

Systematic Modifications of a Simple Tolan: Another Category of Viscosity Sensor

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General experimental and Synthetic details

Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Yields of synthesized compounds were measured after chromatographic purification. ^1H , ^{13}C , and 2D-NMR spectra were measured at 25 °C using 400 MHz spectrometers. HRMS were recorded by EI methods using a magnetic sector-electric sector double focusing analyzer.

General Synthetic procedure

1-(phenylethynyl)naphthalene (3a)

Iodobenzene (0.246 g, 1.206 mmol) was added to the solution of 1-ethynynaphthalene (0.202 g, 1.326 mmol) in a mixture of 6.0 mL of anhydrous DMF and 6.0 mL of triethylamine. The resulting mixture was degassed for 5 min and then $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (0.042 g, 0.060 mmol) and CuI (0.012 g, 0.060 mmol) were added to the solution under Ar. The resulting mixture was heated at 80 °C for 2 h. After cooling the reaction to room temperature, the reaction mixture was poured into water and extracted with DCM. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified over silica gel (eluent: n-Hexane) to afford the compound (92 mg, 33.1%). ^1H NMR (400 MHz, Chloroform-*d*) δ 8.45 (d, J = 8.3 Hz, 1H), 7.86 (dd, J = 8.3, 8.3 Hz, 2H), 7.76 (dd, J = 7.2, 1.2 Hz, 1H), 7.68 – 7.63 (m, 2H), 7.60 (ddd, J = 8.3, 6.8, 1.4 Hz, 1H), 7.53 (ddd, J = 8.1, 6.8, 1.3 Hz, 1H), 7.46 (dd, J = 8.3, 7.2 Hz, 1H), 7.43 – 7.35 (m, 3H); ^{13}C NMR (100 MHz, Chloroform-*d*) δ 133.4, 133.3, 131.8, 130.5, 128.8, 128.5 (two signals), 128.4, 126.9, 126.5, 126.3, 125.4, 123.5, 121.0, 94.4, 87.6; HRMS–EI: m/z $[\text{M}]^+$ calcd for $\text{C}_{18}\text{H}_{12}$: 228.0939; found: 228.0941.

8-(phenylethynyl)quinoline (3b)

Iodobenzene (0.207 g, 1.014 mmol) was added to the solution of 8-ethynylquinoline (0.171 g, 1.116 mmol) in a mixture of 5.1 mL of anhydrous DMF and 5.1 mL of triethylamine. The resulting mixture was degassed for 5 min and then $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (0.036 g, 0.051 mmol) and CuI (0.009 g, 0.051 mmol) were added to the solution under Ar. The resulting mixture was heated at 80 °C for 4 h. After cooling the reaction to room temperature, the reaction mixture was poured into water and extracted with DCM. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified over silica gel (Ethyl Acetate:Hexane = 1:2) to afford the compound (54 mg, 23.3%). ^1H NMR (400 MHz, Chloroform-*d*) δ 8.92 (dd, J = 4.2, 1.8 Hz, 1H), 8.03 (dd, J = 8.3, 1.8 Hz, 1H), 7.86 (dd, J = 7.2, 1.4 Hz, 1H), 7.67 (dd, J = 8.2, 1.4 Hz, 1H), 7.59 – 7.48 (m, 2H), 7.39 (dd, J = 8.2, 7.2 Hz, 1H), 7.31 (dd, J = 8.3, 4.2 Hz, 1H), 7.27 – 7.15 (m, 3H); ^{13}C NMR (100 MHz, Chloroform-*d*) δ 151.2, 148.2, 136.6, 134.3, 132.1, 128.5, 128.3, 126.2, 123.6, 121.7, 95.5, 87.3 (three peaks are overlapped); HRMS–EI: m/z $[\text{M}]^+$ calcd for $\text{C}_{17}\text{H}_{11}\text{N}$: 229.0891; found: 229.0890.

methyl 2-(naphthalen-1-ylethynyl)benzoate (3c)

Methyl 2-iodobenzoate (0.316 g, 1.206 mmol) was added to the solution of 1-ethynynaphthalene (0.202 g, 1.326 mmol) in a mixture of 6.0 mL of anhydrous DMF and 6.0 mL of triethylamine. The resulting mixture was degassed for 5 min and then $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (0.042 g, 0.060 mmol) and CuI (0.012 g, 0.060 mmol) were added to the solution under Ar. The resulting mixture was heated at 80 °C for 5 h. After cooling the reaction to room temperature, the reaction mixture was poured into water and extracted with DCM. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified over silica gel (Ethyl Acetate:Hexane = 1:9) to afford the compound (218 mg, 63.2%). ^1H NMR (400 MHz, Chloroform-*d*) δ 8.59 (d, J = 8.3 Hz, 1H), 8.02 (ddd, J = 7.8, 1.4, 0.5 Hz, 1H), 7.86 (m, 2H), 7.80 (dd, J = 7.1, 1.2 Hz, 1H), 7.77 (ddd, J = 7.8, 1.3, 0.6 Hz, 1H), 7.61 (ddd, J = 8.3, 6.8, 1.4 Hz, 1H), 7.54 (m, 2H), 7.46 (dd, J = 8.3, 7.2 Hz, 1H), 7.41 (ddd, J = 7.9, 7.4, 1.3 Hz, 1H), 3.99 (s, 3H); ^{13}C NMR (100 MHz, Chloroform-*d*) δ 166.9, 134.4, 133.6, 133.3, 131.9, 131.8, 130.9, 130.7, 129.2, 128.3, 128.1, 127.0, 126.6 (two signals), 125.4, 123.9, 121.2, 93.1, 92.8, 52.4; HRMS–EI: m/z $[\text{M}]^+$ calcd for $\text{C}_{20}\text{H}_{14}\text{O}_2$: 286.0994; found: 286.0997.

2-(naphthalen-1-ylethynyl)benzoic acid (4c)

NaOH (2.150 g, 53.750 mmol) was added to a solution of compound **3c** (0.290 g, 1.010 mmol) in the mixed solvent (20 mL of MeOH, 50 mL of THF, and 80 mL of H₂O) at room temperature. The mixture was stirred for 14 h at the same temperature. The reaction mixture was washed with DCM and acidified with 10% aqueous HCl. The aqueous layer was extracted with EtOAc. The organic layer was dried over anhydrous Na₂SO₄ and evaporated to dryness. The residue was purified over silica gel (Ethyl Acetate:Hexane = 1:1) to afford the compound (230 mg, 82.7%). ¹H NMR (400 MHz, Acetone-*d*₆) δ 8.72 (d, *J* = 8.3 Hz, 1H), 8.07 (ddd, *J* = 7.9, 1.5, 0.6 Hz, 1H), 7.98 – 7.91 (m, 2H), 7.86 – 7.78 (m, 2H), 7.68 – 7.55 (m, 3H), 7.55 – 7.49 (m, 2H); ¹³C NMR (100 MHz, Acetone-*d*₆) δ 166.5, 134.3, 133.6, 133.5, 132.3, 132.0, 130.8, 130.7, 129.2, 128.4, 128.3, 127.0, 126.7, 125.5, 123.8, 121.1, 93.2, 92.4 (one carbon signal is overlapped); HRMS–EI: *m/z* [M]⁺ calcd for C₁₉H₁₂O₂: 272.0837; found: 272.0839

