Supplemental Information

A highly sensitive and selective two-photon fluorescent probe for glutathione S-transferase detection and imaging in living cells and tissues

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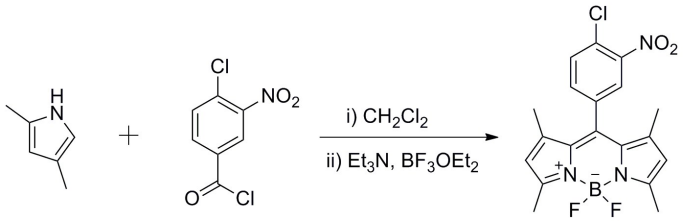
Synthesis of Compounds

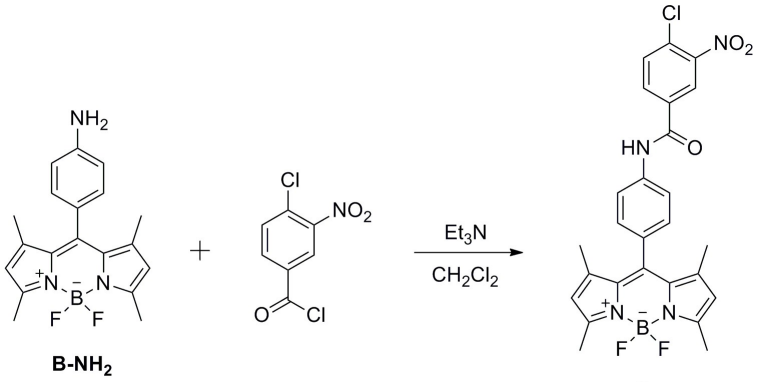
Synthesis of probe 1. Probe **1** was synthesized according to the literature[1]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, *J* = 8.2 Hz, 1H), 7.91 (s, 1H), 7.77 (dd, *J* = 8.2, 1.3 Hz, 1H), 6.06 (s, 2H), 2.57 (s, 6H), 1.43 (s, 6H).



2

Scheme S1. Synthetic scheme for probe **2**.

Probe **2**: To a dry dichloromethane (CH_2Cl_2) solution (150 mL) of 2, 4-dimethylpyrrole (951 mg, 10 mmol) at 0 ˚C under an argon atmosphere, a solution of 4-chloro-3-nitrobenzoyl chloride (770 mg, 3.5 mmol) in dry CH_2Cl_2 (50 mL) was added dropwise over 1 h, the mixture was slowly warmed to room temperature (RT), and stirred for 12 h. With the sequential addition of Et_3N (2.1 mL, 15 mmol) and $BF_3{\cdot}OEt_2$ (3.1 mL, 24.1 mmol), the mixture was stirred at RT for another 12 h. After removing the solvent, the residues were further purified by a silica gel column chromatograph using petroleum ether (PE)/CH_2Cl_2 (1/1 *v/v*) as the mobile phase to afford probe **2** as a red solid (113 mg, yield: 8%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (d, *J* = 2.0 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.50 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.03 (s, 2H), 2.56 (s, 6H), 1.45 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 157.08, 148.59, 142.37, 136.41, 135.13, 133.17, 132.80, 130.91, 127.88, 125.80, 122.11, 15.07, 14.69. HRMS (EI) calcd for 403.1070, found 403.1071.

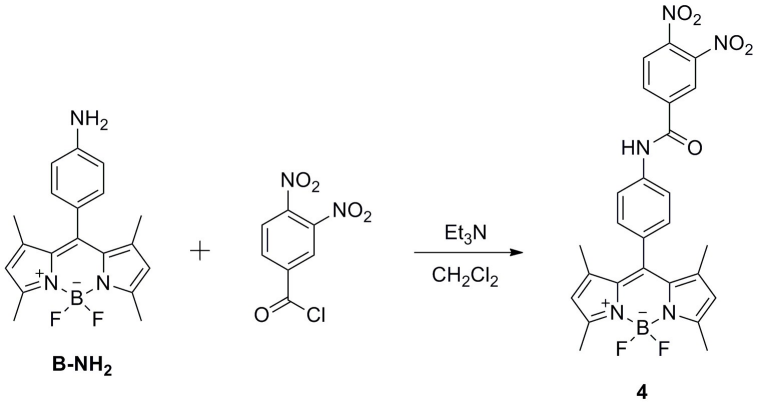


B-NH$_2$ **3**

Scheme S2. Synthetic scheme for probe **3**.

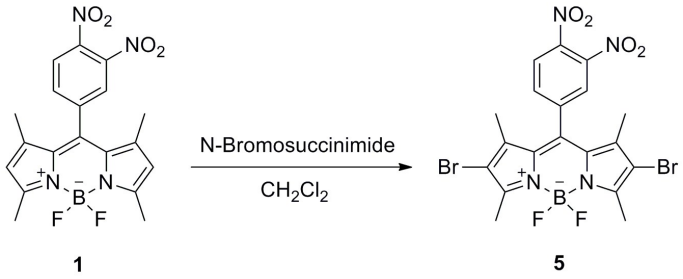
Probe **3**: **B-NH$_2$** was synthesized according to the literature[2]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 (d, *J* = 8.4 Hz, 2H), 6.78 (d, *J* = 8.3 Hz, 2H), 5.97 (s, 2H), 3.83 (s, 2H), 2.54 (s, 6H), 1.49 (s, 6H).

To a solution of **B-NH$_2$** (102 mg, 0.3 mmol) and Et_3N (101 mg, 1 mmol) in 30 mL CH_2Cl_2, a solution of 4-chloro-3-nitrobenzoyl chloride (110 mg, 0.5mmol) in dry CH_2Cl_2 (10 mL) was added at 0˚C, and the reaction mixture was stirred at this temperature for 1 h. After removing the solvent, the residues were further purified by a silica gel column chromatograph using CH_2Cl_2 as the mobile phase to afford probe **3** as an orange solid (133 mg, yield: 85%). ^{1}H NMR (500 MHz, DMSO-*d6*) δ 10.76 (s, 1H), 8.66 (d, *J* = 2.0 Hz, 1H), 8.28 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.99 (dd, *J* = 8.4, 4.2 Hz, 3H), 7.38 (d, *J* = 8.5 Hz, 2H), 6.19 (s, 2H), 2.46 (s, 6H), 1.43 (s, 6H). ^{13}C NMR (125 MHz, DMSO-*d6*) δ 162.83, 154.78, 147.34, 142.67, 141.75, 139.47, 134.86, 132.88, 131.93, 130.87, 129.47, 128.39, 128.11, 124.91, 121.32, 120.69, 14.18. HRMS (ESI positive) calcd for $[M+H]^+$ 523.1520, found 523.1502.



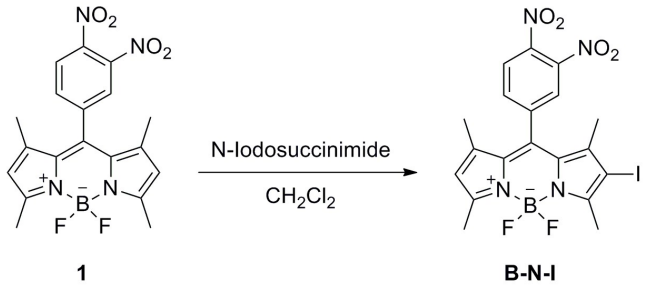
Scheme S3. Synthetic scheme for probe **4**.

Probe **4**: Similar to the synthetic procedures for probe **3**, probe **4** was synthesized using **B-NH$_2$** (102 mg, 0.3 mmol) and 3, 4-dinitrobenzoyl chloride (115.3 mg, 0.5 mmol) as the raw materials. The crude compound was purified over a silica column with CH_2Cl_2 to afford probe **4** as an orange solid (131 mg, yield: 82%). ^{1}H NMR (500 MHz, DMSO-*d6*) δ 10.93 (s, 1H), 8.76 (d, *J* = 1.6 Hz, 1H), 8.51 (dd, *J* = 8.4, 1.6 Hz, 1H), 8.42 (d, *J* = 8.4 Hz, 1H), 7.99 (d, *J* = 8.5 Hz, 2H), 7.41 (d, *J* = 8.5 Hz, 2H), 6.19 (s, 2H), 2.46 (s, 6H), 1.43 (s, 6H). ^{13}C NMR (125 MHz, DMSO-*d6*) δ 162.21, 154.82, 143.30, 142.66, 141.67, 141.60, 139.82, 139.27, 133.85, 130.84, 129.74, 128.48, 125.97, 124.98, 121.34, 120.74, 14.19. HRMS (ESI negative) calcd for $[M-H]^-$ 532.1609, found 532.1622.



