Electronic Supplementary Material (ESI) for Analyst.

Synthesis and Bioimaging of a Biocompatible Hydrogen Sulfide Fluorescent Probe with High Sensitivity and Selectivity

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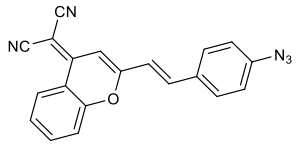
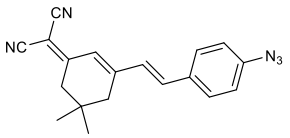
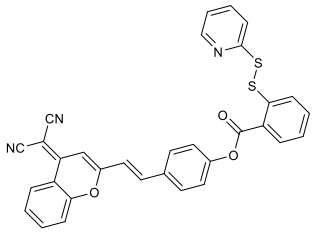
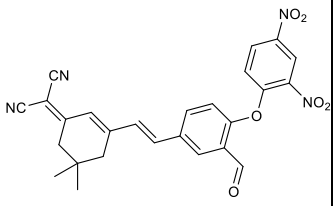
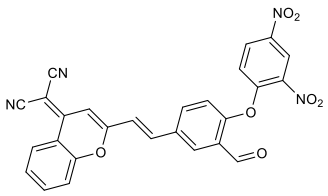
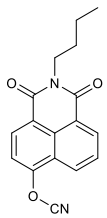
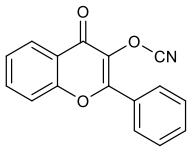
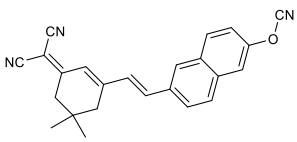
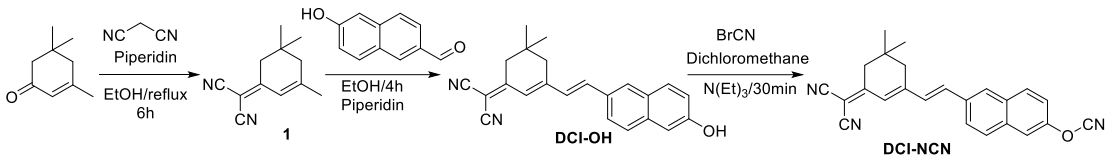
Table 1. Isophoronitrile fluorescent probes reported in the literature

Reference	structure	F.L. intensity	Stokes shift	Detection limit	Response time	Biosystem imaging
1		670 nm	150 nm	3050 nM	60 min	Cells Mice
2		643 nm	171 nm	130 nM	20 min	Not mentioned
3		680 nm	120 nm	1.1 nM	35 min	Hela cells
4		660 nm	170 nm	59 nM	15 min	MC7 cells Mice
5		676 nm	137 nm	83 nM	8 min	Hela cells
6		552 nm	167 nm	240 nM	30 min	Hela cells
7		525 nm	100 nm	250 nM	15 min	A549 cells
This work		618 nm	178 nm	50 nM	15 min	MC38 cells Mice



Scheme 1 synthesis route of compound DCI-NCN

Synthesis of compound 1

Isophorone (0.69 g, 5 mmol) and malononitrile (0.40 g, 6 mmol) were dissolved in absolute ethanol (10 mL), then a catalytic amount of piperidine was added and the mixture was refluxed 6 hours under nitrogen. The reaction was monitored by TLC. After completion of the reaction, the solvent was removed, the precipitate was dissolved in dichloromethane, washed with water and dried over anhydrous sodium sulfate. Finally, the solvent was evaporated under reduced pressure and purified by column chromatography (Cyclohexane: EtOAc=8:1) to give a colorless crystal 0.72g, yield: 78%.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.61 – 6.57 (m, 1H), 2.49 (s, 3H), 2.01 (d, *J* = 1.4 Hz, 4H), 0.99 (s, 6H).^{13}C NMR (101 MHz, Chloroform-*d*) δ 170.36, 159.78, 120.56, 113.17, 112.39, 78.22, 45.67, 42.64, 32.36, 27.80, 25.29.

