Supplementary Information

A fluorogenic probe for tracking GSH flux in developing neurons

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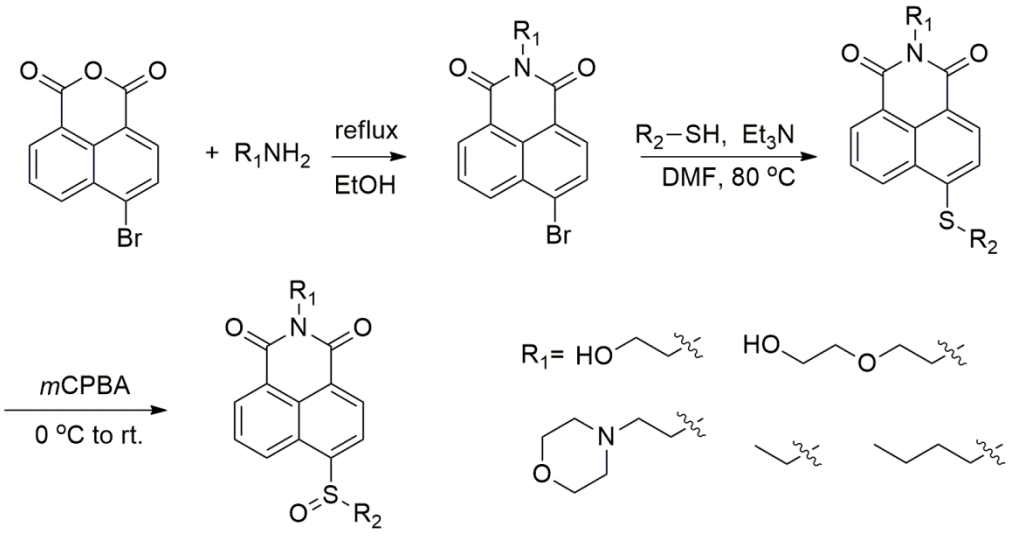
Probe synthesis and characterization

General experimental for probe synthesis

Dry dichloromethane (DCM) was distilled from CaH_2. Other reagents and chemicals for probe synthesis were obtained from commercial suppliers and used without further purification. Reactions were run under a nitrogen atmosphere and monitored by thin-layer chromatography (TLC) carried out on Silica gel 60 F254 plates supplied by Qingdao Puke Separation Material Corporation, and UV light was used as the visualizing agent. Flash column chromatography was performed using 200-300 mesh silica gel supplied by Qingdao Marine Chemical Factory, Qingdao, China. ^{1}H NMR spectra were obtained on a Bruker 500 UltraShieldTM Fourier transform spectrometer (500 MHz) at 25 ^{o}C. ^{13}C NMR spectra were recorded on a Bruker 500 UltraShield^{TM} Fourier transform spectrometer (125 MHz) spectrometer. All NMR spectra were calibrated using the residual solvent ($CDCl_3$) as internal reference (^{1}H NMR = 7.26, ^{13}C NMR = 77.16). All chemical shifts were reported in parts per million (ppm) and coupling constants (J) in Hz. The following abbreviations were used to explain the multiplicities: d = doublet, t = triplet, m = multiplet. High resolution mass spectra (HRMS) were measured on an Agilent 6224 TOF LC/MS spectrometer using ESI-TOF (electrospray ionization-time of flight). UV-Vis spectra were taken on a HITACHI U-3010 Spectrophotometer. Fluorescence measurements were conducted on an Agilent Cary Eclipse Fluorescence Spectrophotometer with slit widths to be 5 and 5 nm for excitement and emission respectively, and the photomultiplier (PMT) detector voltage was set at medium.

Scheme S1. General synthetic route of GP series of probes



Procedures for 4-bromo-1, 8-naphthalimide synthesis

To a stirred solution of 1,8-naphthalic anhydride (1.0 eq) in ethanol, was added the substituted amine (2.0 eq). The reaction was stirred under reflux and monitored by thin-layer chromatography analysis. After the disappearance of starting naphthalic anhydride which required about 3 hours, the solution was cooled to ambient temperature and poured into water to precipitate a solid, which was collected by filtration, washed with water, and dried. Purification of the crude product by flash column chromatography (SiO_2) yielded the product.

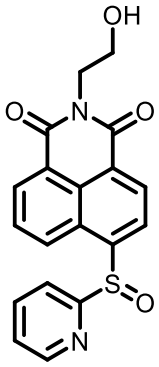
General procedures for sulfenyl naphthalimides synthesis

To a stirred solution of 4-bromo-1, 8-naphthalimide (1.0 eq) and the substituted thiophenol (5.0 eq) in 2-methoxyethanol was added triethylamine (5.0 eq) under nitrogen atmosphere. The reaction was stirred under reflux and monitored by thin-layer chromatography analysis. After the disappearance of the starting naphthalimide which required about 3 hours, the solution was cooled to ambient temperature and poured into water to precipitate a solid, which was collected by filtration, washed with water, and dried. Purification of the crude product by flash column chromatography (SiO_2) to give the product.

General procedures for sulfinyl naphthalimide synthesis

To a stirred solution of the sulfenyl naphthalimide (1.0 eq) in CH_2Cl_2 at 0℃ was added *m*CPBA (1.0 eq) in portions. The reaction was allowed to warm to ambient temperature by removing the ice bath. After completion as shown by thin-layer chromatography analysis which required about 1.0 hr, H_2O was added to quench the reaction and the mixture was diluted with CH_2Cl_2. The biphasic mixture was then transferred to a separatory funnel and the organic layer was washed sequentially with H_2O, saturated $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The remaining residue was purified by flash column chromatography (SiO_2) to give the product.

Probe structure characterization

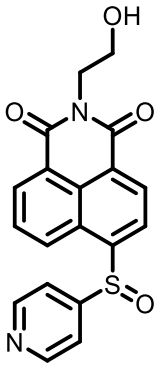


GP1

^{1}H NMR (500 MHz, DMSO-d6): δ 8.90 (d, J = 8.5 Hz, 1H), 8.61 (d, J = 7.6 Hz, 1H), 8.54 (d, J = 7.3 Hz, 1H), 8.47 (d, J = 4.8 Hz, 1H), 8.38 (d, J = 7.7 Hz, 1H), 8.09 (d, J = 8.5 Hz, 2H), 8.00 (t, J = 7.9 Hz, 1H), 7.45 (dd, J = 6.9, 4.9 Hz, 1H), 4.83 (t, J = 6.2 Hz, 1H), 4.12 (t, J = 6.4 Hz, 2H), 3.59 (d, J = 6.1 Hz, 2H).

^{13}C NMR (126 MHz, DMSO): δ 164.90, 163.60, 163.36, 150.35, 147.52, 139.85, 131.62, 130.49, 130.13, 128.70, 128.28, 128.11, 126.22, 125.47, 124.25, 123.47, 119.66, 58.12, 42.46.

ESI-MS (m/z): $[M+H]^+$ calculated:367.0753, found 367.0755.

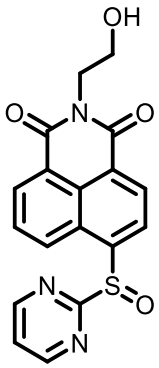


GP2

^{1}H NMR (400 MHz, DMSO-d6): δ 8.83 (d, J = 8.5 Hz, 1H), 8.68 (d, J = 5.0 Hz, 2H), 8.61 (d, J = 7.8 Hz, 1H), 8.52 (d, J = 7.4 Hz, 1H), 8.44 (d, J = 7.7 Hz, 1H), 7.96 (t, J = 8.0 Hz, 1H), 7.79 (d, J = 5.0 Hz, 2H), 4.78 (s, 1H), 4.11 (t, J = 6.7 Hz, 2H), 3.65 – 3.53 (m, 2H).

^{13}C NMR (101 MHz, DMSO-d6): δ 163.47, 163.25, 154.76, 151.23, 147.03, 131.68, 130.56, 129.25, 128.93, 128.35, 127.29, 125.79, 124.65, 123.75, 119.07, 58.14, 42.48.

ESI-MS (m/z): $[M+H]^+$ calculated:367.0753, found 367.0753.

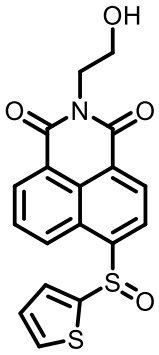


GP3

^{1}H NMR (500 MHz, DMSO-d6): δ 8.89 (d, J = 4.8 Hz, 2H), 8.83 (dd, J = 8.5, 0.9 Hz, 1H), 8.62 (d, J = 7.7 Hz, 1H), 8.52 (dd, J = 7.3, 0.9 Hz, 1H), 8.43 (d, J = 7.7 Hz, 1H), 7.97 (dd, J = 8.4, 7.4 Hz, 1H), 7.57 (t, J = 4.8 Hz, 1H), 4.12 (t, J = 6.4 Hz, 2H), 3.60 (t, J = 6.4 Hz, 2H).

^{13}C NMR (126 MHz, DMSO): δ 172.41, 163.55, 163.34, 160.11, 159.76, 146.35, 131.55, 130.40, 129.88, 128.81, 128.30, 127.98, 125.67, 125.55, 123.56, 123.39, 58.17, 42.49.

ESI-MS (m/z): $[M+H]^+$ calculated:368.0705, found 368.0704.

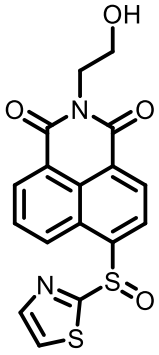


GP4

^{1}H NMR (500 MHz, DMSO-d6): δ 8.74 (d, J = 7.7 Hz, 1H), 8.54 (d, J = 7.7 Hz, 1H), 8.51 (dd, J = 7.3, 1.1 Hz, 1H), 8.46 (dd, J = 8.5, 1.0 Hz, 1H), 8.05 (dd, J = 3.7, 1.3 Hz, 1H), 7.92 – 7.87 (m, 2H), 7.15 (dd, J = 5.0, 3.7 Hz, 1H), 4.78 (t, J = 6.1 Hz, 1H), 4.15 (t, J = 6.5 Hz, 2H), 3.63 (q, J = 6.4 Hz, 2H).

^{13}C NMR (126 MHz, DMSO): δ 163.07, 162.92, 147.56, 145.75, 134.37, 133.60, 130.98, 130.13, 128.52, 128.03, 127.78, 127.73, 126.38, 125.02, 123.25, 122.45, 57.69, 42.03.

ESI-MS (m/z): $[M+H]^+$ calculated:372.0364, found 372.0369.

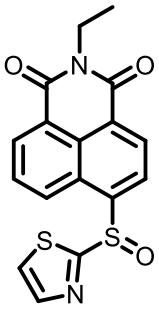


GP5

^{1}H NMR (500 MHz, DMSO-d6): δ 8.68 (dd, J = 8.1, 2.5 Hz, 2H), 8.55 (d, J = 7.2 Hz, 1H), 8.46 (d, J = 7.7 Hz, 1H), 8.08 (d, J = 3.0 Hz, 1H), 8.02 – 7.97 (m, 1H), 7.93 (d, J = 3.0 Hz, 1H), 4.14 (t, J = 6.4 Hz, 2H), 3.62 (t, J = 6.3 Hz, 2H).