(Z)-3-(naphthalen-1-ylmethylene)isobenzofuran-1(3H)-one (5c)

A mixture of 2-(naphthalen-1-ylethynyl)benzoic acid (300 mg, 1.170 mmol), (PPh₃)₂PdCl₂ (36 mg, 0.054 mmol) and CuI (9.0 mg, 0.054 mmol) in DMF (30 ml) was purged with Ar for 10 min. Triethylamine (0.780 mL, 5.40 mmol) was added and the mixture was stirred for 6 h at 80 °C. The mixture was cooled to room temperature and brine solution was added. The solution was extracted with EtOAc. The organic layer was dried over anhydrous Na₂SO₄ and evaporated to dryness. The residue was purified over silica gel (Ethyl Acetate:Hexane = 1:9) to give the cyclized product (210 mg, 70.0%). ¹H NMR (400 MHz, Chloroform-*d*) δ 8.31 (d, *J* = 7.4 Hz, 1H), 8.19 (d, *J* = 8.4 Hz, 1H), 7.97 (dd, *J* = 7.7 Hz, 1H), 7.93 (d, *J* = 7.9 Hz, 1H), 7.89 (d, *J* = 8.2 Hz, 1H), 7.84 (d, *J* = 8.2 Hz, 1H), 7.78 (t, *J* = 7.6 Hz, 1H), 7.63 – 7.45 (m, 4H), 7.21 (s, 1H); ¹³C NMR (100 MHz, Chloroform-*d*) δ 167.3, 145.8, 140.6, 134.6, 133.8, 131.6, 130.1, 129.5, 129.1 (two signals), 128.9, 126.6, 125.9 (two signals), 125.7, 123.8, 123.3, 120.1, 103.1; ν_{\max} (KBr)/cm⁻¹ 1769, 1757; HRMS–EI: *m/z* [M]⁺ calcd for C₁₉H₁₂O₂: 272.0837; found: 272.0838

methyl 2-(quinolin-8-ylethynyl)benzoate (3d)

Methyl 2-iodobenzoate (0.400 g, 1.526 mmol) was added to the solution of 8-ethynylquinoline (0.281 g, 1.832 mmol) in 7.6 mL of triethylamine. The resulting mixture was degassed for 5 min and then Pd(PPh₃)₂Cl₂ (0.032 g, 0.046 mmol) and CuI (0.009 g, 0.046 mmol) were added to the solution under Ar. The resulting mixture was heated at 80 °C for 3 h. After cooling the reaction to room temperature, the reaction mixture was evaporated to dryness. The residue was purified over silica gel (Ethyl Acetate:Hexane = 1:2) to afford the compound (204 mg, 46.4%). ¹H NMR (400 MHz, Chloroform-*d*) δ 9.05 (dd, *J* = 4.3, 1.7 Hz, 1H), 8.16 (ddd, *J* = 8.3, 1.8, 0.5 Hz, 1H), 8.05 (dd, *J* = 7.2, 1.4 Hz, 1H), 8.00 (ddd, *J* = 7.9, 1.4, 0.6 Hz, 1H), 7.87 – 7.77 (m, 2H), 7.56 – 7.47 (m, 2H), 7.44 (dd, *J* = 8.3, 4.2 Hz, 1H), 7.42 – 7.35 (m, 1H), 3.99 (s, 3H); ¹³C NMR (100 MHz, Chloroform-*d*) δ 167.0, 151.1, 148.3, 136.5, 134.6, 134.5, 132.1, 131.7, 130.5, 128.7, 128.4, 128.1, 126.2, 124.1, 123.7, 121.7, 94.2, 92.3, 52.4; HRMS–EI: *m/z* [M]⁺ calcd for C₁₉H₁₃NO₂: 287.0946; found: 287.0945.

(Z)-3-(quinolin-8-ylmethylene)isobenzofuran-1(3H)-one (5d)

NaOH (2.400 g, 60.0 mmol) was added to a solution of compound **3d** (0.345 g, 1.200 mmol) in the mixed solvent (75 mL of MeOH and 35 mL of H₂O) at room temperature. The mixture was stirred for 24 h at the same temperature. The reaction mixture was washed with DCM and the aqueous layer was neutralized (pH=6) with 10% aqueous HCl. The aqueous layer was extracted with DCM. The organic layer was dried over anhydrous Na₂SO₄ and evaporated to dryness. The residue was purified over silica gel (Ethyl Acetate:Hexane = 1:2) to afford the compound (270 mg, 81.6%). ¹H NMR (400 MHz, Chloroform-*d*) δ 8.97 (dd, *J* = 4.2, 1.8 Hz, 1H), 8.75 (dd, *J* = 7.5, 1.4 Hz, 1H), 8.17 (dd, *J* = 8.2, 1.8 Hz, 1H), 8.11 (s, 1H), 8.03 (dt, *J* = 7.8, 0.9 Hz, 1H), 7.95 (dt, *J* = 7.7, 1.0 Hz, 1H), 7.79 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.77 – 7.72 (m, 1H), 7.64 (t, *J* = 7.8 Hz, 1H), 7.55 (td, *J* = 7.5, 0.9 Hz, 1H), 7.45 (dd, *J* = 8.2, 4.2 Hz, 1H); ¹³C NMR (100 MHz, Chloroform-*d*) δ 167.5, 149.6, 145.9, 141.1, 136.9, 134.6, 131.8, 131.2, 129.9, 128.5, 128.2, 127.0, 125.5, 123.6, 121.4, 120.8, 102.1 (one carbon signal is overlapped); ν_{\max} (KBr)/cm⁻¹ 1770; HRMS–EI: *m/z* [M]⁺ calcd for C₁₈H₁₁NO₂: 273.0790; found: 273.0788.

methyl 2-(anthracen-9-ylethynyl)benzoate (3e)

Methyl 2-ethynylbenzoate (0.200 g, 1.25 mmol) was added to the solution of 9-bromoanthracene (0.292 g, 1.14 mmol) in a mixture of 5.7 mL of anhydrous DMF and 5.7 mL of triethylamine. The resulting mixture was degassed for 5 min and then Pd(PPh₃)₂Cl₂ (0.040 g, 0.057 mmol) and CuI (0.011 g, 0.057 mmol) were added to the solution under Ar. The resulting mixture was heated at 80 °C for 12 h. After cooling the reaction to room temperature, the reaction mixture was poured into water and extracted with DCM. The organic layer was dried over anhydrous Na₂SO₄ and evaporated to dryness. The residue was purified over silica gel (Dichloromethane:Hexane = 1:1) to afford the compound (178 mg, 46.5%). ¹H NMR (400 MHz, Chloroform-*d*) δ 8.80 (dd, *J* = 8.7, 1.0 Hz, 2H), 8.45 (s, 1H), 8.07 (ddd, *J* = 7.9, 1.5, 0.6 Hz, 1H), 8.02 (ddt, *J* = 8.3, 1.2, 0.7 Hz, 2H), 7.92 (ddd, *J* = 7.8, 1.4, 0.6 Hz, 1H), 7.65 – 7.55 (m, 3H), 7.52 (ddd, *J* = 8.5, 6.6, 1.3 Hz, 2H), 7.45 (ddd, *J* = 7.9, 7.5, 1.3 Hz, 1H), 4.03 (s, 3H); ¹³C NMR (100 MHz, Chloroform-*d*) δ 167.0, 134.5, 133.1, 131.9, 131.6, 131.3, 130.9, 128.7, 128.2, 128.1, 127.0, 126.8, 125.8, 124.1, 117.4, 99.6, 91.6, 52.5; HRMS–EI: *m/z* [M]⁺ calcd for C₂₄H₁₆O₂: 336.1150; found: 336.1152.