Synthesis of DCI-OH

Isophorone dinitrile 372 mg (2 mmol, 1 equiv), 6- hydroxy-2-naphthaldehyde 345 mg (2 mmol, 1 equiv) was added to a 25 ml reaction flask. After addition of 10 ml of absolute ethanol and 3 drops of piperidine was added, the mixture was stirred at reflux for 8 h. The reaction was monitored by TLC. After the reaction is completed, it is cooled to room temperature, and concentrated to remove the reaction solvent. The crude product was purified by column chromatography (dichloromethane) to give Orange yellow solid 400 mg, yield: 69%.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.03 (s, 1H), 8.03 (s, 1H), 7.82 (d, *J* = 8.7 Hz, 1H), 7.77 (d, *J* = 8.8 Hz, 1H), 7.70 (d, *J* = 8.7 Hz, 1H), 7.44 (q, *J* = 16.1 Hz, 2H), 7.11 (d, *J* = 12.7 Hz, 2H), 6.90 (s, 1H), 2.62 (s, 2H), 2.59 (s, 2H), 1.03 (s, 6H).^{13}C NMR (100 MHz, DMSO-d_6) δ 170.67, 157.15, 156.63, 138.67, 135.79, 131.04, 130.66, 129.63, 128.82, 128.05, 127.23, 124.73, 122.69, 119.73, 114.46, 113.66, 109.54, 76.09, 42.77, 38.68, 32.13, 27.93.

Synthesis of probe DCI-NCN.

To a solution of DCI-OH (125 mg, 0.34 mmol) in dichloromethane (10 ml) was added cyanogen bromide (35.7 mg, 0.34mmol) in dichloromethane (2 ml) dropwise, followed by the addition of 3 drops of triethylamine at 0°C. The resulting mixture was stirred for 30 min at room temperature. The reaction was monitored by TLC. After completion of the reaction, solvent was removed in vacuo and the residue was purified by column chromatography to give 90 mg yellow solid, yield: 77%.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (dd, *J* = 5.3, 3.8 Hz, 2H), 7.88 (d, *J* = 8.7 Hz, 1H), 7.79 (dd, *J* = 5.7, 2.4 Hz, 2H), 7.41 (dd, *J* = 9.1, 2.7 Hz, 1H), 7.21 (d, *J* = 16.1 Hz, 1H), 7.13 (d, *J* = 16.1 Hz, 1H), 6.92 (s, 1H), 2.63 (s, 2H), 2.52 (s, 2H), 1.11 (s, 6H).^{13}C NMR (100 MHz, $CDCl_3$) δ 169.06, 151.19, 135.99, 134.16, 131.70, 131.47, 130.39, 128.51, 128.37, 125.54, 124.22, 115.81, 113.34, 112.61, 111.88, 108.56, 43.01, 39.30, 32.08, 28.05. HRMS(ESI): m/z[M+CH_3OH_2] $^{+}$ calcd for 398.1849, found 398.1841

