Supporting Information

Naphthalimide Appended Rhodamine Derivative: Through Bond Energy Transfer for Sensing of Hg^{2+} Ions

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Instruments and experimental procedures

General information

All reagents were purchased from Aldrich and were used without further purification. THF (AR grade) was used to perform analytical studies. UV-vis spectra were recorded on a SHIMADZU UV-2450 spectrophotometer, with a quartz cuvette (path length, 1 cm). The fluorescence spectra were recorded with a SHIMADZU 5301 PC spectrofluorimeter. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL-FT NMR-AL 300 MHz using $CDCL_3$ as solvent and tetramethylsilane SiMe4 as internal standards. Data are reported as follows: chemical shifts in ppm (δ), multiplicity (s = singlet, d = doublet, q = quartet, br = broad singlet, m = multiplet, dd = doublet of doublet), coupling constants (Hz), integration, and interpretation. Silica Gel 60 (60-120 mesh) was used for column chromatography. Fluorescence quantum yield[1] was determined using optically matching solutions of rhodamine B (Φ_{fr} = 0.65 in ethanol) as standard at an excitation wavelength of 540 nm and quantum yield is calculated using the equation:

$$\Phi_{fs} = \Phi_{fr} \quad X \quad \frac{1\text{-}10^{-AsLs}}{1\text{-}10^{-ArLr}} \quad X \quad \frac{N_s^2}{N_r^2} X \quad \frac{D_s}{D_r}$$

Φ_{fs} and Φ_{fr} are the radiative quantum yields of sample and the reference respectively, A_s and A_r are the absorbance of the sample and the reference respectively, Ds and Dr the respective areas of emission for sample and reference. L_s and L_r are the lengths of the absorption cells of sample and reference respectively. N_s and N_r are the refractive indices of the sample and reference solutions (pure solvents were assumed respectively).

[1] Deams, J. N.; Grosby, G. A. *J. Phys. Chem*. **1971**, *75*, 991.

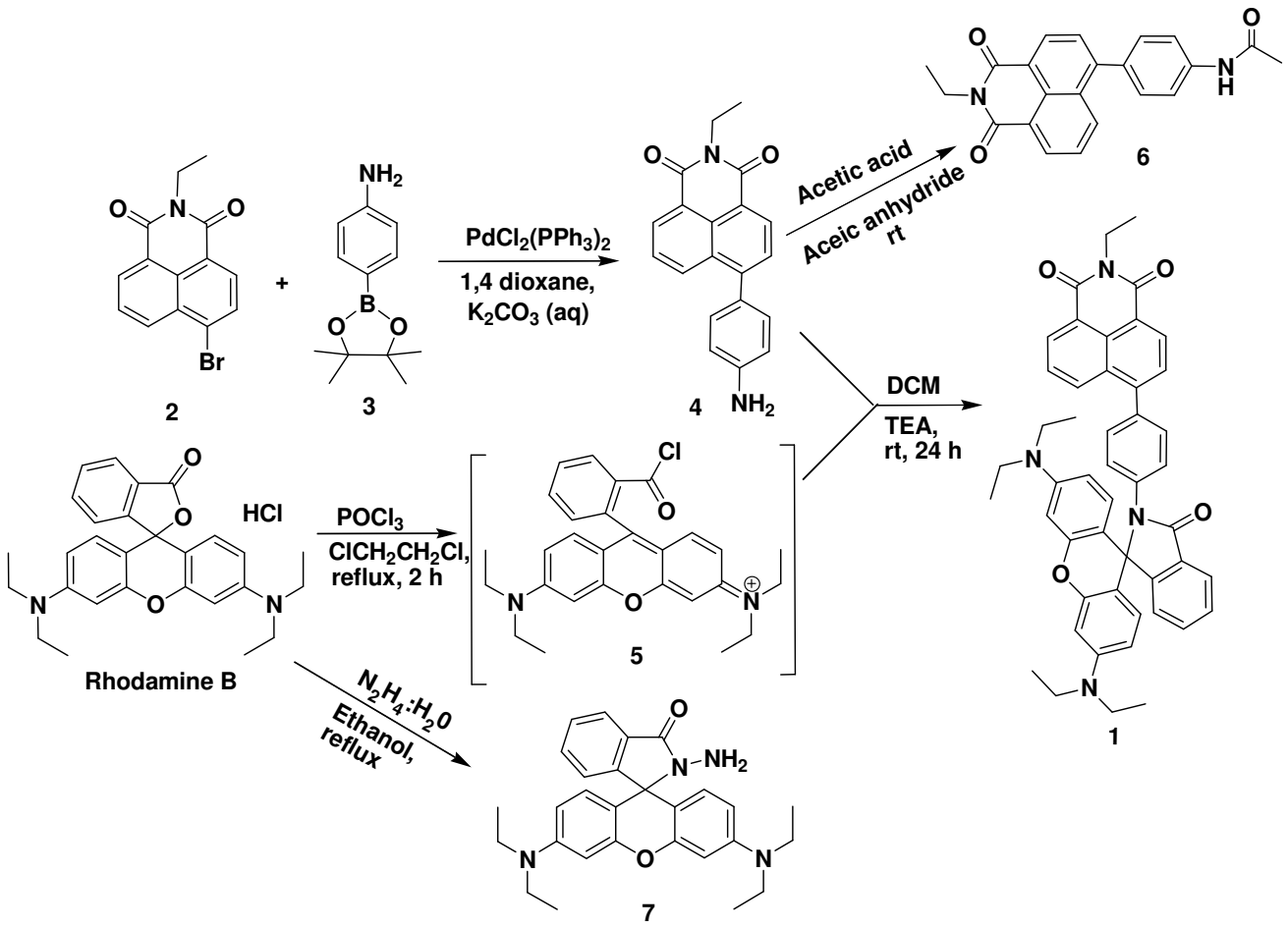
Procedure for metal ion sensing

Solutions of compound **1** and metal perchlorates were prepared in THF and THF:H_2O (9.5:0.5) mixture. In titration experiments, each time a 3 ml solution of **1** (5 μM) was filled in a quartz cuvette (path length, 1 cm) and metal ions were added into the quartz cuvette by using a micro-pippet. For fluorescence measurements, excitation was provided at 360 nm, and emission was collected from 350 to 650 nm.

Procedure for fluorescence imaging

The prostate cancer (PC3) cell lines were incubated with receptor **1** (1 μM in THF:H_2O (9.5:0.5, v/v) buffered with HEPES, pH = 7.0) in RPMI-1640 medium for 20 min at 37ºC and washed with phosphate buffered saline (PBS) buffer (pH 7.4) to remove excess of receptor **1**. The cells were then treated with mercury perchlorate (10 μM) in RPMI-1640 medium and incubated for further 20 min at 37ºC and washed with PBS buffer. The cells were imaged by confocal fluorescence microscope with excitation wavelength 488 nm.

Synthetic routes and characteristic data



Scheme 1. The synthetic route for the compound **1**

Compound **2**[2], **3**[3] and **7**[4] were synthesized according to the literature procedure.

Synthesis of (2):

To a solution of 4-bromo-1, 8-naphthalic anhydride (0.5 g, 1.8 mmol) in dioxane (20 mL) was added an excess of ethyl amine, and the reaction mixture was refluxed for 24 hours. The reaction mixture was then cooled and poured into ice cold water for precipitation. Precipitate, thus formed was filtered, washed with methanol, and dried to give 0.45 g (82 %) of compound **2** as a creamy solid. ^{1}H NMR

[2] Gunnlaugsson, T.; Kruger, P. E.; Jensen, P.; Ali, H, D, P.; Hussey, G. M. *J. Org. Chem*. **2005,** *70*, 10875.
[3] Bhalla, V.; Tejpal, R.; Kumar, M.; Puri, R. K.; Mahajan, R. K. *Tetrahedron. Lett*. **2009**, *50*, 2649.
[4] Dujols, V.; Ford, F.; Cazarnik, A. W. *J. Am. Chem. Soc*. **1997**, *119*, 7386.

($CDCl_3$, 300 MHz, ppm) 1.32 (t, 3 H, *J* = 7.02), 4.19-4.26 (q, 2 H), 7.83 (t, 1 H, *J* = 9), 8.02 (d, 1 H, *J* = 9), 8.40 (d, 1 H, *J* =9), 8.55 (d, 1 H, *J* = 9), 8.65 (d, 1 H, *J* = 7.2). ^{13}C NMR ($CDCl_3$, 300 MHz, ppm) 9.70, 32.04, 118.76, 119.63, 124.45, 125.40, 126.56, 127.04, 127.47, 127.54, 128.35, 129.57, 159.81. m.p. 158-160 ºC.

Synthesis of (3):

To a suspension of $[PdCl_2(PPh_3)_2]$ (5.08 g, 39.73 mmol) in dioxane (15 mL) were added 4-bromoaniline (2.5 g, 14.45 mmol), 4, 4, 5, 5-tetramethyl-1, 3, 2-dioxaborolane (5.08 g, 39.73), and triethylamine (5.83 g, 57.8 mmol) under argon. After stirring for 5 h at 80 ºC, the dioxane was removed under vacuum and the residue so obtained was treated with water, extracted with dichloromethane, and dried over anhydrous Na_2SO_4. The organic layer was evaporated, and the compound was purified by column chromatography using dichloromethane as an eluent to give 2.3 g, (75 %) of compound **3** as brown solid. . ^1H NMR ($CDCl_3$, 300 MHz, ppm) 1.32 (s, 12 H), 3.83 (s, 2 H), 6.61 (d, 2 H, *J* = 6), 7.62 (d, 2 H, *J* = 6). ^{13}C NMR ($CDCl_3$, 300 MHz, ppm) 24.82, 83.28, 114.06, 136.39, 149.26. m.p. 160 ºC.