^{13}C NMR (126 MHz, DMSO): δ 174.02, 163.02, 162.80, 145.73, 144.61, 131.19, 130.08, 128.72, 128.55, 127.82, 126.92, 126.39, 125.45, 123.13, 123.04, 57.67, 42.03.

ESI-MS (m/z): $[M+H]^+$ calculated: 373.0317, found 373.0318.

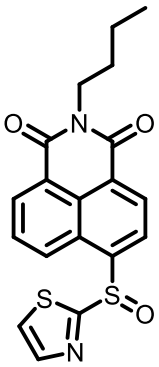


GP6

^{1}H NMR (500 MHz, DMSO-d6): δ 8.68 (d, J = 8.0 Hz, 2H), 8.55 (d, J = 7.2 Hz, 1H), 8.46 (d, J = 7.7 Hz, 1H), 8.09 (d, J = 3.1 Hz, 1H), 8.00 (t, J = 7.9 Hz, 1H), 7.93 (d, J = 3.1 Hz, 1H), 4.06 (q, J = 7.0 Hz, 2H), 1.19 (t, J = 7.1 Hz, 3H).

^{13}C NMR (126 MHz, DMSO): δ 174.48, 163.17, 162.94, 146.28, 145.13, 131.72, 130.62, 129.29, 129.08, 128.25, 127.42, 126.95, 125.85, 123.53, 35.45, 13.45.

ESI-MS (m/z): $[M+H]^+$ calculated:357.0368, found 357.0373.

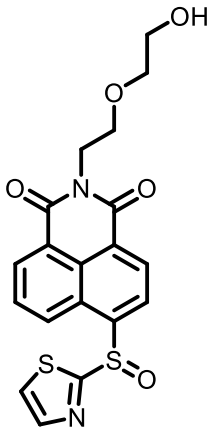


GP7

^{1}H NMR (500 MHz, DMSO-d6): δ 8.70 – 8.69 (m, 1H), 8.68 – 8.66 (m, 1H), 8.55 (dd, J = 7.3, 1.1 Hz, 1H), 8.46 (d, J = 7.7 Hz, 1H), 8.08 (d, J = 3.1 Hz, 1H), 8.00 (dd, J = 8.5, 7.3 Hz, 1H), 7.93 (d, J = 3.1 Hz, 1H), 4.02 (dd, J = 8.2, 6.6 Hz, 2H), 1.60 (ddt, J = 9.0, 7.6, 3.6 Hz, 2H), 1.34 (q, J = 7.4 Hz, 2H), 0.91 (t, J = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, DMSO): δ 174.50, 163.39, 163.15, 146.29, 145.12, 131.76, 130.67, 129.29, 129.07, 128.28, 127.45, 126.89, 125.81, 123.57, 123.51, 40.52, 30.00, 20.24, 14.17.

ESI-MS (m/z): $[M+H]^+$ calculated 385.0681, found 385.0684.

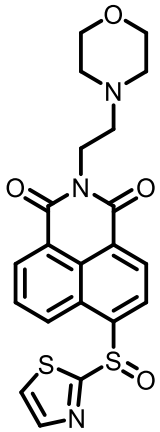


GP8

1H NMR (500 MHz, DMSO-d6): δ 8.73 – 8.69 (m, 2H), 8.58 (dd, J = 7.4, 3.0 Hz, 1H), 8.48 (dd, J = 7.7, 2.6 Hz, 1H), 8.10 (d, J = 3.0 Hz, 1H), 8.05 – 8.00 (m, 1H), 7.94 (d, J = 3.0 Hz, 1H), 4.61 – 4.55 (m, 1H), 4.23 (t, J = 6.5 Hz, 2H), 3.64 (t, J = 6.5 Hz, 2H), 3.43 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d6): δ 174.48, 163.44, 163.22, 146.43, 145.14, 131.85, 130.76, 129.42, 129.12, 128.30, 127.46, 126.95, 125.74, 123.57, 123.44, 72.58, 67.24, 60.61.

ESI-MS (m/z): $[M+H]^+$ calculated 417.0579, found 417.0581.



GP9

^{1}H NMR (500 MHz, Chloroform-d): δ 8.66 (d, 1H), 8.58 (d, J = 7.3 Hz, 1H), 8.44 (d, J = 7.7 Hz, 1H), 7.82 (d, J = 7.8 Hz, 1H), 7.78 – 7.73 (m, 2H), 7.35 (d, J = 3.4 Hz, 1H), 4.76 – 4.71 (m, 2H), 4.45 – 4.38 (m, 2H), 3.74 (d, J = 12.2 Hz, 2H), 3.60 – 3.56 (m, 2H), 3.36 – 3.30 (m, 4H).

^{13}C NMR (126 MHz, Chloroform-d): δ 163.74, 163.56, 159.94, 144.28, 139.53, 132.24, 131.43, 131.18, 131.15, 131.03, 128.81, 127.91, 122.96, 122.82, 122.50, 67.88, 64.84, 61.61, 34.14.

ESI-MS (m/z): $[M+H]^+$ calculated 442.0895, found 442.0893.

Fluorescent analysis methods

Deionized water was used to prepare all aqueous solutions. All the photophysical characterization experiments were carried out at room temprature. Phosphate buffer saline (PBS,

10 mM, pH 7.4) was purged with nitrogen for 5 min before use. **GP1-GP9** was dissolved in DMSO to make a 5 mM stock solution. GSH and other reactive bio-relevant species were prepared by dissolving commercial reagents in H_2O. To test the fluorescent responses of **GP1-GP9** towards GSH or other reactive species, aliquots of probe stock solution were diluted with PBS (with 1‰ cetrimonium bromide) and treated with analytes to make sure both probes and analytes were kept at desired final concentrations. After quick and vigorous shaking, the mixture was allowed standing in the dark at room temperature for desired time and the fluorescent spectra were then recorded under excitation at 405 nm. The emission spectra were scanned from 410 to 650 nm. All fluorometric experiments were performed in triplicate, and data shown were the average.

Biological methods

Primary mouse cortex neurons culture

Primary cortex neurons were derived from embryonic 16.5 (E16.5) mouse. And the experimental methods were in accordance with the guidelines of the IACUC of Shanghai and the National Research Council Guide for the Care and Use of Laboratory Animals. Cortical tissues were dissected from embryos and digested in HBSS containing 0.25% trypsin at 37 ^{o}C for 15 min. Neurons were plated on poly-l-lysine-coated 96-well plates and cultured with Neurobasal medium with 2% B-27 supplement, 2 mM Glutamax, and 1% horse serum (ThermoFisher Scientific) in humidified incubator at 37 °C with 5% CO_2.

SH-SY5Y Cell culture

SH-SY5Y cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained at 37 °C in a humidified atmosphere containing 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine.

Fluorescence imaging of GSH in different cells

SH-SY5Y cells or neurons were seeded into 96-well plates microplate with optically clear bottom (Greiner bio-one, Germany) 24h at the density of 2-10 $X10^4$ cells each well. For direct fluorescent quantitation, cells were incubated with probes (10μM) for 30 min in culture medium.

For endogenous GSH detection, SH-SY5Y cells were incubated with NEM (Sigma, E3876) of 100μM and 200μM for 30min and changed to fresh medium containing GP5 10μM for further 30min.For exogenous GSH detection, SH-SY5Y cells were firstly incubated with NEM (200μM) before loaded with probe GP5 (10μM) 30 min, then 100μM and 200μM GSH (Sigma, G4251) was added to fresh medium for culture another 30 min.

For detect endogenous GSH through development of cultured primary cortex neurons, GP5 (10μM) was add to culture medium for indicated time-day in vitro(DIV) 0.25, 0.5, 2, 4, 7 for staining 30min. Before that, all neurons were placed in normal conditional medium for development.

After treatment with probe, all above were washed with PBS twice before image. The fluorescence images were recorded by Operetta high content imaging system (PerkinElmer, USA) at the excitation wavelengths of 360-440nm. The fluorescence was quantified by columbus

analysis system (Perkinelmer, US).

Cell viability assay

Cell viability was detected by MTS assay as the guidelines. Briefly, cultured cells were added 10 μL per well of MTS/PMS (20:1, Promega Corp) solution each well containing 100 μL of culture medium. After incubation at 37 °C under 5% CO_2 for 4 h in cell incubator, the absorbance of the solutions was measured at 490 nm by an M5 microplate reader (Molecular Device, USA).

Neuron polarity quantification and analysis

For the determination of polarized neurons, neuron was plated onto each well in low density of 2 $X10^4$ cells for 8h with conditional medium. Then cultured medium was change to fresh containing 100μM BSO. After treatment with or without BSO 40h, the cultured primary cortex neurons were staining with GP5 and photo as described above. Neuronal polarity was assessed by determining the percentage of polarized cells within 15 images in 3 wells taken at 40X magnification. A polarized neuron was considered if the longest neurite was least twice as long as any sister neurite.

Statistical analysis

Unpaired T-test was performed to analyze the results using GraphPad Prism software. Results (Fig. 4-7) are presented as mean ± S.D. Statistical significance was determined at $P < 0.05$(*); $P < 0.01$(**); $P < 0.001$(***).

Supplementary Figures

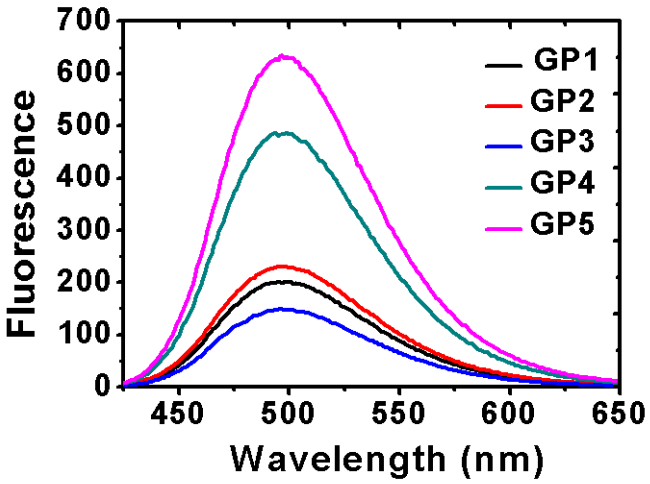


Fig. S1 Fluorescence spectra of **GP1-GP5** (5 μM) after being treated with GSH (2 mM, 60 min) in PBS (10 mM, pH7.4).

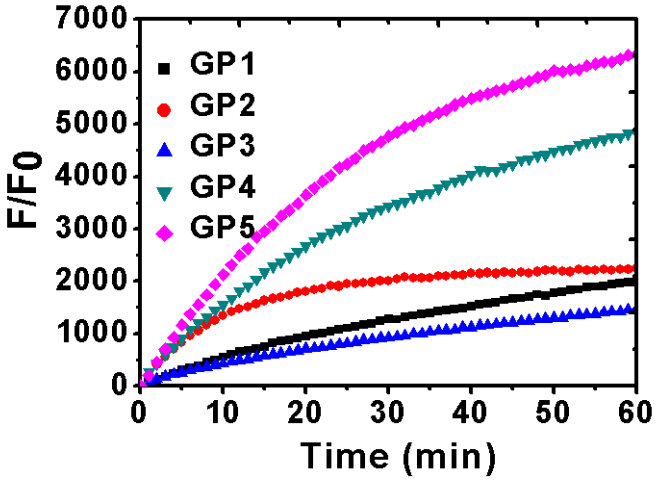


Fig. S2 Time-dependent fluorescence changes of probe **GP1**-**GP5** in response to GSH (2 mM). F_0 represents probe emission at 496 nm before the addition of GSH.

