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Supporting Information

An "Off-On" Fluorescent Probe for the Detection of Cysteine/Homocysteine and Its Imaging in Living Cells

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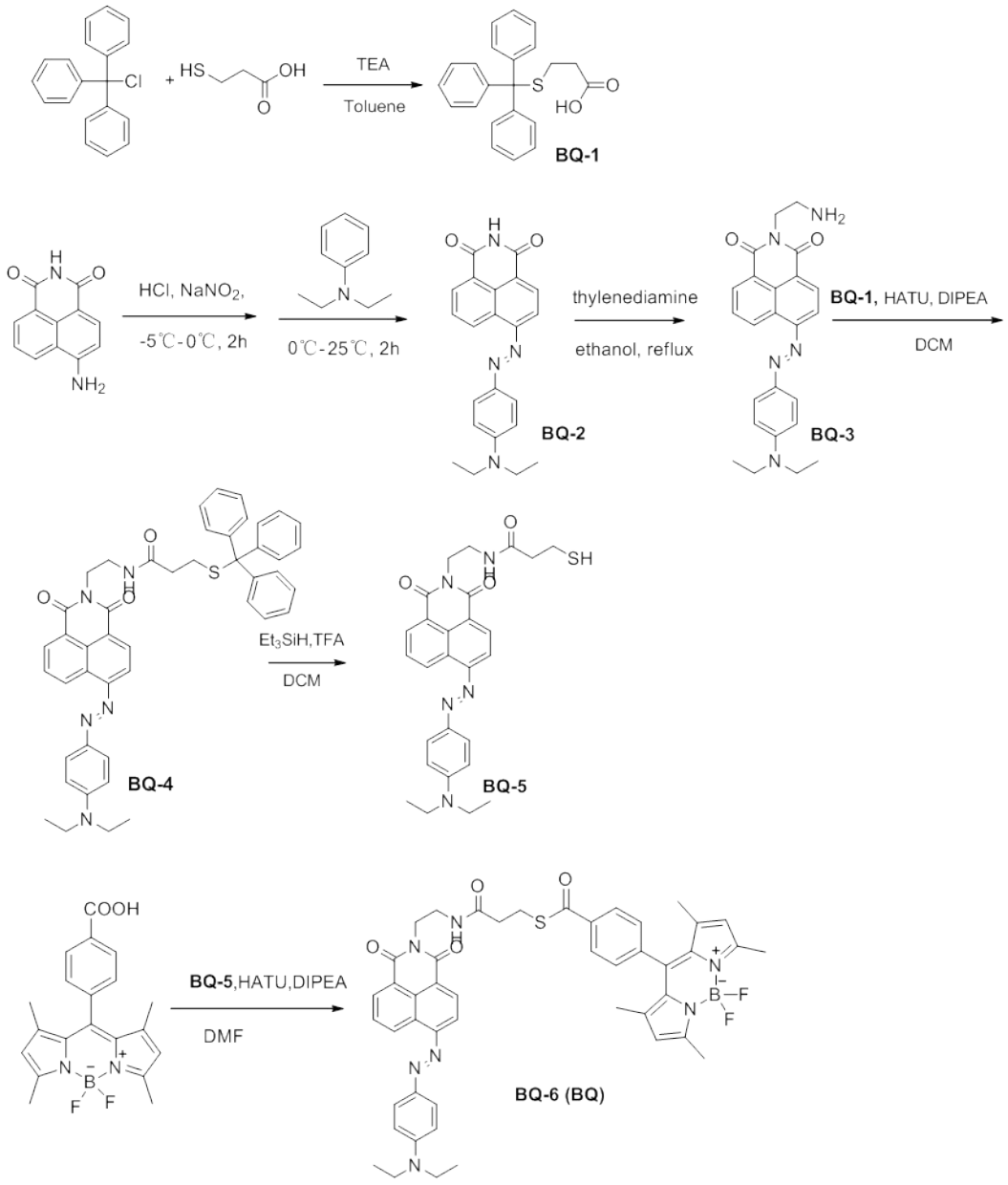
1. Materials and Instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Bruker AV-400 NMR spectrometer, using TMS as an internal standard. High resolution mass spectrometric (HRMS) analyses were measured on a HP 5989A. UV-visible spectra were measured on a Cary 100 UV-Vis spectrophotometer. Fluorescence spectra were measured on a Cary Eclipse (Varian, Inc.) fluorescence spectrophotometer. The pH measurements were measured on pH-Meter PB-20. TLC analyses were performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Hailang Company.

2. Synthesis



Scheme S1 Synthesis of probe **BQ**

Synthesis of compound BQ-1

Trityl chloride (460 mg, 1.65mmol) was dissolved in 30 mL dry toluene, and triethylamine (0.225 mL, 1.65 mmol) was added dropwise under N_2 protection at the ice bath. After 3 -mercapto propionic acid (130.7 μL, 1.5 mmol) was added, the reaction mixture was stirred for 20 min at the ice bath. After further stirring for 5 h at room temperature, the reaction solution was concentrated under reduced pressure, and the resulting residue was purified by column chromatography (PE: Ethyl acetate , 10:1, v/v) to yield product **BQ-1** as a pale yellow solid (330 mg, yield 67%). ^{1}H NMR (400 MHz，$CDCl_3$) δ 7.35 (d, *J* = 7.8 Hz, 6H), 7.21 (t, *J* = 7.2 Hz, 6H), 7.15 (t, *J* = 7.2 Hz, 3H), 4.34(t, *J* = 5.2 Hz, 2H), 3.59 (t, *J* =5.2 Hz, 2H); ^{13}C NMR (100 MHz，$CDCl_3$) δ 172.7, 144.3, 129.1, 128.0, 126.7, 66.1, 32.8, 26.6..

Synthesis of compound BQ-2

To a solution of concentrated HCl (2 mL) and water (2 mL) in a 50 mL round bottom flask was added 4-Amino-1,8-naphtalic anhydride (213 mg, 1 mmol) and the mixture was stirred at 0 °C for 30 min. Deionized water (0.5 mL) containing $NaNO_2$ (96 mg, 1.4 mmol) was added to the solution, and the solution was further stirred at 0 °C for 2 h. Deionized water (0.5 mL) containing N,N-Diethyl aniline (214 mg, 1.4 mmol) was slowly introduced to the solution, and the solution was further stirred at 0 °C for 1 h. The reaction solution was added saturated sodium acetate solution (20 mL) and stirred at 0 °C for 2 h. The mixture was then extracted by DCM, and organic phase was concentrated under reduced pressure, and the resulting residue was purified by column chromatography (DCM) to afford product **BQ-2** as a dark purple solid (76 mg, 20% yield). ^{1}H NMR (400 MHz，$CDCl_3$,) δ 9.30 (dd, *J* = 8.4, 0.8 Hz, 1H), 8.67-8.62 (m, 2H), 8.00-7.97 (m, 3H), 7.86 (t, *J* = 8.0 Hz, 1H), 6.78(d, *J*= 9.2, 2H), 3.54 (q, *J* = 7.2 Hz, 4H), 1.29 (t, *J* = 7.2 Hz, 6H).

Synthesis of compound BQ-3

Compound **BQ-2** (186.5 mg, 0.5 mmol) was dissolved in ethanol. Ethylenediamine (2 mL) was added to the solution and the mixture was stirred under reflux for 2 h. The reaction solution was then concentrated under reduced pressure, and the resulting residue was purified by column chromatography (DCM: methanol, 100:1, v/v) to afford product **BQ-3** as a dark purple solid (115 mg, 56% yield). ^{1}H NMR (400 MHz，$CDCl_3$) δ 9.26 (dd, *J* = 8.4, 0.8 Hz, 1H), 8.67-8.63 (m, 2H), 8.03 (d, *J* = 9.2 Hz, 2H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.84 (t, *J* = 7.4 Hz, 1H), 6.79 (d, *J* = 9.2 Hz, 2H), 4.42 (t, *J* = 7.2 Hz, 4H),3.53 (t, *J* = 7.2 Hz, 2H), 3.51 (q, *J* = 7.2 Hz, 4H), 1.29 (t, *J* = 7.2 Hz, 6H); HRMS (ES+) calcd for $C_{24}H_{25}N_5O_2$ ($[M+H]^+$) 416.2087, found 416.2088.