Synthesis of (4):

To a solution of **2** (0.50 g, 1.64 mmol) and **3** (0.41 g, 1.18 mmol) in dioxane (8 ml) were added K_2CO_3 (0.45 g, 3.28 mmol), distilled water (2 mL), and $[Pd(Cl)_2(PPh_3)_2]$ (0.28 g, 0.41 mmol) under nitrogen, and the reaction mixture was refluxed overnight. The dioxane was then removed under vacuum, and the residue so obtained was treated with water, extracted with dichloromethane, and dried over anhydrous Na_2SO_4. The organic layer was evaporated, and the compound was purified by column chromatography using hexane/ethyl acetate (1:1) as an eluent to give 0.40 g (70 %) of compound **4** as orange colored solid. ^1H NMR ($CDCl_3$, 300 MHz, ppm) 1.35 (t, 3 H, *J* = 6.0), 3.90 (s, 2 H), 4.23-4.30 (q, 2 H), 6.82-6.86 (m, 2 H), 7.30-7.34 (m, 2 H), 7.65-7.71 (m, 2 H), 8.35-8.38 (m, 1

H), 8.60-8.63 (m, 2 H). ^{13}C NMR ($CDCl_3$, 300 MHz, ppm) 13.77, 35.85, 115.38, 121.39, 123.31, 126.86, 127.90, 129.15, 129.26, 130.57, 131.31, 131.40, 131.51, 133.34, 147.30, 147.67, 164.46, 164.67. m. p. 224-226 ºC. TOF MS ES+, *m/z*: 317.66 $(M+1)^+$. Anal. calcd. for $C_{20}H_{16}N_2O_2$: C, 75.93; H, 5.1; N, 8.86. Found: C, 75.67; H, 4.89; N, 8.69.

Synthesis of (1):

To synthesize compound **1,** a solution of rhodamine B (0.07g, 0.15 mmol) in 1,2-dichloroethane (10 mL) was stirred, and phosphorus oxychloride (0.23 mL) was added dropwise over 5 at room temperature. The solution was refluxed for two hours. The reaction mixture was cooled and evaporated in vacuo to give rhodamine B acid chloride, which was impure and used in the next step directly. The crude acid chloride was dissolved in the dry dichloromethane (10 ml) and added dropwise over 1 hour to a solution of 4 (0.05g, 0.158 mmol) and TEA (0.78 mmol) in dichloromethane (10 mL) at room temperature. After 24 hours, the solvent was removed under pressure, and the residue left was dissolved in chloroform, extracted with water, dried over anhydrous Na_2SO_4. The organic layer was evaporated, and recrystallized with methanol to give 0.06 g (55%) of compound **1** as light pink colored solid. ^{1}H NMR ($CDCl_3$, 300 MHz, ppm) 1.15 (t, 12 H, J = 9.0), 1.33 (t, 3 H, J = 9), 3.29-3.36 (q, 8 H), 4.24-4.29 (q, 2 H), 6.31-6.36 (m, 4 H), 6.68 (d, 2 H, J = 9.0), 7.08-7.10 (m, 2 H), 7.16-7.19 (m, 1 H), 7.26-7.29 (m, 2 H) 7.50-7.54 (m, 2 H), 7.59-7.66 (m, 2 H), 8.03-8.05 (m, 1 H), 8.18-8.22 (dd, 1 H, J = 1.2), 8.56-8.61 (m, 2 H). ^{13}C NMR ($CDCl_3$, 300 MHz, ppm) 12.96, 13.78, 35.80, 44.74, 68.05, 98.37, 106.66, 108.78, 122.04, 123.33, 123.88, 124.34, 127.01, 128.13, 128.68, 129.14, 130.61, 130.98, 131.44, 133.29, 136.78, 137.89, 146.83, 149.32, 153.46, 164.24, 164.70, 168.39. m. p. 222ºC. TOF MS ES+, *m/z*: 741.35 $(M+1)^+$. Anal. calcd. for $C_{48}H_{44}N_4O_4$: C, 77.81; H, 5.99; N, 7.56. Found: C, 77.53; H, 5.71; N, 7.32.

Synthesis of (6):

To a solution of **4** (0.05 g, 0.158 mmol) in acetic acid (5.0 mL) was added an excess of acetic anhydride in ice cold condition, and the resulting reaction mixture was stirred at room temperature for 2 hours. The reaction mixture was then poured into ice cold water for precipitation. Precipitate, thus formed was filtered, washed with water, and dried to give 0.04 g (80 %) of compound **6** as a yellow colored solid. ^{1}H NMR (DMSO-d_6, 400 MHz, ppm) 1.32 (t, 3 H, *J* = 6.0), 2.59 (s, 3 H), 4.20-4.25 (q, 2 H), 7.44 (d, 2 H, *J* = 6.0), 7.69-7.75 (m, 2 H) 7.82 (d, 2 H, *J* = 6.0), 8.32 (d, 1 H, *J* = 6.0), 8.58 (d, 2 H, *J* = 3.0), 9.91 (s, 1 H). ^{13}C NMR (DMSO-d_6, 400 MHz, ppm) 12.92, 23.90, 30.48, 34.79, 119.18, 120.84, 122.29, 126.36, 127.23, 128.13, 129.48, 129.80, 130,19, 130.46, 132.25, 132.86, 139.23, 146.13, 163.19, 163.40, 168.74. m. p. 214ºC. TOF MS ES+, *m/z*: 359.47 $(M+1)^+$.

Synthesis of (7):

This compound was synthesized following literature procedure.[4] Yield 80 %; ^{1}H NMR ($CDCl_3$, 300 MHz, ppm) 1.16 (t, 12 H, *J* = 6.0), 3.30-3.37 (q, 8 H), 3.60 (br, 2 H), 6.26-6.30 (m, 2 H) 6.41-6.47 (m, 4 H), 7.08-7.13 (m, 1 H), 7.41-7.47 (m, 2 H), 7.91-7.94 (m, 1 H).

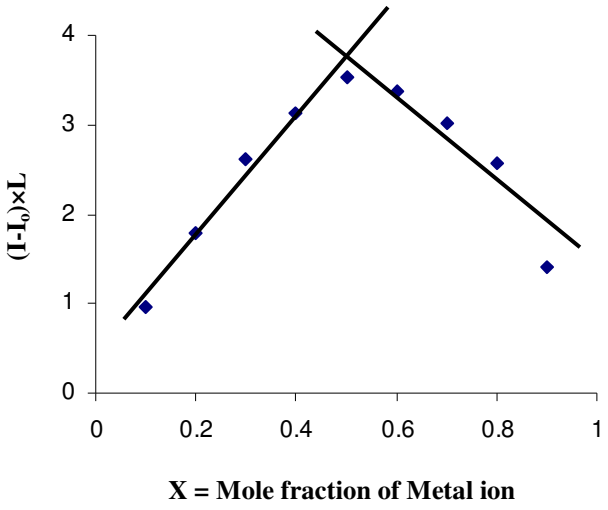


Figure S1. Job's plot of **1** with Hg^{2+} ions in THF representing stoichiometry 1:1.

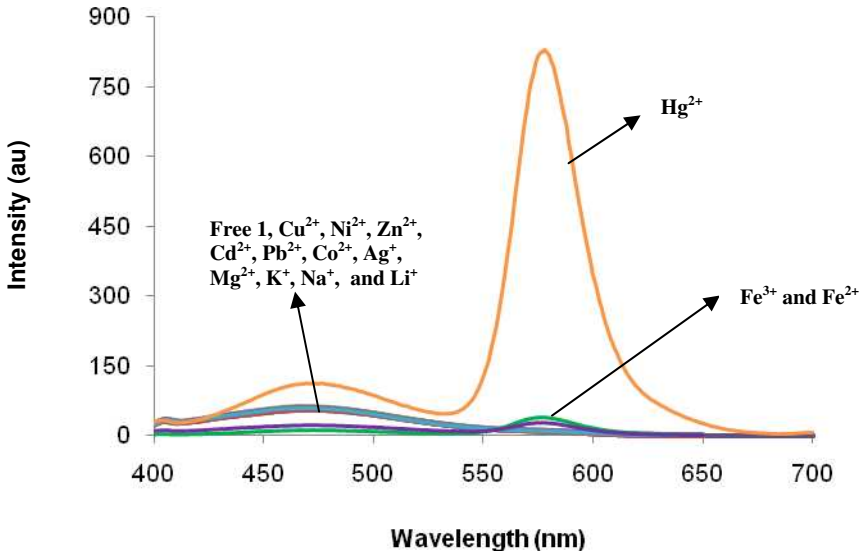


Figure S3. Fluorescence spectra of **1** (5 μM) in response to the presence of different metal ions (350 equiv each) in THF:H_2O (95:05, v/v) buffered with HEPES, pH = 7.0; λ_{ex} = 360 nm.

