Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

Simultaneous Sensing of Cysteine/Homocysteine and Glutathione with a Fluorescent Probe Based on Single Atom Replacement Strategy

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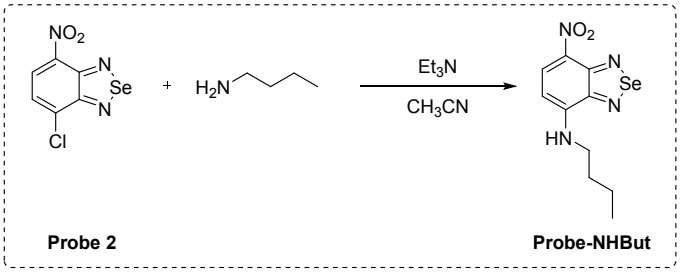
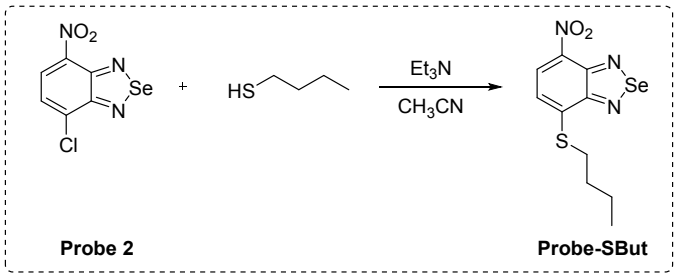
I. Experimental Section



Scheme S1. Synthesis of the Probe-NHBut.

Synthesis of N-butyl-7-nitrobenzo[c][1,2,5]selenadiazol-4-amine

Nitrobenzoselenadiazoles (0.381 mmol) and butan-1-amine (0.571 mmol) were dissolved in acetonitrile, a small amount of triethylamine was added, and the mixture was stirred under reflux at 80 °C overnight. The plate was confirmed to be complete. If the starting material had been reacted, the solvent was evaporated under reduced pressure and recrystallized from ethanol to give a red solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.68 (d, J = 8.8 Hz, 1H), 6.49 (s, 1H), 6.20 (d, J = 8.7 Hz, 1H), 3.45 (td, J = 7.2, 5.8 Hz, 2H), 1.80 (p, J = 7.3 Hz, 2H), 1.52 (h, J = 7.4 Hz, 2H), 1.01 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.6, 152.4, 147.4, 135.6, 129.9, 97.2, 43.3, 30.8, 20.2, 13.8.



Scheme S2. Synthesis of the Probe-SBut.

Synthesis of 4-(butylthio)-7-nitrobenzo[c][1,2,5]selenadiazole

Nitrobenzoselenadiazoles (0.190 mmol) and butane-1-thiol (0.286 mmol) were dissolved in acetonitrile, a small amount of triethylamine was added, and the mixture was stirred under reflux at room temperature overnight. The plate was confirmed to be complete. If the starting material had been reacted, the solvent was evaporated under reduced pressure and recrystallized from ethanol to give a orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (d, J = 8.0 Hz, 1H), 7.17 (d, J = 8.1 Hz, 1H), 3.18 (t, J = 7.5 Hz, 2H), 1.84 (q, J = 7.8 Hz, 2H), 1.58 (d, J = 9.7 Hz, 2H), 1.01 (t, J = 7.6 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.2, 150.9, 146.3, 128.9, 118.2, 31.2, 29.8, 22.2, 13.6.

Calculation of the detection limit (LOD)

$$\mathrm{LOD} = 3\sigma/S$$

$$\sigma = \sqrt{\frac{\sum(\bar{x} - x_i)^2}{n-1}}$$

σ: the standard deviation of the blank solution.

$\bar{x}$ is the mean of the blank measures; x_i is the values of blank measures; n is the number of tested blank measure (n = 10)

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

MTT assay

HepG2 cells cytotoxicity was evaluated by MTT assay. HepG2 cells were cultivated in a 96-well plate until 60-70% confluence, and then incubated with different concentrations of Probe 1 (0-20 μM) for 24 h. Then 20 μL MTT was added for 4 h at 37°C. Absorbance was measured at 490 nm on SpectraMax i3 (Molecular Devices, USA). All experiments were repeated three times, and the data were presented as the normalized percentage of control HepG2 cells.

II. Supplementary Spectra

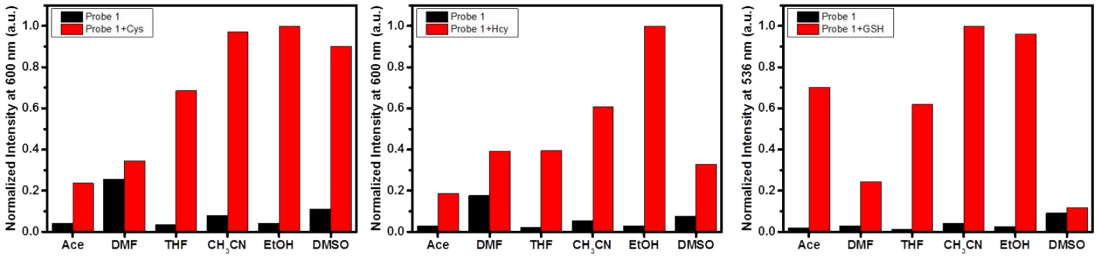


Fig. S1. Fluorescence response of Probe 1 (10 μM in various solvent-PBS, pH = 7.4, v/v, 5:5) in the presence of 10 equiv. of Cys, Hcy and GSH at room temperature. The fluorescence intensity changes at 536 nm (λ_{ex} = 446 nm), 600 nm (λ_{ex} = 510 nm).

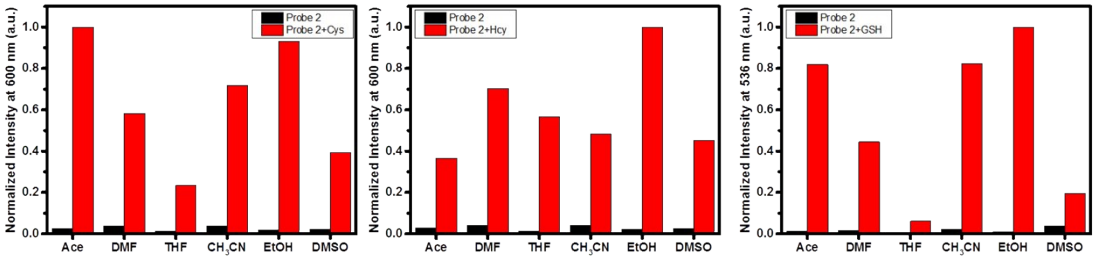


Fig. S2. Fluorescence response of Probe 2 (10 μM in various solvent-PBS, pH: 7.4, v/v, 5:5) in the presence of 10 equiv. of Cys, Hcy and GSH at room temperature. The fluorescence intensity changes at 536 nm (λ_{ex} = 446 nm), 600 nm (λ_{ex} = 510 nm).

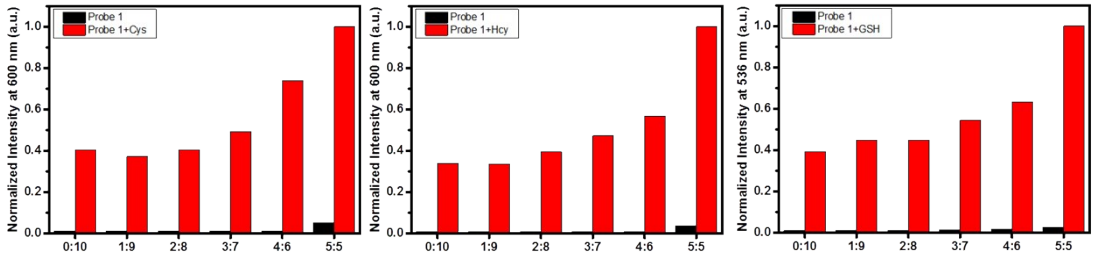


Fig. S3. The effect of volume ratio of EtOH/PBS (10 mM, pH: 7.4) on the fluorescence intensities changes of free Probe 1 (10 μM, black column) and Probe 1 (10 μM) in the presence of 10 equiv. of Cys, Hcy or GSH (100 μM, red column) for 30 min. The fluorescence intensity changes at 536 nm (λ_{ex} = 446 nm), 600 nm (λ_{ex} = 510 nm).

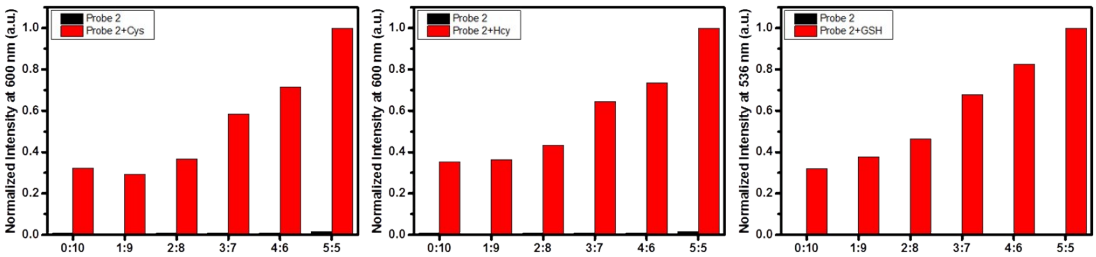


Fig. S4. The effect of volume ratio of EtOH/PBS (10 mM, pH: 7.4) on the fluorescence intensities changes of free Probe 2 (10 μM, black column) and Probe 2 (10 μM) in the presence of 10 equiv. of

Cys, Hcy or GSH (100 μM, red column) for 30 min. The fluorescence intensity changes at 536 nm (λ_{ex} = 446 nm), 600 nm (λ_{ex} = 510 nm).