Synthesis of compound BQ-4

Compound **BQ-1** (34.81 mg, 0.1 mmol) was dissolved in dry DCM. DIPEA (17.5 μL, 0.1 mmol), HATU (38 mg, 0.1 mmol) and **BQ-3** (41.52 mg, 0.1 mmol) were added to the solution and the mixture was stirred at room temperature overnight under Ar protection. The reaction solution was then concentrated under reduced pressure, and the resulting residue was purified by column chromatography (DCM) to afford product **BQ-4** as a dark purple solid (32.9 mg, 51.3% yield). ^{1}H NMR (400 MHz，$CDCl_3$) δ 9.19 (d, *J* = 8.4 Hz, 1H), 8.52-8.56

(m, 2H), 8.01 (d, *J* = 9.2 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.75 (t, *J* = 8.2, 1H), 7.35 (d, *J* = 7.6 Hz, 6H), 7.21 (t, *J* = 7.0 Hz, 6H), 7.15 (t, *J* = 7.2 Hz, 3H), 6.77 (d, *J* = 9.2, 2H), 4.34 (t, *J* = 5.2 Hz, 2H), 3.59 (t, *J* = 5.2 Hz, 2H), 3.50 (q, *J* = 7.6 Hz, 4H), 2.44 (t, *J* = 7.6 Hz, 2H), 1.97 (t, *J* = 7.6 Hz, 2H), 1.27 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (100 MHz，$CDCl_3$,) δ 171.2, 165.0, 164.6, 152.2, 151.4, 144.7, 144.3, 132.2, 132.6, 131.1, 129.6, 129.2, 129.1, 127.9, 126.9, 126.7, 126.6, 121.9, 121.1, 112.2, 111.2, 66.7, 53.4, 45.0, 39.3, 35.6, 27.5, 12.7. HRMS (ES+) calcd for $C_{46}H_{43}N_5O_3S$ ($[M+Na]^+$) 768.2984 found 768.2983.

Synthesis of compound BQ-5

Compound **BQ-4** (50 mg, 0.067 mmol) was dissolved in 10 mL dry CH_2Cl_2, and triethyl silane (15.3 μL, 0.1 mmol) was added dropwise under N_2 protection with the ice bath. After trifluoroacetic acid (370 μL, 5 mmol) was added, the reaction mixture was stirred for 10 min with the ice bath. After further stirring for 2 h at room temperature, the reaction solution was concentrated under reduced pressure, and the resulting residue was purified by column chromatography (CH_2Cl_2: methanol, 50:1, v/v) to afford product **BQ-5** as a dark purple solid (62.3 mg, 67% yield). ^{1}H NMR (400 MHz，$CDCl_3$) δ 9.24 (d, *J* = 8.4 Hz, 1H), 8.62-8.59 (m, 2H), 8.02-7.83 (m, 4H), 6.78 (d, *J* = 8.8 Hz, 2H), 4.43 (t, *J* = 5.2 Hz, 2H), 3.71-3.52 (m, 6H), 2.72 (t, *J* = 5.6 Hz, 2H), 2.45 (t, *J* = 5.6 Hz, 2H), 1.28 (t, *J* = 7.2 Hz, 6H); HRMS (ES+) calcd for $C_{27}H_{29}N_5O_3S$ ($[M+H]^+$) 504.2061 found 504.2069.

Synthesis of compound BQ-6 (probe BQ)

BODIPY (36.8 mg, 0.1 mmol) was dissolved in dry DMF. DIPEA(17.6 μL, 0.1 mmol), HATU (38 mg, 0.1 mmol) and **BQ-5**（50.3 mg，0.1 mmol） were added to the solution and the mixture was stirred at room temperature overnight under Ar protection. The reaction solution was concentrated under reduced pressure, and the resulting residue was purified by column chromatography (CH_2Cl_2: methanol, 100:1, v/v) to yield product **BQ-6 (**probe **BQ)** as a dark purple solid (32 mg, 36.7% yield). ^{1}H NMR (400 MHz，$CDCl_3$) δ 9.18 (d, *J* = 8.4 Hz, 1H), 8.58 (d, *J* = 7.2 Hz, 1H), 8.56 (d, *J* = 8.0 Hz, 1H), 7.90 (d, *J* = 9.2 Hz, 2H), 7.88-7.85 (m, 3H), 7.75 (t, *J* = 8.0 Hz, 1H), 7.27 (d, *J* = 8.2 Hz, 2H), 6.69 (d, *J* = 9.2 Hz, 2H), 5.87 (s, 2H), 4.38 (q, *J* = 5.2 Hz, 2H), 3.65 (q, *J* = 5.2 Hz, 2H), 3.44 (q, *J* = 7.2 Hz, 4H), 3.24 (t, *J* = 6.8 Hz, 2H), 2.51 (t, *J* = 6.8 Hz, 2H),1.18-1.22(m, 18H); ^{13}C NMR (100 MHz，$CDCl_3$,) δ 191.3, 170.9, 165.2, 164.8, 156.0, 152.3, 151.5, 144.3, 142.9, 140.0, 137.2, 132.4, 131.7, 131.2, 130.9, 129.3, 129.2, 128.6, 127.9, 126.9, 122.1, 121.5, 121.1, 112.3, 111.3, 45.0, 39.8, 39.3, 36.1, 29.7, 24.8, 14.6, 12.7; HRMS (ES+) calcd for $C_{47}H_{46}BF_2N_7O_3S$ ($[M+Na]^+$) 876.3291 found 876.3295.

3. Method

3.1 Preparation of the test solution.

A stock solution of probe **BQ** (1.0×10^{-3} M) was prepared in DMSO. Milli-Q Water was used to prepare all aqueous solutions. The test solution is potassium phosphate buffer (10 mM, pH 7.4) containing 45% acetonitrile (PBS-CH_3CN buffer). The solutions of various testing species were prepared from Cys, Hcy, GSH, Glu, Met, Thr, Tys, Leu, Ser, His, respectively. The resulting solution was shaken well and incubated for 30 min at room temperature before recording the spectra.

3.2 Culture of Hela liver cells and fluorescence imaging

Cell culture

Hela cells were obtained from American Type Culture collection, and grown in DMEM (High glucose) supplemented with 10% FBS. Cells were incubated in a 5% CO_2 humidified incubator at 37 ^oC and typically passaged with sub-cultivation ratio of 1:4 every two days.

Live-cell imaging

Hela cells were grown in the exponential phase of growth on 35-mm glass-bottom culture dishes (Φ 20 mm) for 1-2 days to reach 70-90% confluency. These cells were used for fluorescence imaging experiments. The cells were washed with fresh DMEM (without FBS) for three times, and then incubated with probe BQ in 2 mL DMEM (the final concentration of probe is 2 μM, containing 2% DMSO as the co-solvent) under an atmosphere of 5% CO_2 and 95% air for 30 min at 37°C. Cells were washed twice with 1mL fresh DMEM at room temperature, and then followed by addition of 1mL DMEM and observed under confocal microscopy (Leica TCS SP5 II Confocal Laser Scanning Microscope, 63x1.4 oil), with excitation by 476 nm laser and 500-700 nm emission light was collected. For the Cys treated samples, the cells were washed with DMEM for three times, and then incubated with probe in 2 mL DMEM (the final concentration of probe is 2 μM, containing 2% DMSO as the co-solvent) under an atmosphere of 5% CO_2 and 95% air for 10 min at 37°C. Then the cells were loaded with different concentrations of Cys (50 μM, 200 μM and 1 mM), which was further incubated for another 20 min. After the cells were washed twice with 1 mL DMEM at room temperature, 1 mL DMEM was added and observed under confocal microscopy, with excitation by 476 nm laser and 500-700 nm emission light was collected. The same conditions was used for conducting live cell assays for Hcy and GSH, respectively.

4. Figures

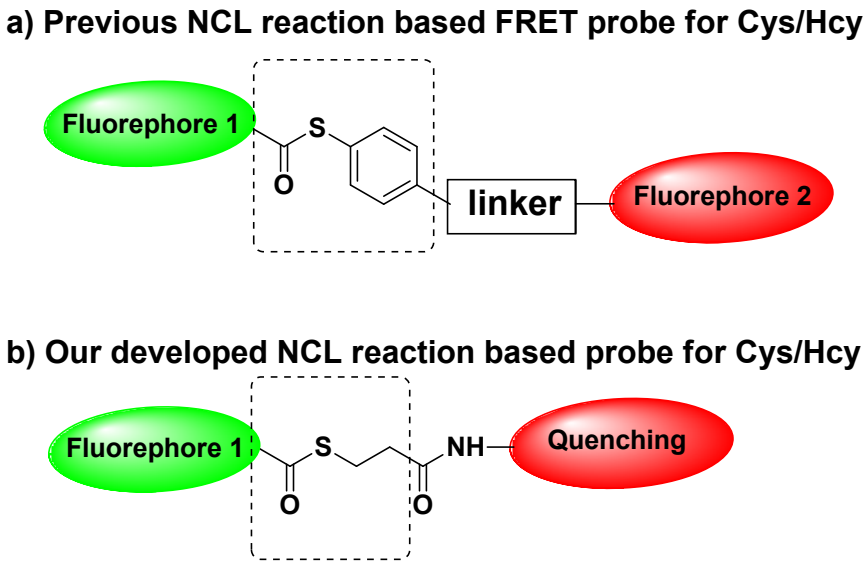


Figure S1. a) Previous NCL reaction based FRET probe for Cys/Hcy.[1-3] b) Our developed NCL reaction based probe for Cys/Hcy.