2-(anthracen-9-ylethynyl)benzoic acid (4e)

NaOH (1.200 g, 29.8 mmol) was added to a solution of compound **3e** (0.400 g, 1.190 mmol) in the mixed solvent (48 mL of MeOH, 24 mL of THF, and 24 mL of H₂O) at room temperature. The mixture was stirred for 24 h at the same temperature. The reaction mixture was washed with DCM and acidified with 10% aqueous HCl. The aqueous layer was extracted with DCM. The organic layer was dried over anhydrous Na₂SO₄ and evaporated to dryness. The residue was purified over silica gel (Ethyl Acetate:Hexane = 1:1) to afford the compound (380 mg, 100%). ¹H NMR (400 MHz, Acetone-*d*₆) δ 8.92 (dd, *J* = 8.7, 1.1 Hz, 2H), 8.61 (s, 1H), 8.14 (ddd, *J* = 7.9, 1.4, 0.6 Hz, 1H), 8.13 – 8.08 (m, 2H), 8.05 (ddd, *J* = 7.8, 1.3, 0.6 Hz, 1H), 7.71 (td, *J* = 7.6, 1.4 Hz, 1H), 7.64 (ddd, *J* = 8.7, 6.6, 1.3 Hz, 2H), 7.60 – 7.52 (m, 3H); ¹³C NMR (100 MHz, Acetone-*d*₆) δ 166.6, 134.5, 133.1, 132.2, 131.8, 131.4, 130.9, 128.8, 128.5, 128.4, 127.1, 127.0, 126.0, 124.0, 117.2, 99.9, 91.2; HRMS–EI: *m/z* [M]⁺ calcd for C₂₃H₁₄O₂: 322.0994; found: 322.0996

3-(anthracen-9-yl)-1*H*-isochromen-1-one (5e)

A mixture of 2-(anthracen-9-ylethynyl)benzoic acid (350 mg, 1.050 mmol), (PPh₃)₂PdCl₂ (35.0 mg, 0.050 mmol) and CuI (10.0 mg, 0.050 mmol) in DMF (50 mL) was purged with Ar for 10 min. Triethylamine (0.750 mL, 5.0 mmol) was added and the mixture was stirred for 1 h at 80 °C. The mixture was cooled to room temperature and brine solution was added. The resulted solution was extracted with EtOAc. The organic layer was dried over anhydrous Na₂SO₄ and evaporated to dryness. The residue was purified over silica gel (Hexane: DCM = 4:6) to afford the compound (250 mg, 71.4%). ¹H NMR (400 MHz, Chloroform-*d*) δ 8.59 (s, 1H), 8.45 (d, *J* = 8.0 Hz, 1H), 8.08 – 8.00 (m, 4H), 7.82 (d, *J* = 7.9 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.56 (d, *J* = 7.9, 1H), 7.52 – 7.43 (m, 4H), 6.81 (s, 1H); ¹³C NMR (100 MHz, Chloroform-*d*) δ 163.1, 152.5, 137.2, 135.2, 131.2, 130.7, 130.0, 129.7, 128.8, 128.7, 126.9, 126.8, 126.0, 125.5 (two signals), 120.9, 110.0; ν_{max} (KBr)/cm⁻¹ 1720; HRMS–EI: *m/z* [M]⁺ calcd for C₂₃H₁₄O₂: 322.0994; found: 322.0996

2D NMR spectra

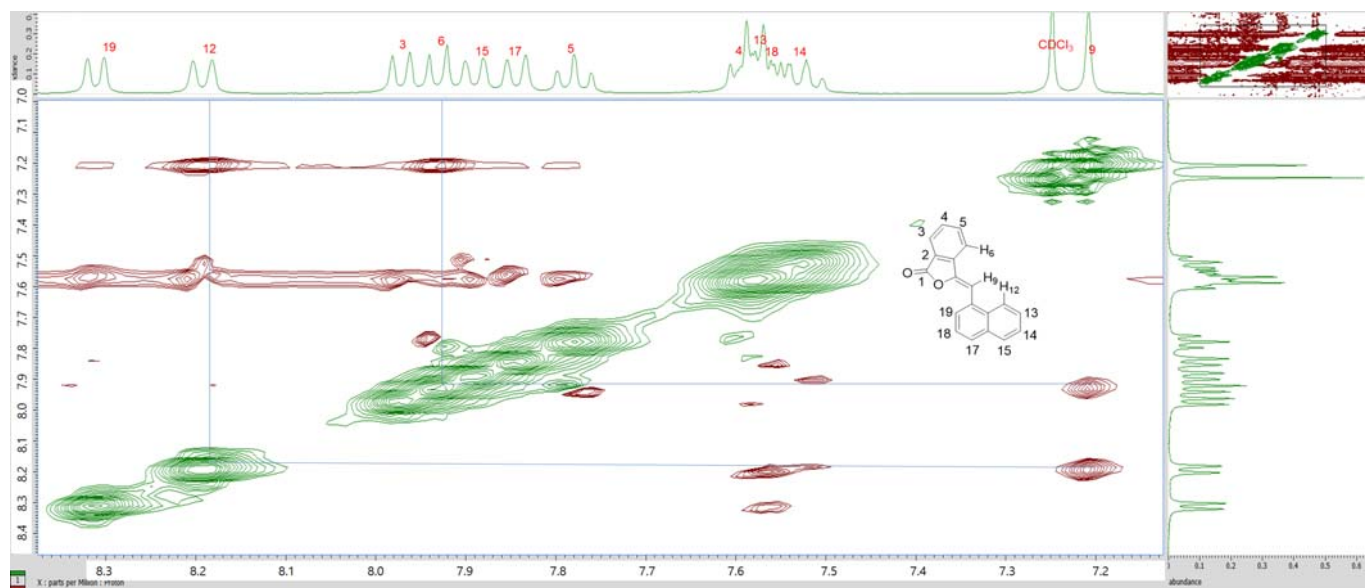


Figure S1. 2D NOESY spectrum of **5c** in CDCl_3 at 25 °C (400 MHz)

