Supporting Information

A lysosome-targeted probe for the real-time detection of hypobromous acid in living human cancer cells

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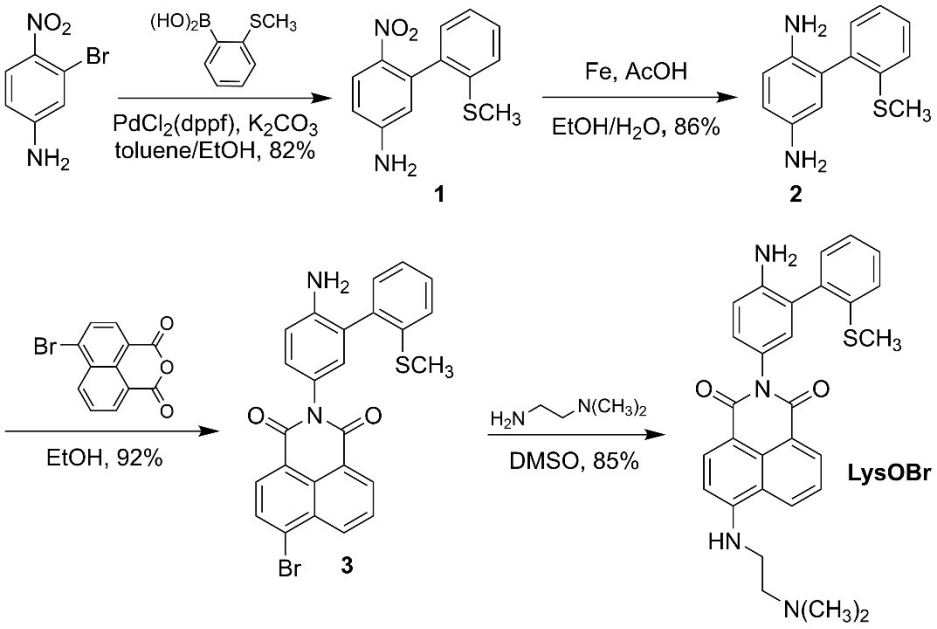
1. Reagents and instruments

Unless otherwise stated, all reagents used for reactions were purchased from commercial suppliers and without further purification. Column chromatographic purification was performed with Merck silica gel 60 (particle size 0.040-0.063 mm). ^{1}H NMR and ^{13}C NMR spectra were taken with Bruker Ascend III 400 and Avance III 600 instruments at 298.6 K in $CDCl_3$ and DMSO (d_6). Multiplicities of signals are described as follows: s - singlet, d - doublet, t - triplet, m - multiplet. Coupling constants (J) are given in Hz. ESI-MS data were acquired on Agilent Technologies 6230 TOF LC/MS with ESI source. UV measurements were carried out on HITACHI U-3900 Spectrophotometer. Fluorescence measurements were carried on Edinburgh FLS980 spectrophotometer, using 450W Xenon lamp. Absolute quantum yields were measured using an integrating sphere detector from Edinburgh Instruments. The ultra-pure water was obtained from Direct-Q5 purifier. HeLa Cells fluorescence imaging was studied by a Nikon A1 confocal laser scanning microscope.

Abbreviations:

RT: Room Temperature
$PdCl_2$(dppf): 1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II)
Ar: Argon
PE: Petroleum Ether
EtOH: Ethanol
EtOAc: Ethylacetate
DCM: Dichloromethane
DMSO: Dimethyl Sulfoxide
MeOH: Methanol

2. Synthesis procedure of probe LysOBr



Scheme S1. Synthetic route of probe **LysOBr**

Synthesis of compound 1

To a solution of 4-amino-2-bromonitrobenzene (655 mg, 3 mmol) and 2-methylthiophenylboronic acid (612 mg, 3.6 mmol) in a mixture of toluene: EtOH (1:3, 40 mL), K_2CO_3 (2 M, 5 mL) was added, and the mixture was degassed 3 times by evacuating the flask and backfilling with Ar. Then, $PdCl_2$(dppf) (125 mg, 0.15 mmol) was added, and the mixture was stirred at 80^{o} C for 12 hours, the reaction mixture was allowed to cool to RT, diluted with DCM (15 mL), and filtered through Celite. The filtrate was extracted with DCM (3 x 20 mL). The combined organic phases were washed with saturated aqueous NaCl (40 mL) and concentrated under reduced pressure. The residue was purified by column chromatography using PE/EtOAC (v/v 50/1) to afford compound **1** as a pale yellow solid (640 mg, 82 %), ^{1}H NMR (400 MHz, Chloroform-d) δ 8.06 (d, J = 8.9 Hz, 1H), 7.37

– 7.31 (m, 1H), 7.31 – 7.25 (m, 1H), 7.20 (td, *J* = 7.4, 1.3 Hz, 1H), 7.10 (dd, *J* = 7.5, 1.5 Hz, 1H), 6.77 (d, *J* = 9.0 Hz, 1H), 6.56 (m, 1H), 4.74 (s, 2H), 2.35 (s, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 149.1, 140.0, 138.4, 138.3, 136.7, 128.4, 128.2, 127.5, 125.9, 125.2, 118.1, 114.5, 16.1.

Synthesis of compound 2

Compound **1** (600 mg, 2.3 mmol) was dissolved in a mixture of EtOH: H_2O (1:2, 30 mL). Iron powder (390 mg, 7 mmol) and acetic acid (5 mL) were then added, and the resulting mixture was stirred at 60 °C for 12 hours. After the reaction was completed, the slurry was extracted with diethyl ether (3 x 30 mL). The combined organic phases were washed with aqueous NaOH (1 M, 3 x 50 mL) and dried over $MgSO_4$. Filtration and removal of the solvent under reduced pressure to get compound **2** (450 mg, 86%) as red oil without further purification, ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.34 (m, 1H), 7.24 (d, *J* = 7.9 Hz, 1H), 7.21 – 7.16 (m, 2H), 6.70 – 6.62 (m, 2H), 6.52 – 6.46 (m, 1H), 3.48 (s, 4H), 2.37 (s, 3H). ^{13}C NMR (150 MHz, Chloroform-*d*) δ 138.4, 137.7, 137.4, 136.4, 130.3, 128.3, 127.6, 124.9, 124.5, 118.1, 117.3, 117, 15.3.

Synthesis of compound 3

Compound **2** (230 mg, 1 mmol) and 4-bromo-1,8-naphthalic anhydride (280 mg, 1 mmol) were dissolved in EtOH (10 mL) and stirred under reflux for 24 hours. After the reaction was completed, cooled and filtrated, the residue and recrystallized in EtOH to get the compound **3** as a yellow solid (450 mg, 92%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.57 (d, *J* = 7.3 Hz, 1H), 8.54 (d, *J* = 8.5 Hz, 1H), 8.33 (d, *J* = 7.8 Hz, 1H), 8.21 (d, *J* = 7.8 Hz, 1H), 8.00 (t, *J* = 7.9 Hz, 1H), 7.45 - 7.39 (m, 2H), 7.28 (t, *J* = 7.4 Hz, 1H), 7.22 (d, *J* = 7.5 Hz, 1H), 7.11 (dd, *J* = 8.5, 2.4 Hz, 1H), 6.93 - 6.90 (m, 2H), 4.73 (s, 2H), 2.43 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d6) δ 163.2, 145.2, 138.2, 136.5, 132.3, 131.4, 131.2, 130.8, 130.2, 130.1, 129.7, 128.9, 128.8, 128.6, 128.5, 128.3, 125.0, 124.7, 124.2, 123.8, 123.3, 122.5, 114.8, 14.6.