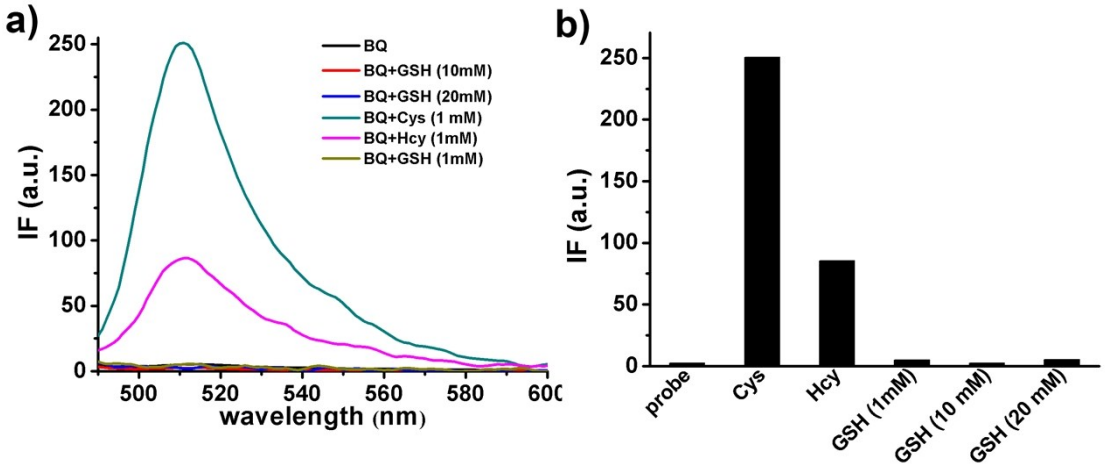


Figure S2. Fluorescence spectra of **BQ** (1 μM) after addition of Cys (1 mM), Hcy (1 mM), or GSH (1 mM, 10 mM and 20 mM, respectively) in PBS-CH_3CN (10 mM, pH 7.4, 45% acetonitrile) for 30 min at room temperature with λ_{ex} = 470 nm.

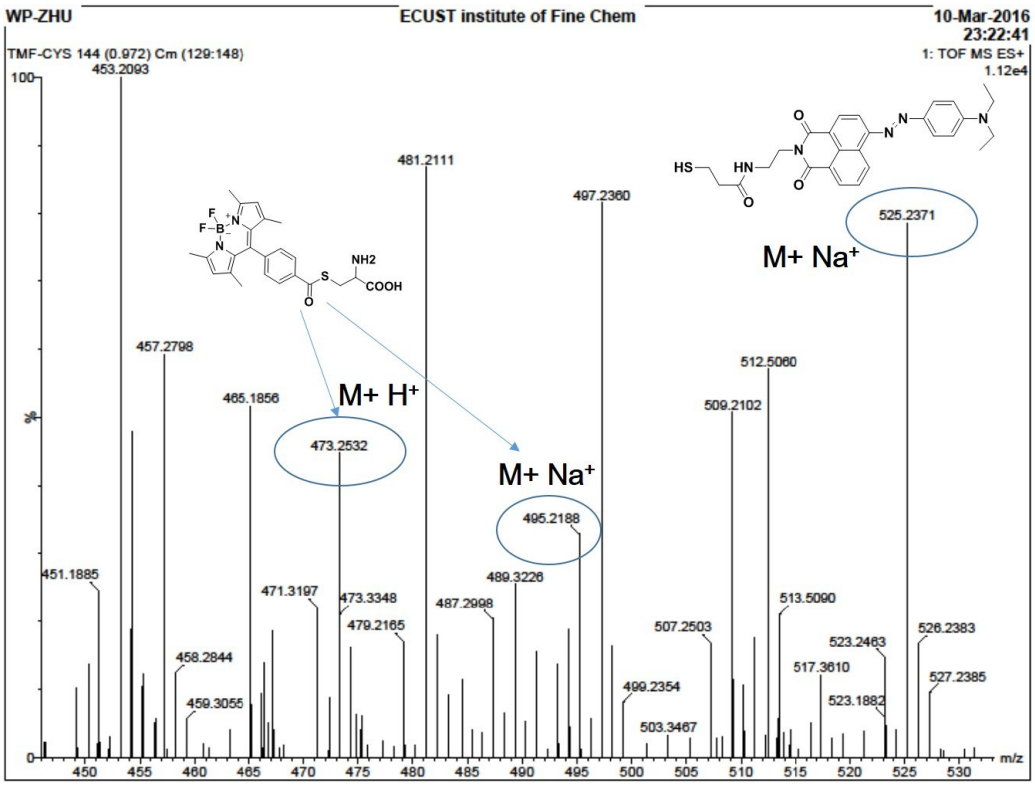


Figure S3. Mass spectrometry of BQ (1 μM) after addition of Cys (1 mM) and then incubation for 30 min at 37℃ in potassium phosphate buffer (10 mM, pH 7.4) containing 45% acetonitrile.

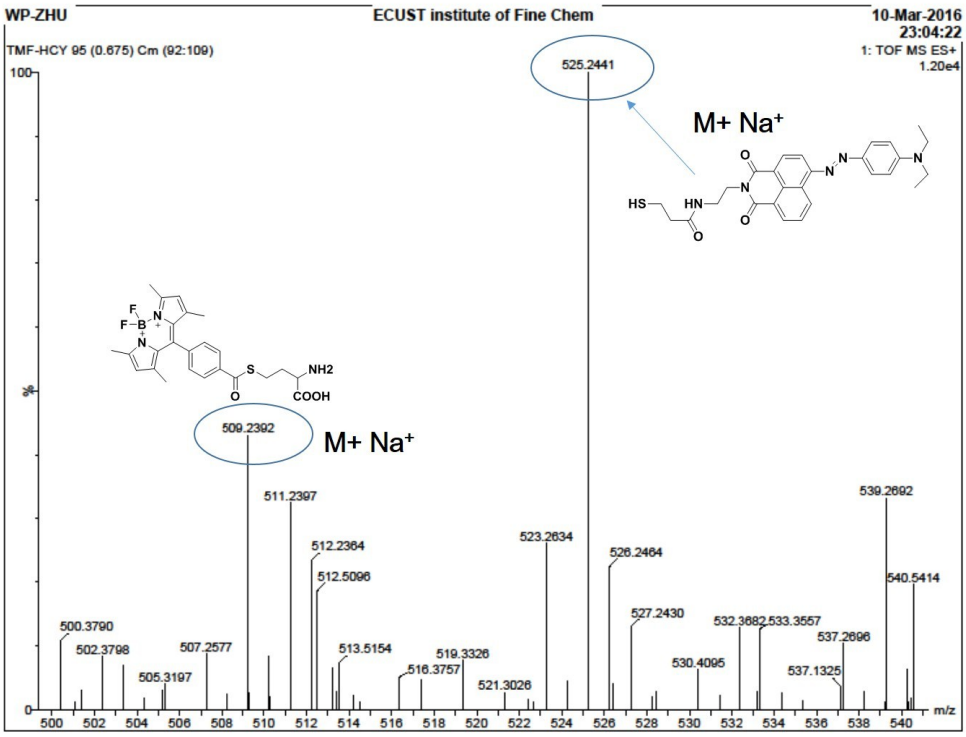


Figure S4. Mass spectrometry of **BQ** (1 μM) after addition of Hcy (1 mM) and then incubation for 30 min at 37℃ in potassium phosphate buffer (10 mM, pH 7.4) containing 45% acetonitrile.