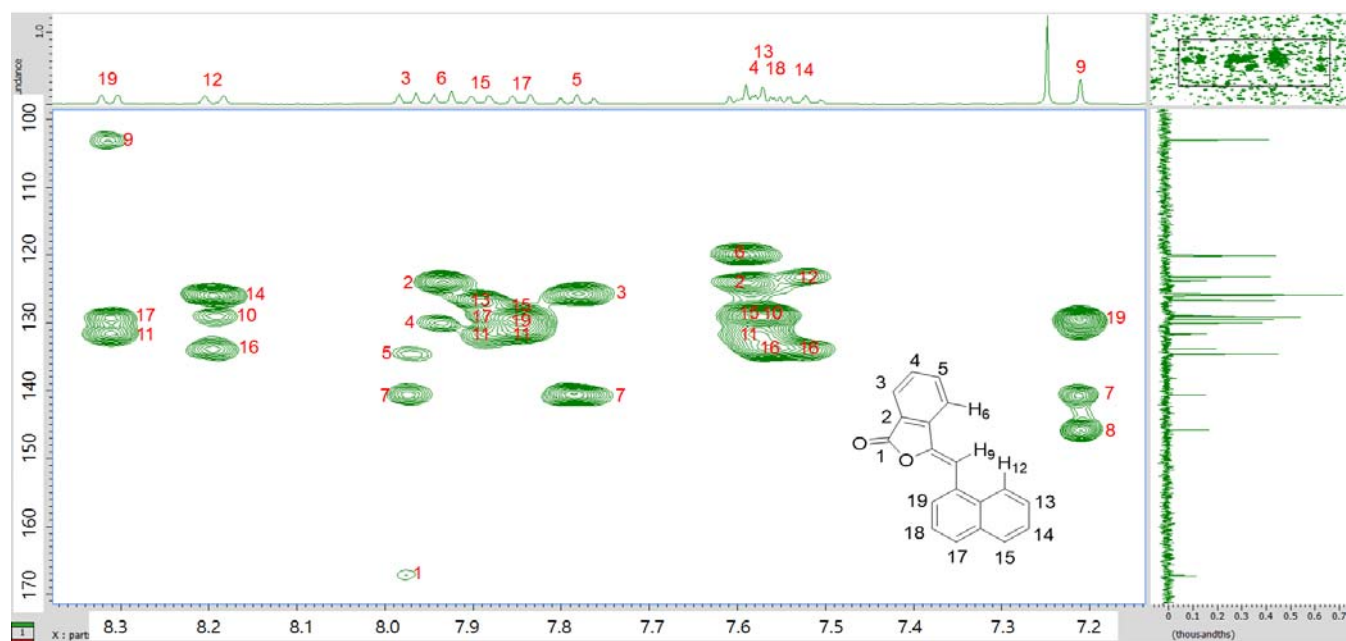


Figure S2. 2D HMBC spectrum of **5c** in CDCl_3 at 25 °C (400 MHz)

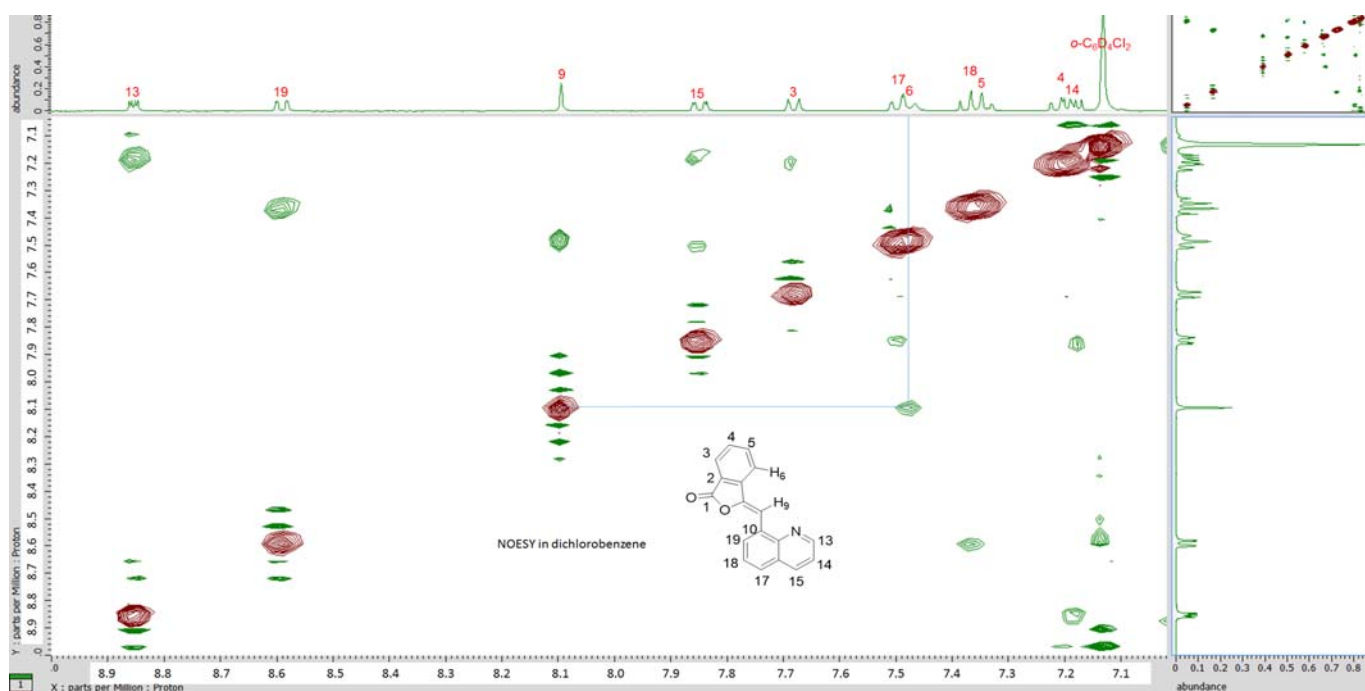


Figure S3. 2D NOESY spectrum of **5d** in dichlorobenzene- d_4 at 25 °C (400 MHz)