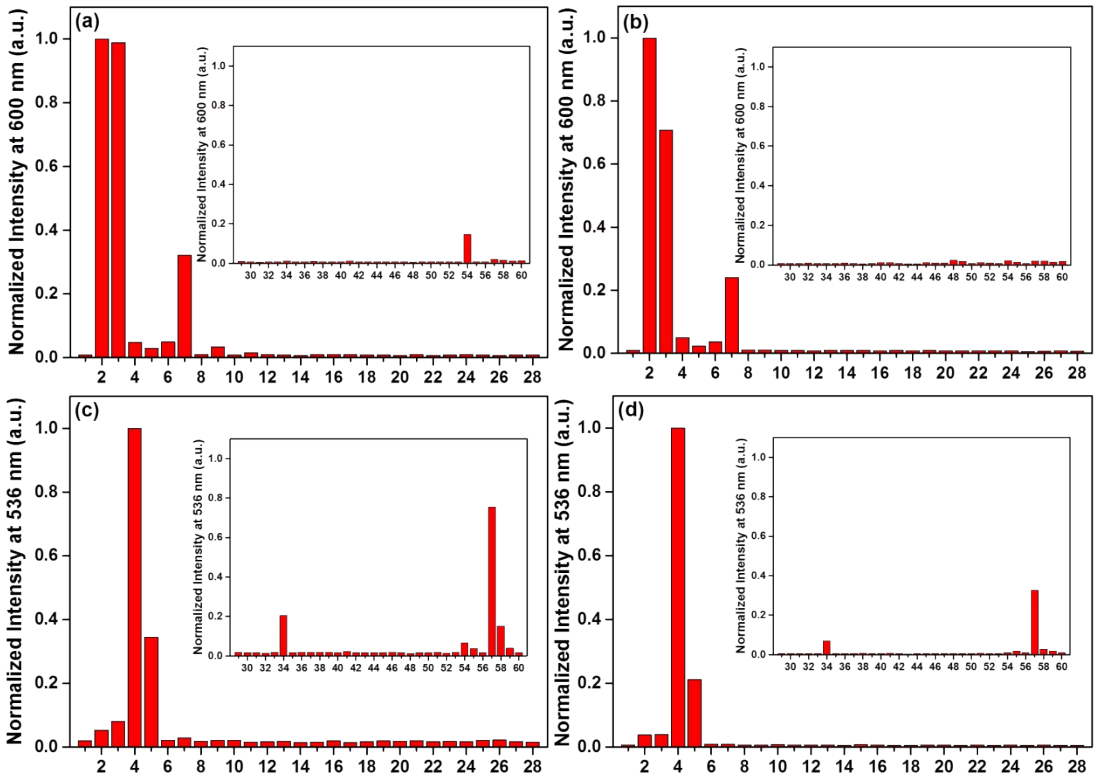


Fig. S5. Fluorescence spectra of Probe 1 (a, c) and Probe 2 (b, d) (10 μM) upon addition of various amino acids (100 μM) and representative ions (100 μM) in EtOH-PBS (pH: 7.4, 10 mM, v/v, 5/5) at room temperature. Each spectrum was recorded 30 min after addition of the corresponding species. They are in turn: probe, Cys, Hcy, GSH, NAC, L-Thr, Gly, L-Asp, L-His, L-Try, L-Glu, Fe^{3+}, Zn^{2+}, Cu^{2+}, Pb^{2+}, Mg^{2+}, Ag^{+}, Ca^{2+}, Ba^{2+}, Na^{+}, Ni^{2+}, K^{+}, Co^{2+}, Ti^{2+}, Al^{3+}, Cr^{3+}, Mn^{2+}, NH_4^{+}, Zr^{4+}, Sn^{2+}, Li^{+}, Bi^{+}, Cd^{2+}, $S_2O_3^{2-}$, Br^{-}, F^{-}, CO_3^{2-}, CH_3COO^{-}, $Cr_2O_7^{2-}$, $H_2PO_4^{-}$, CrO_4^{2-}, HPO_4^{2-}, Cl^{-}, I^{-}, SCN^{-}, SO_4^{2-}, $S_2O_5^{2-}$, IO_4^{-}, ClO_4^{-}, SO_3^{2-}, NO_2^{-}, NO_3^{-}, HSO_3^{-}, N_3^{-}, S^{2-}, HS^{-}, Mercaptoacetic acid, 3-Mercaptopropionic acid, GSSG, Na_2S_2. λ_{ex} = 510 nm (a, b), 446 nm (c, d).

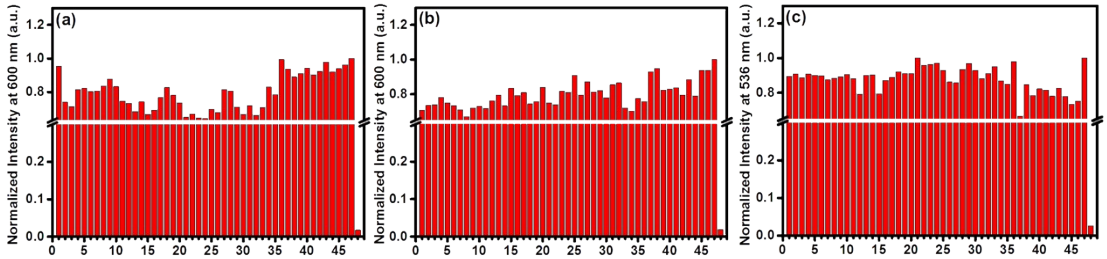


Fig. S6. Fluorescence response of Probe 1 (10 μM) to (a) Cys, (b) Hcy, (c) GSH in the presence of various analytes (100 μM) (including: NAC, L-Thr, Gly, L-Asp, L-His, L-Try, L-Glu, Fe^{3+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Na^{+}, K^{+}, Al^{3+}, Cr^{3+}, Mn^{2+}, NH_4^{+}, Zr^{4+}, Sn^{2+}, Li^{+}, Bi^{+}, Cd^{2+}, $S_2O_3^{2-}$, Br^{-}, F^{-}, CH_3COO^{-}, $Cr_2O_7^{2-}$, $H_2PO_4^{-}$, HPO_4^{2-}, I^{-}, SCN^{-}, SO_4^{2-}, $S_2O_5^{2-}$, IO_4^{-}, ClO_4^{-}, SO_3^{2-}, NO_2^{-}, NO_3^{-}, N_3^{-}, S_2^{-}, HS^{-}, GSSG, Na_2S_2, Thioglycolic acid, 3-Mercaptopropionic acid, probe, only probe). λ_{ex} = 510 nm (a, b), 446 nm

(c).

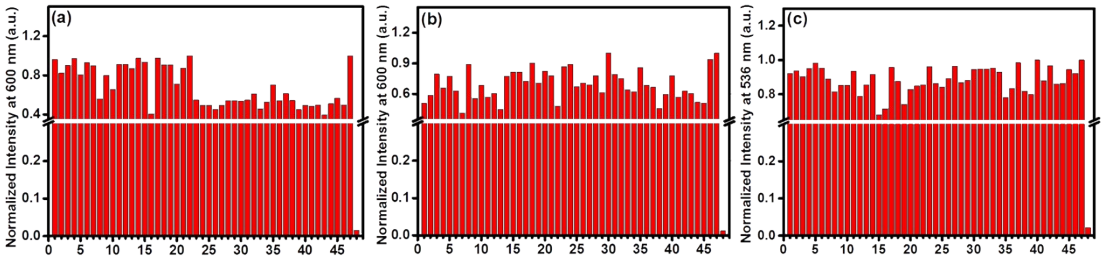


Fig. S7. Fluorescence response of Probe 2 (10 μM) to (a) Cys, (b) Hcy, (c) GSH in the presence of various analytes (100 μM) (including: NAC, L-Thr, Gly, L-Asp, L-His, L-Try, L-Glu, Fe^{3+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Na^{+}, K^{+}, Al^{3+}, Cr^{3+}, Mn^{2+}, NH_4^{+}, Zr^{4+}, Sn^{2+}, Li^{+}, Bi^{+}, Cd^{2+}, $S_2O_3^{2-}$, Br^{-}, F^{-}, CH_3COO^{-}, $Cr_2O_7^{2-}$, $H_2PO_4^{-}$, HPO_4^{2-}, I^{-}, SCN^{-}, SO_4^{2-}, $S_2O_5^{2-}$, IO_4^{-}, ClO_4^{-}, SO_3^{2-}, NO_2^{-}, NO_3^{-}, N_3^{-}, S_2^{-}, HS^{-}, GSSG, Na_2S_2, Thioglycolic acid, 3-Mercaptopropionic acid, probe, only probe). λ_{ex} = 510 nm (a, b), 446 nm (c).

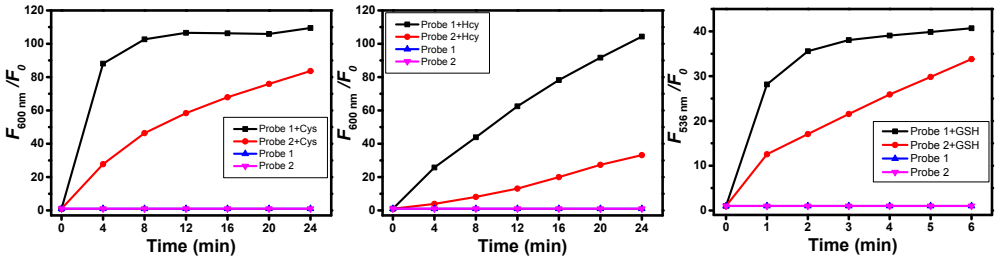


Fig. S8. Time-dependent fluorescence intensity changes at 600 nm (Cys, Hcy) and 536 nm (GSH) against reaction time of Probe 1 and Probe 2 (10 μM) toward Cys, Hcy and GSH (100 μM). The fluorescence intensity changes at 536 nm (λ_{ex} = 446 nm), 600 nm (λ_{ex} = 510 nm).