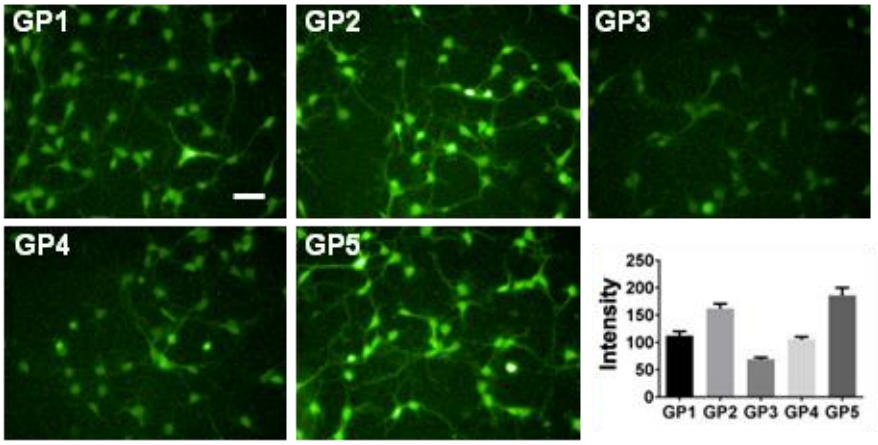


Fig. S3 Imaging GSH in primary cortex neurons (DIV2) with **GP1**-**GP5**, and the sensitivity comparison.

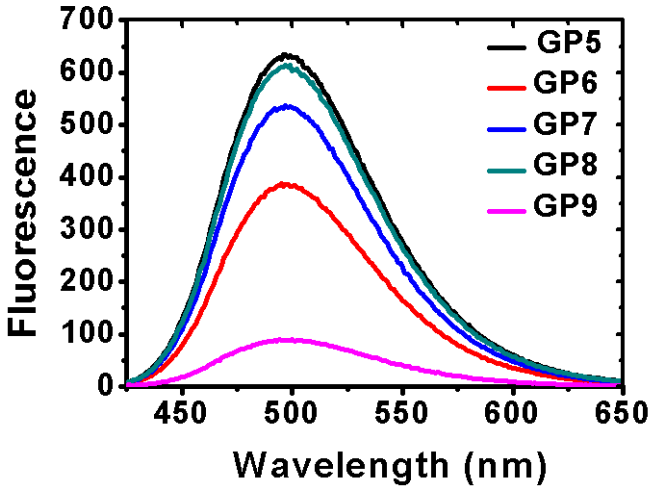


Fig. S4 Fluorescence spectra of **GP5**-**GP9** (5 μM) after being treated with GSH (2 mM, 60 min) in PBS (10 mM, pH7.4).

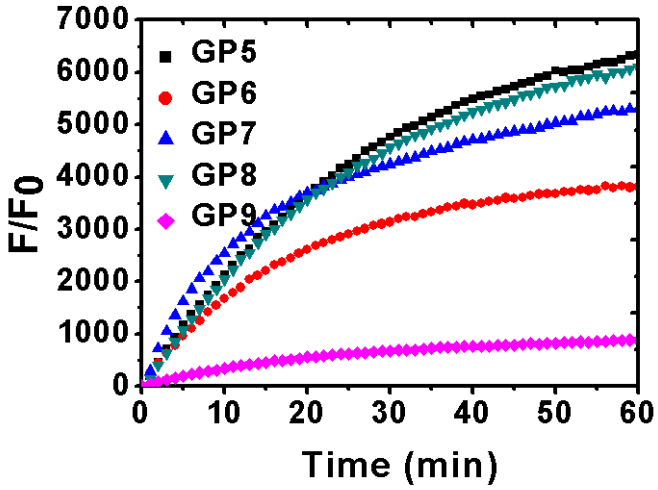


Fig. S5 Time-dependent fluorescence intensity change of probe **GP5-GP9** in response to GSH (2 mM). F_0 represents probe emission at 496 nm before treatment with GSH.

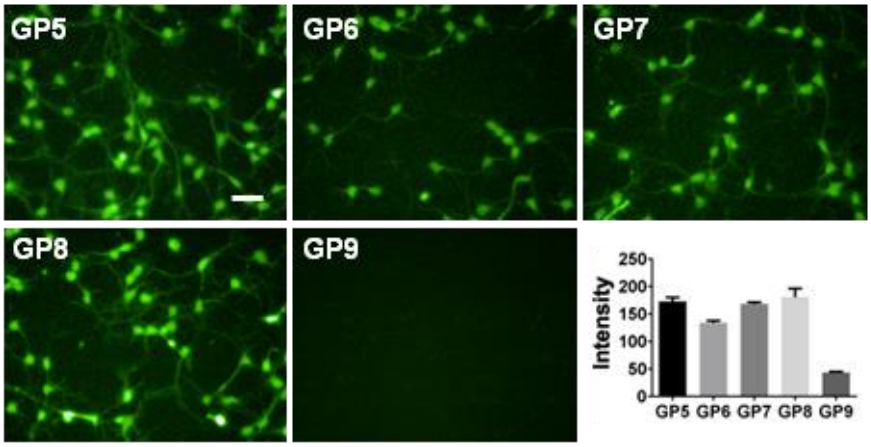


Fig. S6 Imaging GSH in primary cortex neurons (DIV2) with **GP5-GP9**, and the sensitivity comparison.

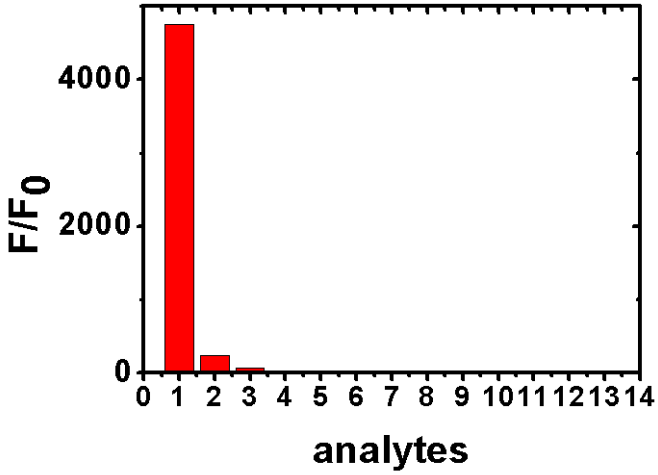


Fig. S7 Fluorescence changes of **GP5** in response to various analytes. Aliquots of **GP5** (5 μM) in PBS were incubated with various analytes for 60 min, then its intensity at 496 nm was measured. The analytes tested were 1) GSH; 2) Cys; 3) Hcy; 4) Ala; 5) Gly; 6) H_2O_2; 7) $ONOO^-$; 8) NO^{2-}; 9) Fe^{3+}; 10) Cu^{2+}; 11) Ca^{2+}; 12) $S_2O_3^{2-}$; 13) SO_4^{2-}; 14) SO_3^{2-}. All analytes were kept at or above their biologically relevant concentrations (All were 500 μM except $ONOO^-$ (20 μM), Cys (300 μM) and Hcy (15 μM).

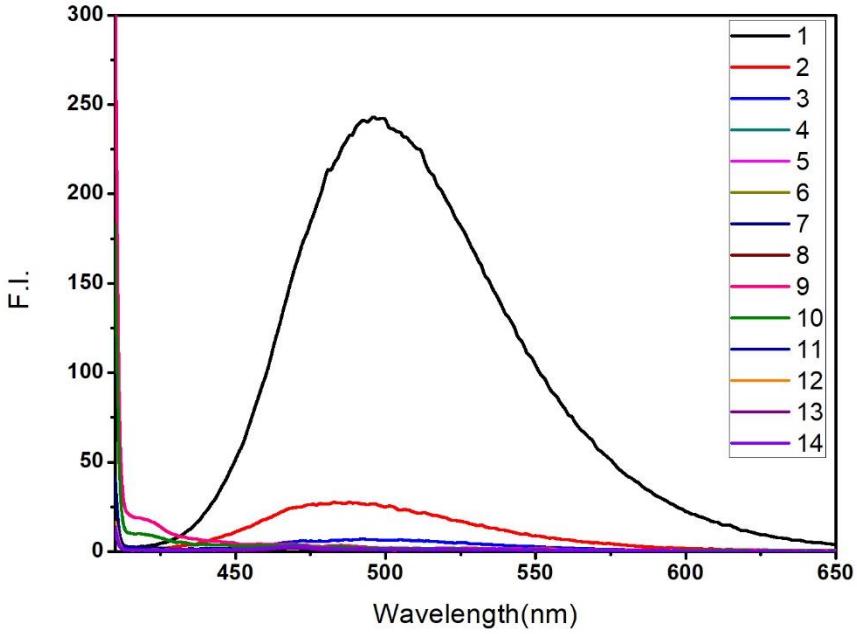


Fig. S8 Selectivity evaluation of **GP5** towards GSH. Fluorescent intensity change of **GP5** in response to various analytes. Aliquots of **GP5** (5 μM) in PBS were incubated with various analytes for 60 min, then its fluorescence spectra was recorded. The analytes tested were 1) GSH; 2) Cys; 3) Hcy; 4) Ala; 5) Gly; 6) H_2O_2; 7) $ONOO^-$; 8) NO^{2-}; 9) Fe^{3+}; 10) Cu^{2+}; 11) Ca^{2+}; 12) $S_2O_3^{2-}$; 13) SO_4^{2-}; 14) SO_3^{2-}. All analytes were kept at or above their biologically relevant concentrations (all were 500 μM except $ONOO^-$ (20 μM), Cys (300 μM) and Hcy (15 μM).

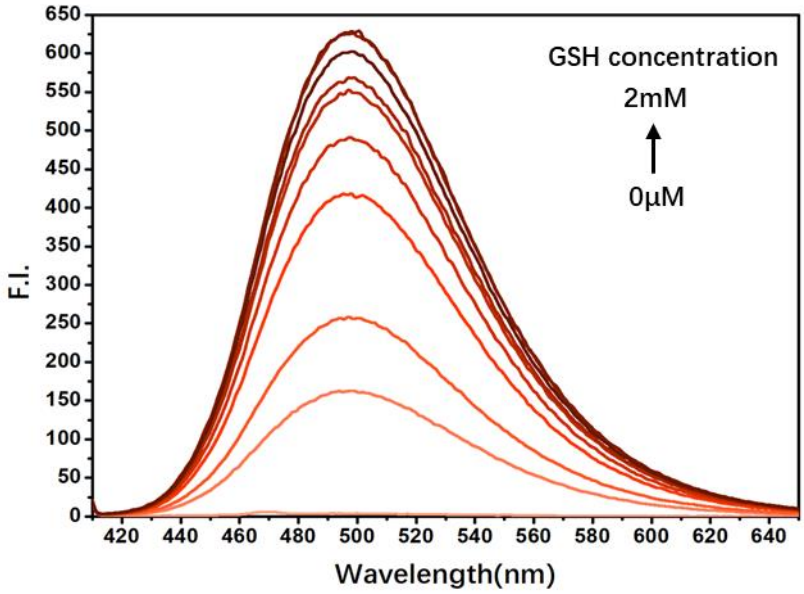


Fig. S9 Fluorescence changes of **GP5** on addition of various GSH concentrations.