optimal configuration

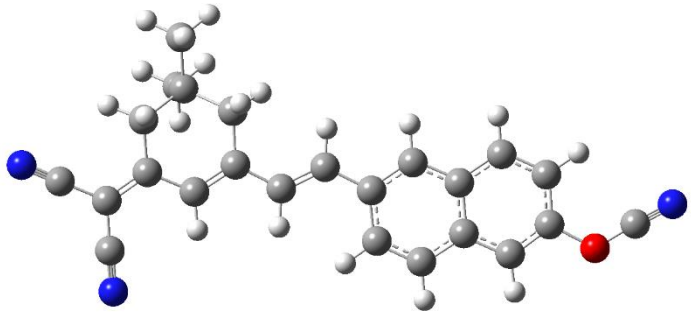


Fig. S1 The optimal configuration of DCI-NCN

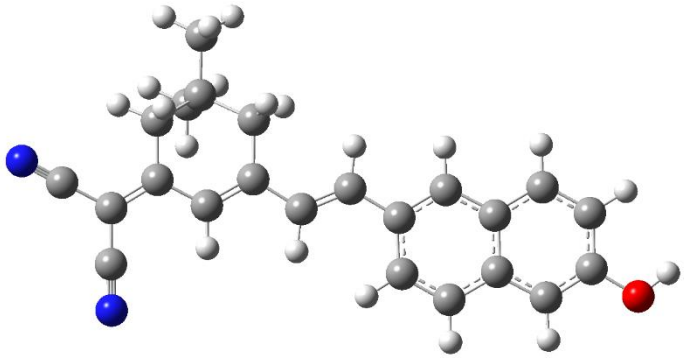


Fig. S2 The optimal configuration of DCI-OH

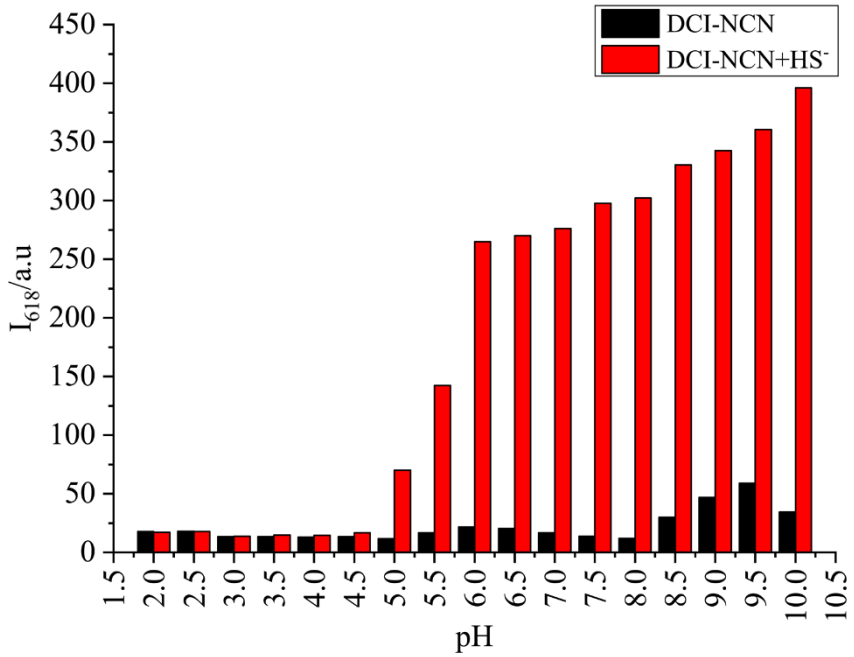


Fig. S3 Fluorescence intensity changed at 618 nm with Na_2S upon different pH with λex at 440 nm at 25℃.