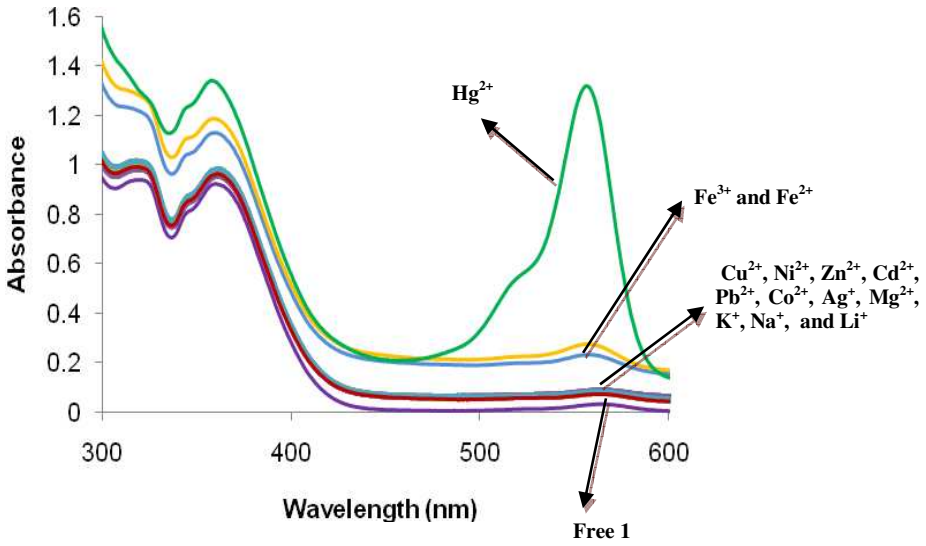


Figure S4 UV-vis spectra of **1** (5 μM) in response to the presence of different metal ions (100 equiv each) in THF:H_2O (95:05, v/v) buffered with HEPES, pH = 7.0; λ_{ex} = 360 nm.

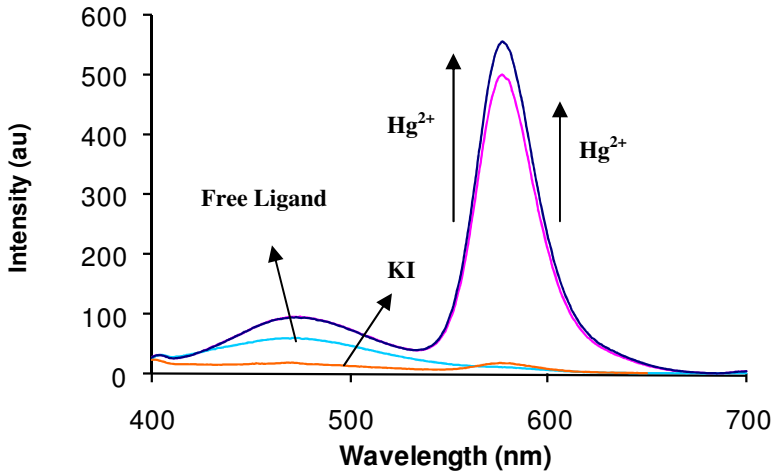


Figure S5. Fluorescence spectra showing reversibility of Hg^{2+} coordination to receptor **1** by KI; sky blue line, free **1** (5 μM), blue line, **1** + 250 equiv Hg^{2+}, orange line, **1** + 250 equiv Hg^{2+} + 400 equiv KI, pink line, **1** + 250 equiv Hg^{2+} + 400 equiv KI + 600 equiv Hg^{2+}, in THF:H_2O (95:05, v/v) buffered with HEPES, pH = 7.0; λ_{ex} = 360 nm.

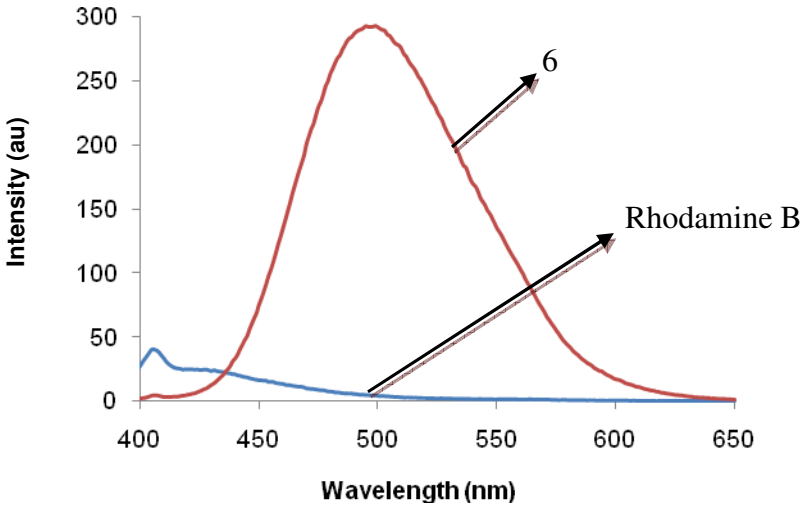


Figure S6. Fluorescence spectra of equimolar mixture of **6** (Red) and ring opened rhodamine B (Blue) in THF:H_2O (95:05, v/v) buffered with HEPES, pH = 7.0; λ_{ex} = 360 nm. The concentration is 5.0 μM for both **1** and rhodamine B.

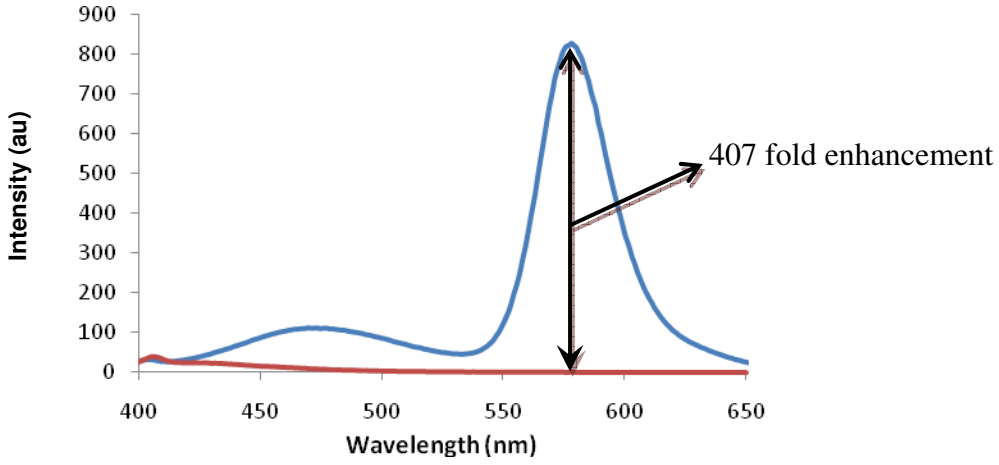
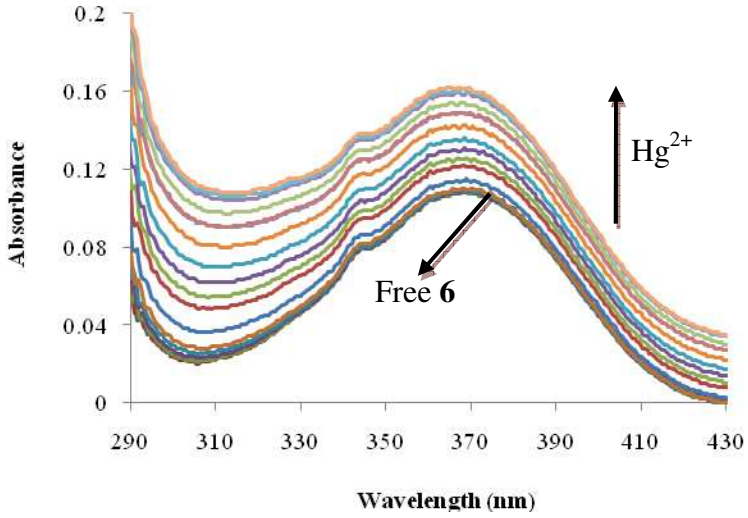
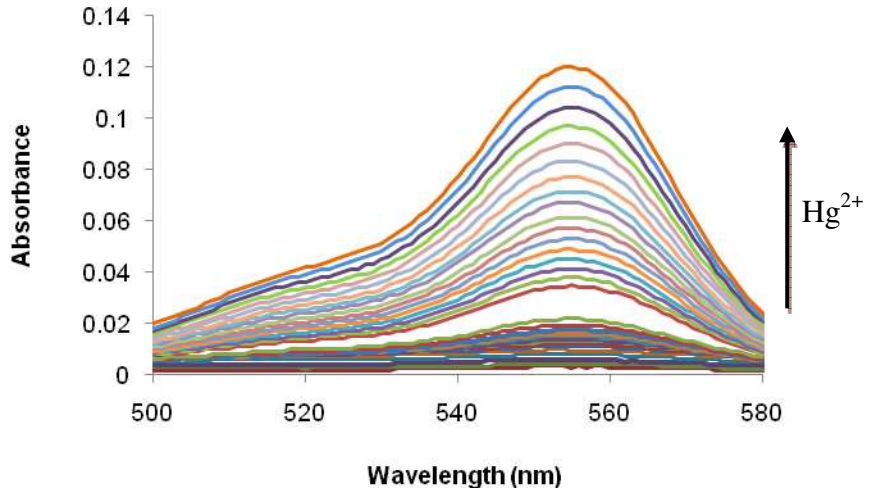


Figure S8. Fluorescence emission (blue) of **1** (5 μM) in the presence of Hg^{2+} ions and ring-opened rhodamine ((5 μM, red) with an excitation at 360 nm in THF:H_2O (95:5, v/v); buffered with HEPES, pH = 7.0; equation used ($I-I_0/I_0$); I_0 = fluorescence intensity of ring-opened rhodamine at 578 nm; I = final fluorescence intensity at 578 nm of receptor **1** after the addition of Hg^{2+} ions; λ_{ex} = 360 nm.

