Electronic Supplementary Material (ESI) for Chemical Communications

Rational design of a ratiometric fluorescent probe with a large emission shift for the facile detection of Hg^{2+}

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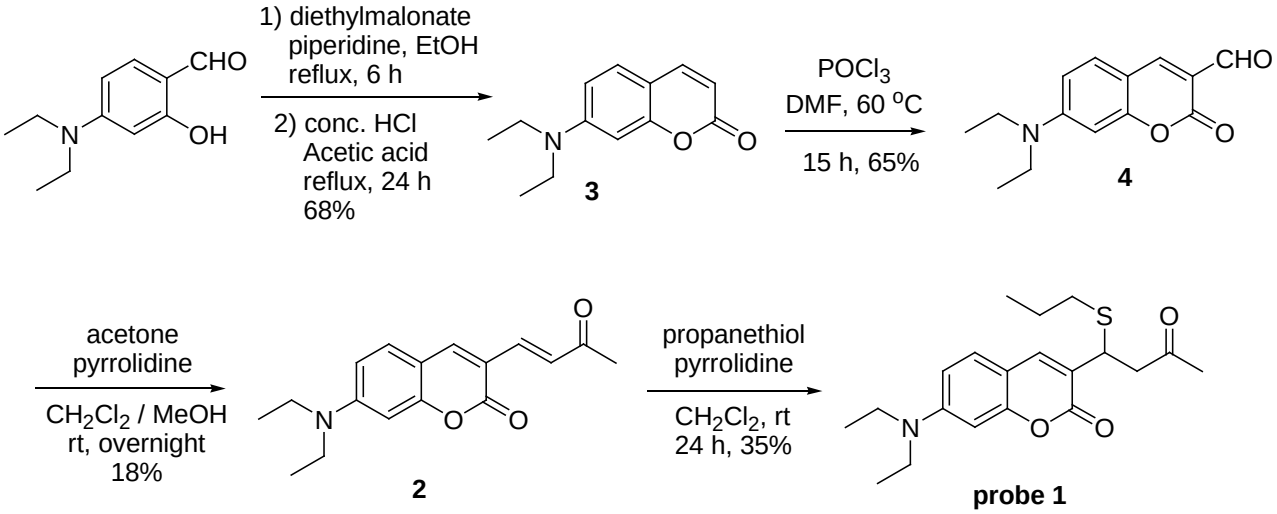
General Information: Commercial reagents were used as received, unless otherwise stated. Merck 60 silica gel was used for chromatography, and Whatman silica gel plates with fluorescence F_{254} were used for thin-layer chromatography (TLC) analysis. ^{1}H and ^{13}C NMR spectra were recorded on Bruker tardis (sb300). 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 Zeiss LSM510 META Confocal Microscope.

Scheme S1. Synthesis of Probe **1**



7-Diethylaminocoumarin (3). To a solution of 4-diethylaminosalicylaldehyde (2.0 g, 10.35 mmmol) in 30 mL of EtOH was added piperidine (1.0 mL, 10.12 mmol) and diethylmalonate (3.1 mL, 20.32 mmol), then heated to reflux and stirred for 6 h. EtOH was evaporated under reduced pressure, then concentrated HCl (20.0 mL) and acetic acid (30.0 mL) was added. The mixture was heated to reflux and stirred for 24 h. The solution was cooled to room temperature, and adjusted to be basic by 40% aq. NaOH, and a large amount of precipitate was formed and filtered. The crude product was further purified by column chromatograph and obtained as a yellow solid (1.54 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.52 (d, J = 9.3 Hz, 1H), 7.23 (d, J = 8.7 Hz, 1H), 6.55 (dd, J = 8.7 Hz, 2.4 Hz, 1H), 6.47 (d, J = 2.4 Hz, 1H), 6.02 (d, J = 9.3 Hz, 1H), 3.40 (q, J = 4.9 Hz, 4H), 1.20 (t, J = 6.9 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.27, 156.74, 150.68, 143.69, 128.76, 109.16, 108.65, 108.26, 97.51, 44.78, 12.43. HR-ESI-Mass (m/z): [M + H^+] calcd. for $C_{13}H_{15}NO_2$: 218.1181, obsd: 218.1175.

