Electronic Supplementary Information (ESI)

A near-infrared colorimetric fluorescent chemodosimeter for the detection of glutathione in living cells

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1. General Methods

All chemical reagents and solvents were analytical grade and purchased from commercial suppliers. 2-(2-(4-hydroxystyryl)-4H-chromen-4-ylidene) malononitrile and 4-(2-(4-(dicyanomethylene)-4H-chromen-2-yl)vinyl) phenyl 2,4-dinitro benzenesulfonate were prepared by the established literature procedure.[1] ^{1}H NMR and ^{13}C NMR spectra were recorded on the Bruker AV-300 and AV-400 spectrometer with chemical shifts reported in ppm (in $CDCl_3$, TMS as internal standard) at room temperature. Mass spectra were measured on a Bruker microTOF mass spectramenter and a Waters LCT Premier XE spectrometer.

UV-vis absorption spectra were recorded on a Varian Cary 100 spectrophotometer. Fluorescence spectra were measured with a Varian CARY Eclipse Fluorescence spectrophotometer. Spectral-grade solvents were used for measurements of UV-vis absorption and fluorescence.

2-(2-(4-hydroxystyryl)-4H-chromen-4-ylidene) malononitrile (DCPO)

2-(2-methyl-4H-chromen-4-ylidene)malononitrile (208 mg, 1.00 mmol) and 4-hydroxybenzaldehyde (387.5 mg, 1.10 mmol) dissolved in 30 mL toluene. And then add 1.21 ml piperidine and 0.5 ml acetic acid. A dean-stark head was fitted and reaction mixture was heated under reflux for 8h. After the completion of the reaction, the mixture was allowed to cool to room temperature and then condensed under reduced pressure. A red solid was obtained (200 mg, 40.0%). ^{1}H NMR(400MHz, DMSO-d_6, ppm):δ = 10.16 (s, 1H), 8.73(dd,J_1 = 8.4 Hz, J_2 = 1.2 Hz,1H), 7.91 (m, 1H), 7.79 (d, J = 7.6 Hz,1H), 7.70(d, J = 16.0 Hz,1H), 7.60 (m, 3H,Ph-H),7.28 (d, J = 16.0

Hz,1H), 6.96(s, 1H), 6.85 (d, J = 8.8 Hz,1H). ^{13}C NMR(100 MHz, DMSO-d_6, ppm): δ= 165.3, 164.1, 158.1, 157.3, 144.5, 140.5, 135.6, 131.3, 129.8, 124.2, 122.6, 122.4, 121.3, 121.3, 121.2, 110.9, 64.3.HRMS (ESI) calcd for $C_{20}H_{12}N_2O_2$ [M^+-H] 311.0843, found 311.0821.

4-(2-(4-(dicyanomethylene)-4H-chromen-2-yl)vinyl)phenyl2,4-dinitrobenzenesulfonate (probe 1)

To a stirred solution of 2-(2-methyl-4H-chromen-4-ylidene)malononitrile (100 mg, 0.32 mmol) in dry CH_2Cl_2 (15 ml) was added pyridine 0.5 ml. The mixture was then cooled to 0℃ and a solution of 2,4-dinitrobenzensulfonyl chloride (256.10 mg, 0.96 mmol) in dry CH_2Cl_2 (5 ml) was added dropwise. After being stirred at 0℃for 30 minutes, the mixture was then stirred at room temperature for 3h. After the completion of the reaction, the mixture was condensed under reduced pressure. The residue was purified with column chromatography (silica gel, DCM–MeOH, 20:1,v/v), and a red solid was obtained (50 mg, 29.0%). ^{1}H NMR(300MHz, DMSO-d_6, ppm):δ = 10.160(s, 1H), 8.727(dd, J_1 = 8.4 Hz, J_2 = 1.2 Hz,1H), 7.913(m, 1H), 7.791(d, J = 7.6 Hz,1H), 7.697(d, J = 16.0 Hz,1H), 7.604(m, 3H,Ph-H),7.279(d, J = 16.0 Hz,1H), 6.957(s, 1H), 6.850(d, J = 8.8 Hz,1H). ^{13}C NMR(75 MHz, DMSO-d_6, ppm): δ= 157.9, 153.3, 152.3, 151.9, 149.6, 148.5, 136.8, 135.9,135.4, 134.0, 131.1, 130.4, 127.9, 126.6, 125.0, 123.1, 121.7, 121.5, 119.4, 117.4, 117.3, 116.0, 107.7, 61.4.HRMS (ESI) calcd for C26H14N4O8S [M^+-H] 541.0454, found 541.0472.