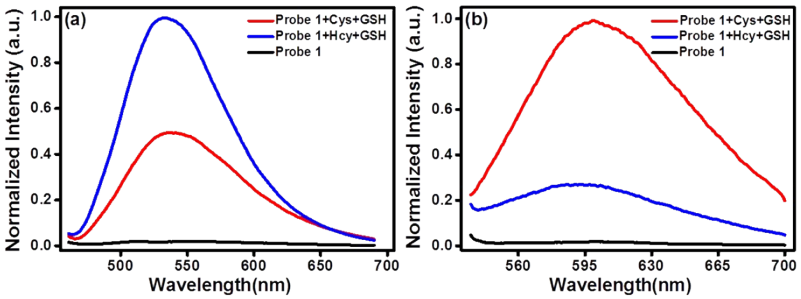


Fig. S9. Fluorescence spectra of Probe 1 (10 μM) upon addition of various analytes (100 μM) in EtOH-PBS (pH 7.4, 10 mM, v/v, 5/5) at room temperature. (a) λ_{ex} = 446 nm , (b) λ_{ex} = 510 nm.

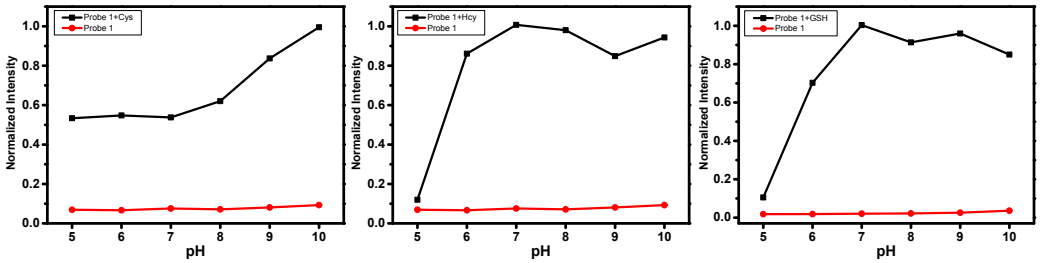


Fig. S10. The influence of pH on fluorescence responses of Probe 1 (10 μM) in EtOH-PBS (pH = 7.4, 10 mM, v/v, 5/5) at room temperature, the pH were adjusted by NaOH (aq, 1M) or HCl (aq, 1M), fluorescence responses are shown before (red line) and after (black line) the addition of Cys, Hcy, GSH (100 μM), respectively. The fluorescence intensity changes at 536 nm (λ_{ex} = 446 nm), 600 nm (λ_{ex} = 510 nm).

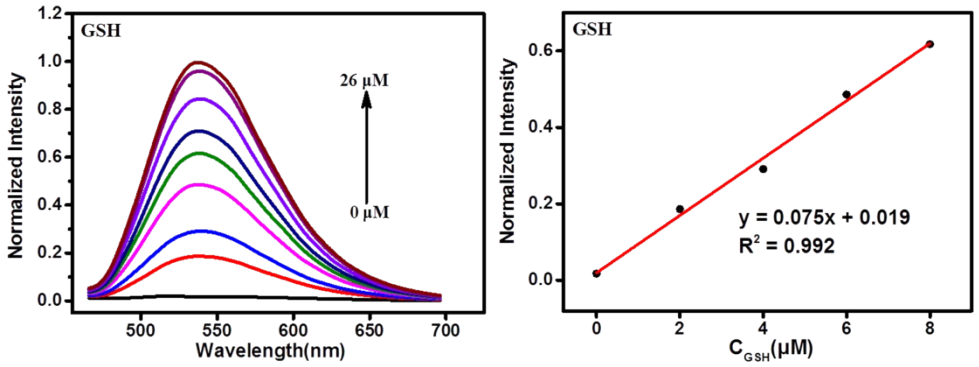


Fig. S11. Fluorescence intensity spectra (left) of Probe 1 (10 μM) in the presence of 0 μM to 26 μM of GSH in EtOH-PBS (pH = 7.4, 10 mM, v/v, 5/5) at room temperature. Each spectrum was recorded after 30 min. λ_{ex} = 446 nm, λ_{em} = 536 nm. The linear changes of the fluorescence intensity (right) of Probe 1 at 536 nm and as a function of GSH concentration.

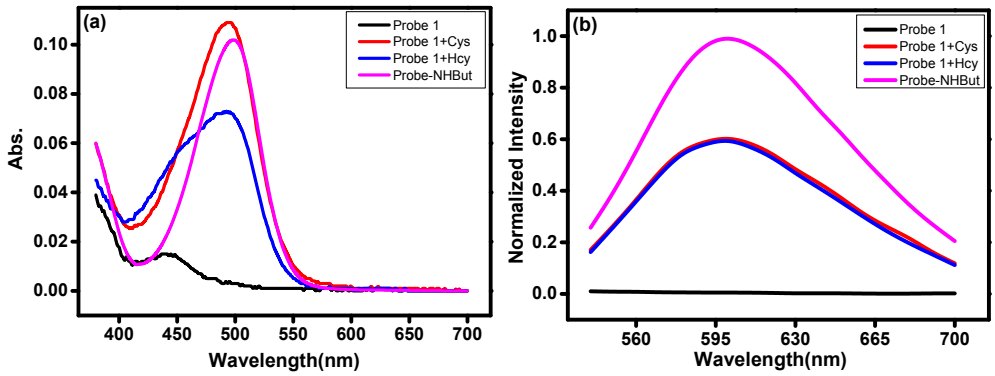


Fig. S12. (a) Absorption spectra, (b) Fluorescence spectra of Probe 1 (10 μM) upon addition of 10 equiv. Cys/Hcy for 30 min and Probe-NHBut (10 μM) in EtOH-PBS (pH 7.4, 10 mM, v/v, 5/5) at room temperature. λ_{ex} = 510 nm.

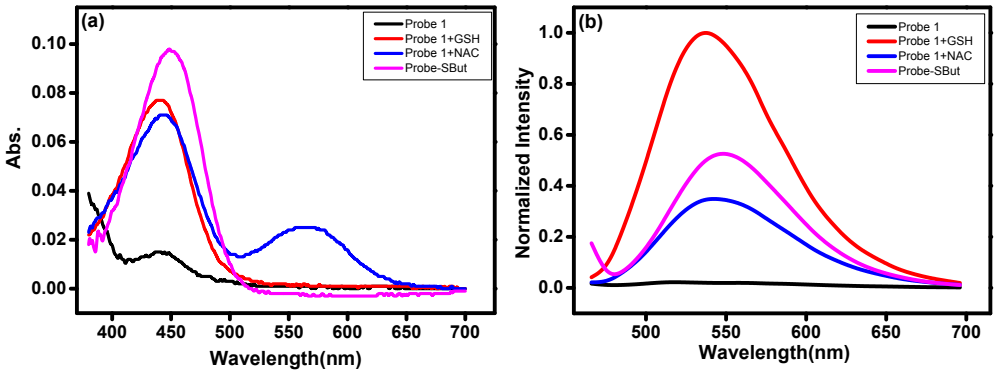


Fig. S13. (a) Absorption spectra, (b) Fluorescence spectra of probe Probe 1 (10 μM) upon addition of 10 equiv. Cys/Hcy for 30 min and Probe-SBut (10 μM) in EtOH-PBS (pH 7.4, 10 mM, v/v, 5/5) at r.t. λ_{ex} = 446 nm.

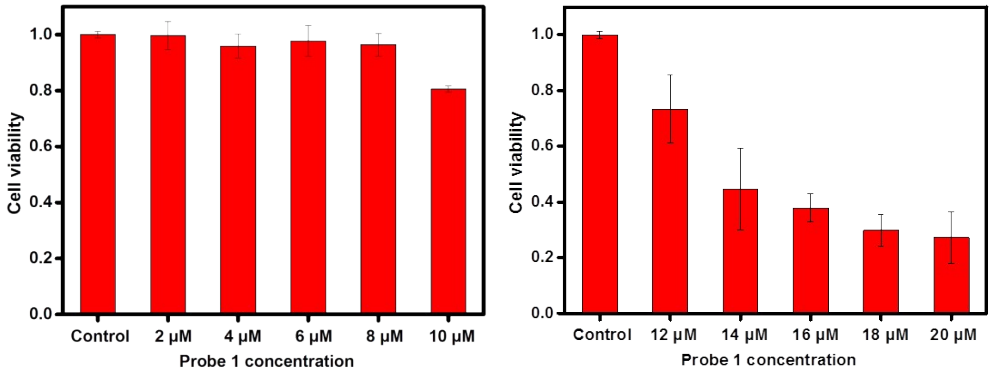


Fig. S14. MTT assay for the viability of HepG2 cells treated with various concentrations of Probe 1 (0-20 μM).

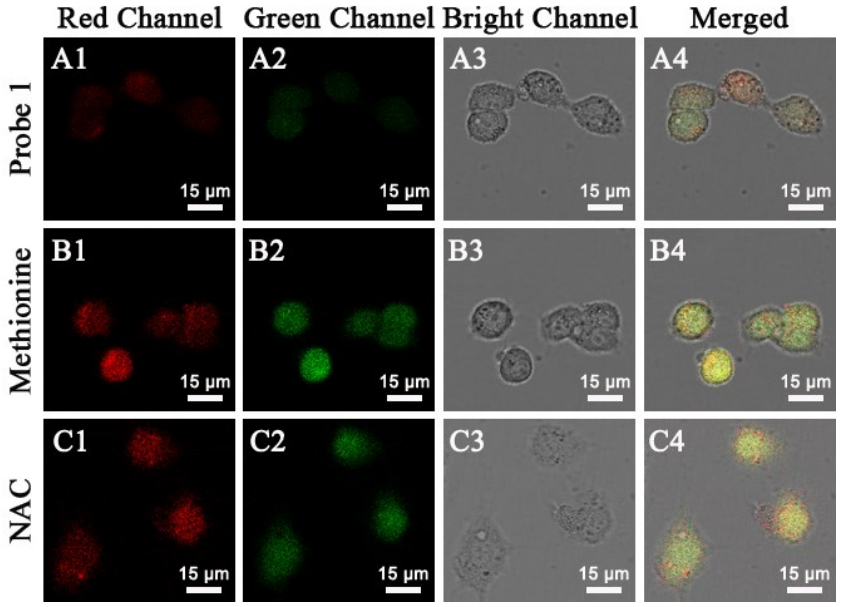


Fig. S15. Confocal fluorescence images of endogenous Cys/Hcy, GSH in HepG2 cells. (A1-A4) HepG2 cells were pretreated with Probe 1 (5 μM, 60 min), then imaged. (B1-B4) HepG2 cells were

pretreated with Methionine (100 μM, 2 h), subsequently incubated with Probe 1 (5 μM, 60 min), then imaged. (C1-C4) HepG2 cells were pretreated with NAC (100 μM, 2 h), subsequently incubated with Probe 1 (5 μM, 60 min), then imaged. (λ_{ex} = 514 nm, λ_{em} = 560-650 nm for the red channel; λ_{ex} = 458 nm, λ_{em} = 500-560 nm for the green channel). Scale bar: 15 μm.

