Supporting Information for

A New Strategy to Construct FRET Platform for Ratiometric Sensing Hydrogen Sulfide

Longwei He,[a] Weiying Lin,*[, a, b] Qiuyan Xu,[a] and Haipeng Wei[a]

[a] State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, Hunan 410082, P. R. China.

[b] Institute of Fluorescent Probes for Biological Imaging, School of Chemistry and Chemical Engineering, School of Biological Science and Technology, University of Jinan, Jinan, Shandong 250022, P.R. China.

E-mail: Weiyinglin2013@163.com

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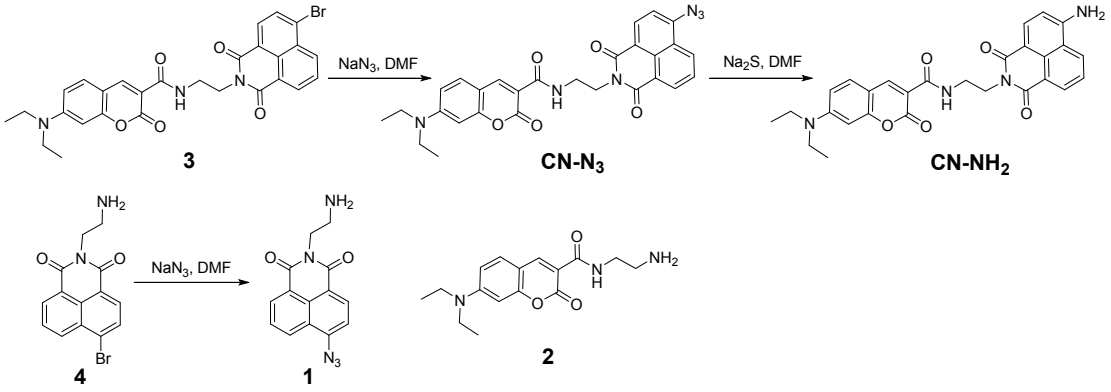
Materials and instruments: Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments; High resolution mass spectrometric (HRMS) analyses were measured on a Finnigan MAT 95 XP spectrometer; NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard; Electronic absorption spectra were obtained on a LabTech UV Power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell; The fluorescence imaging of cells was performed with OLYMPUS FV1000 (TY1318) confocal microscopy; The ratiometric fluorescence images were acquired using Image-Pro Plus software (a powerful 2D and 3D image processing, enhancement, and analysis software with extensive measurement and customization features); The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

PC-3 cells culture. PC-3 cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C.

Imaging of exogenous H_2S in living cells. PC-3 cells were incubated with 5.0 μM **CN-N_3** for 20 minutes in an atmosphere of 5% CO_2 and 95% air, and then treated with 20 μM NaHS for 10 min. Subsequently, the cells were imaged using OLYMPUS FV1000 (TY1318) confocal microscope with an excitation filter of 405 nm and emission channels of 450−480 nm (blue channel) and 520−560 nm (green channel).

Imaging of endogenous H_2S in living cells. PC-3 cells were incubated with 200 μM cysteine for 60 minutes in an atmosphere of 5% CO_2 and 95% air, and then treated with 5.0 μM **CN-N_3** for 10 min. Subsequently, the cells were imaged using OLYMPUS FV1000 (TY1318) confocal microscope with an excitation filter of 405 nm and emission channels of 450−480 nm (blue channel) and 520−560 nm (green channel).

