

Electronic Supplementary Information (ESI)

for

A new ratiometric fluorescent probe for detection of thiophenols

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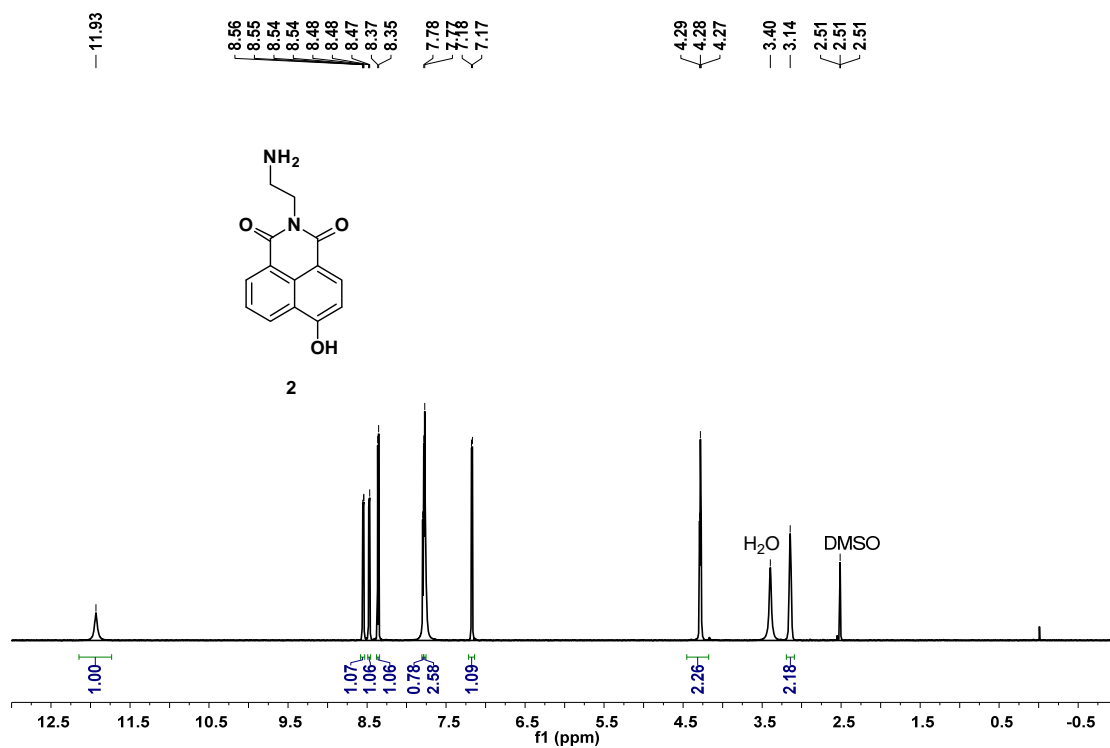
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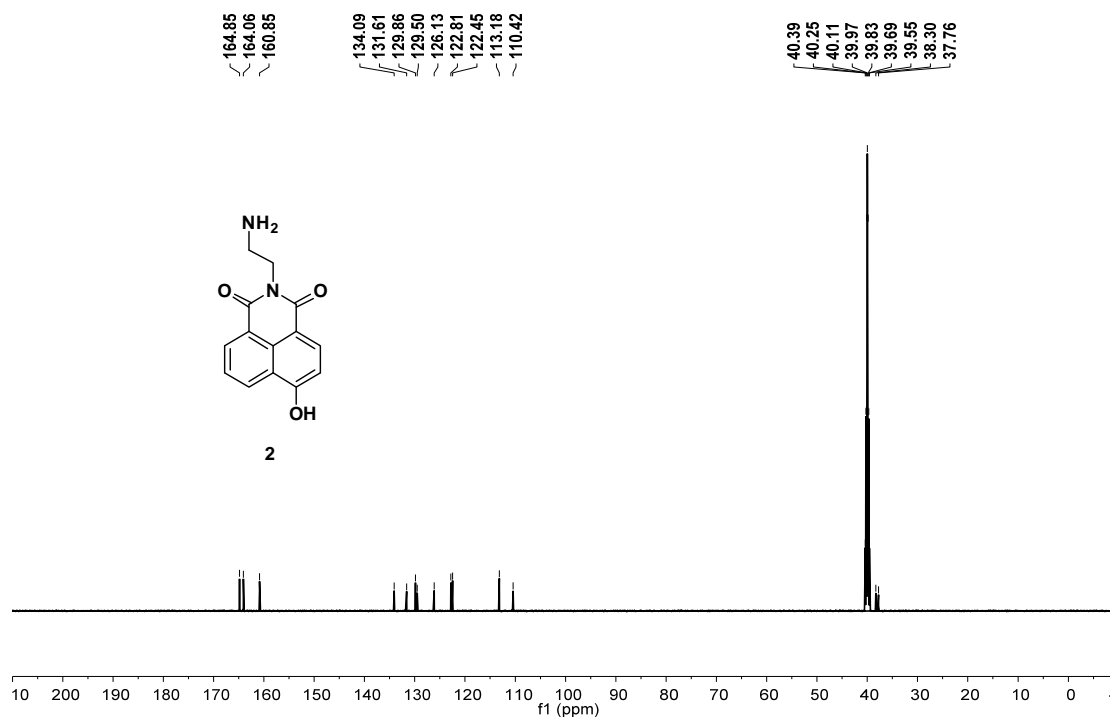
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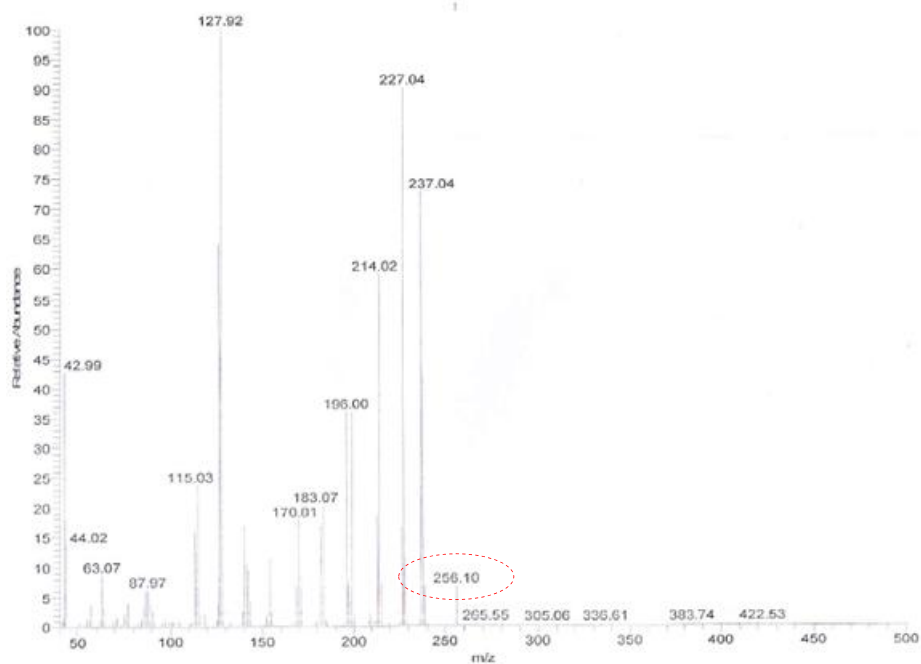
1. Structure characterizations of compound 2, CN and probe CN-DNP