Scheme S4. Synthetic scheme for probe **5**.

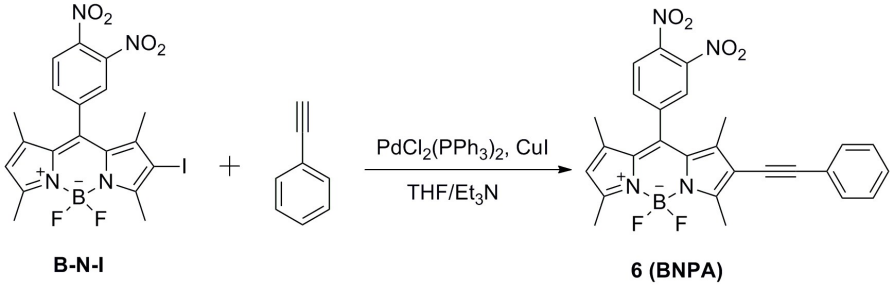
Probe **5**: To a solution of **1** (124 mg, 0.3 mmol) in 30 mL CH_2Cl_2, N-bromosuccinimide (142 mg, 0.8 mmol) was added, and the reaction mixture was stirred at RT for 3 h. After removing the solvent, the residues were further purified by a silica gel column chromatograph using PE/CH_2Cl_2 (3/2 *v/v*) as the mobile phase to afford probe **5** as a red solid (147 mg, yield: 86%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 (d, *J* = 8.2 Hz, 1H), 7.90 (d, *J* = 1.7 Hz, 1H), 7.76 (dd, *J* = 8.2, 1.7 Hz, 1H), 2.63 (s, 6H), 1.44 (s, 6H).^{13}C NMR (125 MHz, $CDCl_3$) δ 156.42, 143.75, 142.75, 140.94, 139.47, 134.96, 133.37, 129.49, 126.30, 125.43, 113.24, 14.58, 13.95. HRMS (EI) calcd for 569.9521, found 569.9512.



Scheme S5. Synthetic scheme for **B-N-I**.

B-N-I: To a solution of **1** (124 mg, 0.3 mmol) in 30 mL CH_2Cl_2, N-iodosuccinimide (90 mg, 0.4 mmol) was added, and the reaction mixture was stirred at RT overnight until the starting material was consumed. After removing the solvent, the residues were further purified by a silica gel column chromatograph using PE/CH_2Cl_2 (2/1 *v/v*) as

the mobile phase to afford **B-N-I** as a red solid (120 mg, yield: 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 8.2 Hz, 1H), 7.90 (d, *J* = 1.6 Hz, 1H), 7.76 (dd, *J* = 8.2, 1.7 Hz, 1H), 6.13 (s, 1H), 2.64 (s, 3H), 2.58 (s, 3H), 1.45 (s, 3H), 1.44 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 160.06, 156.99, 143.84, 143.70, 142.58, 142.43, 141.46, 134.51, 133.47, 130.93, 129.90, 126.18, 125.47, 123.62, 85.73, 17.58, 16.02, 15.41, 14.96. HRMS (EI) calcd for 540.0277, found 540.0286.



B-N-I **6 (BNPA)**

Scheme S6. Synthetic scheme for probe **6 (BNPA)**.

Synthesis of probe 6 (BNPA): In a 50 mL Schlenk tube were added **B-N-I** (108 mg, 0.2 mmol), phenylacetylene (102 mg, 1 mmol), $PdCl_2(PPh_3)_2$ (14 mg, 0.02 mmol), CuI (3.8 mg, 0.02 mmol). 8 mL of freshly distilled THF and 4 mL of Et_3N were added and resulting suspension was excessively deaerated by bubbling with Argon for 40 min. After degassing, the reaction mixture was stirred at 55 ˚C for 2 h. Solvents were removed at reduced pressure and the residue was washed with water (150 mL) and extracted into CH_2Cl_2. The organic layer was removed and separation by column chromatography on silica gel using PE/CH_2Cl_2 (2/1 *v/v*) as the eluent to afford **BNPA** as a brown solid (22 mg, yield: 21%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, *J* = 8.2 Hz, 1H), 7.93 (s, 1H), 7.78 (d, *J* = 8.2 Hz, 1H), 7.46 (d, *J* = 3.6 Hz, 2H), 7.37 – 7.29 (m, 3H), 6.12 (s, 1H), 2.72 (s, 3H), 2.60 (s, 3H), 1.56 (s, 3H), 1.46 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.75, 158.84, 143.68, 143.24, 142.55, 141.34, 135.01, 133.48, 131.45, 131.35, 129.30, 128.42, 128.38, 126.12, 125.51, 123.31, 123.07, 116.83, 99.99, 97.05, 80.93, 15.36, 14.93, 14.04, 13.75. HRMS (EI) calcd for 514.1624, found 514.1621.

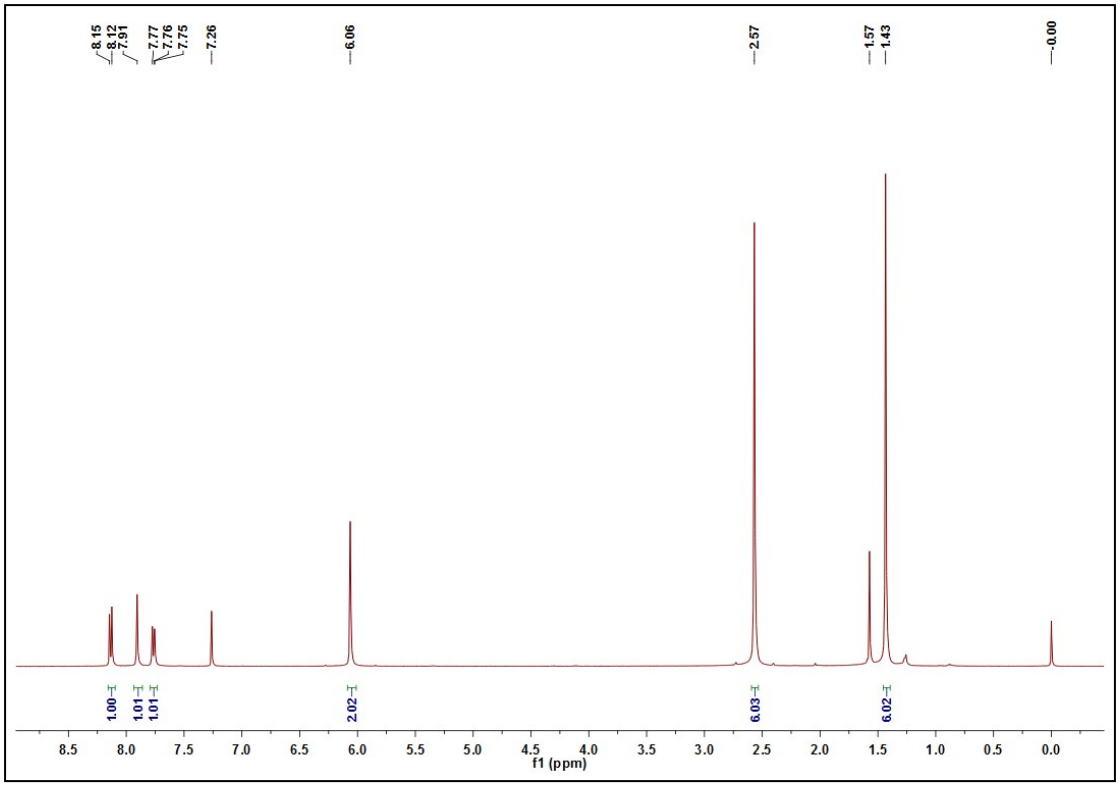


Fig. S1. ^{1}H NMR spectrum of probe **1** in $CDCl_3$.