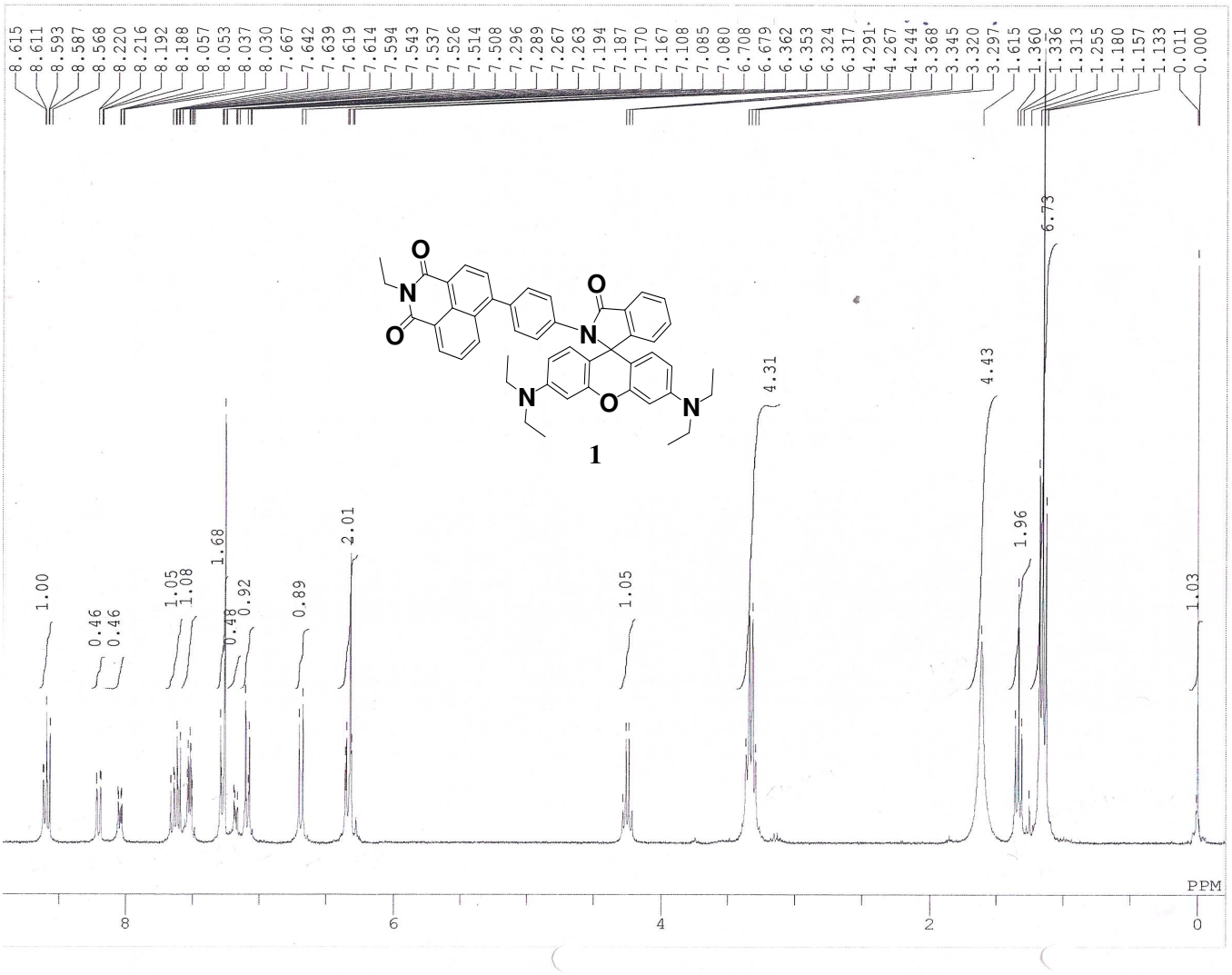


UV-vis spectra of **6** (5 µM) in THF:H_2O (9.5:0.5, v/v) buffered with HEPES, pH = 7.0; in the presence of Hg^{2+} ions (100 equiv).



UV-vis spectra of **7** (5 µM) in THF:H_2O (9.5:0.5, v/v) buffered with HEPES, pH = 7.0; in the presence of Hg^{2+} ions (100 equiv).

^{1}H NMR ($CDCl_3$, 300 MHz, ppm) spectrum of **1**



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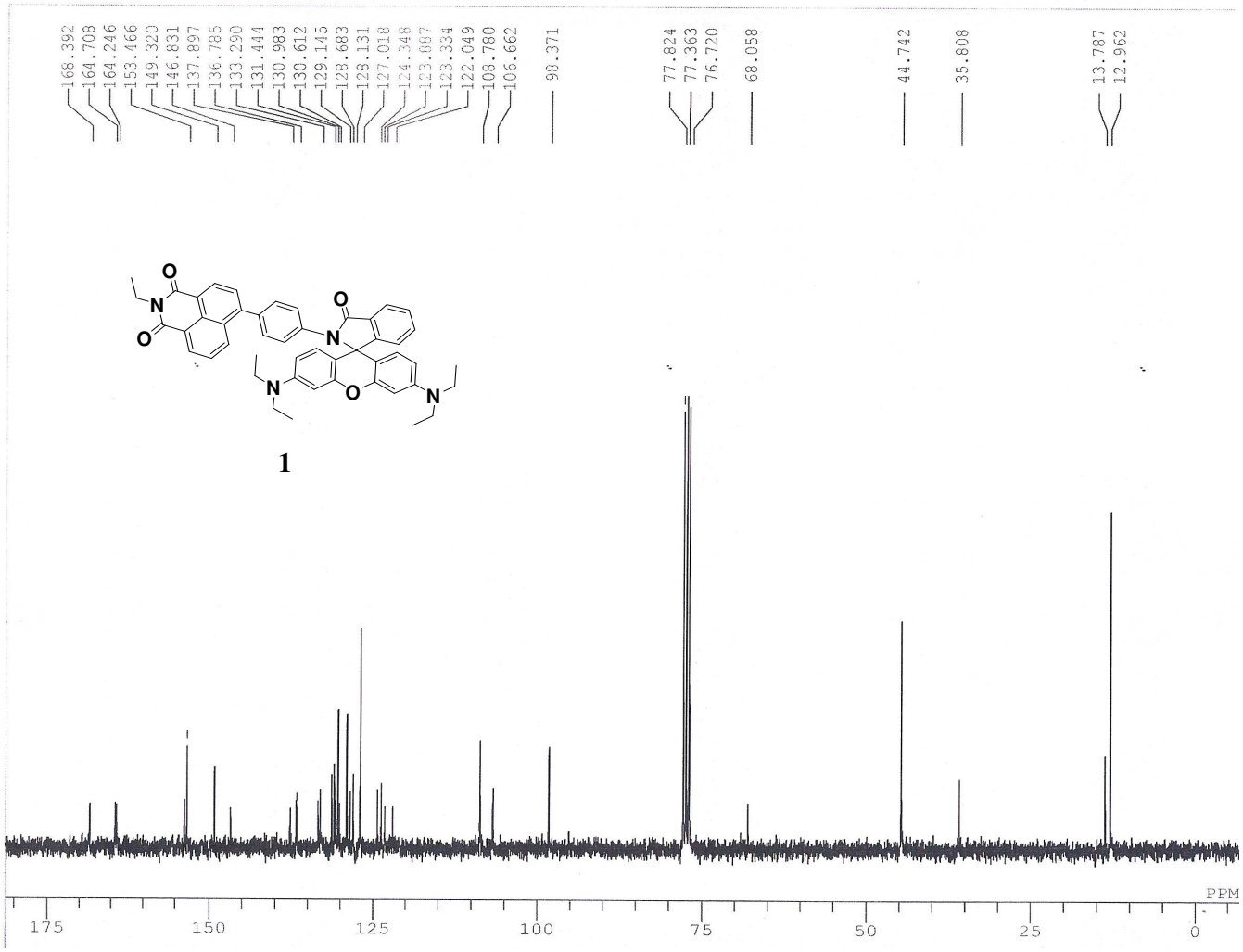
^{13}C NMR ($CDCl_3$, 300 MHz, ppm) spectrum of **1**