Synthesis. Compounds **2**, **3**, and **4** were prepared according to the reported methods previously.[1]



Synthesis of compound CN-N_3. The mixture of Compound **3** (28 mg, 0.05 mmol) and sodium azide (16 mg, 0.25 mmol) in 3 mL of dry N,N-dimethylformide (DMF) was heated to 105 ℃ for 3 h. After cooling to room temperature, the reaction mixture was poured into 50 mL of water and then extracted three times with dichloromethane. The organic phase was collected, washed with brine, and dried with anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the solid residue was purified by flash chromatography column using methanol/dichloromethane (v/v 1:50) to afford a yellow solid as compound **CN-N_3** (20 mg, yield 76.2%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.21-1.24 (t, *J* = 7.2 Hz, 6H), 3.41-3.46 (q, *J* = 7.2 Hz, 4H), 3.82-3.86 (q, *J* = 6.0 Hz, 2H), 4.47-4.50 (t, *J* = 6.0 Hz, 2H), 6.45-6.46 (1H), 6.60-6.63 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.36-7.38 (d, *J* = 9.2 Hz, 1H), 7.43-7.45 (d, *J* =8.0 Hz, 1H), 7.70-7.73 (t, *J* = 7.8 Hz, 1H), 8.40-8.42 (d, *J* = 8.4, 1H), 8.57-8.59 (d, *J* = 8.0, 1H), 8.62-8.63 (2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.43, 30.02, 38.30, 39.55, 45.05, 92.59, 96.55, 108.39, 109.82, 110.37, 114.68, 118.85, 122.53, 124.36, 126.84, 128.76, 129.29, 131.08, 131.87, 132.38, 143.40, 148.06, 152.44, 157.60, 162.52, 163.57, 164.10. MS (EI) *m/z* 524.2 $[M]^+$. HRMS (EI) *m/z* calcd for $C_{28}H_{24}N_6O_5$ (M^+): 524.2054. Found 524.2040.

Synthesis of compound CN-NH_2. Sodium sulfide (12 mg, 0.15 mmol) was added to the solution of compound **CN-N_3** (16 mg, 0.03 mmol) in 3 mL DMF. The mixture was stirred for 4 h. The reaction mixture was poured into 50 mL of water and then extracted three times with dichloromethane. The organic phase was collected, washed with brine, and dried with anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the solid residue was purified by flash chromatography column using methanol/dichloromethane (v/v 1:20) to afford a yellow solid as compound **CN-NH_2** (13 mg, yield 87.4%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.12-1.16 (t, *J* = 7.0 Hz, 6H), 3.30-3.35 (q, *J* = 7.0 Hz, 4H), 3.82-3.85 (t, *J* = 6.8 Hz, 2H), 4.44-4.48 (t, *J* = 6.8 Hz, 2H), 6.06 (s, 1H), 6.09-6.12 (d, *J* = 9.6 Hz, 1H), 6.78-6.80 (d, *J* = 8.4 Hz, 1H), 6.93-6.95 (d, *J* =8.8 Hz, 1H), 7.52-7.56 (t, *J* = 7.8 Hz, 1H), 8.07-8.09 (1H), 8.34-8.36 (d, *J* = 8.0, 1H), 8.53-8.54 (d, *J* = 7.2, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.73,

29.70, 40.51, 44.47, 54.27, 98.40, 103.16, 108.46, 109.44, 111.37, 111.40, 119.97, 122.68, 124.75, 127.28, 128.76, 129.88, 131.61, 133.10, 134.00, 149.75, 151.87, 163.99, 164.66. MS (EI) *m/z* 498.2 $[M]^+$. HRMS (EI) *m/z* calcd for $C_{28}H_{26}N_4O_5$ (M^+): 498.1898. Found 498.1890.

Synthesis of compound 1. The mixture of compound **4** (32 mg, 0.1 mmol) and sodium azide (32 mg, 0.5 mmol) in 3 mL of dry N,N-dimethylformide (DMF) was heated to 105 ℃ for 3 h. After cooling to room temperature, the reaction mixture was poured into 50 mL of water and then extracted three times with dichloromethane. The organic phase was collected, washed with brine, and dried with anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the solid residue was purified by flash chromatography column using methanol/dichloromethane (v/v 1:20) to afford a yellow solid as compound **1** (24 mg, yield 85.7%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.70-3.74 (m, 2H), 4.40-4.42 (q, *J* = 5.4 Hz, 2H), 7.45-7.47 (d, *J* = 8.0 Hz, 1H), 7.72-7.76 (t, *J* = 7,6 Hz, 1H), 8.43-8.45 (d, *J* = 8.4, 1H), 8.55-8.57 (d, *J* = 8.0, 1H), 8.61-8.63 (d, *J* = 7.2, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.07, 39.27, 114.73, 118.34, 122.13, 124.36, 126.92, 129.23, 132.09, 132.58, 143.98, 161.53, 164.16, 164.62. MS (EI) *m/z* 281.1 $[M]^+$. HRMS (EI) *m/z* calcd for $C_{14}H_{11}N_5O_2$ (M^+): 281.0997. Found 281.0794.