IR(cm^{-1}) 712, 855, 978, 1140, 1346, 1457, 1497, 1536, 1553, 1603, 2210, 3032

Reference

1. Li, Y. M.; Zhang, X. L. ; Zhu, B. C.; Yan, J. L.; Xu, W. P. *Analytical Sciences* **2010**, *26*,

1077–1080.

2. Kinetics of fluorescence enhancement profile and detection limit

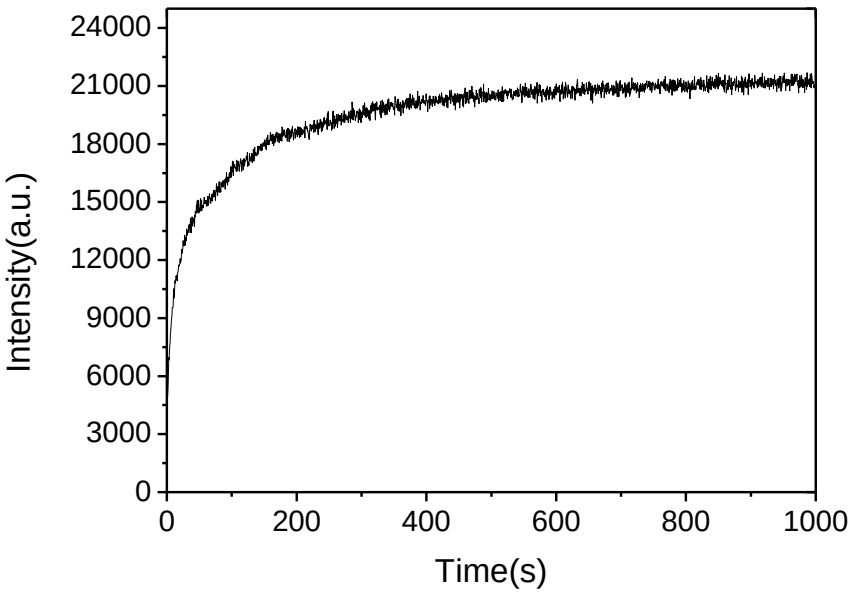

Figure S1. Kinetics of fluorescence enhancement profile of probe **1** (1×10^{-5} M) at 690 nm in the presence of GSH (5 equiv) upon excitation at 560 nm. All fluorescence changes were measured at 37 °C in PBS buffer (pH 7.4).

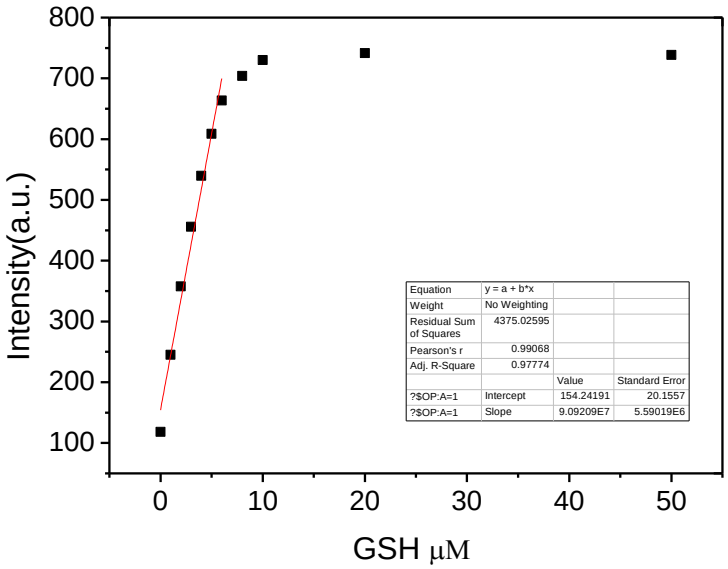

Figure S2. Response of fluorescence signals to GSH concentrations, a linear regression curve was then fitted to these fluorescent intensity data. The Standard Deviation was obtained by fluorescence responses to be σ = 0.5327, therefore, the detection limit was calculated by the formula ($3\sigma/k$) and gave a result as 1.8×10^{-8} M.