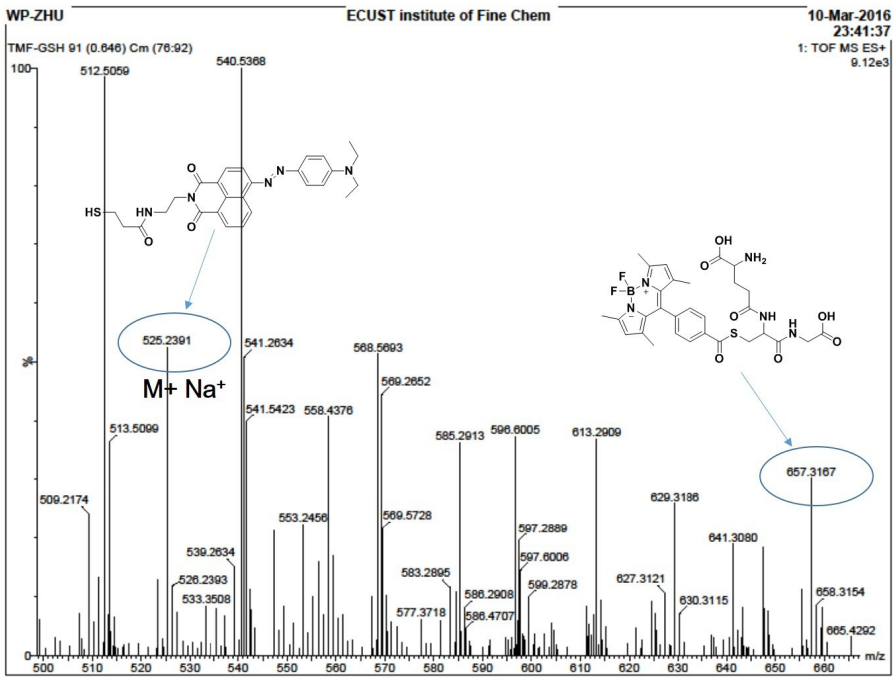


Figure S5. Mass spectrometry of **BQ** (1 μM) after addition of GSH (1 mM) and then incubation for 30 min at 37℃ in potassium phosphate buffer (10 mM, pH 7.4) containing 45% acetonitrile.

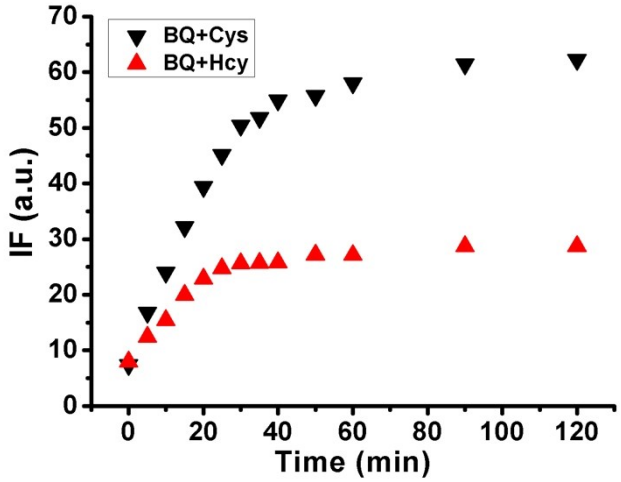


Figure S6. Time-dependent fluorescence spectra of **BQ** (1 μM) upon addition of Cys (200 μM) and Hcy (200 μM), respectively, in PBS-CH_3CN (10 mM, pH 7.4, 45% acetonitrile) for 30 min at room temperature with λ_{ex} = 470 nm.

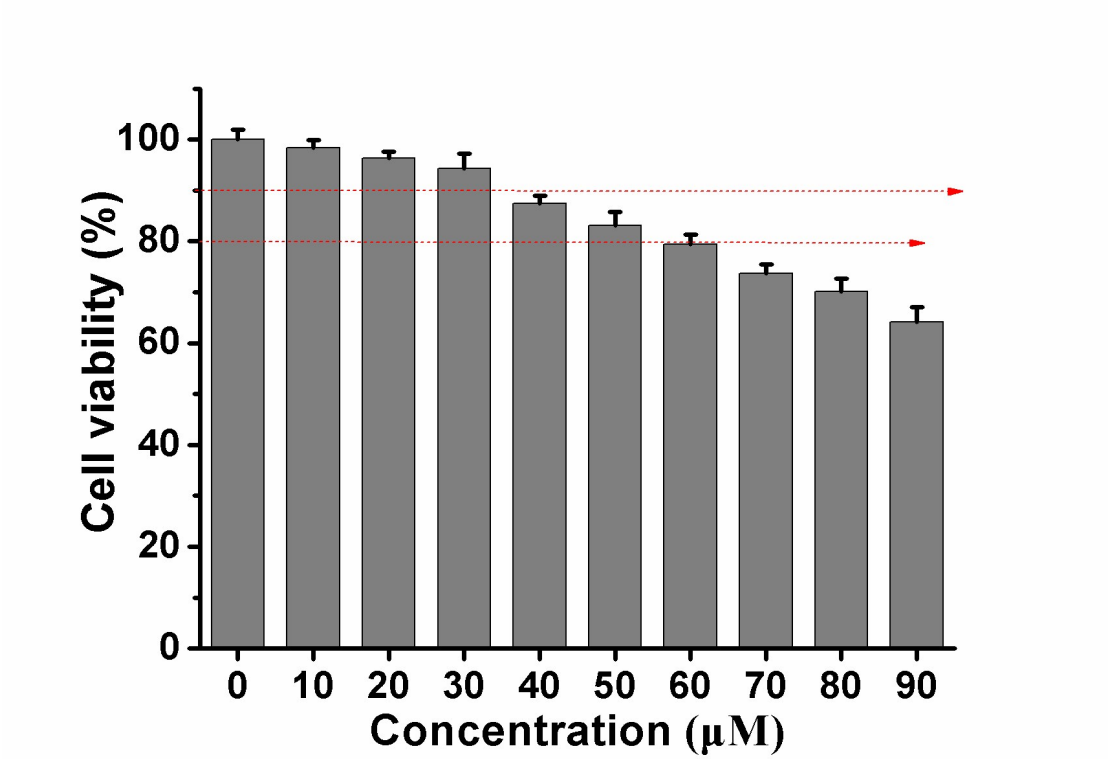


Figure S7. Cell toxicity of probe **BQ** (from 0 to 90 μM) when the incubation time was 12 h. Error bar represents s.d.. The cell viability was tested by CCK-8 assay.

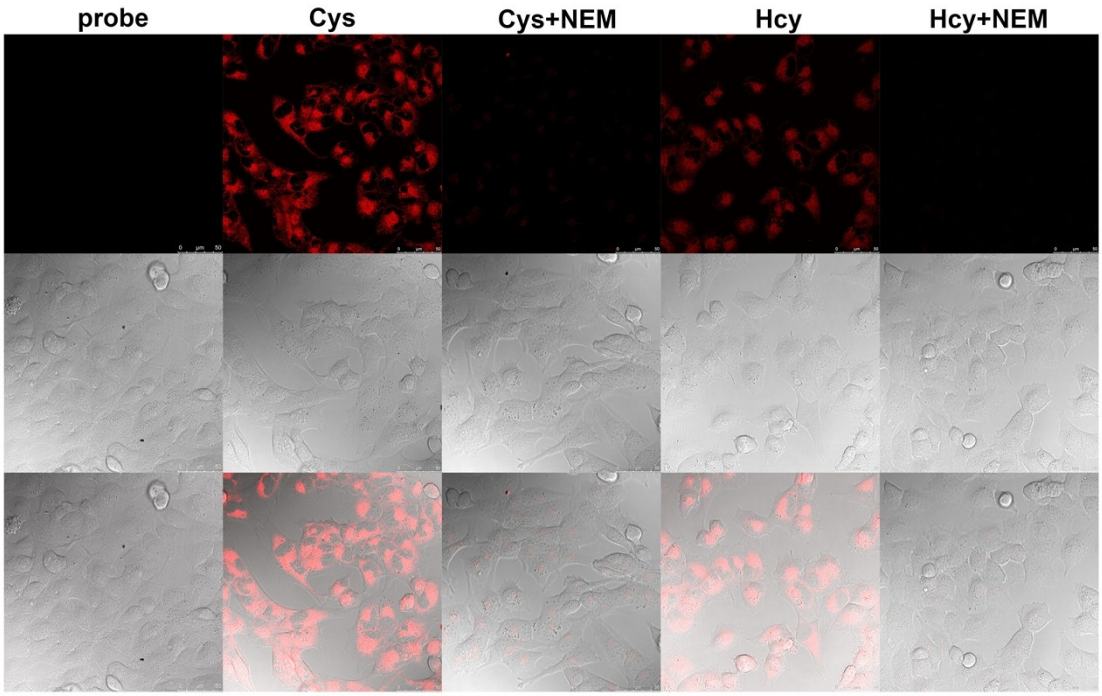


Figure S8. Confocal fluorescence and bright-field images of probe **BQ** in live HeLa cells. The Hela cells were first treated with NEM (1 mM) for 30 min. Then, fluorescence image of Hela cells after addition of **BQ** (2 μM) (Probe channel). (Cys/(Cys+NEM) and Hcy/(Hcy+NEM) channel) Fluorescence image of Hela cells/(NEM treated Hela cells) with **BQ** (2 μM) for first 10 min, then following by addition of Cys (1 mM) and Hcy (1 mM) for another 20 min, respectively.

