Supporting Information

A "double-locked" probe for detection of hydrogen sulfide in viscous system

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Contents:

1. General Experimental Section

Materials. Solvents were dried by distillation before use. All other reagents were of commercial quality and used without further purification. HeLa cells and PC 12 cells were purchased from the Committee on Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). Sartorius ultrapure water (18.2 MΩ cm) water was used throughout the analytical experiments. Cells were cultured in phenol red-free Dulbecco's modified Eagle's medium (DMEM) supplemented with penicillin / streptomycin, and 10% fetal bovine serum in a 5% CO_2 incubator at 37 °C.

Instruments. F-4600 Fluorescence Spectrometer (Hitachi, Inc. Japan) with a 1.0 cm quartz cells at the slits of 10/10 nm. High-resolution mass spectral analyses were carried out on Bruker maxis UHR-TOF Ultra High Resolution Quadrupole-time of flight mass spectrometer (Bruker Co., Ltd., Germany). ^{1}H-NMR and ^{13}C-NMR spectra were taken on a Bruker Advance 400-MHz spectrometer, δ values are in ppm relative to TMS. The fluorescence images of cells were taken using a TCS SP8 confocal laser scanning microscopy (Leica Co., Ltd. Germany) with an objective lens (×40). All pH measurements were performed with a pH-3c digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass-calomel electrode. Absorbance was measured in a TRITURUS microplate reader in the MTT assay.

Fluorescence analysis. Fluorescence spectra were obtained with F-4600 Fluorescence Spectrometer (Hitachi, Inc. Japan) with a 1.0 cm quartz cells at the slits of 10/10 nm. After dilution **DCO-H_2S-V** to 10 μM with the PBS / glycerol (pH = 7.4, PBS: Gly = 1: 9) solution, various amounts of NaHS were added. Fluorescence emission spectra of **DCO-H_2S-V** (10 μM) under different solution viscosities in the presence of NaHS (20 μM) was measured. Excitation was at 460 nm.

Cell culture. HeLa cells and PC 12 cells were maintained following the protocols provided by the American Type Tissue Culture Collection. Cells were first grown in a circular petri dish (60 mm) using high glucose Dulbecco's Modified Eagle Medium (DMEM, 4.5 g of glucose/L) supplemented with 10% fetal bovine serum (FBS), $NaHCO_3$ (2 g/L) and 1% antibiotics (penicillin/streptomycin, 100 U/mL). Cultures were maintained in a humidified incubator at 37°C, in 5% CO_2 / 95% air. One

day before imaging, cells were passed and plated on 18 mm glass coverslips in culture dish. The culture medium was refreshed every 24 h. All cells used were in the exponential growth phase.

MTT assay. HeLa Cells (10^6 cell mL^{-1}) were dispersed within replicate 96-well microtiter plates to a total volume of 200 μL $well^{-1}$. Plates were maintained at 37 °C in a 5% CO_2 / 95% air incubator for 4 h. **DCO- H_2S-V** and **DCON** were diluted to different concentrations of solution with medium and added to each well after the original medium has been removed. HeLa cells were incubated with **DCO-H_2S-V** and **DCON** for 4 h. The concentrations of **DCO-H_2S-V** and **DCON** were 1 μM to 500 μM, respectively. MTT solution (5.0 mg mL^{-1} in PBS) was then added to each well. After 4 h, the remaining MTT solution was removed and DMSO (150 μL) was added to each well to dissolve the formazan crystals. Absorbance was measured at 490 nm in a TRITURUS microplate reader.

Confocal imaging. Fluorescence imaging studies were performed with a TCS SP8 confocal laser scanning microscope (Germany Leica Co., Ltd) with an objective lens (×40). Excitation of probe-loaded cells at 488 nm was carried out with an Argon laser, and emission was collected using a META detector between 520 and 700 nm. Prior to imaging, the medium was removed. Cell imaging was carried out after washing cells with PBS (pH 7.4, 0.10 M) three times. HeLa cells and PC12 cells were cultured in phenol red-free Dulbecco's modified Eagle's medium (DMEM) supplemented with penicillin/streptomycin, and 10% fetal bovine serum in a 5% CO_2 incubator at 37 °C. Cells were plated on confocal dish and allowed to adhere for 24 h. The HeLa cell experiment was divided into four groups. The first group was incubated with **DCO-H_2S-V** (10 μM) only for 20 min. The second group was pretreated with nystatin (10 μM) for 1 h and then incubated with **DCO-H_2S-V** (10 μM) for another 20 min. The third group was pretreated with NaHS (40 μM) for 30 min and then incubated with **DCO-H_2S-V** (10 μM) for another 20 min. The fourth group was pretreated with nystatin (10 μM) for 1 h and subsequently treated with NaHS (40 μM) for 30 min, then incubated with **DCO-H_2S-V** (10 μM) for another 20 min. The PC 12 cell experiment was divided into two groups. The first group was incubated with **DCO-H_2S-V** (10 μM) only for 20 min. The second group was pretreated with glutamate (1mM) for 4 h and then incubated with **DCO-H_2S-V** (10 μM) for another 20 min. Cells were washed three times by PBS buffer before imaging experiments.