Synthesis of compound LysOBr

Compound **3** (250 mg, 0.5 mmol), N,N-dimethylethylenediamine (110 μL, 1 mmol) were dissolved in dry DMSO and stirred at 90 °C for 12 hours. After completion of the reaction, the resulting solution was poured into ice water and a yellow precipitate was isolated by filtration. The resulting solid was purified by column chromatography using eluent CH_2Cl_2/MeOH (v/v 100/1). Compound **LysOBr** was obtained as a yellow solid (210 mg, 85%), ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.71 (d, *J* = 8.5 Hz, 1H), 8.48 (d, *J* = 7.3 Hz, 1H), 8.31 (d, *J* = 8.5 Hz, 1H), 7.75 (t, *J* = 7.9 Hz, 1H), 7.64 (t, *J* = 5.5 Hz, 1H), 7.43 - 7.39 m, 2H), 7.28 (td, *J* = 7.4, 1.4 Hz, 1H), 7.21 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.02 (dd, *J* = 8.4, 2.5 Hz, 1H), 6.91 – 6.84 (m, 2H), 6.80 (d, *J* = 2.4 Hz, 1H), 4.66 (s, 2H), 3.54 (q, *J* = 6.3 Hz, 2H), 2.67 (t, *J* = 6.7 Hz, 2H), 2.43 (s, 3H), 2.30 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d6) δ 164.2, 163.4, 150.4, 144.8, 138.2, 136.7, 134.1, 130.7, 130.2, 129.7, 129.0, 128.3, 128.2, 125.0, 124.7, 124.7, 124.3, 124.2, 122.5, 120.2, 114.7, 108.3, 103.8, 56.9, 45.3, 40.9, 14.6.

Structural characterization of the reaction product of LysOBr with HOBr

Probe **LysOBr** (50 mg, 0.1 mmol) was dissolved in MeOH, and the solution was added HOBr (15 mg, 0.15 mmol) at room temperature. Atrer 10 minutes, the reaction mixture was extracted with dichloromethane and dried by anhydrous sodium sulfate. Filtration and removal of the solvent under reduced pressure to get crude solid, and was further purified by column chromatography using eluent CH_2Cl_2/MeOH (v/v 50/1).Compound **LysOBr[O]** was obtained as a yellow solid (43 mg, 91%), ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71 (d, *J* = 8.6 Hz, 1H), 8.46 (d, *J* = 6.4 Hz, 1H), 8.30 (d, *J* = 9.3 Hz, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.93 (d, *J* = 2.1 Hz, 1H), 7.75 (t, *J* = 7.8 Hz 1H), 7.61 (m, 3H), 7.52 (t, *J* = 8.0 Hz, 1H), 7.13 – 7.04 (m, 2H), 6.85 (d, *J* = 2.4 Hz,1H), 3.52 (q, *J* = 6.3 Hz, 2H), 2.64 (t, *J* = 6.5 Hz, 2H), 2.41 (s, 3H), 2.27 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.68, 162.86, 149.92, 148.85, 133.68, 131.05, 130.71, 130.19, 130.08, 129.26, 127.95, 127.43, 126.52, 124.60, 124.39, 124.27, 123.79, 123.56, 121.96, 119.62, 119.13, 107.63, 103.30, 56.30, 44.70, 40.35, 31.80.

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

HOBr solution was prepared by dissolving Br_2 in ultrapure water, then $AgNO_3$ solution was added drop by drop until the red solution to the colorless endpoint and then filtered. The Lambert-Beer's law was used to calculate the concentration of HOBr (ε_{260} = 160 L mol^{-1} cm^{-1}) [1]. Other ROS and RNS were prepared freshly according the published method [2]. The stock H_2O_2, NaClO and TBHP slolutions were purchased from Sigma-Aldrich. $OONO^-$ was prepared by adding NaOH solution to 3-morpholinosydnonimine hydrochloride ((SIN-1). The mixture was stirred for 15 min, and concentration was calculated by measuring the absorbance at 302 nm (ε_{302} = 1670 M^{-1} cm^{-1}). ·OH and ·*t*BuO were prepared by Fenton reaction, the molar ratio of $FeSO_4$:H_2O_2 and $FeSO_4$:TBHP was 1:10. ROO·and NO·were diluted from the commercially available 2, 2-

azobis (2-amindinopropane) dihydrochloride and SNP (sodium nitroferricyanide(III) dihydrate) to ultrapure water. $^1O_2$ was prepared by dissolving NaOCl to a solution of H_2O_2. O_2^- was prepared by dissolving KO_2 to DMSO and stirring for 20 min.

4. General UV and fluorescence spectra measurements

A stock solution of 1 mM **LysOBr** in DMSO was freshly prepared. The measured solutions of probe with HOBr were prepared by diluting 20 μL probe with PBS buffer solution (10 mM, pH 5), and then appropriate volume of HOBr stock solution was added to ensure a final volume of 2 mL. The resulting solutions were shaken well and measured after 5 minutes. The same method was applied to the selectivity experiments. And the different pH solutions were adjusted by adding minimum volumes of NaOH (0.2 M) or HCl (0.2 M) with fresh PBS (10 mM, pH = 7.4). The fluorescence spectrums were collected with the excited wavelength of 420 nm and the slit widths of 2.0 nm.

5. MTT Assay

HeLa cells were incubated in Dulbeccos modified Eagles medium (DMEM) supplement with 10% (V/V) Fetal Fovine Serum (FBS, Gibco), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 ^oC with 5 % CO_2 in appropriate humidity. Before MTT test, HeLa cells (10^5 cells/well) were dispersed in a 96-well cell culture plate, and filled to 200 μL per well. 24 hours after incubation, the medium was removed and the cells were supplemented with medium containing **LysOBr** of different concentrations (0, 2, 4, 6, 8, 10 μM) and cultured for 12 hours. Cells incubated with no probe were used as blank control. After removal of the medium, 100 μL MTT solutions (0.5 mg/ mL) was added to each well away from light. After 4 hours, the MTT solutions were removed, and 200 μL of DMSO was added to each well to fully dissolve the formed formazan crystals by a shaker. Finally, a microplate reader was used to measure the absorbance at 490 nm.

