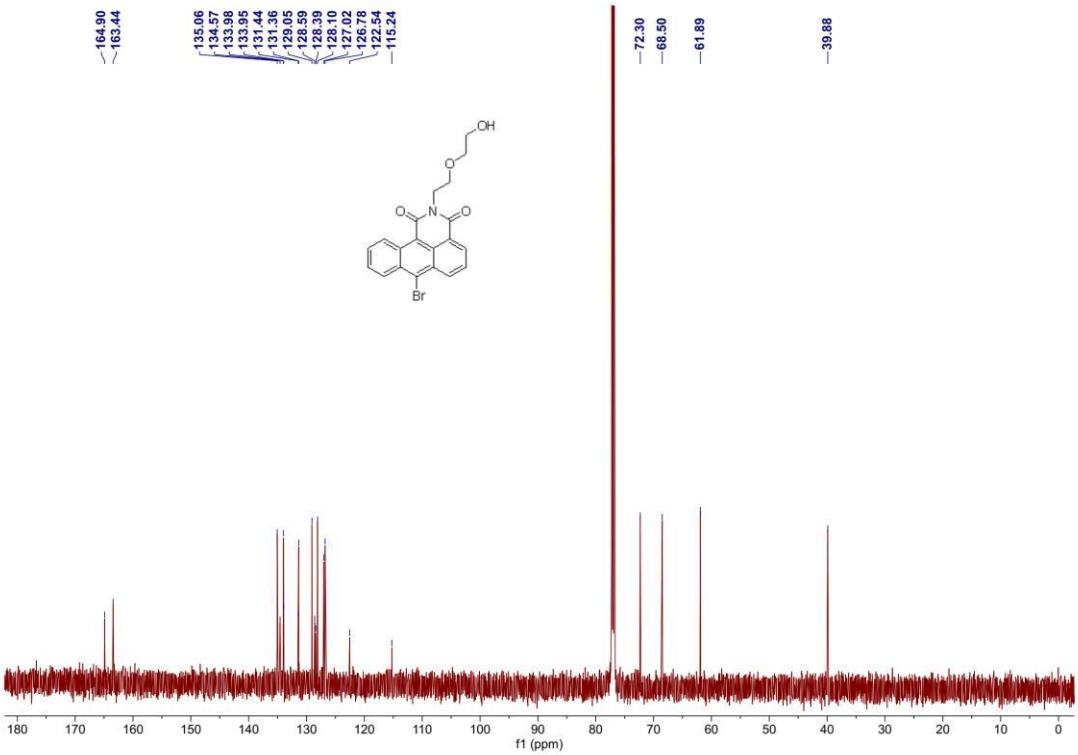


Figure. S7. ^{13}C NMR (151 MHz) spectrum of **1** in $CDCl_3$.

