Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

A fluorescent probe capable of detection of H_2S at a submicromolar range and in cellular imaging

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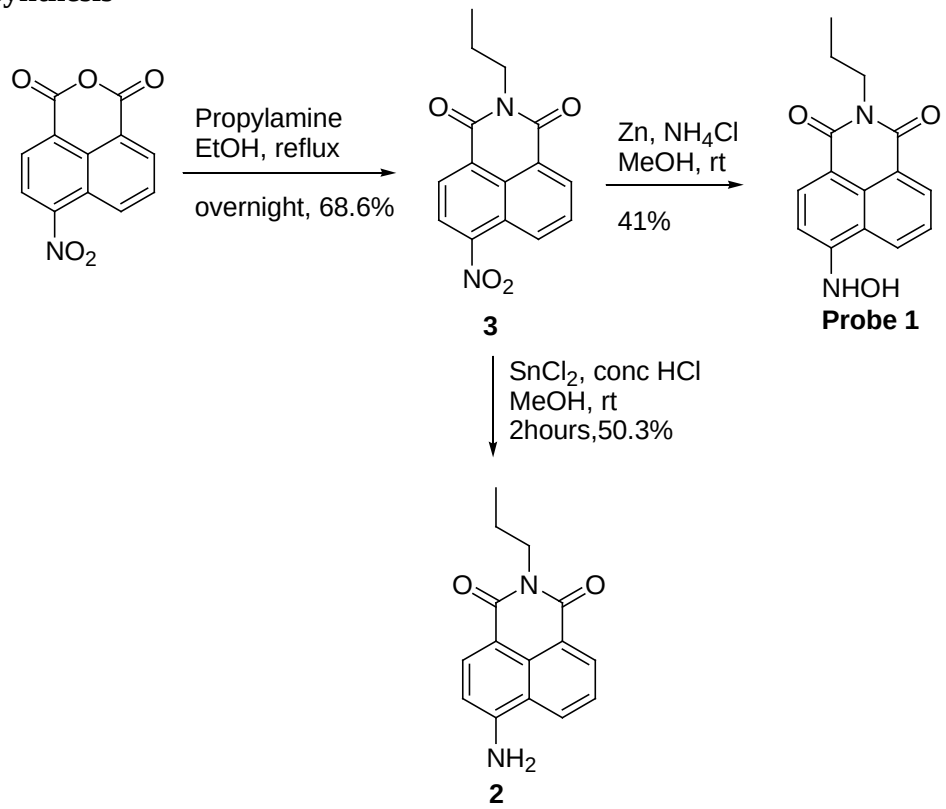
General Information: Commercial reagents were used as received, unless otherwise stated. Merck 60 silica gel was used for chromatography, and Whatman silica gel plates with fluorescence F_{254} were used for thin-layer chromatography (TLC) analysis. ^{1}H and ^{13}C NMR spectra were recorded on Bruker tardis (sb300) and Bruker Avance 500. Data for ^{1}H are reported as follows: chemical shift (ppm), and multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). Data for ^{13}C NMR are reported as ppm.

Spectroscopic materials and methods: Millipore water was used to prepare all aqueous solutions. The pH was recorded by a Beckman Φ^{TM} 240 pH meter. UV absorption spectra were recorded on a Shimadzu UV-2410PC UV-Vis spectrophotometer. Fluorescence emission spectra were obtained on a SHIMADZU spectrofluorophotometer RF-5301pc. Cell imaging experiments were carried out by Olympus IX71 fluorescence microscopy.

1. Probe synthesis



6-Nitro-2-propyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (3). To 4-nitro-1,8-naphthalic anhydride (300 mg, 1.23mmol) in 20 mL of EtOH was added propylamine (203 µL, 2.46 mmol), then heated to reflux and stirred overnight. The solvent was removed by rotavapor, then the product was purified through column chromatograph, and obtained as straw yellow solid (240 mg, 69%). ^{1}H NMR ($CDCl_3$): δ 8.72 (d, *J* = 8.5 Hz, 1H), 8.63 (d, *J* = 7.5 Hz, 1H), 8.59 (d, *J* = 8.0 Hz, 1H), 8.32 (d, *J* = 8.0 Hz, 1H), 7.91 (t, *J* = 8.0 Hz, 1H), 4.08 (t, *J* = 7.5 Hz, 2H), 1.75-1.68 (m, 2H), 0.98 (t, *J* = 7.5 Hz, 3H),. ^{13}C NMR (500 MHz, $CDCl_3$): δ 156.06, 155.24, 142,29, 125.18, 122.76, 122.58, 122.03, 121.81, 119.80, 116.75, 116.40, 115.82, 35.19, 14.18, 4.38.