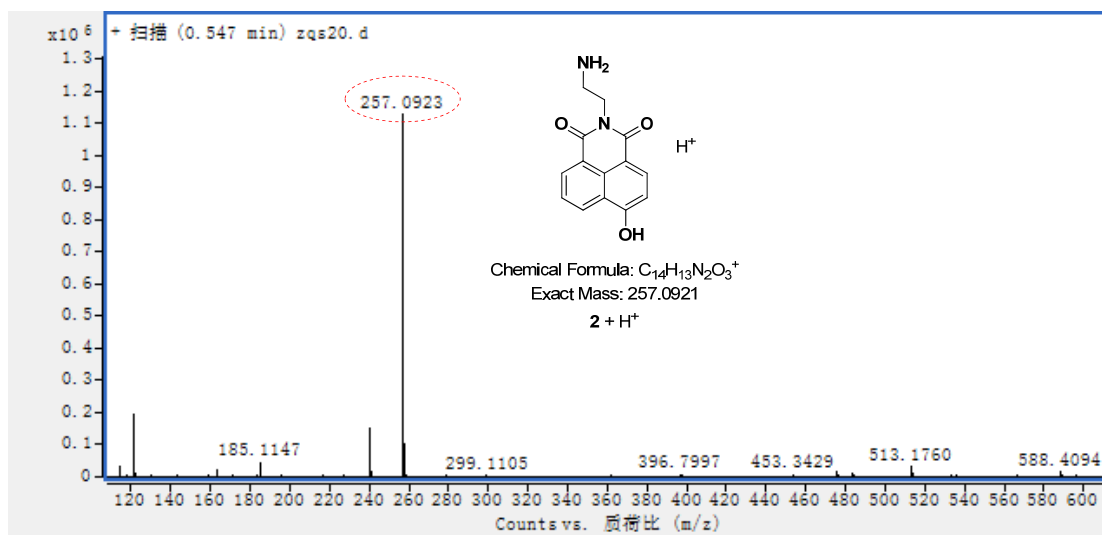
¹H-NMR spectrum of compound 2 in *d*₆-DMSO



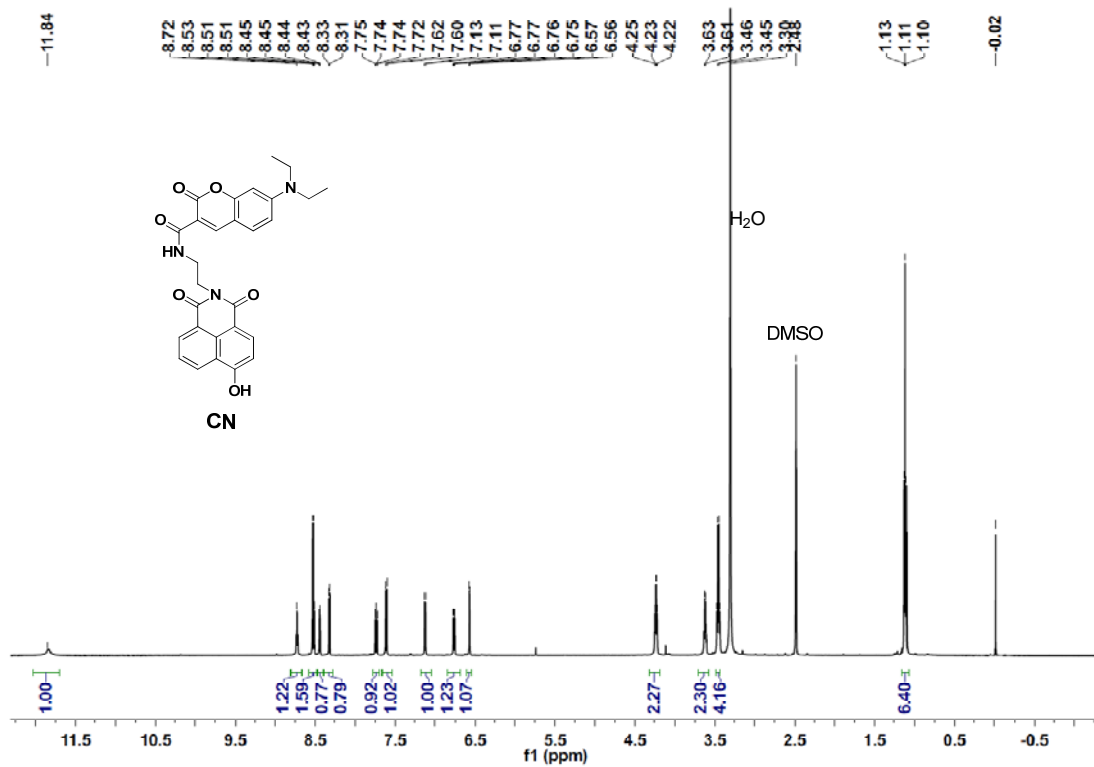
¹³C-NMR spectrum of compound 2 in *d*₆-DMSO