III. NMR chart

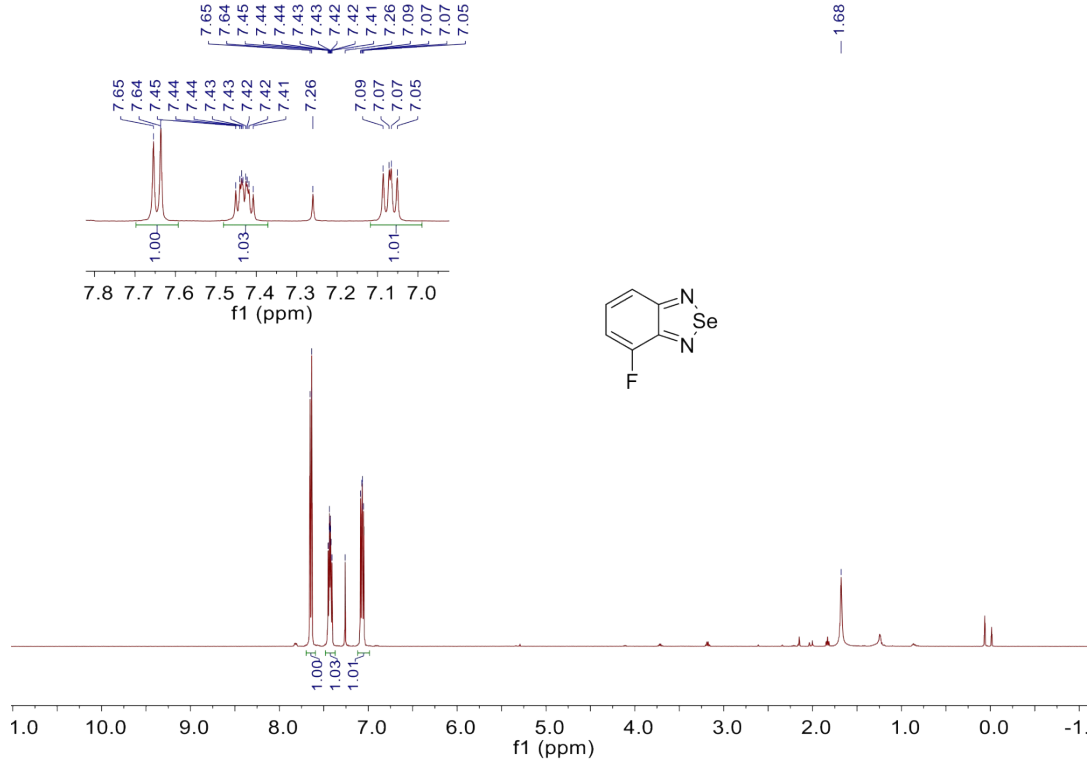


Fig. S16. ^{1}H NMR spectrum of compound 1 in $CDCl_3$.

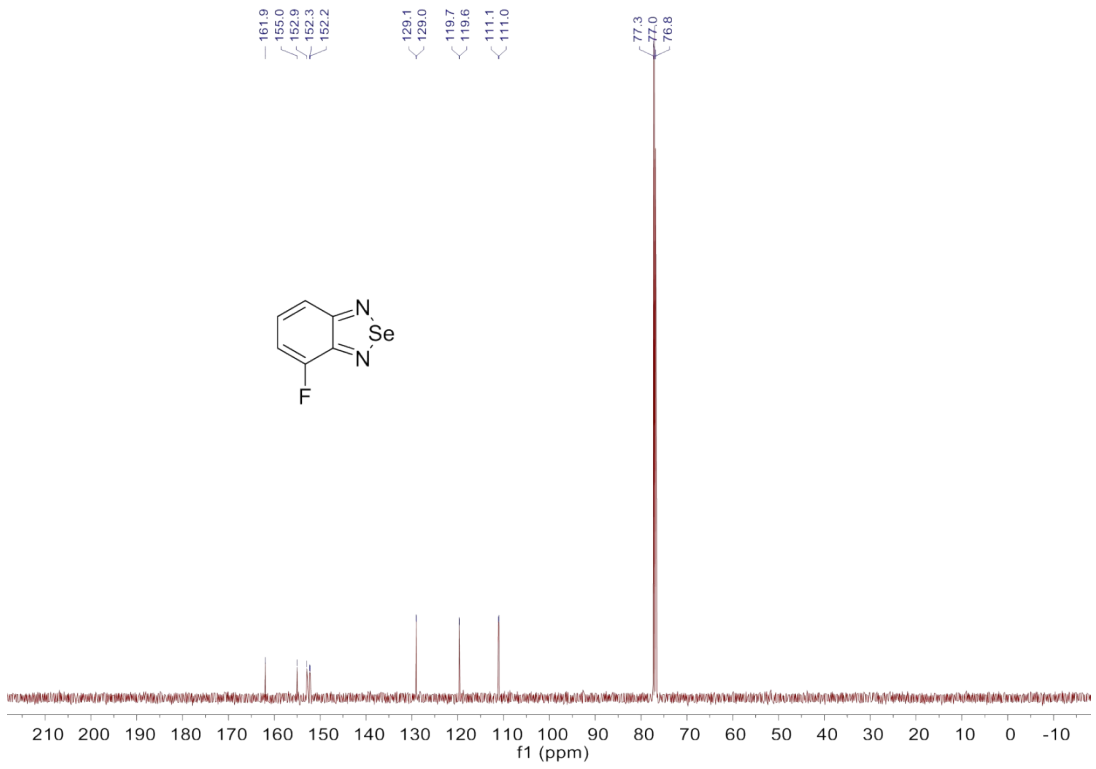


Fig. S17. ^{13}C NMR spectrum of compound 1 in $CDCl_3$.

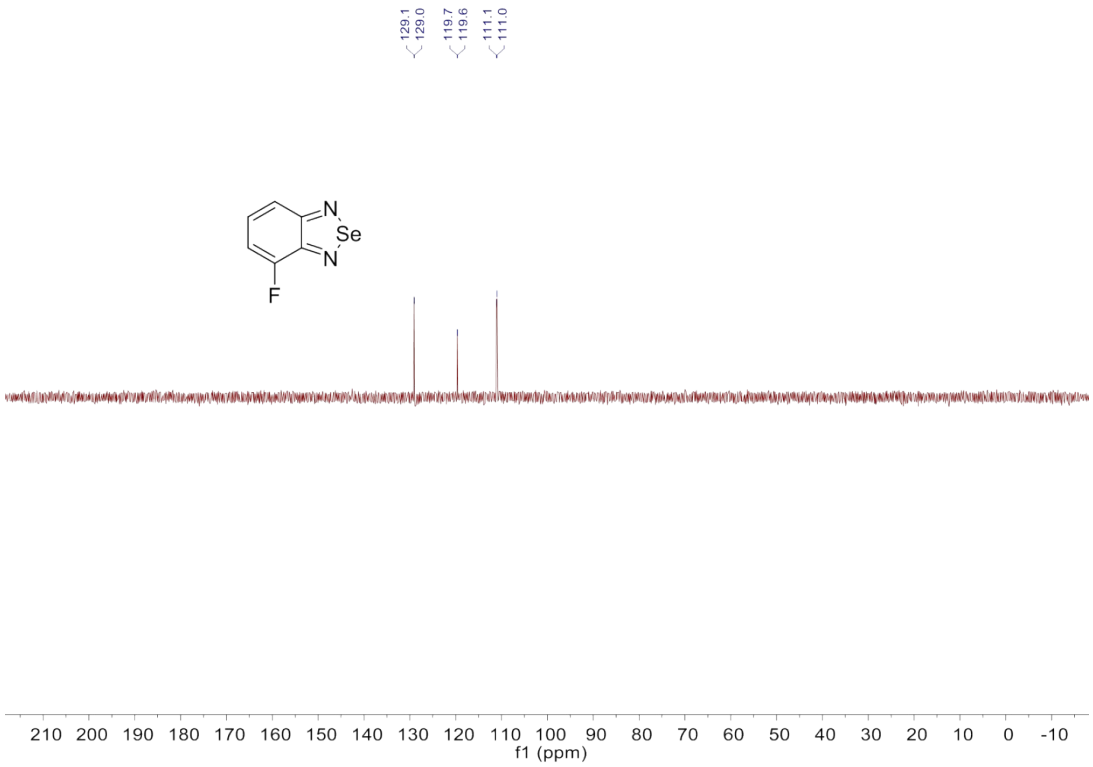


Fig. S18. ^{13}C-DEPT135 NMR spectrum of compound 1 in $CDCl_3$.