6-Amino-2-propyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (2). To a solution of compound (**3**) (240 mg, 0.84 mmol) in 10 mL of MeOH was added $SnCl_2$ (960 mg, 5.06 mmol) followed 2 mL

of conc. HCl. The mixture was stirred at room temperature for 2 hours. The solvent was removed under rotavapor, then 20 mL of H_2O was added, and the pH was adjust to basic by aq. NaOH (2M). The product was extracted with DCM (30 mL × 3), dried over Na_2SO_4, further purified through column chromatograph and obtained as yellow solid (108 mg, 50.3%).^{1}H NMR ($CDCl_3$): δ 8.60 (d, *J* = 6.9 Hz, 1H), 8.41 (d, *J* = 8.1 Hz, 1H), 8.10 (d, *J* = 8.4 Hz, 1H), 7.65 (t, *J* = 8.4 Hz, 1H), 6.88 (d, *J* = 8.1 Hz, 1H), 4.95 (s, 2H), 4.13 (t, *J* = 7.5 Hz, 2H), 1.82-1.69(m, 2H), 1.00 (t, *J* = 7.4 Hz, 3H),. ^{13}C NMR (500 MHz, $CDCl_3$ with 2 drops of CD_3OD): δ.164.70, 164.18, 149.41, 133.83, 131.45, 129.77, 127.01, 124.83, 122.95, 119.96, 111.70, 109.34, 41.69, 21.37, 11.47. HRMS (m/z): calcd. for $C_{15}H_{15}N_2O_2$ $[M + H]^+$: 255.1134, found, 255.1142.

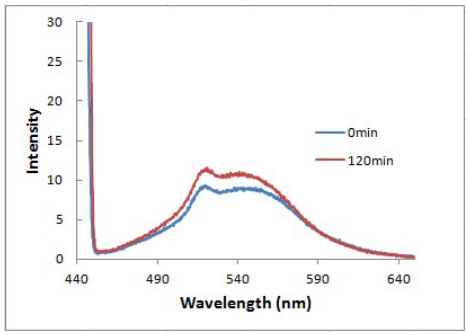
6-(Hydroxyamino)-2-propyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (probe 1). To a solution of compound (**3**) (62 mg, 0.22 mmol) in 10mL MeOH was added Zinc powder (32 mg, 0.49 mmol) and NH_4Cl (14 mg, 0.26 mmol), then stirred at room temperature for 1 hours. Another 10mL CH_2Cl_2 and 10 mL of MeOH was added, the solid was removed by filtration, then the solvent was removed by rotavapor. The product was purified through column chromatograph, and obtained as yellow solid (24mg, 41%). ^{1}H NMR (DMSO-d_6): δ 10.46 (s, 1H), 9.31 (s, 1H), 8.43-8.40 (m, 2H), 8.33 (d, *J* = 8.4 Hz, 1H), 7.68(t, *J* = 8.0 Hz, 1H), 7.19 (d, *J* = 8.4 Hz, 1H), 3.96 (t, *J* = 7.5 Hz, 2H), 1.67-1.55 (m, 2H), 0.98 (t, *J* = 7.4 Hz, 3H),. ^{13}C NMR (300 MHz, CD_3OD): δ166.13, 165.73, 154.52, 135.38, 132.06, 130.45, 129.05, 125.89, 123.41, 120,11, 112,21, 106.61, 42.62, 22.43, 11.74. HRMS (m/z): calcd. for $C_{15}H_{15}N_2O_3$ $[M + H]^+$: 271.1083, found, 271.1094.