References

(1) (a) M. Ikeda, T. Yoshii, T. Matsui, T. Tanida, H. Komatsu and I. Hamachi, *J. Am. Chem. Soc.*, 2011, **133**, 1670; (b) X. Zhou, F. Su, H. Lu, P. Senechal-Willis, Y. Tian, R. H. Johnson and D. R. Meldrum, *Biomaterials*, 2012, **33**, 171.

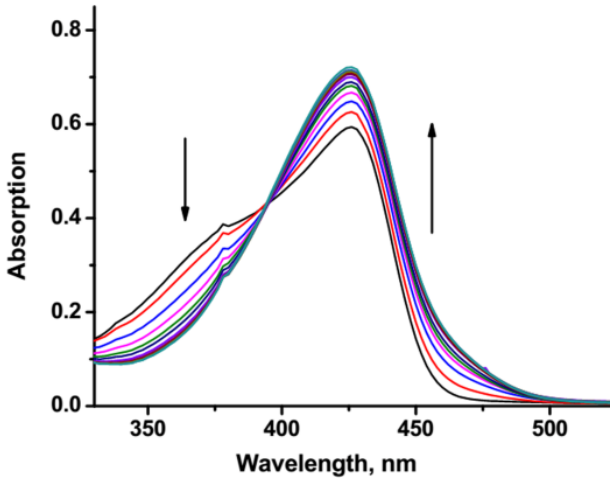


Fig. S1 Absorption spectra of 10 μM **CN-N_3** with 0–5.0 eq. of NaHS in 25 mM phosphate buffer (pH 7.4, containing 50% ethanol).