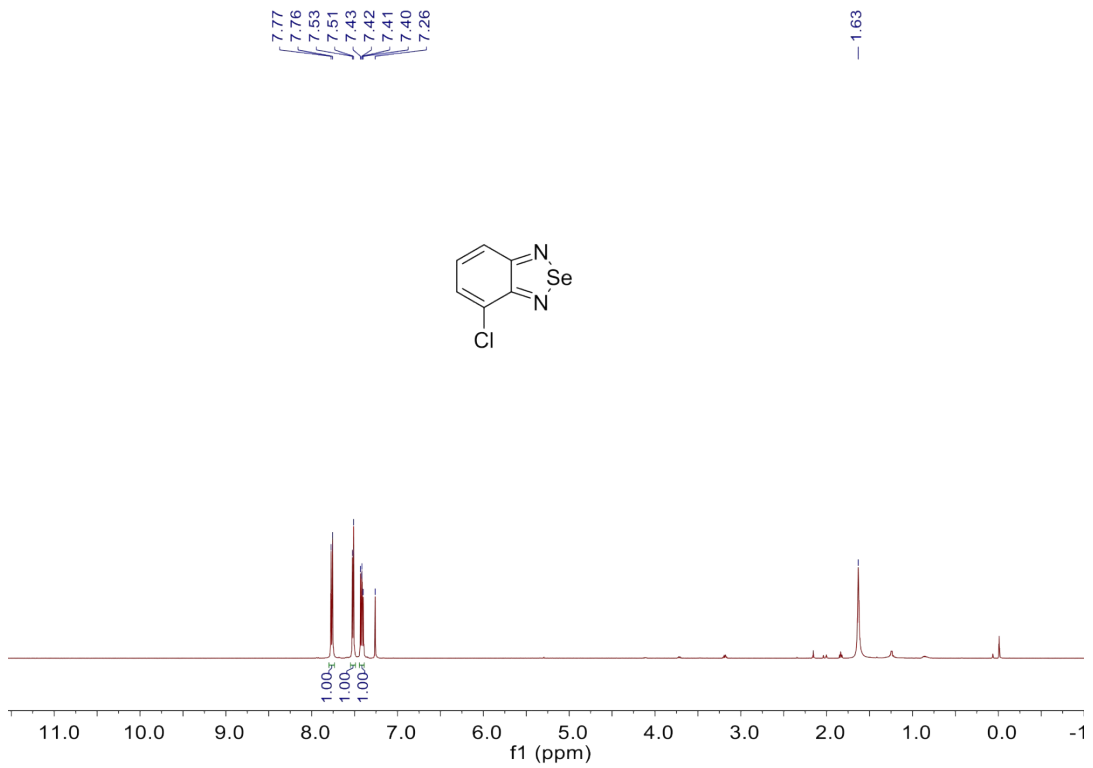


Fig. S19. ^{1}H NMR spectrum of compound 2 in $CDCl_3$.

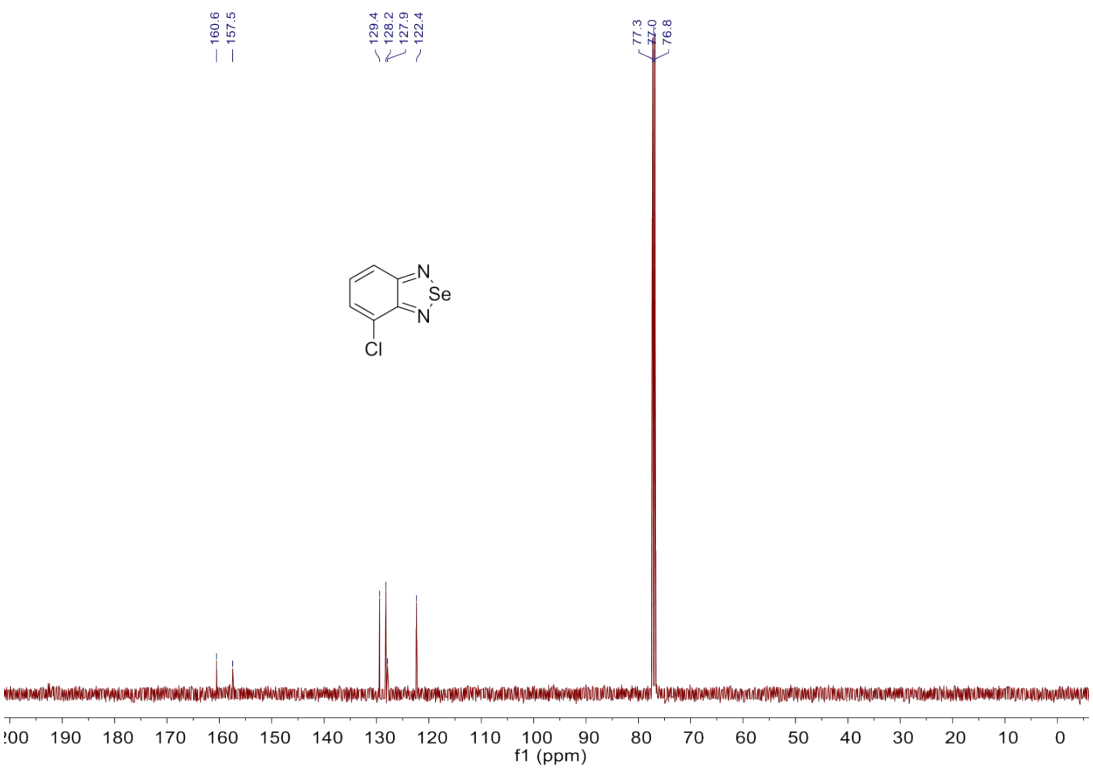


Fig. S20. ^{13}C NMR spectrum of compound 2 in $CDCl_3$.

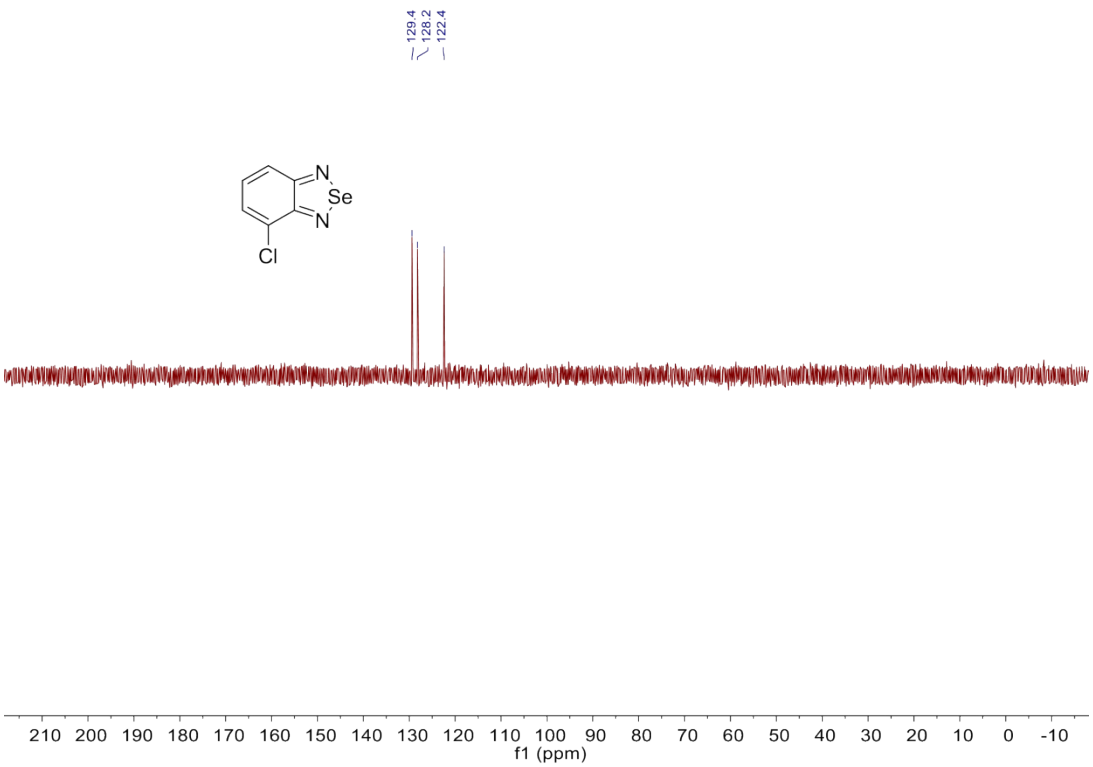


Fig. S21. ^{13}C-DEPT135 NMR spectrum of compound 2 in $CDCl_3$.

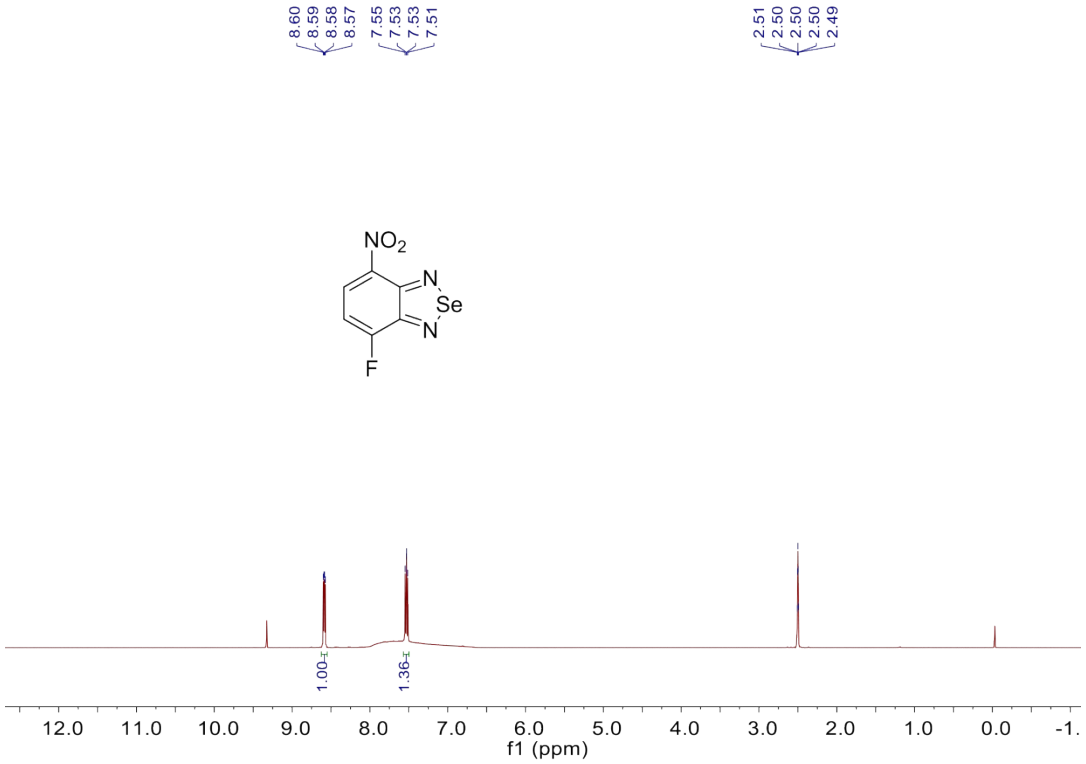


Fig. S22. ^{1}H NMR spectrum of Probe 1 in DMSO-d_6.