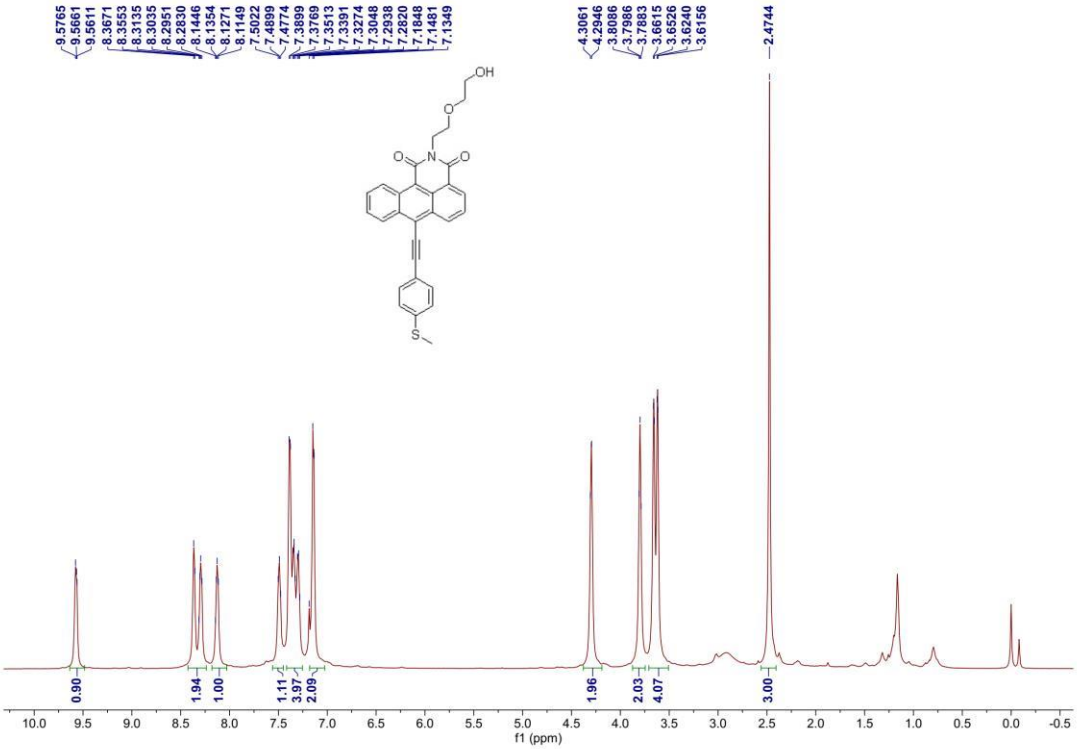


Figure. S8. ^{1}H NMR (600 MHz) spectrum of **2** in $CDCl_3$.

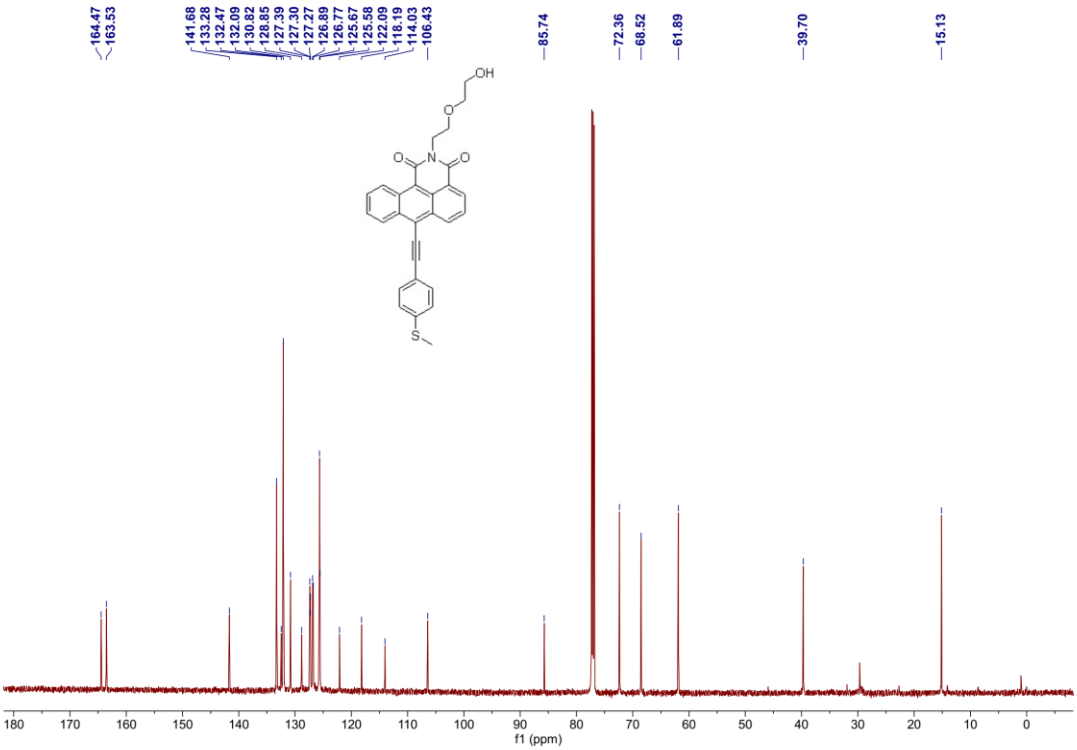


Figure. S9. ^{13}C NMR (151 MHz) spectrum of **2** in $CDCl_3$.