3. Mass spectra of probe 1 and probe 1 + GSH systems.

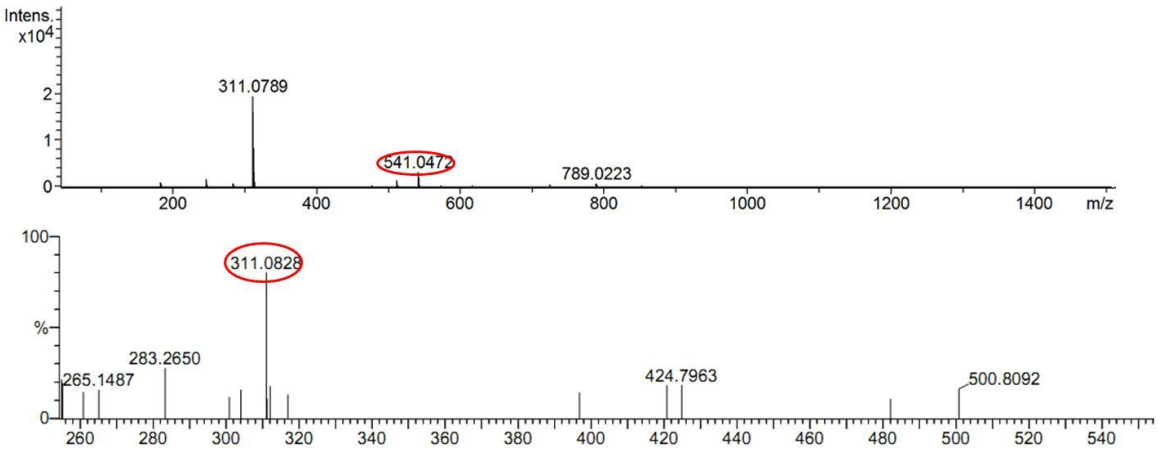

Figure S3. Mass spectra of probe **1** and probe **1** + GSH systems.