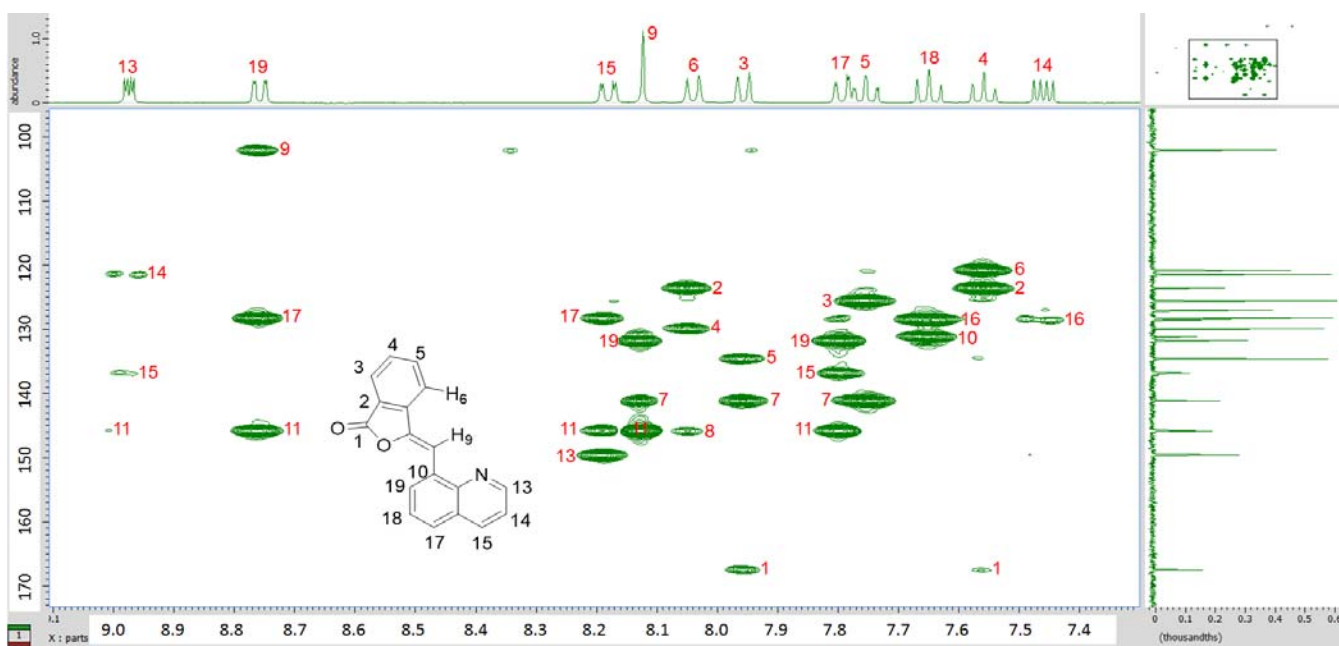


Figure S4. 2D HMBC spectrum of **5d** in CDCl_3 at 25 °C (400 MHz)

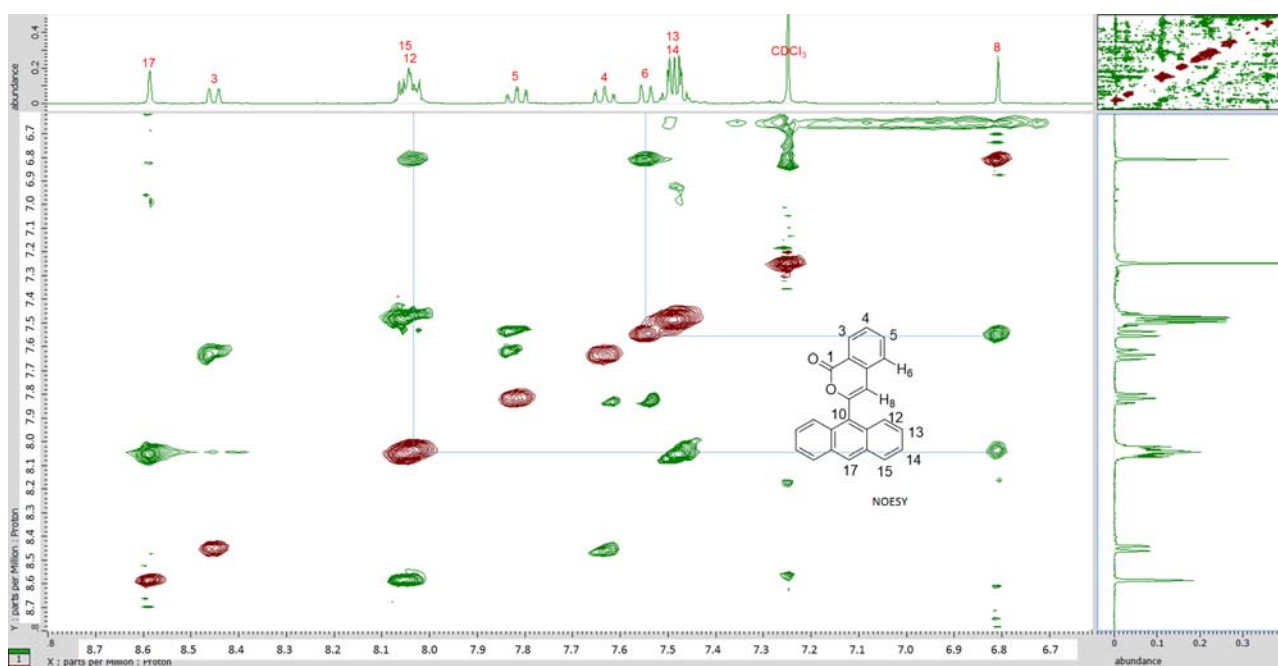


Figure S5. 2D NOESY spectrum of **5e** in CDCl_3 at 25 °C (400 MHz)

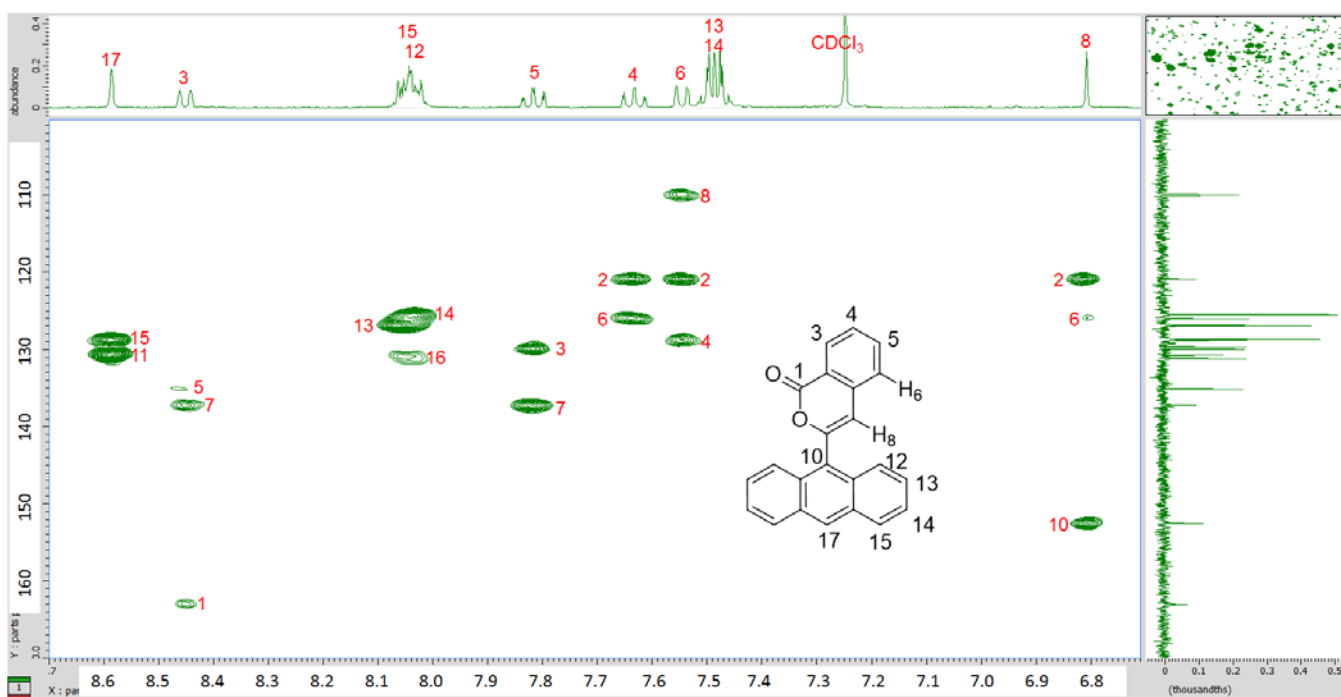
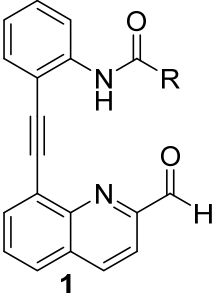
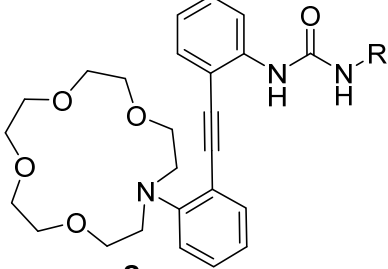