2. Determination of the fluorescence quantum yield

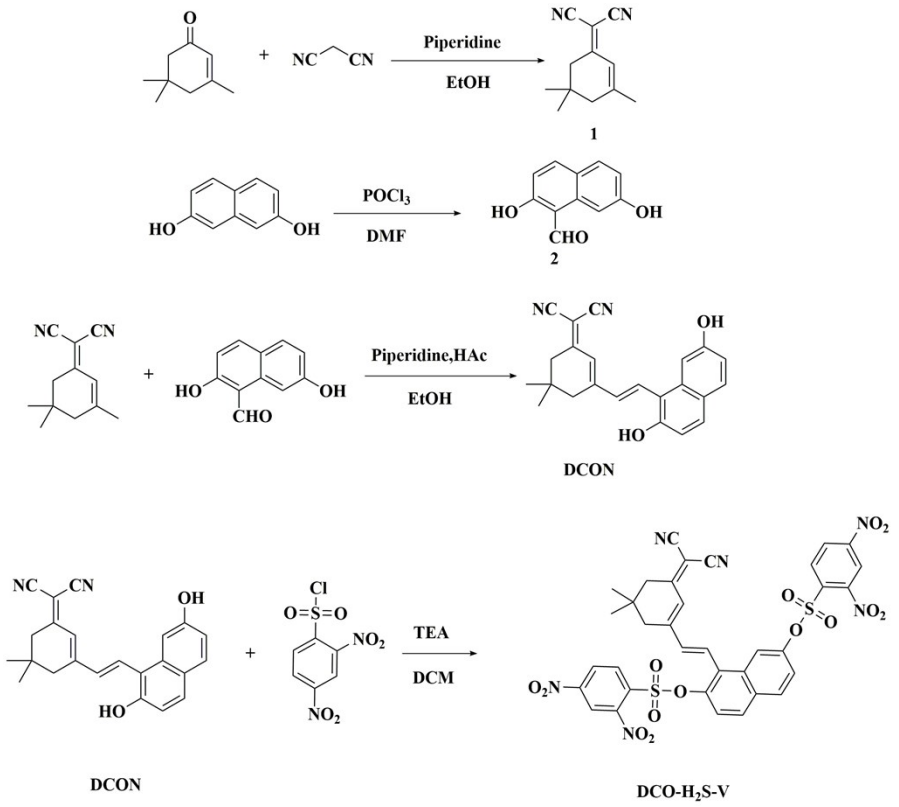
Fluorescence quantum yield (Φ) was determined by using rhodamine B (Φ = 0.89, in ethanol) as the fluorescence standard. The quantum yield was calculated using the following equation.

$$\Phi_x = \Phi_{st}\left(D_x/D_{st}\right)\left(A_{st}/A_x\right)\left(\eta_x^{\,2}/\eta_{st}^{\,2}\right)$$

Where Φ_{st}, D and A refer to the reported quantum yield of the standard, the area under the emission spectra and the absorbance at the excitation wavelength. η stands for the refractive index of the solvent used. Subscripts x and st represents sample and standard, respectively. Absorbance of sample and reference at their respective excitation wavelengths was controlled to be lower than 0.05. (Ref. Anal. Chem. 2021, 93, 3241-3249).

The quantum yield of **DCO-H_2S-V** is lower than 0.001 in PBS and glycerol solution due to the effective fluorescence quenching effect of the 2, 4-dintrobenzensulfonyl moiety on the fluorophore core. The fluorescence quantum yield of **DCON** was increased from 0.0034 in PBS to 0.141 in glycerol.

