Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information

A smart mitochondria-targeting TP-NIR fluorescent probe for selective and sensitive sensing H_2S in living cells and mice

Qiaomei Yang[a], Liyi Zhou[a,*], Longpeng Peng[a], Gangqiang Yuan[a], Haiyuan Ding[a], Libin Tan[a] and Yizhuang Zhou[b,*]

Hunan Key Laboratory of Processed Food for Special Medical Purpose, National Engineering Laboratory for Deep Process of Rice and Byproducts, College of Food Science and Engineering, Central South University of Forestry and Technology, Changsha, Hunan 410004, China.

*To whom correspondence should be addressed.

Fax: +86-0731-85623096, Central South University of Forestry and Technology, Changsha, Hunan 410004, China. E-mail: zhouly0817@163.com (L. Zhou), zhouyizhuang3@163.com

Materials and apparatus

Unless otherwise specified, all chemicals were obtained from commercial suppliers and used without further purification. Thin layer chromatography (TLC) was carried out using silica gel 60 F254, and column chromatography was conducted over silica gel (200-300 mesh), both of them were obtained from Qingdao Ocean Chemicals (Qingdao, China). Water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore, USA). LC-MS analyses were performed using an Agilent 1100 HPLC/MSD spectrometer. Mass spectra were performed using an LCQ advantage ion trap mass spectrometer (Thermo Finnigan). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-400 spectrometer using TMS as an internal standard. All chemical shifts are reported in the standard δ notation of parts per million. UV-vis absorption spectra were recorded in 1.0 cm path length quartz cuvettes on a Shimadzu 2450 UV-visible spectrometer. Fluorescence images of HeLa cells were obtained using an Olympus FV1000-MPE multiphoton laser scanning confocal microscope (Japan). Fluorescence measurements were carried out on a G9800A fluorescence spectrometer (USA) with excitation and emission slits set at 5.0 nm, respectively. The pH was measured with a PHS-3C pH meter. Animal imaging was carried out on a PhotoIMAGERTMRT imager with excitation and emission wavelengths set at 633 nm and 660 nm, respectively.

Fluorescent quantum yields and TP absorption active cross section

The quantum yields of **MNIR-H_2S** and **MNIR-H_2S+H_2S** were calculated by comparison with rhodamine 6 G (R=0.95 in ethanol) as a reference using the following equation (1):

$$\Phi_F = I_{AR}(n/n_R)^2\Phi_{FR}/I_{RA} \quad (1)$$

Where F is the quantum yield, I is the integrated area under the fluorescence spectra, A is the absorbance, n is the refractive index of the solvent, and R refers to the reference rhodamine 6G.

The two-photon excited fluorescence was measured by using a Ti: sapphire femtosecond oscillator (Spectra Physics Mai Tai) as the excitation source. The output laser pulses have a tunable central wavelength from 690 nm to 1020 nm with pulse duration of less than 100 fs and a repetition rate of 80.5 MHz. The laser beam was focused onto the samples using a lens with a focus length of 3.0 cm. The emission was collected at an angle of 90^{o} to the direction of the excitation beam to minimize the scattering. The emission signal was directed into a CCD (Princeton Instruments, Pixis 400B) coupled monochromator (IsoPlane160) with an optical fiber. A 750 nm short pass filter was placed before the spectrometer to minimize the scattering from the excitation light. The two-photon absorption (TPA) cross section (δ) of the sample (s) at each wavelength was calculated according to equation (2), and rhodamine B in CH_3OH was used as the reference (r).

$$\delta=\delta_r(S_s\Phi_r\varphi_rC_r)/(S_r\Phi_s\varphi_sC_s) \quad (2)$$

where S is the integrated fluorescence intensity, Φ is the fluorescence quantum yield, C is the concentration of sample (s) and reference (r), and φ is the collection efficiency of the experimental set up. The uncertainty in the measurement of cross sections is ~15%. The detailed calculation is given in Table S1.