6. Confocal fluorescence imaging

Confocal Fluorescence Imageing were collected on a Nikon A1 confocal laser scanning microscope using a 20× objective. To detect the endogenous HOBr, fluorescence images of Hela cells were performed by incubating with **LysOBr** (5 μM), and then treated with HOBr (30 μM), NaBr (100 μM) or NaBr (100 μM)/N-acetylcysteine (100 μM). The cells were washed by PBS (pH 7.4) for three times before imaging and fluorescence was collected in green channel (520/560 nm) with the excitation wavelength at 405 nm. To determine the subcellular localization of **LysOBr**, **LysOBr** (2 μM) and Lyso-Tracker DND Red (1 μM) were co-incubated in cells for 30 min, then washed by PBS for three times and treated with HOBr (5 μM) for 30 min. The fluorescence was collected in a range of 520 - 560 nm upon excitation at 405 nm and the range of 580-600 nm upon excitation at 552 nm.

7. Determination of the detection limit

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

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

Where σ is the standard deviation of blank measurement, k is the slope of the equation between fluorescence intensity and the concentrations of HOBr. We measured the fluorescence intensity of the probe **LysOBr** without HOBr ten times to obtain the standard deviation, and the slope k was obtained according to the linear equation of the fluorescence intensity of **LysOBr** at 530 nm with the increasing concentration of HOBr.

8. Absorption responses of LysOBr toward HOBr

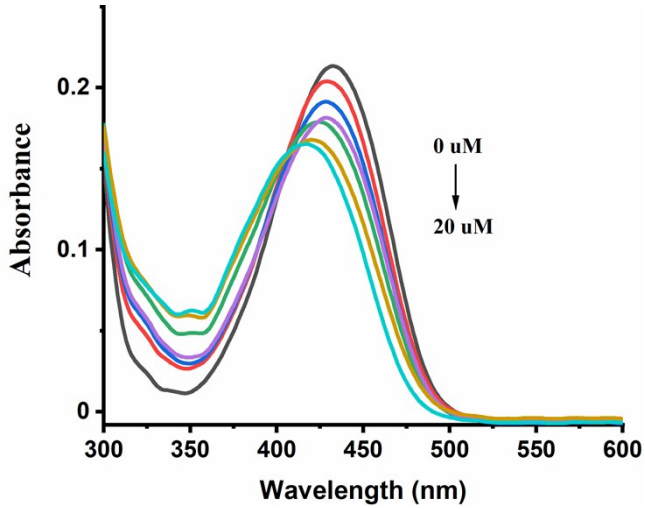
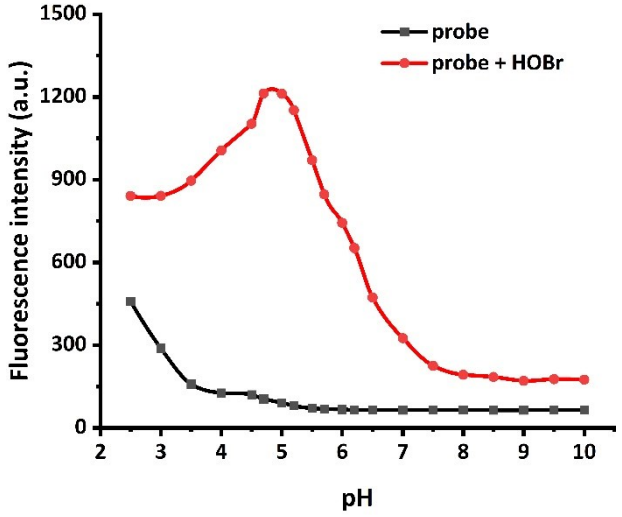


Fig. S1. The absorbance intensities of **LysOBr** (10 μM) with different amount of HOBr (0-20 μM).



9. The pH dependence of LysOBr with HOBr

Fig. S2. pH effect on the fluorescence intensities of **LysOBr** in the presence or absence of HOBr (10 μM) (ex/em: 420/530 nm, slit: 2/2 nm).

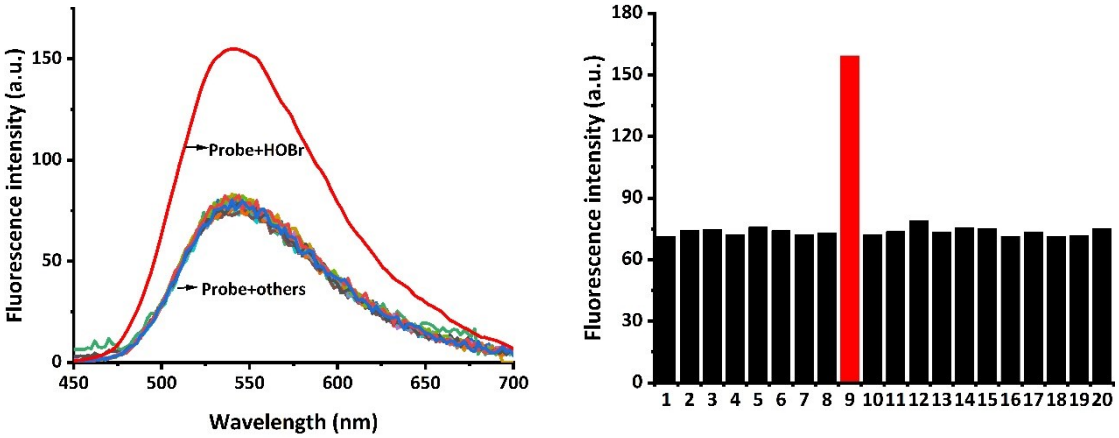


Fig. S3. The fluorescence spectra and fluorescence intensity of **LysOBr** (10 μM) in the presence of various bio-related analytes (1 − 20: H_2O_2, •OH, O_2^-, ROO•, NO•, TBHP, •OtBu, $ONOO^-$, HOBr, HOCl, Ca^{2+}, Mg^{2+}, Cu^{2+}, Hg^{2+}, Cu^+, Fe^{3+}, Zn^{2+}, Fe^{2+} (100 μM), Hcy (500 μM), Cys (500 μM), GSH (1 mM)) in PBS solution (ex/em: 420/530 nm, slit: 2/2 nm , pH 7, 1% DMSO).

10.The effects of interference of common metal ions and biothiols on monitoring HOBr

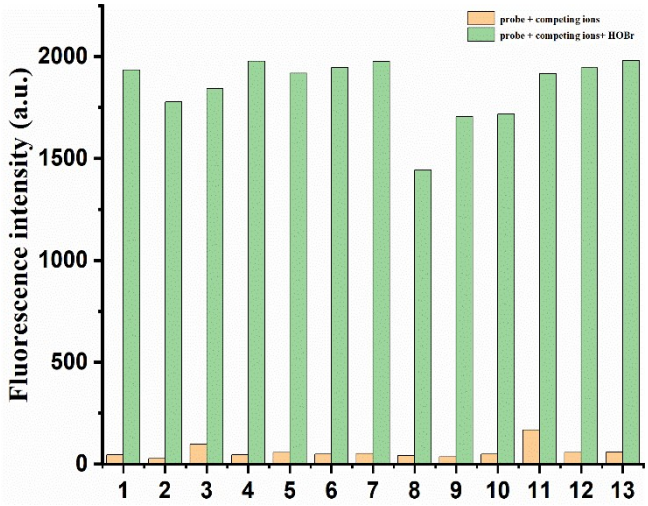


Fig. S4. The competitive fluorescence responses of probe **LysOBr** (10 μM) toward various analytes (100 μM) and HOBr (30 μM) 1. Ca^{2+}, 2. Mg^{2+}, 3. Cu^{2+}, 4. Cu^+, 5, Fe^{3+}, 6. Zn^{2+}, 7. Fe^{2+}, 8. Hcy (500 μM), 9. Cys (500 μM), 10. GSH (1 mM), 11. Br^-, 12. H_2S, 13. Ag^+. (ex/em: 420/530 nm, slit: 2/2 nm, pH 5).