3. Synthesis of Fluorescent Probe DCO-H_2S-V



Scheme S1. Synthesis of **DCO-H_2S-V**

Synthesis of compound 1: Isophorone (1.5 mL, 10 mmol) and malononitrile (0.66 g, 10 mmol) were dissolved in 20 mL anhydrous ethanol, catalytic amount of piperidine was added. The mixture was stirred at 60 ℃ for 8 h. After cooling, the reaction mixture was poured into ice water (100 mL), the precipitated solid was filtered to give product **1** (1.11 g, yield 60%).^{1}H NMR (400 MHz, DMSO-d_6): δ 6.56 (s, 1H), 2.53 (s, 2H), 2.23 (s, 2H), 2.05 (s, 3H), 0.96 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 171.9, 162.9, 119.8, 113.9, 113.1, 76.5, 45.3, 42.4, 32.4, 27.7, 25.4. HRMS: Calculated for [$C_{12}H_{14}N_2$], [M-H]$^-$: 185.1157, found 185.1103.

Synthesis of compound 2: Phosphorus oxychloride (20 mL, 210 mmol) was slowly transfered into 20 mL anhydrous DMF which was placed in ice-water bath, 2,7-dihydroxynaphthalene (2 g, 12.4 mmol) which was dissolved in 10 mL anhydrous DMF was added into the reaction mixture. The mixture was stirred at 55 ℃ for 4 h. After cooling, the reaction mixture was poured into ice water (300 mL), the precipitated solid was filtered to give product **2** (1.16 g, yield 50%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.71 (s, 1H), 8.35 (d, *J* = 1.6 Hz, 1H), 7.94 (d, *J* = 8.9 Hz, 1H), 7.68 (d, *J* = 8.8 Hz, 1H), 7.06-6.81 (m, 4H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 192.4, 165.4, 159.4, 138.6, 134.2, 130.9, 122.5, 116.2, 115.2, 112.1, 105.7. HRMS: Calculated for [$C_{11}H_8O_3$], [M-H]$^-$: 187.0473, found 187.0382.

Synthesis of DCON: Compound **1** (220 mg, 1.2 mmol) and Compound **2** (225 mg, 1.2 mmol) were dissolved in 5 mL anhydrous ethanol, then 0.5 mL piperidine and 0.5 mL acetic acid was added. The mixture was stirred at 90 ℃ for 4 h. After cooling to room temperature, the solvent was removed and extracted with water and dichloromethane, the organic phase was dried with anhydrous sodium sulfate, after the solvent was removed under reduced pressure. The residue was purified using silica gel column chromatography with DCM/EtOH (50/1), yielding a red solid (85 mg, 20%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.47 (s, 1H), 9.77 (s, 1H), 7.67 (t, *J* = 12.4 Hz, 3H), 7.54-7.38 (m, 2H), 7.00 (d, *J* = 8.8 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 1H), 6.73 (s, 1H), 2.66 (d, *J* = 17.2 Hz, 4H), 1.08 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 170.7, 157.7, 157.3, 156.7, 134.9, 132.9, 132.5, 131.8, 130.9, 123.2, 121.9, 115.6, 115.3, 114.6, 113.8, 113.3, 105.5, 75.5, 49.1, 42.9, 38.4, 32.2, 27.9. HRMS: Calculated for [$C_{23}H_{20}N_2O_2$], [M-H]$^-$: 355.1525, found 355.1414.

Synthesis of DCO-H_2S-V: **DCON** (10 mg, 0.03 mmol) were dissolved in 1.5 mL dichloromethane, a drop of triethylamine was added and stirred for ten minutes. 2,4-dinitrobenzenesulfonyl chloride (24 mg, 0.09 mmol) which was dissolved in 1 mL dichloromethane was added into the reaction

mixture. The mixture was stirred at room temperature for 2 h. After the red solid was filtered to give **DCO-H_2S-V** (12 mg, yield 49%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.02 (d, *J* = 15.1 Hz, 2H), 8.48 (d, *J* = 8.4 Hz, 2H), 8.26 (d, *J* = 8.7 Hz, 1H), 8.10 (dd, *J* = 12.3, 5.8 Hz, 3H), 7.80 (s, 1H), 7.53 (d, *J* = 9.0 Hz, 1H), 7.39 (d, *J* = 7.7 Hz, 1H), 7.10 (d, *J* = 16.4 Hz, 1H), 6.76 (d, *J* = 16.4 Hz, 1H), 6.60 (s, 1H), 2.43 (s, 2H), 2.29 (s, 2H), 0.95 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 170.4, 153.9, 151.9, 148.5, 148.3, 147.9, 145.3, 139.2, 134.1, 133.7, 132.1, 131.8, 131.2, 127.8, 124.7, 123.2, 121.9, 121.4, 121.1, 118.4, 113.9, 112.8, 79.2, 55.3, 42.5, 38.1, 31.9, 27.7. HRMS: Calculated for [$C_{35}H_{24}N_6O_{14}S_2$], [M-H]$^-$: 815.0708, found 815.0677.