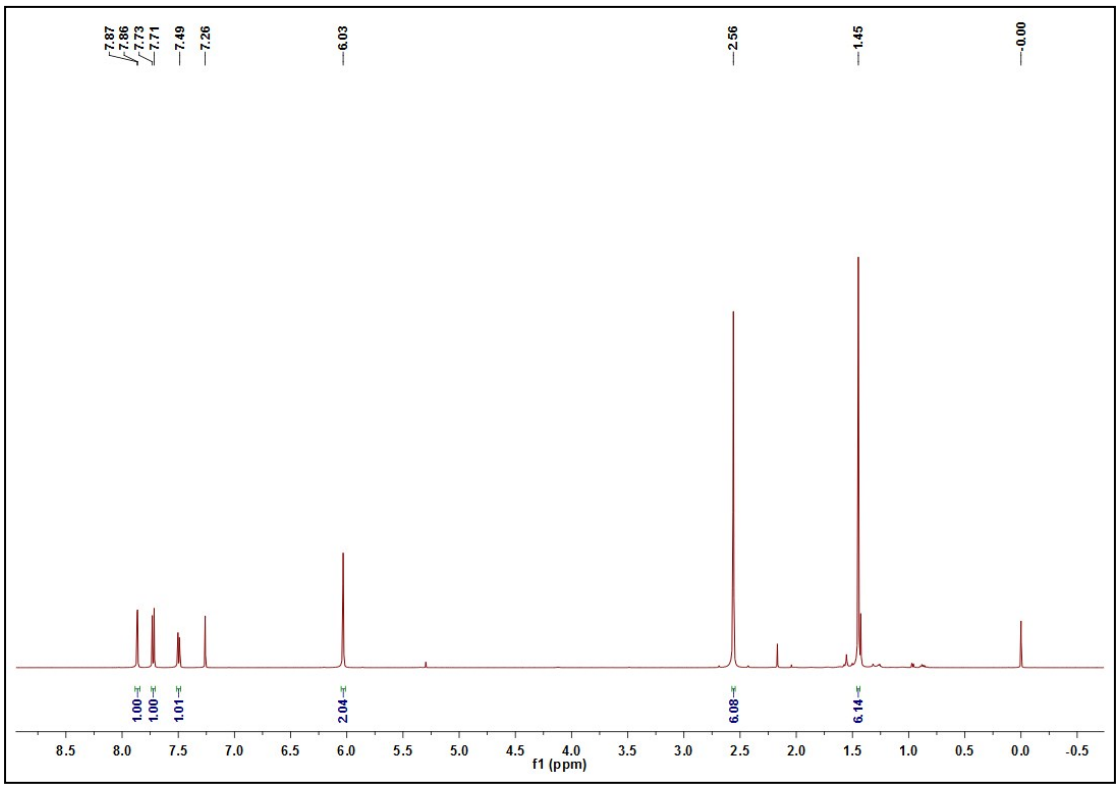


Fig. S2. ^{1}H NMR spectrum of probe **2** in $CDCl_3$.

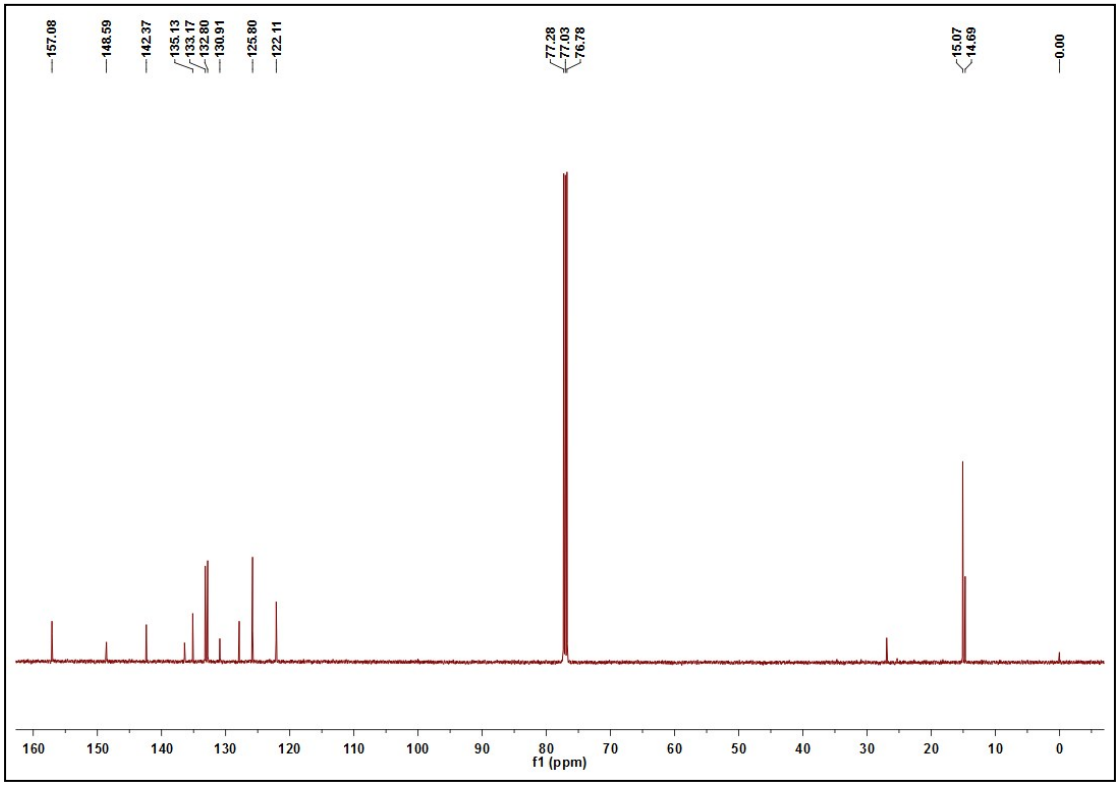


Fig. S3. ^{13}C NMR spectrum of probe **2** in $CDCl_3$.

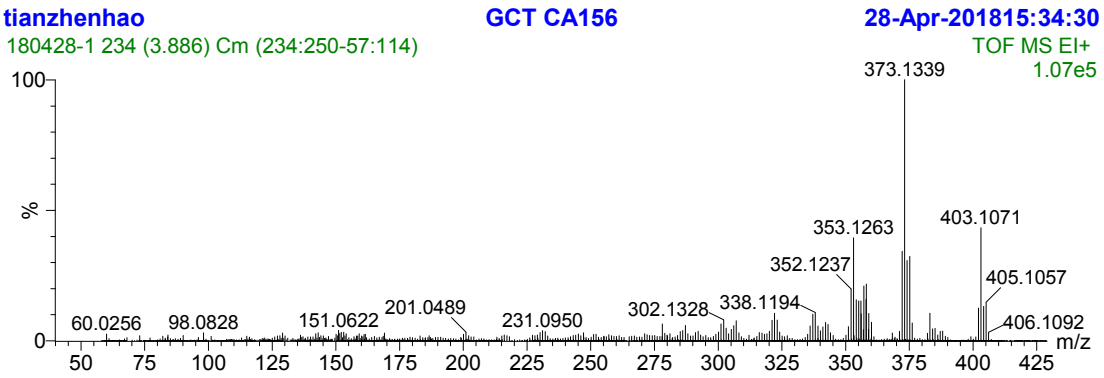


Fig. S4. HRMS spectrum of probe **2**.