11. Photostability of probe LysoOBr after reaction with HOBr

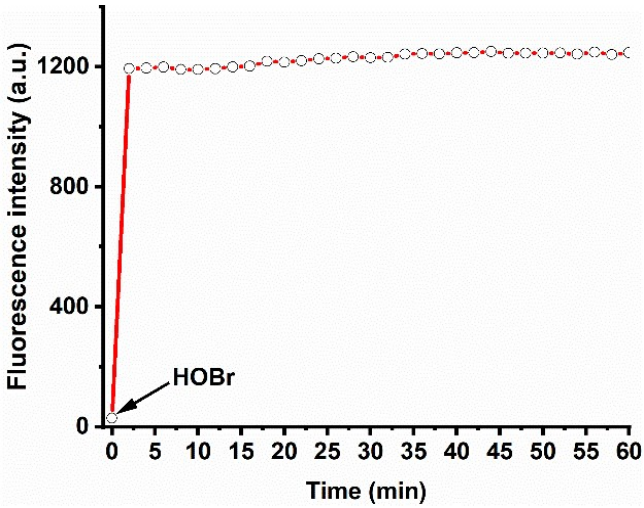


Fig. S5. Fluorescen intensity of probe **LysOBr** (10 μM) with HOBr (10 μM) under a continuous irradiation by 450w xenon lamp.

12. Cytotoxicity assays

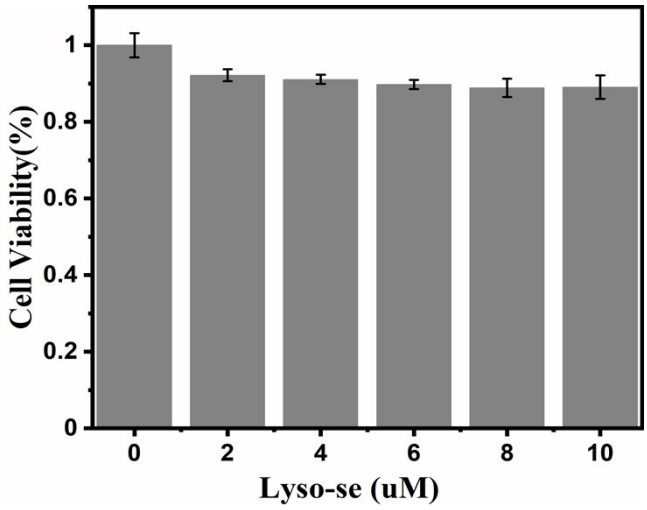


Fig. S6.. Cell viability of HeLa cells treated with different concentrations of **LysOBr** (0, 2, 4, 6, 8 and 10 μM) for 12 h.

13. NMR spectra

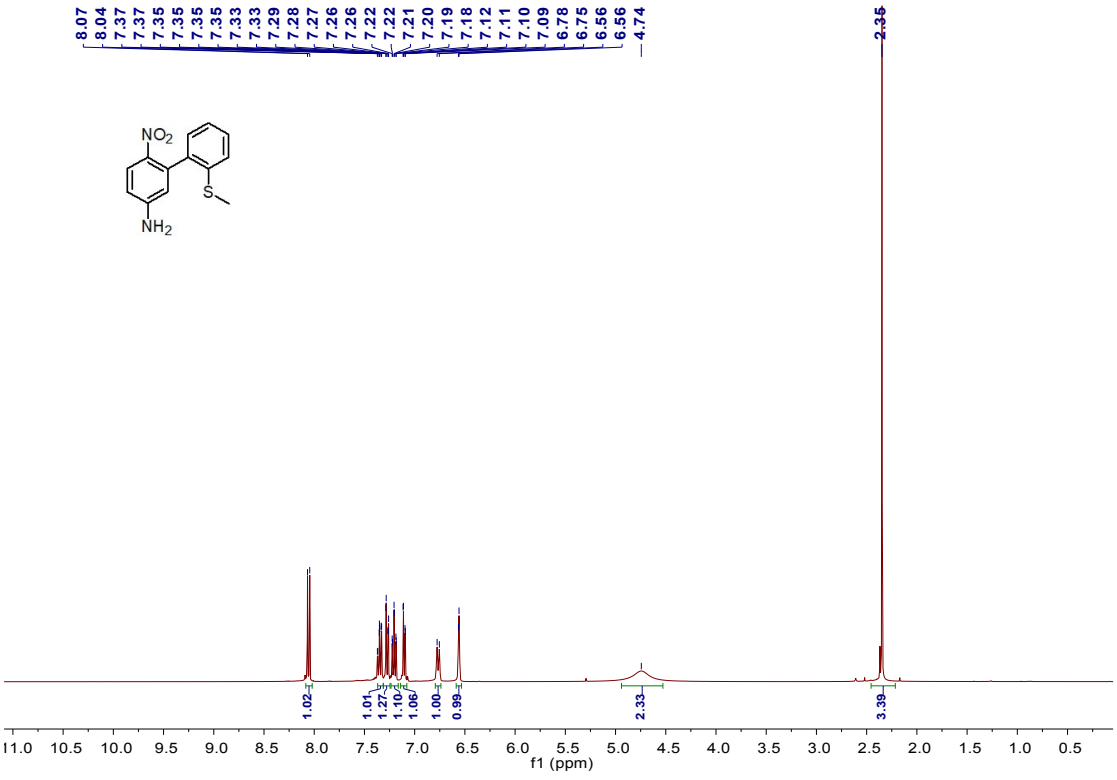


Fig. S7. ^{1}H NMR (400 MHz) spectrum of **1** in $CDCl_3$.