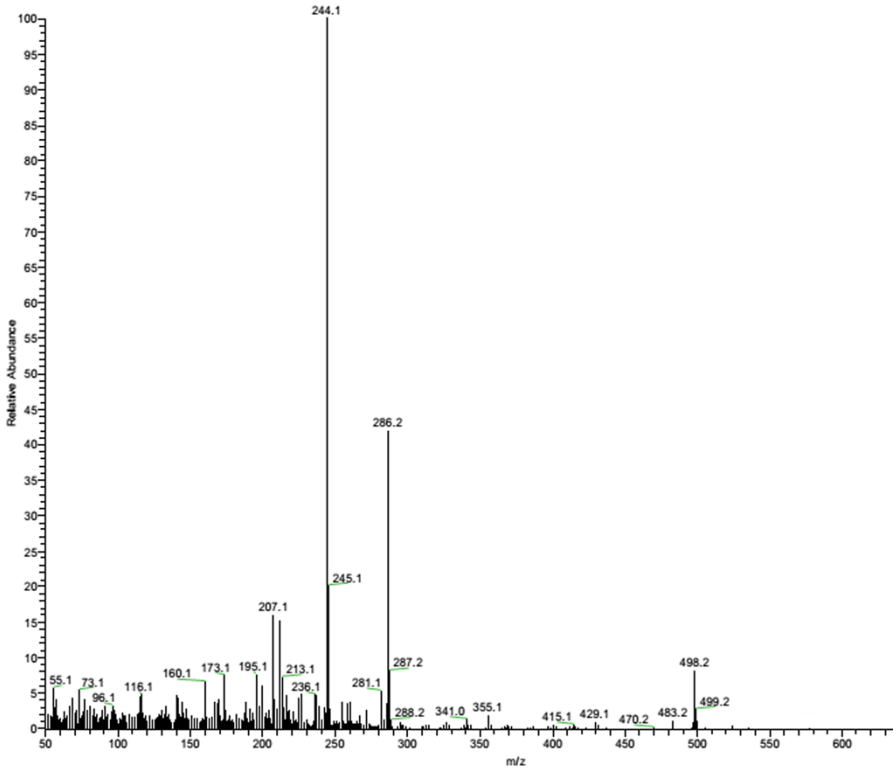


Fig. S2 EI-MS spectrum of compound **CN-NH_2** obtained and isolated from reaction mixture of **CN-N3** with NaHS.

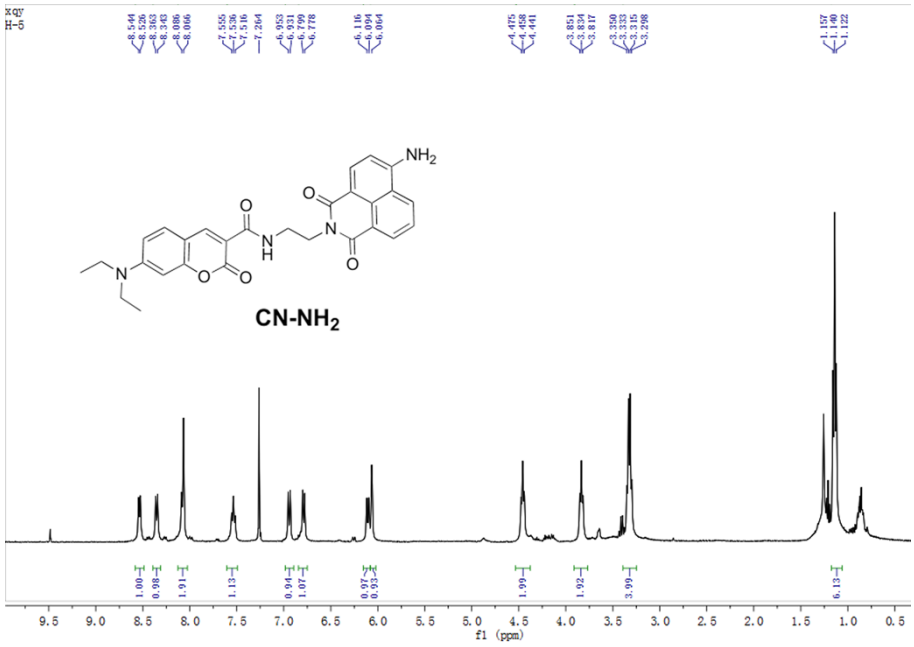


Fig. S3 The ^{1}H NMR spectrum of **CN-NH$_2$** in $CDCl_3$.