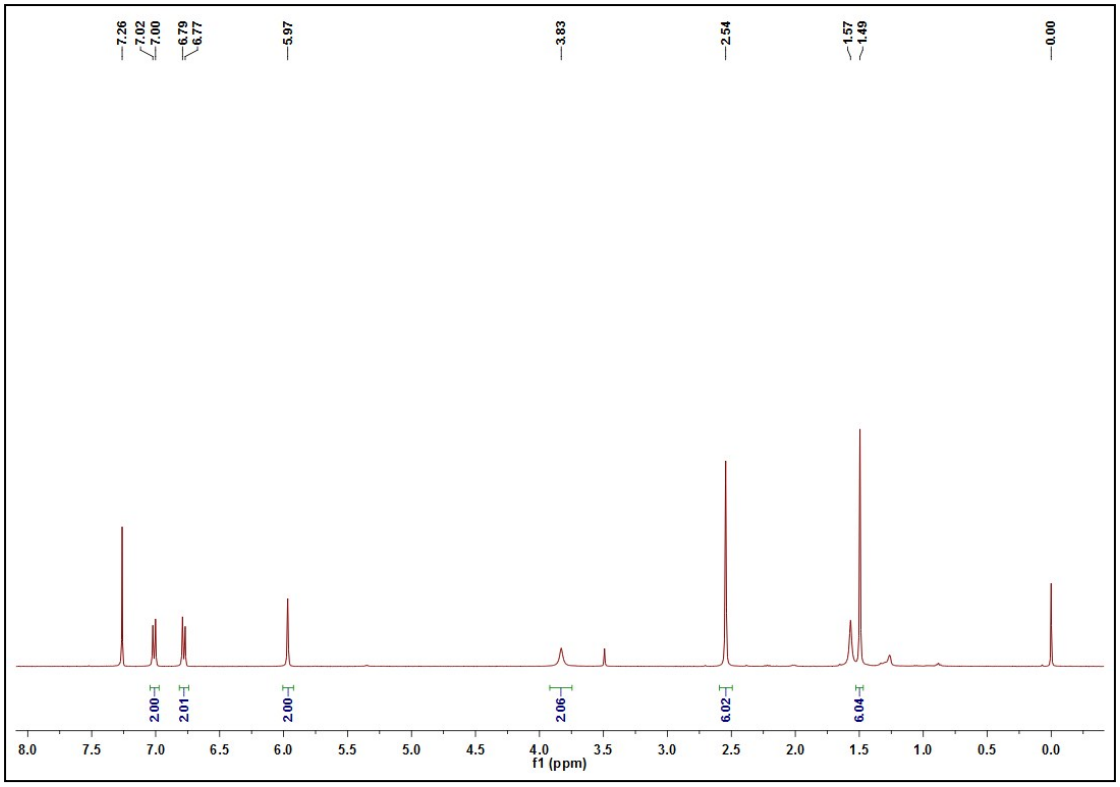


Fig. S5. ^{1}H NMR spectrum of **B-NH_2** in $CDCl_3$.

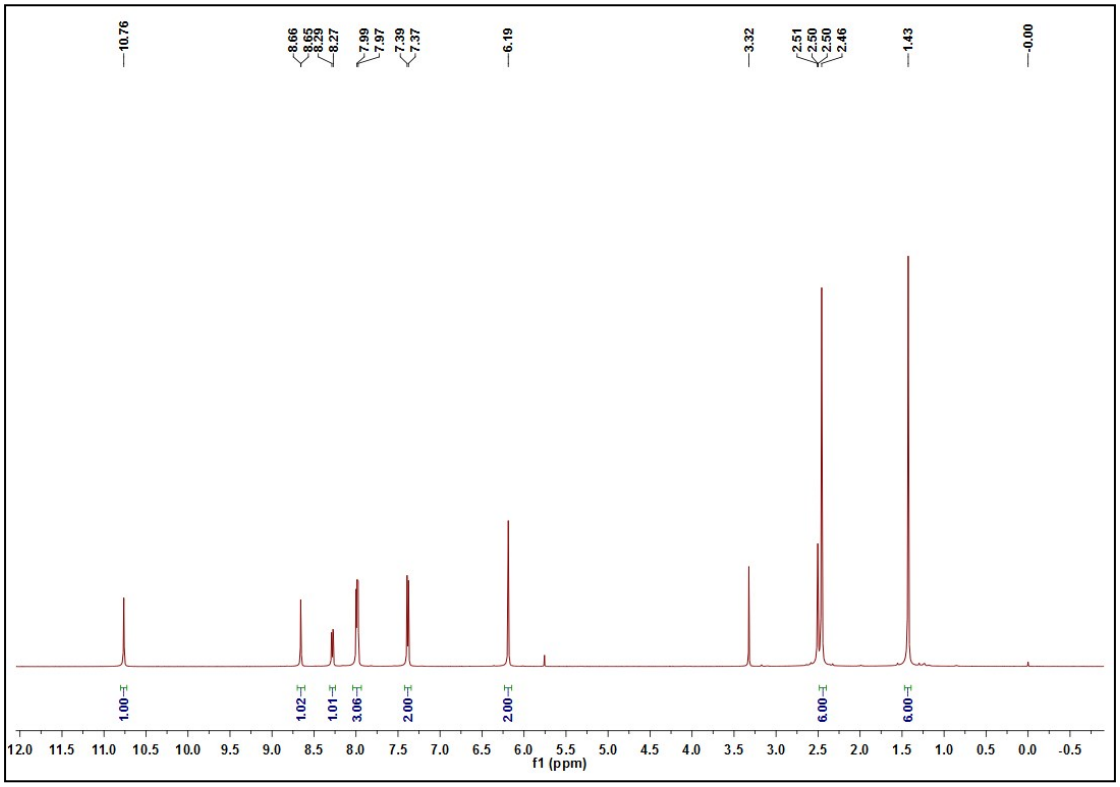


Fig. S6. ^{1}H NMR spectrum of probe **3** in DMSO-*d6*.

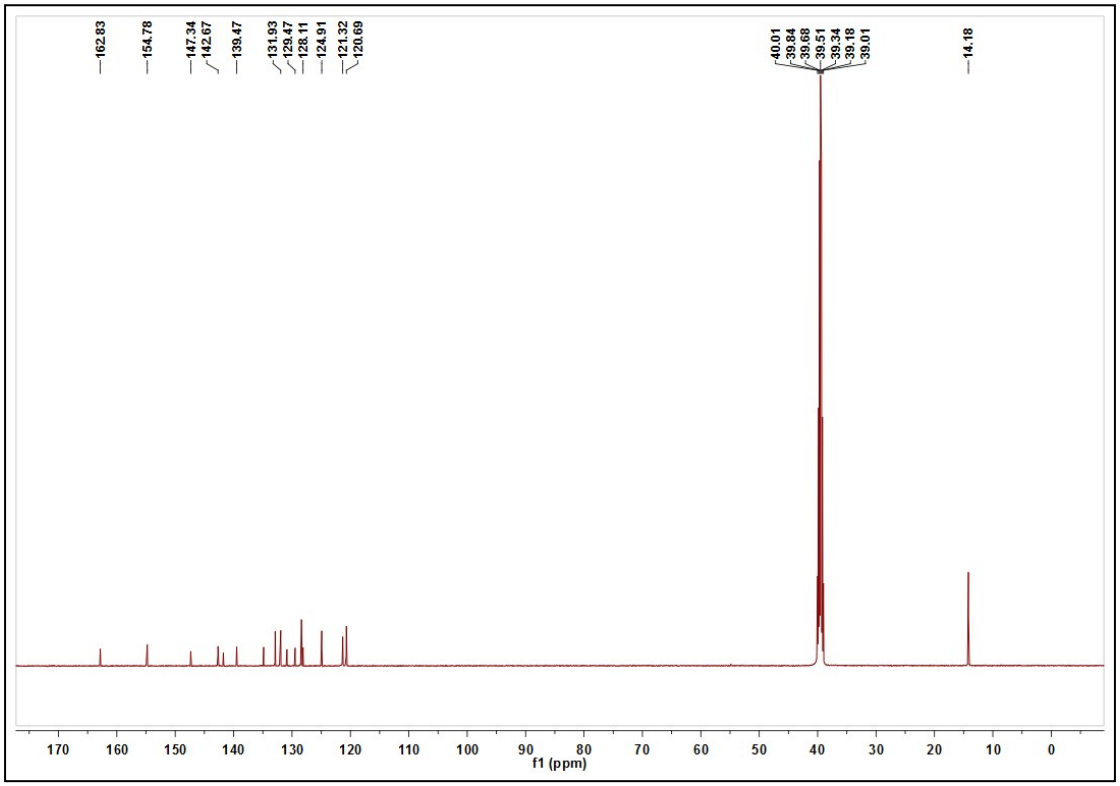


Fig. S7. ^{13}C NMR spectrum of probe **3** in DMSO-*d6*.