4. Recognition mechanism of DCO-H_2S-V toward H_2S

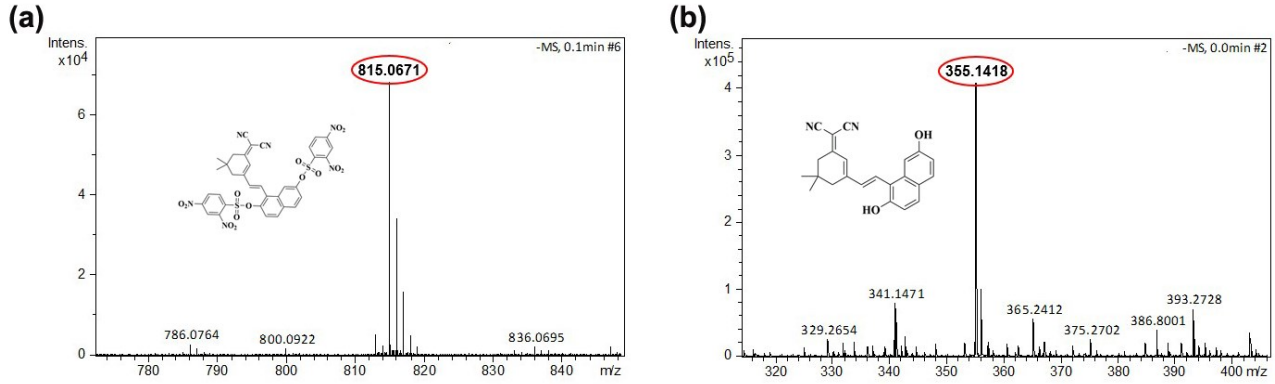


Figure S1. The MS peak of **DCO-H_2S-V** (10 μM) before (a) and after (b) reaction with NaHS (40 μM) in PBS. The peak at m/z =355.1418 [M-H]$^-$ indicates the generation of fluorophore **DCON**.