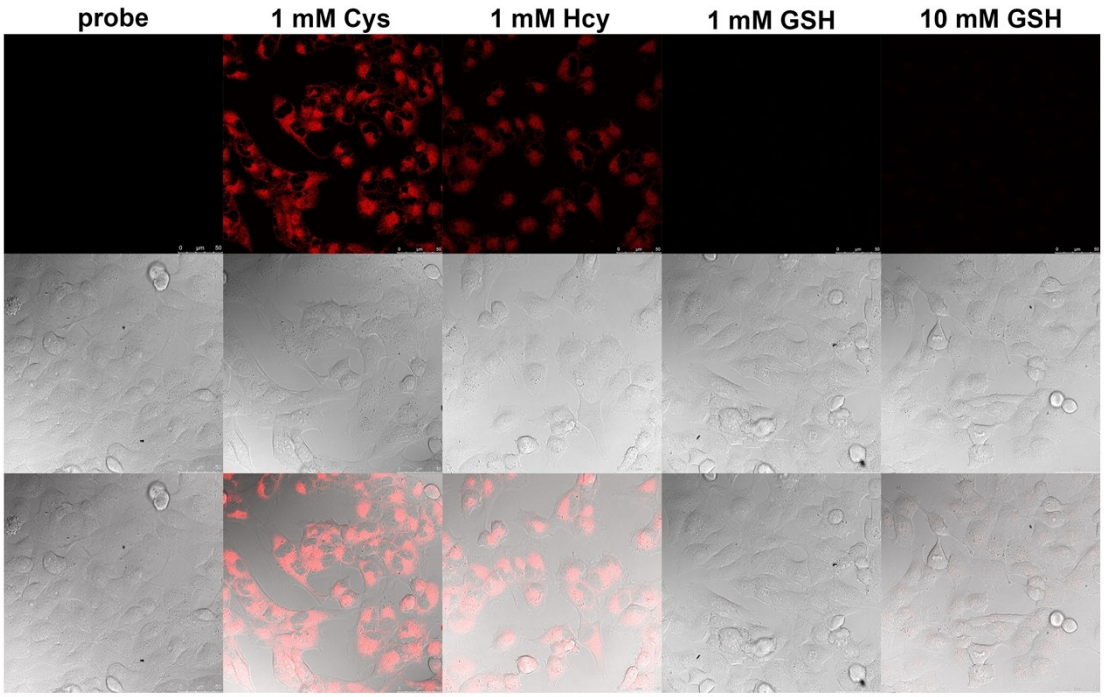
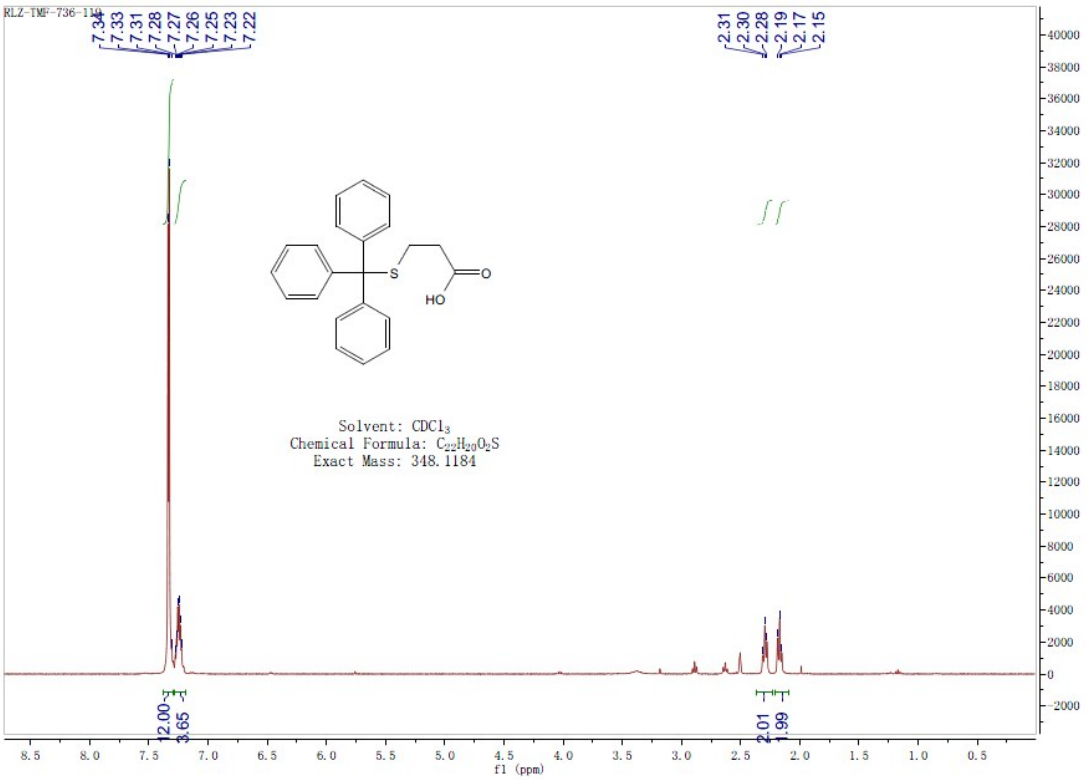


Figure S9. Confocal fluorescence and bright-field images of probe **BQ** in live HeLa cells. (Probe channel) Fluorescence image of Hela cells after addition of **BQ** (2 μM). (Cys, Hcy and GSH channel) Fluorescence image of Hela cells treated with **BQ** (2 μM) for first 10 min, then

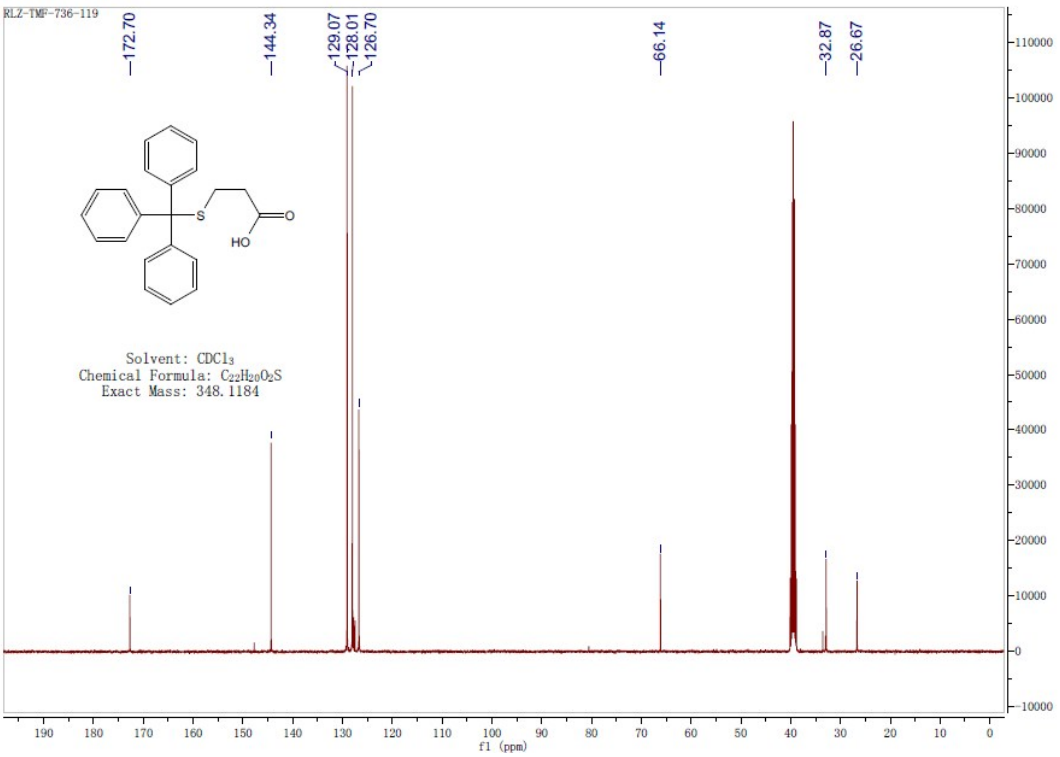
following by addition of Cys (1 mM), Hcy (1 mM) and GSH (1 mM or 10 mM) for another 20 min, respectively.