Table S1. The details of fluorescent quantum yields and TP active absorption cross-section of **MNIR-H_2S and MNIR-H_2S+H_2S**

Φ (**MNIR-H_2S**)	Φ (**MNIR-H_2S+H_2S**)	δ (**MNIR-H_2S**)/GM	δ (**MNIR-H_2S+H_2S**)/GM
0	**0.35**	**0 (810 nm)**	**40 (810 nm)**

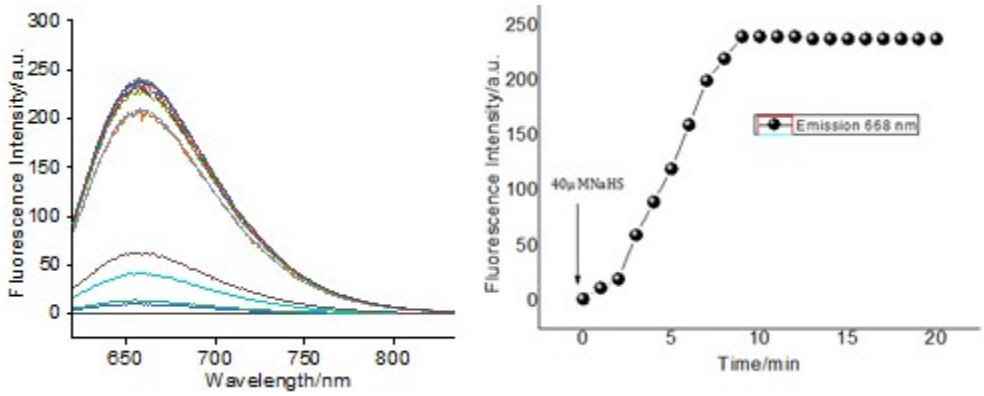


Fig.S1. Time course of fluorescence intensities of **MNIR-H_2S** (1.0 μM) at 668 nm treated with 40.0 μM of NaHS (pH 7.4).

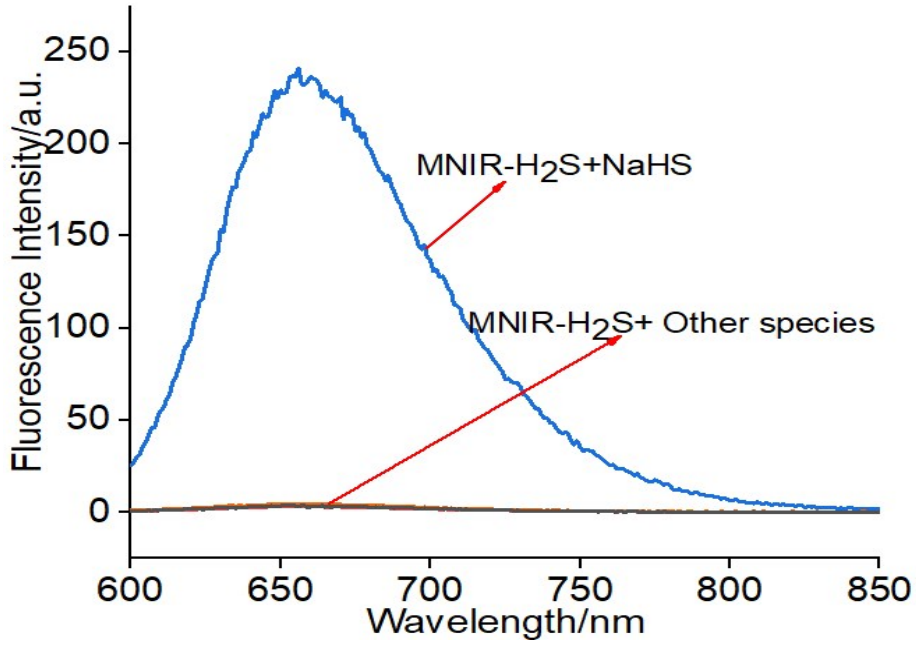


Fig.S2. Selectivity experiments: 1 μM MNIR-H_2S incubated with various analyzed species/NaHS (100 μM other species and 40 μM NaHS) in PBS buffered (10 mM, pH7.4, water/DMSO=99/1, v/v), λ_{ex}=622 nm, Legend: (1) GSH, (2) Cys, (3) Vitamin C, (4) K^+, (5) Ca^{2+}, (6) Na^+, (7) Mg^{2+}, (8) Zn^{2+}, (9) Fe^{3+}, (10) Gly, (11) Cu^{2+}, (12) Blank, (13) H_2S, (14) Pb^{2+}, (15) Glu.