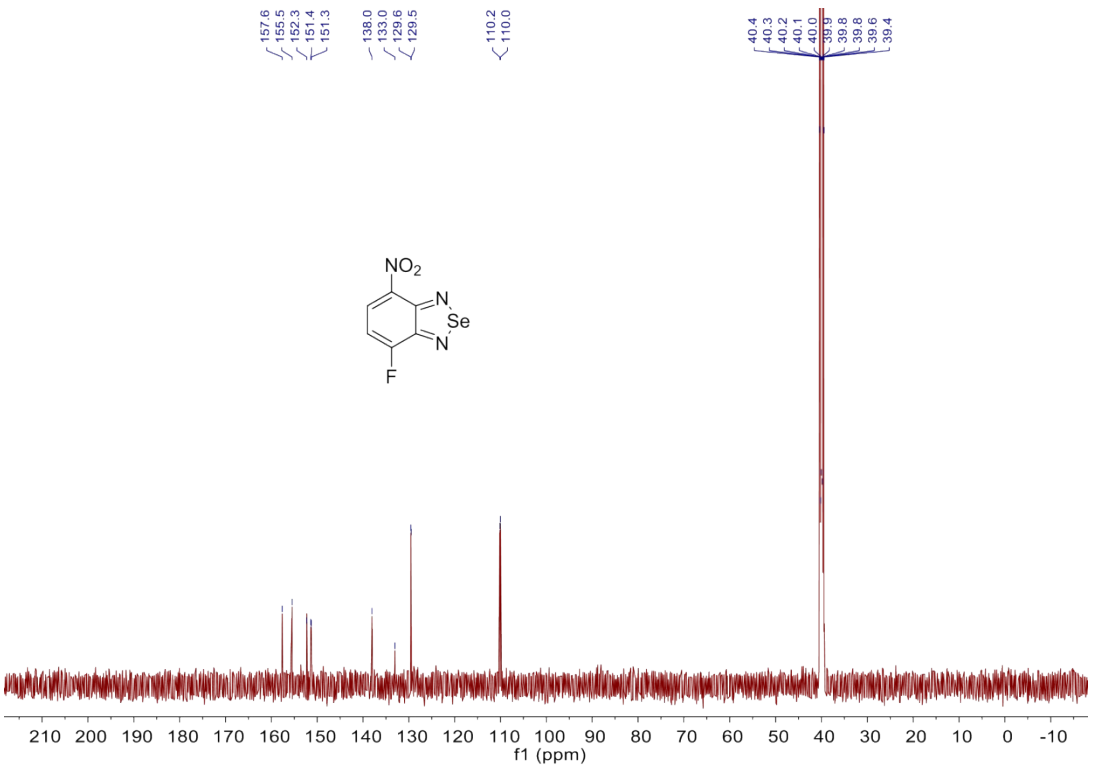


Fig. S23. ^{13}C NMR spectrum of Probe 1 in DMSO-d_6.

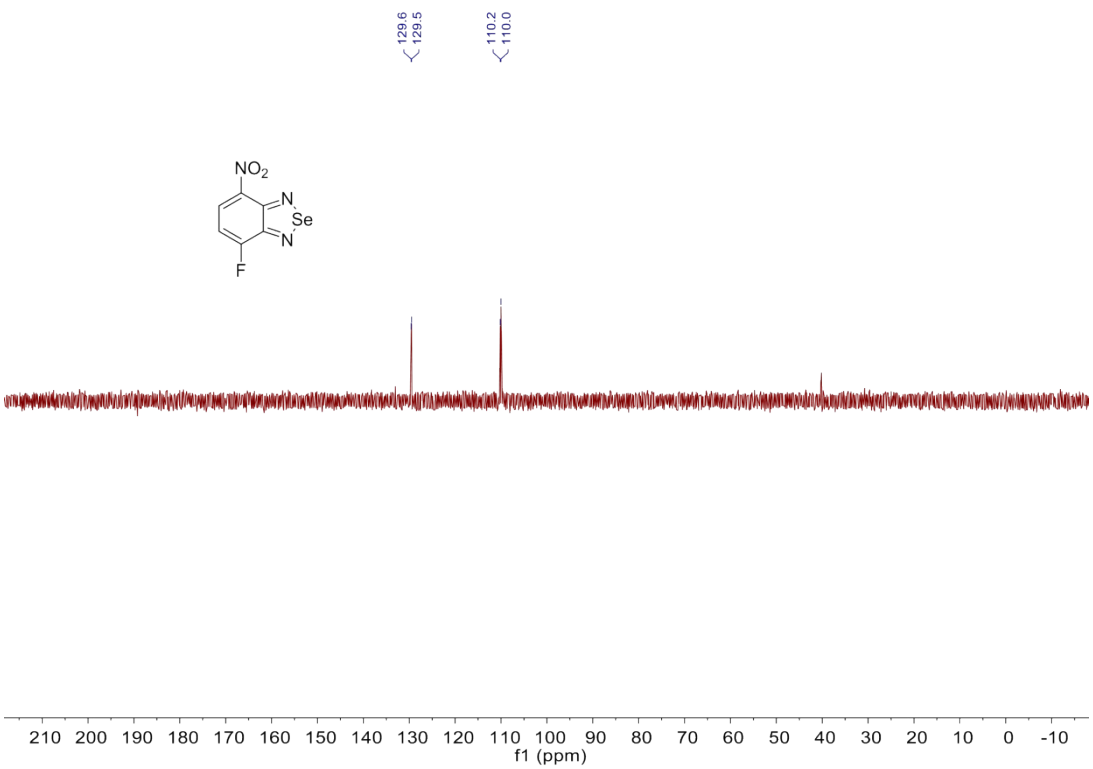


Fig. S24. ^{13}C-DEPT135 NMR spectrum of Probe 1 in DMSO-d_6.

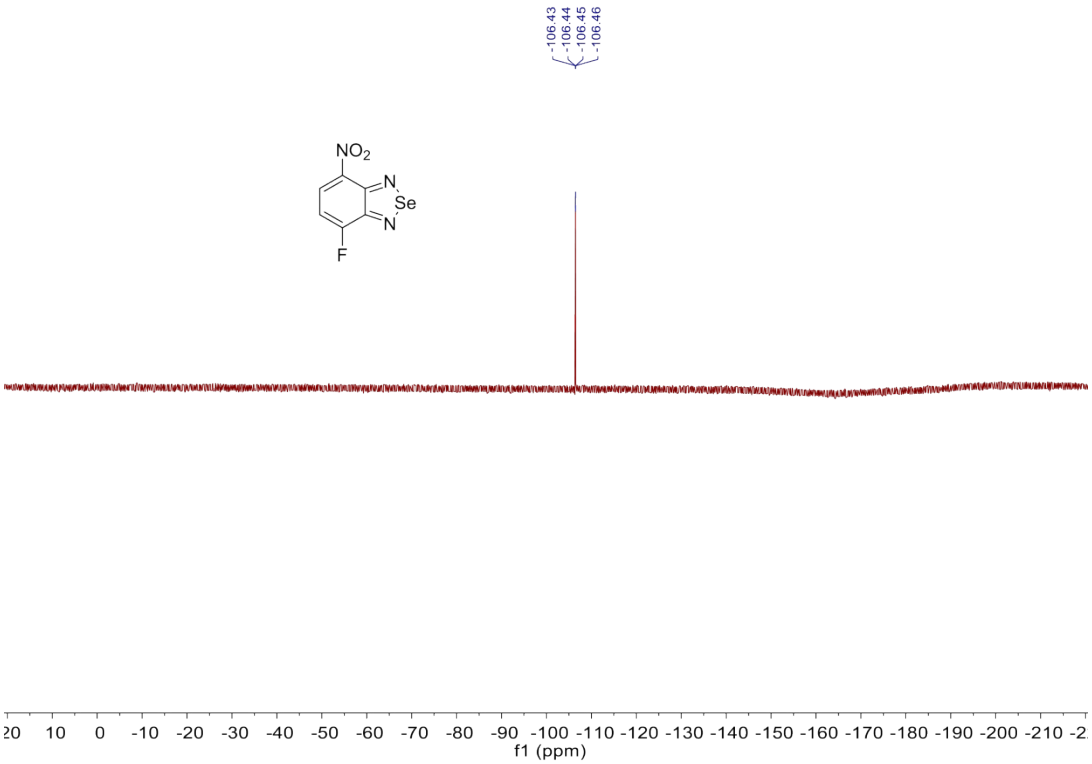


Fig. S25. ^{19}F NMR spectrum of Probe 1 in DMSO-d_6.