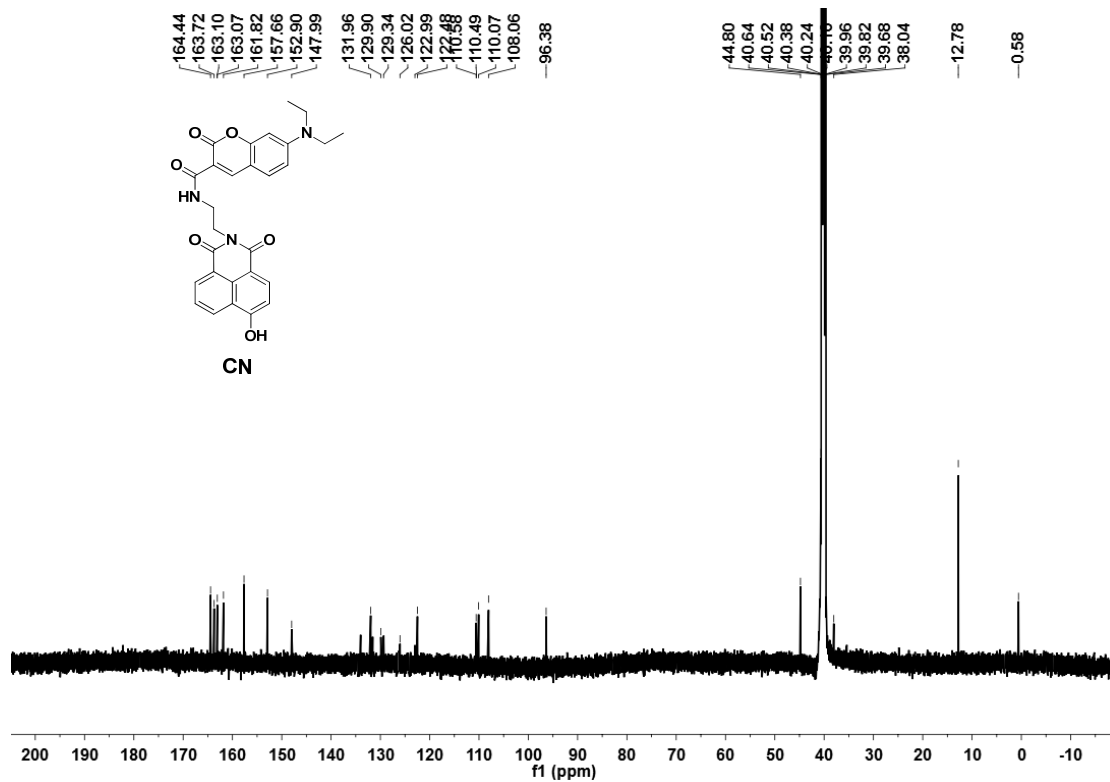
MS (EI) spectrum of compound 2



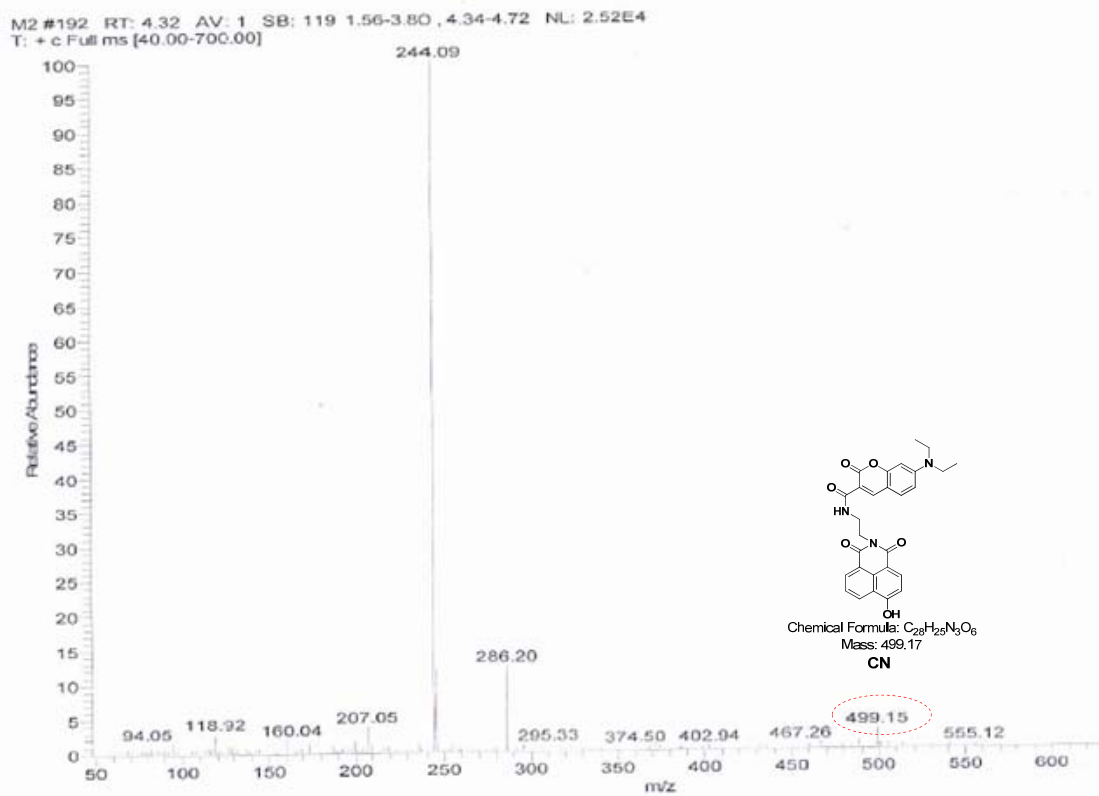
HR-MS spectrum of compound 2



