Supplementary Information

1,3,5-Triphenylbenzene fluorophore as a selective Cu^{2+} sensor in aqueous media

Sakan Sirilaksanapong,[a] Mongkol Sukwattanasinitt,[b] Paitoon Rashatasakhon[b]*

[a]*Program of Petrochemical and Polymer Science, Faculty of Science, Chulalongkorn University, Bangkok, Thailand 10330*

[b]*Organic Synthesis Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand 10330*

paitoon.r@chula.ac.th

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1. General information

All chemicals were reagent grades purchased from Sigma-Aldrich. Organic solvents for reaction work up and chromatography were commercial grades, which were distilled prior to use. Thin layer chromatography (TLC) was performed on aluminum sheets precoated with silica gel (Merck Kiesegel 60 F_{254}) (Merck KgaA, Darmstadt, Germany). Column chromatography was performed on silica gel (Merck Kieselgel 60G) (Merck KGaA, Darmstadt, Germany). All ^{1}H- and ^{13}C-NMR spectra were obtained on a Varian Mercury NMR spectrometer, which operated at 400 MHz for ^{1}H and 100 MHz for ^{13}C nuclei (Varian Company, CA, USA). Elemental (CHN) analyses were performed on PE 2400 seriesII (Perkin-Elmer, USA). Absorption spectra were measured by a Varian Cary 50 UV-Vis spectrophotometer. Fluorescence spectra were obtained from a Varian Cary Eclipse spectrofluorometer.

2. Synthetic procedures

1,3,5-Tris-(4-iodophenyl)benzene(4)

A solution of 4-iodoacetophenone (1.0 g, 4.08 mmol) and CF_3SO_3H (90 μL, 1.00 mmol) in 3 mL of Toluene was stirred under reflux conditions for 3 h. The volatile solvents were removed by evaporation and the residue was purified by column chromatography on silica gel using hexane/CH_2Cl_2 (4:1) as the eluent. The product was further purified by crystallization in EtOH/CH_2Cl_2 (1:1) to afford **4** as white needles (0.65 g, 70 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (d, J = 8.1 Hz, 6H), 7.68 (s, 3H), 7.40 (d, J = 8.1 Hz, 6H). This spectral data agreed with the results reported in the literature [a].

[a] Kotha, S.; Kashinath, D.; Lahiri, K.; Sunoj, B. R. *Eur. J. Org. Chem.* **2004**, 4003-4013.

1,3,5-Tris-(4-ethynyl-phenyl)-benzene) (5)

To a degassed solution of **4** (1.0 g, 1.46 mmol) in THF (50 mL) and Et_3N (50 mL) were added $Pd(PPh_3)_2Cl_2$ (154 mg, 220 μmol), PPh_3 (116 mg, 440 μmol) and CuI (84 mg, 440 μmol). Trimethylsilylacetylene (0.7 mL, 4.8 mmol) was slowly added and the reaction mixture was stirred for 5 hours at room temperature. Then the solution was partitioned between CH_2Cl_2 (300 mL) and 1 M HCl (200 mL). The organic phase was separated, washed with saturated NH_4Cl and dried over Na_2SO_4. After evaporation of CH_2Cl_2, the crude product was dissolved in THF (20 mL) and MeOH (80 mL), then K_2CO_3 (1.4 g, 10.13 mmol) was added. The reaction mixture was stirred for 3 hours. CH_2Cl_2 was added and the organic phase was washed with water, separated, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (hexane/CH_2Cl_2; 10:3) to afford **5** (0.54 g, 85%) as colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.76 (s, 3H), 7.65 (d, J = 8.4 Hz, 6H), 7.61 (d, J = 8.3 Hz, 6H), 3.16 (s, 3H). ^{13}C NMR (200 MHz, $CDCl_3$): δ = 141.7, 141.1, 132.7, 127.2, 125.3, 121.6, 83.4, 78.1. These spectral data agreed with the results reported in the literature [b].

[b] Simpson, C. D.; Mattersteig, G.; Martin, K.; Gherghel, L.; Bauer, R. E.; Rader, H. J.; Mullen, K. *J. Am. Chem. Soc.* **2004**, 126, 3139-3147

Triester 6

To a degassed solution of **4** (100 mg, 0.14 mmol) in THF (10 mL) and Et_3N (10 mL) were added $Pd(PPh_3)_2Cl_2$ (15 mg, 22 μmol), PPh_3 (12 mg, 44 μmol) and CuI (8.4 mg, 44 μmol). A solution of ethyl 4-ethynylbenzoate (100 mg, 0.58 mmol) in THF (2 mL) was added dropwise then stirred overnight. After removal of solvents under reduced pressure, the residue was extracted with H_2O and CH_2Cl_2. The organic phase was washed with brine and dried over Na_2SO_4. The CH_2Cl_2 was removed under reduced pressure and the residue was purified by column chromatography on silica gel using hexane/CH_2Cl_2 (1:4) as the eluent. The triester **6** (93 mg, 78%) was obtained as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (d, J = 8.4 Hz, 6H), 7.82 (s, 3H), 7.70 (dd, J = 8.17 and 20.26 Hz, 12H), 7.62 (d, J = 8.4 Hz, 6H), 4.40 (q, J = 7.1 Hz, 6H), 1.42 (t, J = 7.1 Hz, 9H); ^{13}C NMR (200 MHz, $CDCl_3$): δ = 166.1, 141.7, 141.0, 132.3, 131.5, 129.9, 129.5, 127.8, 127.3, 125.3, 122.2, 92.1, 89.7, 61.2, 14.3. Anal. Calcd. for $C_{57}H_{42}O_6$ (822.94): C 83.19, H 5.14; found C 83.27, H 5.08.

Fluorophore 1

To a solution of **6** (93 mg, 0.69 mmol) in THF (4 mL) and EtOH (4 mL) was added saturated KOH aqueous solution (0.1 mL) and the mixture was refluxed for 6 h. The volatile solvents were evaporated and the residue dissolved in water (5 mL). The solution was cooled with ice and acidified to pH 3 by 1 M HCl. The suspension was centrifuged and the precipitate was vacuum dried to afford **1** as a yellow solid (79 mg, 90%). ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 8.03 (s, 3H), 7.99 (d, J = 8.9 Hz, 12H), 7.72 (t, J = 8.0 Hz, 12H). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 166.7, 140.7, 140.3, 132.1, 131.5, 130.6, 129.6, 127.6, 126.6, 124.8, 121.2, 91.9, 89.5. HRMS (ESI) calcd for $C_{51}H_{30}O_6$ (M-H$^+$) 737.1964; found 737.1991.

Hexaester 7

This compound was prepared from **4** (100 mg, 0.14 mmol) and diethyl 5-ethynylisophthalate (120 mg, 0.51 mmol) using the same procedure described for **6**. After purification by a column chromatography using hexane/CH_2Cl_2 (1:2) as the eluent, hexaester **7** (110 mg, 87%) was obtained as a pale yellow solid. ^{1}H NMR (400 MHz, CDCl$_3$): δ = 8.64 (t, J =1.6 Hz, 3H) 8.40 (d, J = 1.6 Hz, 6H), 7,84 (s, 3H), 7.72 (dd, J = 20.9 Hz, J = 8.5 Hz, 12H), 4.44 (q, J = 7.1 Hz, 12H), 1.44 (t, J = 7.1 Hz, 18H); ^{13}C NMR (100 MHz, CDCl$_3$): δ = 165.2, 141.7, 141.1, 136.4, 132.3, 131.3, 130.1, 127.4, 125.3, 124.2, 122.0, 90.9, 88.5, 61.6, 14.3. These spectral data agreed with the results reported in the literature [c].

[c] Yan, Y.; Telepeni, I.; Yang, S.; Lin, X.; Kockelmann, W.; Dailly, A.; Blake, A. J.; Lewis, W.; Walker, G. S.; Allan, D. R.; Barnett, S. A.; Champness, N. R.; Schröder, M. *J. Am. Chem. Soc.* **2010**, *132*, 4092-4094.

Fluorophore 2

This compound was prepared by hydrolysis of **7** (110 mg) using the method described in the synthesis of **1**. After the suspension was centrifuged and the precipitate was vacuum dried, **2** was obtained as a brown solid (95 mg, 96%). ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 8.45 (t, J = 1.5 Hz, 3H), 8.28 (d, J = 1.5 Hz, 6H), 8.06 (s, 3H), 8.04 (d, J = 8.4 Hz, 6H), 7.77 (d, J = 8.3 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d$_6$): δ = 166.0, 140.7, 140.3, 135.3, 132.7, 132.2, 129.2, 127.6, 124.8, 123.1, 121.1, 90.7, 88.5. These spectral data agreed with the results reported in the literature [c] above.

Trisalicylate ester 8

A mixture of ethyl 5-iodosalicylate (0.25 g, 0.872 mmol) and THF/*i*Pr_2NH (3mL/1 mL) was degassed with N_2 gas. $Pd(PPh_3)_2Cl_2$ (5.5 mg, 2 mol%) and CuI (3 mg, 6 mol%) were added and the mixture stirred at 25 °C for 10 min. A solution of **5** (0.1 g, 0.26 mmol) in anhydrous THF (1 mL) was then added dropwise and mixture stirred 3.5 h at 50 °C. Upon completion, volatile materials were removed under reduced pressure, and the residue added to water (10 mL), which was extracted with CH_2Cl_2. The organic phase was washed with water and then brine, and dried over $NaSO_4$. The CH_2Cl_2 was removed under reduced pressure and the resulting solid purified by column chromatography on silica gel using CH_2Cl_2/hexane (1:1) to afford the salicylate ester **8** (0.168 g, 73%) as a pale grey solid. ^1H NMR (400 MHz, $CDCl_3$): δ 11.02 (s, 3H), 8.08 (s, 3H), 7.81 (s, 3H), 7.67 (dd, J = 26.66, 7.48 Hz, 15H), 6.99 (d, J = 7.84 Hz, 3H), 4.45 (q, J = 6.63 Hz, 6H), 1.46 (t, J = 6.91 Hz, 9H) ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.7, 141.8, 140.5, 138.5, 133.4, 132.0, 127.3, 125.1, 122.7, 118.0, 115.0, 114.2, 112.8, 89.4, 88.0, 61.8, 14.2. Anal. Calcd. for $C_{57}H_{42}O_9$ (870.94): C 78.61, H 4.86; found C 78.82, H 4.81.

Fluorophore 3

This compound was prepared by hydrolysis of **9** (110 mg) under the method described above as synthesize compound **1**. After the suspension was centrifuged and the precipitate was vacuum dried, **3** was obtained as a off-white solid (100 mg, 98%). ^1H NMR (400 MHz, DMSO-d_6) δ 8.00 (dd, J = 13.89, 5.59 Hz, 12H), 7.69 (t, J = 8.49 Hz, 9H), 7.02 (d, J = 8.44 Hz, 3H), 2.08 (s, 3H) ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.9, 161.2, 140.7, 139.7, 137.9, 133.5, 131.7, 127.4, 124.5, 121.8, 117.9, 113.9, 112.9, 89.4, 87.99. Anal. Calcd. for $C_{51}H_{30}O_9$ (786.78): C 77.85, H 3.84; found C 77.58, H 3.82.