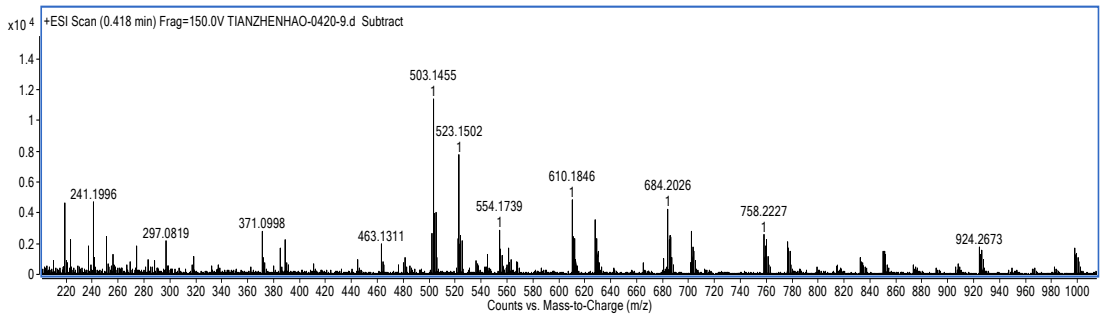


Fig. S8. HRMS spectrum of probe **3**.

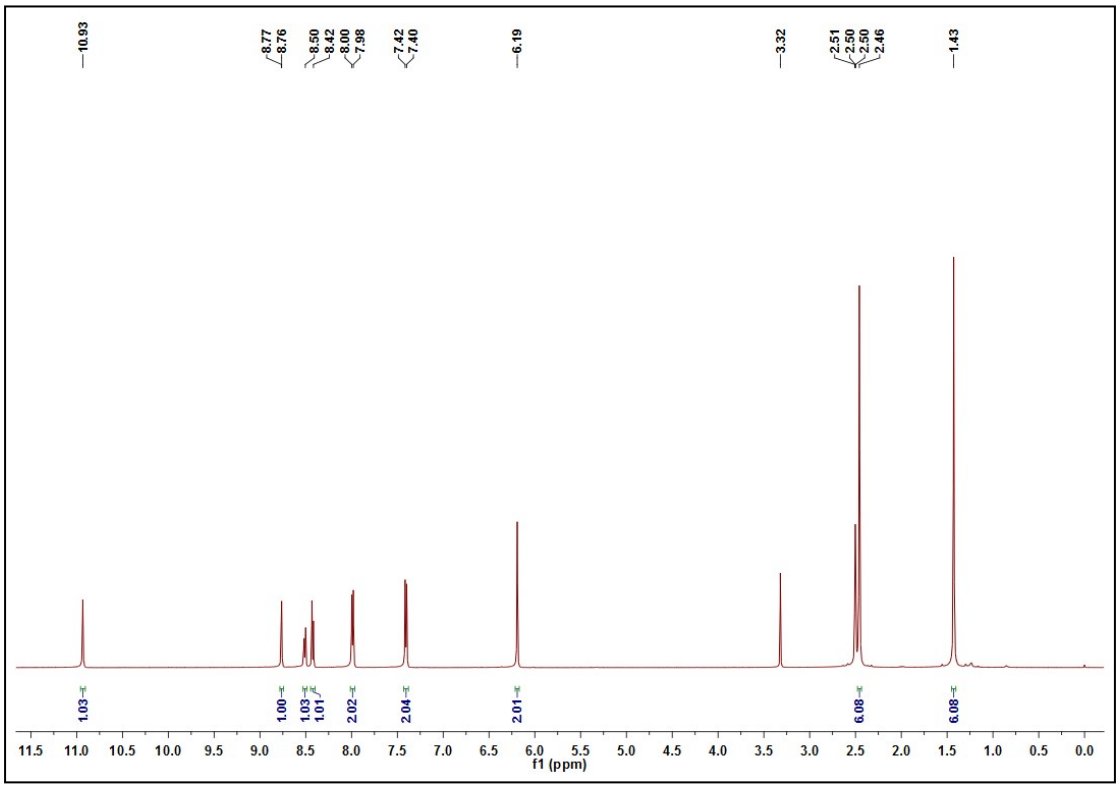


Fig. S9. ^{1}H NMR spectrum of probe **4** in DMSO-*d6*.

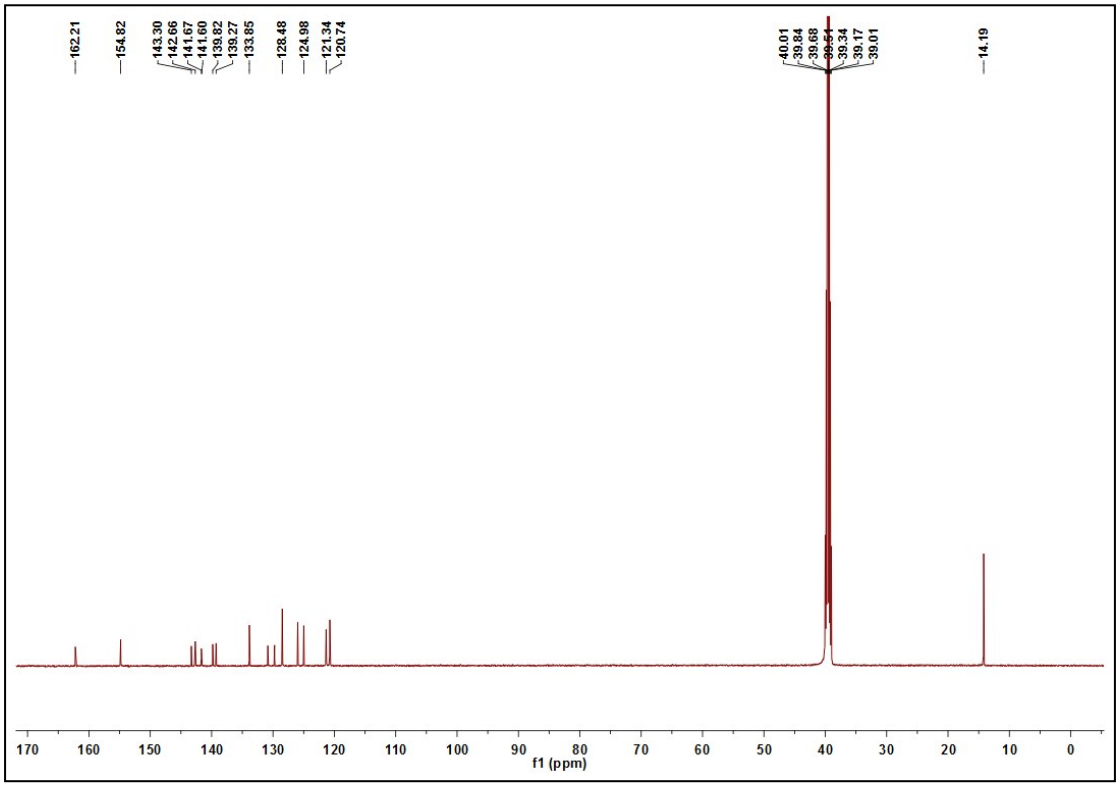


Fig. S10. ^{13}C NMR spectrum of probe **4** in DMSO-*d6*.

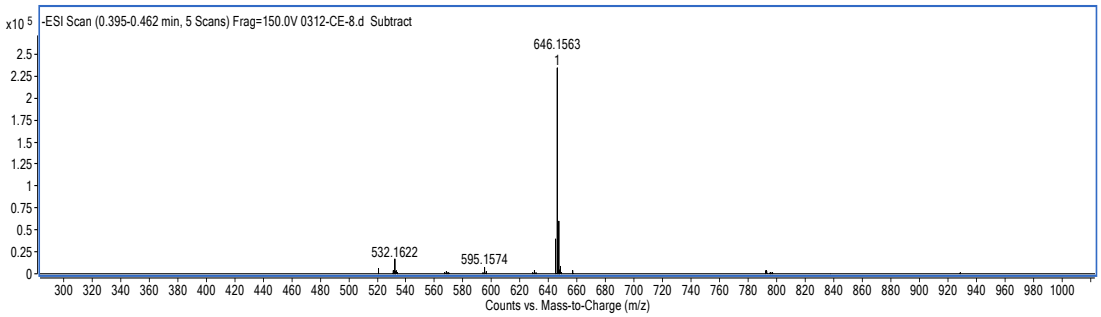


Fig. S11. HRMS spectrum of probe **4**.