5. Effects of pH

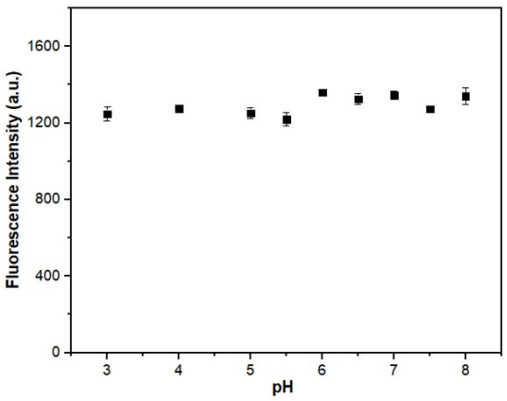
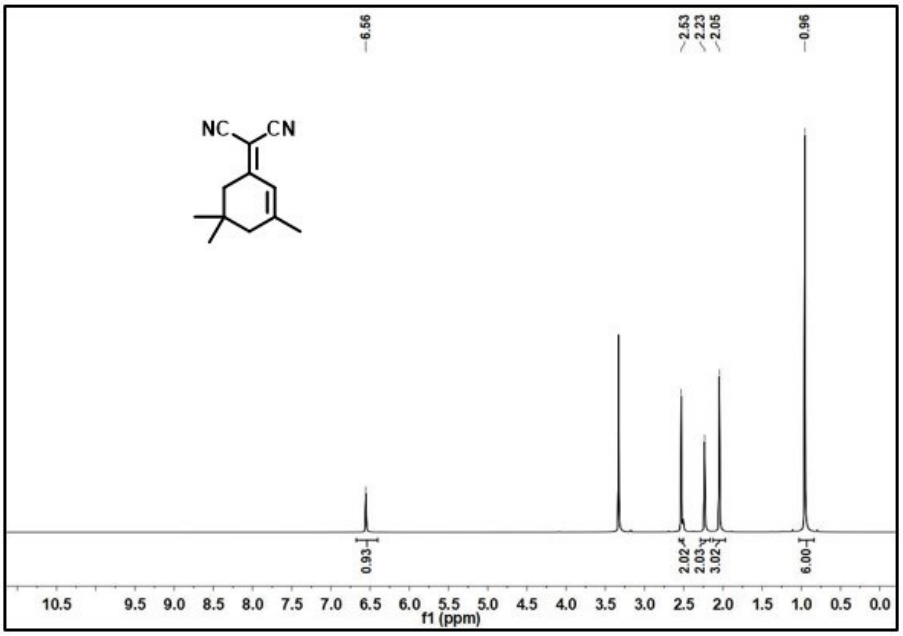
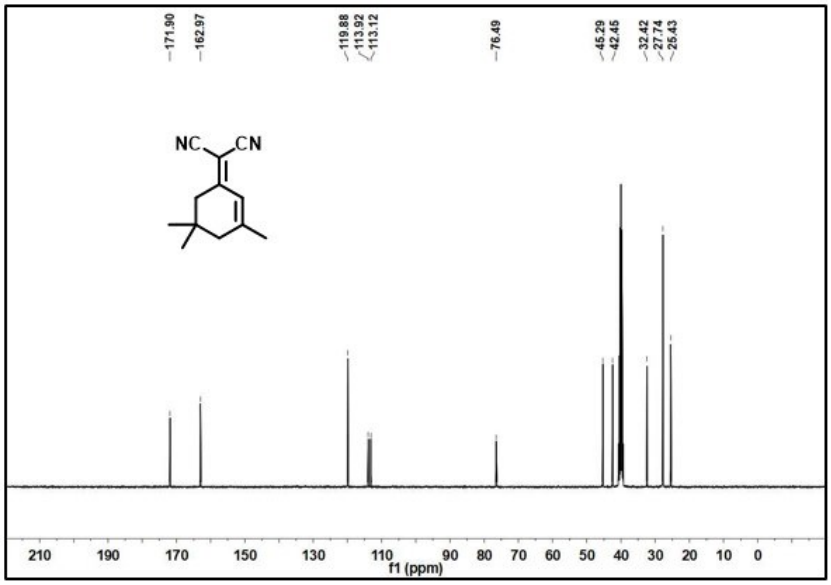


Figure S2. Fluorescence intensity of **DCO-H_2S-V** (10 μM) in PBS: glycerol =2: 8 with different values of pH. $\lambda_{ex/em}$ = 460 / 630 nm.

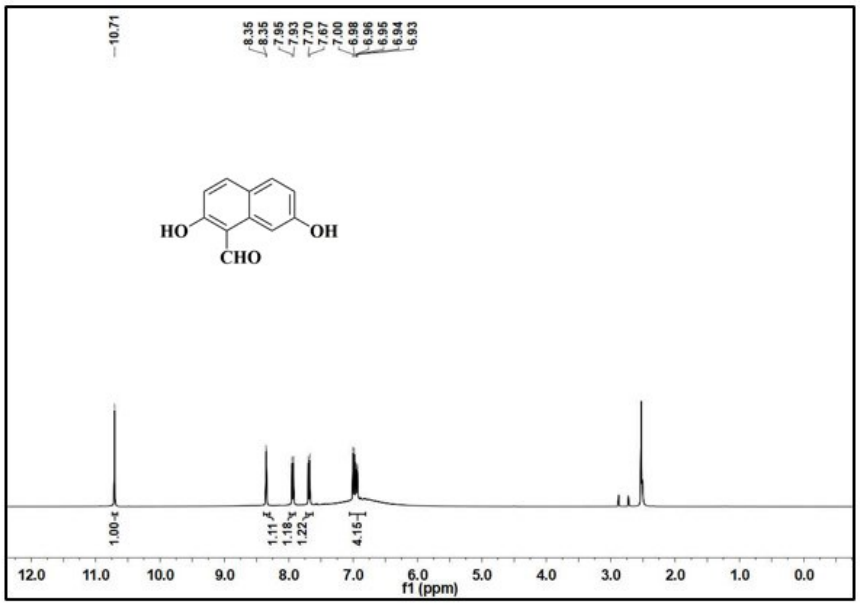
6. Spectrogram of DCO-H_2S-V and intermediates