4. pH dependency of DCPO and probe 1 and pK_a value of DCPO

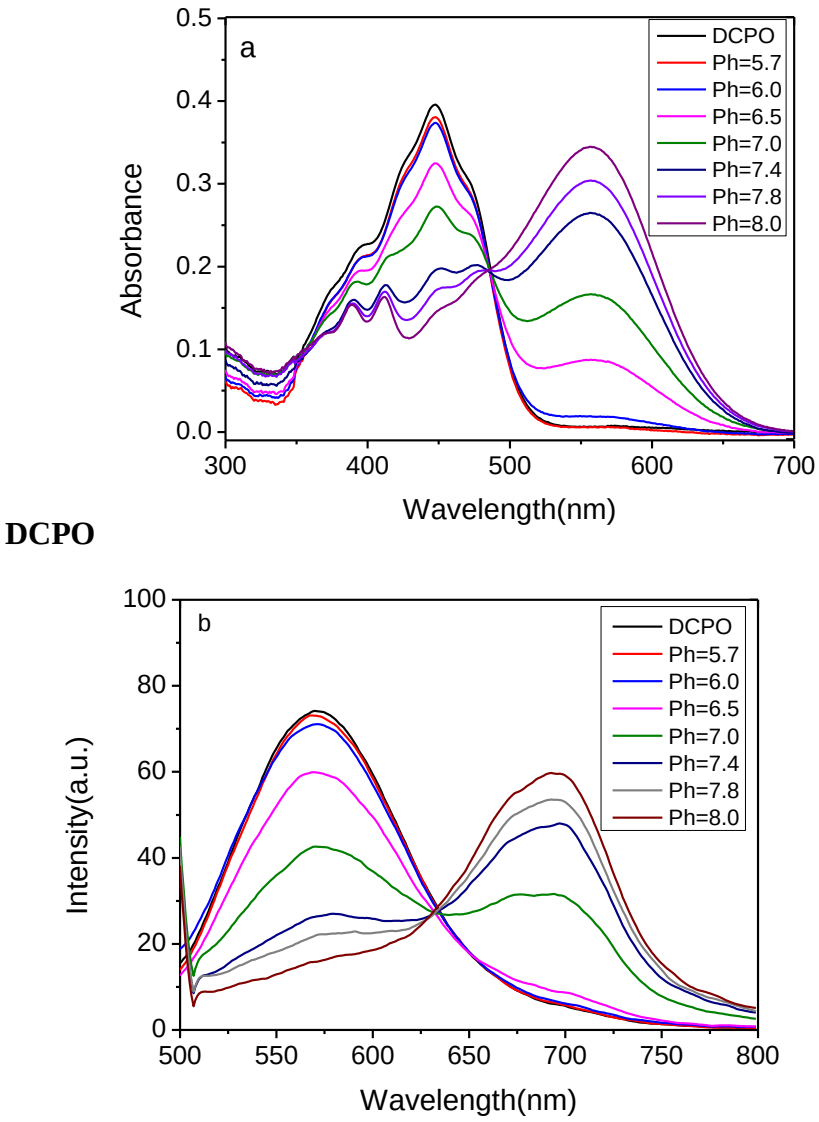

Figure S4. (a) Absorption spectra of DCPO in buffer solution as a function of pH. (b) Corresponding emission spectra (λex = 486 nm). The pH was adjusted by NaH_2PO_4 and Na_2HPO_4.

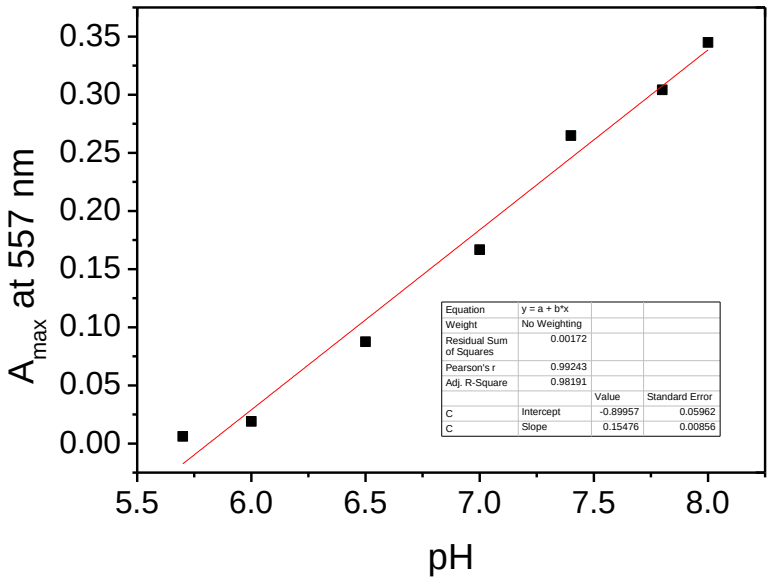

Figure S5. pH-dependent absorbance at 557 nm of DCPO. The pK_a of DCPO was calulated using: $\log[(I_{Fmax} - I_F) / (I_F - I_{Fmin})] = pH - pKa$, giving a p$K_a$ of 8.70.