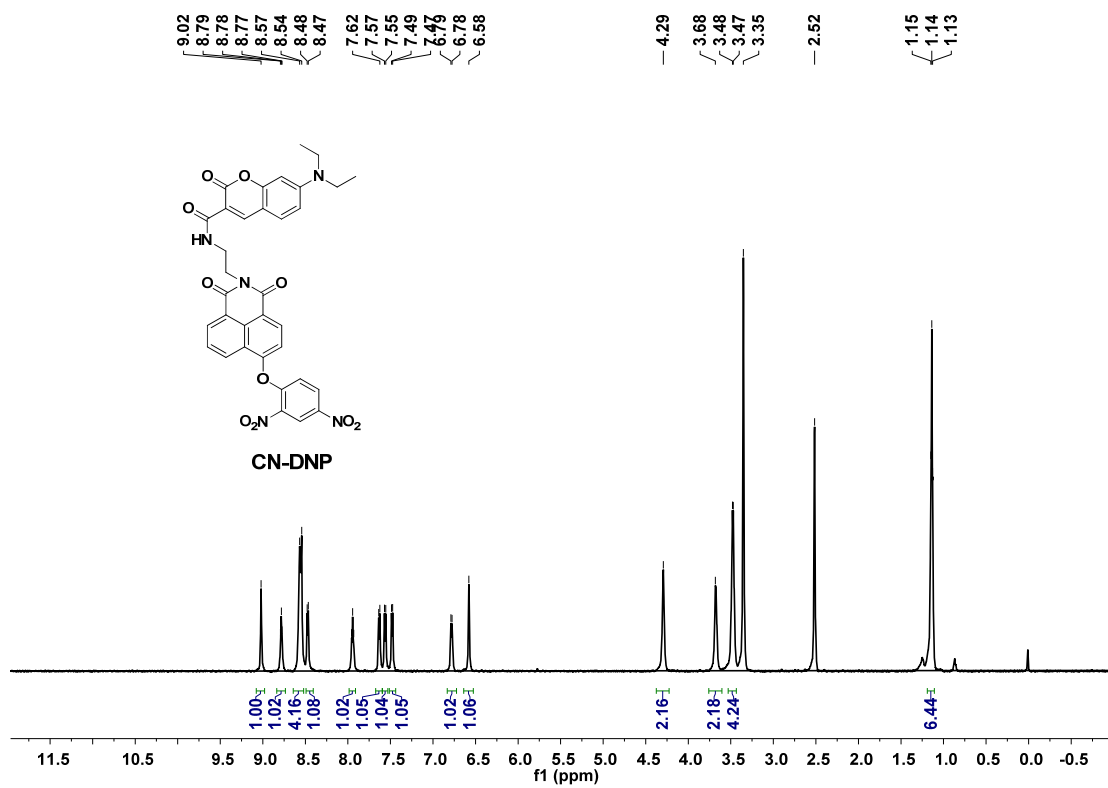
$^1\text{H-NMR}$ spectrum of CN in d_6 -DMSO



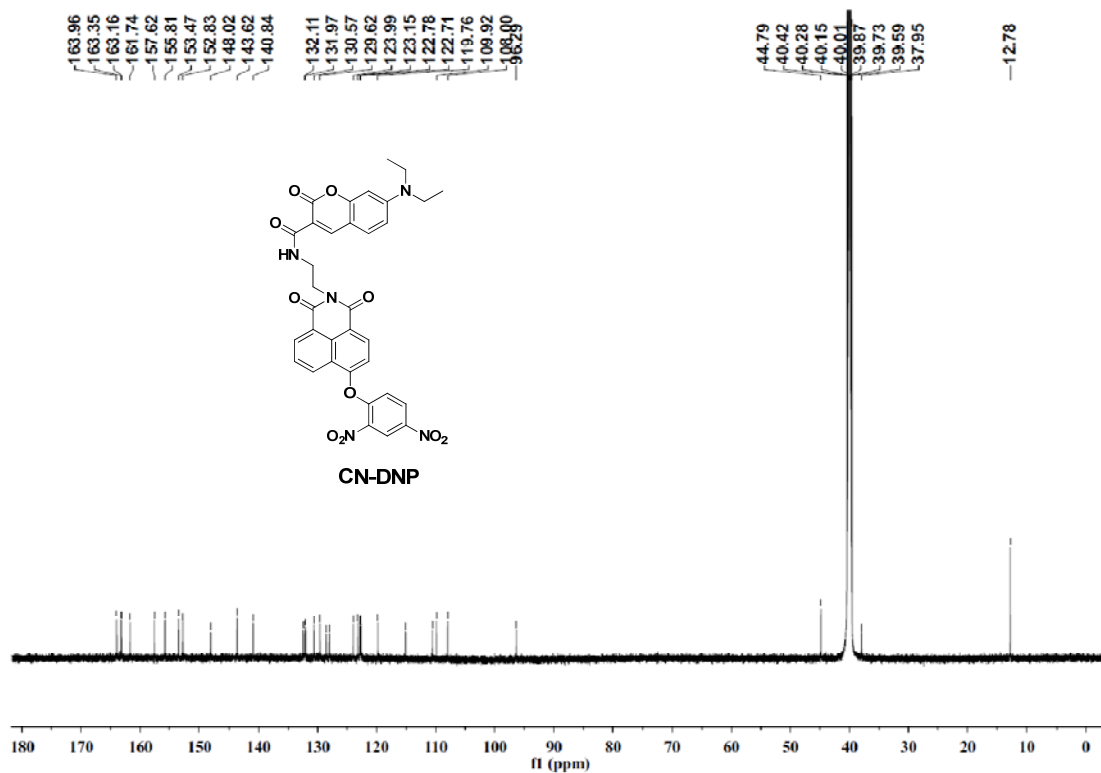
$^{13}\text{C-NMR}$ spectrum of CN in d_6 -DMSO