5. NMR and HR-MS spectra

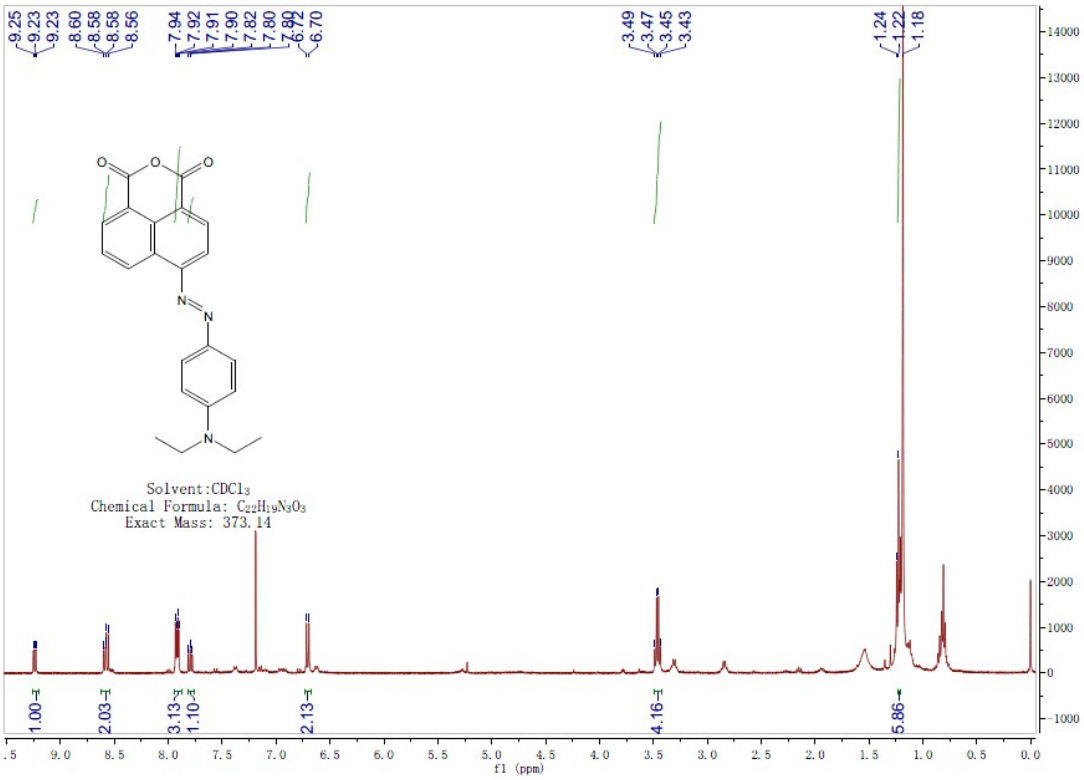
^{1}H NMR of compound BQ-1



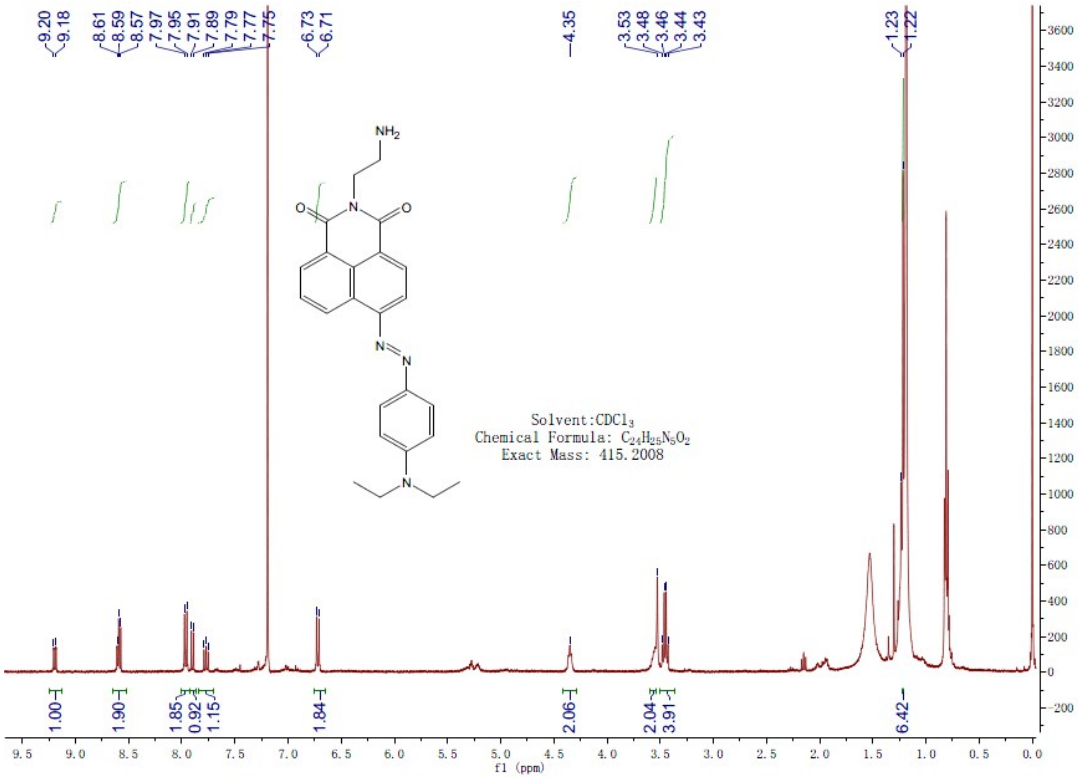
^{13}C NMR of compound BQ-1



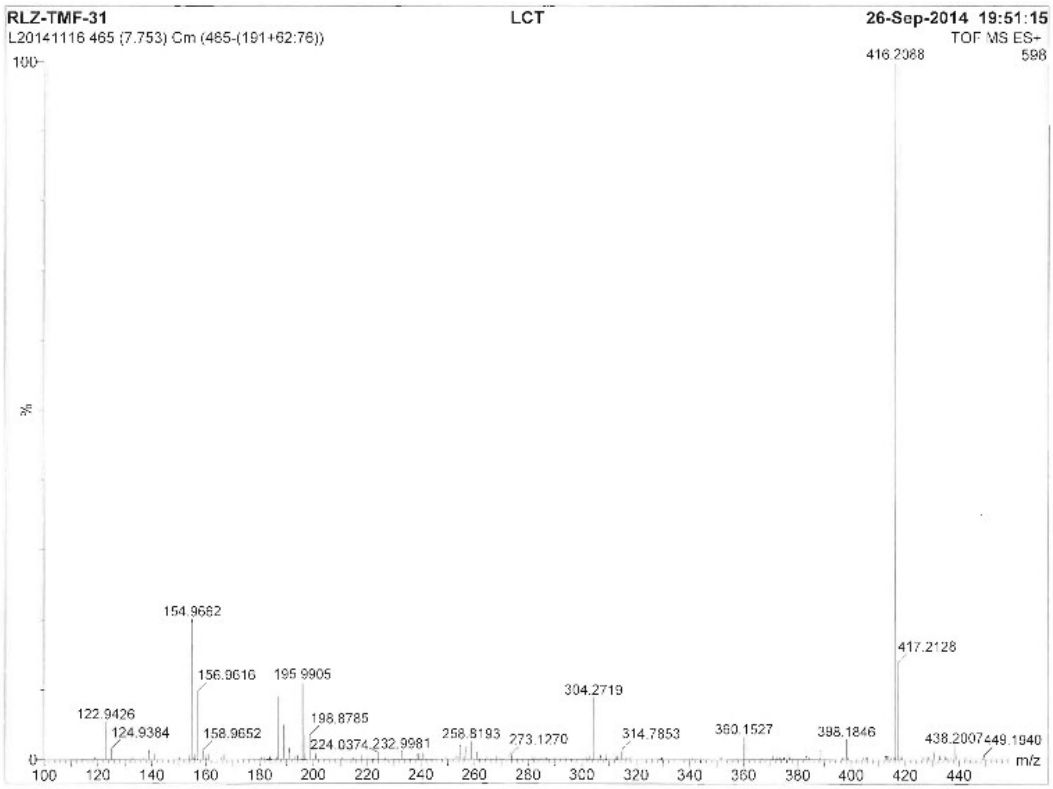
^{1}H NMR of compound BQ-2



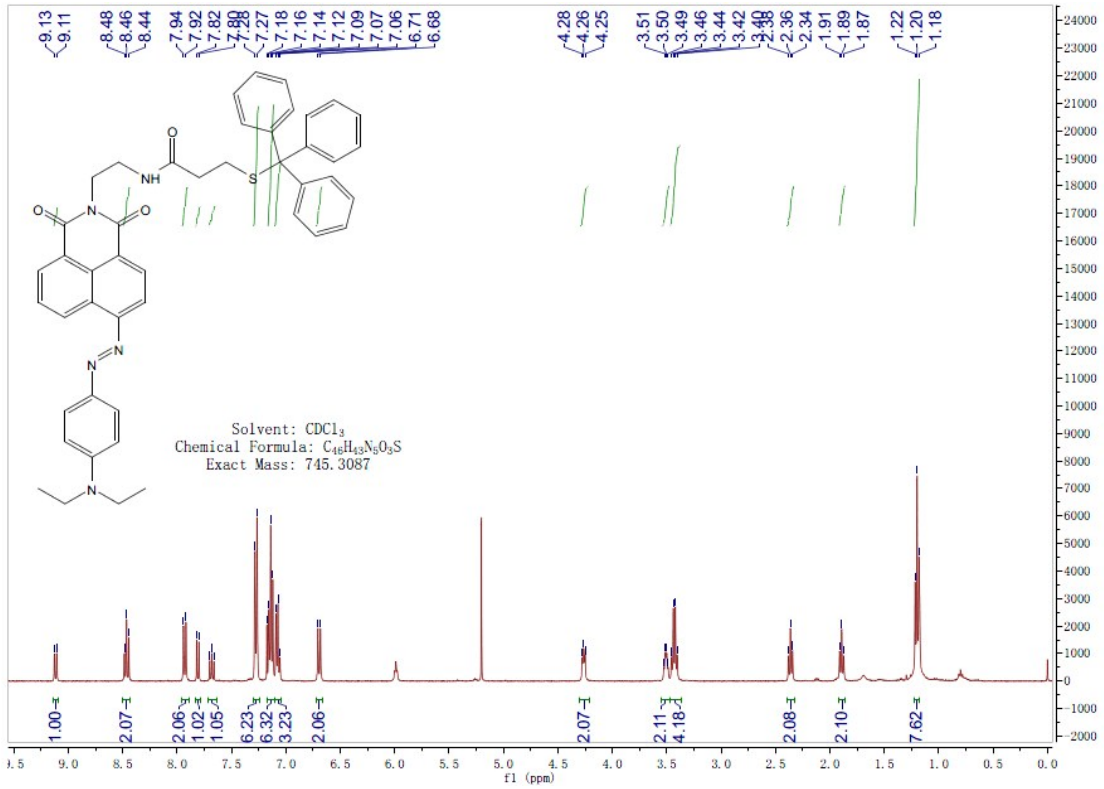
^{1}H NMR spectrum of compound BQ-3



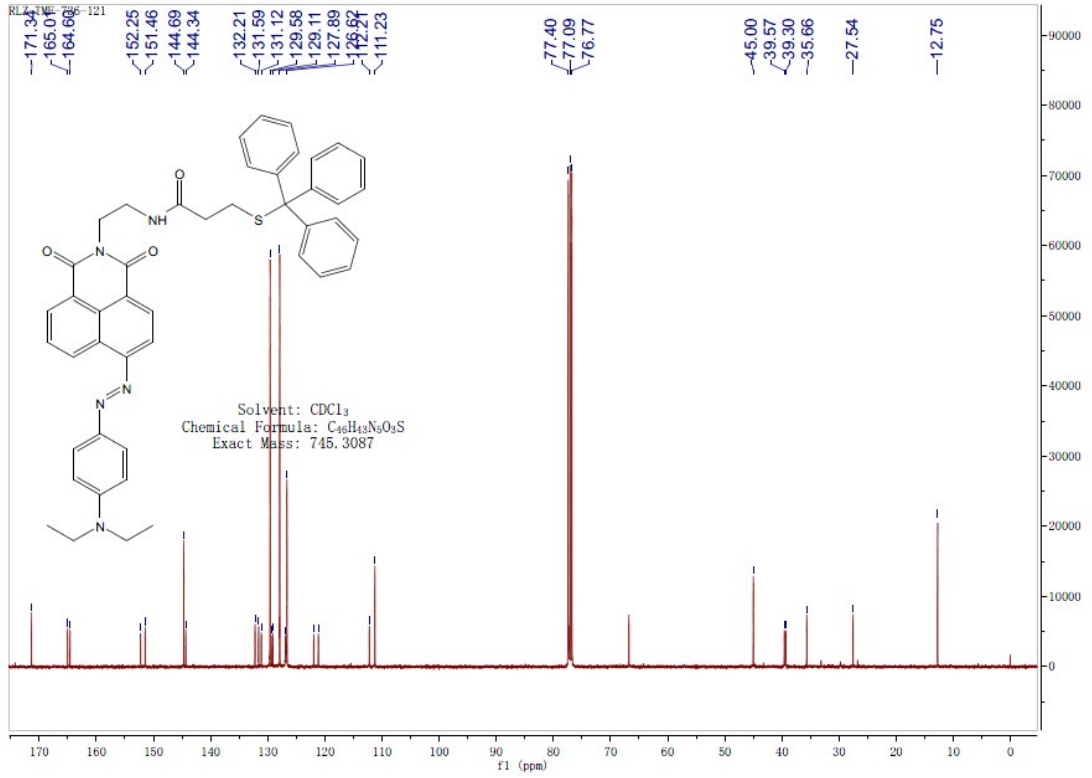
HR-MS spectrum of compound BQ-3