2. Fluorescence quantum yields were calculated according to the equation below by reference to fluorescein in basic ethanol (Φ = 0.97).[1]

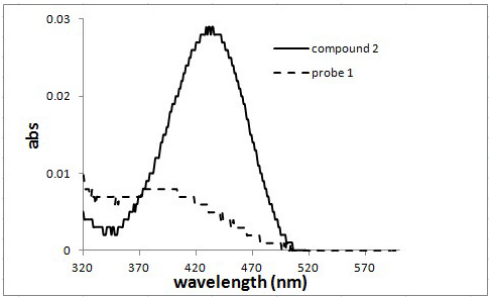
$$\Phi_X = \Phi_S * [A_S / A_X] * [F_X / F_S] * [n_X / n_S]^2$$

where, Φ_S is the reported quantum yield of the standard fluorescein. $\mathbf{A_S}$ is the absorbance at the excitation wavelength of the standard. $\mathbf{A_X}$ is the absorbance at the excitation wavelength of the measured compound. $\mathbf{F_X}$ is the area under the emission spectra of the measured compound. $\mathbf{F_S}$ is the area under the emission spectra of the srandard. $\mathbf{n_X}$ is the refractive index of the solvent used. $\mathbf{n_S}$ is the refractive index of the solvent of the standard.

3. **Probe stability study.**
Figure S1. Probe **1** with concentration of 2.0 μM was incubated in 100 mM pH 7.4 phosphate buffer solution for 120 min. Only negligible fluorescence change was observed.

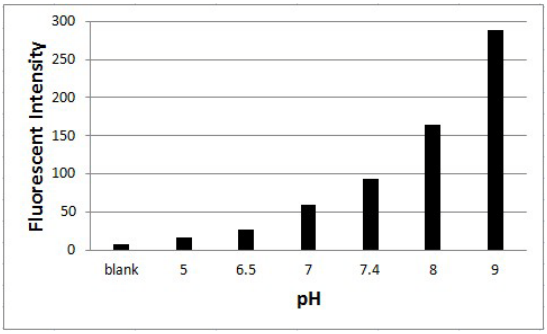


4. **Figure S2**. Absorbance of probe **1** and compound **2** (2.0 μM respectively).

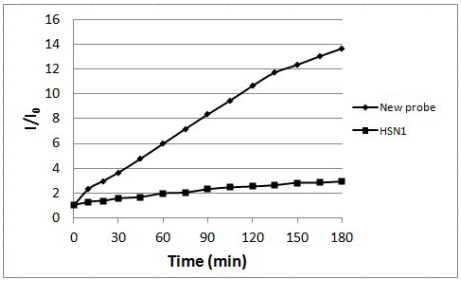


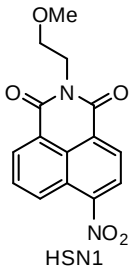
5. Evidence of detection mechanism
   38 mg probe **1** was treated with 5 eq. $Na_2S•9H_2O$ at pH 7-8 aquous solution, then the mixture was sitrred at rt for 10 h. The only fluorescent compound was successfully separated with yield 11.4%, and the obtained HNMR spectra was consistent with compund **2** synthesized above; 73.6% of the probe **1** was recovered

6. **Figure S3.** The effect of medium pH on probe **1** toward H_2S. Fluorescence intensity was recorded at 120 min when probe **1** (2.0 μM) reacted with Na_2S (10.0 μM) in 0.1 M phosphate buffer (containing 0.2% DMSO). λ_{ex} = 440 nm, fluorescent intensity is taken at λ_{em} = 544 nm.



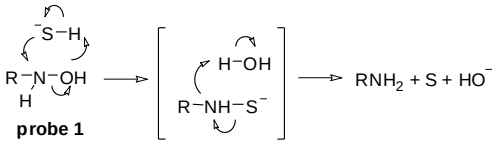
7. Comparison with the reported nitro-reduction based probe.
   Figure S4. Probe **1** (e.g., New probe) or **HSN1** (2.0 μM) was treated with 10 μM Na_2S in 100 mM pH 7.4 phosphate buffer solution at 25 °C. T he fluorescence was recorded at 544 nm with an excitation at 440 nm.



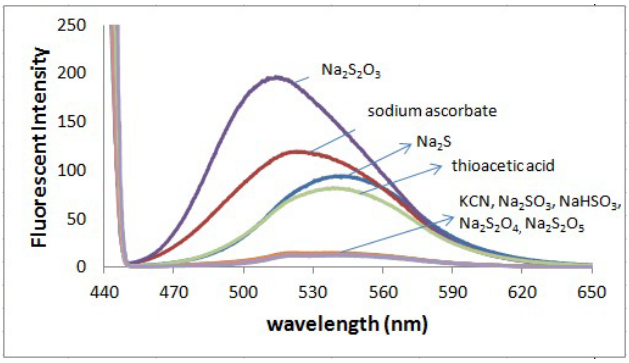


HSN1

8. **Figure S5**. A possible mechamism for the reduction of hydroxylamine to amine by H_2S.