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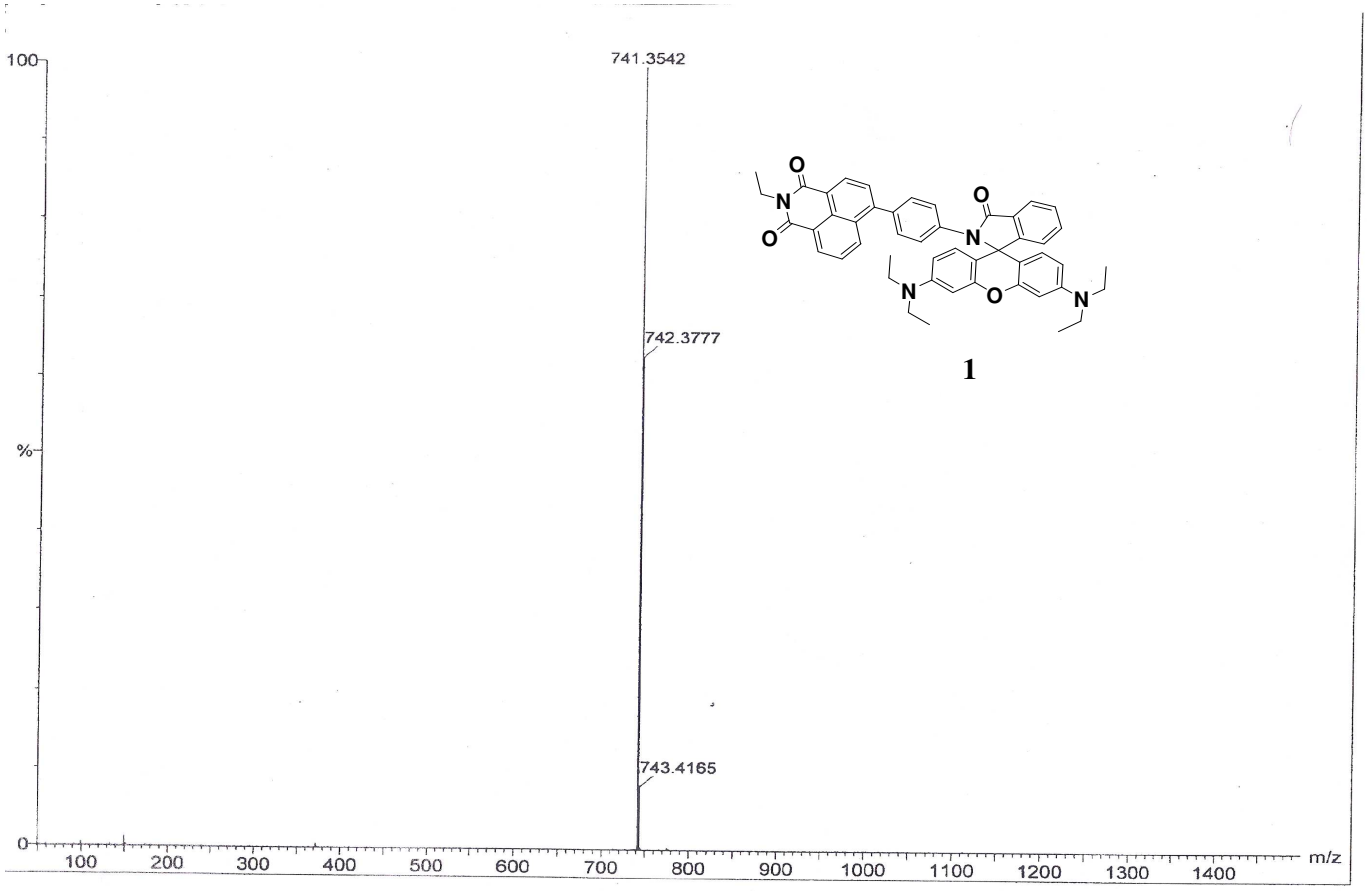
Mass spectrum of **1**



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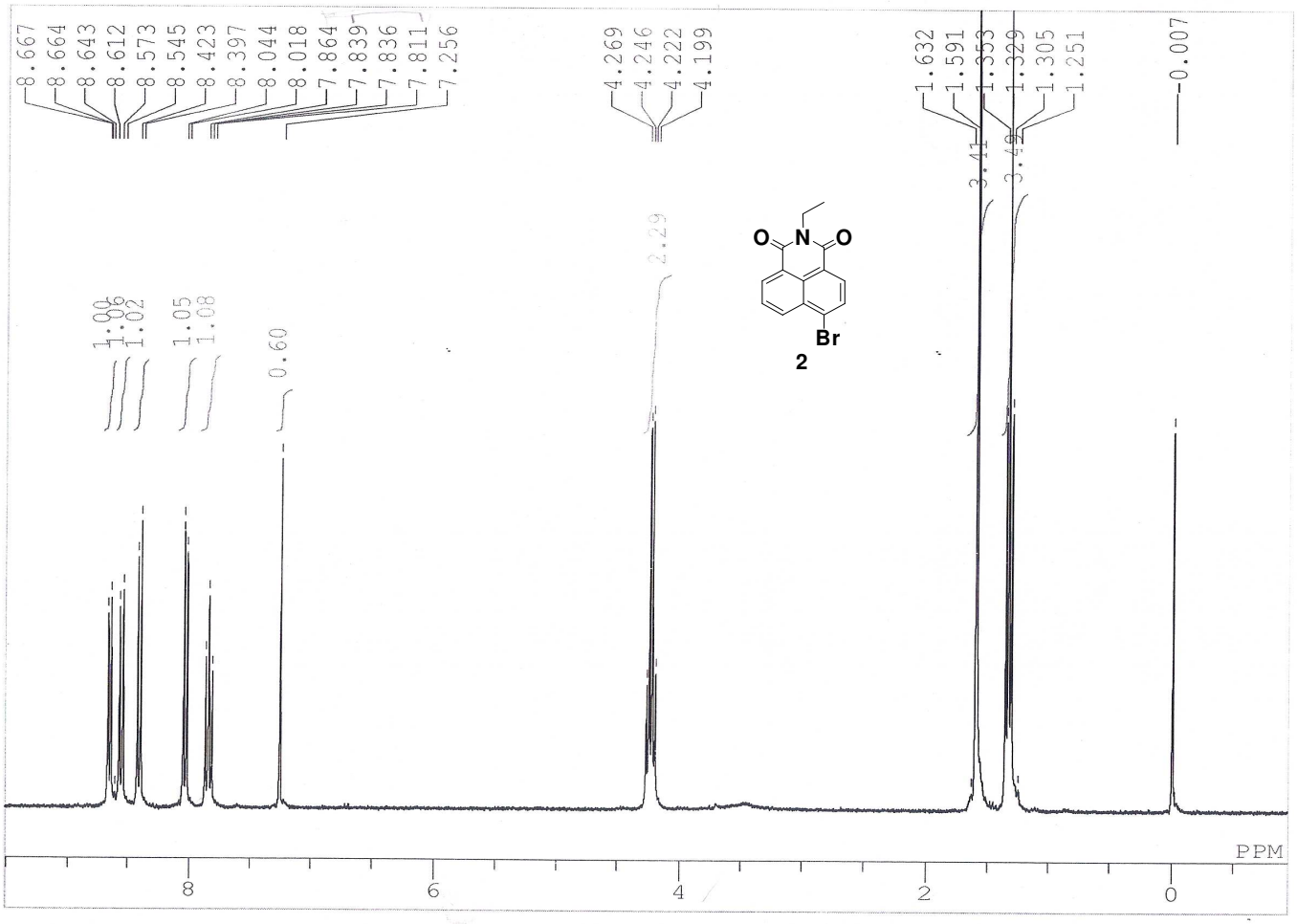
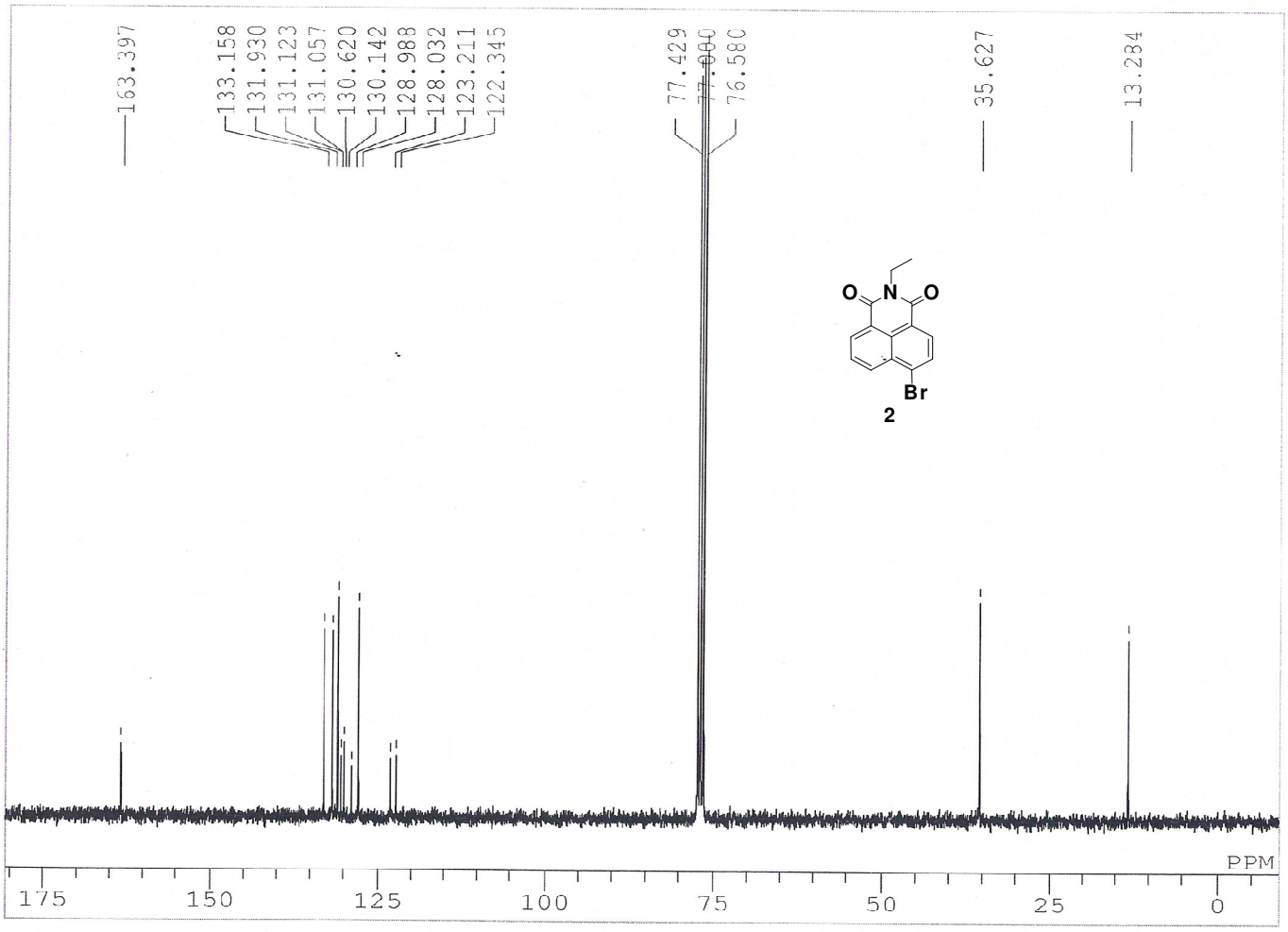
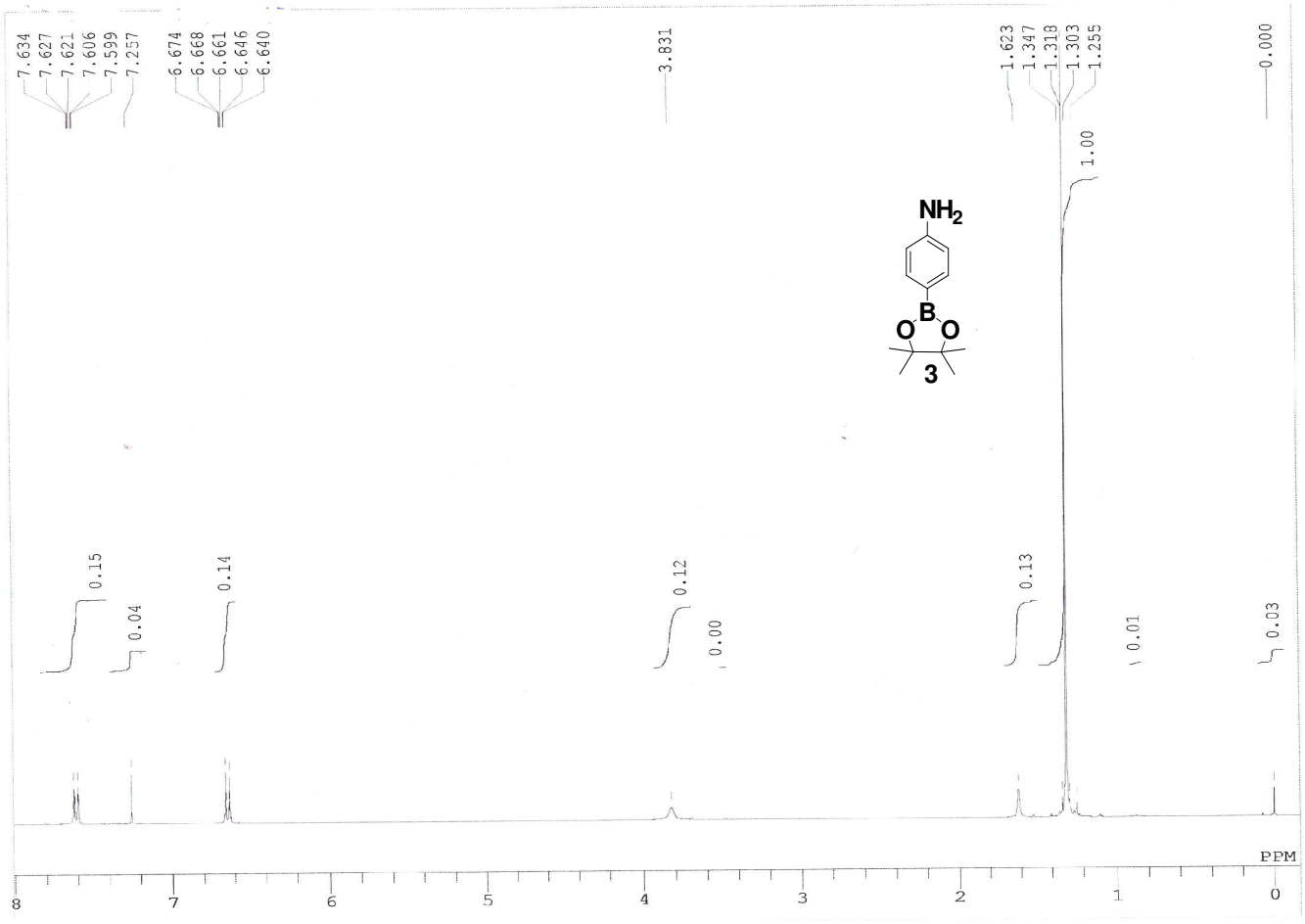
^{1}H NMR ($CDCl_3$, 300 MHz, ppm) spectrum of **2**