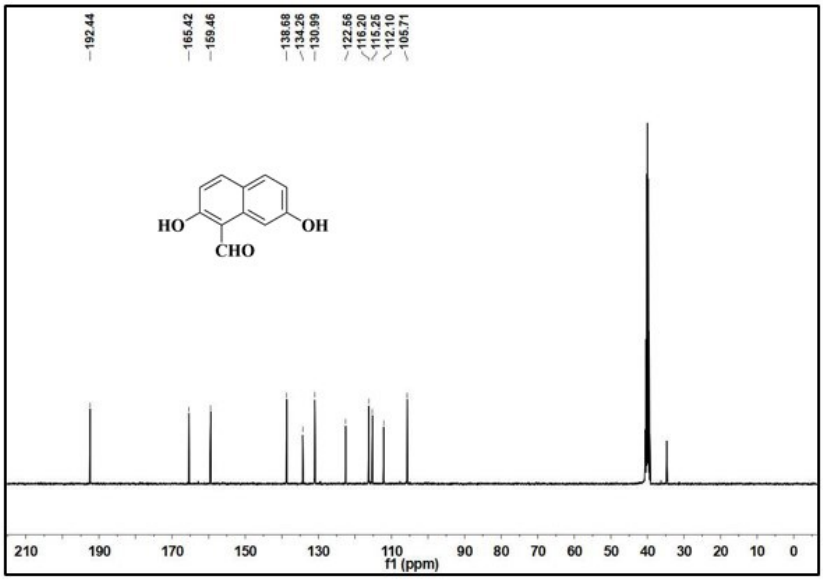
^{1}H NMR of compound **1**



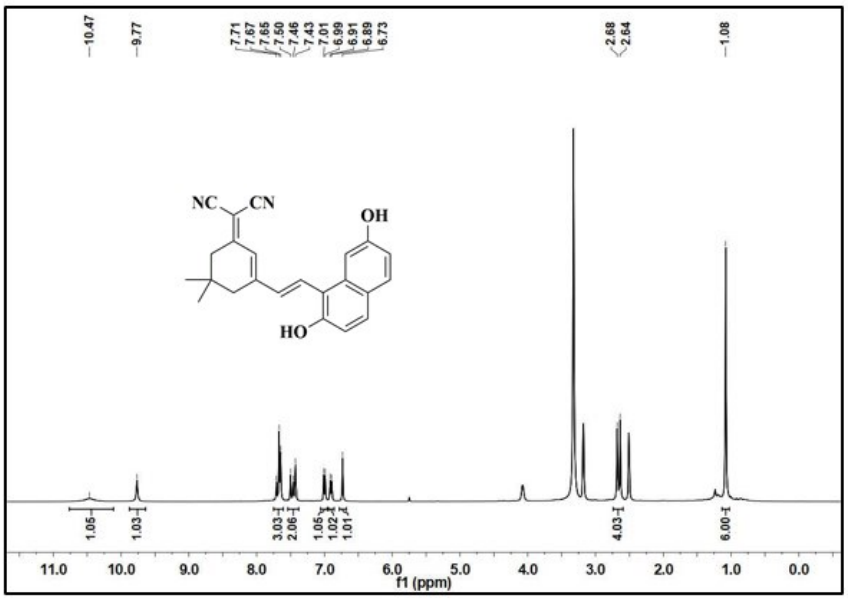
^{13}C NMR of compound **1**



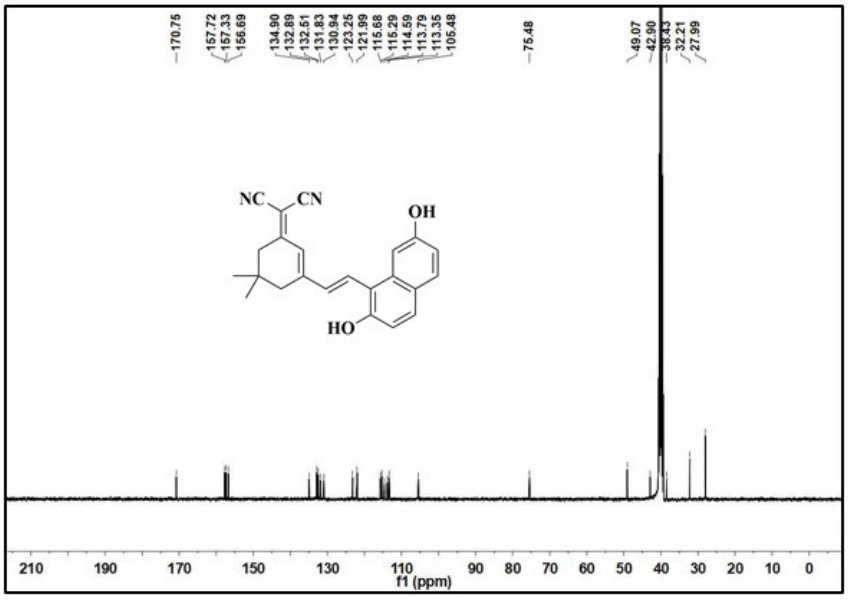
^{1}H NMR of compound **2**



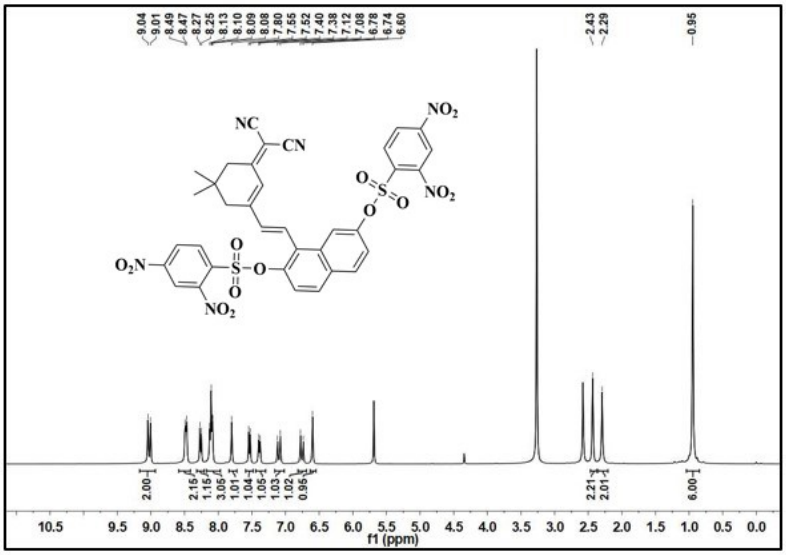