3. Photophysical data

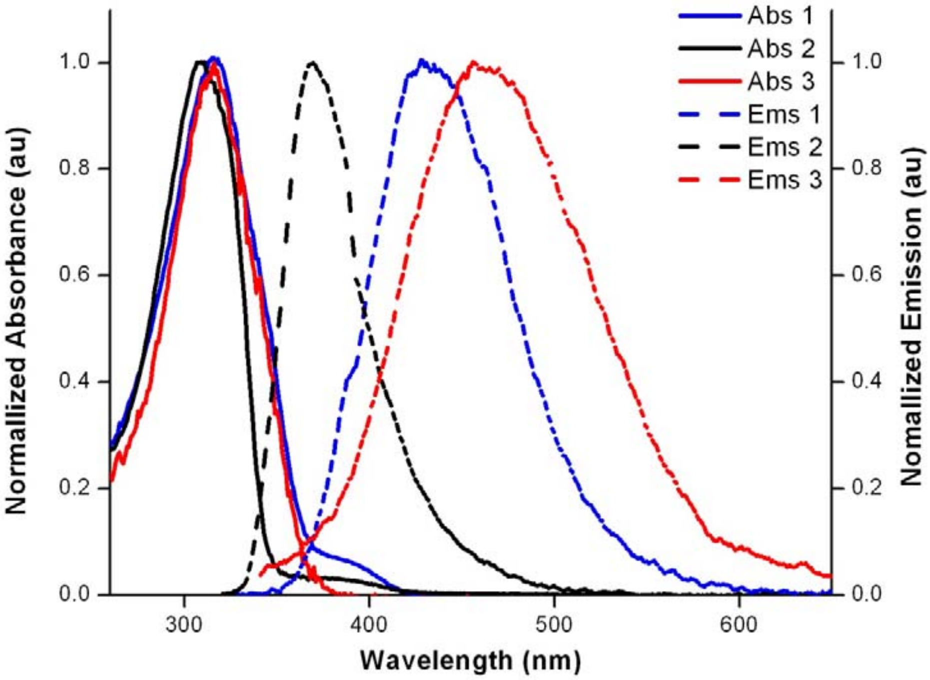


Fig. S1 Normalized absorption and emission spectra of compound **1**, **2** and **3** in 10 mM phosphate buffer pH 8

Metal ion sensing experiments

Stock solutions of **1**, **2** and **3** (10 mM) in phosphate buffer pH 8.0 and stock solution of LiCl, NaOAc, KCl, MgCl, $Ca(OAc)_2$, $Sr(NO_3)_2$, $Ba(NO_3)_2$, $Mn(OAc)_2$, $FeSO_4$, $Fe(NO_3)_3$, $Co(OAc)_2$, $Ni(OAc)_2$, $Cu(OAc)_2$, $Zn(OAc)_2$, $ZrSO_4$, AgOAc, $CdSO_4$, $Hg(OAc)_2$, and $Pb(OAc)_2$ (0.1 mM) in Milli-Q water were prepared. Then, the emission spectrum of **1-3** (diluted to 1 μM using phosphate buffer) was recorded from 300 nm to 700 nm at a controlled temperature using an excitation wavelength at 318, 310, 316 nm, respectively. The fluorescent intensities at the maximum emission wavelengths were used as the control values. For the sensing experiments, solutions containing each fluorophore (1 μM) and each metal ion (10 μM) were prepared by dilution of the stock solutions with phosphate buffer to a final volume of 5 mL. After settling for 3-5 minutes, the fluorescent spectra of each mixed solutions were acquired.

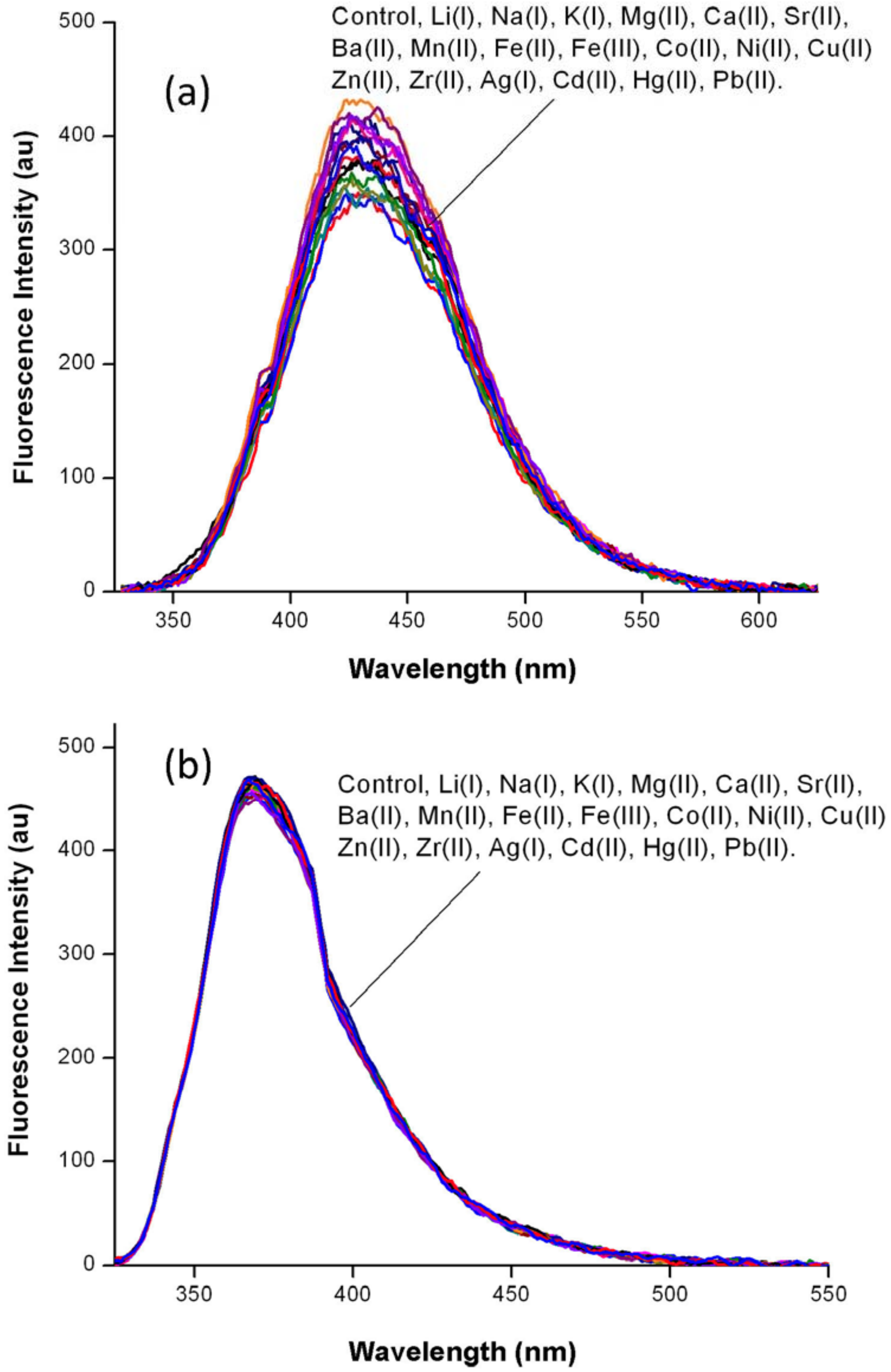


Fig. S2 Fluorescence spectra of (a) **1** (1 μM) and (b) **2** (1 μM) in the presence of various metal ion (10 μM)

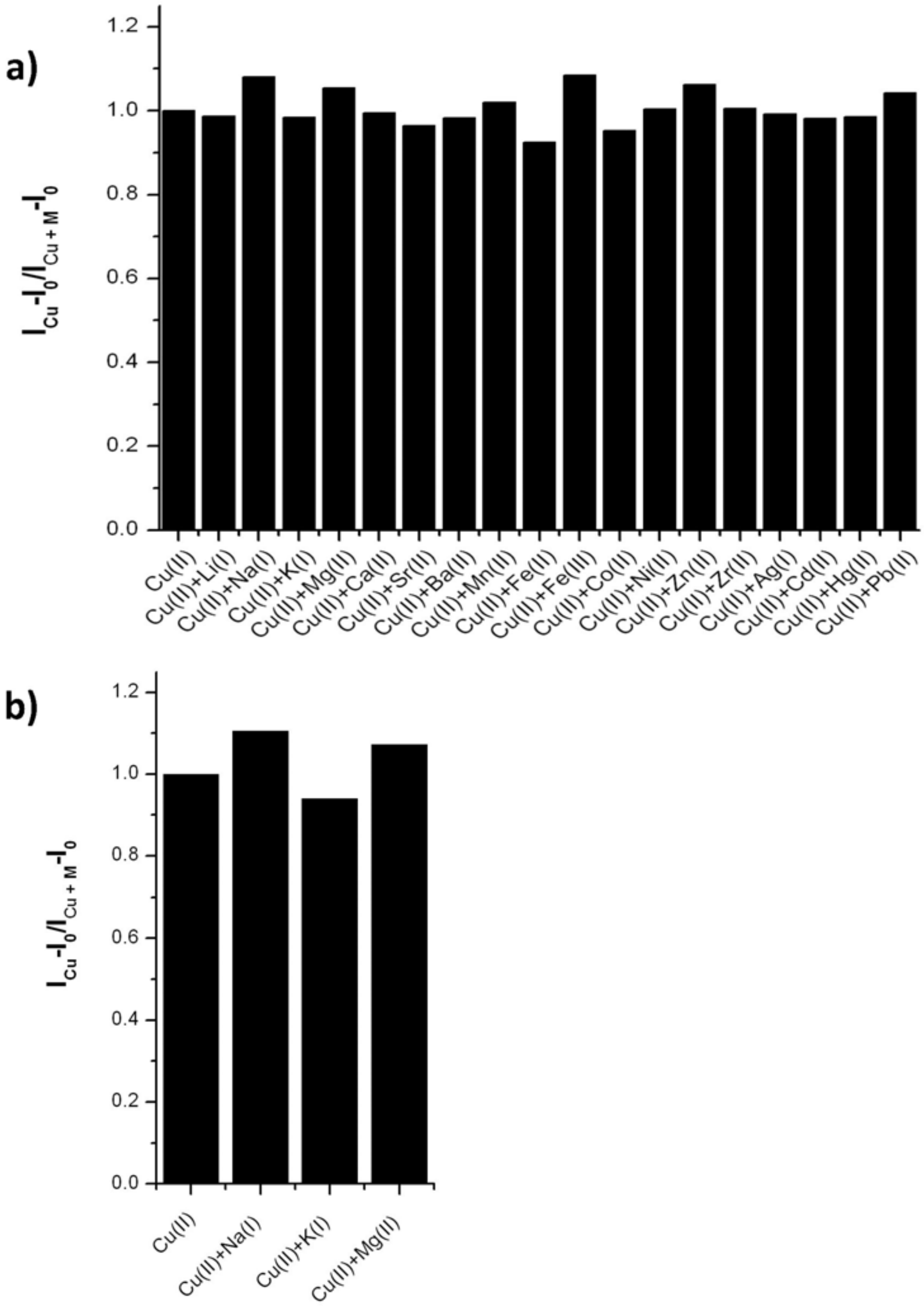


Fig. S3 Fluorogenic response of **3** (1μM) in the presence of Cu^{2+} (1μM) with a) interfering metal ions (10 μM) and b) interfering metal ions (1 mM) in phosphate buffer