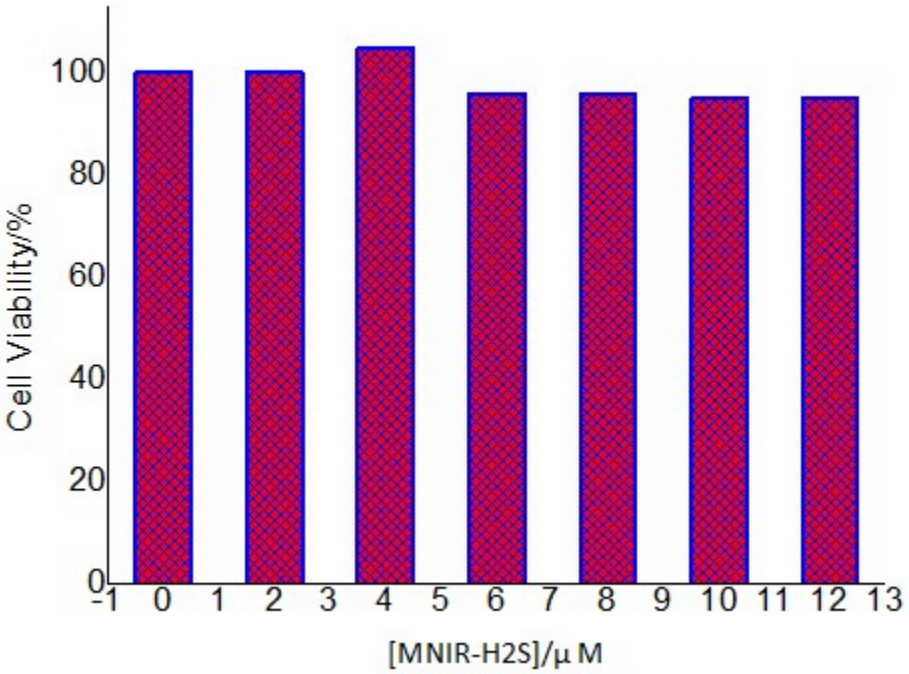
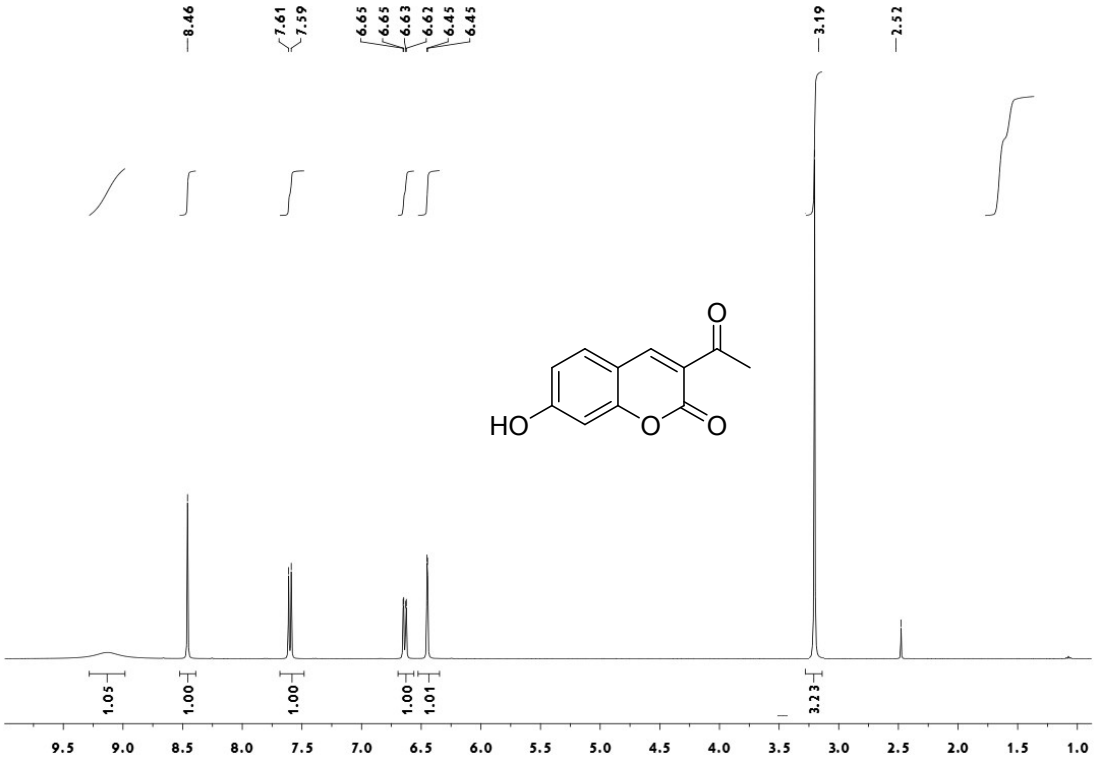