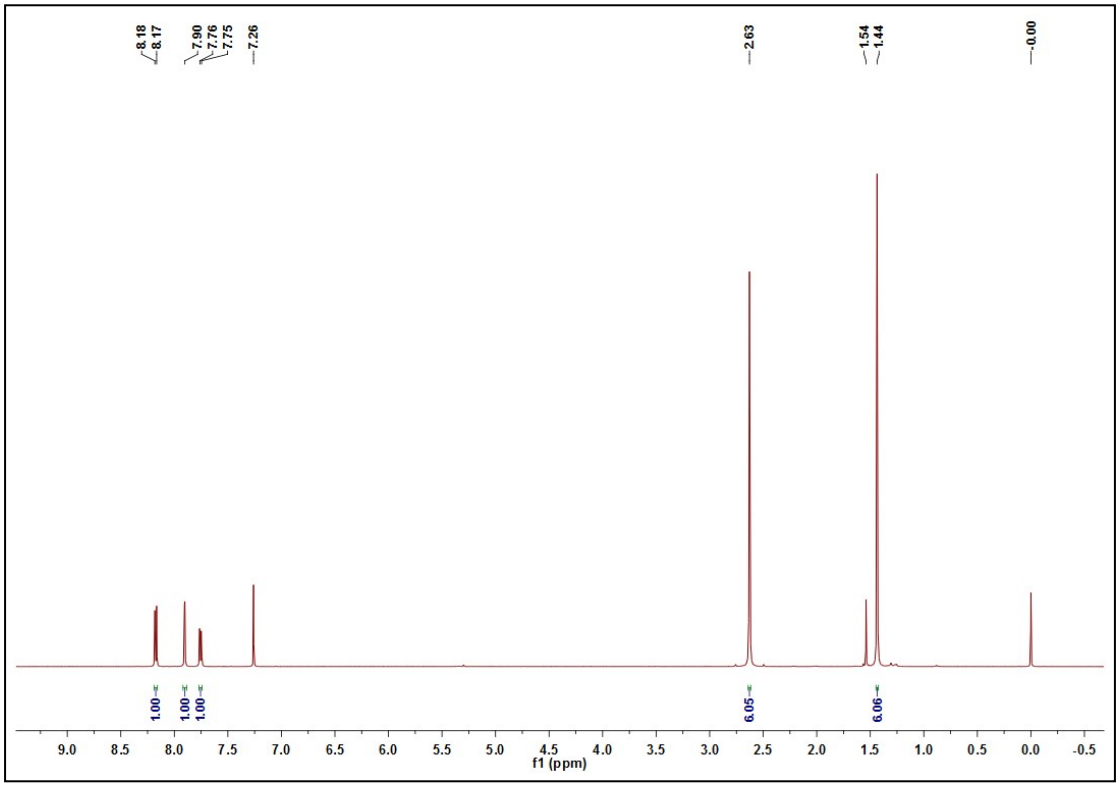


Fig. S12. ^{1}H NMR spectrum of probe **5** in $CDCl_3$.

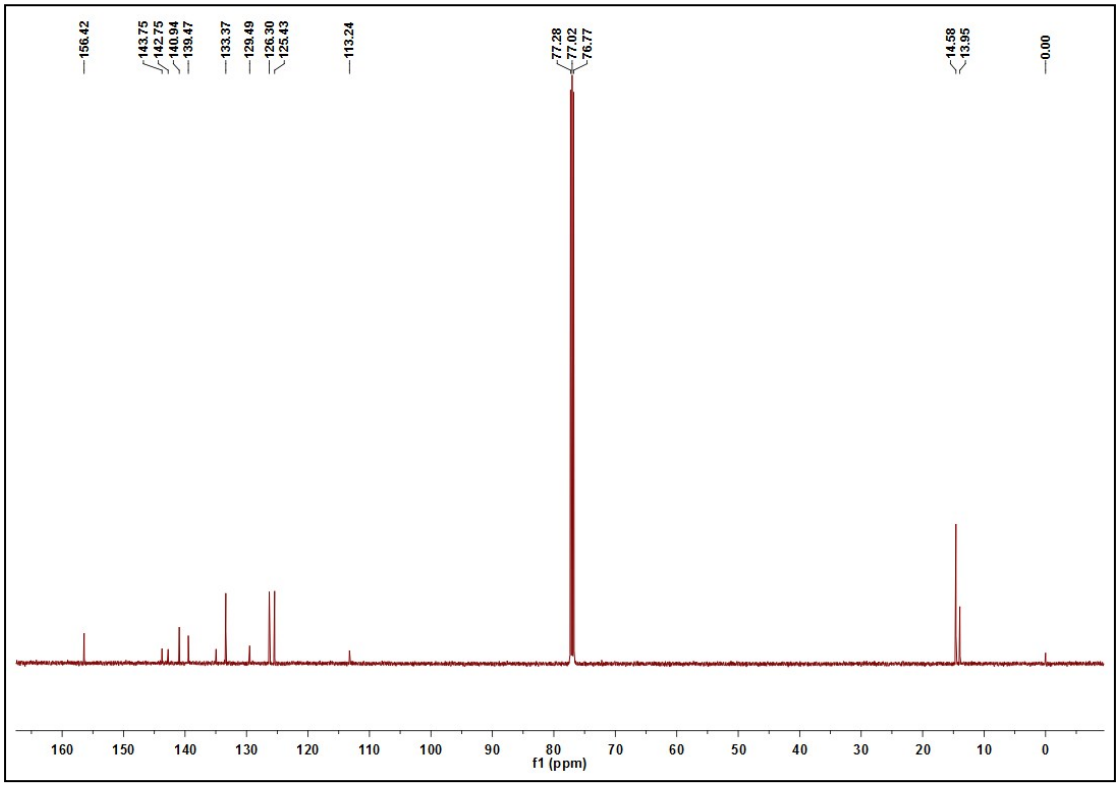


Fig. S13. ^{13}C NMR spectrum of probe **5** in $CDCl_3$.

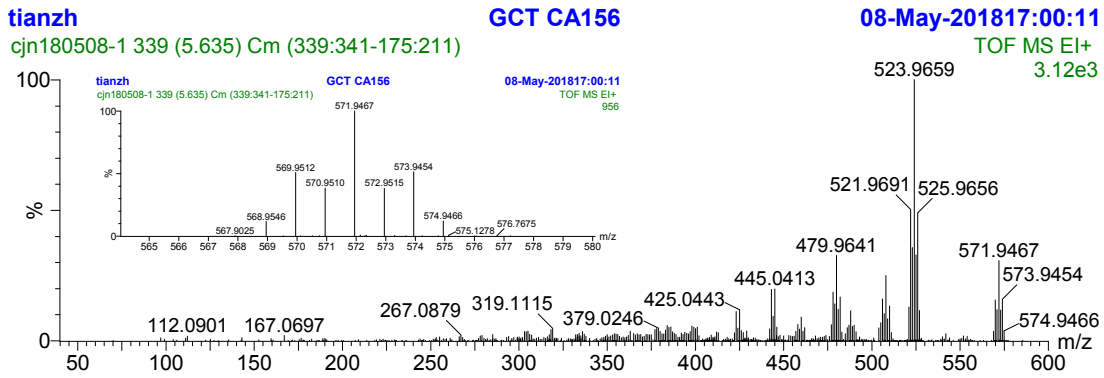


Fig. S14. HRMS spectrum of probe **5**.