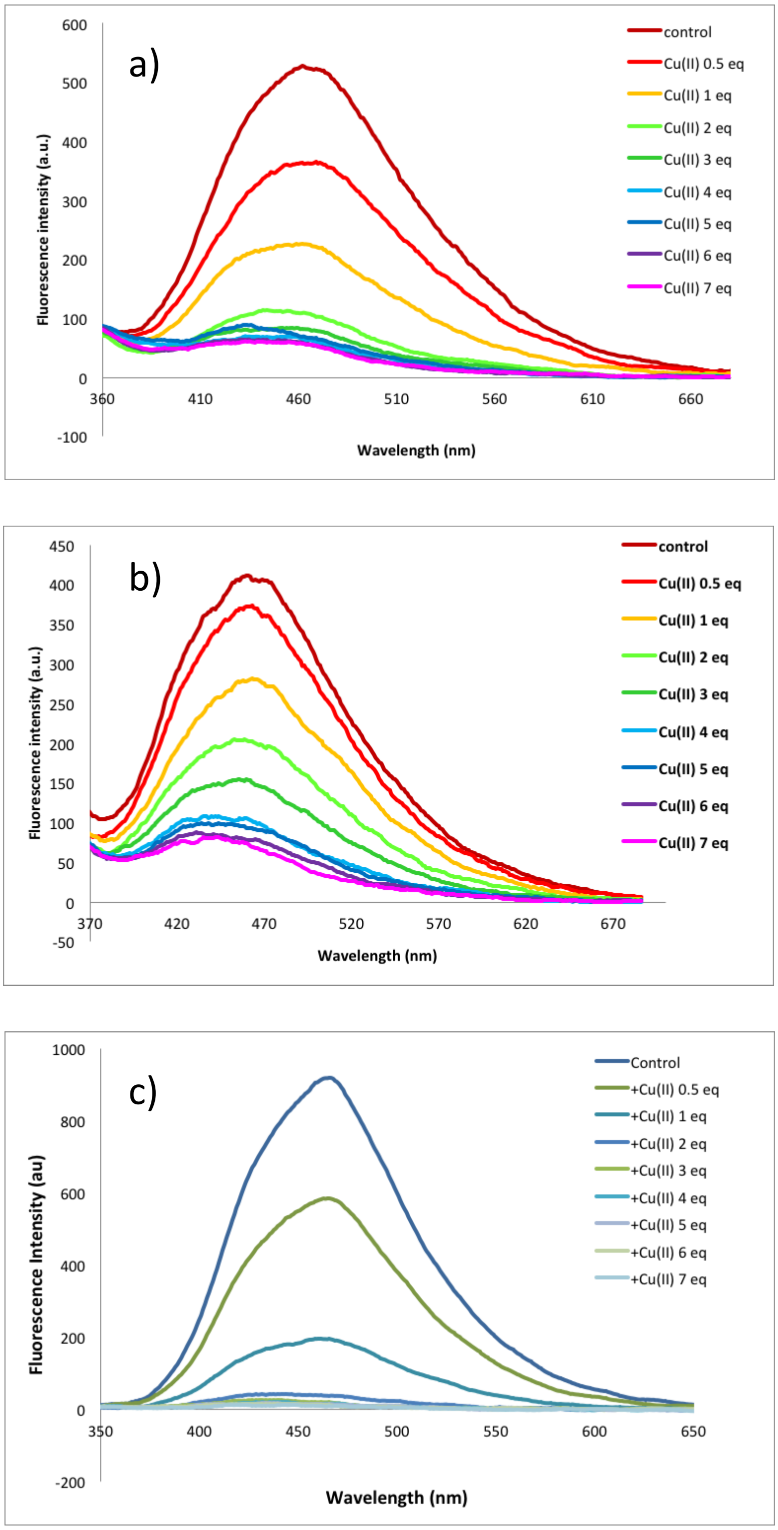


Fig. S4 Fluorescence spectra of **3** (1 μM) in the presence of various concentration of Cu(II); a) at 30 °C, b) at 50 °C, and c) at 30 °C with Triton X-100 (30 μM)

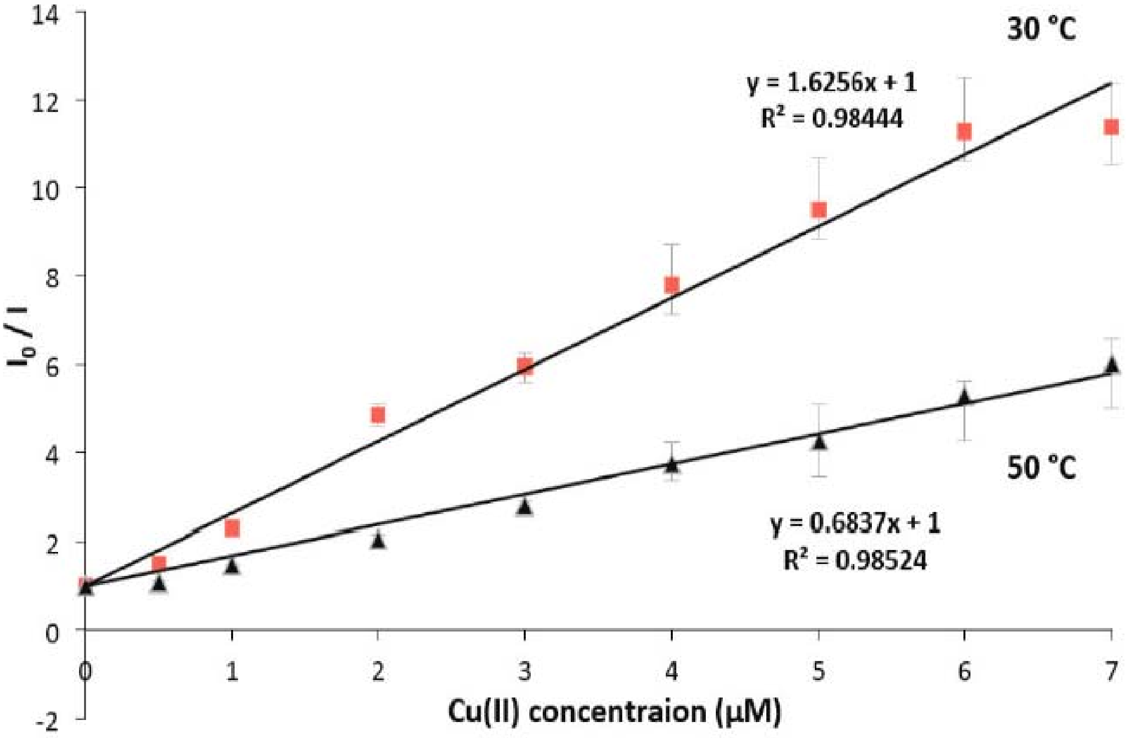


Fig. S5 Stern-Volmer plots for the fluorescence quenching of **3** by Cu^{2+} at 30 and 50 °C

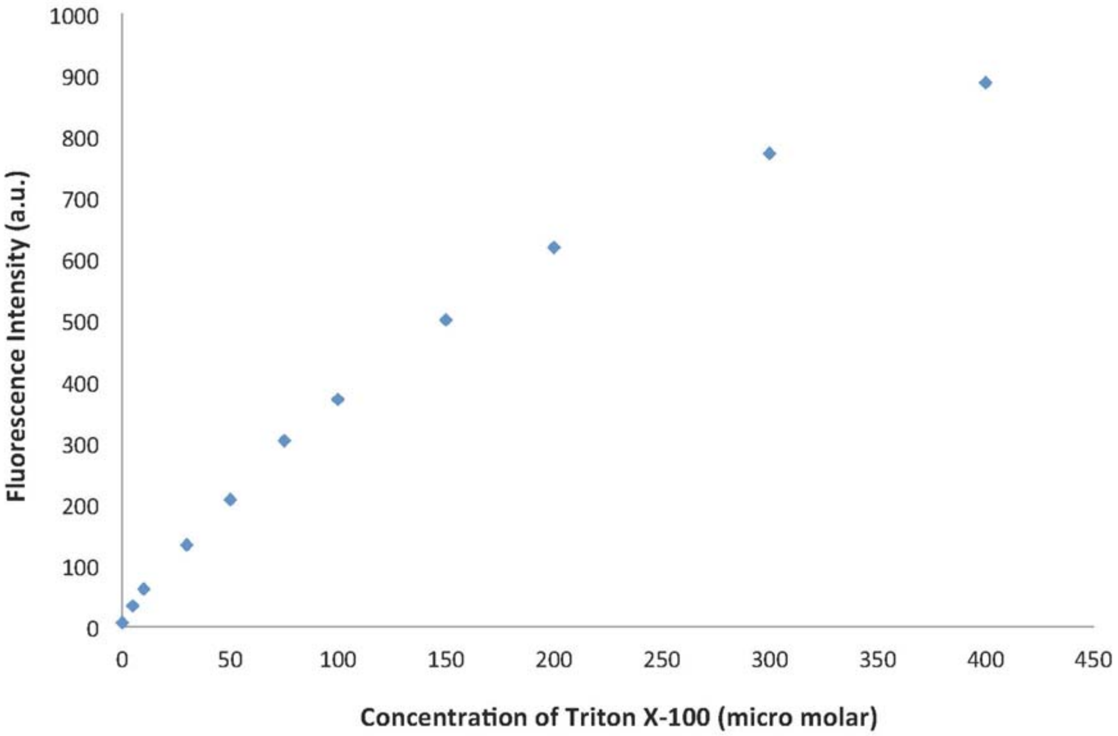


Fig. S6 Fluorescent intensity of **3** (1 μM) in various concentration of Triton X-100.

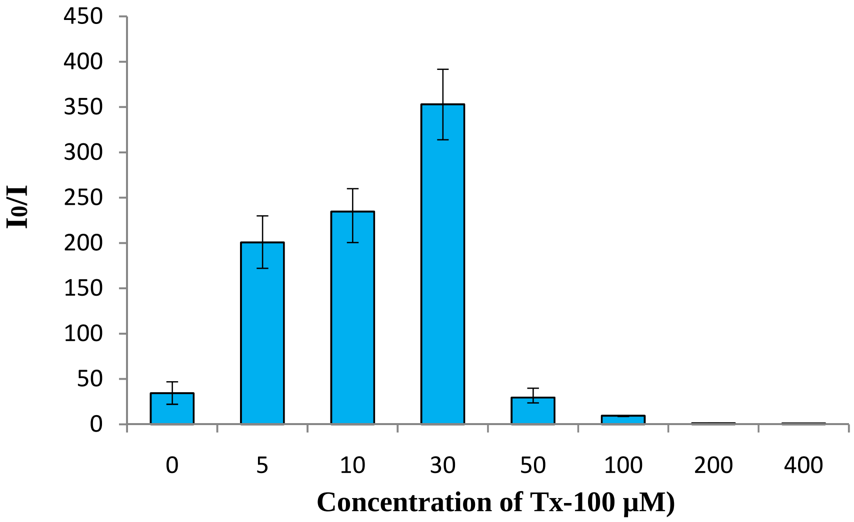


Fig. S7 Quenching efficiencies of **3** (1 μM) with Cu(II) (10 μM) under various concentrations of Triton X-100 (0-400 μM).