Figure S6. 2D HMBC spectrum of **5e** in CDCl_3 at 25 °C (400 MHz)

Spectroscopic studies

Table S1. Basic photophysical properties of tolan derivatives.^a

Product	^{abc} λ_{MAX}	^{em} λ_{MAX}	Φ_{F} (%) ^b	I_{60}/I_0 ^c
 1	336 325 (in MeOH)	491 423 (in MeOH)	2.1	1.99
 2	344	423	39	1.02

^aAll measurements were conducted in CH₃CN at 25 °C unless stated.

^bfluorescence quantum yields were measured in a relative way as compared to the fluorescence quantum yield of 4-methyl-7-aminocoumarin in CH₃CN.

^c I_{60}/I_0 is the observed fluorescence intensity ratio where I_{60} is a measured intensity in 60% glycerol and I_0 in 0% glycerol in MeOH at their ^{em} λ_{max} .

Table S2. Physical and photophysical properties of **5d** in solvents

5d	polarity ^a	Viscosity ^b (cPs)	^{abc} λ_{MAX}	^{em} λ_{MAX}	Φ_{F} (%)
Acetonitrile	37.5	0.37	356	429	0.305
1,2-Dichloroethane	10.5 (20 °C)	0.79	361	429	0.357
Dichloromethane	9.08	0.45 (15 °C)	361	430	0.384
Methanol	32.6 (25 °C)	0.55	357	432	0.412
Dimethyl sulfoxide	48.9	2.24	362	436	0.636

^aThe dielectric constants of the solvents. ^bthe viscosity of the solvent at 20 °C.

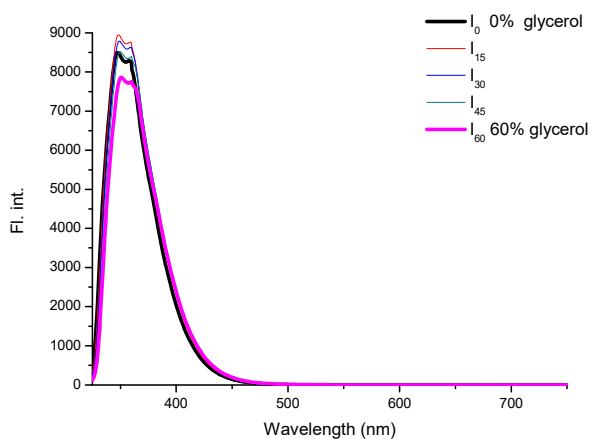


Figure S7. Fluorescence spectra changes of **3a** (5 μM) in methanol–glycerol mixtures of varied viscosity.

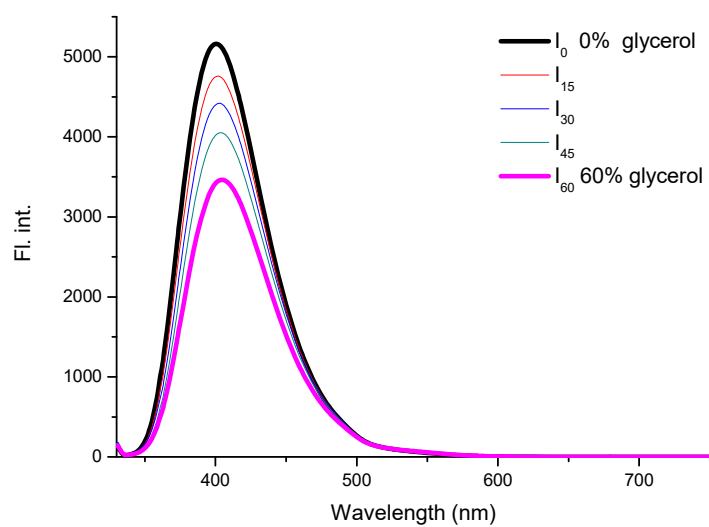


Figure S8. Fluorescence spectra changes of **3b** (5 μM) in methanol–glycerol mixtures of varied viscosity.

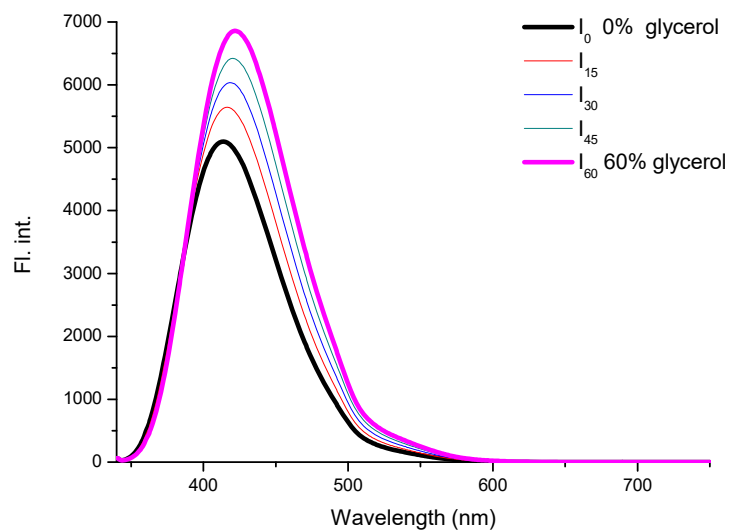


Figure S9. Fluorescence spectra changes of **3c** (5 μM) in methanol–glycerol mixtures of varied viscosity.