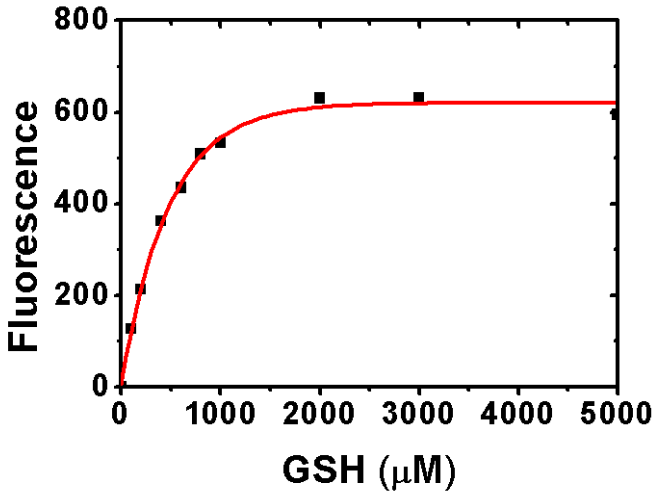
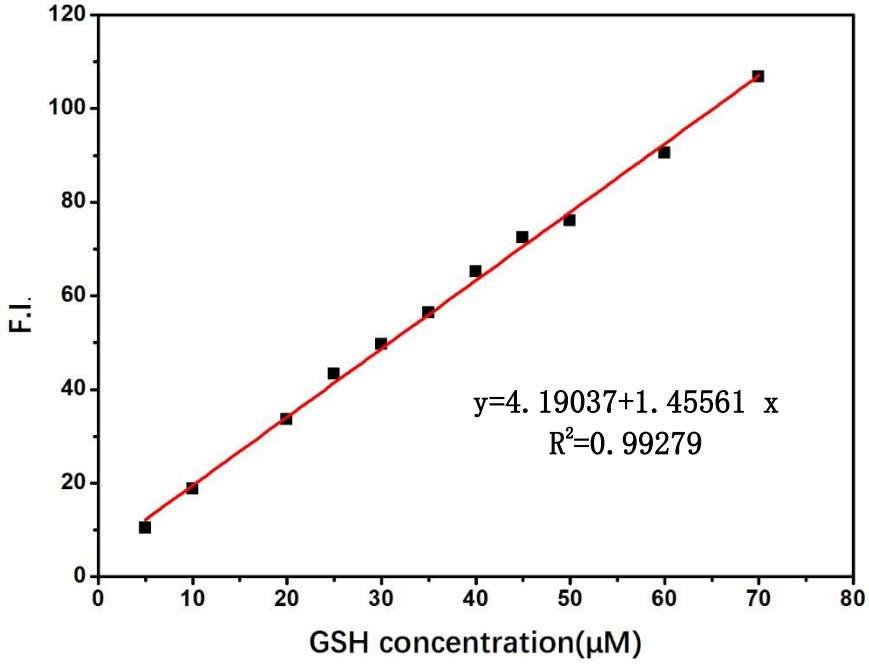


Fig. S10 GSH concentration-dependent fluorescence intensity changes of **GP5** (5 μM) at 496 nm. **GP5** was incubated with various doses of GSH for 60 min and then its emission intensity at 496 nm was plotted against GSH dosage. All of the experiments performed in PBS (10 mM, pH 7.4) at room temperature. The excitation wavelength was 405 nm.



LOD=3σ/S

σ: the standard deviation of the blank solution

S: the slope of the linear calibration plot between the fluorescence emission intensity and the concentration of GSH.

Fig. S11 LOD calculation. Linear calibration plot between the fluorescence emission intensity and the concentration of GSH.

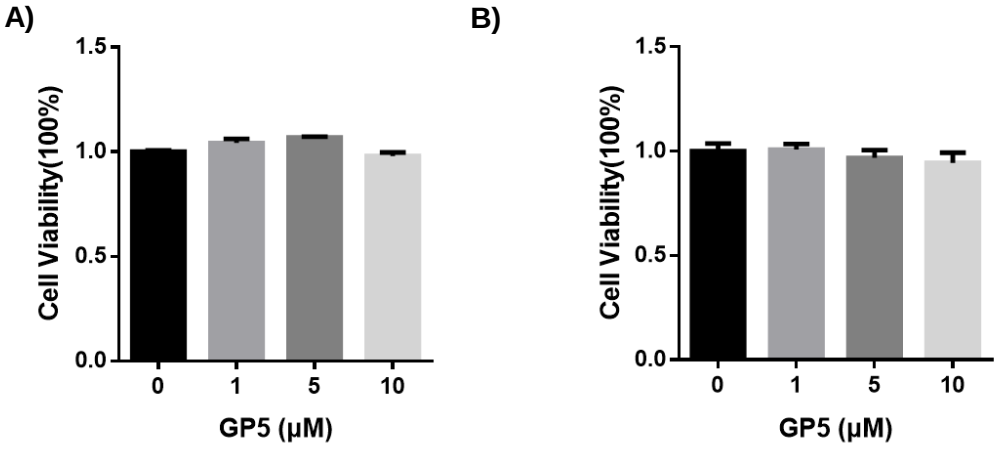


Fig. S12 Probe **GP5** had no effect on cell growth. (A) SH-SY5Y cells were incubated with increasing concentrations of probe **GP5** for 1 h, and then the medium was changed to fresh one. Cell viability was then tested after 47 h of incubation by MTS assay. (B) SH-SY5Y cells were incubated with increasing concentrations of probe **GP5** for 4 h, and then the medium was changed to fresh one. Cell viability was tested by MTS assay 44h later.

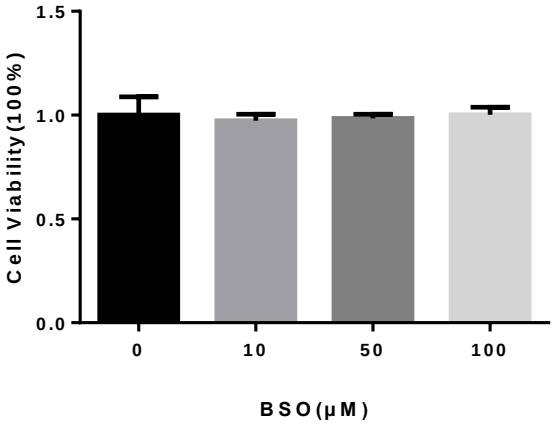
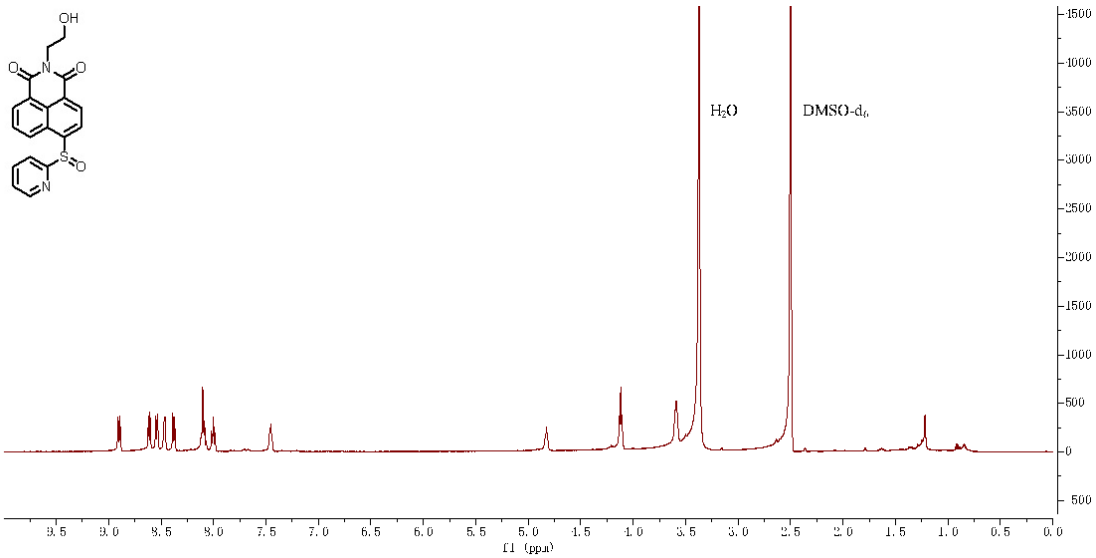
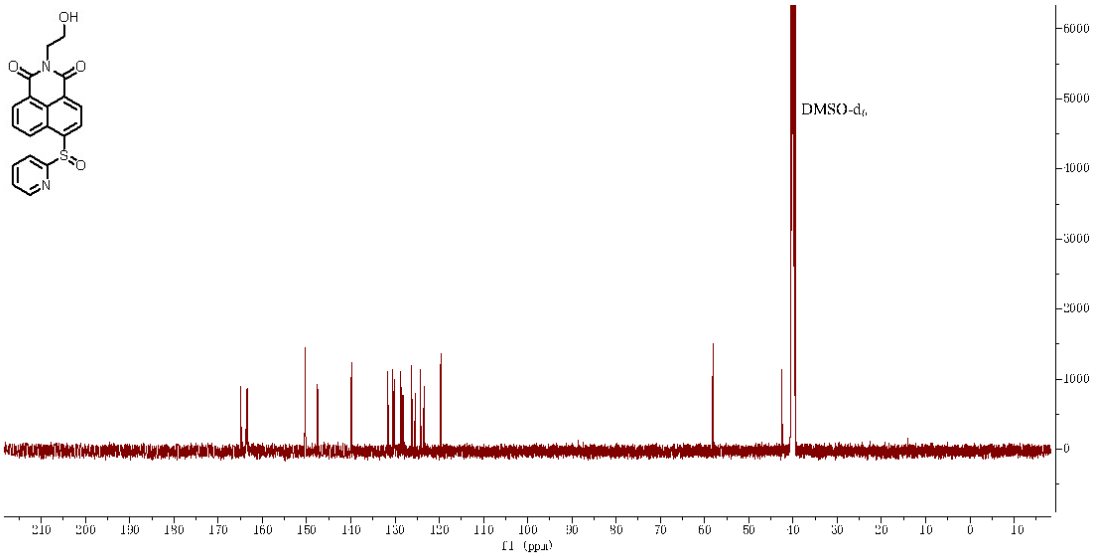


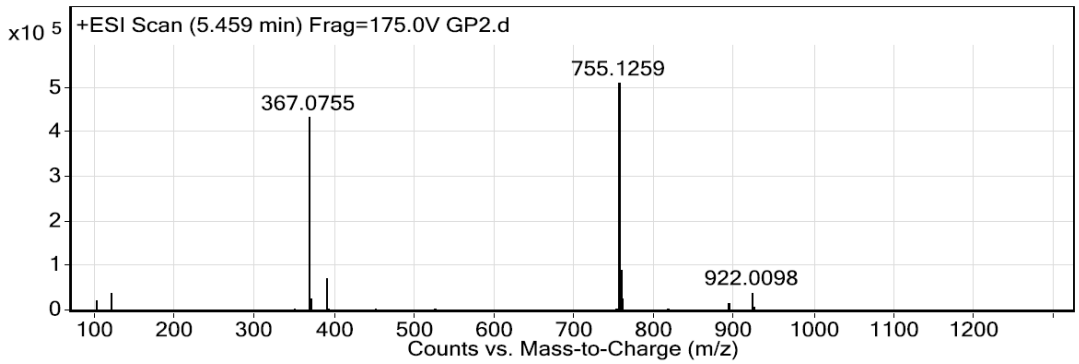
Fig. S13 BSO has no toxicity in PCN. PCN were incubated with increasing concentrations of BSO for 40 h after insertion into plate. Cell viability was then tested by MTS assay.