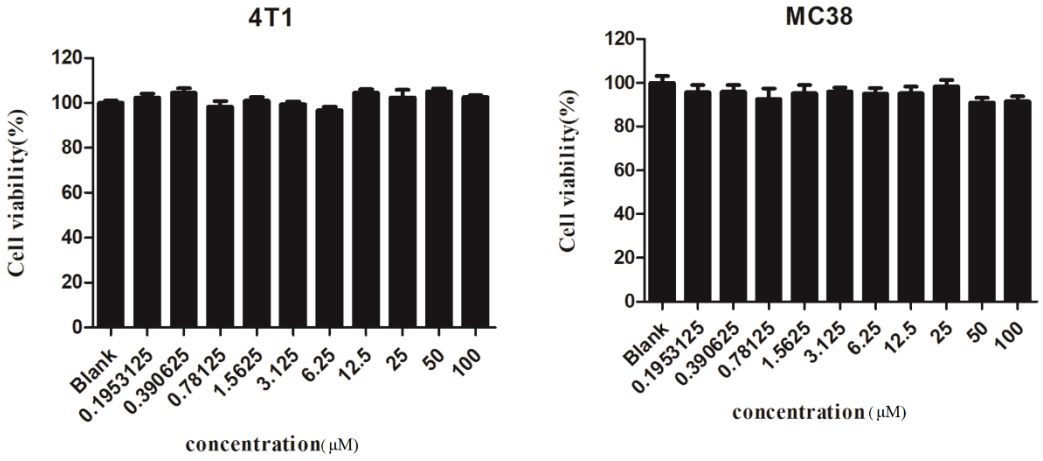


Fig. S4 Cytotoxicity of probe DCI-NCN to 4T1 and MC38 cells

Spectral Characterization

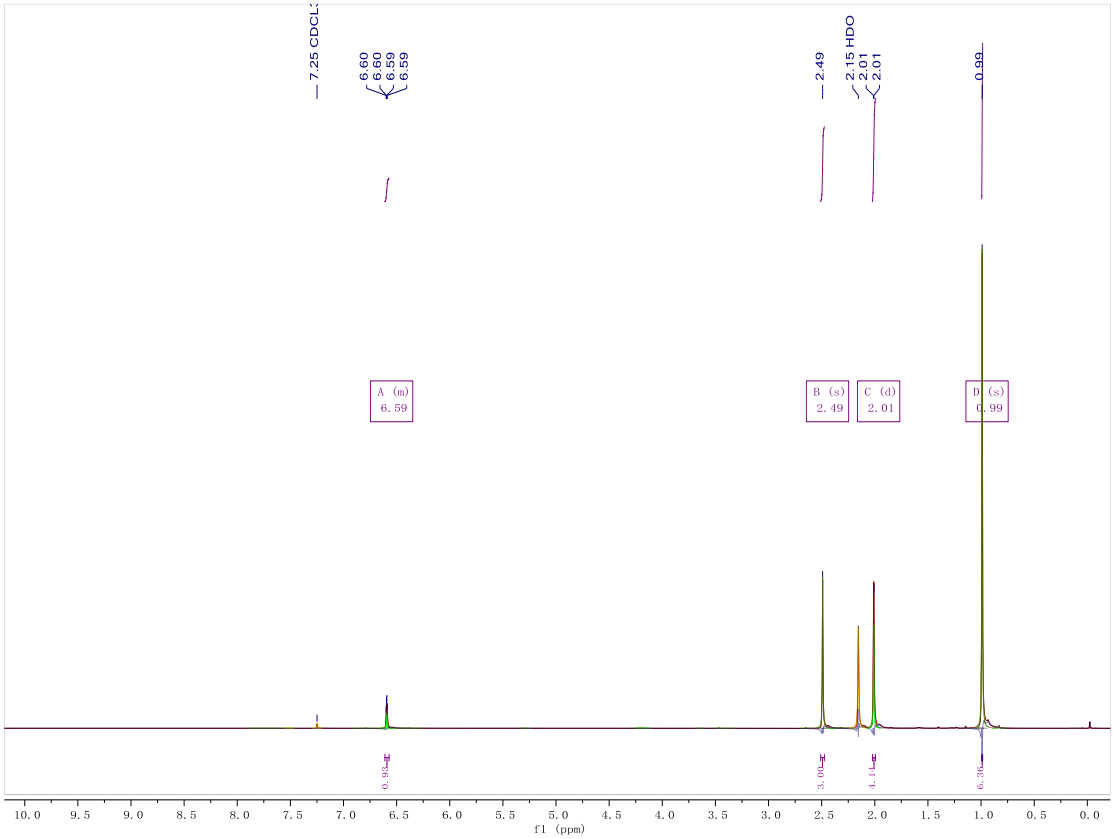