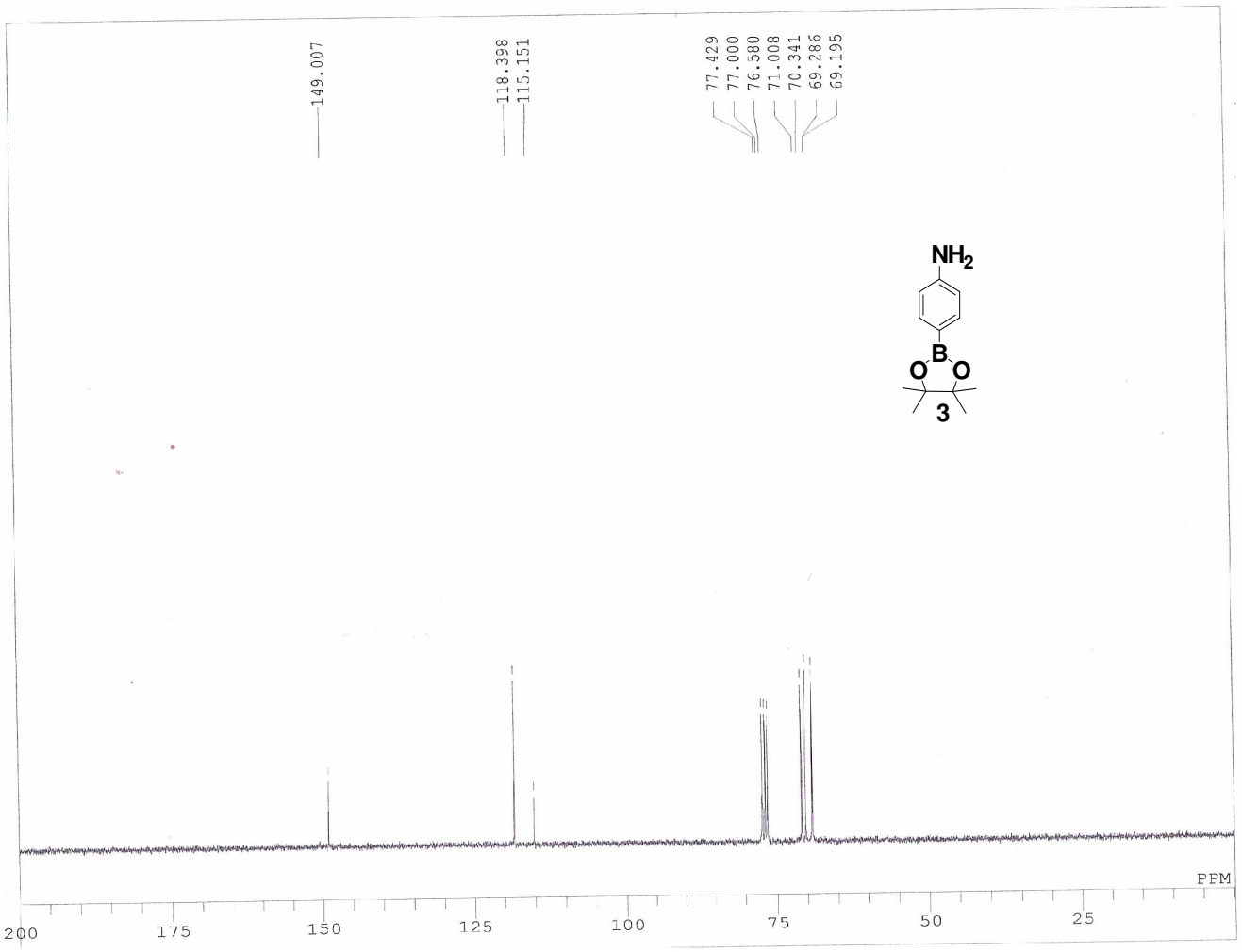
^{13}C NMR ($CDCl_3$, 300 MHz, ppm) spectrum of **2**