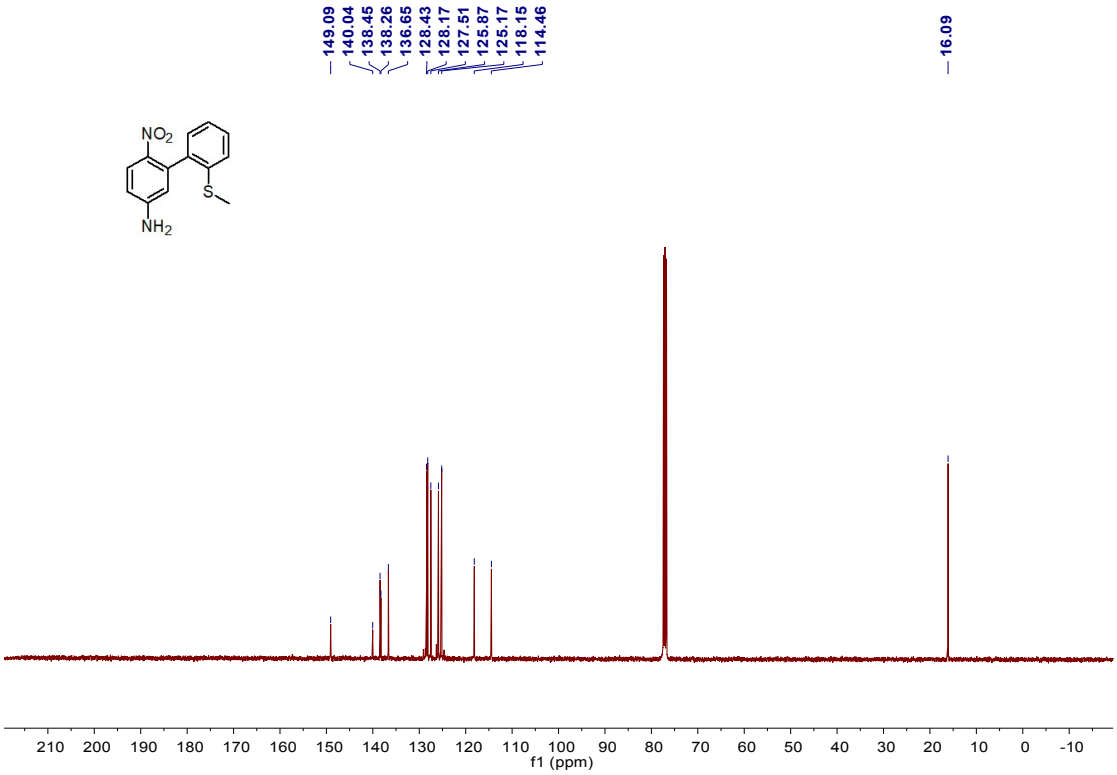


Fig. S8. ^{13}C NMR (100 MHz) spectrum of **1** in $CDCl_3$.

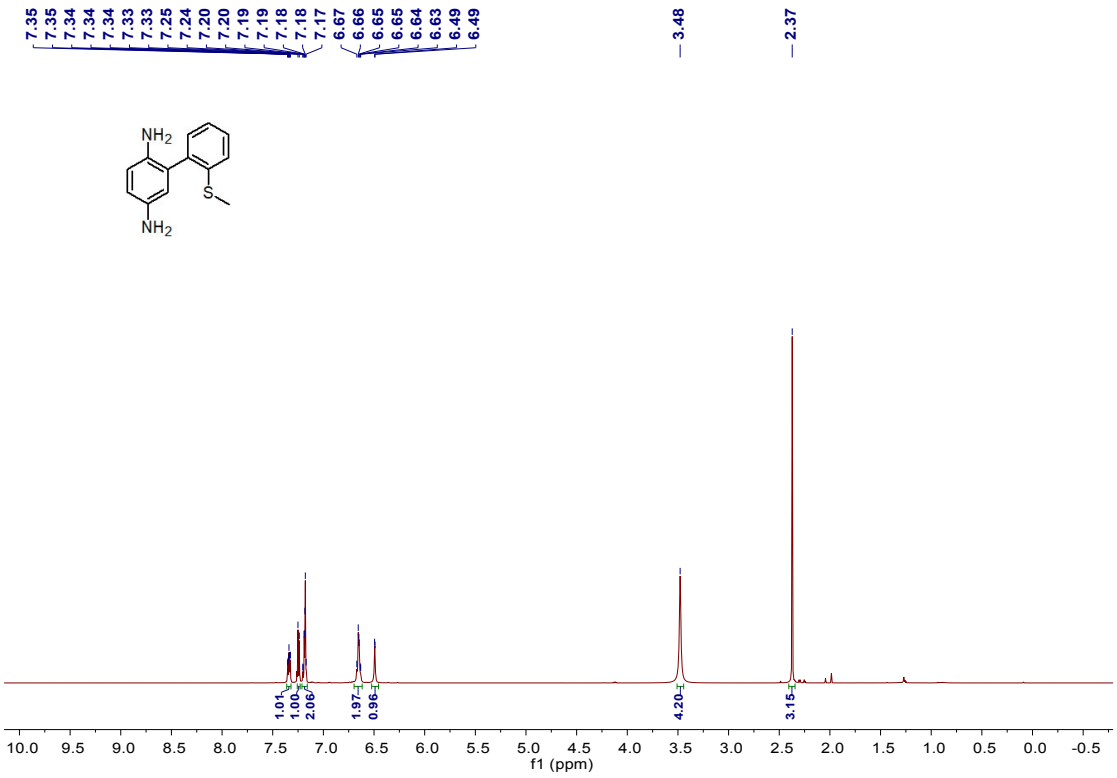


Fig. S9. ^{1}H NMR (600 MHz) spectrum of **2** in $CDCl_3$.

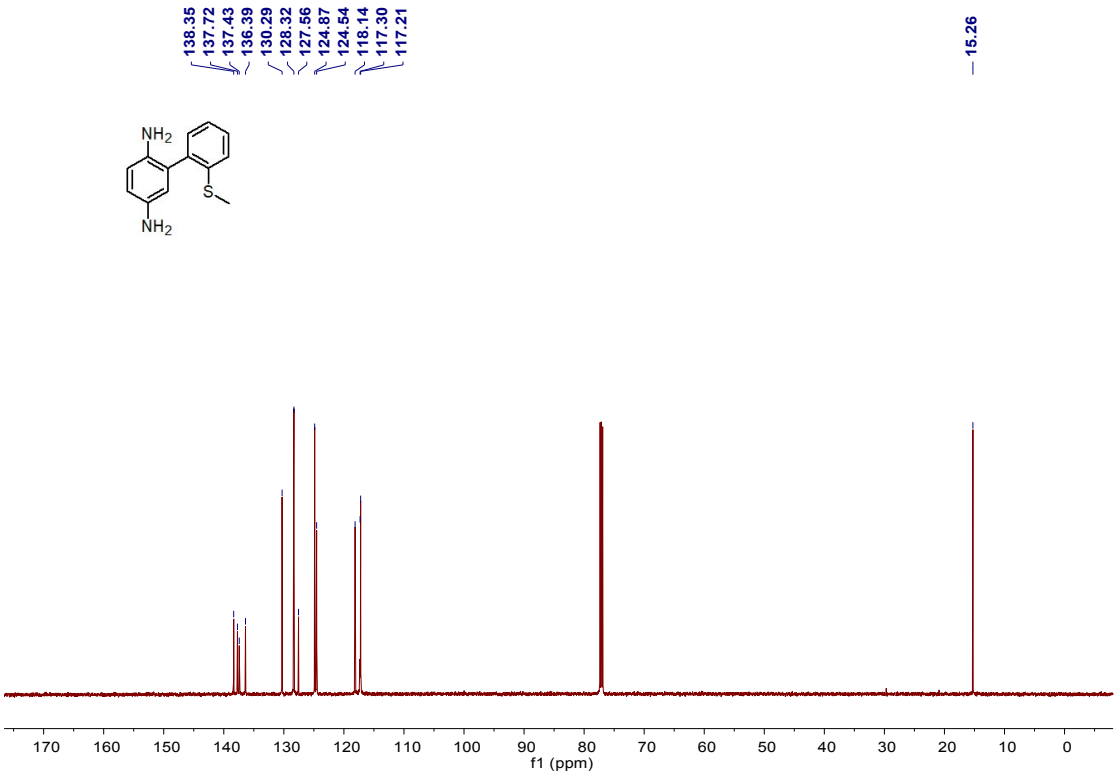


Fig. S10. ^{13}C NMR (150 MHz) spectrum of **2** in $CDCl_3$.