Fig. S5 [1]HNMR spectrum of the compound 1

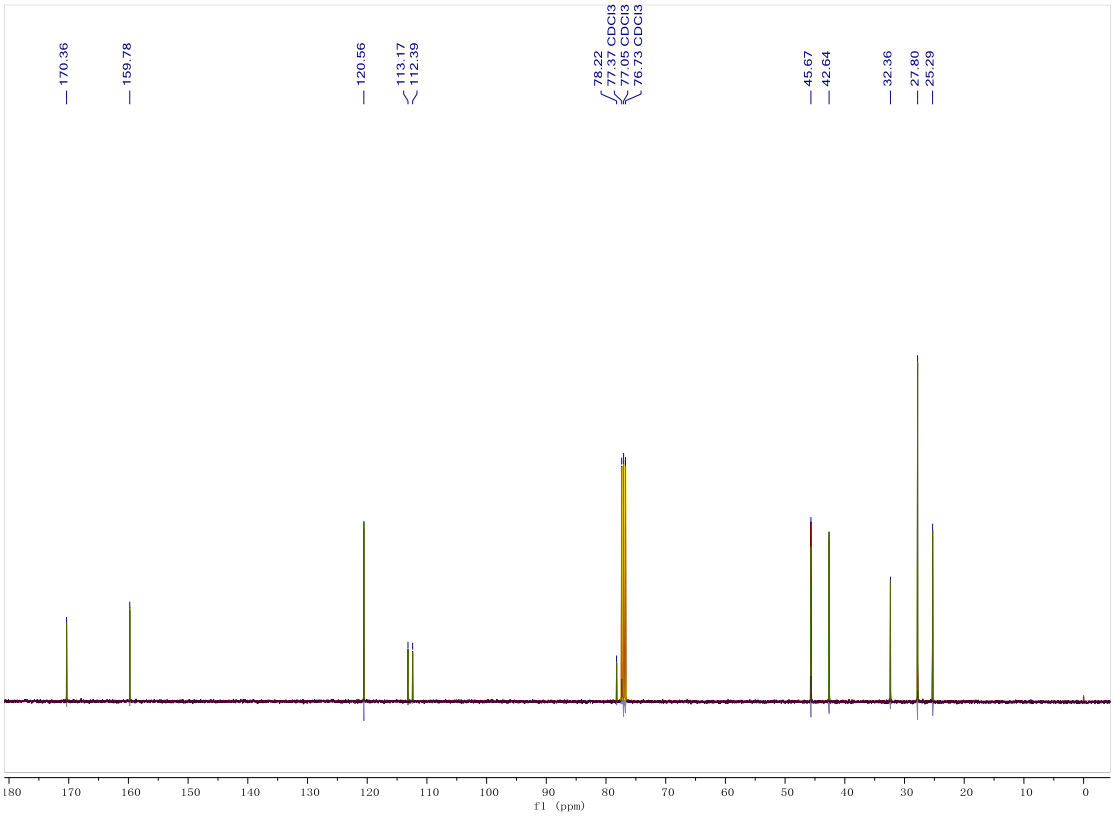


Fig. S6 [13]CNMR spectrum of the compound 1

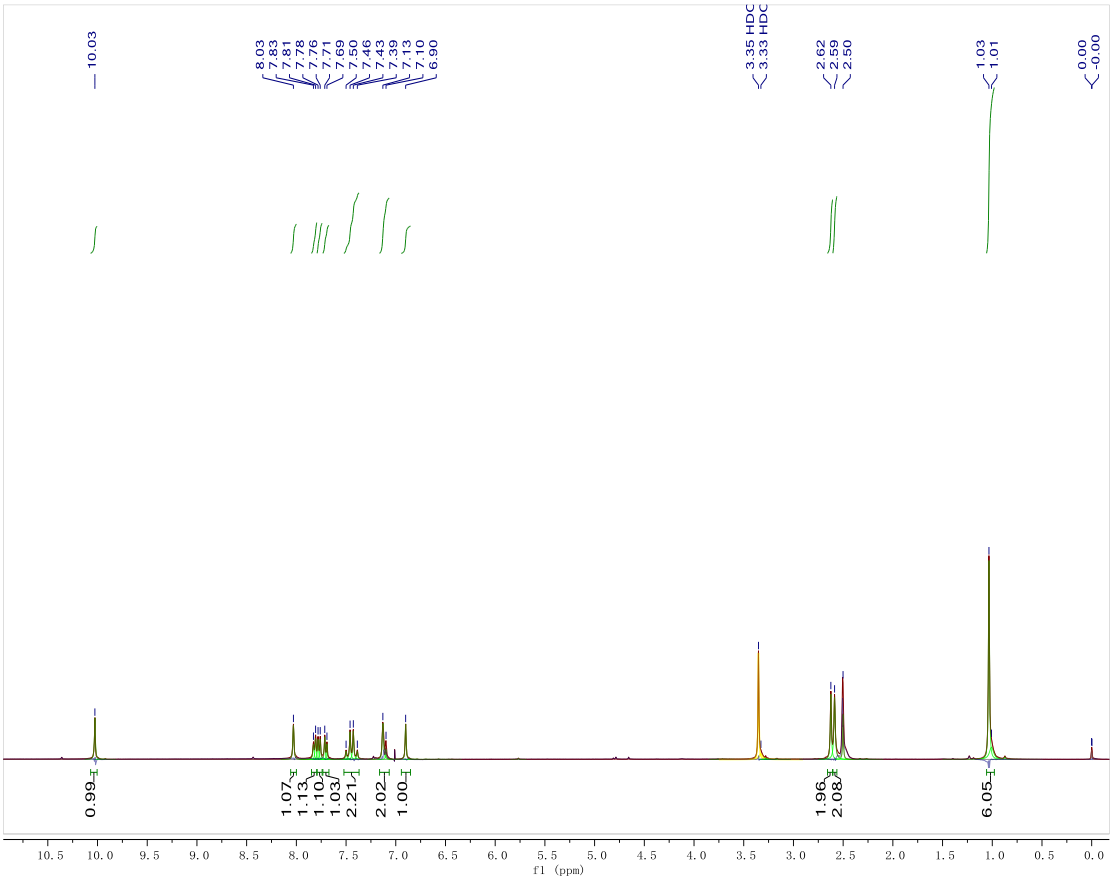


Fig. S7 [1]HNMR spectrum of the compound DCI-OH