9. **Figure S6**. The detection of other potential reductants.



Probe **1** (2.0 μM) was treated with 50 μM different compounds above respectively for 2 h.

10. Imaging experiments.

a. Primary culture of rat cortical astrocytes

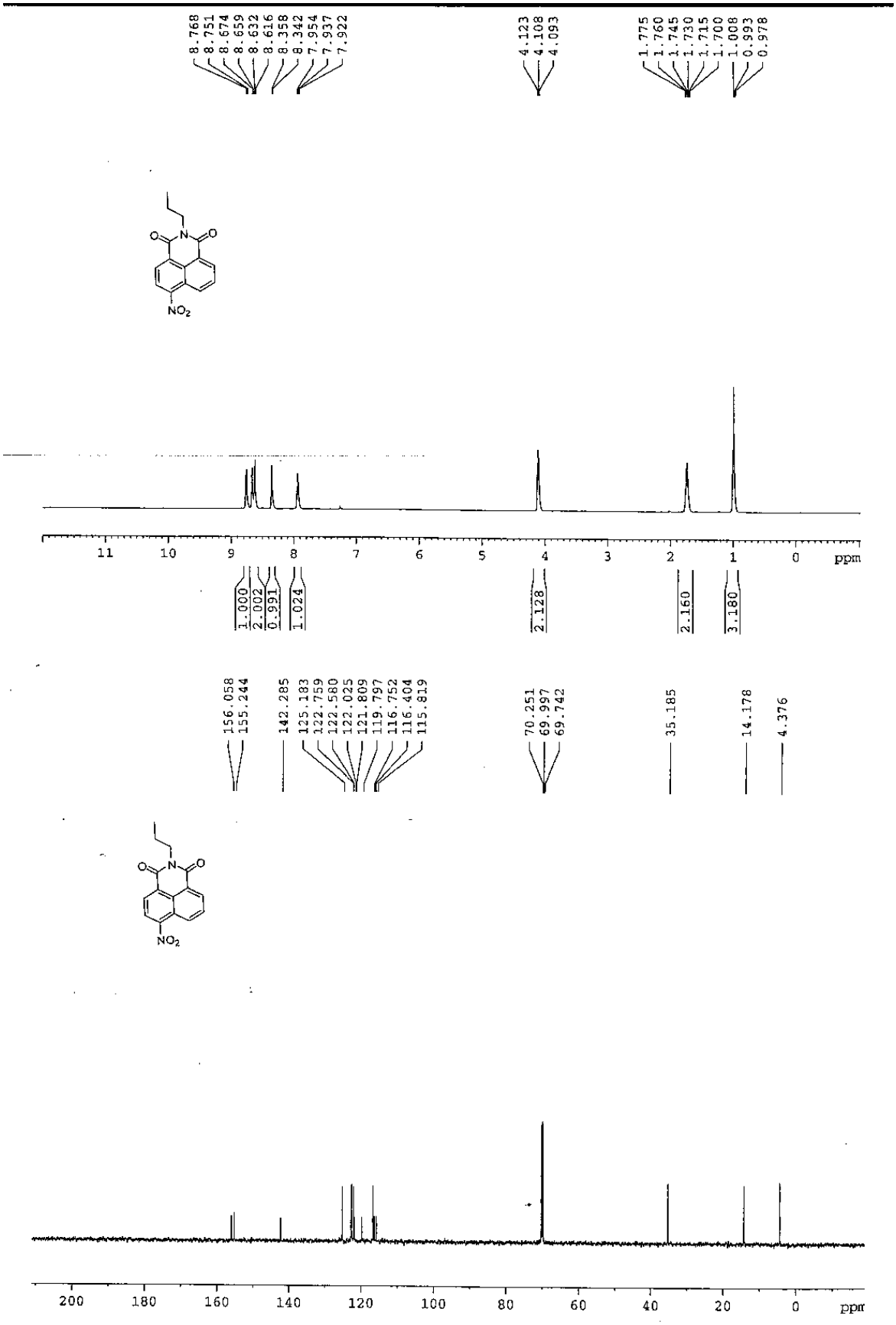
Primary cortical astrocytes were isolated from the cortices of postnatal day 1 rat brains. The cells were harvested from tissue under sterile conditions, placed through one round of enzymatic dissociation and expansion in astrocyte growth medium (85% Dulbecco's Modified Eagle medium containing 4.5 g/L glucose, and 15% Fetal Bovine Serum).