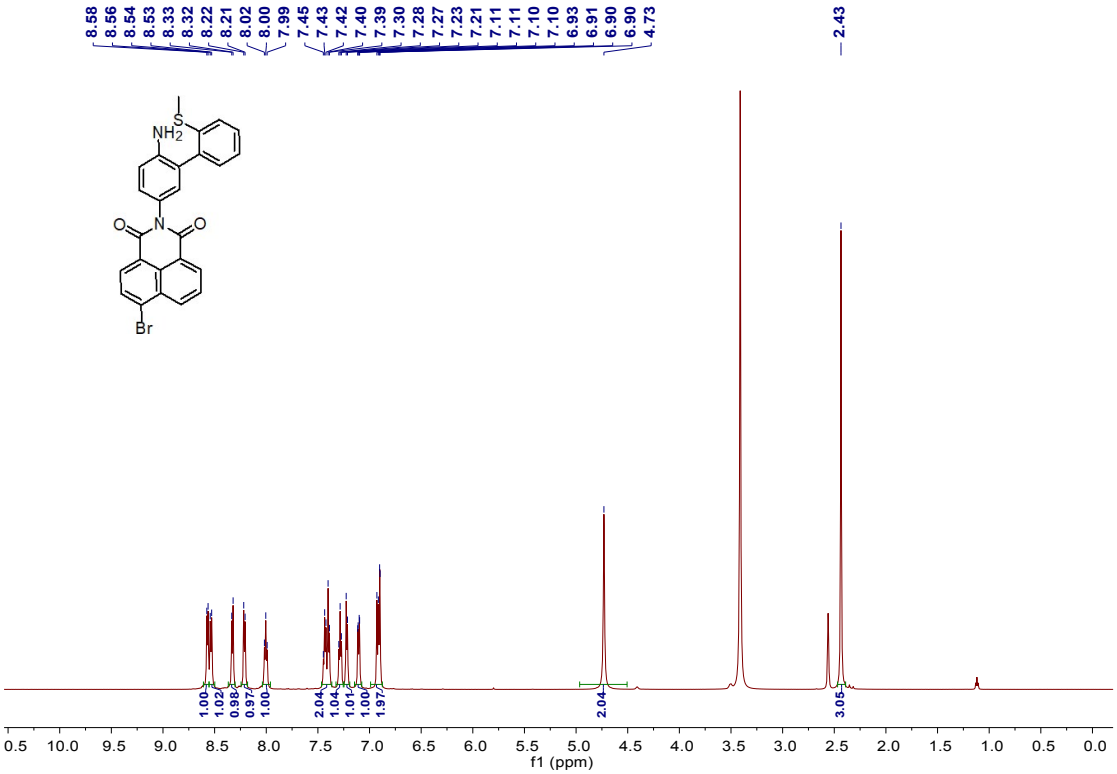


Fig. S11. [1]H NMR (600 MHz) spectrum of **3** in DMSO-d_6.

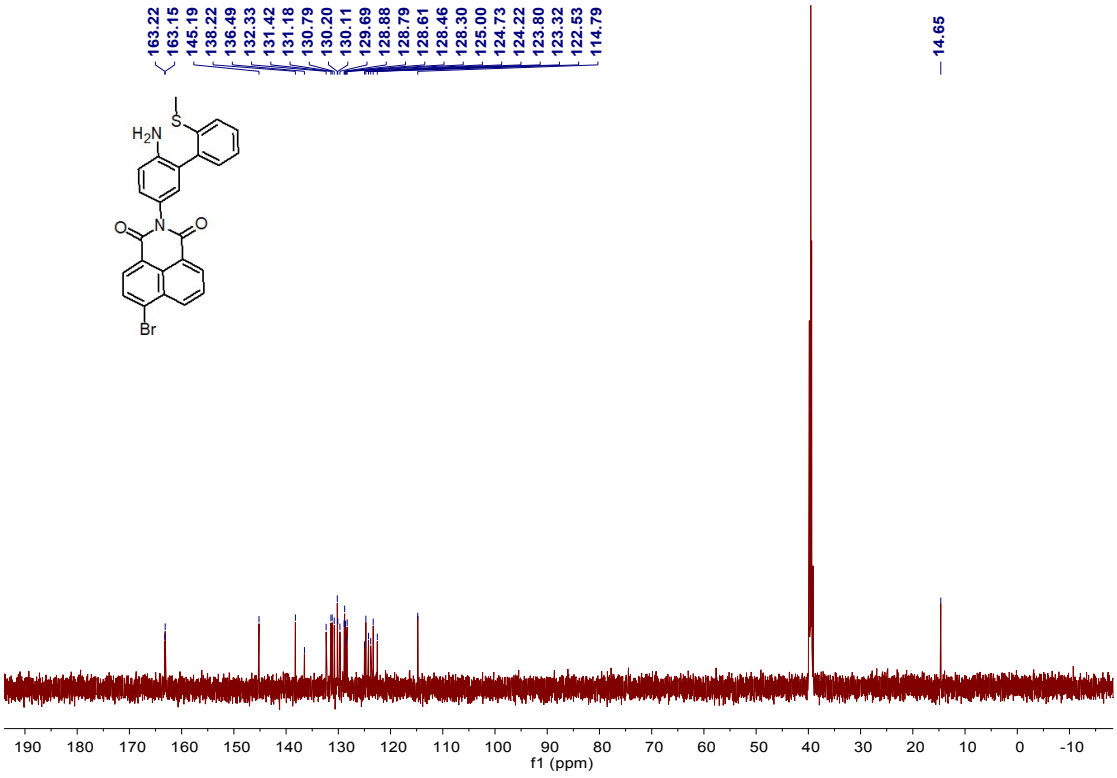


Fig. S12. [13]C NMR (150 MHz) spectrum of **3** in DMSO-d_6.