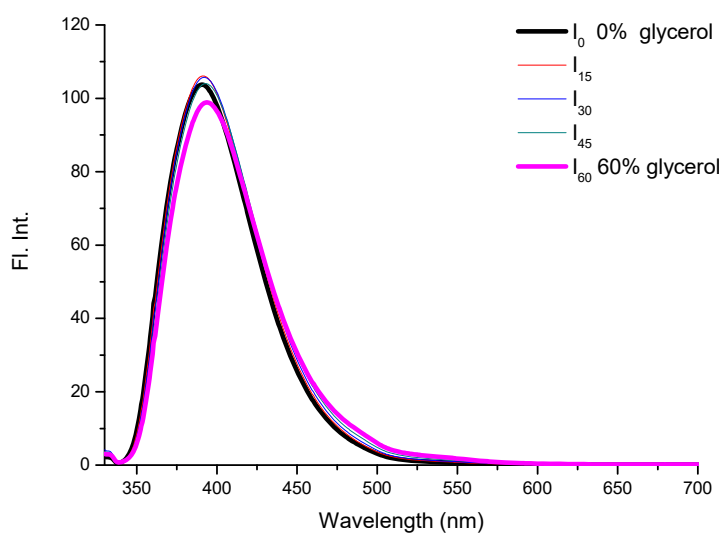


Figure S10. Fluorescence spectra changes of **3d** (5 μM) in methanol–glycerol mixtures of varied viscosity

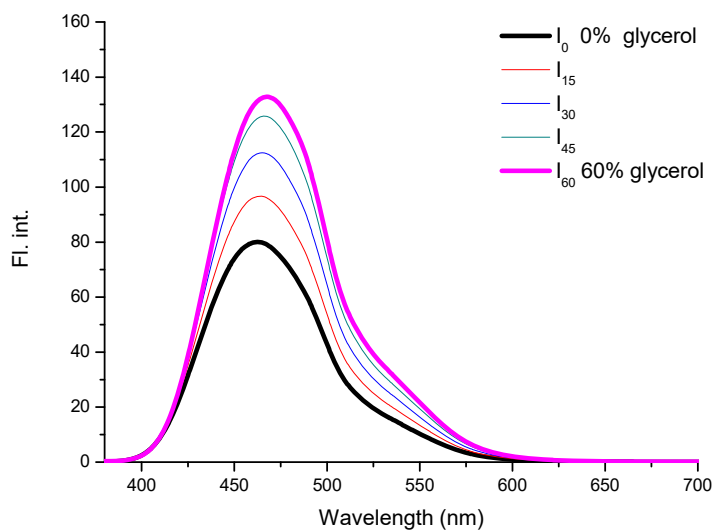


Figure S11. Fluorescence spectra changes of **5c** (5 μM) in methanol–glycerol mixtures of varied viscosity

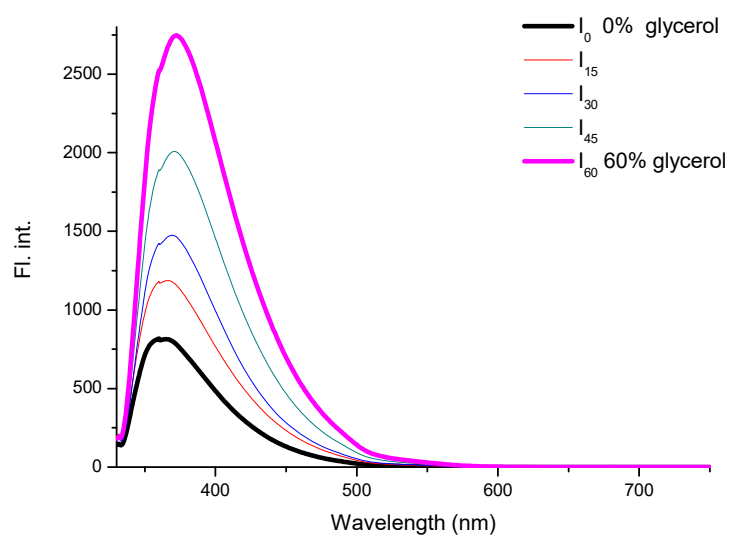


Figure S12. Fluorescence spectra changes of **4c** (5 μM) in methanol–glycerol mixtures of varied viscosity

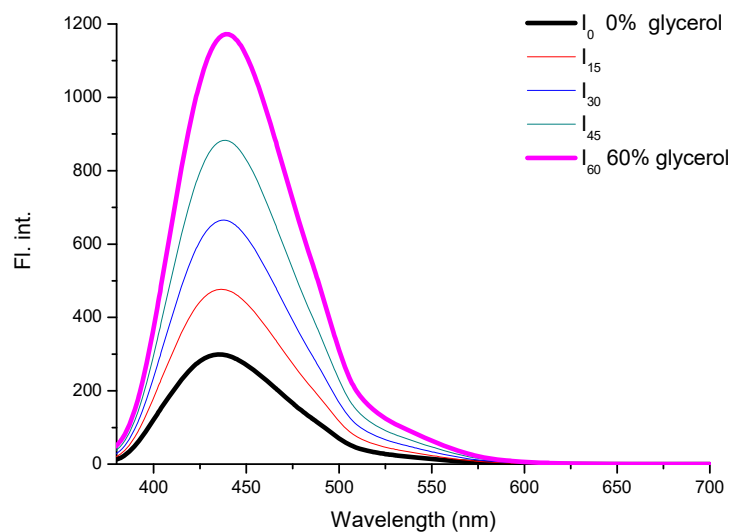


Figure S13. Fluorescence spectra changes of **5d** (5 μ M) in methanol-glycerol mixtures of varied viscosity.

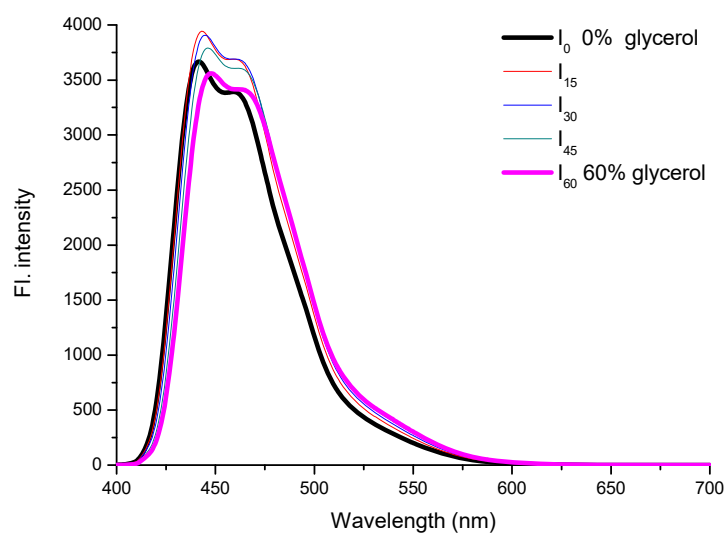


Figure S14. Fluorescence spectra changes of **3e** (5 μ M) in methanol-glycerol mixtures of varied viscosity.