b. IntracellularH_2S detection

Astrocyte cells were plated onto polylysine-coated glass coverslips. Cells were washed with Dulbecco's Modified Eagle medium (DMEM) and incubated with growth medium containing 2.0 μM probe for 1h at 37 ºC. After washing the cells with DMEM, different concentration of Na_2S was added into the growth media of the cells and incubated for 2h at 37 ºC. Following a through wash, the coverslips were placed onto Olympus IX71 fluorescence microscopy and imaged with a GFP dichroic mirror.

References

1. Seybold, P. G.; Garterman, M.; Callis, J. *Photochem. Photobiol*. **1969**, *9*, 229-242.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1.760
1.745
1.730
1.715
1.700
1.008
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0.978
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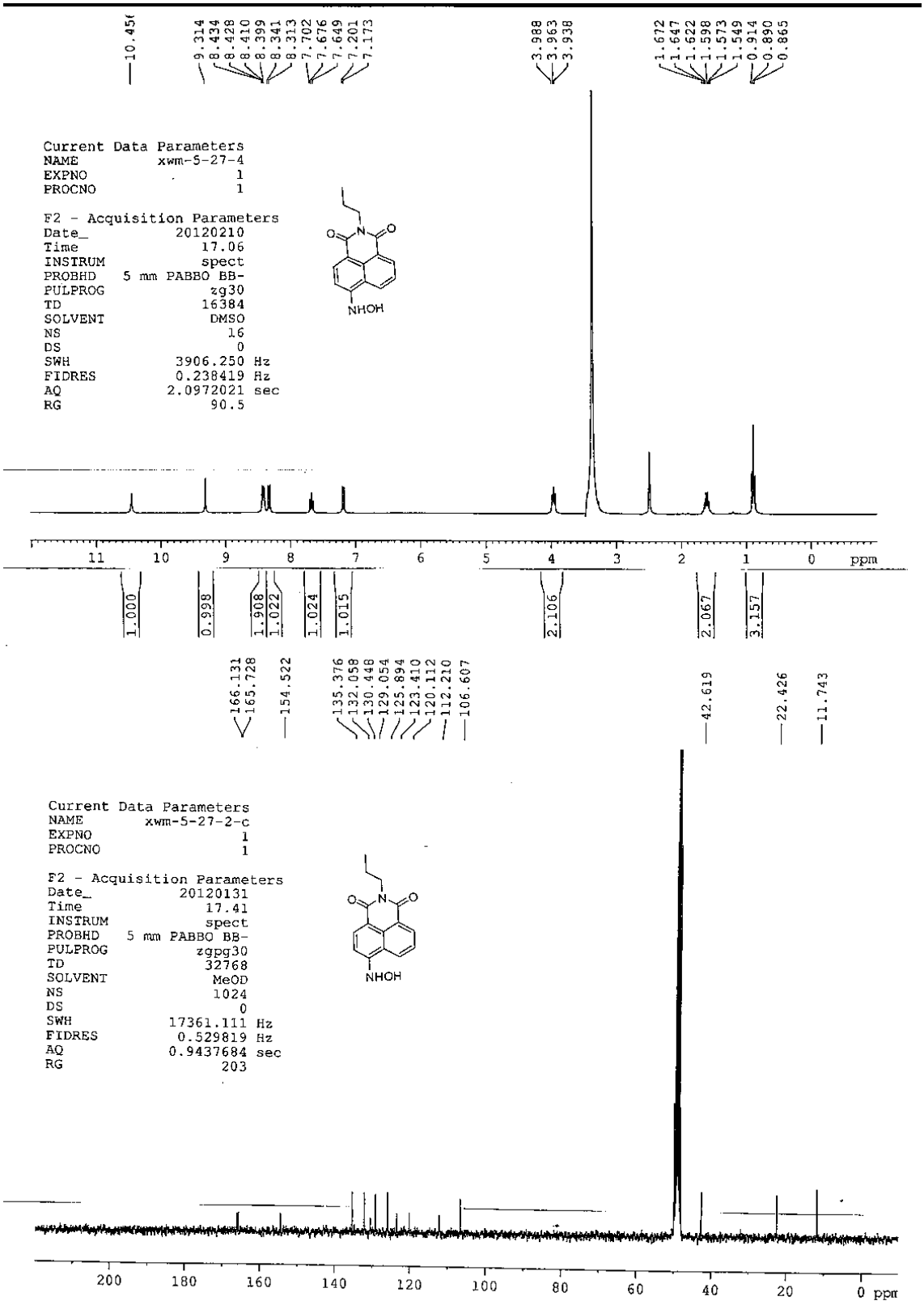
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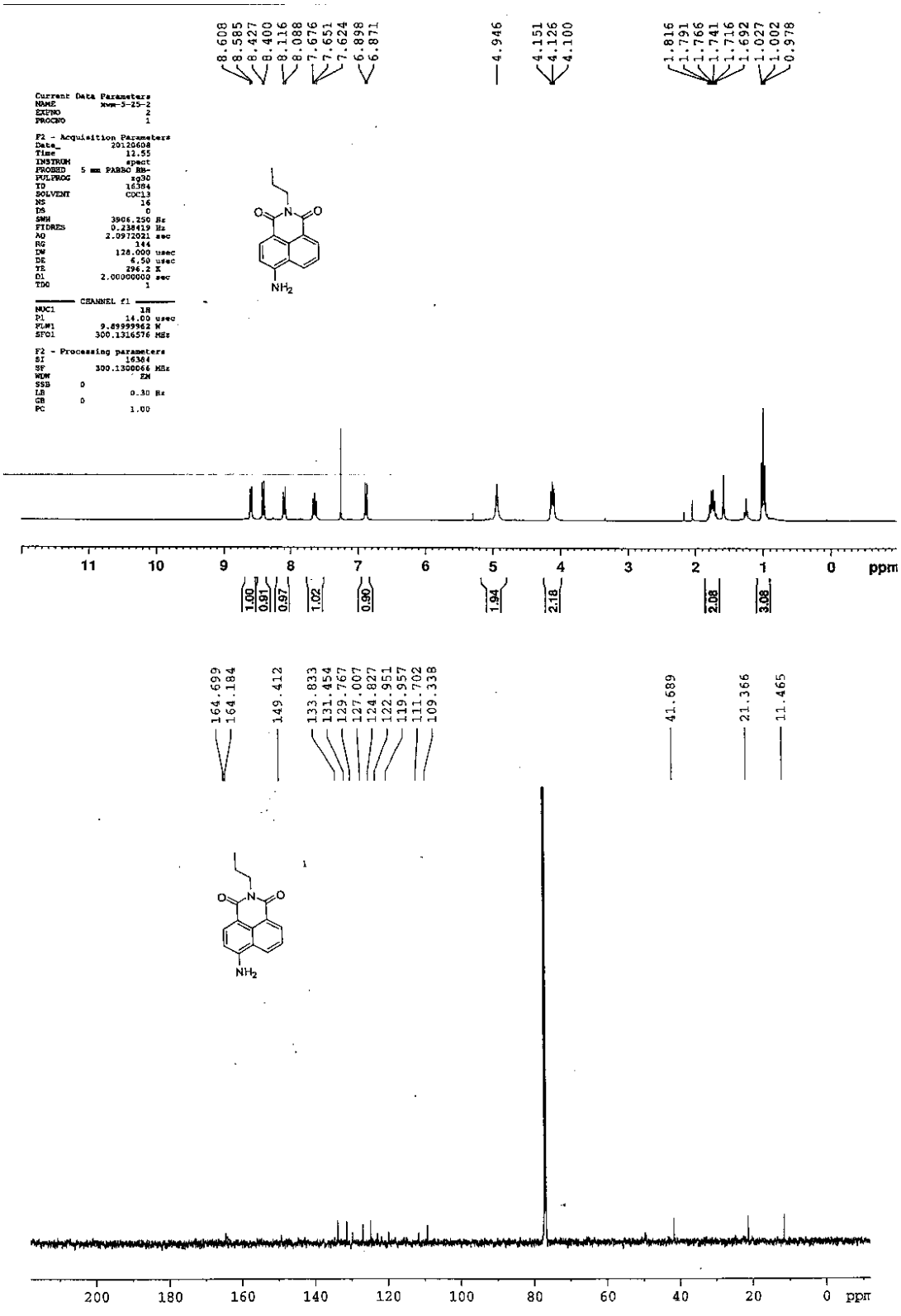
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