^{13}C NMR of compound **2**



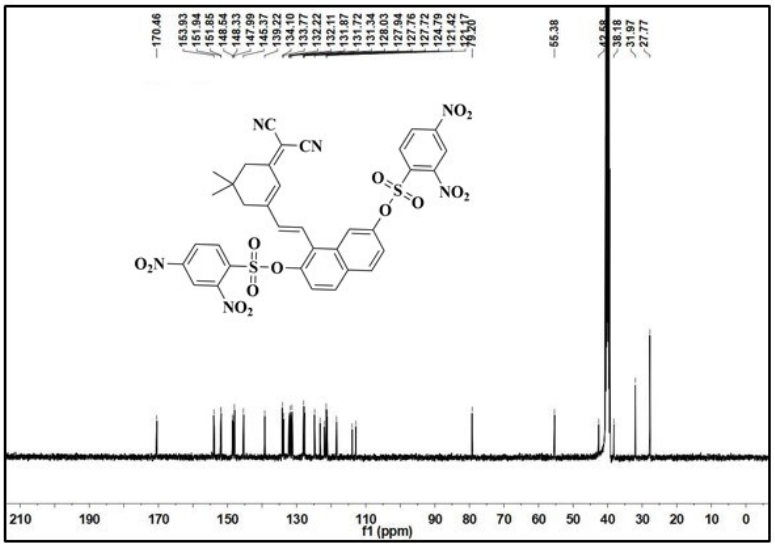
^{1}H NMR of compound **DCON**



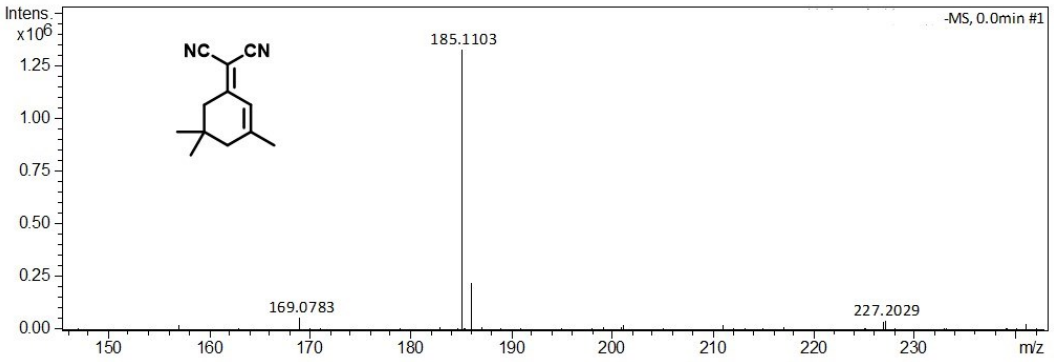
^{13}C NMR of compound **DCON**



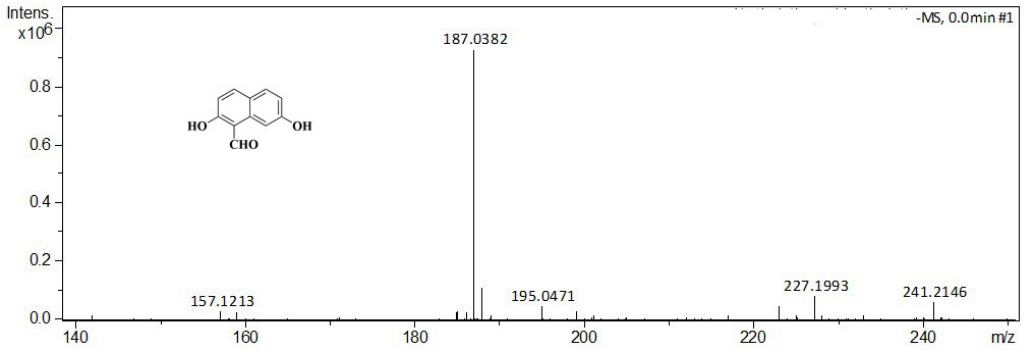
^{1}H NMR of probe **DCO-H_2S-V**