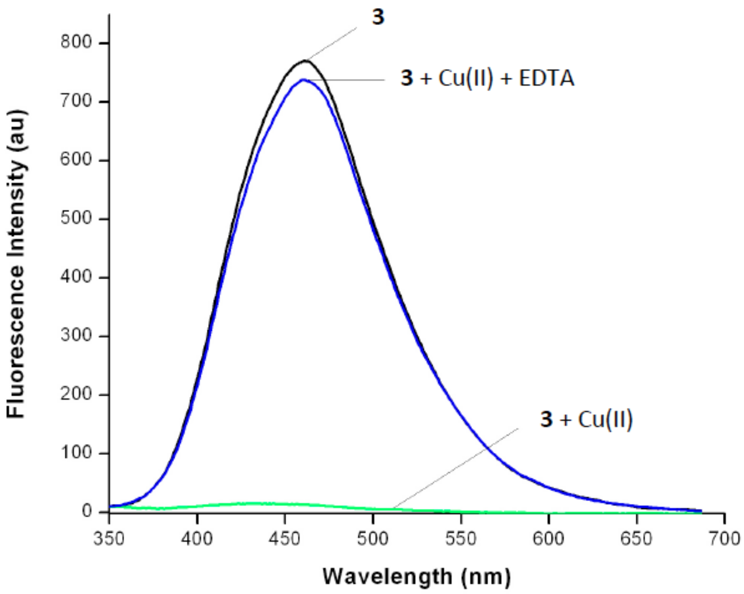
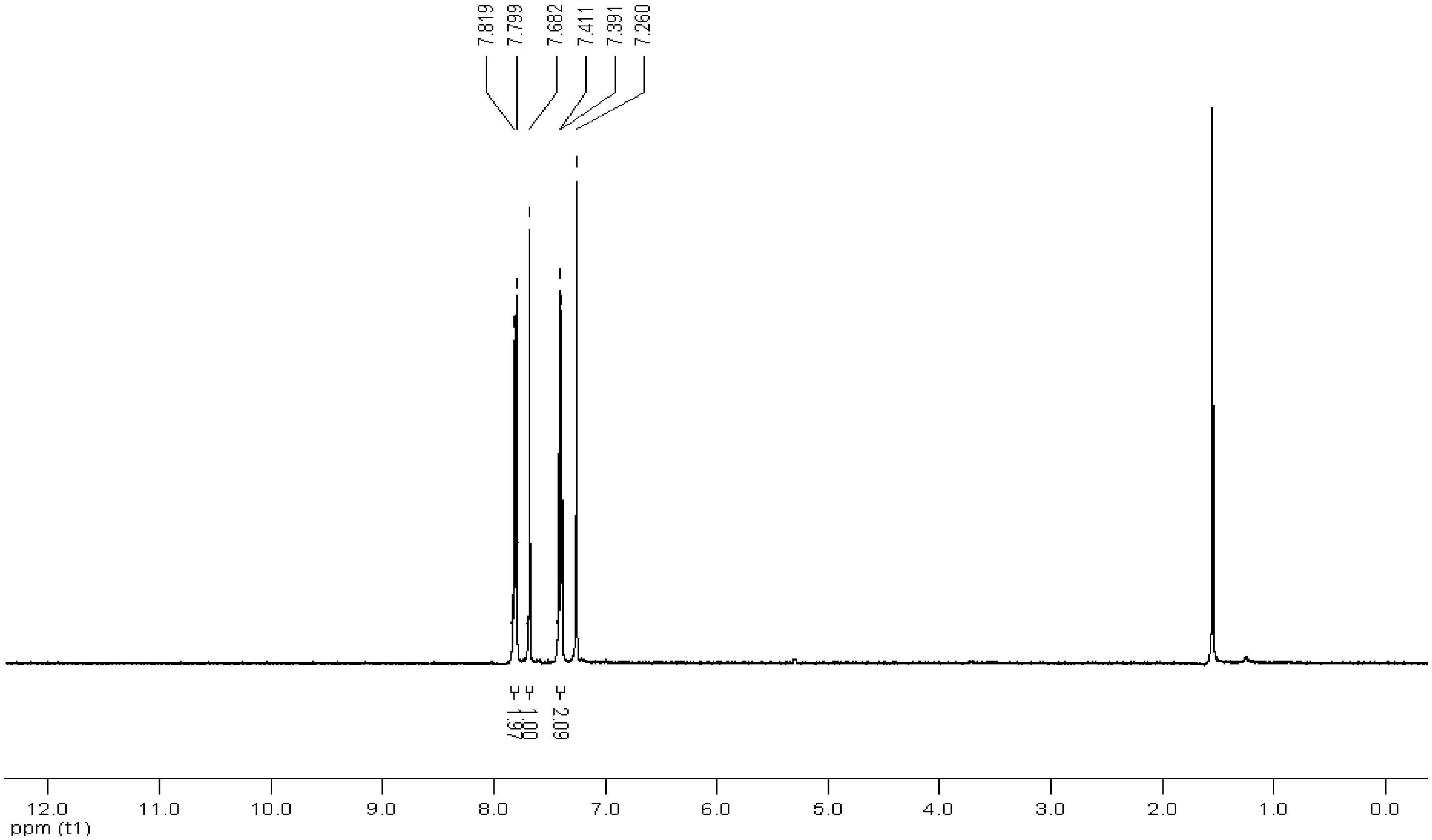


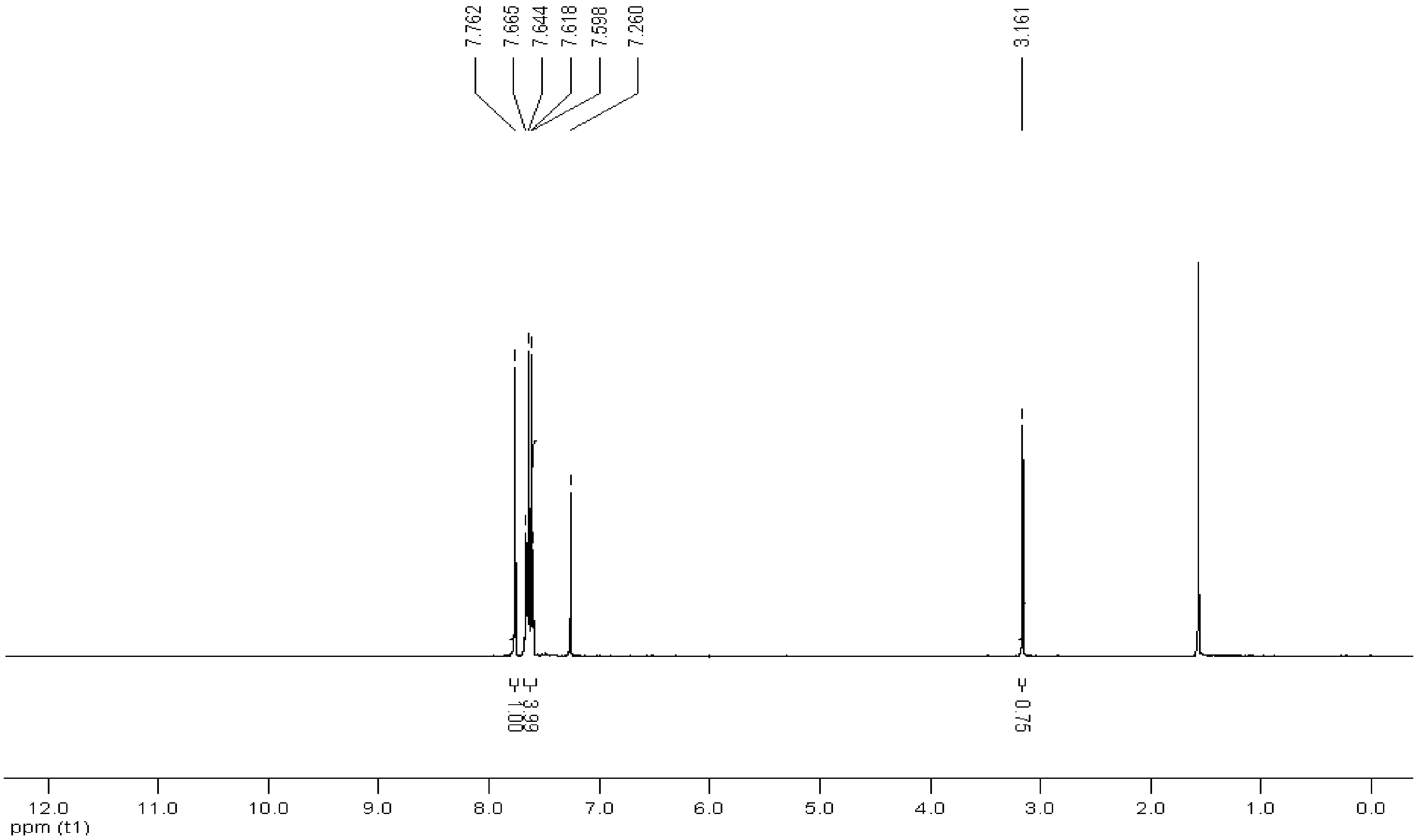
Fig. S8 Fluorescent signal quenching by Cu(II) and signal restoration by EDTA. [**3** (1 μM), Cu(II) (10 μM), Triton X-100 (30 μM), EDTA (20 μM)]

4) Spectroscopic data (^{1}H and ^{13}C NMR)