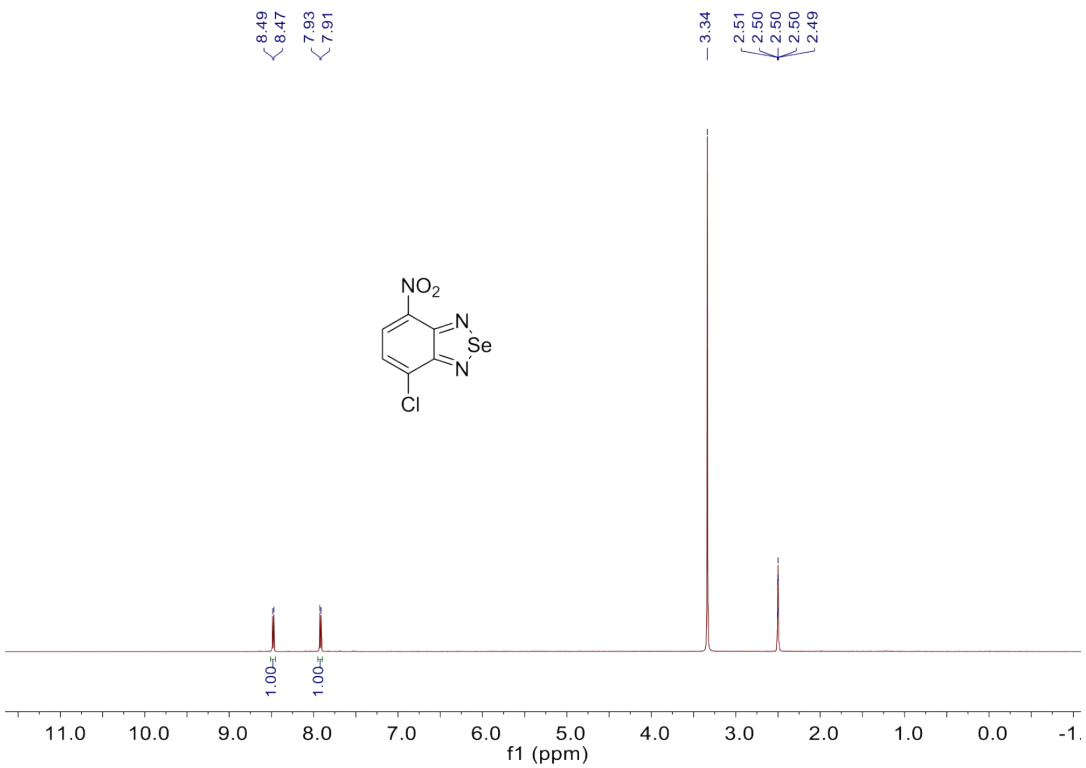


Fig. S26. ^{1}H NMR spectrum of Probe 2 in DMSO-d_6.

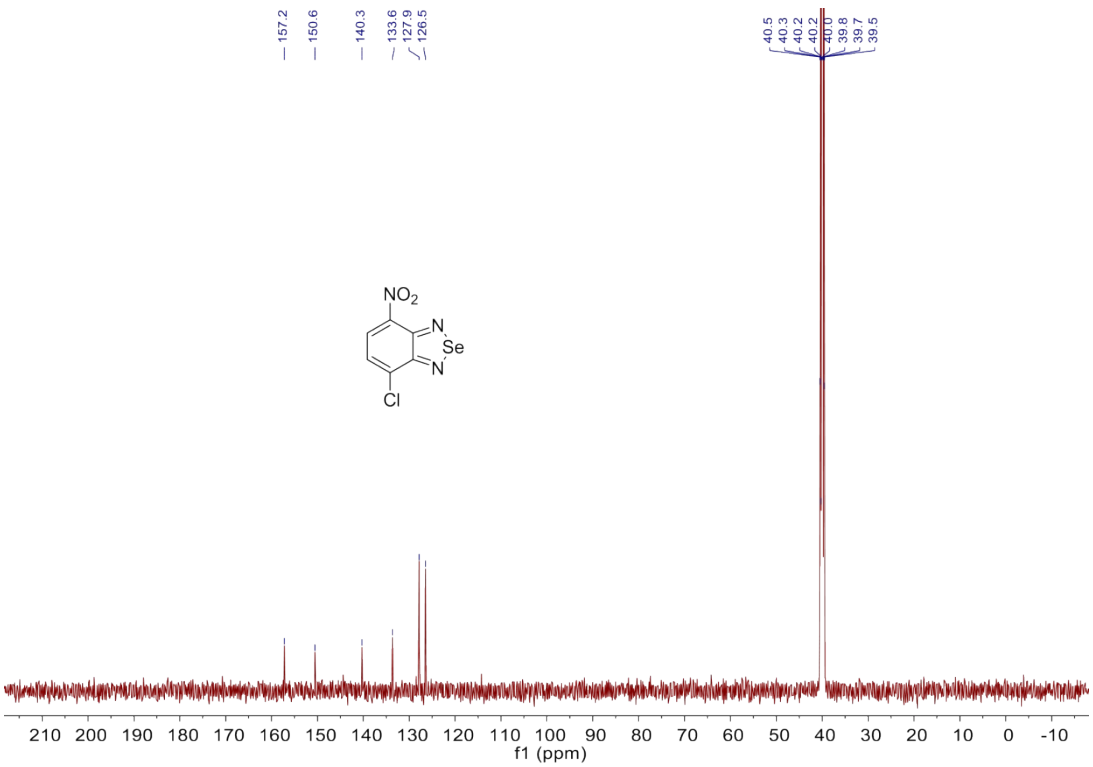


Fig. S27. ^{13}C NMR spectrum of Probe 2 in DMSO-d_6.

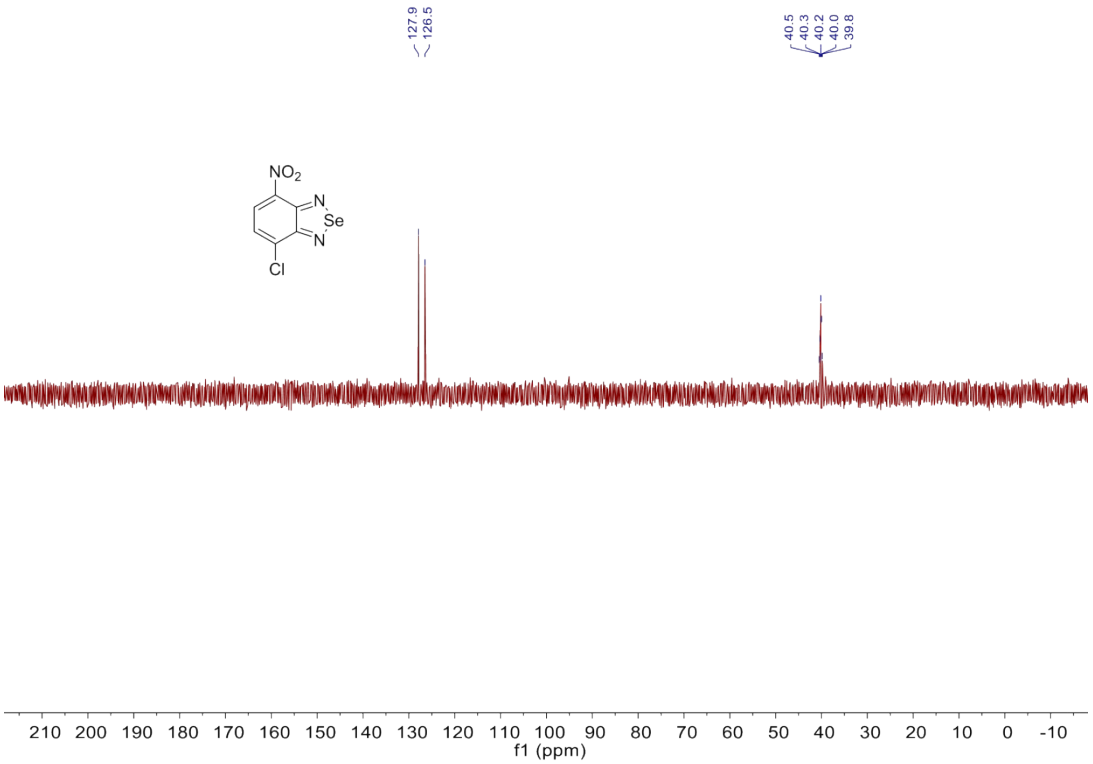


Fig. S28. ^{13}CDEPT135 NMR spectrum of Probe 2 in DMSO-d_6.