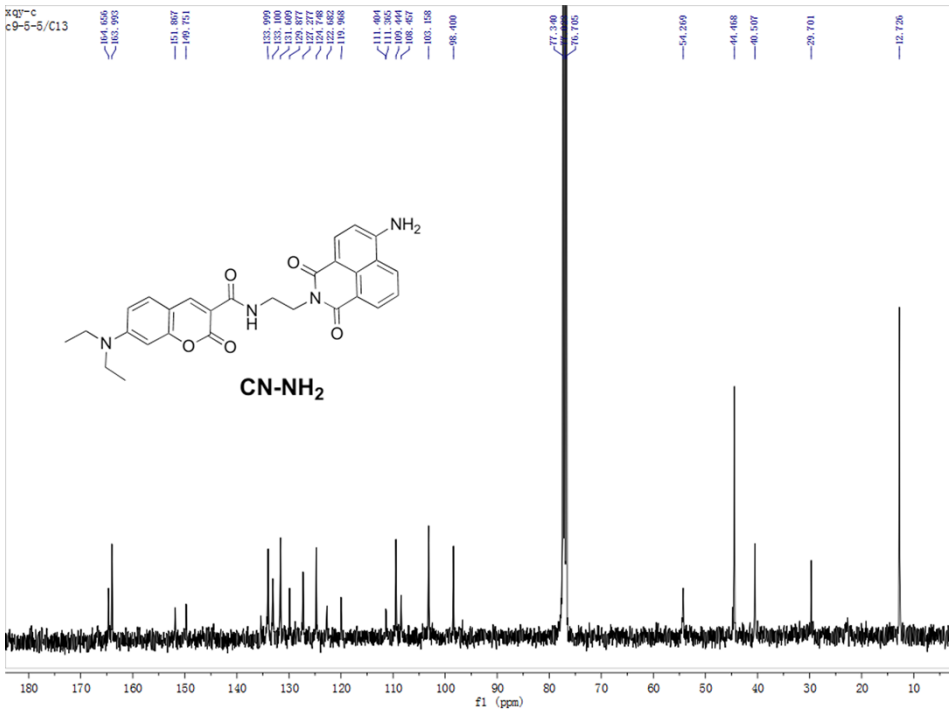


Fig. S4 The ^{13}C NMR spectrum of **CN-NH$_2$** in $CDCl_3$.

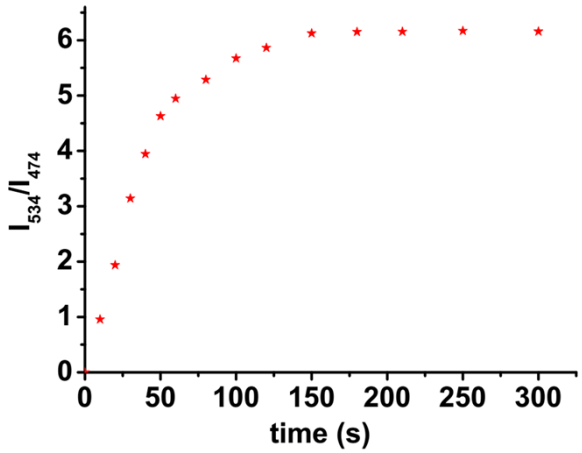


Fig. S5 Time-dependent (0 to 6 min) fluorescence intensity ratio (I_{534}/I_{474}) responses of sensor **CN-N$_3$** (10 μM) to NaHS (50 μM) in PBS buffer (pH 7.4, containing 50% ethanol).

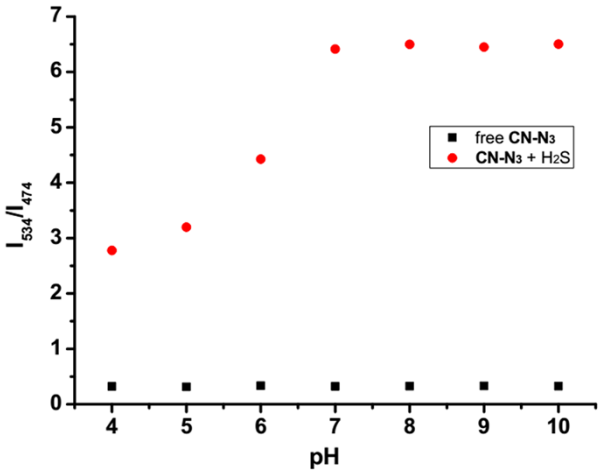


Fig. S6 The pH influence on the fluorescence intensity ratio (I_{534}/I_{474}) of **CN-N$_3$** (10 μM) in the absence (■) or presence (●) of NaHS (50 μM) in PBS buffer (containing 50% ethanol).