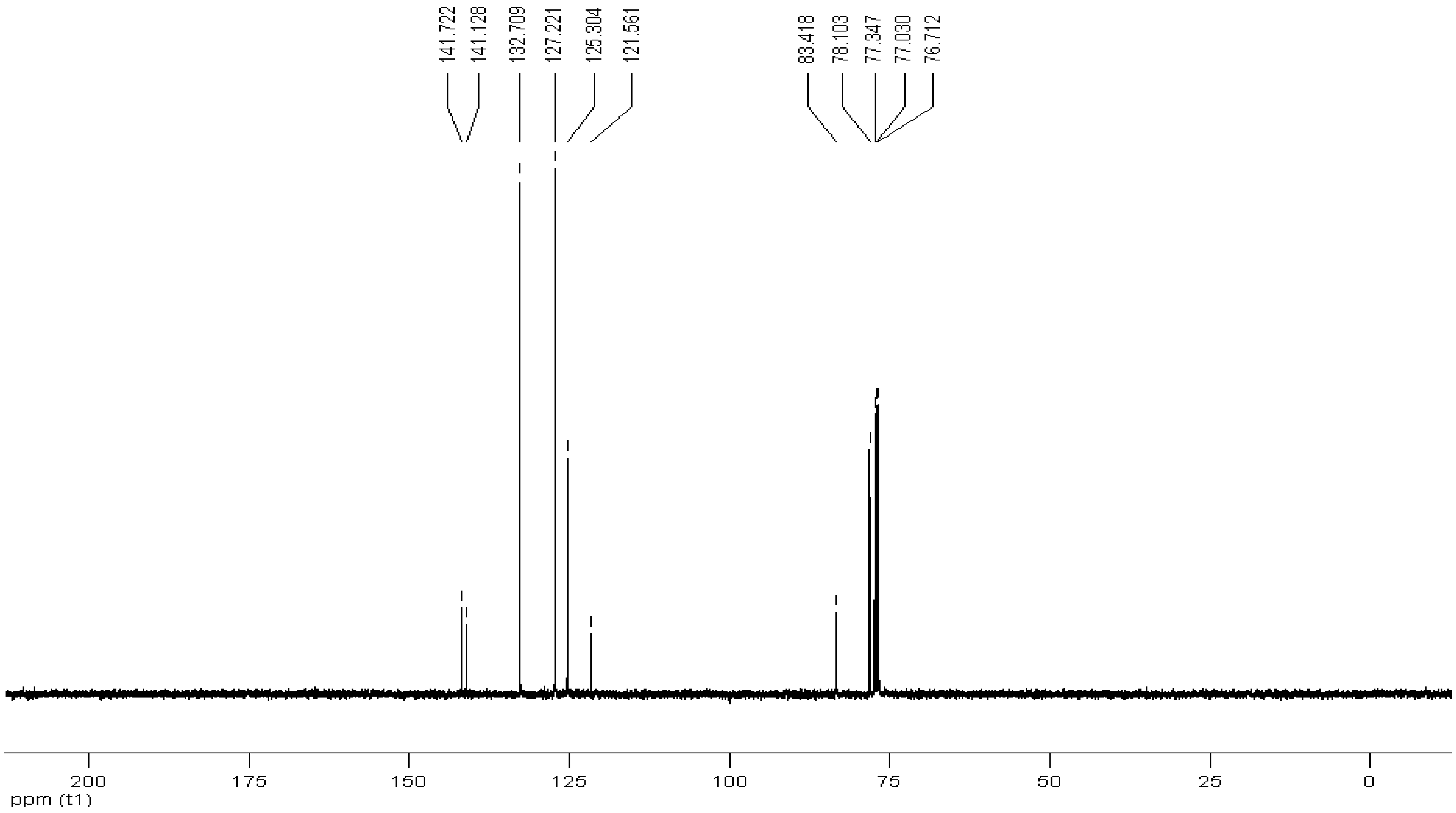
^{1}H NMR of 1,3,5-Tris-(4-iodophenyl)benzene(4)



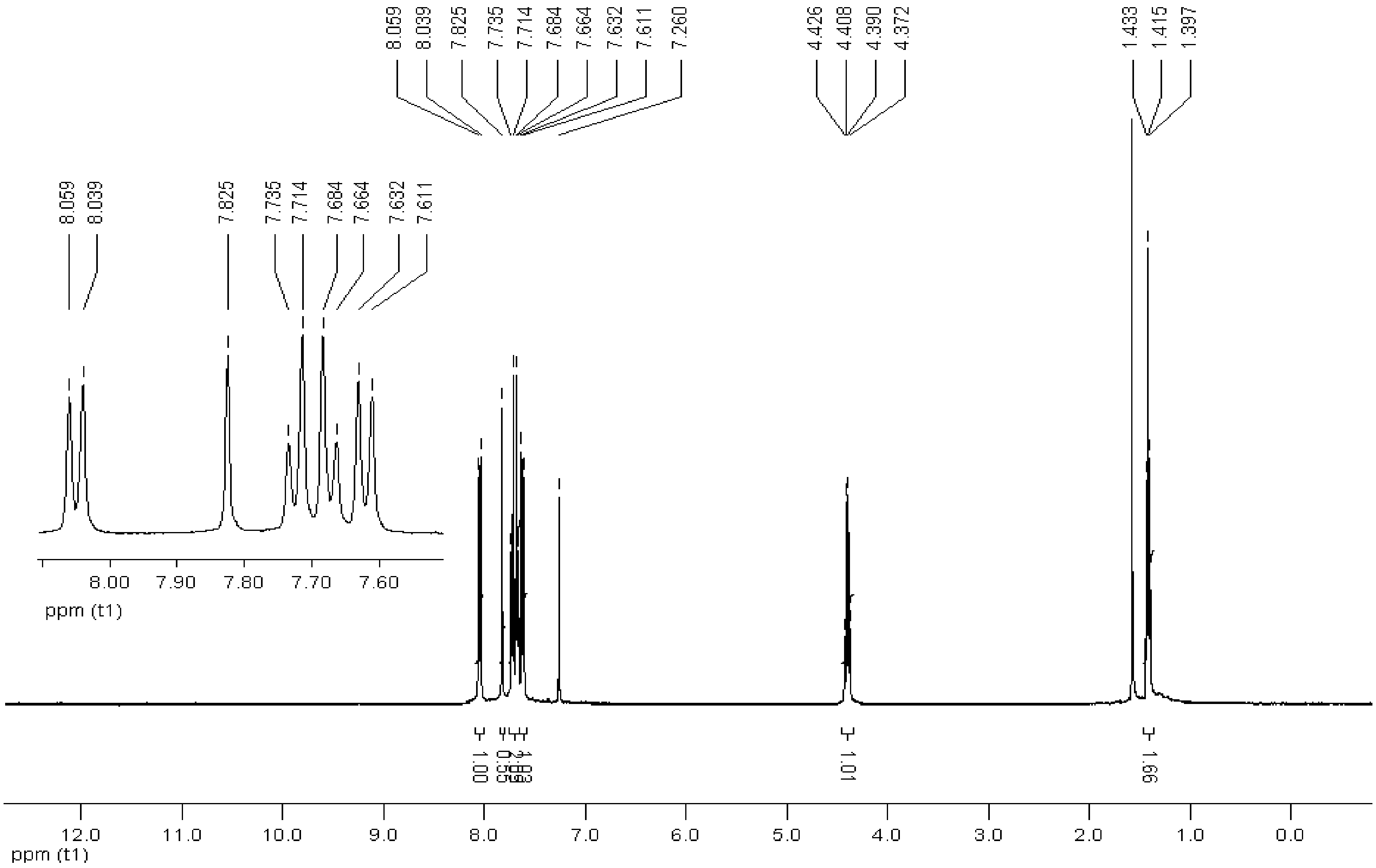
^{1}H NMR of *1,3,5-Tris-(4-ethynyl-phenyl)-benzene)* (5)



^{13}C NMR of *1,3,5-Tris-(4-ethynyl-phenyl)-benzene)* (5)



^{1}H NMR of Triester 6



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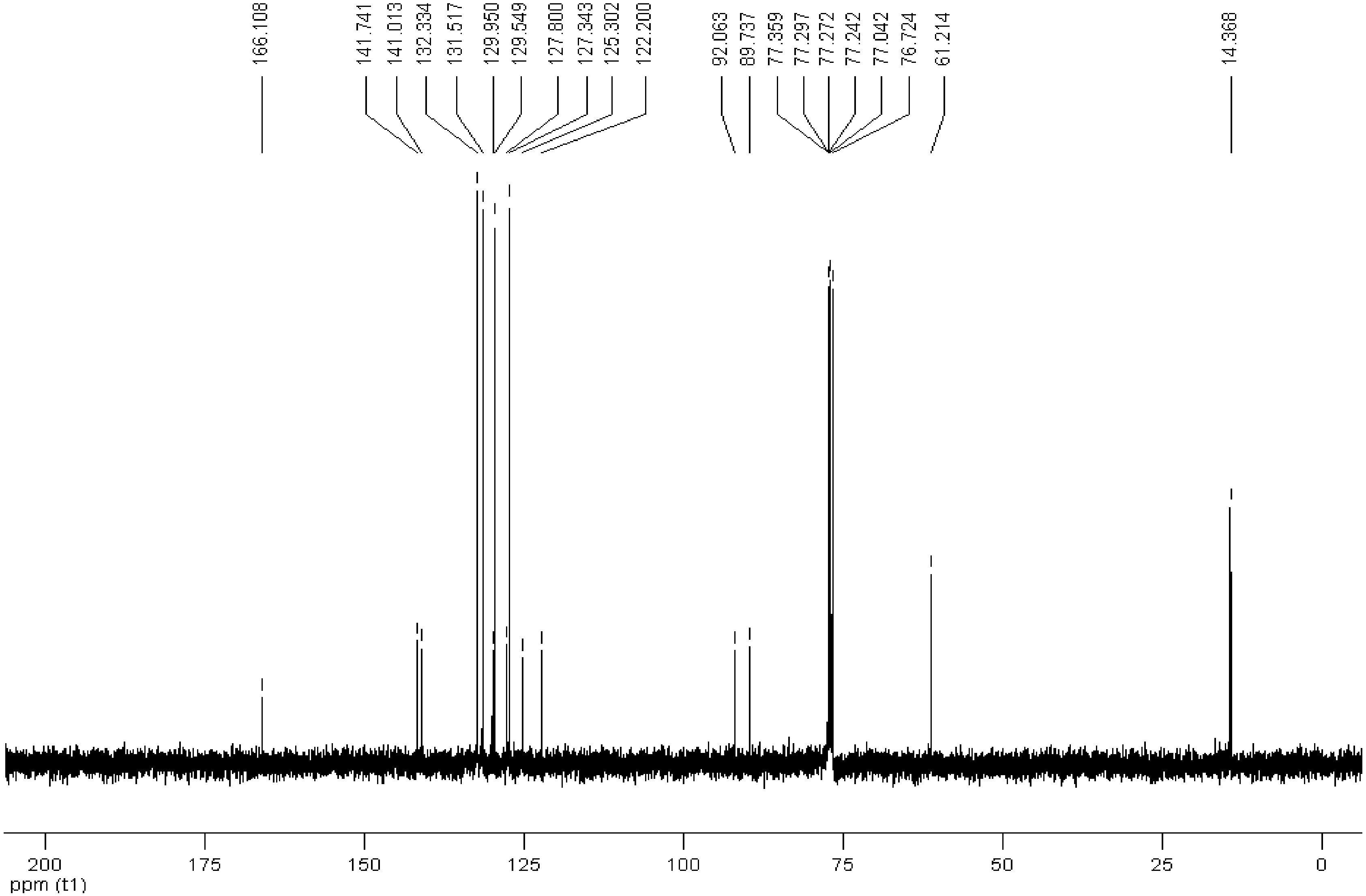
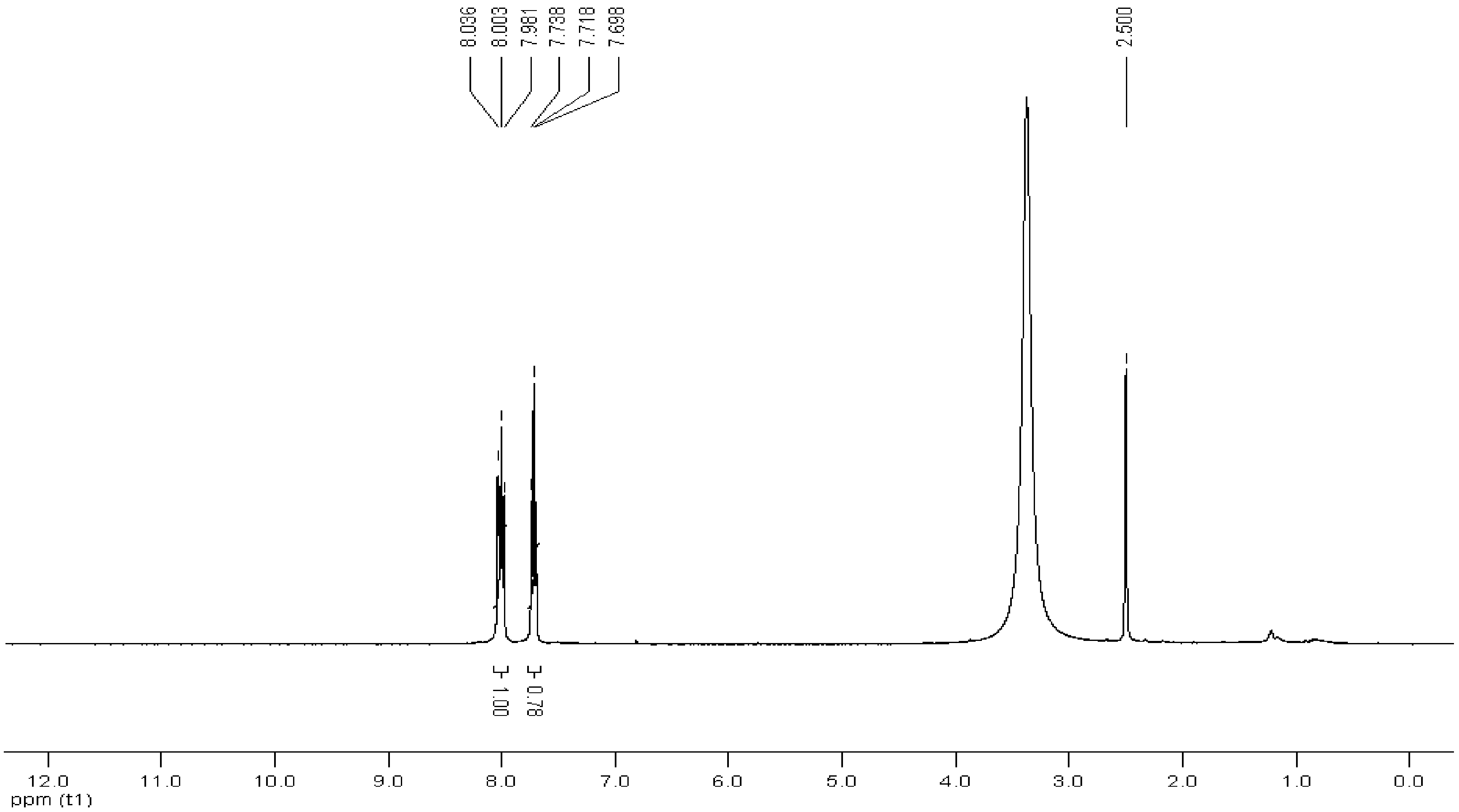