MS (EI) spectrum of compound CN

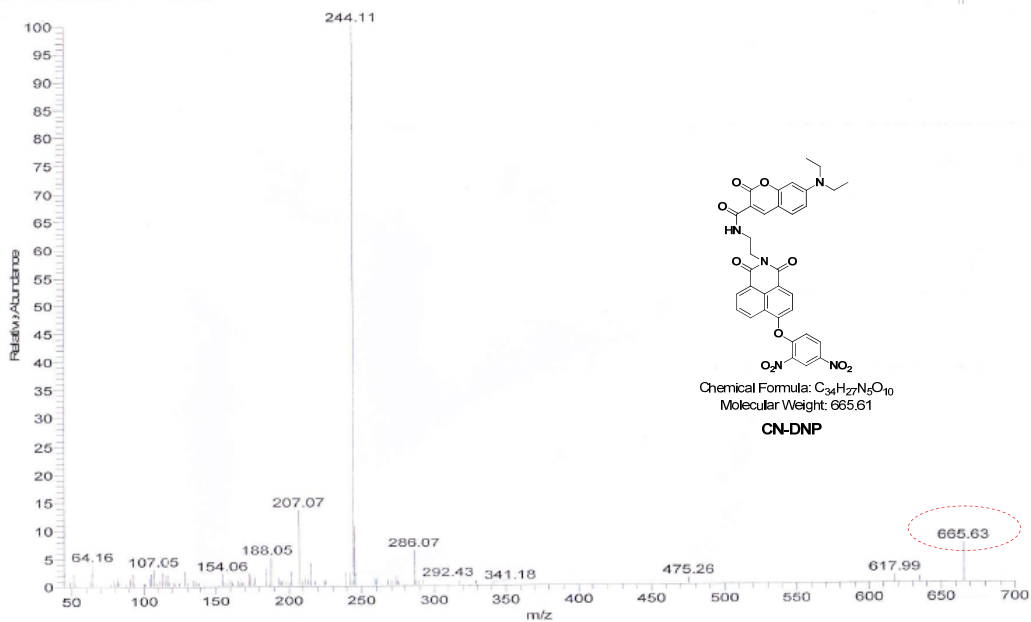


$^1\text{H-NMR}$ spectrum of probe CN-DNP in d_6 -DMSO

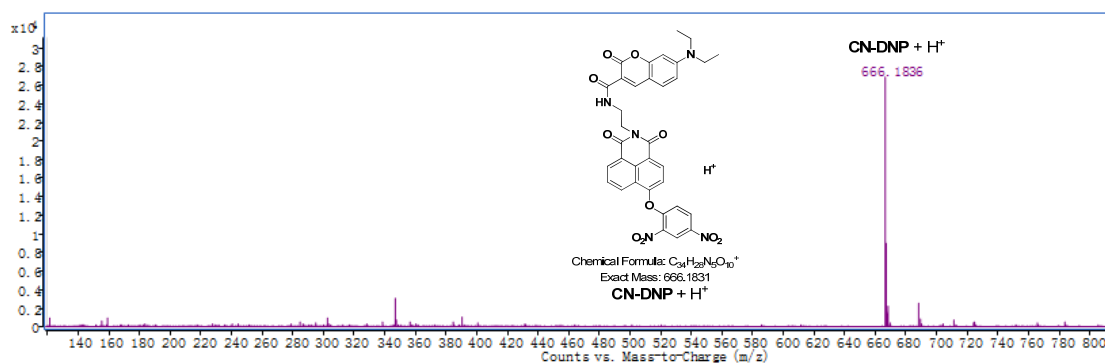


^{13}C -NMR spectrum of probe **CN-DNP** in d_6 -DMSO

M3 #177 RT: 3.98 AV: 1 SB: 164 0.29-3.65, 4.45-4.72 NL: 8.09E3
T: + c Full ms [40.00-700.00]



MS (EI) spectrum of compound **CN-DNP**



HR-MS spectrum of compound **CN-DNP**

2. Additional data

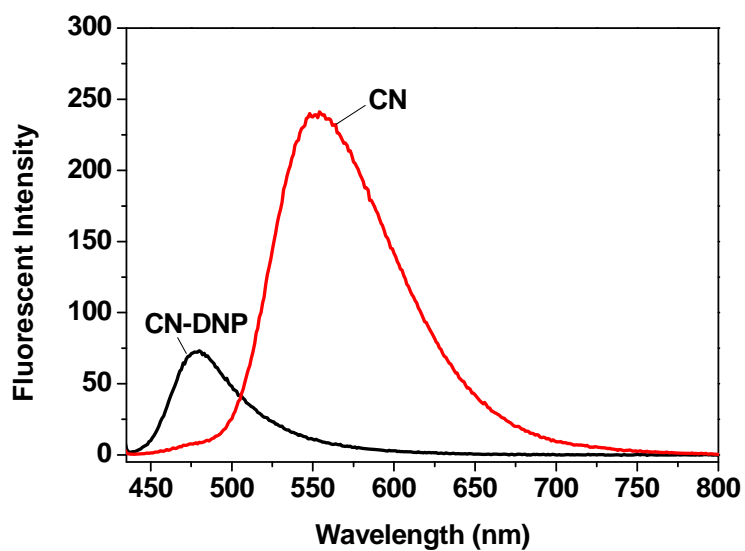


Figure S1. Fluorescence spectra of probe **CN-DNP** (10 μM) and compound **CN** (10 μM) in PBS buffer (50 mM, pH 7.4) with 40% DMF at 37 $^{\circ}\text{C}$. $\lambda_{\text{ex}} = 425 \text{ nm}$, slit width: $d_{\text{ex}} = d_{\text{em}} = 5 \text{ nm}$.