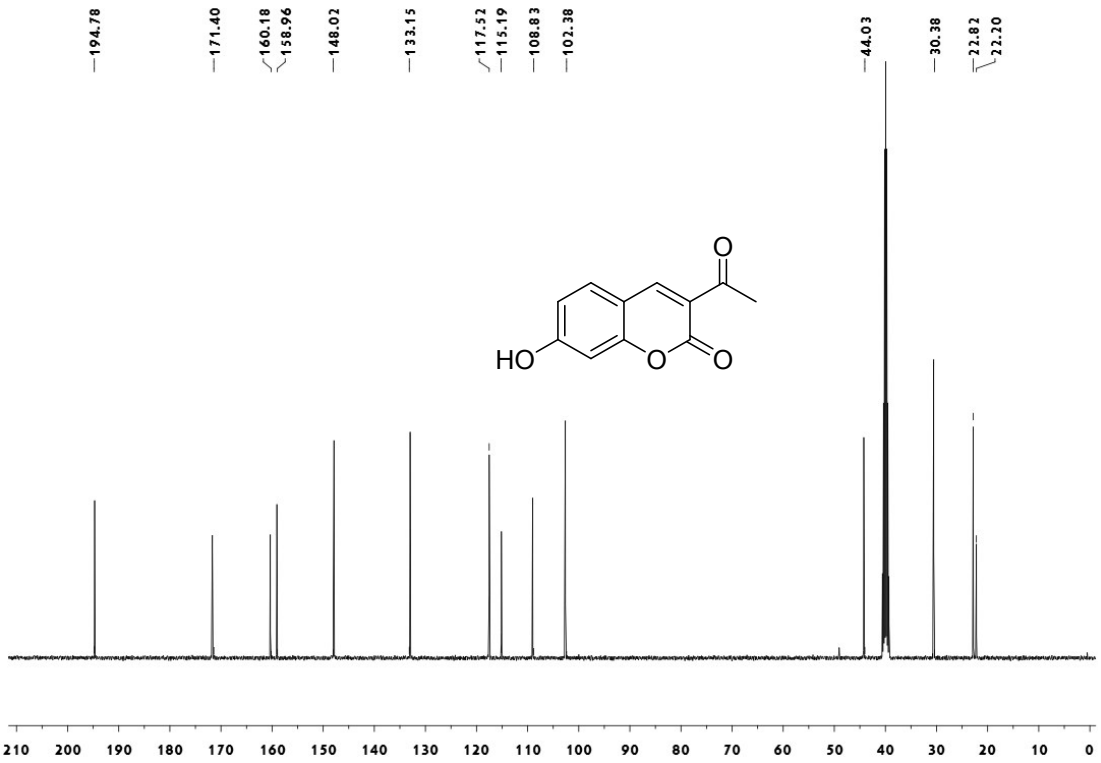
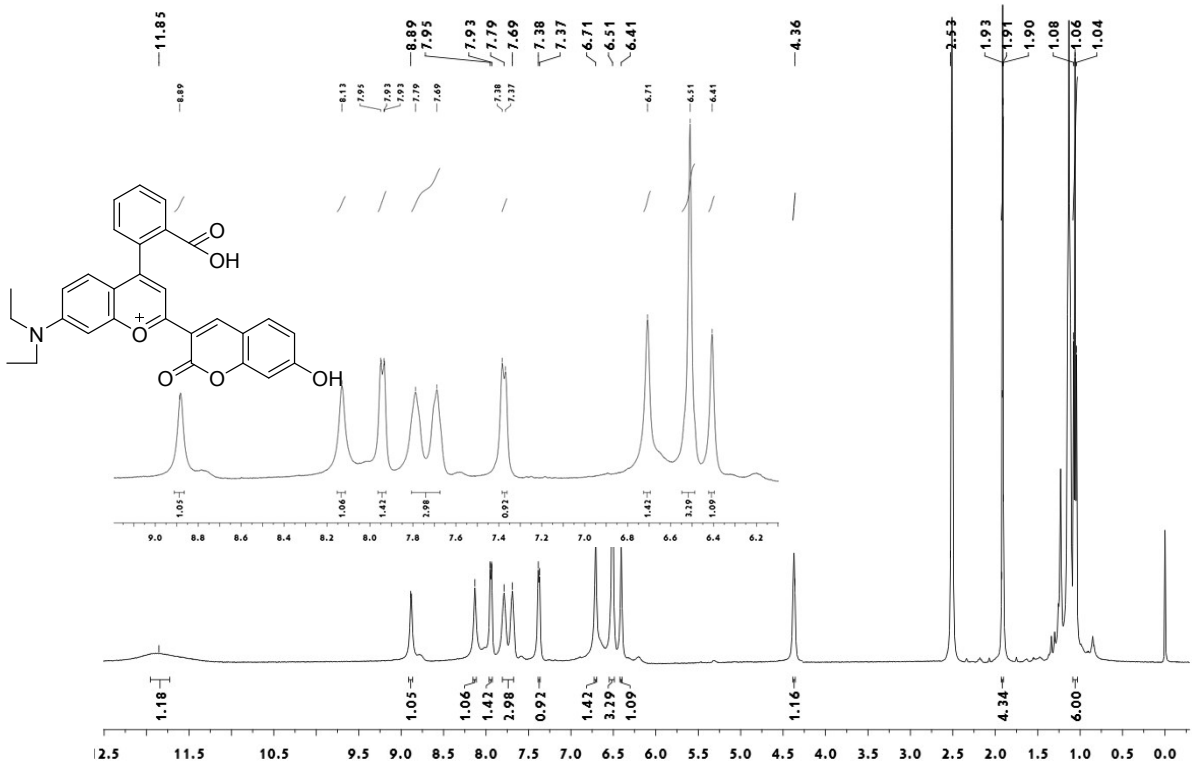