^{1}H NMR of Fluorophore 1



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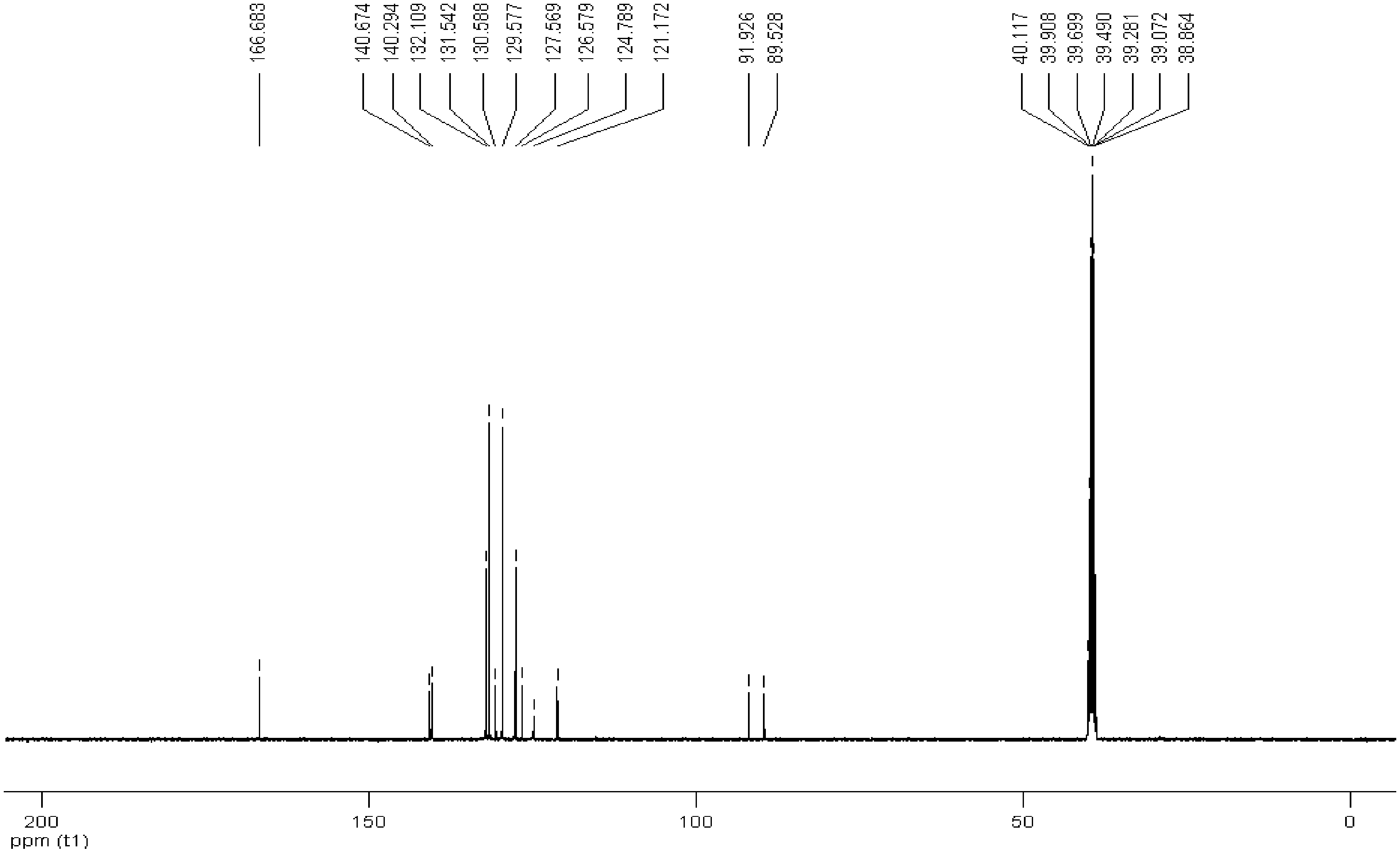
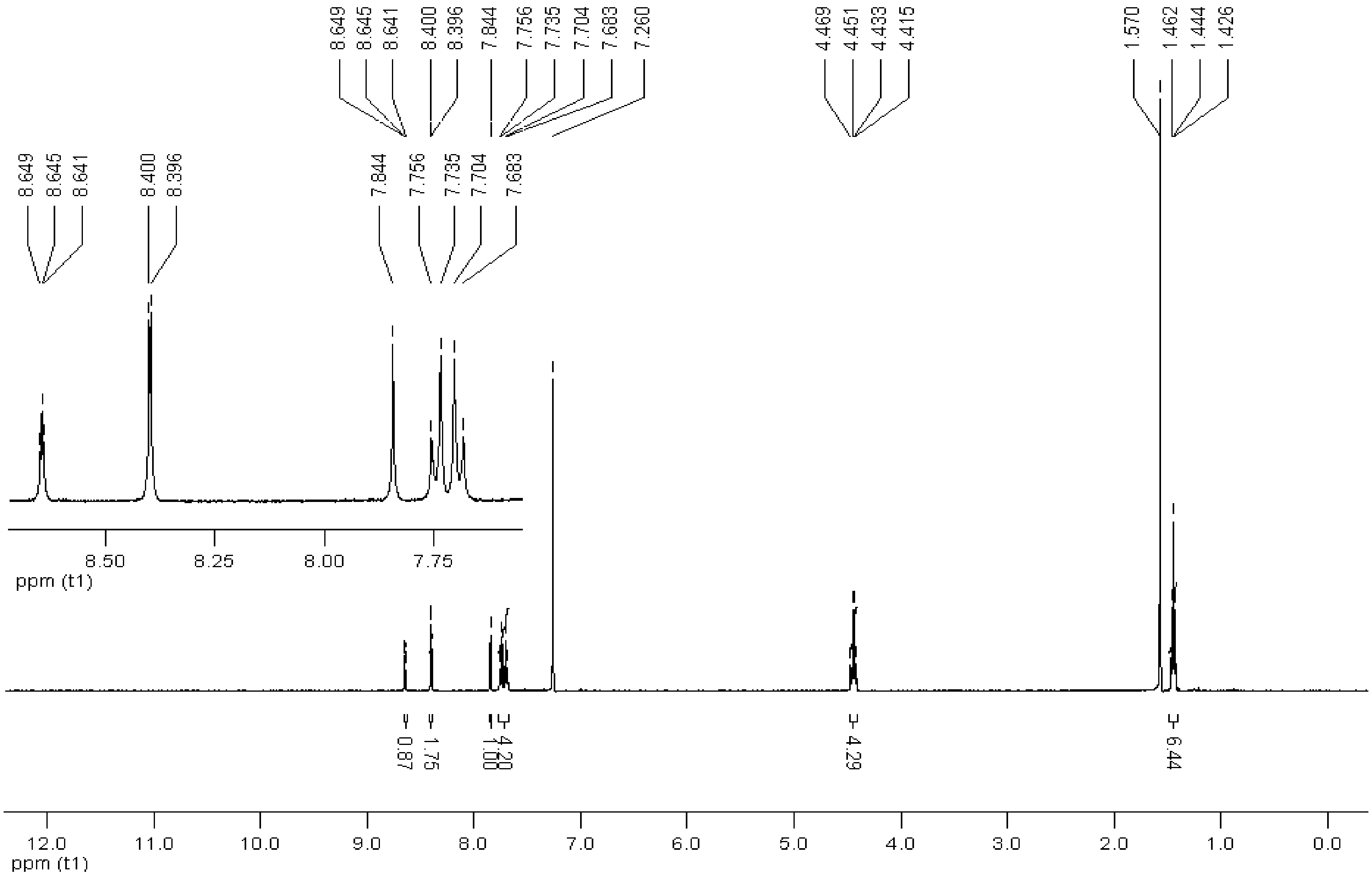