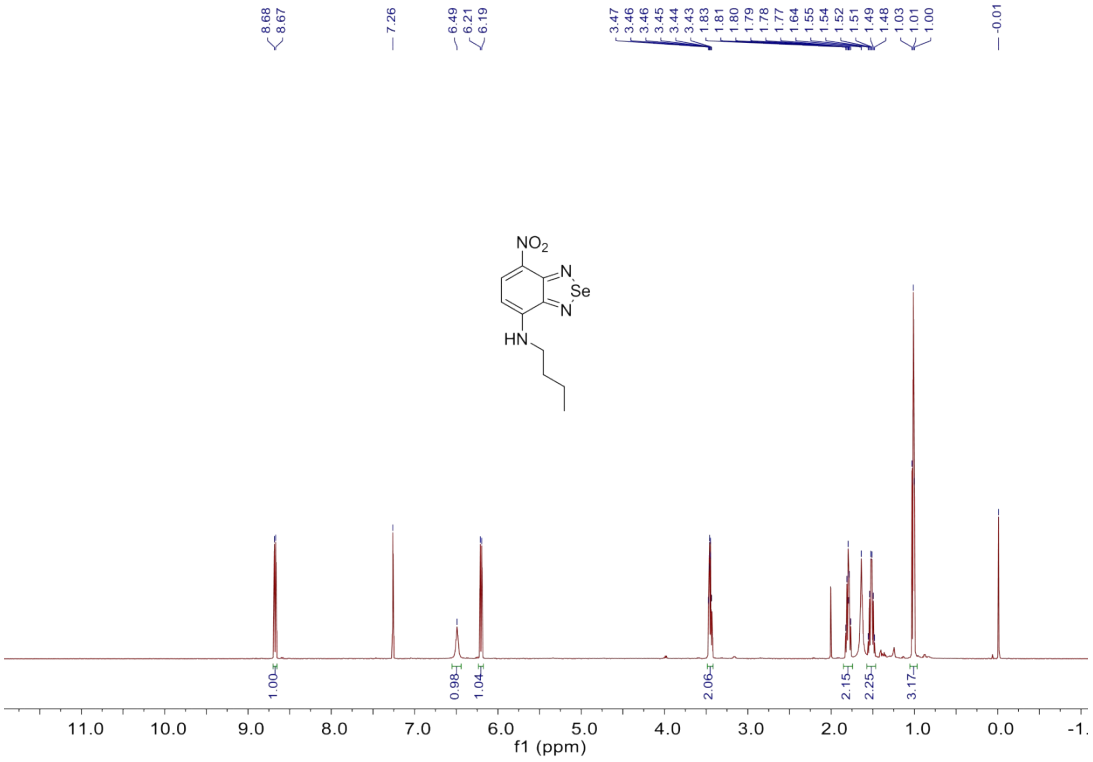


Fig. S29. ^{1}H NMR spectrum of Probe-NHBut in $CDCl_3$.

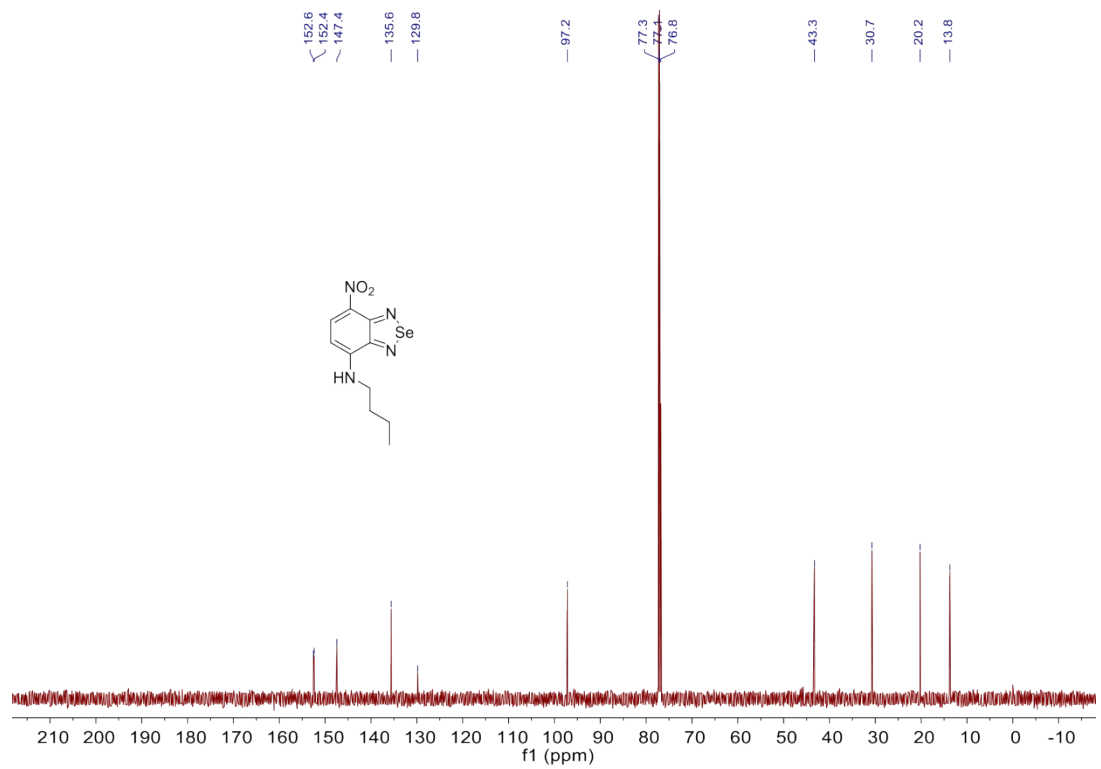


Fig. S30. ^{13}C NMR spectrum of Probe-NHBut in $CDCl_3$.

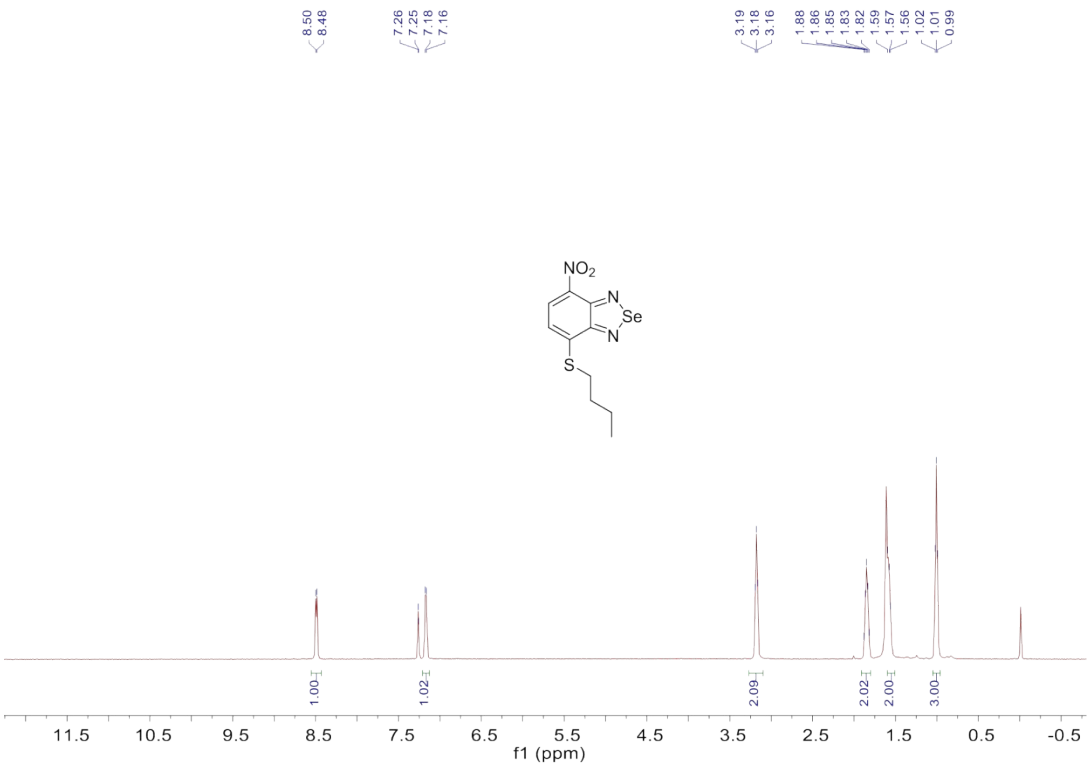


Fig. S31. ^{1}H NMR spectrum of Probe-SBut in $CDCl_3$.

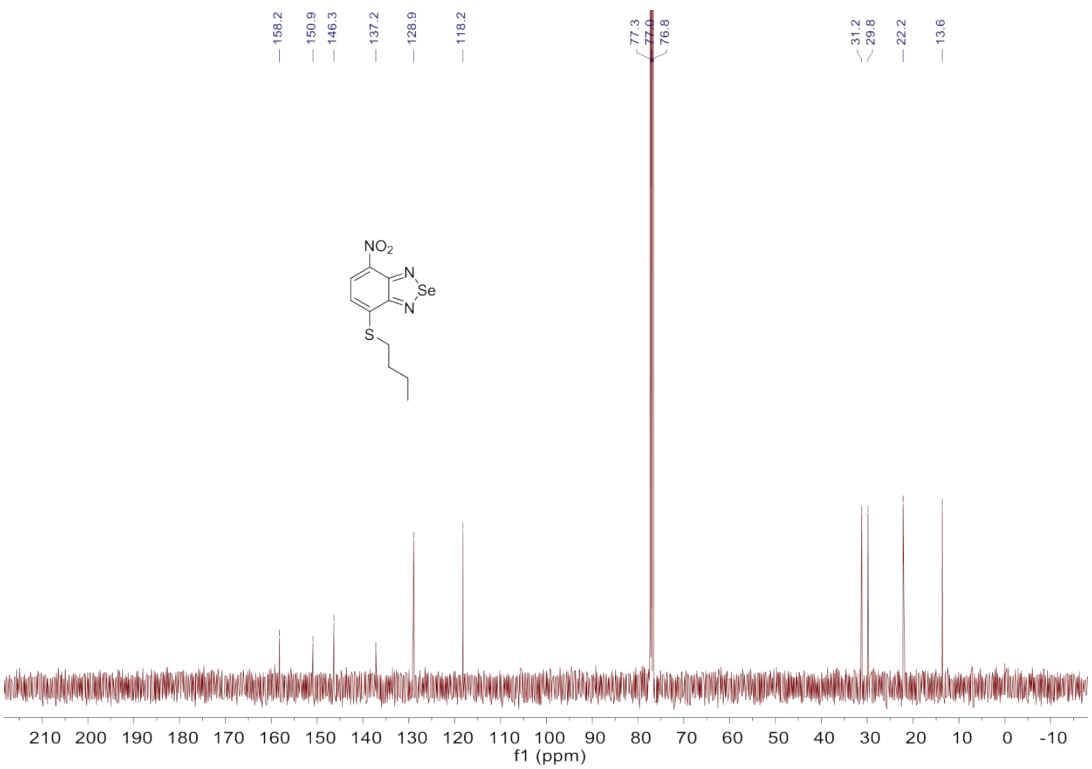


Fig. S32. ^{13}C NMR spectrum of Probe-SBut in $CDCl_3$.