7-(Diethylamino)-2-oxo-2H-chromene-3-carbaldehyde (4). DMF (2.0 mL, 25.83 mmmol) was added dropwise to $POCl_3$ (2.0 mL, 21.46 mmmol) at room temperature, and stirred for 30 min. This solution was combined with a solution of compound **3** (650mg, 2.99mM) in 8 mL of DMF. The mixture was heated to 60 °C, and then stirred for 15 h. After cooled to room temperature, the

mixture was poured into 50 mL of ice water, and pH was adjusted to neutral, and a large amount of solid was precipitated. The product was filtered, washed with H_2O, dried with oil pump, and obtained as a yellow solid (475 mg, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.12 (s, 1H), 8.24 (s, 1H), 7.40 (d, *J* = 9.0 Hz, 1H), 6.63 (d, *J* = 9.0 Hz, 1H), 6.48(s, 1H), 3.47 (q, *J* = 7.1 Hz, 4H), 1.25 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 187.94, 161.88, 158.94, 153.46, 145.36, 132.51, 114.34, 110.17, 108.24, 97.12, 45.28, 12.45. HR-ESI-Mass (m/z): [M + H^+] calcd. for $C_{14}H_{15}NO_3$: 246.1130, obsd: 246.1141.

(*E*)-7-(diethylamino)-3-(3-oxobut-1-enyl)-2H-chromen-2-one (2). To a solution of compound **4** (43 mg, 0.18 mM) in 5 mL of CH_2Cl_2 (1:1) was added acetone (19 µL, 0.26 mmol) and 1 drop of pyrrolidine. The mixture was stirred at room temperature overnight. After removal of solvents under reduced pressure, the residue was purified by column chromatograph and a yellow solid was obtained (9 mg, 18%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77 (s, 1H), 7.46 (d, *J* = 15.9 Hz, 1H), 7.31 (d, *J* = 9.0 Hz, 1H), 7.13 (d, *J* = 15.9 Hz, 1H), 6.61 (d, *J* = 9.0 Hz, 1H), 6.49 (s, 1H), 3.44 (q, *J* = 7.1 Hz, 4H), 2.35 (s, 3H) 1.23 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.82, 160.46, 156.75, 151.94, 144.11, 137.80, 129.97, 127.12, 114.54, 109.51, 108.76, 97.00, 45.04, 28.36, 12.46. HR-ESI-Mass (m/z): [M + H^+] calcd. for $C_{17}H_{19}NO_3$: 286.1443, obsd: 286.1450.

Probe (1). To a solution of compound **2** (9 mg, 0.03 mmol) in 5 mL of CH_2Cl_2 was added propanethiol (12 µL, 0.13mmol) and one drop of pyrrolidine. The mixture was stirred at room temperature for 24 h. After removal of solvents under reduced pressure, the reaction mixture was purified by column chromatograph and a yellow liquid was obtained (4 mg, 35%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.63 (s, 1H), 7.27 (d, *J* = 8.7 Hz, 1H), 6.57 (dd, *J* = 8.7 Hz, 2.4 Hz, 1H), 6.49 (d, *J* = 2.4 Hz, 1H), 4.33 (t, *J* = 7.2 Hz, 1H), 3.41 (q, *J* = 7.1 Hz, 4H), 3.14 (dd, *J* = 16.8 Hz, 6.9 Hz, 1H), 2.96 (dd, *J* = 16.8 Hz, 7.5 Hz, 1H), 2.48 (m, 2H), 2.17 (s, 3H), 1.59 (m, 2H), 1.20 (t, *J* = 7.2 Hz, 6H), 0.94 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 205.80, 161.57, 155.87, 150.43, 140.24, 128.77, 121.01, 108.85, 108.30, 97.16, 48.18, 44.83, 40.55, 34.32, 30.17, 22.66, 13.53, 12.42. HR-ESI-Mass (m/z): [M + H^+] calcd. for $C_{20}H_{27}NO_3S$: 362.1790, obsd: 362.1794

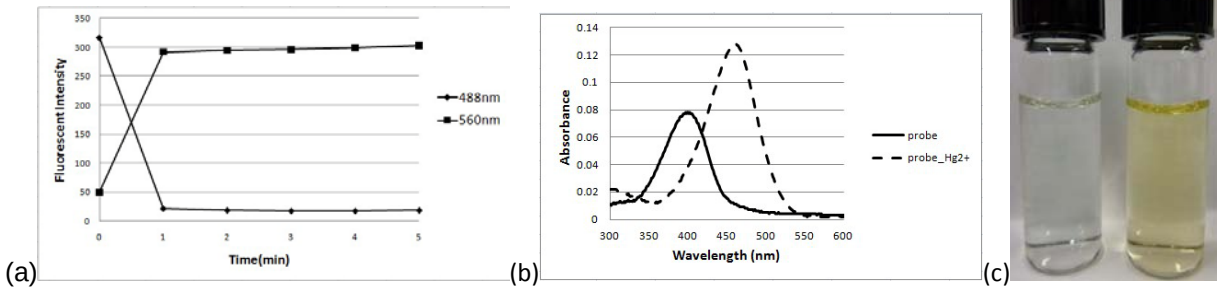


Figure S1. (a) Emission of probe **1** (5.0 µM) at 488 nm and 560 nm after addition of Hg^{2+} (10.0 µM, λ_{ex} = 435 nm). Data were recorded every minute. (b) UV/Vis spectra of probe **1** at 5.0 µM (solid line) and the probe at 5.0 µM with 10.0 µM Hg^{2+} (dashed line). (c) Color change observed by naked eyes. All experiments were carried out in a 0.1 M phosphate buffer solution containing 0.5% acetonitrile (pH = 7.4).