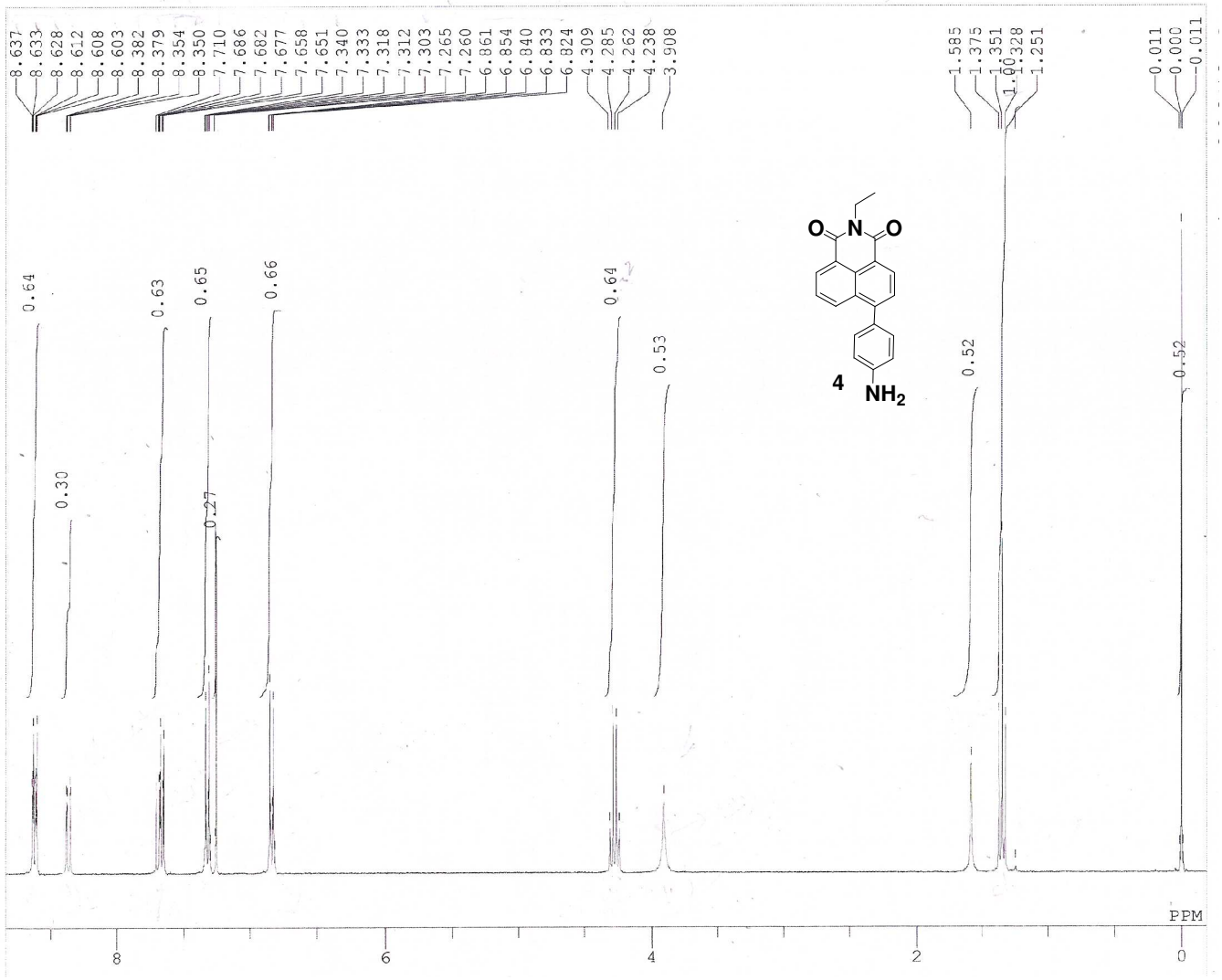
^{1}H NMR ($CDCl_3$, 300 MHz, ppm) spectrum of **3**



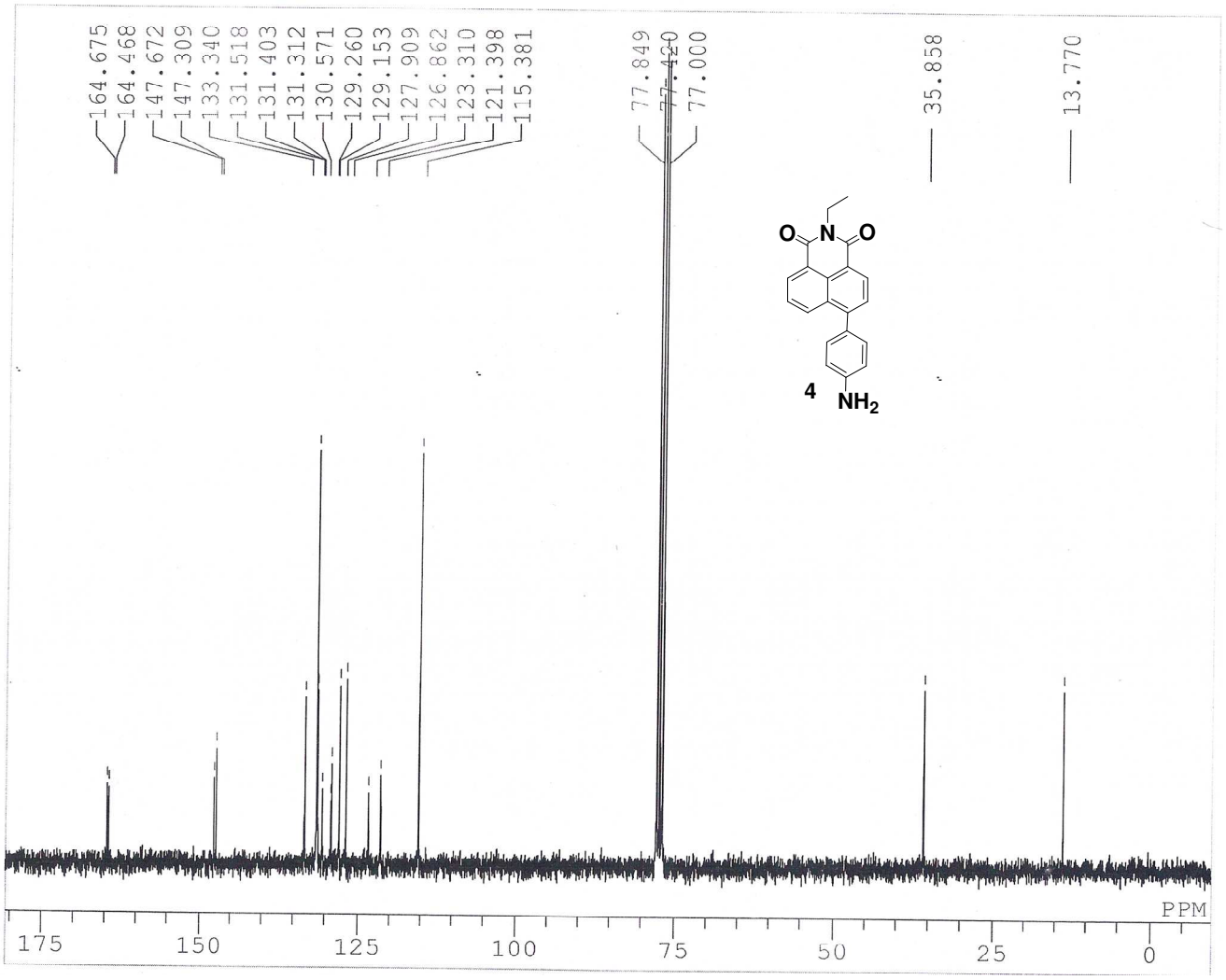
^{13}C NMR ($CDCl_3$, 300 MHz, ppm) spectrum of **3**



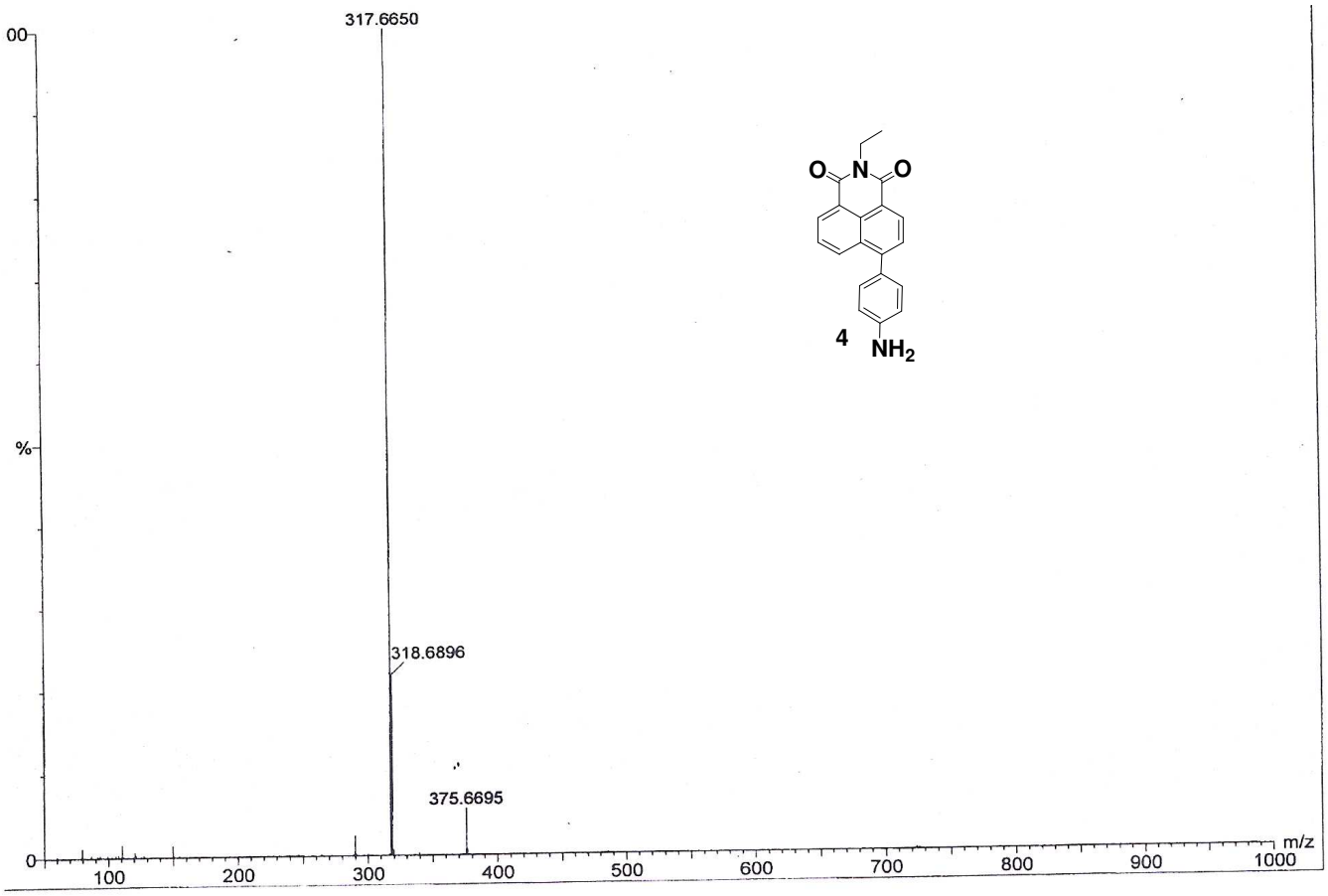
^{1}H NMR ($CDCl_3$, 300 MHz, ppm) spectrum of **4**