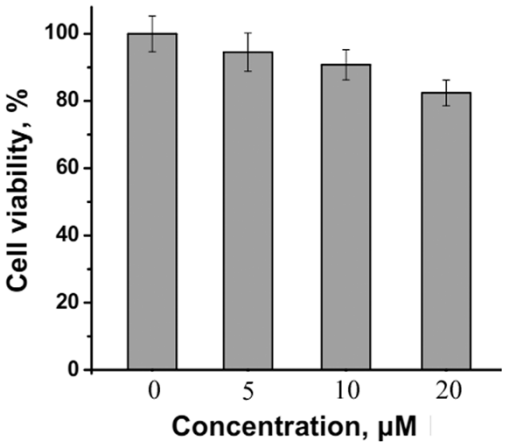


Figure S7. Cytotoxicity of probe **CN-N_3** evaluated on living cells by the standard MTT assay. The cells were incubated with the compound for 24 h.

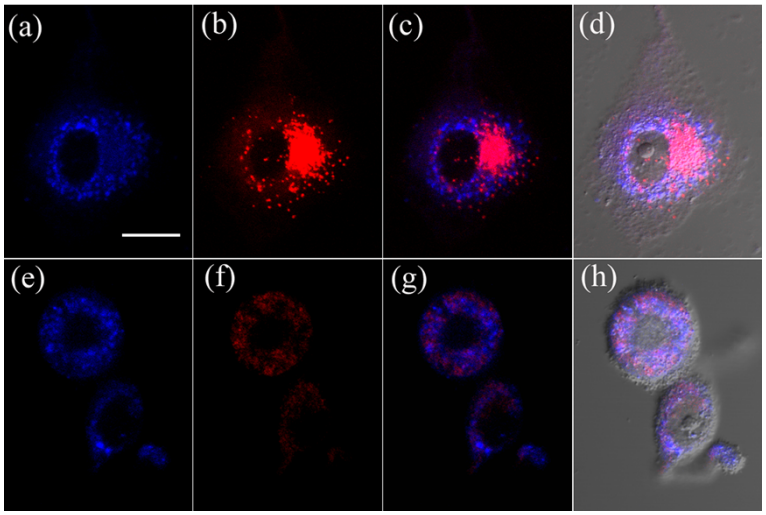


Fig. S8 Confocal fluorescence images of **CN-N_3** (5 μM) incubated with 1 μM LysoTracker Red (a-d) or 1 μM Mitotracker Red FM (e-h) in living PC-3 cells. Images were acquired using (a, e) 405 nm excitation and emission channel of 450−480 nm (blue) and (b, f) 559 nm excitation and emission channel of 650−700 nm (red); (c, g) merged images of blue and red channels; (d, h) merged bright field and images (c, g). Scale bar = 20 μm.