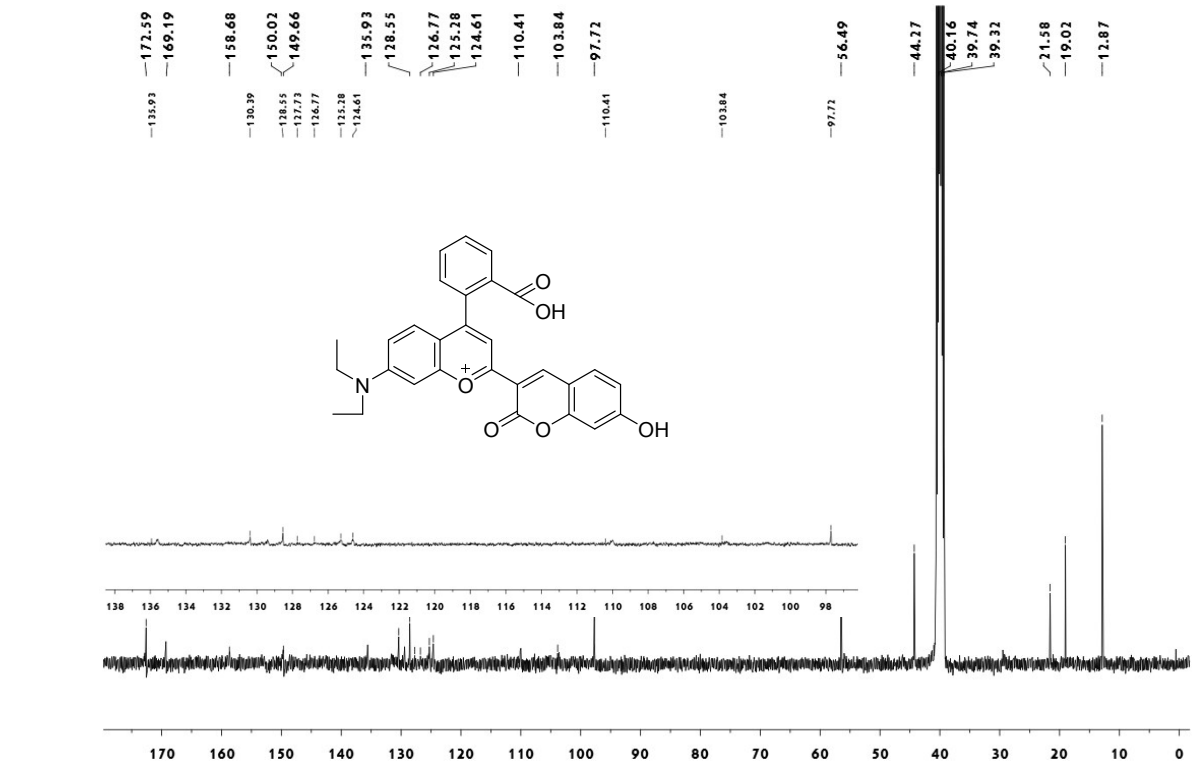
Fig.S3. Cytotoxicity of **MNIR-H_2S** against HeLa cells, as determined by MTT assay: HeLa cells treated with probe (0-12.0 μM)