NMR and HRMS traces

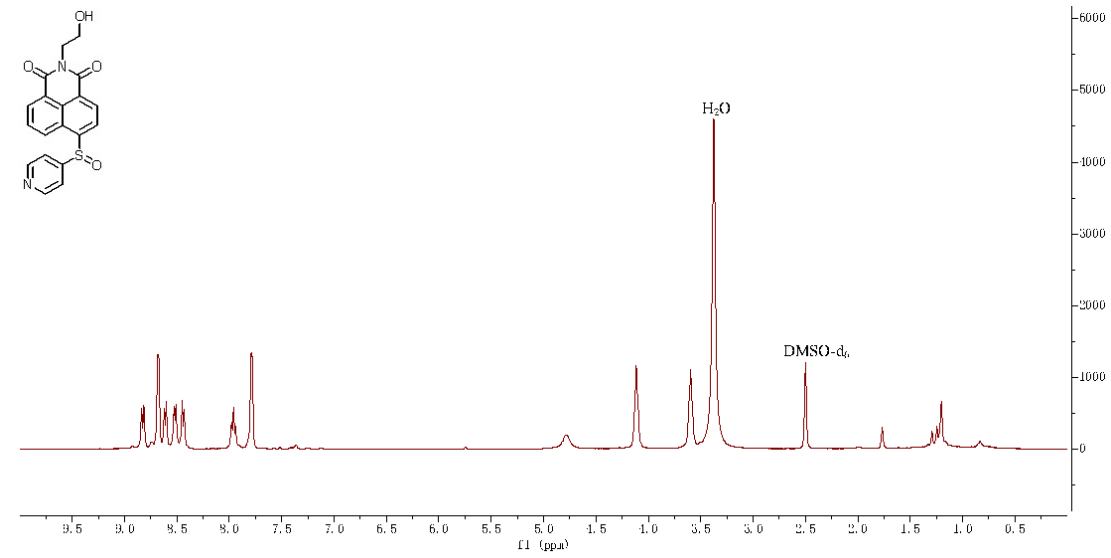
Probe GP1

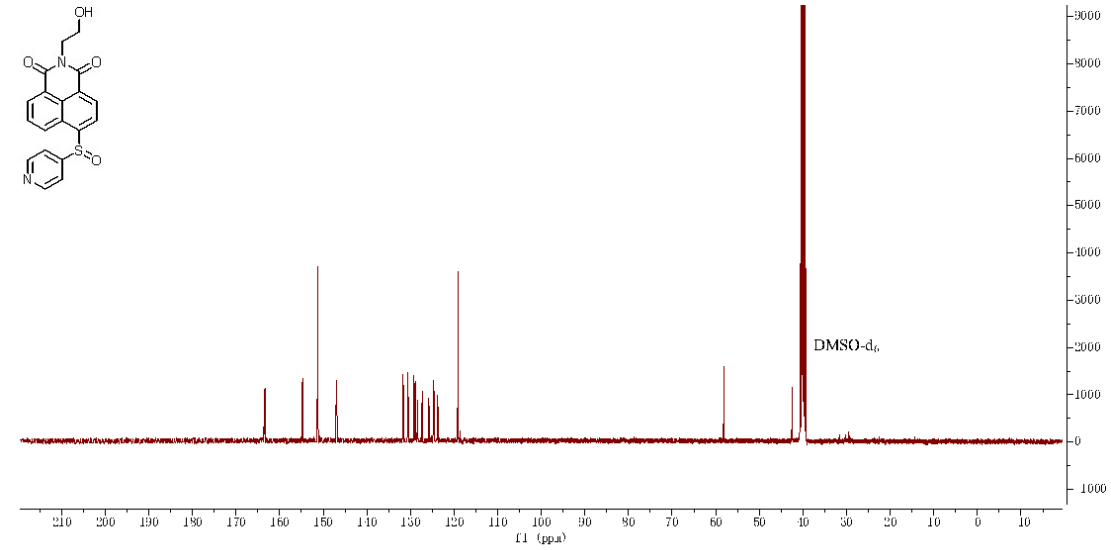


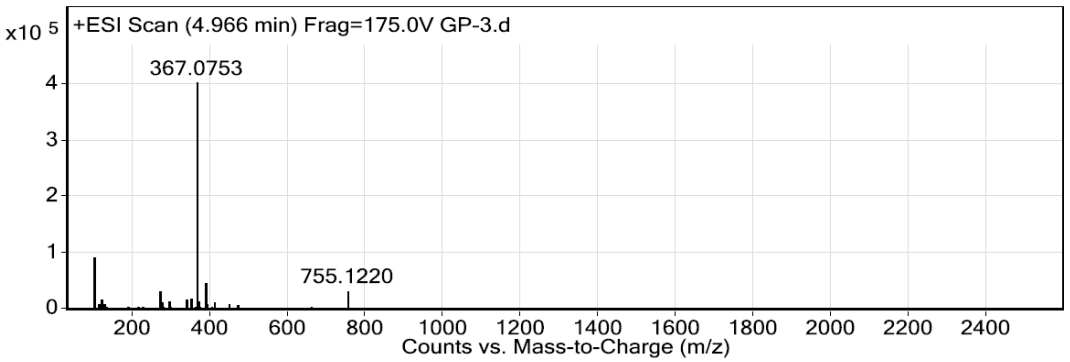




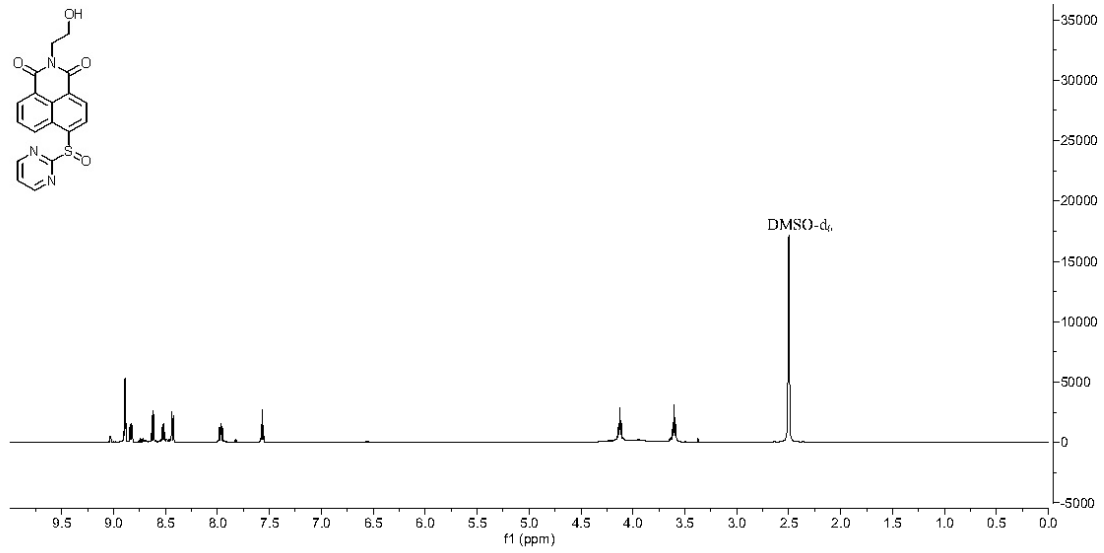
Probe GP2

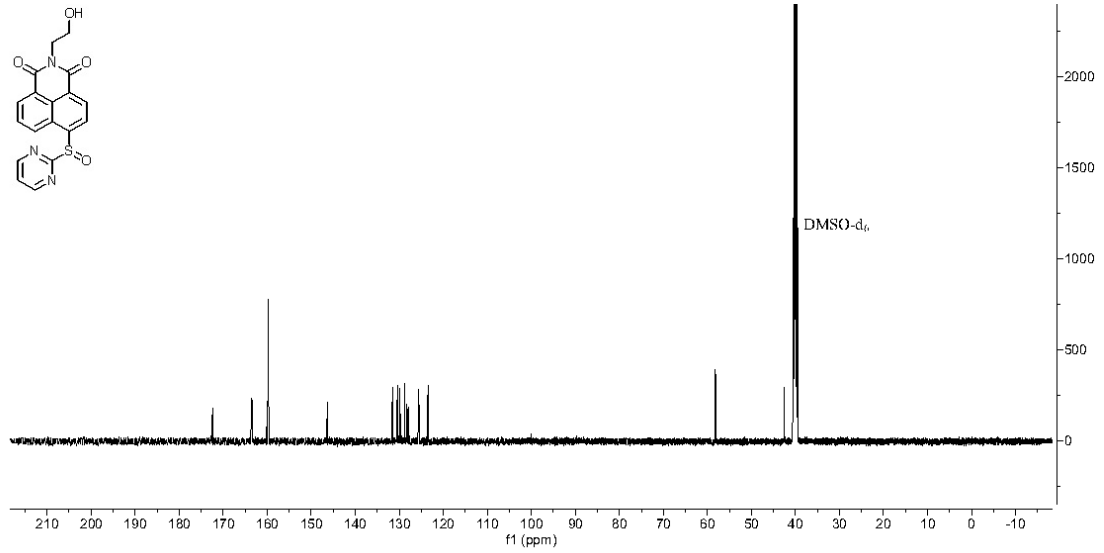

OH
O
N
O
S
O
N
H2O
DMSO-d6
f1 (ppm)

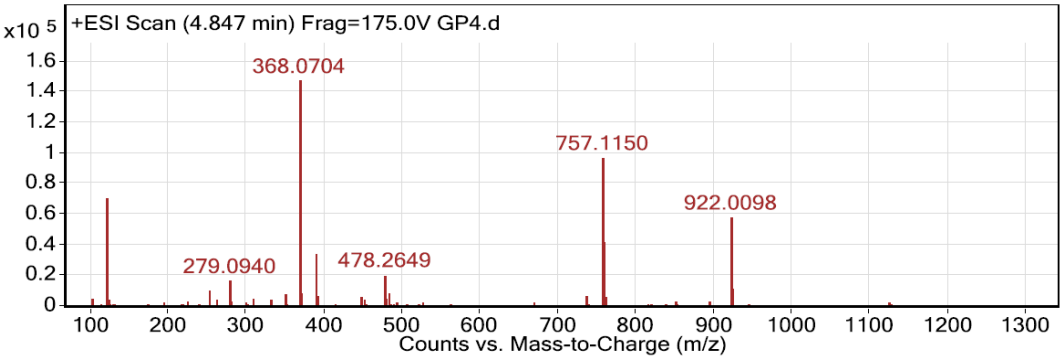

OH
O
N
O
S
O
N
DMSO-d6
f1 (ppm)


x10 5
+ESI Scan (4.966 min) Frag=175.0V GP-3.d
367.0753
755.1220
Counts vs. Mass-to-Charge (m/z)