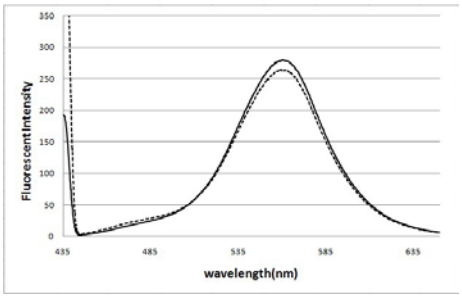


Figure S2. The solid line was the emission of probe **1** (5.0 μM) after addition of Hg^{2+} (2.5 μM) in 0.1M phosphate buffer solution (containing 0.5% acetonitrile) at pH 7.4; the dashed line was the emission of compound **2** (5.0 μM), λ_{ex} = 435 nm.

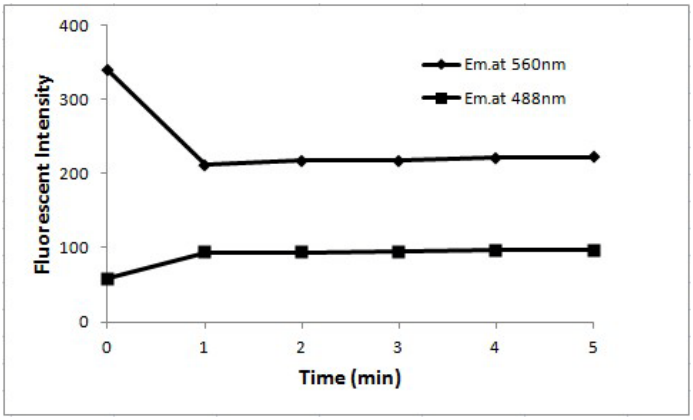


Figure S3. Probe **1** response time at 0.75 μM Hg^{2+}. In this experiment, 0.75 μM Hg^{2+} was added to 5.0 μM probe **1**, and the results were the same as 10 μM Hg^{2+}.

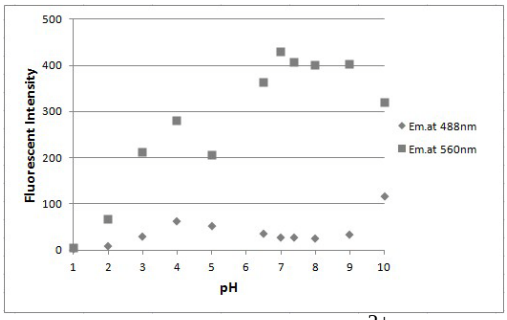


Figure S4. The response of 5.0 μM probe **1** to 10.0 μM Hg^{2+} was investigated at different pHs. At pH 1, all fluorescence was quenched; at pH 2 to 5, the probe can respond to Hg^{2+} with suppressed fluorescence; at pH 6.5 to 9, similar results were observed; at pH 10, the response begun to decrease.

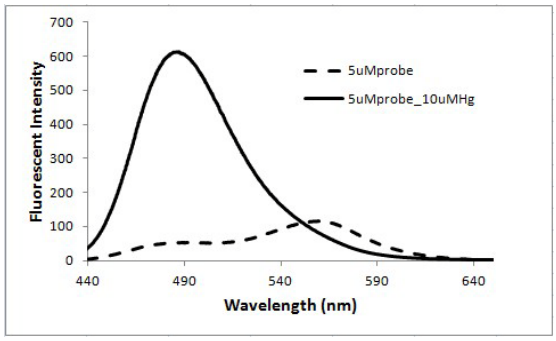


Figure S5. When the probe was excited at 405 nm in a buffer soulation, we found that the yellow emission was much weaker than the blue one. This can explain why the yellow emission was weak in the imaging experiment.

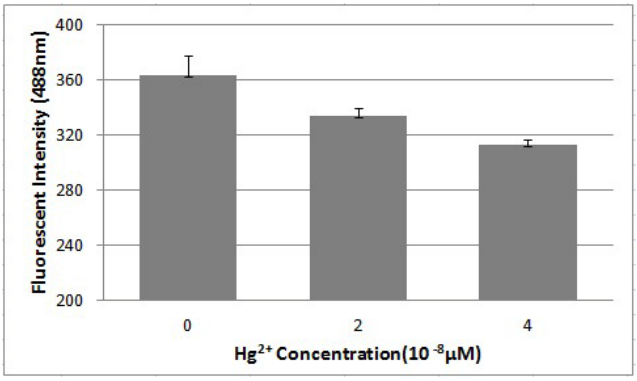


Figure S6. Detection limit of probe **1**.