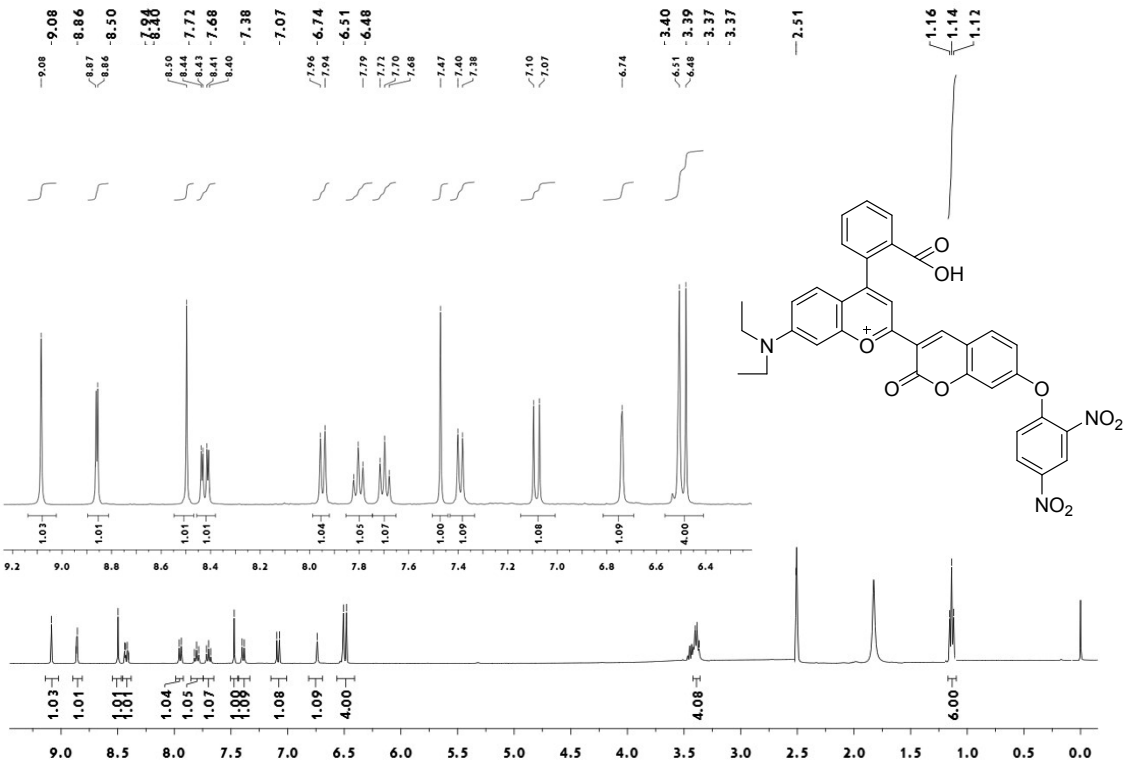
2. ^{1}H NMR, ^{13}C NMR and MS Spectra:

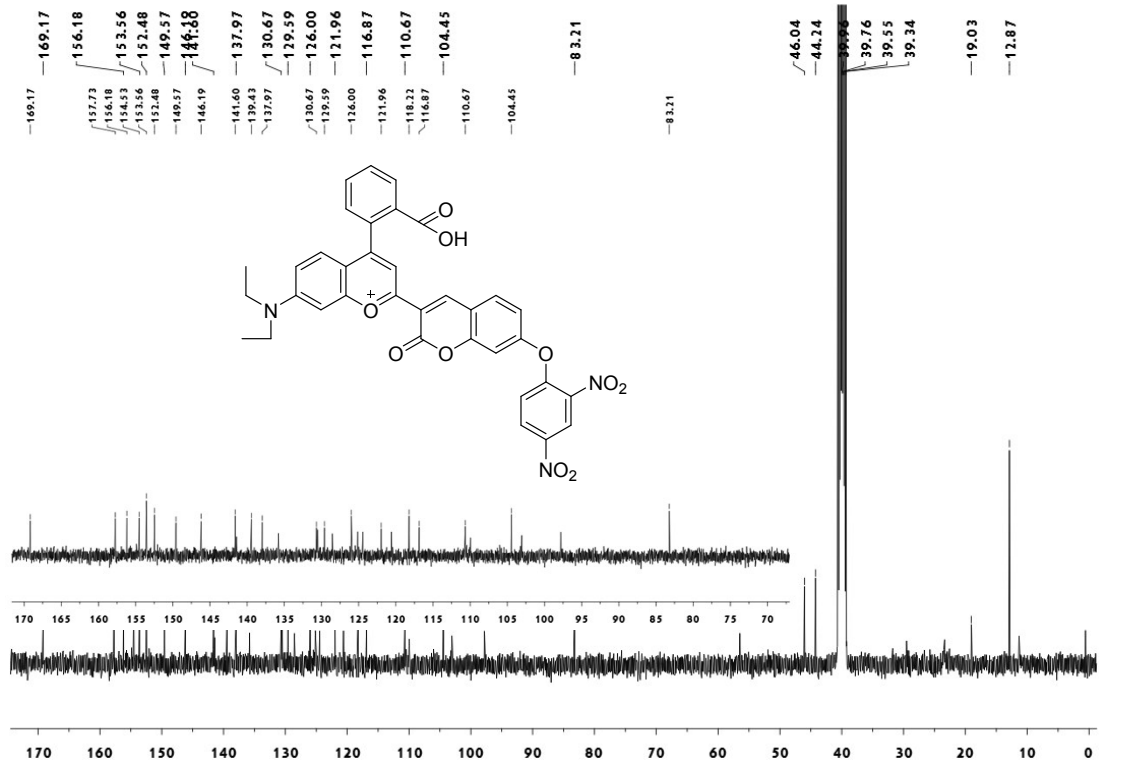





11.85
8.89
7.95
7.93
7.79
7.69
7.38
7.37
6.71
6.51
6.41
4.36
2.53
1.93
1.91
1.90
1.08
1.06
1.04
O
OH
N
O
O
O
OH
1.18
1.05
1.06
1.42
2.98
0.92
1.42
3.29
1.09
1.16
4.34
6.00


172.59
169.19
158.68
150.02
149.66
135.93
128.55
126.77
125.28
124.61
110.41
103.84
97.72
56.49
44.27
40.16
39.74
39.32
21.58
19.02
12.87
O
OH
N
O
O
O
OH


9.08
8.86
8.50
7.72
7.68
7.38
7.07
6.74
6.51
6.48
3.40
3.39
3.37
2.51
1.16
1.14
1.12
O
OH
N
O
O
O
O
NO2
NO2


169.17
156.18
153.56
152.48
149.57
137.97
130.67
129.59
126.00
121.96
116.87
110.67
104.45
83.21
46.04
44.24
39.76
39.55
39.34
19.03
12.87
O
OH
N
O
O
O
O
NO2
NO2