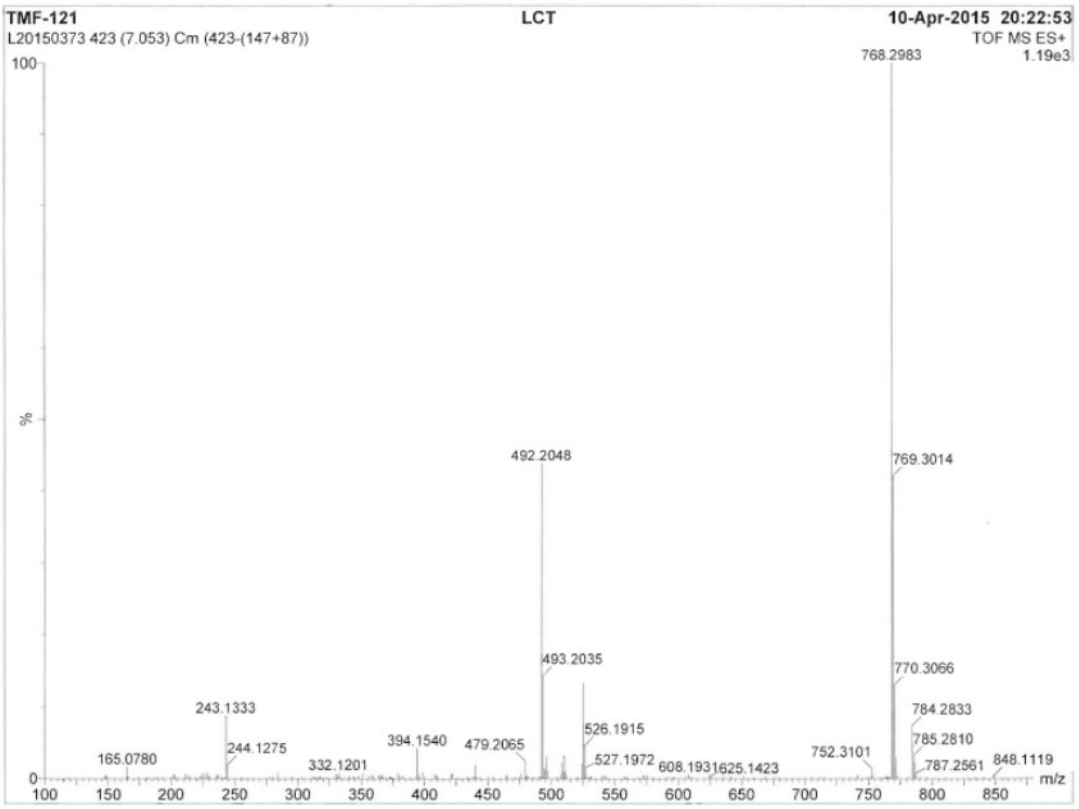
^{1}H NMR spectrum of compound BQ-4



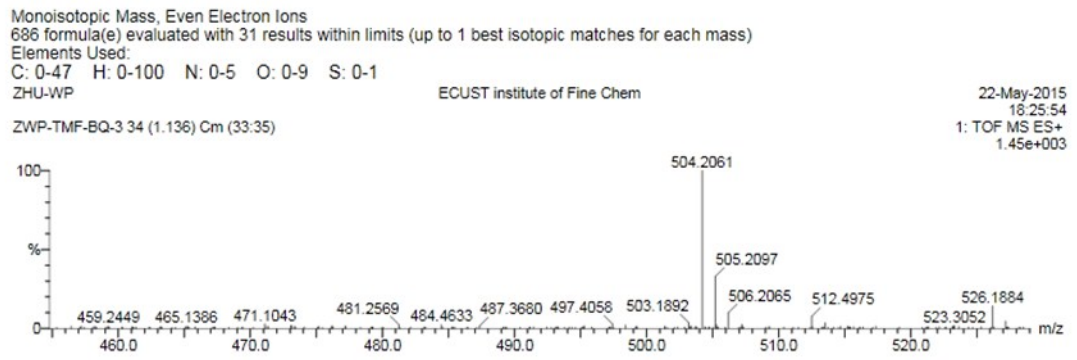
^{13}C NMR spectrum of compound BQ-4



HR-MS spectrum of compound BQ-4

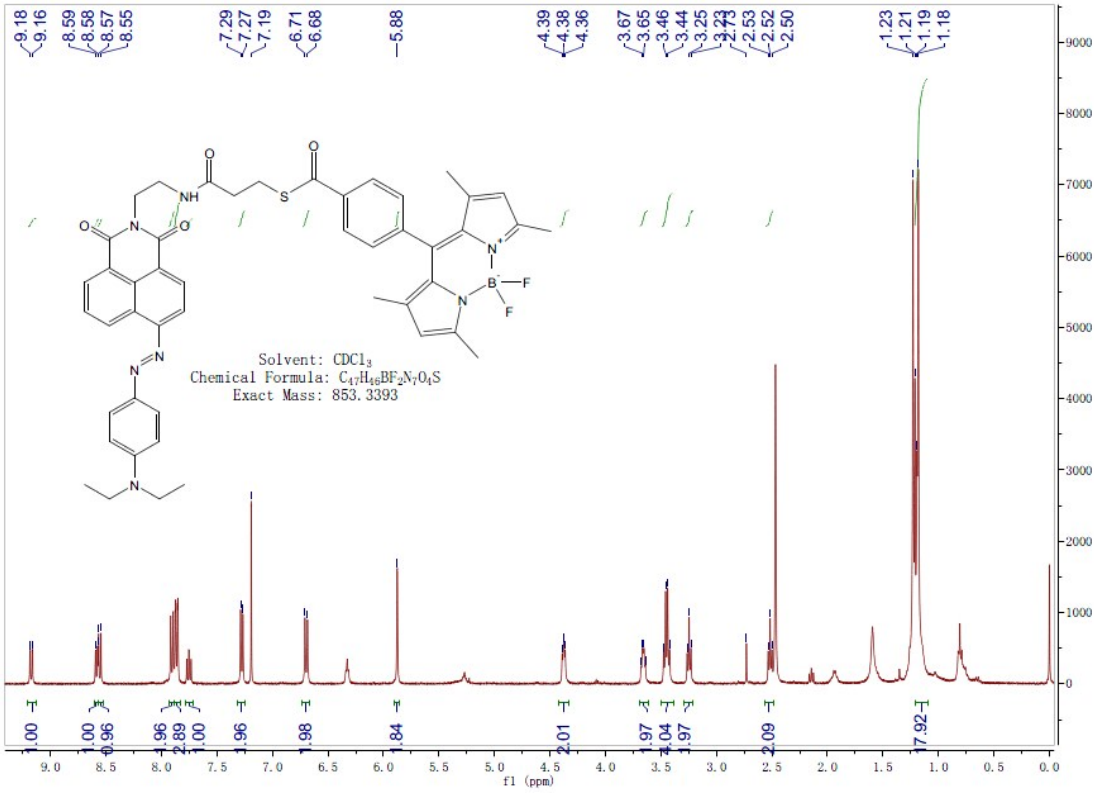


HR-MS spectrum of compound BQ-5

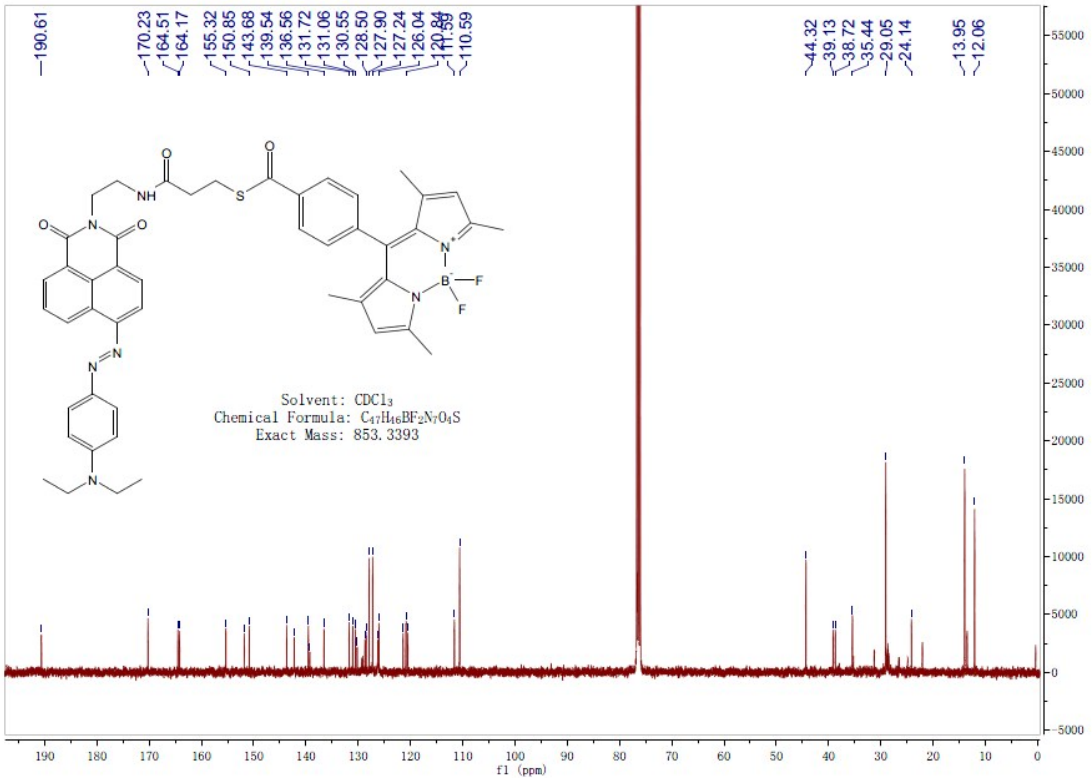


Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