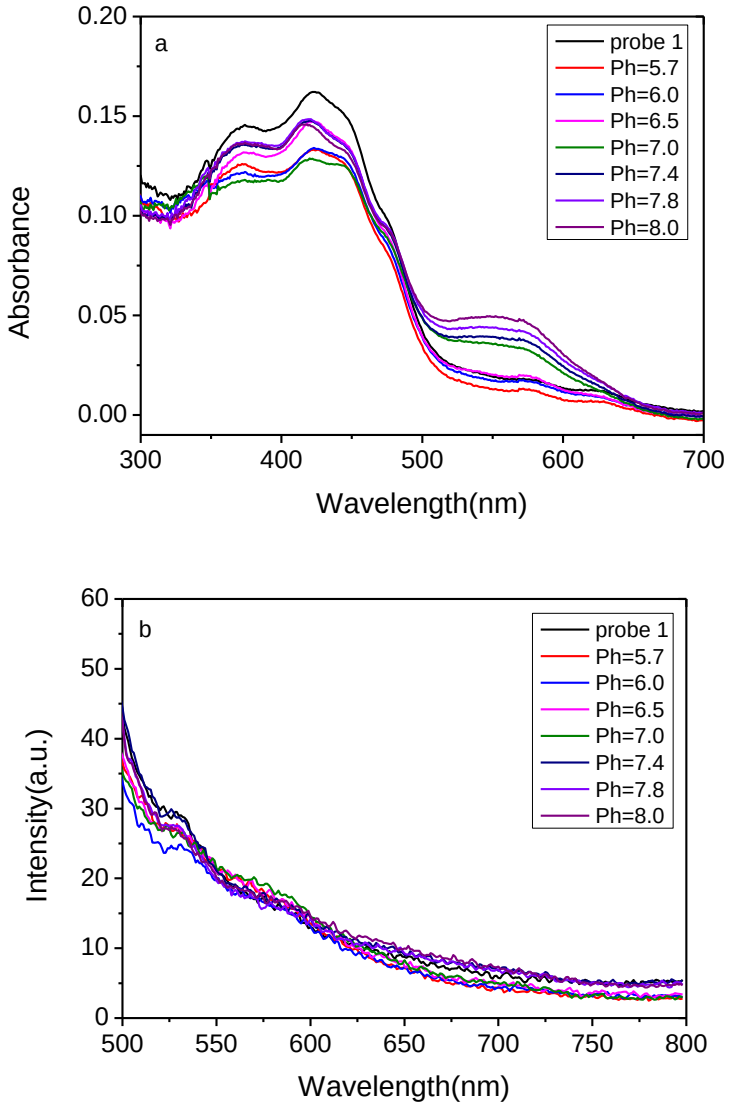

Figure S6. (a) Absorption spectra of probe **1** in buffer solution as a function of pH. (b) Corresponding emission spectra (λex = 486 nm). The pH was adjusted by NaH_2PO_4 and Na_2HPO_4.

5 Fluorescence spectra of probe 1 in the presence of different concentrations of GSH when excited at 446 nm.

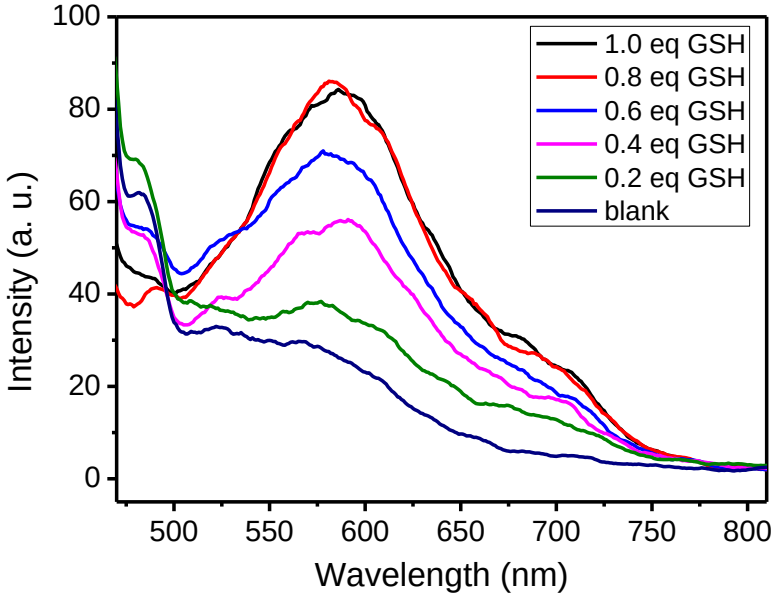

Fig. S7 Fluorescence spectra of probe 1 (1×10^{-5} M) in the presence of different concentrations of GSH when excited at 446 nm.

6. Cell culture experiments

Human cervical carcinoma cell line Hela cells were employed for in vitro cell imaging. The cells were cultivated at 37°C in RPMI 1640 with 10% FCS. To evaluate the cell imaging ability, Hela cells were seeded in a 1.5 inches imaging plate with an amount of 30000 cells mL^{-1}. The probe **1** was co-cultivated with Hela cells at a concentration of 20 μM for 0.5 h at 37°C, 5% CO_2 and 95% humidity. 2.5% DMSO was used in the cell culture process. Before the confocal laser scanning microscopy (Nikon A1R) observation, the cells were washed 3 times with commercial available PBS buffer solution (pH = 7.4). Finally, 2 mL of PBS solution was added and the cells were visualized under a CLSM. The fluorescence images were taken under 60 × oil-immersion objective. Red fluorescence of probe **1** was excited at 488 nm. The emission wavelengths were ranged from 660 – 740 nm.