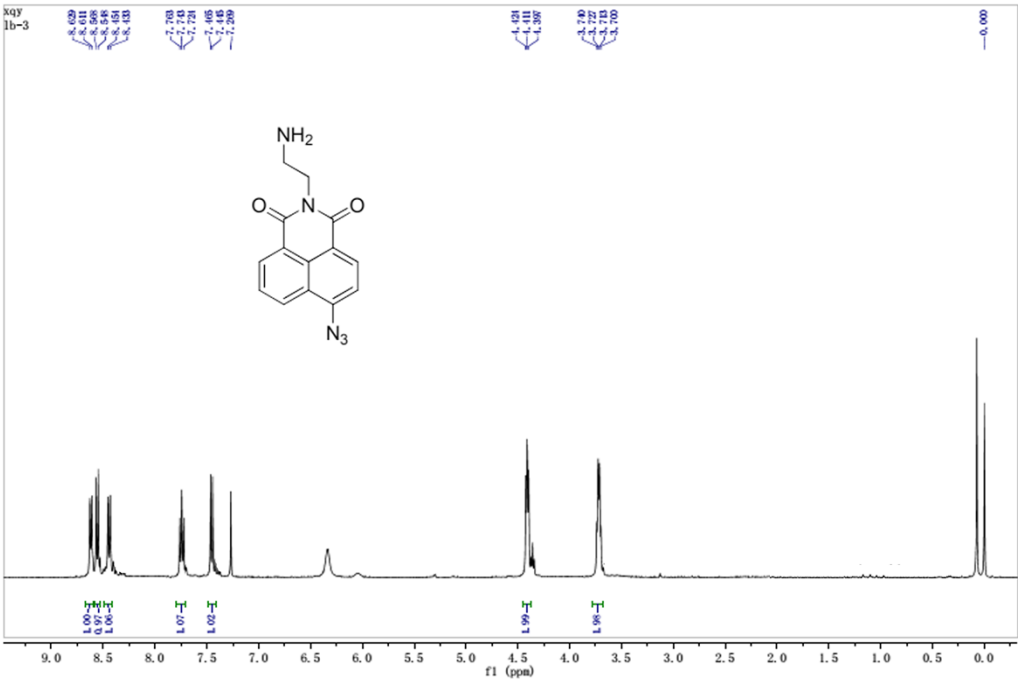


Fig. S9 ^{1}H NMR spectrum of control compound **1**.

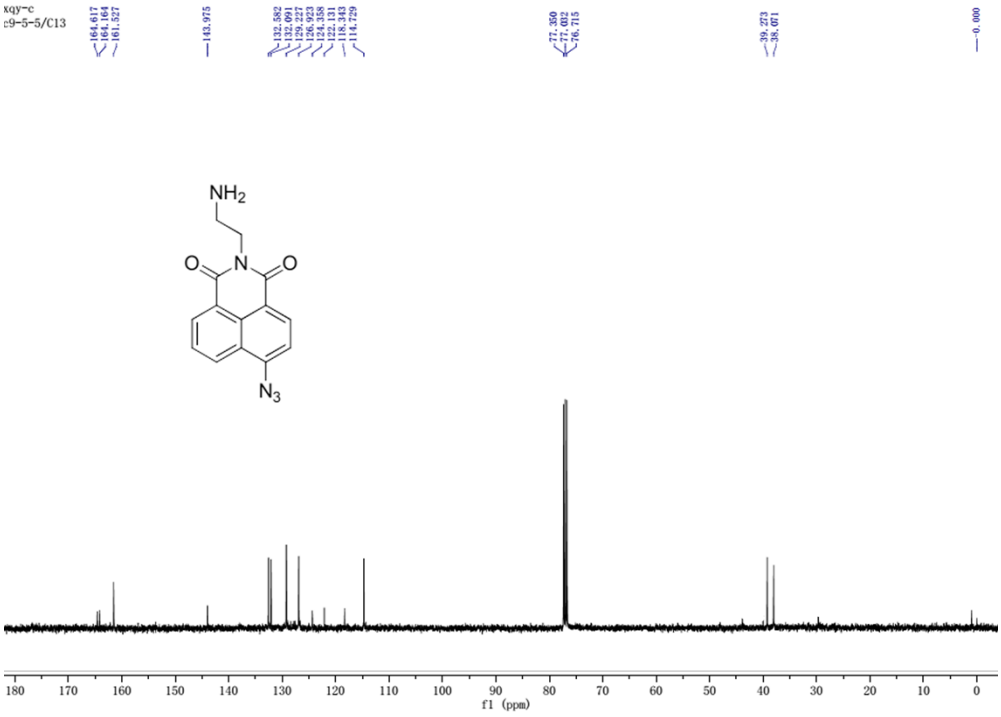


Fig. S10 ^{13}C NMR spectrum of control compound **1**.