^{13}C NMR of probe **DCO-H_2S-V**



HR MS of compound **1**



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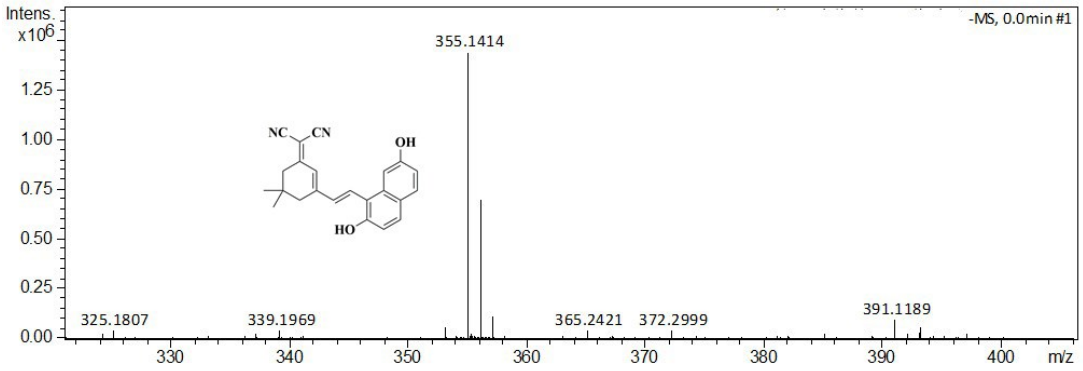
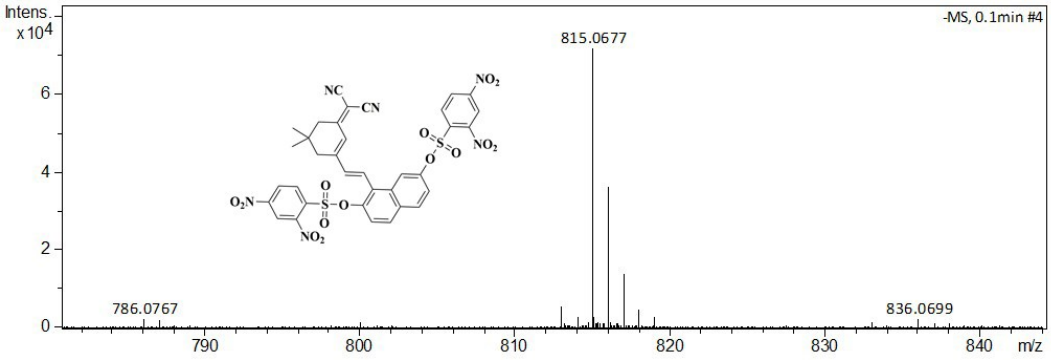


HRMS of compound **DCON**



HRMS of probe **DCO-H_2S-V**