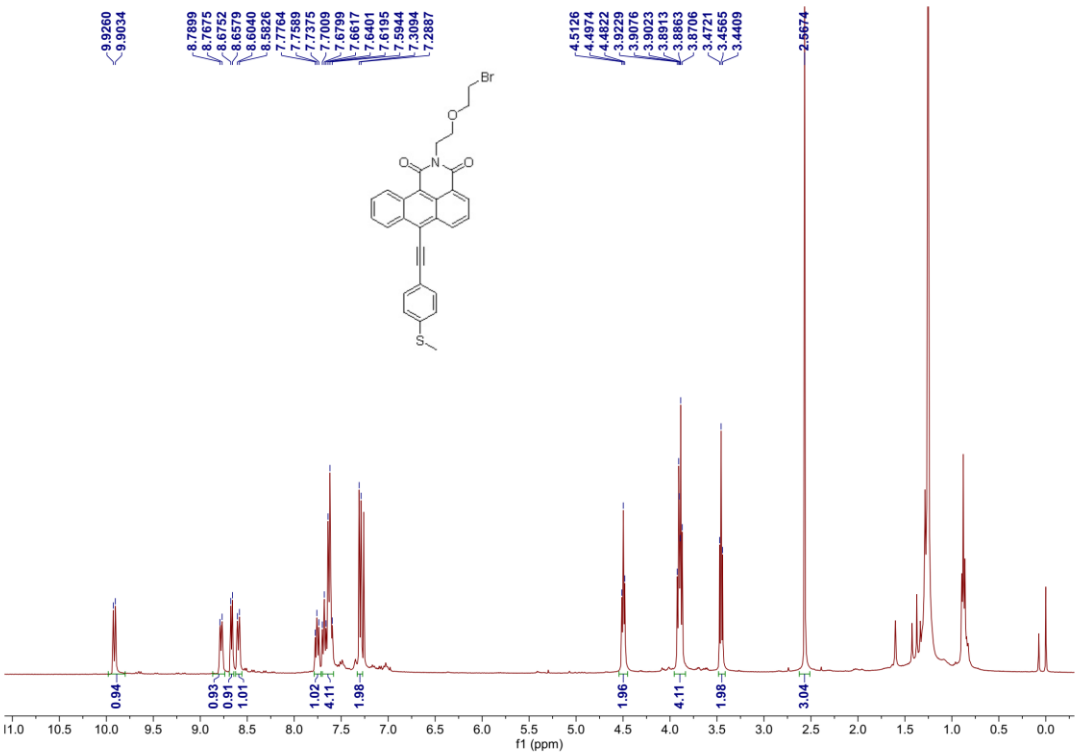


Figure. S10. ^{1}H NMR (400 MHz) spectrum of **3** in $CDCl_3$.