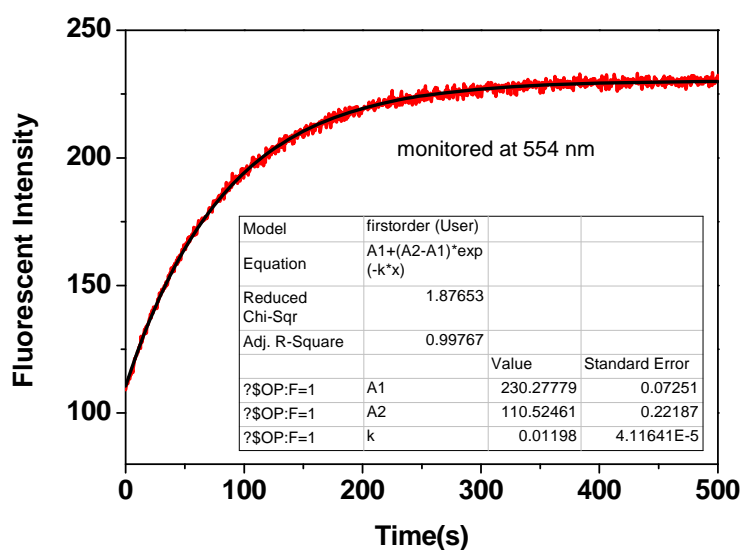
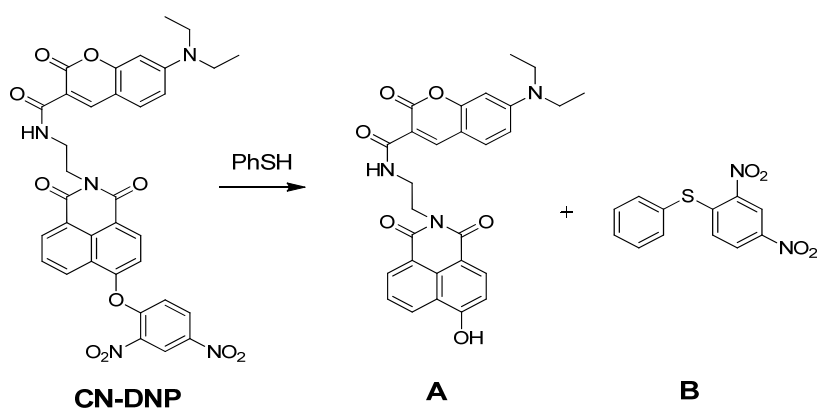


Figure S2. Fluorescence kinetic of probe **CN-DNP** (10 μM) with PhSH (30 μM) in PBS buffer (50 mM, pH 7.4) with 40% DMF at 37 $^{\circ}\text{C}$ (Red spots). The reaction was monitored every 0.5 second at 554 nm with $\lambda_{\text{ex}} = 425$ nm, slit width: $d_{\text{ex}} = d_{\text{em}} = 5$ nm. The kinetic curve is fitted (black line) by a first-order reaction scheme with rate constant k_{obs} determined to be about 0.012 s^{-1} as shown in the figure.

Data for investigation of the sensing mechanism



To examine the sensing mechanism of probe **CN-DNP** to thiophenol, the following experiment was performed. Probe **CN-DNP** (10 mg) was dissolved in PBS buffer with 40% DMF (5 mL), and then thiophenol (5 mg, ~ 3 eq.) was added into the solution. After stirring 1 hours at 37 $^{\circ}\text{C}$, the mixture was diluted with water, and extracted by dichloromethane. The organic layer was evaporated under reduced pressure, and the crude product was purified by flash column chromatography to

afforded two solid products **A** and **B**, which are proved to be **CN** and a known thioether, respectively (see below, Figure S3 and S4).