7. ^{1}H NMR and ^{13}C NMR spectra

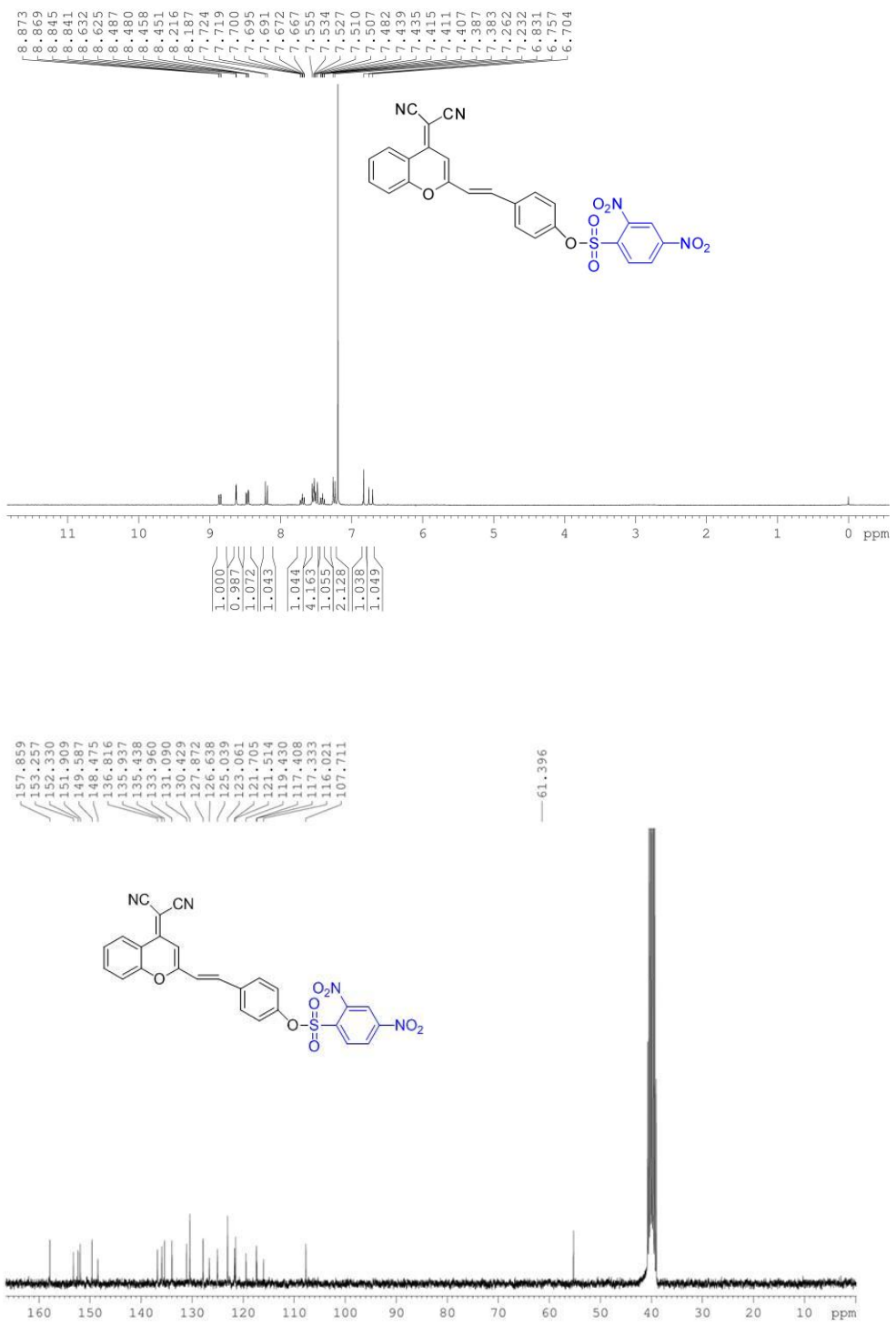

Figure S8. ^{1}H NMR and ^{13}C NMR spectra of probe **1**.