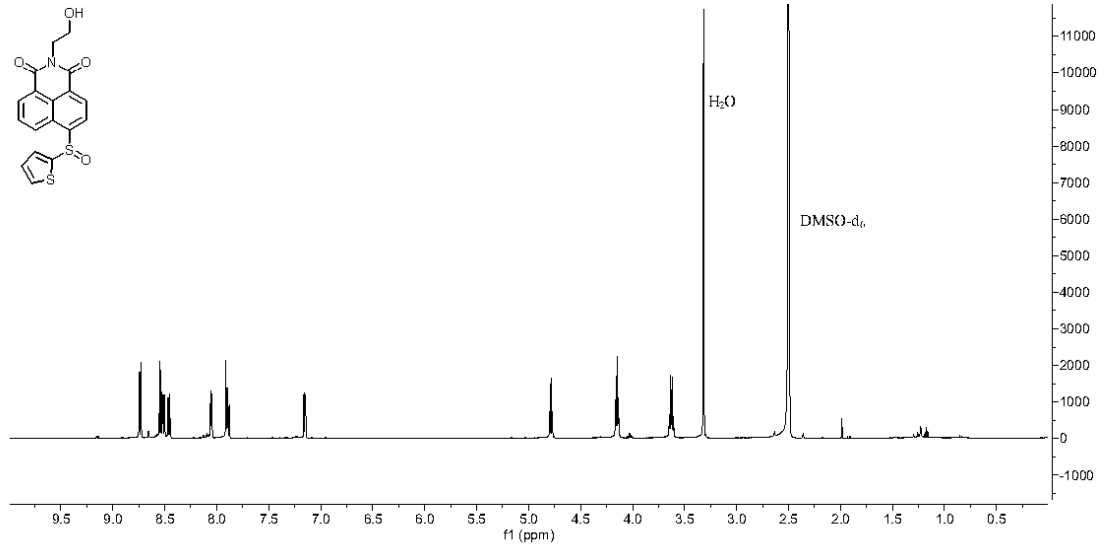
Probe GP3

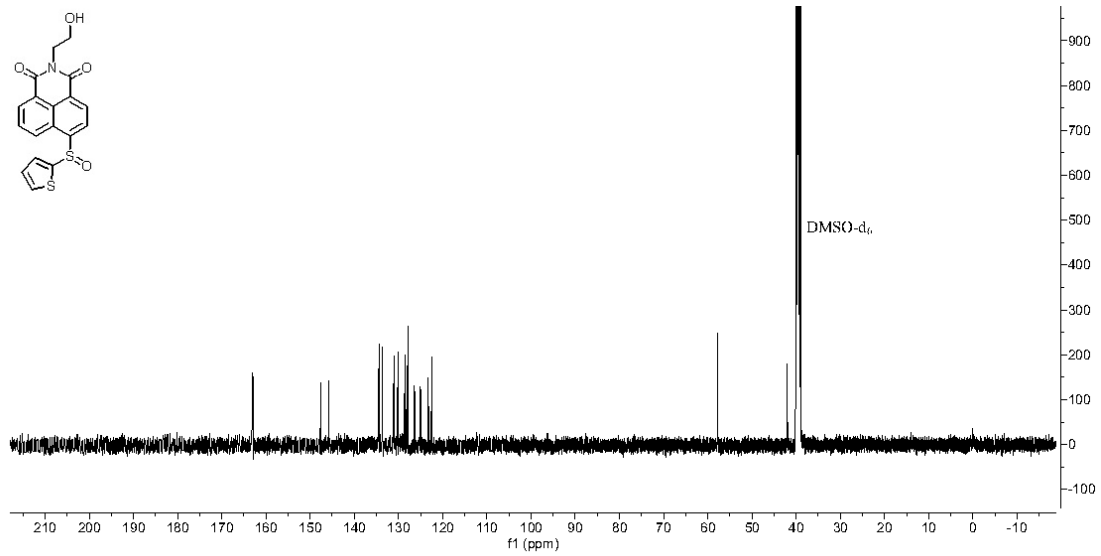

OH
O
N
O
N
S
O
N
DMSO-d6
35000
30000
25000
20000
15000
10000
5000
0
-5000
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

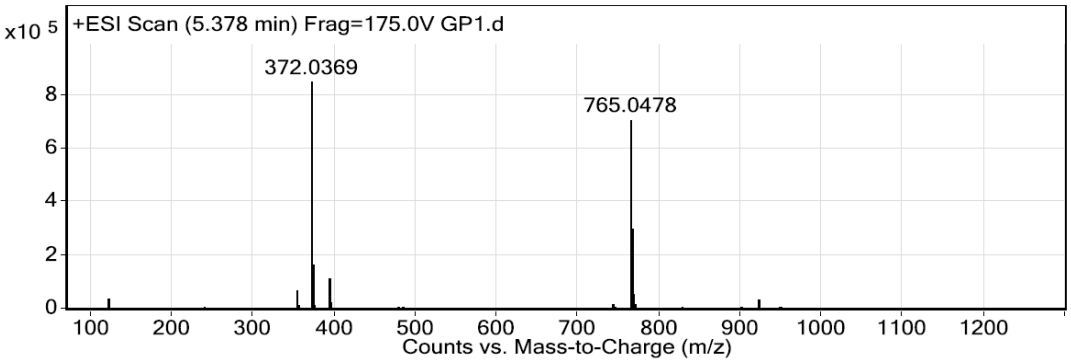

OH
O
N
O
N
S
O
N
DMSO-d6
2000
1500
1000
500
0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


x10 5
+ESI Scan (4.847 min) Frag=175.0V GP4.d
368.0704
757.1150
922.0098
279.0940
478.2649
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
100 200 300 400 500 600 700 800 900 1000 1100 1200 1300
Counts vs. Mass-to-Charge (m/z)

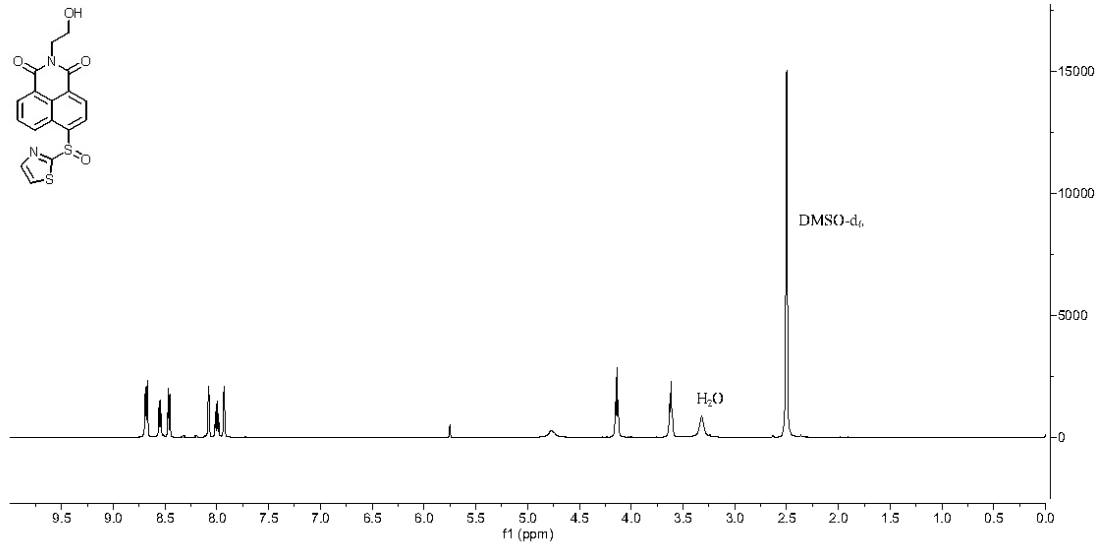
Probe GP4

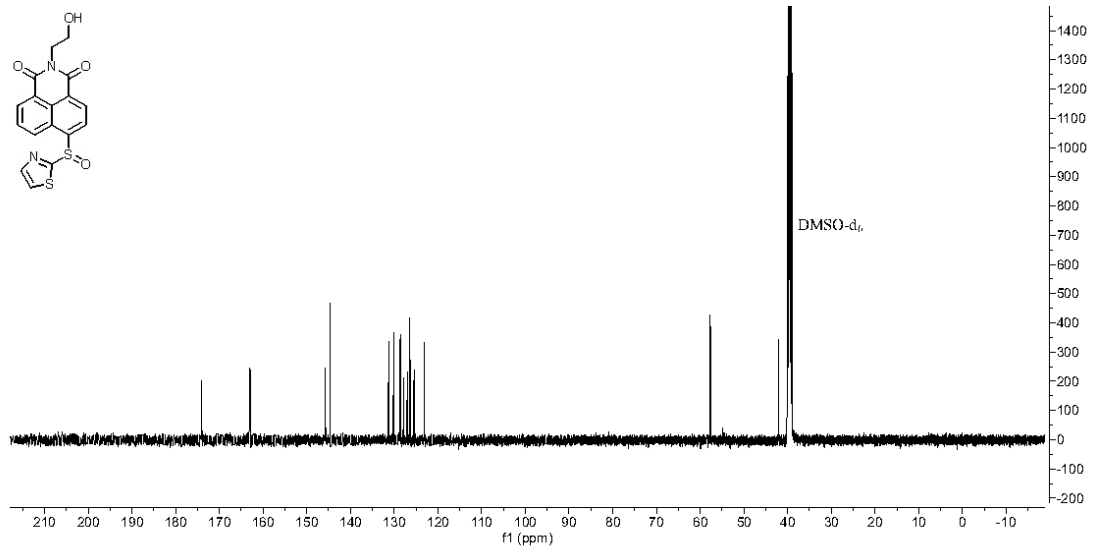

OH
O
N
O
S
O
S
H2O
DMSO-d6
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

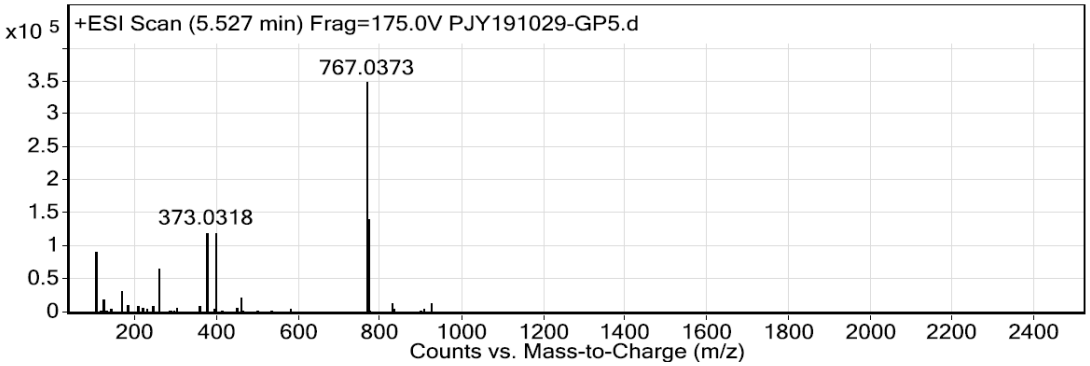

OH
O
N
O
S
O
S
DMSO-d6
900
800
700
600
500
400
300
200
100
0
-100
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


x10 5
+ESI Scan (5.378 min) Frag=175.0V GP1.d
372.0369
765.0478
8
6
4
2
0
100 200 300 400 500 600 700 800 900 1000 1100 1200
Counts vs. Mass-to-Charge (m/z)