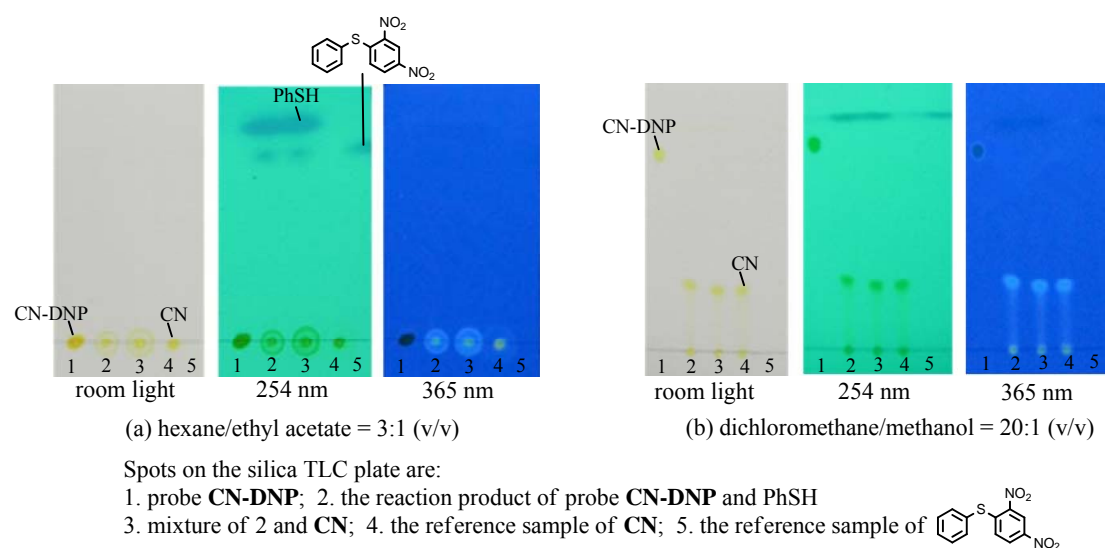
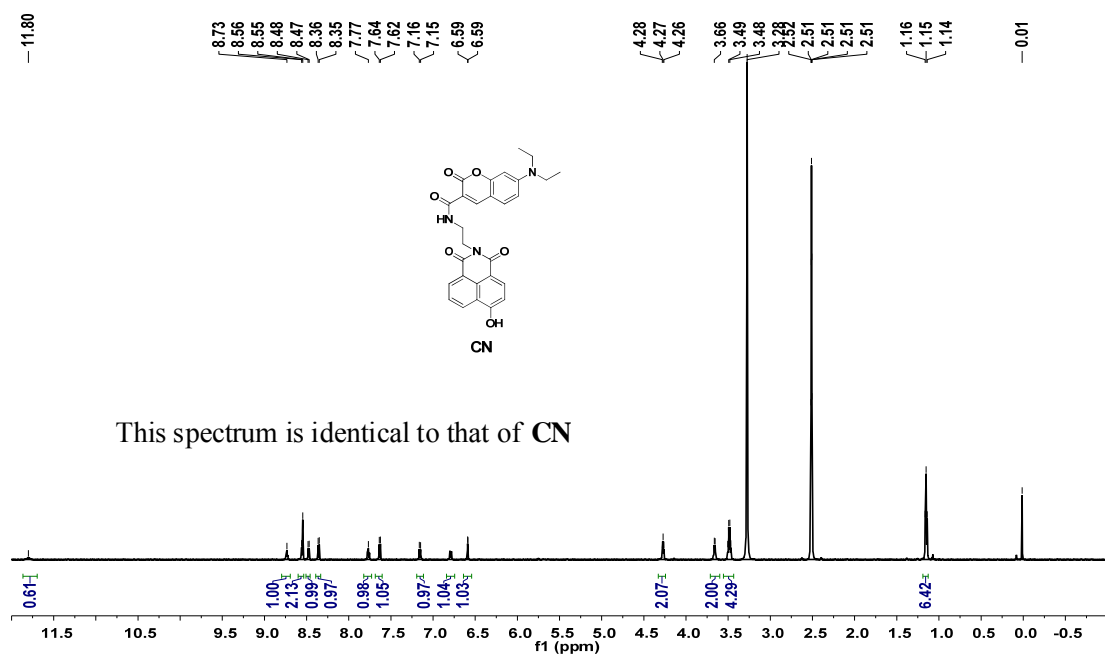
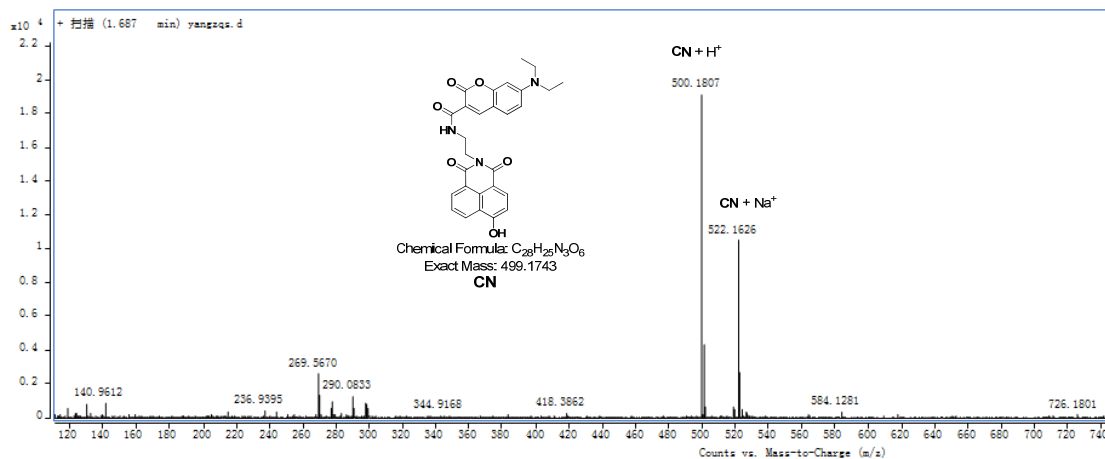


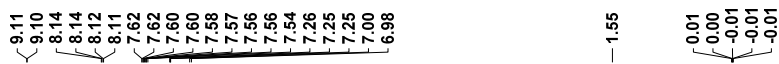
Figure S3. Thin layer chromatography (TLC) analysis of the reaction of probe **CN-DNP** with 3 equiv. of thiophenol in PBS buffer (50 mM, pH 7.4, with 40% DMF, v/v) under different light and different eluent conditions. The eluent for TLC: (a) hexane/ethyl acetate = 3:1 (v/v) and (b) dichloromethane/methanol = 20:1 (v/v). The result of TLC analysis clearly indicates that the reaction of probe **CN-DNP** with PhSH produced **CN** and **B**. Compound **B** has been reported in our previous published paper (Yu. D., Huang. F., Ding. S., Feng. G. *Anal. Chem.*, 2014, **86**, 8835–8841).



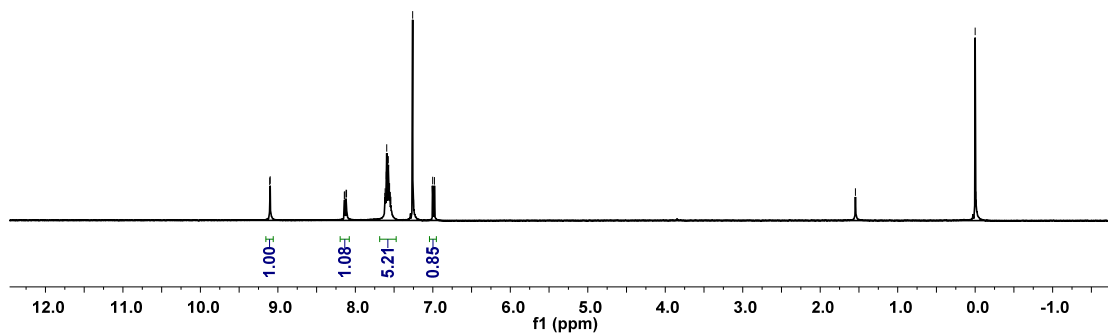
¹H-NMR spectrum of the isolated product **A** in *d*₆-DMSO



HR-MS spectrum of the isolated product **A**, which shows the expected MS of CN



This spectrum is identical to the reported spectrum of O=[N+]([O-])c1ccc(Sc2ccccc2)cc1



$^1\text{H-NMR}$ spectrum of product **B** in CDCl_3

Figure S4. $^1\text{H NMR}$ and HR-MS spectra of the isolated product **A** and **B** from the reaction of probe **CN-DNP** with thiophenol.