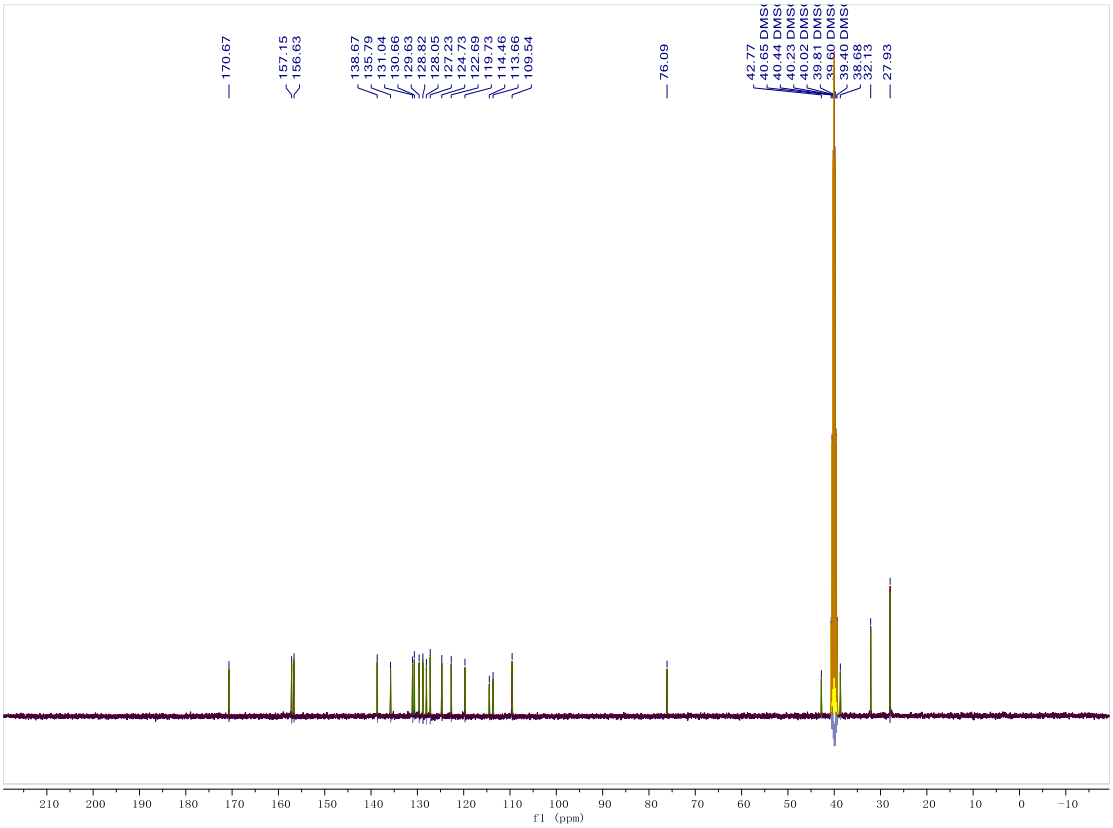


Fig. S8 [13]CNMR spectrum of the compound DCI-OH

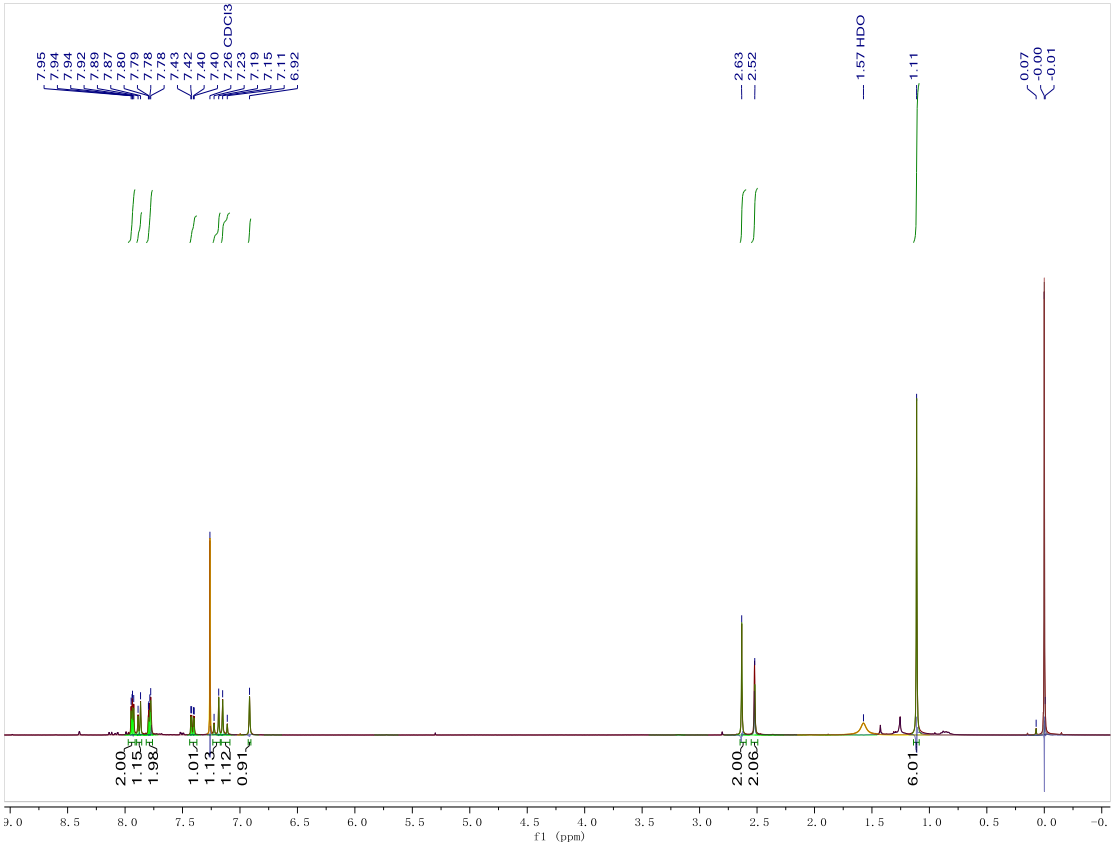


Fig. S9 ^{1}HNMR spectrum of the compound DCI-OH