The salt forms used for analysis are: $HgCl_2$, $AgNO_3$, F_3CSO_3Cu, $CuCl_2$, $NiCl_2{\cdot}6H_2O$, $Zn(NO_3)_2{\cdot}6H_2O$, $Cd(NO_3)_2{\cdot}4H_2O$, $Pb(NO_3)_2$, $AlCl_3$, $Co(NO_3)_2{\cdot}6H_2O$, $CrCl_3$, Cs_2CO_3, $MgSO_4$, $CaCl_2$, LiCl, K_2CO_3, $BaCl_2$, $MnSO_4{\cdot}H_2O$, $FeSO_4{\cdot}7H_2O$, $FeCl_3$.

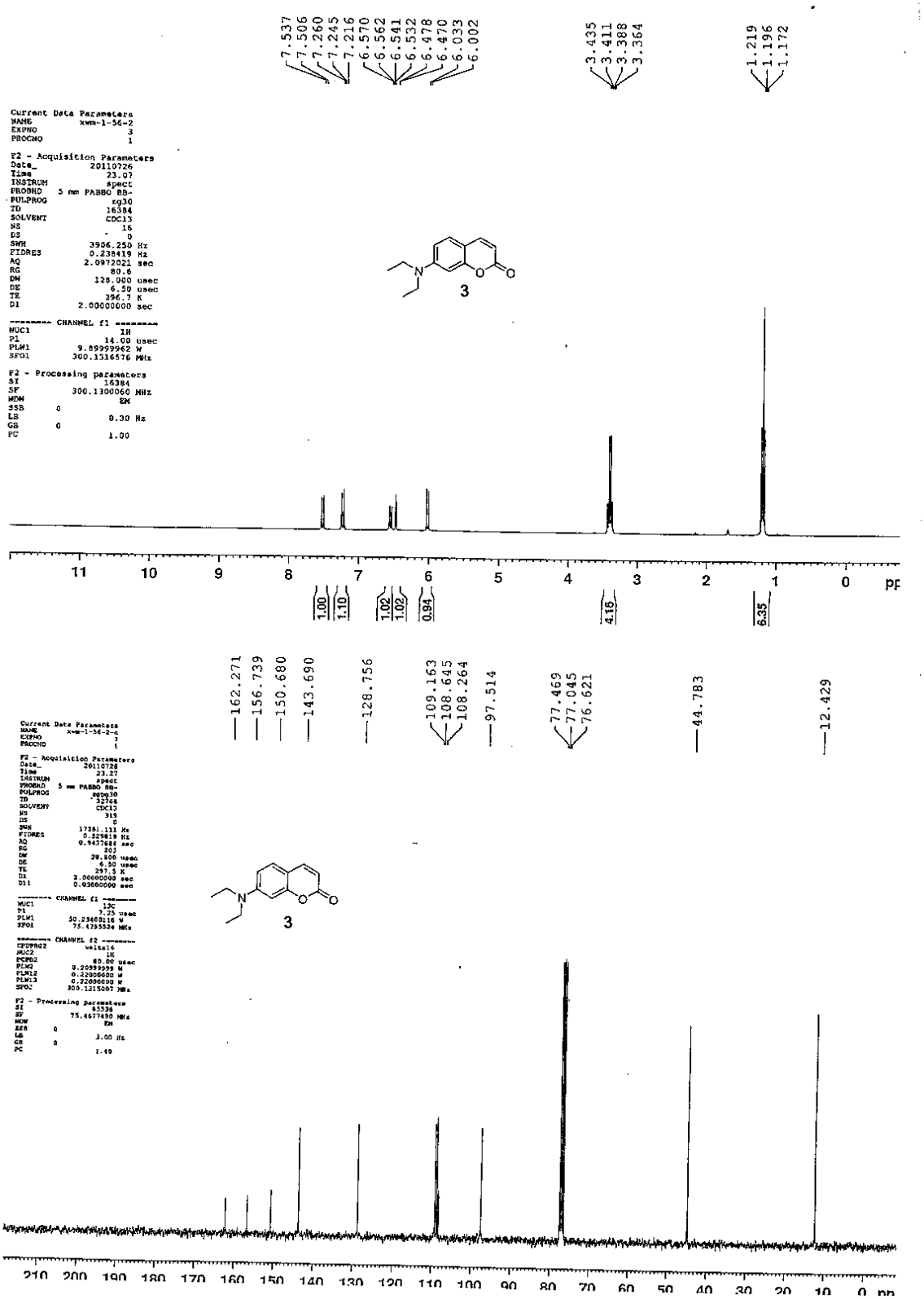
Imaging of C8D1A astrocyte cell line incubated with probe 1 and Hg^{2+}

Cell incubation. C8D1A cells (mouse astrocyte cell line) were seeded on micro cover glasses (VWR, USA) in 6-well plate at a density of 5×10^3 cells per well in DMEM culture media (Invitrogen, USA) with 10% fetal bovine serum (Invitrogen, USA). After 24 h, growth medium was discarded and the cells were washed four times with PBS. Probe was stored as a 1mM stock solution at 4 °C.