^{1}H NMR of Hexaester 7



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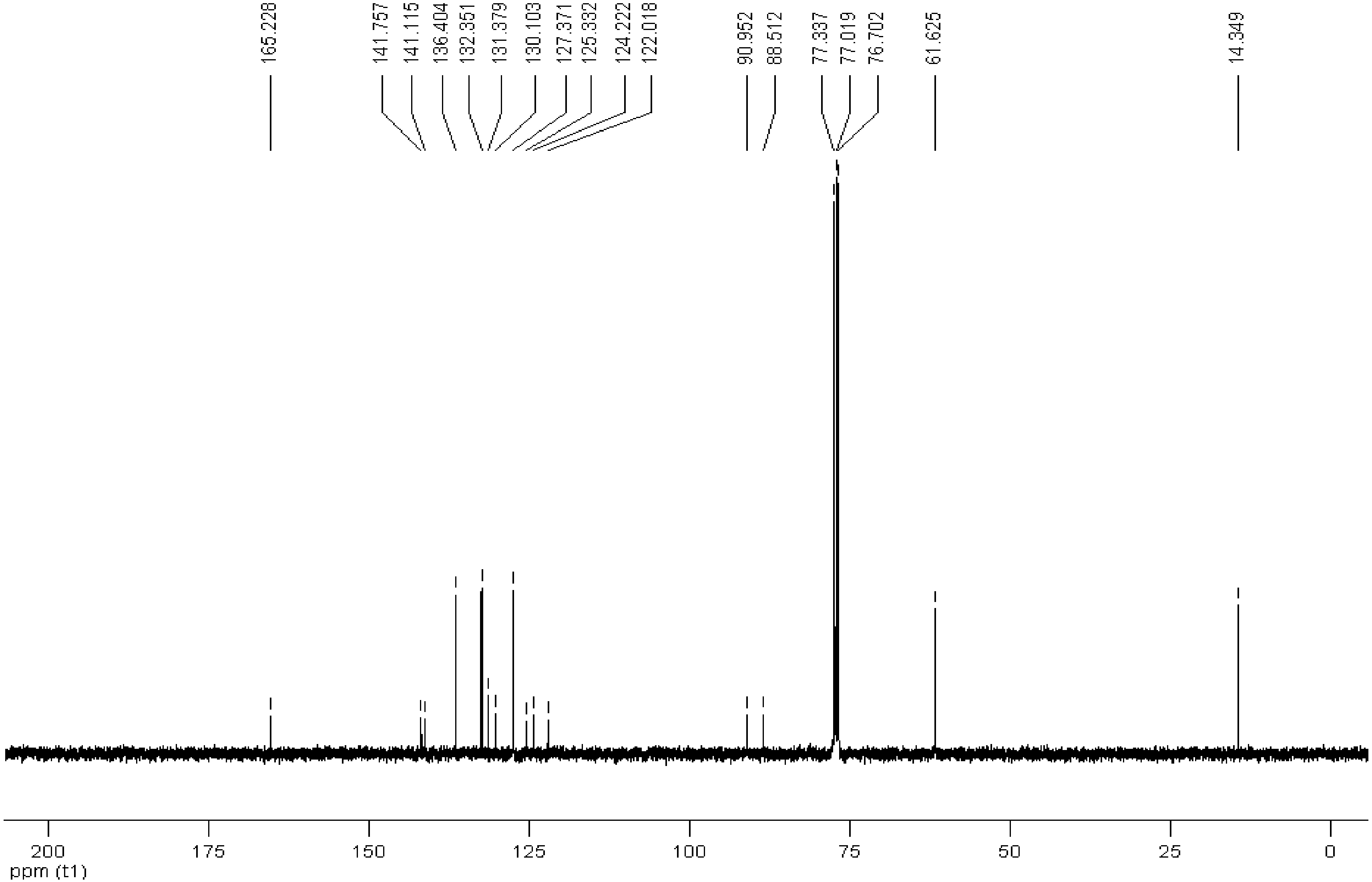
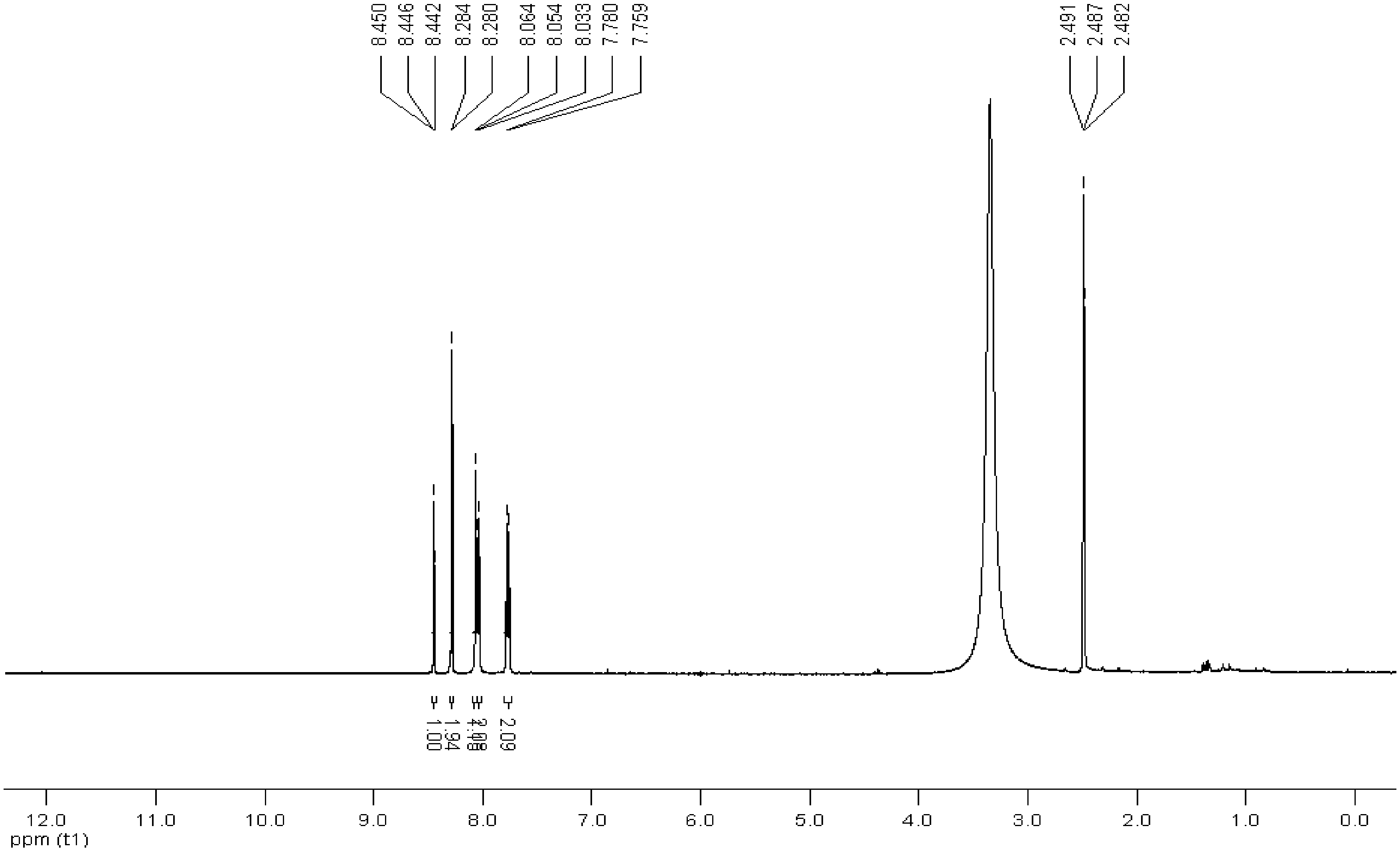


^{1}H NMR of Fluorophore 2



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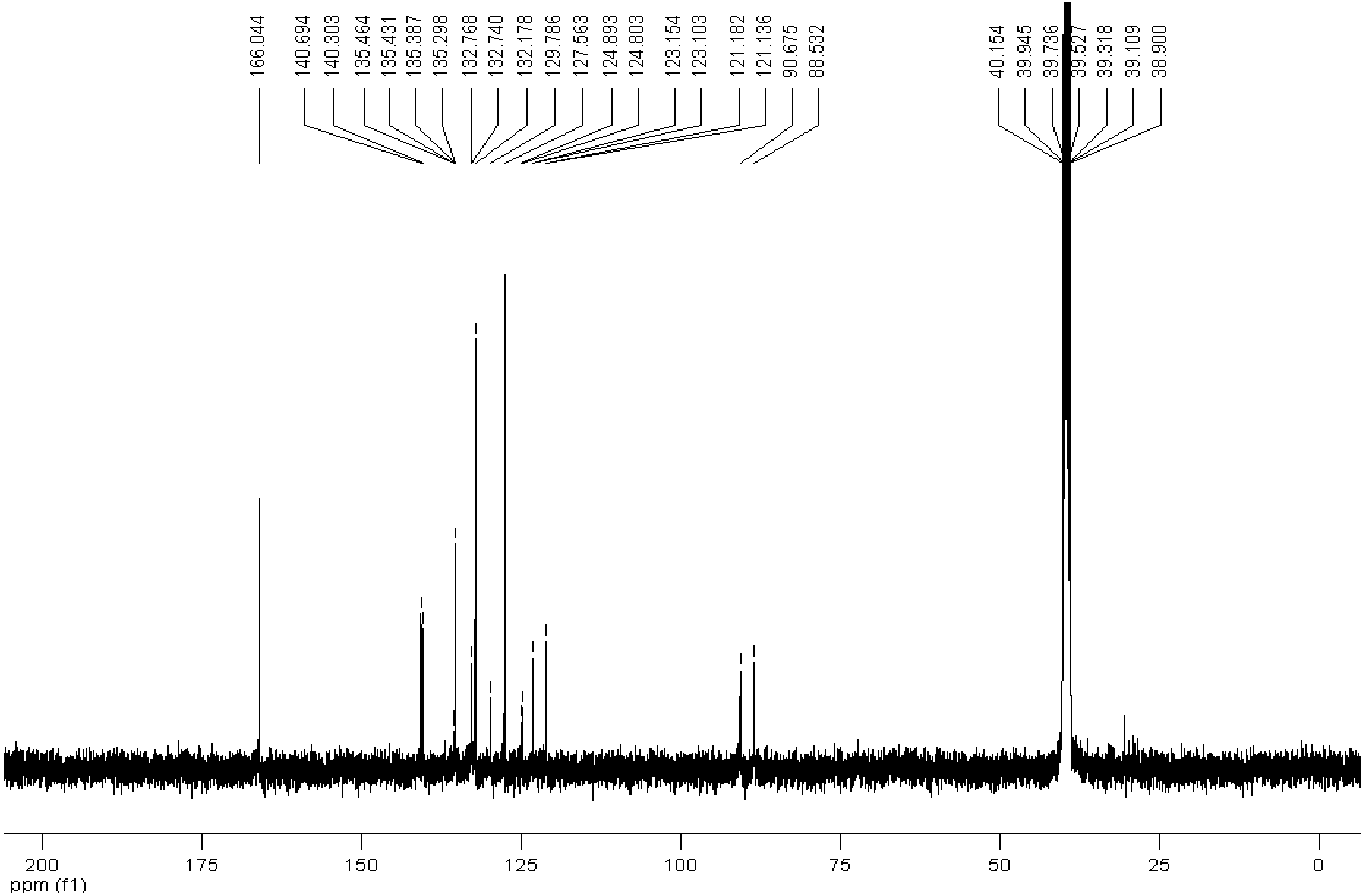
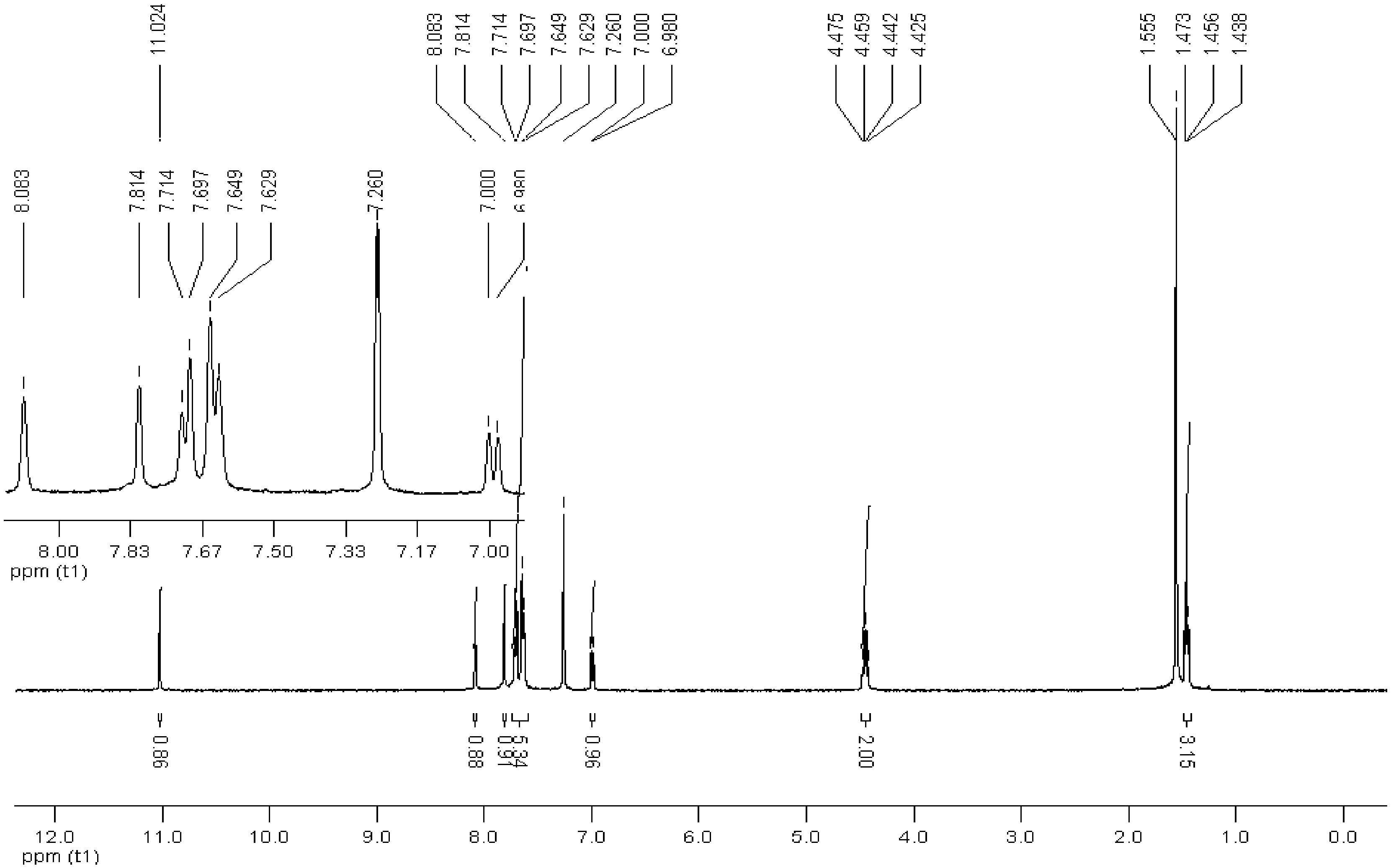