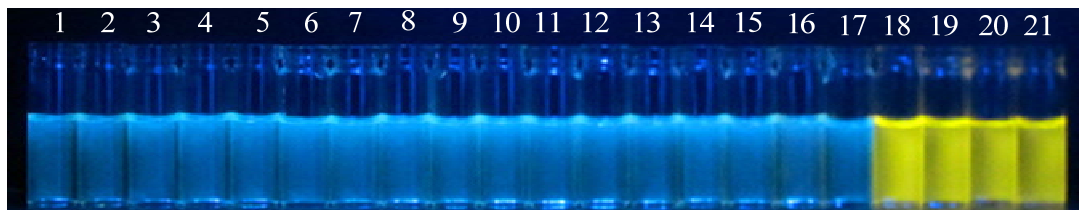


Figure S5. Emission color changes of probe **CN-DNP** (10 μM) upon addition of various analytes (100 μM) in PBS buffer (50 mM, pH 7.4) with 40% DMF at 37 $^{\circ}\text{C}$. Analytes from left to right: 1. none, 2. Cys, 3. Hcy, 4. GSH, 5. NaN_3 , 6. NaCl, 7. NaHS, 8. Na_2SO_3 , 9. NaHSO_3 , 10. KBr, 11. KI, 12. KCN, 13. NaSCN, 14. $\text{C}_6\text{H}_5\text{OH}$, 15. $\text{C}_6\text{H}_5\text{NH}_2$, 16. $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{SH}$, 17. *p*- NO_2 - $\text{C}_6\text{H}_4\text{SH}$, 18. *p*- OCH_3 - $\text{C}_6\text{H}_4\text{SH}$, 19. *p*- CH_3 - $\text{C}_6\text{H}_4\text{SH}$, 20. *p*- NH_2 - $\text{C}_6\text{H}_4\text{SH}$ and 21. $\text{C}_6\text{H}_5\text{SH}$. The photo is taken under a 365 nm UV light.

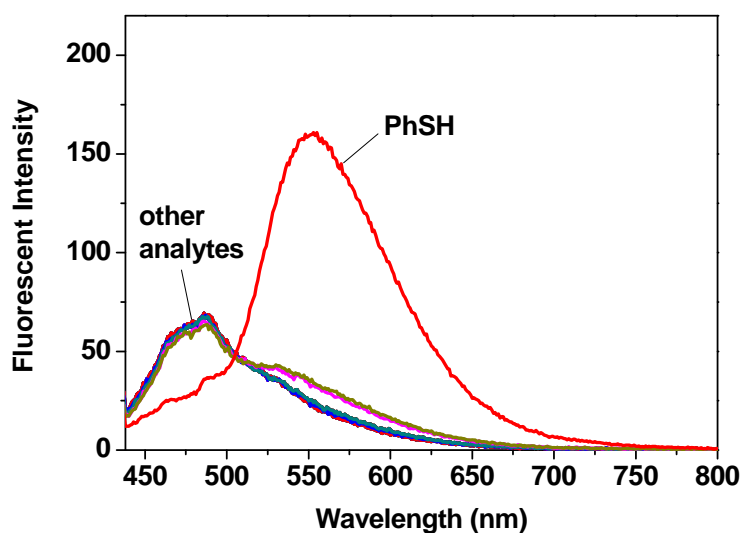


Figure S6. Fluorescence spectra changes of probe **CN-DNP** (10 μM) upon addition of various analytes (100 μM , except GSH 1 mM) in DMF-PBS buffer (50 mM, pH 7.4, containing 40% DMF and 10% FBS. FBS is fetal bovine serum.). Other analytes are: none, Cys, Hcy, GSH, NaN_3 , NaCl, NaHS, Na_2SO_3 , NaHSO_3 , NaF, KBr, KI, KCN, NaSCN, $\text{C}_6\text{H}_5\text{OH}$, $\text{C}_6\text{H}_5\text{NH}_2$, NaClO, H_2O_2 , NaNO_2 , $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{SH}$. All data were collected at 37 $^{\circ}\text{C}$ with $\lambda_{\text{ex}} = 425$ nm and slit width: $d_{\text{ex}} = d_{\text{em}} = 5$ nm.

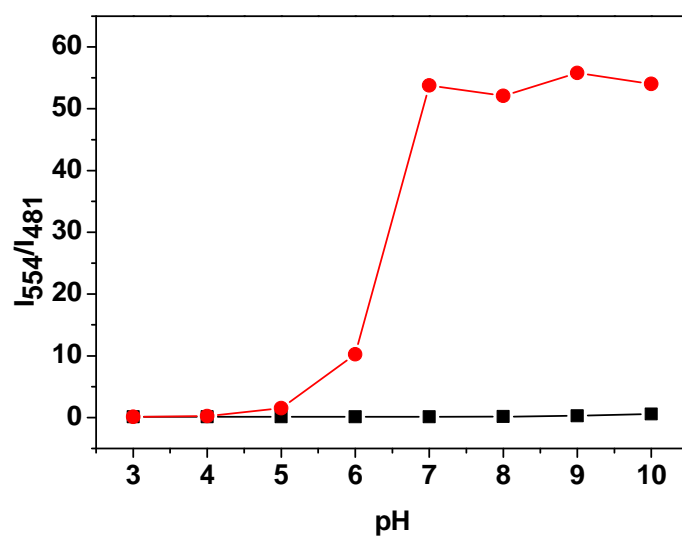


Figure S7. Fluorescence emission ratios (I_{554}/I_{481}) of **CN-DNP** (10 μM) as a function of pH values in the absence (the black point) or presence (the red point) of thiophenol (30 μM). The excitation wavelength was 425 nm.