For control group, C8D1A cells were incubated with 5µM Probe in PBS for 30 min at 37 °C, then washed with PBS three times. For Hg^{2+} imaging, cover glasses were incubated with a solution of 5µM Probe (or 5µM Hg^{2+}) in PBS for 30 min at 37 °C. Afterwards, PBS was used to remove the remaining probe (or Hg^{2+}) by washing three times; the cells were further treated with 5µM Hg^{2+} (or 5µM probe) in PBS for 30 min, then washed with PBS three times.

Confocal Imaging. The fluorescence measurements of C8D1A cells were performed by live cell confocal imaging by Zeiss LSM510 META Confocal Microscope with a 63× objective. Fluorophores were excited by using a 405 nm laser diode, and emissions were obtained at double channels, 475 – 525nm and 530 – 600 nm. Images were acquired with the Zeiss LSM software.

NMR spectra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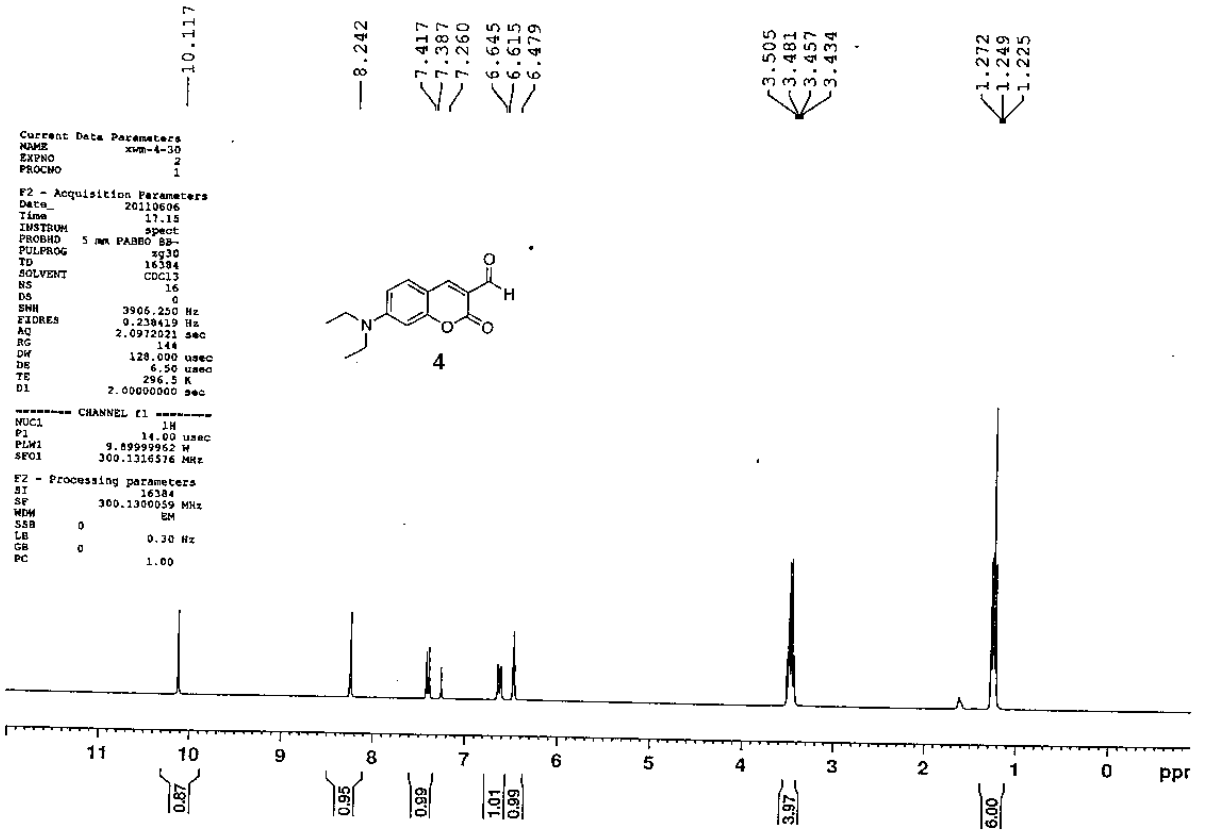
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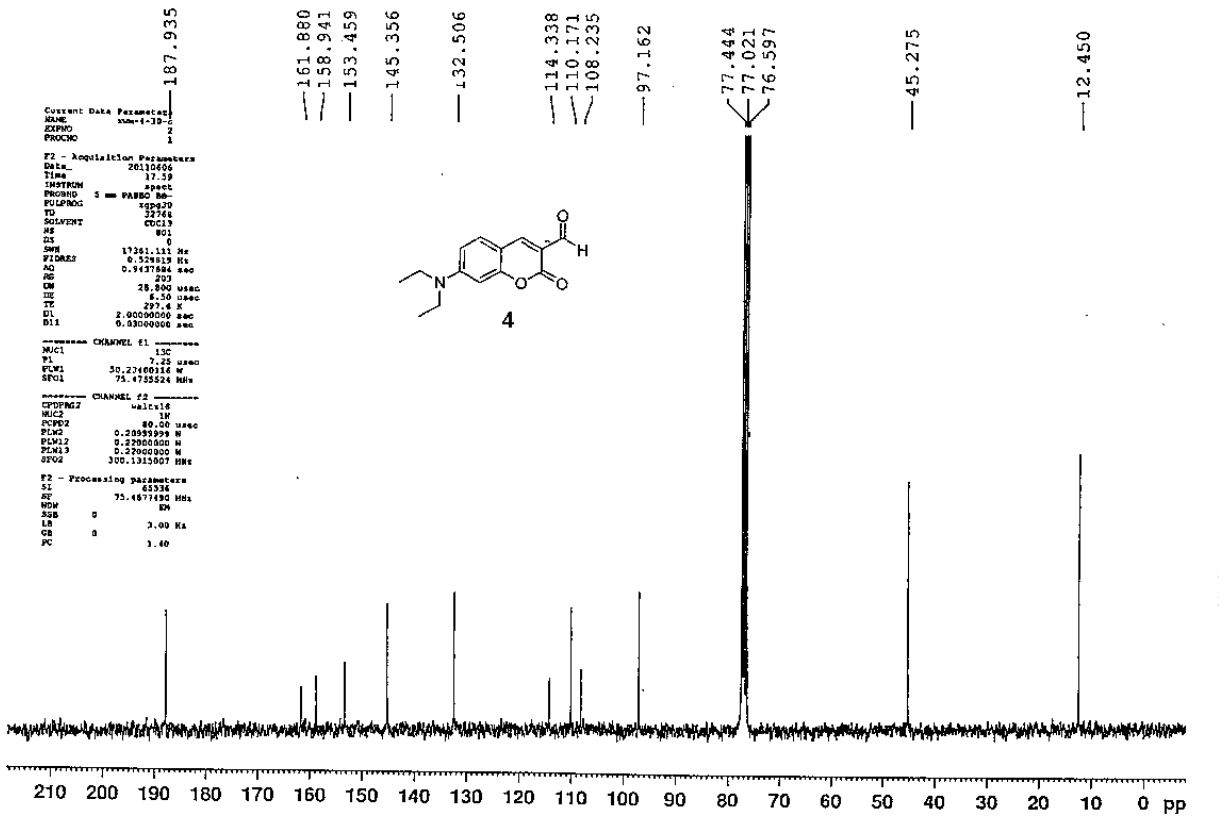