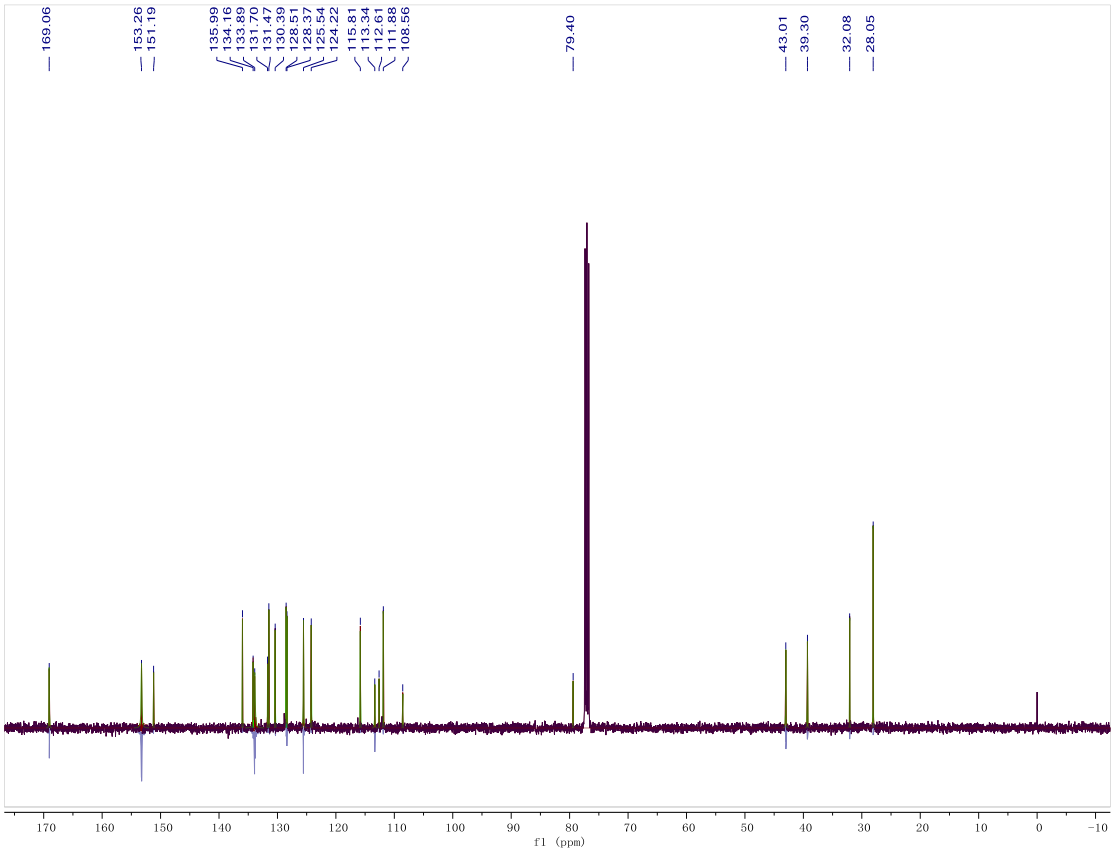


Fig. S10 ^{13}CNMR spectrum of the compound DCI-NCN

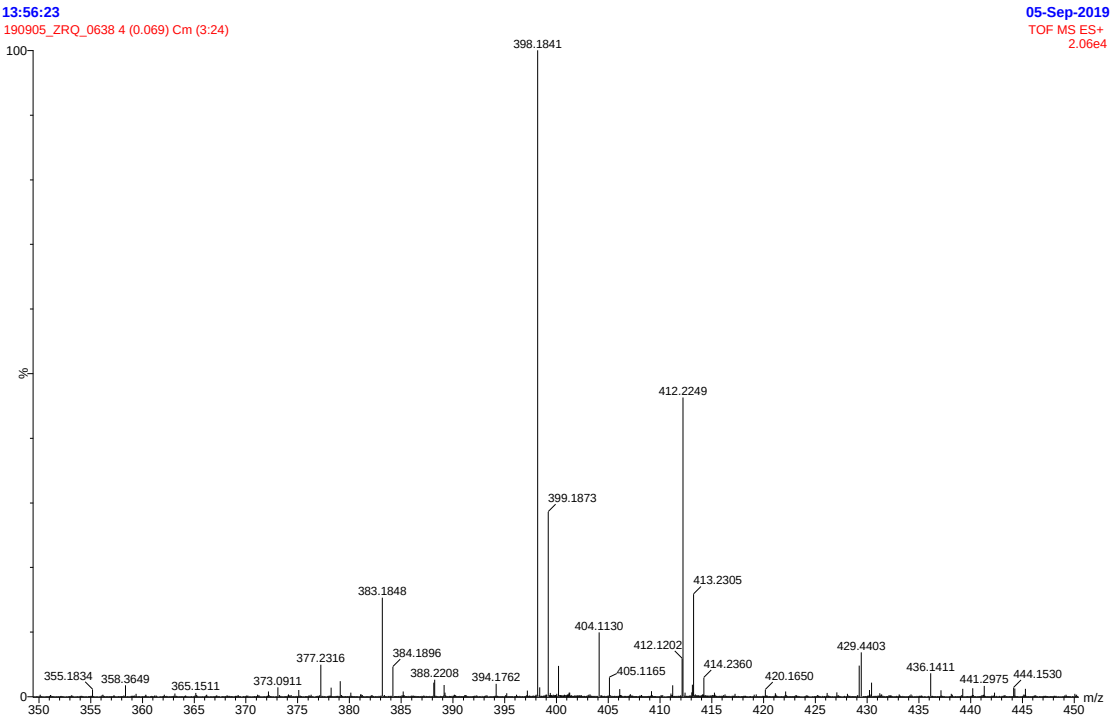


Fig. S11 HRMS spectrum of the compound DCI-NCN

Reference

1. W. Sun, J. Fan, C. Hu, J. Cao, H. Zhang, X. Xiong, J. Wang, S. Cui, S. Sun and X. Peng, *Chem Commun (Camb)*, 2013, **49**, 3890-3892.
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