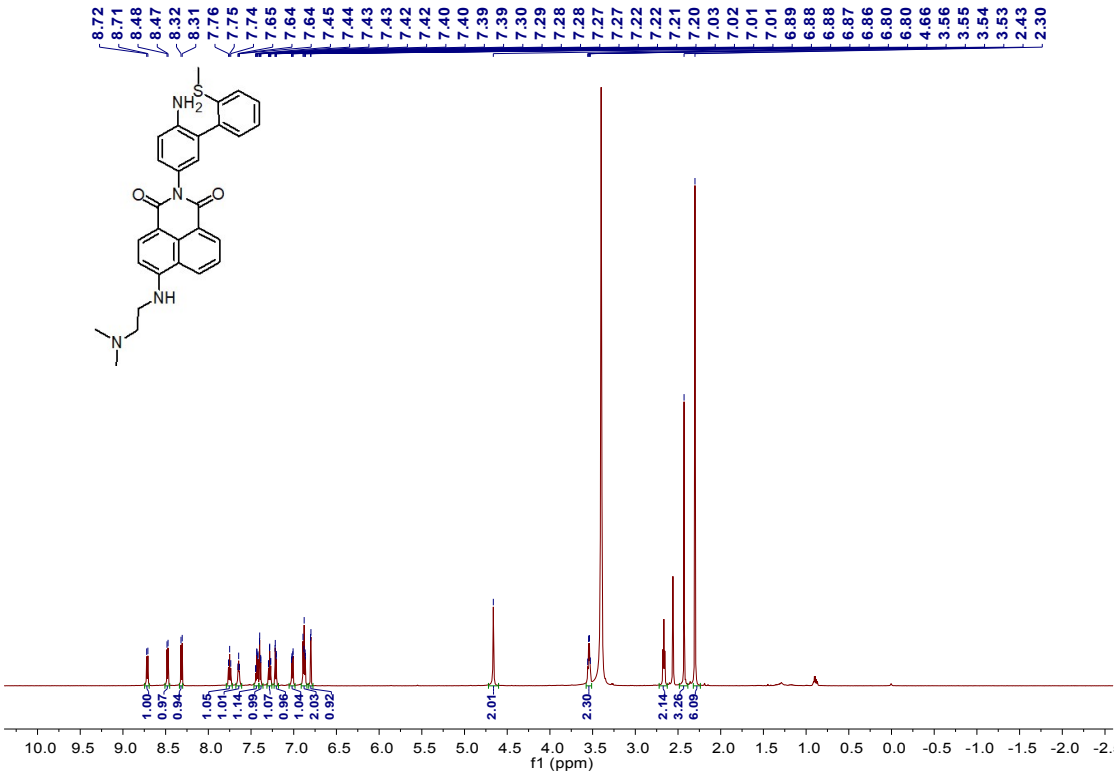


Fig. S13. ^{1}H NMR (600 MHz) spectrum of **LysOBr** in DMSO-d_6.

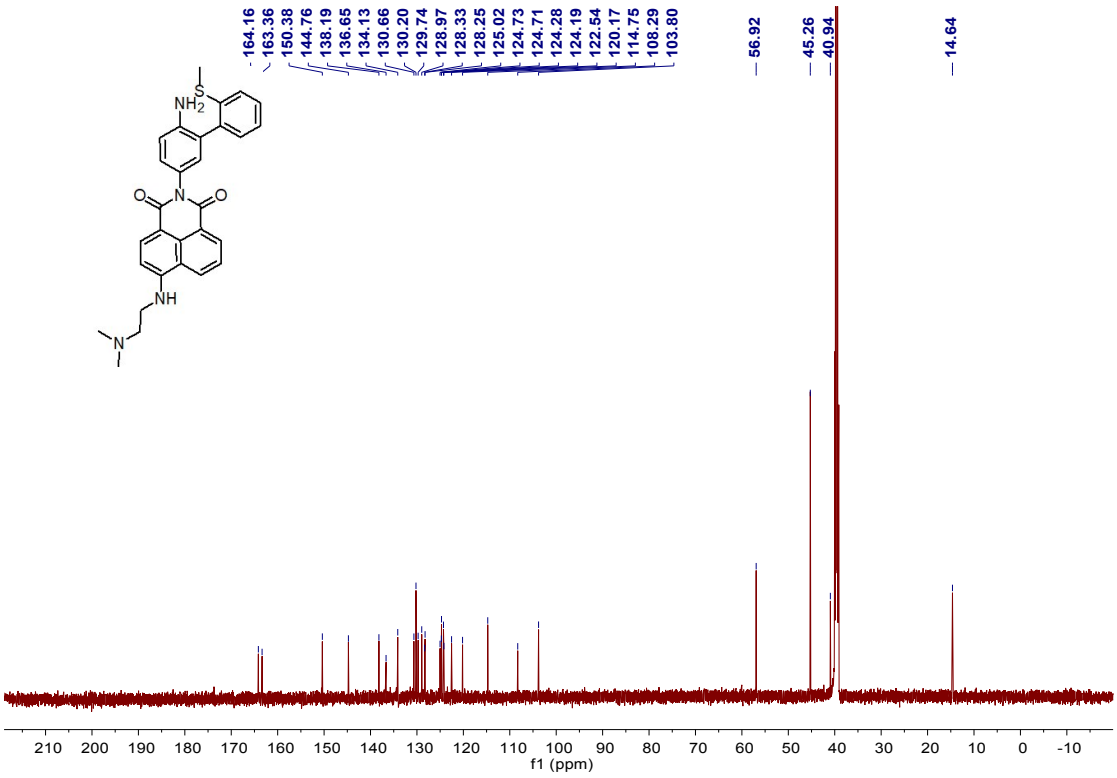


Fig. S14. ^{13}C NMR (150 MHz) spectrum of **LysOBr** in DMSO-d_6.

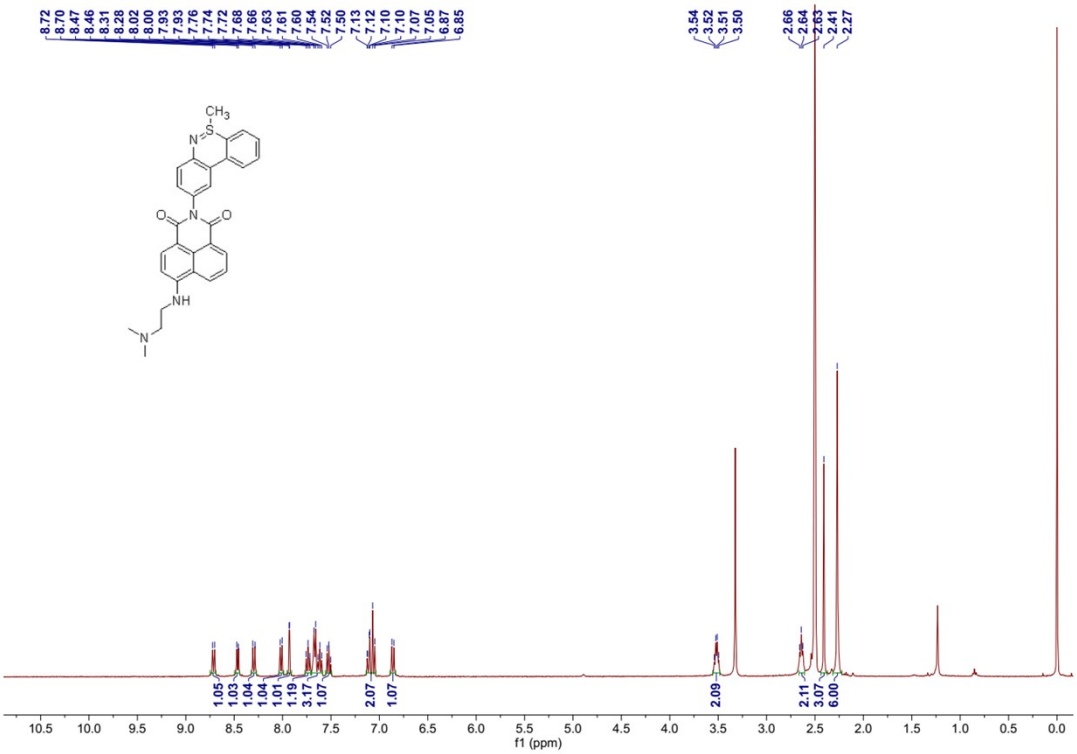


Fig. S15. ^{1}H NMR (400 MHz) spectrum of **LysOBr[O]** in DMSO-d_6.