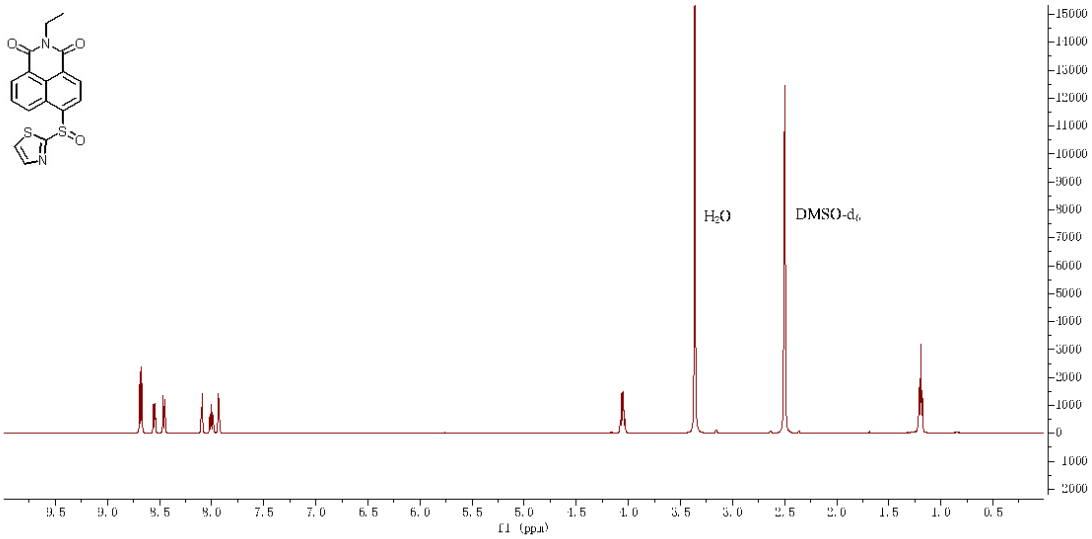
Probe GP5

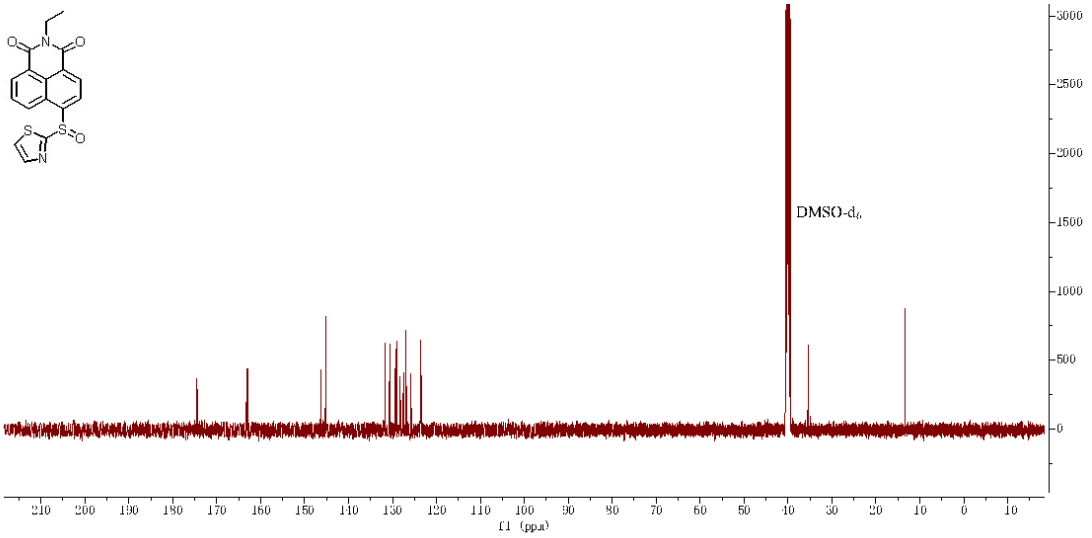

OH
O
N
O
N
S
O
S
DMSO-d6
H2O
15000
10000
5000
0
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

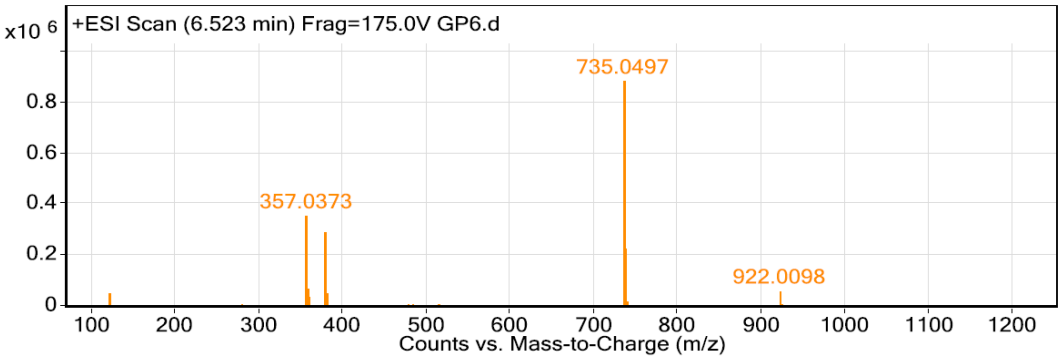

OH
O
N
O
N
S
O
S
DMSO-d6
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
-200
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


x10 5
+ESI Scan (5.527 min) Frag=175.0V PJY191029-GP5.d
767.0373
373.0318
3.5
3
2.5
2
1.5
1
0.5
0
200 400 600 800 1000 1200 1400 1600 1800 2000 2200 2400
Counts vs. Mass-to-Charge (m/z)

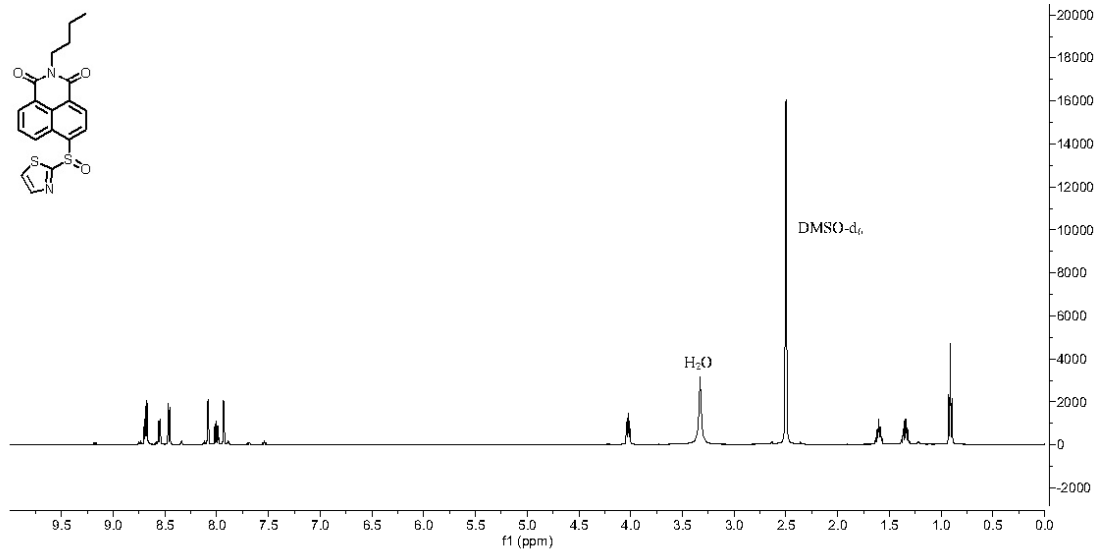
Probe GP6

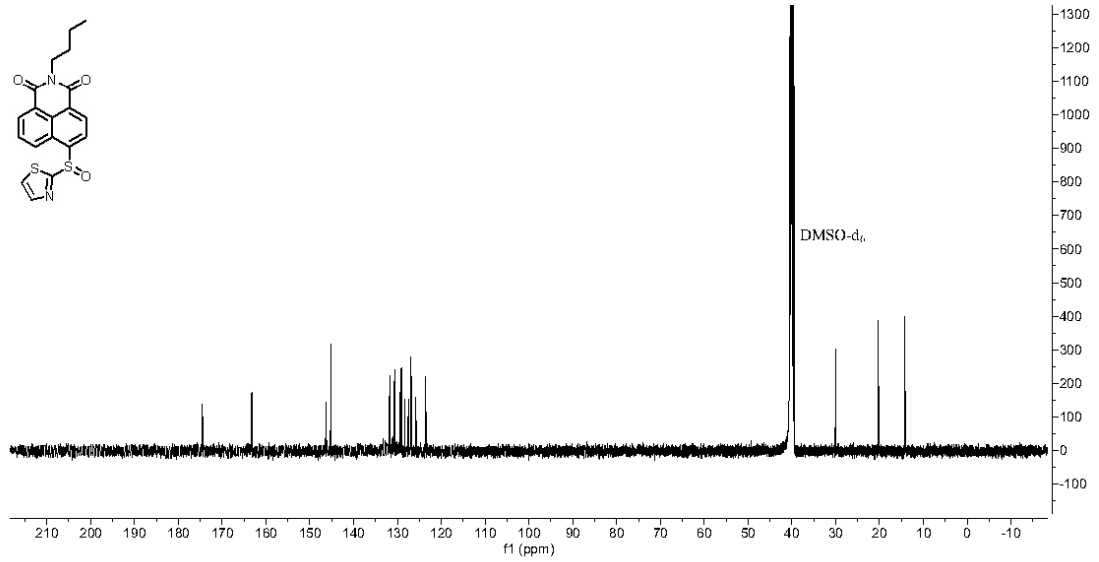

H2O
DMSO-d6
f1 (ppm)

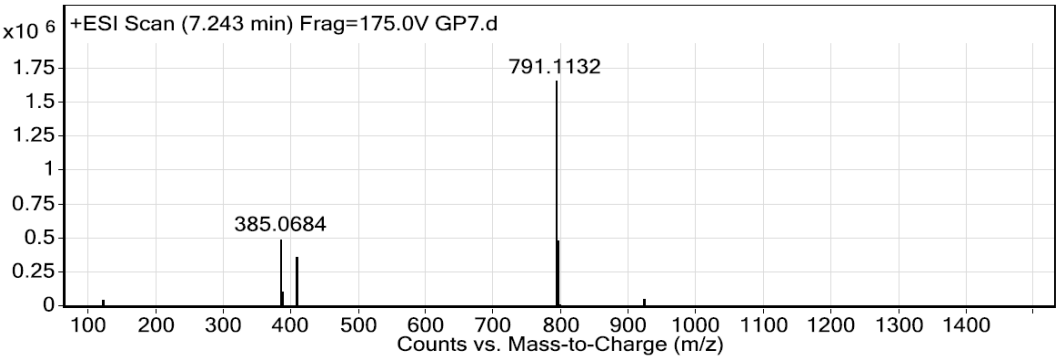

DMSO-d6
f1 (ppm)


x10 6
+ESI Scan (6.523 min) Frag=175.0V GP6.d
735.0497
357.0373
922.0098
Counts vs. Mass-to-Charge (m/z)