504.2061	504.2069	-0.8	-1.6	15.5	48.7	0.0	C27 H30 N5 O3 S

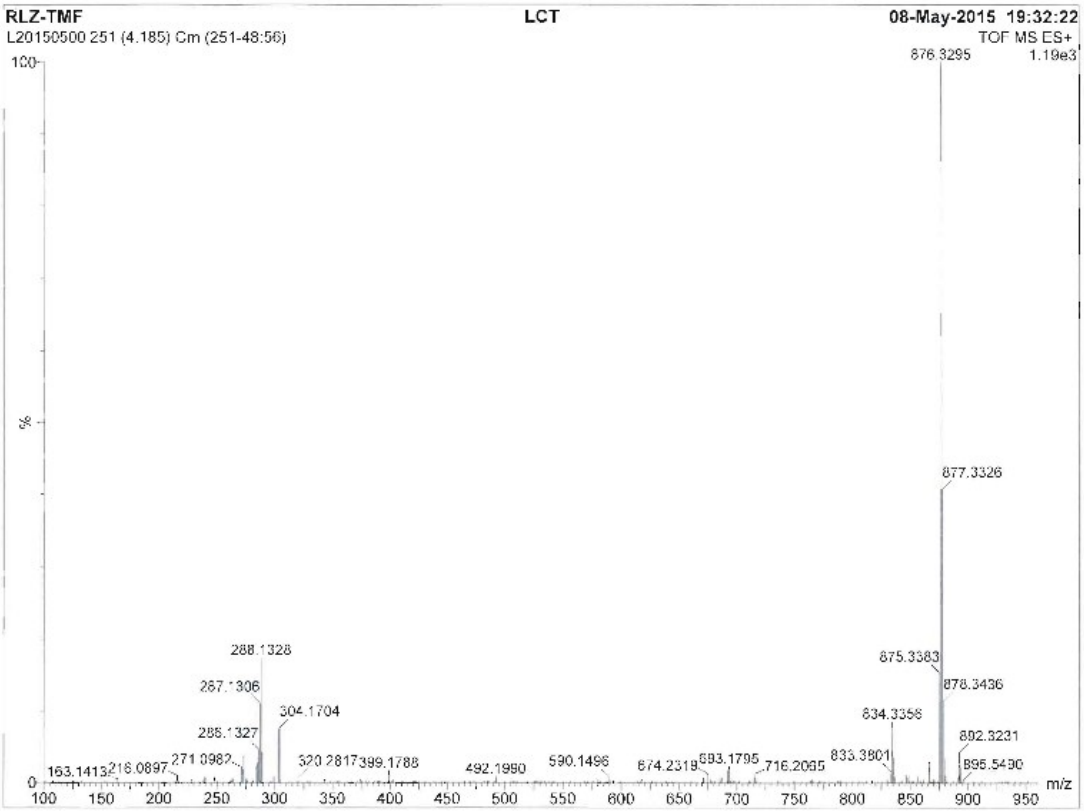
^{1}H NMR spectrum of compound BQ



^{13}C NMR spectrum of compound BQ



HR-MS spectrum of compound BQ



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3. X.-F. Yang, Q. Huang, Y. Zhong, Z. Li, H. Li, M. Lowry, J. O. Escobedo and R. M. Strongin, *Chemical Science*, 2014, **5**, 2177-2183.