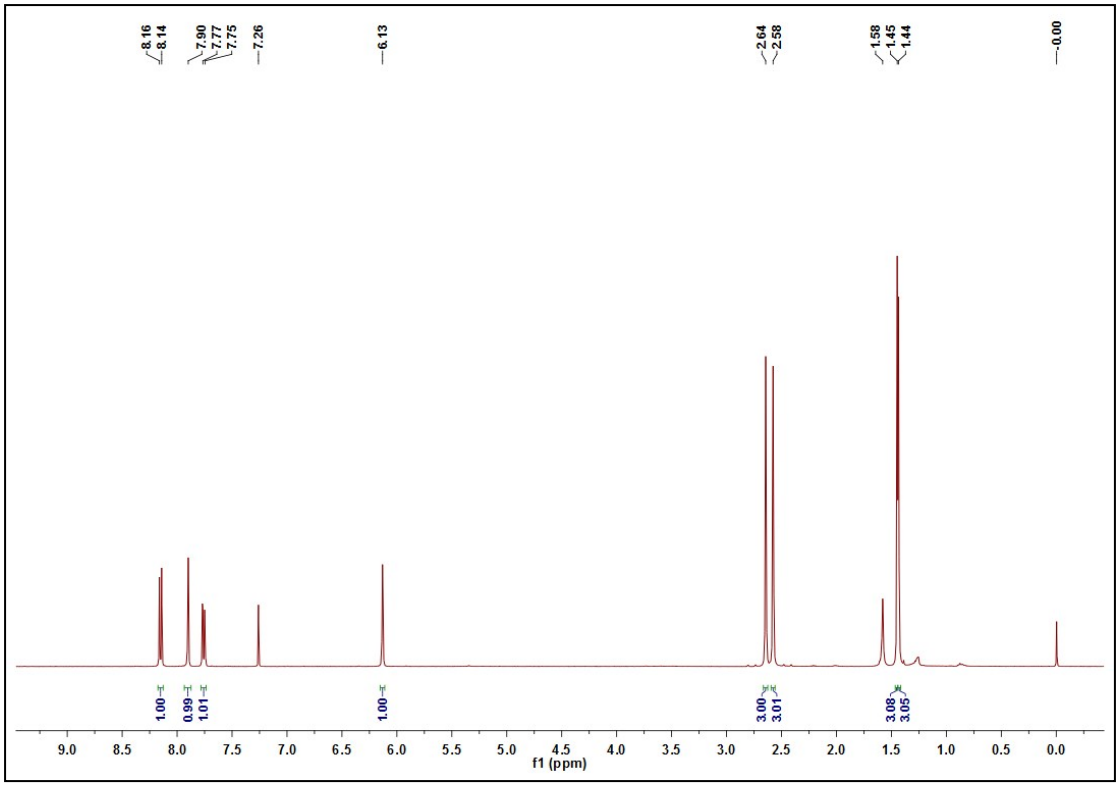


Fig. S15. ^{1}H NMR spectrum of **B-N-I** in $CDCl_3$.

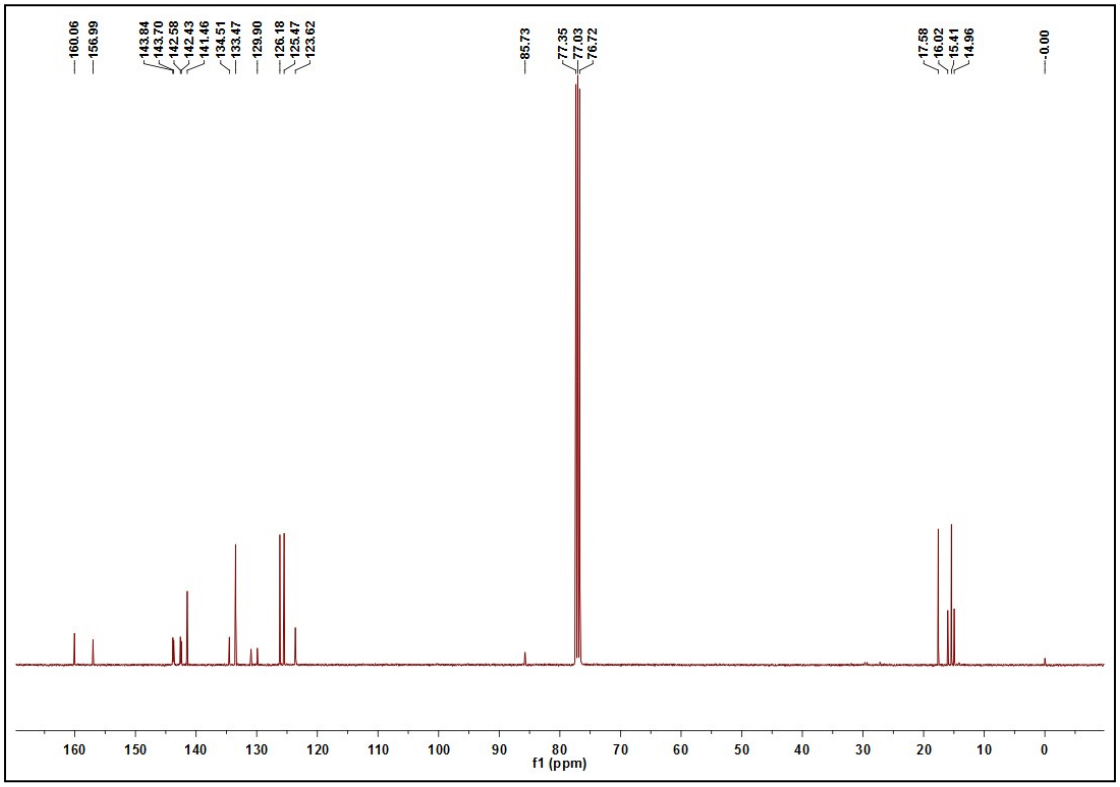


Fig. S16. ^{13}C NMR spectrum of **B-N-I** in $CDCl_3$.

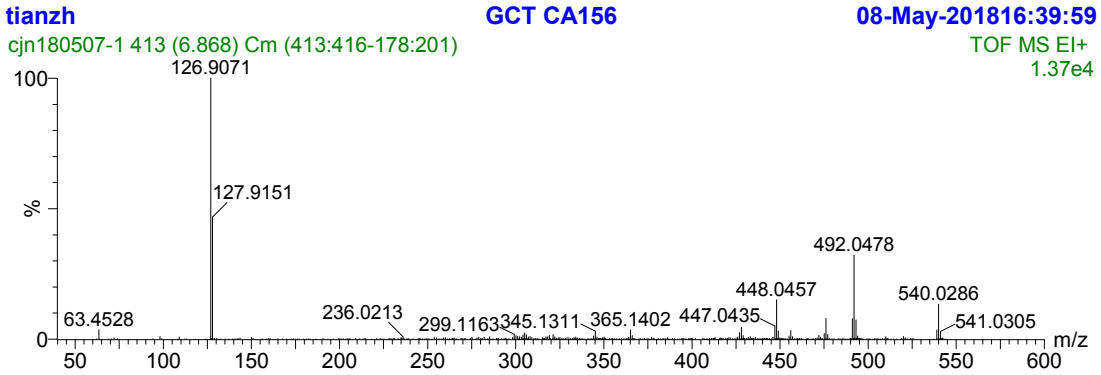


Fig. S17. HRMS spectrum of **B-N-I**.