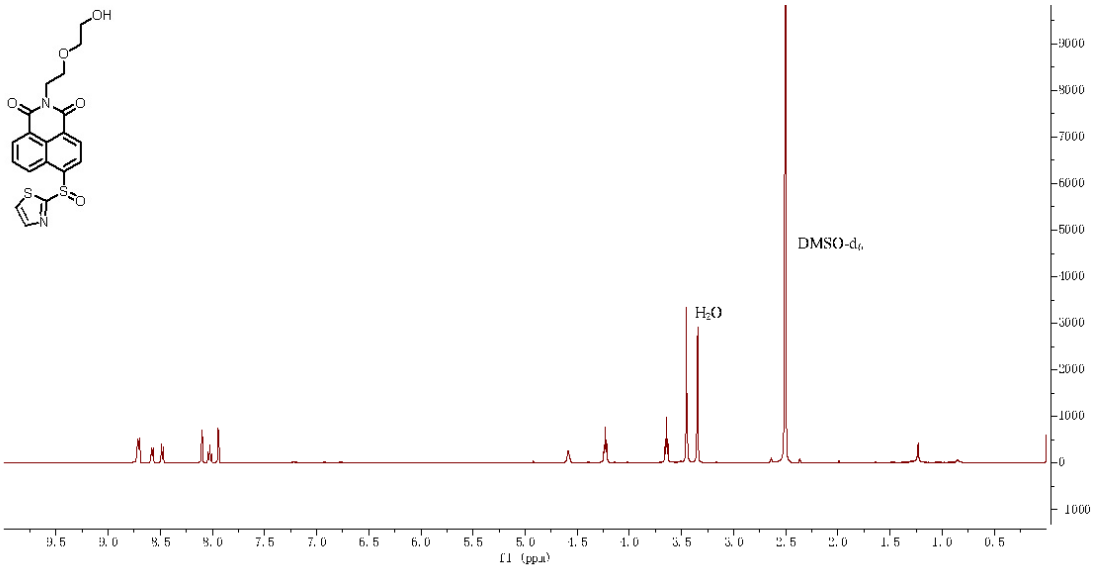
Probe GP7

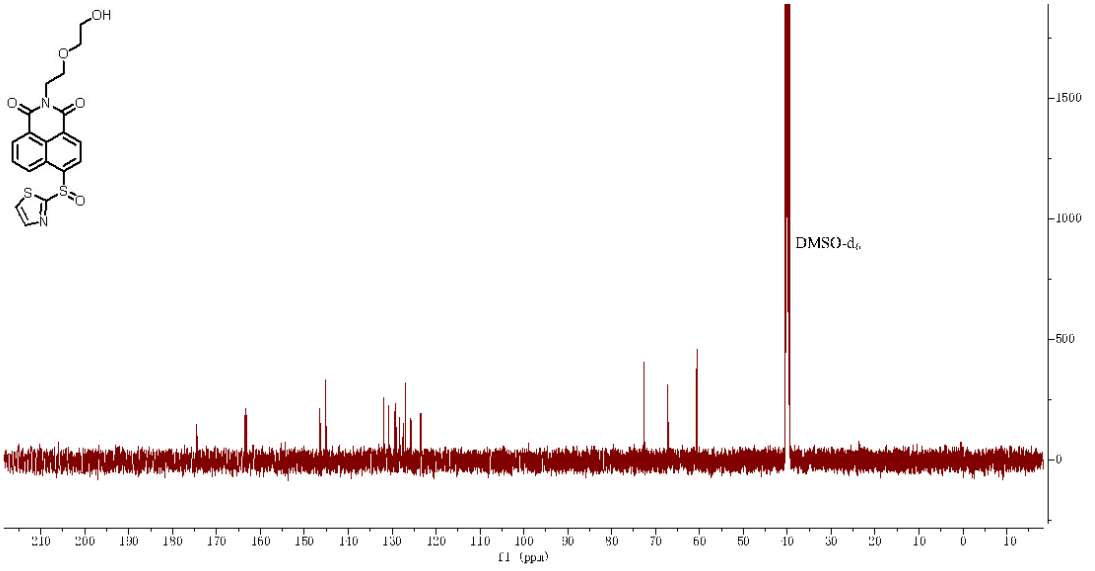

DMSO-d6
H2O
f1 (ppm)

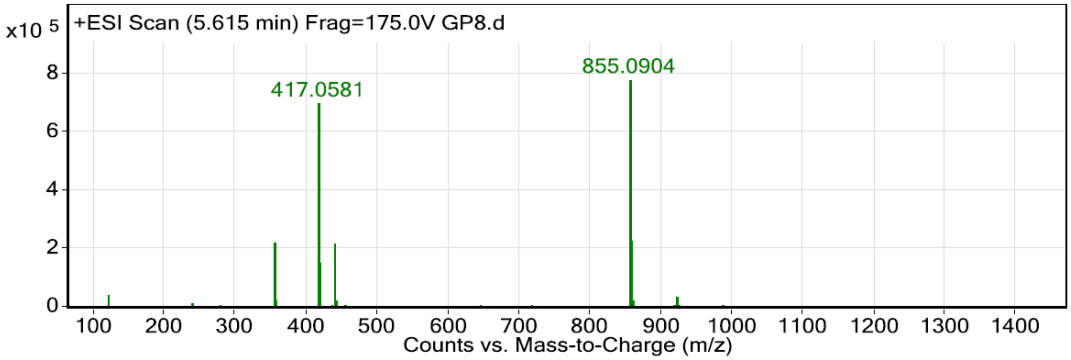

DMSO-d6
f1 (ppm)


+ESI Scan (7.243 min) Frag=175.0V GP7.d
x10 6
791.1132
385.0684
Counts vs. Mass-to-Charge (m/z)

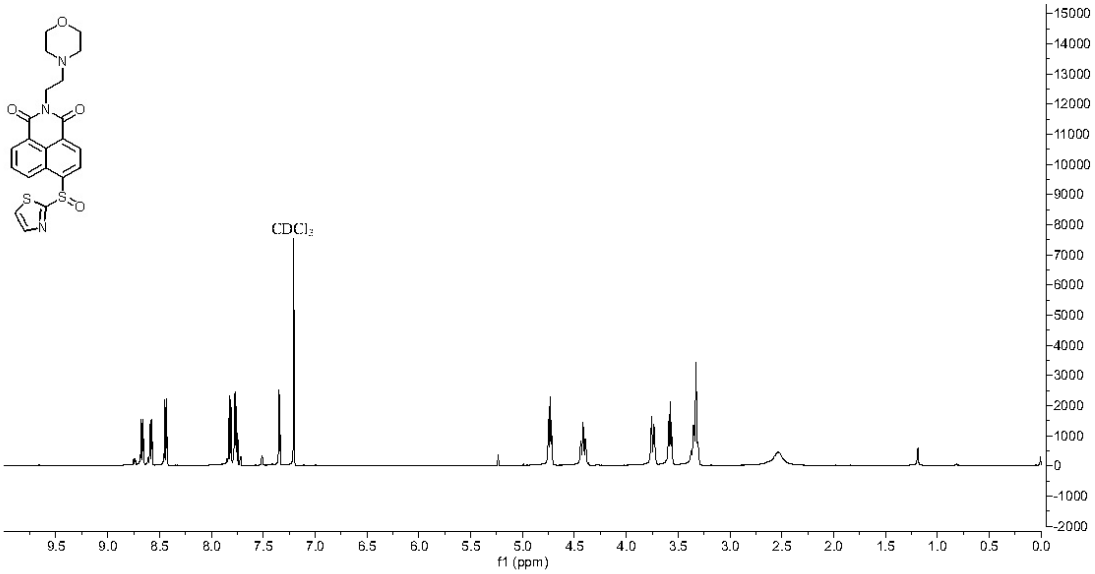
Probe GP8

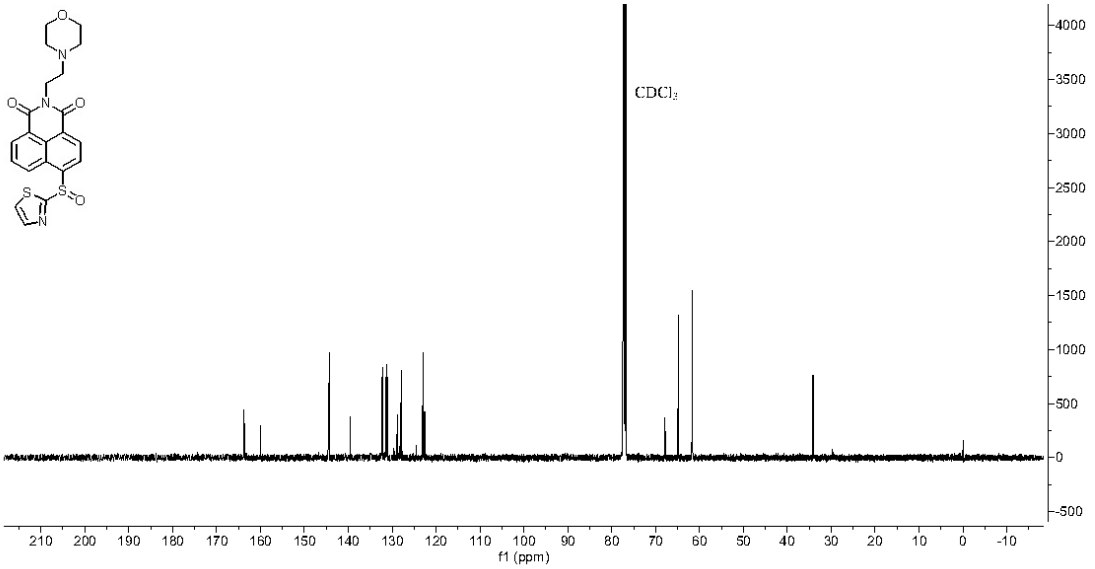

DMSO-d6
H2O
f1 (ppm)

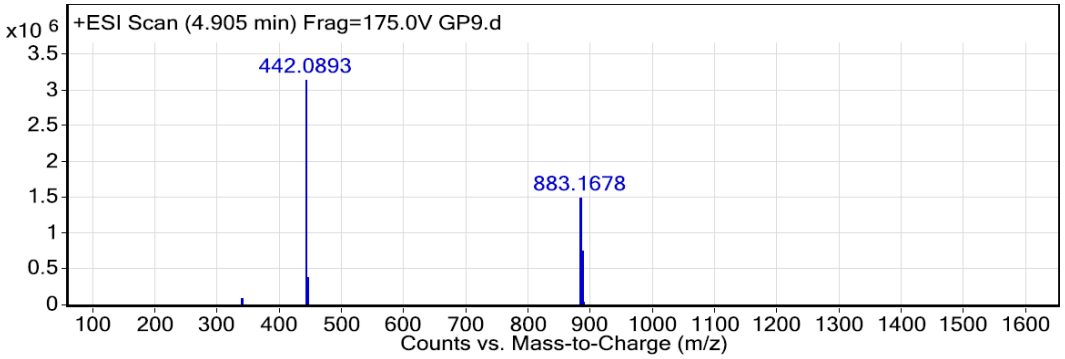

DMSO-d6
f1 (ppm)


x10 5
+ESI Scan (5.615 min) Frag=175.0V GP8.d
417.0581
855.0904
Counts vs. Mass-to-Charge (m/z)

Probe GP9


CDCl3
f1 (ppm)


CDCl3
f1 (ppm)


+ESI Scan (4.905 min) Frag=175.0V GP9.d
x10 6
442.0893
883.1678
Counts vs. Mass-to-Charge (m/z)