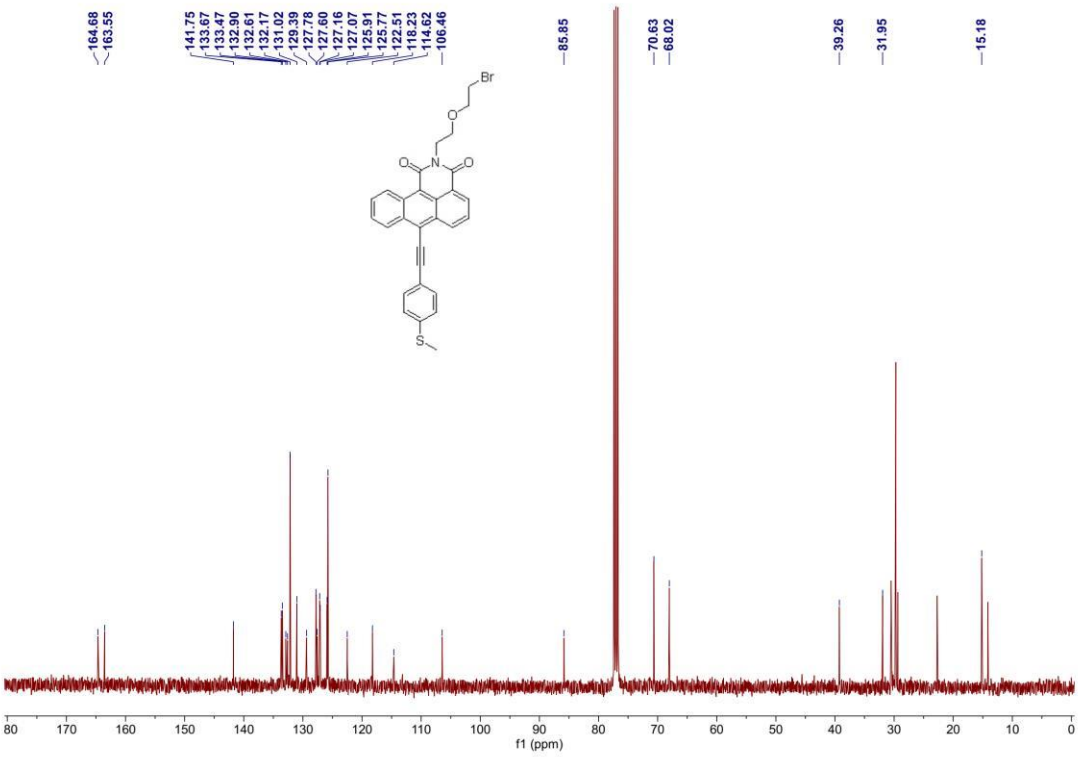


Figure. S11. ^{13}C NMR (101 MHz) spectrum of **3** in $CDCl_3$.

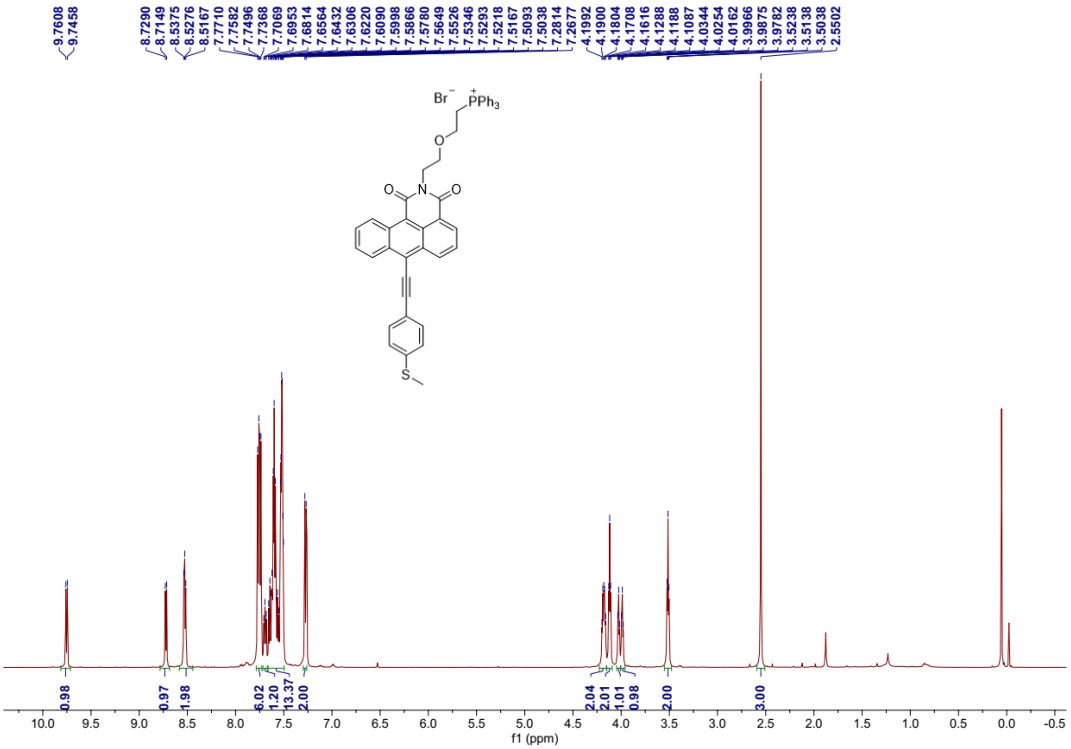


Figure. S12. ^{1}H NMR (400 MHz) spectrum of **mito-ACS** in $CDCl_3$.