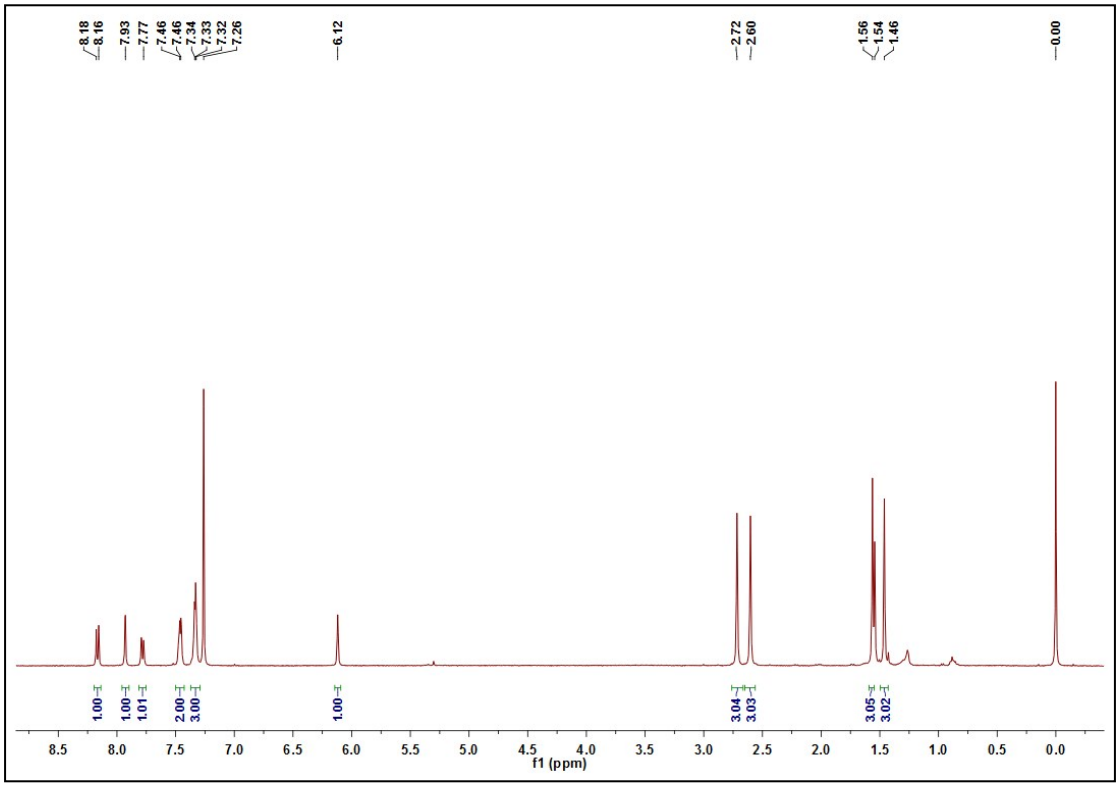


Fig. S18. ^{1}H NMR spectrum of probe **6** in $CDCl_3$.

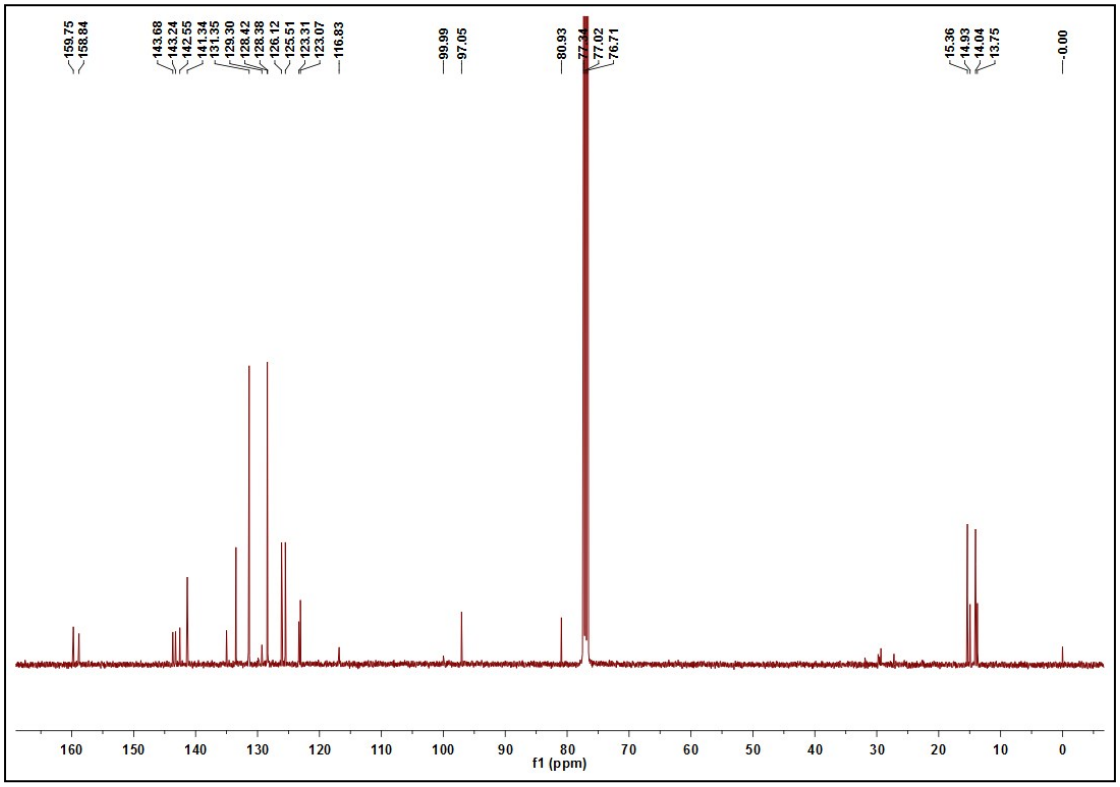


Fig. S19. ^{13}C NMR spectrum of probe **6** in $CDCl_3$.

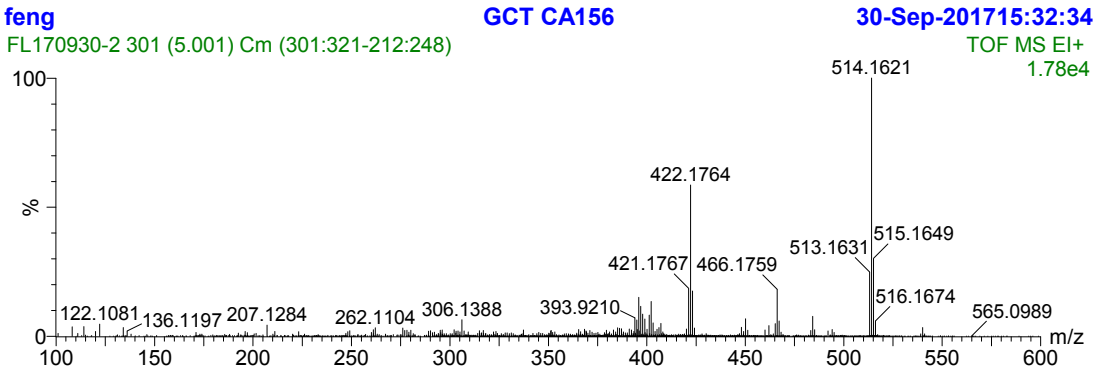


Fig. S20. HRMS spectrum of probe **6**.

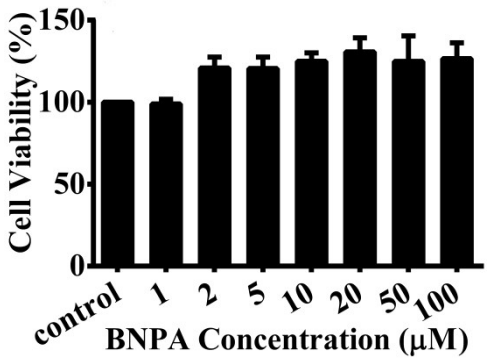


Fig. S21. Cell viability of HepG-2 cells in the presence of different concentrations of **BNPA**.

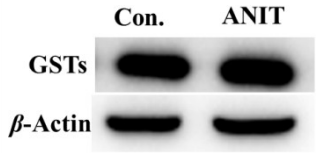


Fig. S22. The western blot assay of GSTs for the normal and ANIT-Induced liver.

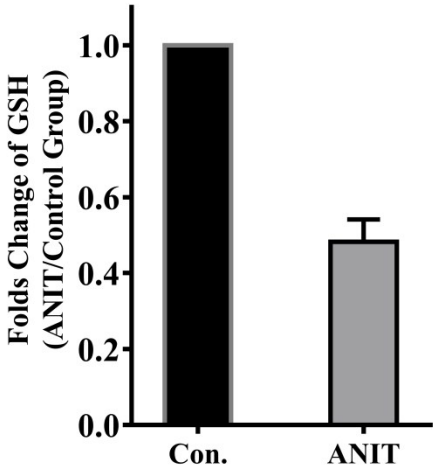


Fig.S23. The GSH change for the normal liver and ANIT-Induced liver.

Reference

1. T. Tachikawa, N. Wang, S. Yamashita, S. C. Cui and T. Majima, *Angew. Chem. Int. Edit.*, 2010, **49**, 8593-8597.
2. Y. Li, X. Wang, J. Yang, X. L. Xie, M. M. Li, J. Y. Niu, L. L. Tong and B. Tang, *Anal. Chem.*, 2016, **88**, 11154-11159.