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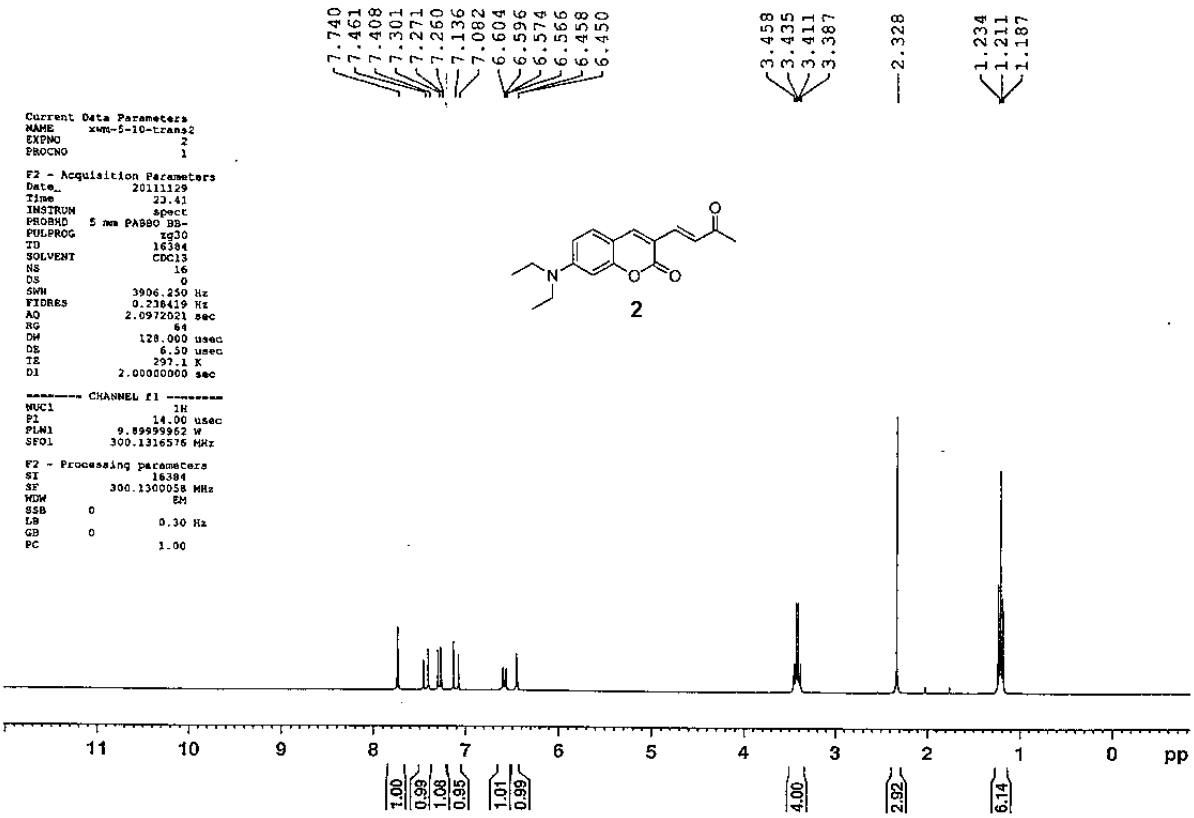
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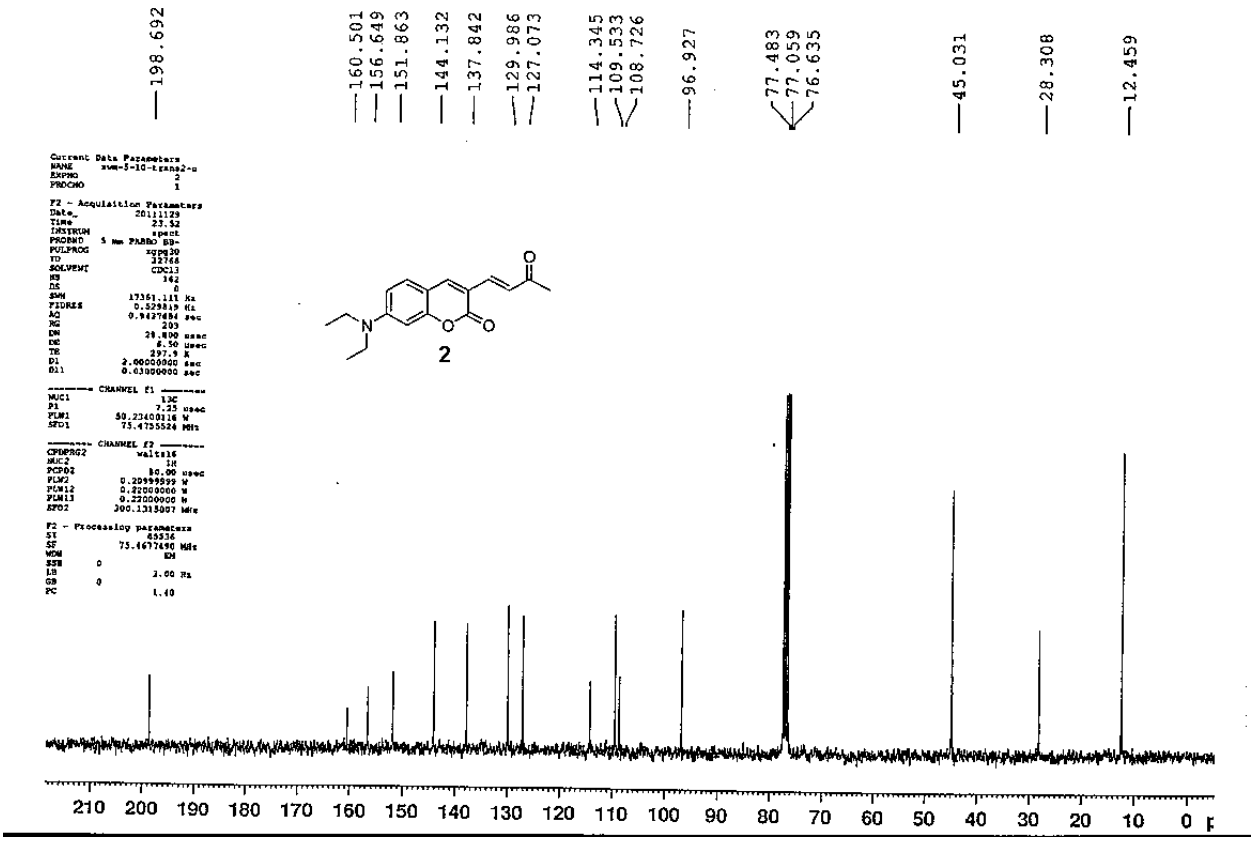
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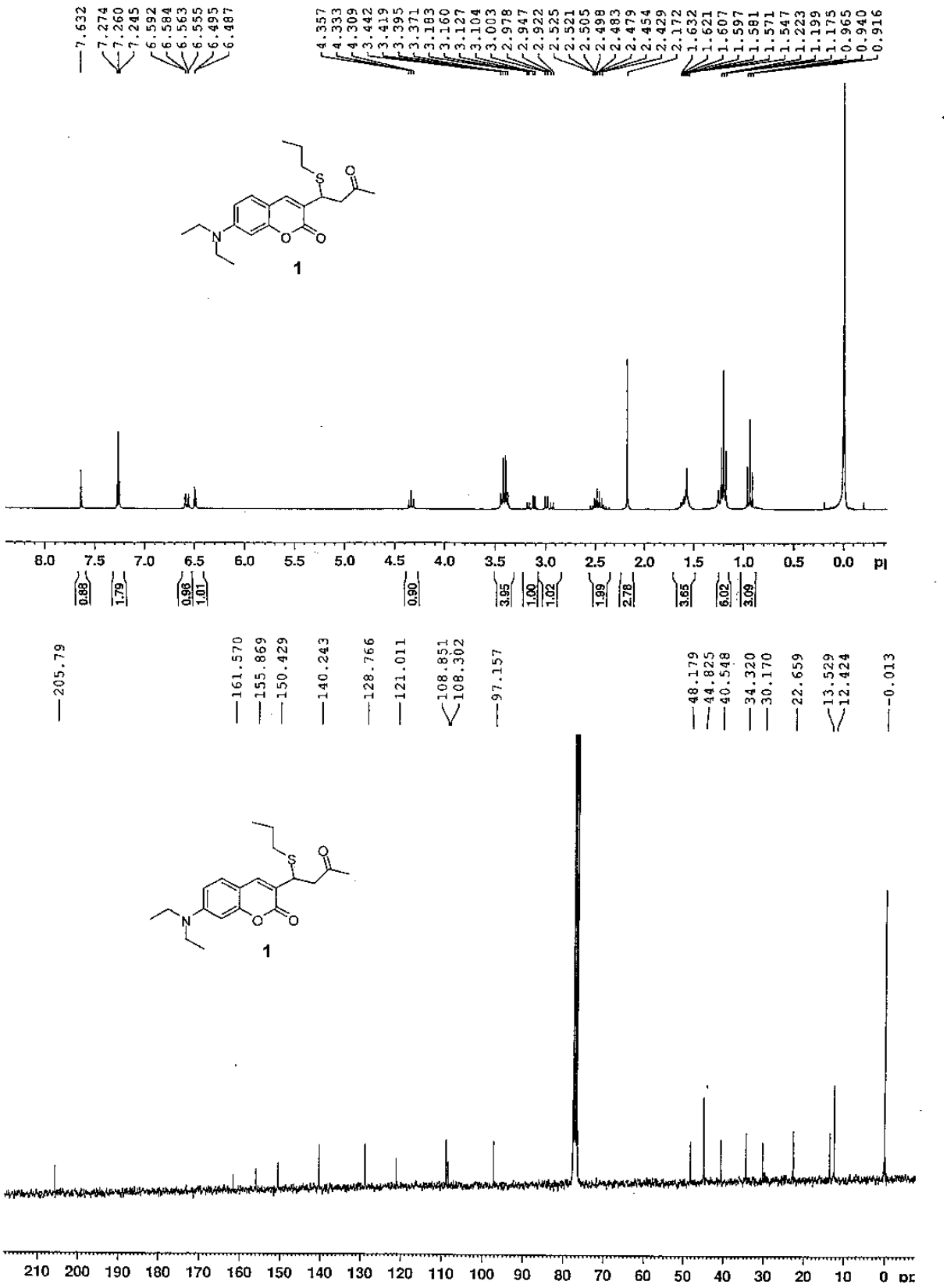
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