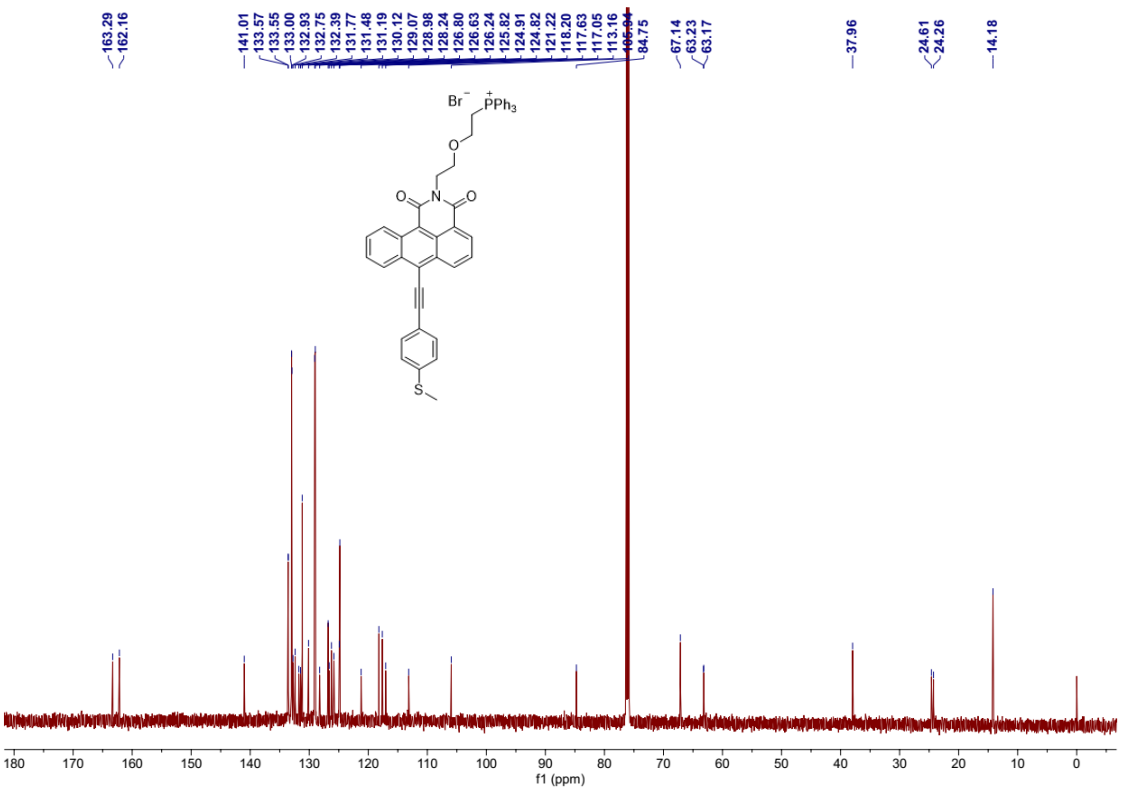


Figure. S13. ^{13}C NMR (101 MHz) spectrum of **mito-ACS** in $CDCl_3$.

1. Zhu, B.; Gao, C.; Zhao, Y.; Liu, C.; Li, Y.; Wei, Q.; Ma, Z.; Du, B.; Zhang, X., A 4-hydroxynaphthalimide-derived ratiometric fluorescent chemodosimeter for imaging palladium in living cells. *Chem. Commun.* **2011,** 47, (30), 8656-8658.
2. Zeng, L.; Xia, T.; Hu, W.; Chen, S.; Chi, S.; Lei, Y.; Liu, Z., Visualizing the Regulation of Hydroxyl Radical Level by Superoxide Dismutase via a Specific Molecular Probe. *Anal Chem* **2018,** 90, (2), 1317-1324.