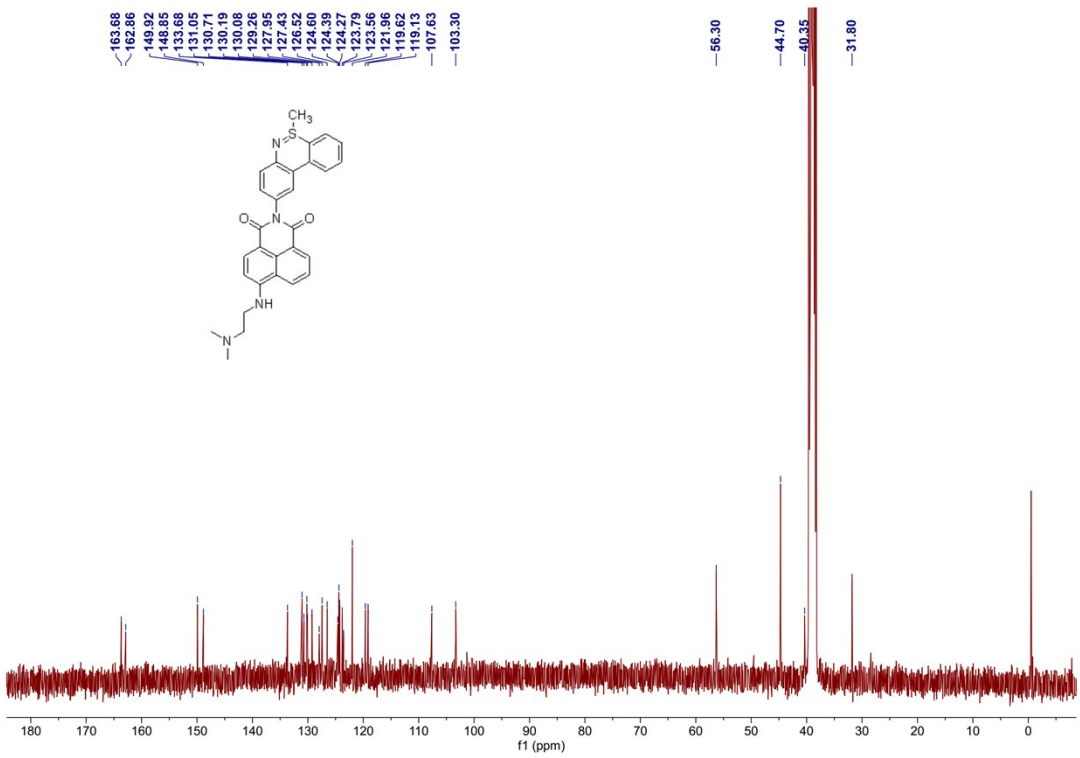


Fig. S16. ^{13}C NMR (100 MHz) spectrum of **LysOBr[O]** in DMSO-d_6.

14. ESI-MS spectra of LysOBr and LysOBr[O]

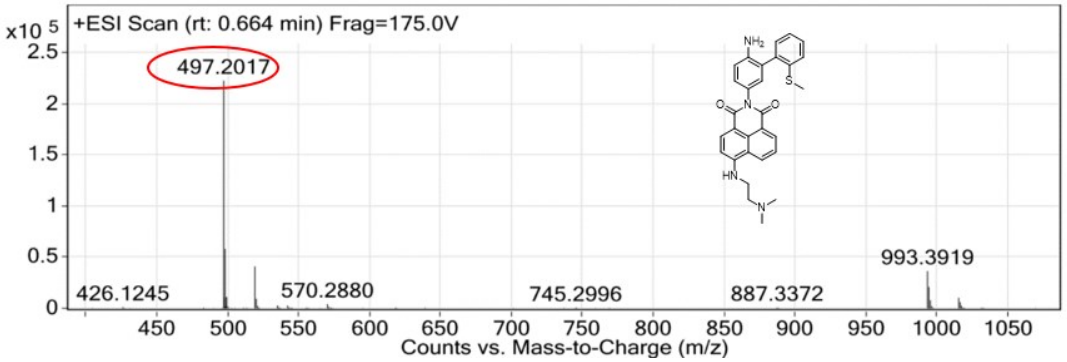


Fig. S17. ESI-MS spectrum of **LysOBr**.

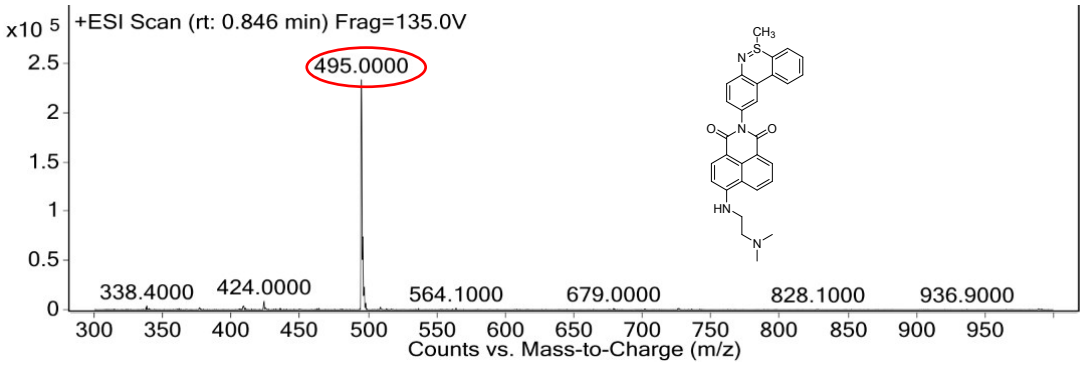


Fig. S18. ESI-MS spectrum of **LysOBr[O]**

[1] K. Xu, D. Luan, X. Wang, B. Hu, X. Liu, F. Kong, B. Tang, *Angew. Chem. Int. Ed. Engl*. 2016, **55** 12751-12754.
[2] S. Maity, S. Das, C.M. Sadlowski, J. Zhang, G.K. Vegesna, N. Murthy. *Chem. Commun*, 2017, **53**, 10184-10187.
[3] B. Zhu, C. Gao, Y. Zhao, C. Liu, Y. Li, Q. Wei, Z. Ma, B. Du, X. Zhang. *Chem. Commun*. 2011, **47**, 8656-8658.