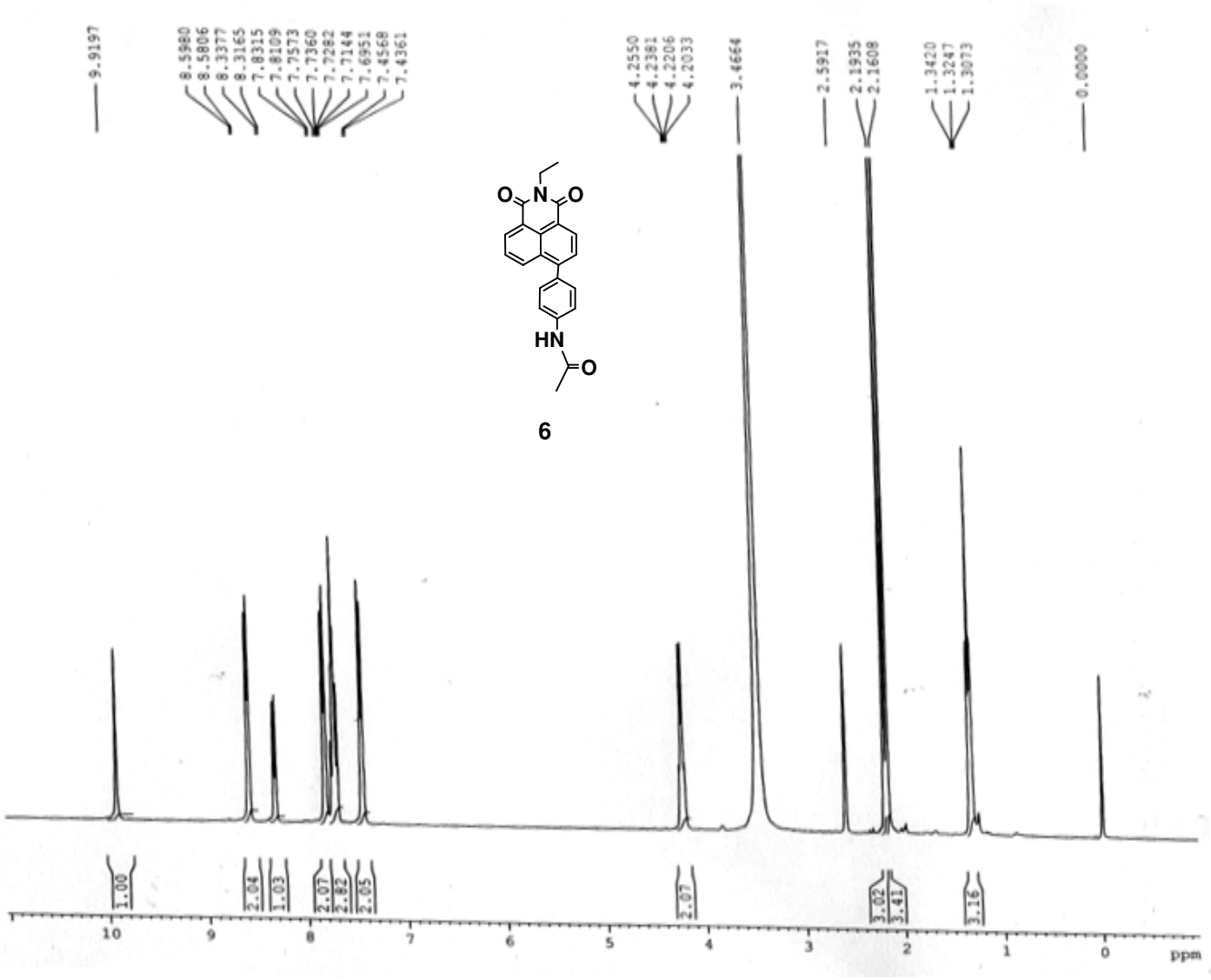
^{13}C NMR ($CDCl_3$, 300 MHz, ppm) spectrum of **4**



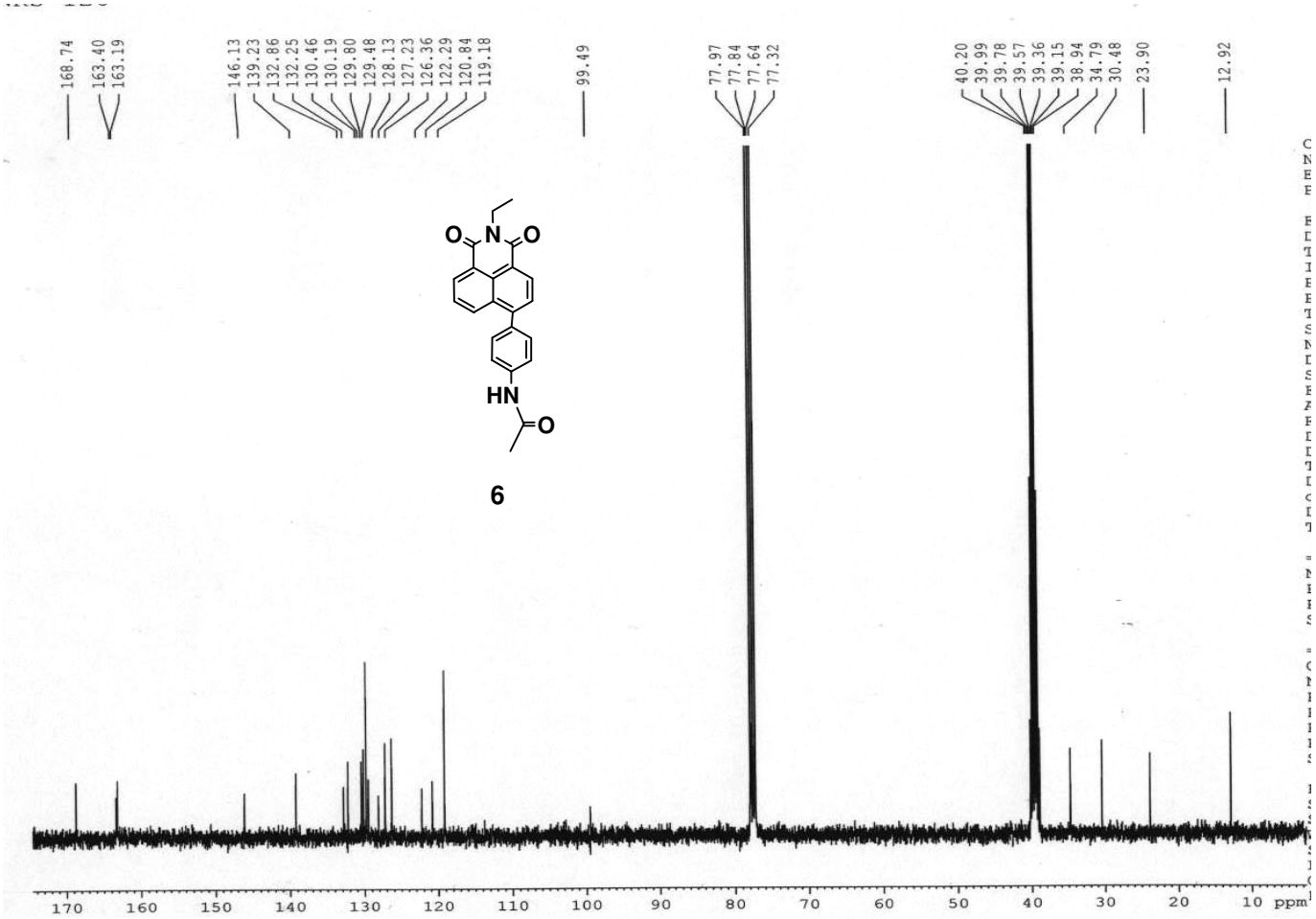
Mass spectrum of **4**



^{1}H NMR (DMSO-d_6, 400 MHz, ppm) spectrum of **6**



^{13}C NMR (DMSO-d_6, 400 MHz, ppm) spectrum of **6**



Mass spectrum of **6**