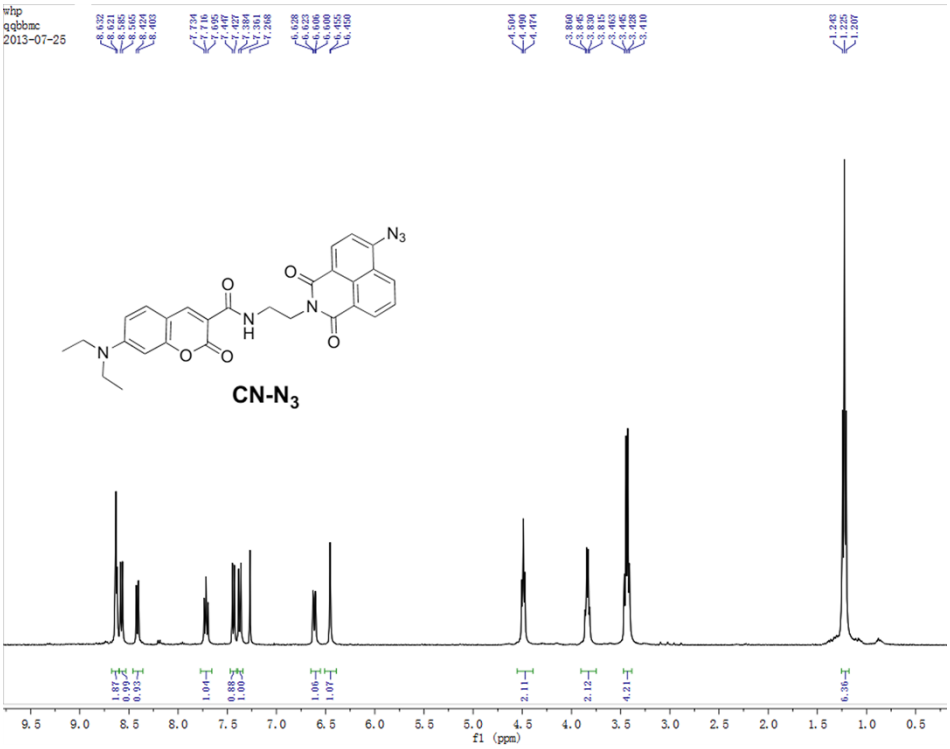


Fig. S11 ^{1}H NMR spectrum of compound **CN-N$_3$**.

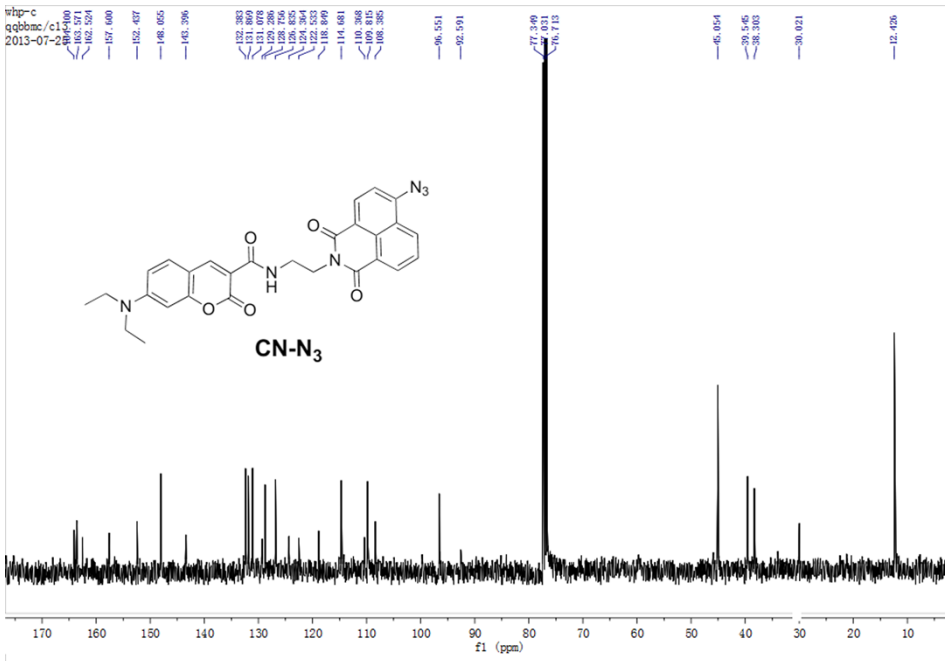


Fig. S12 ^{13}C NMR spectrum of compound **CN-N$_3$**.