^{1}H NMR of Trisalicylate ester 8



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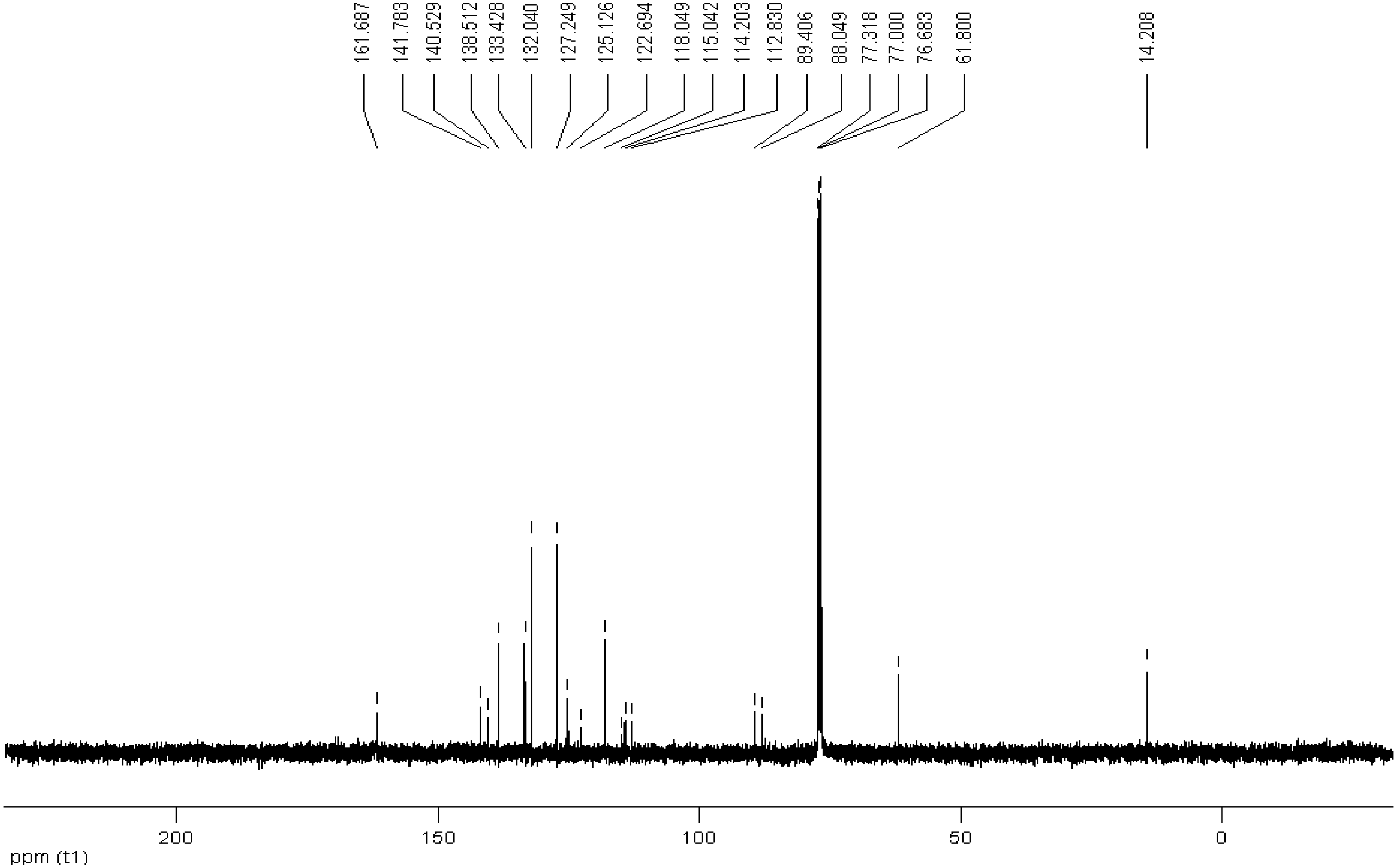
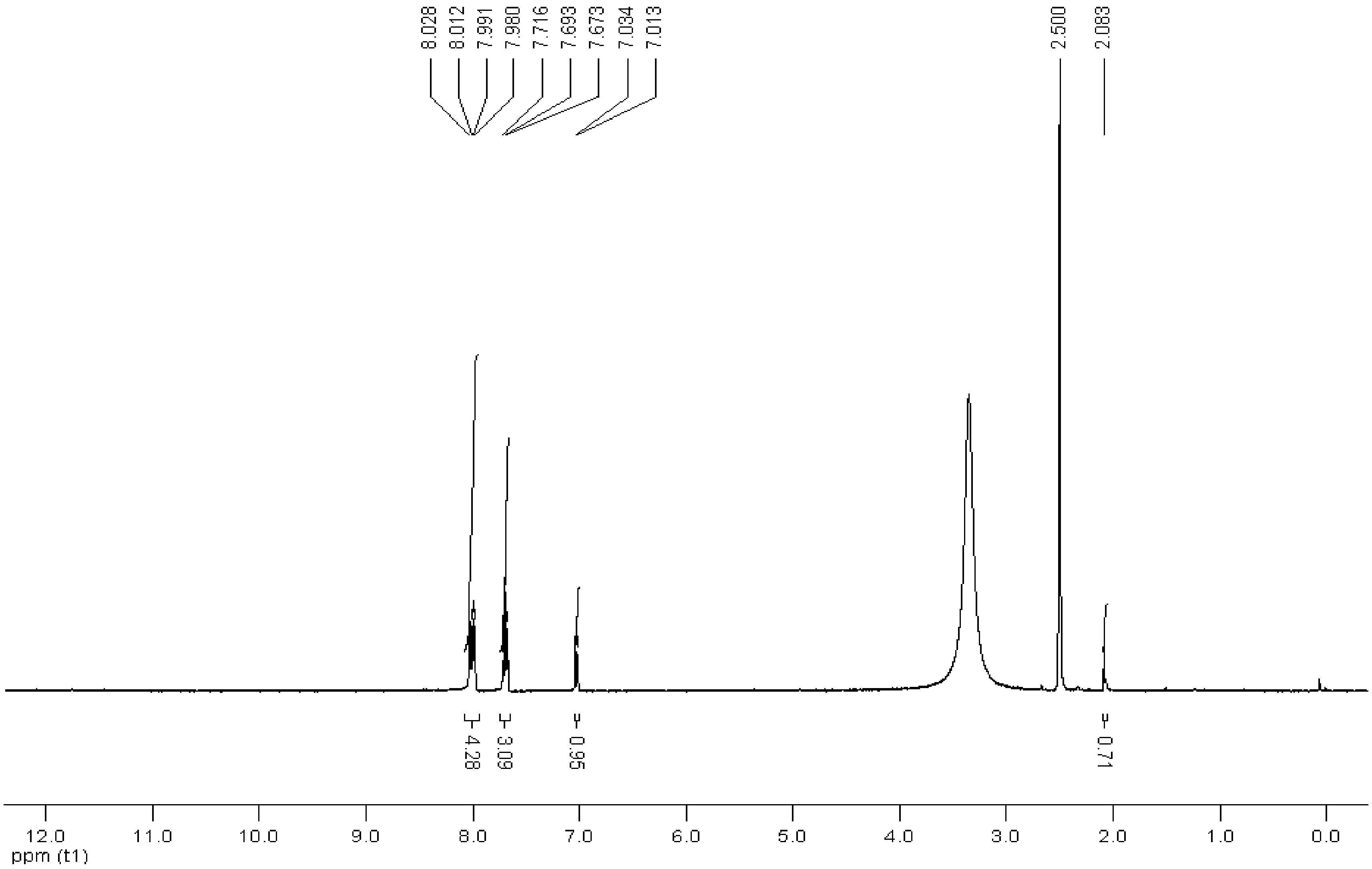


^{1}H NMR of Fluorophore 3



^{13}C NMR of Compound 3