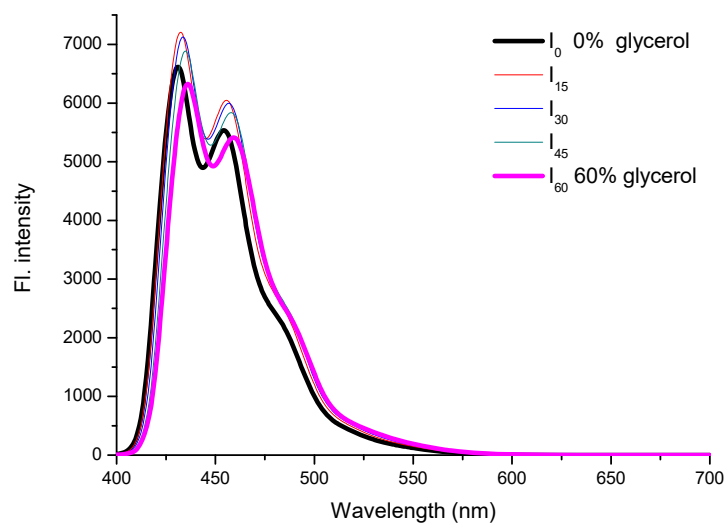


Figure S15. Fluorescence spectra changes of **4e** (5 μM) in methanol–glycerol mixtures of varied viscosity.

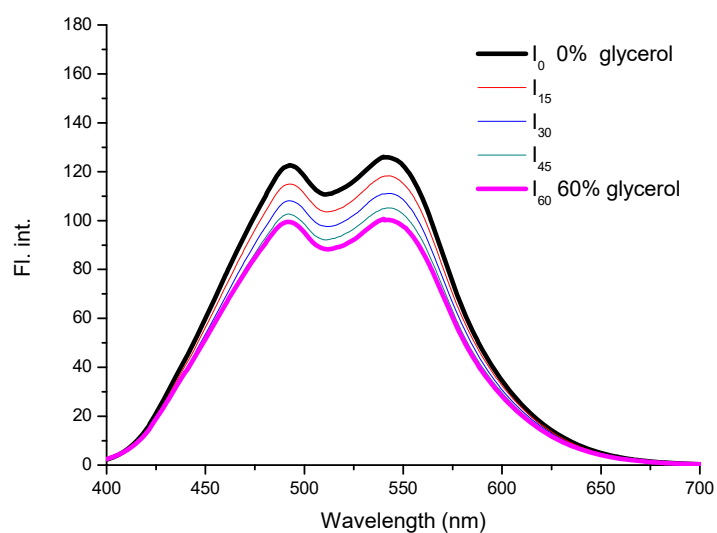


Figure S16. Fluorescence spectra changes of **5e** (5 μM) in methanol–glycerol mixtures of varied viscosity.

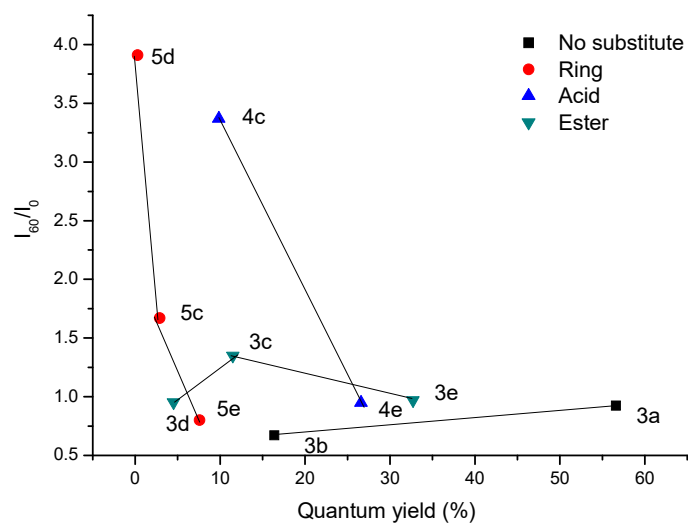


Figure S17. I_{60}/I_0 values of tolans against their fluorescence quantum yields in CH_3CN at 25 °C. All data comes from Table 1 in the current paper.

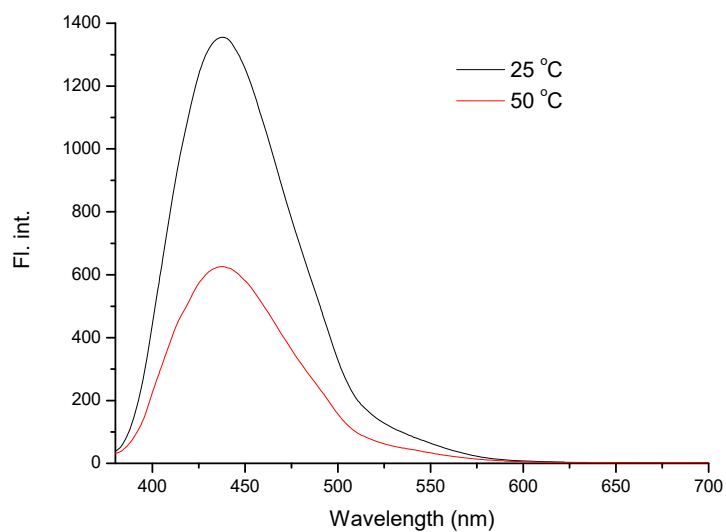


Figure S18. Fluorescence spectra changes of **5d** (5 uM in DMSO) upon the excitation at 363 nm at two different temperatures.

Fluorescence spectra changes of 5d at a various pHs

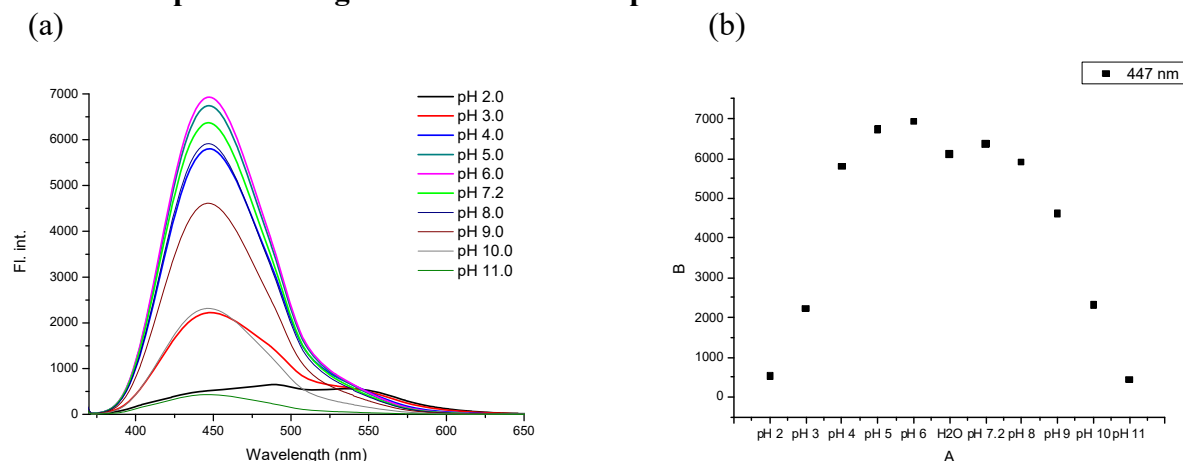


Figure S19. (a) Fluorescence spectra changes and (b) intensity changes of **5d** (5 μ M) at 447 nm at a various pHs in 90% HEPES (10 mM) buffer containing 10% MeOH

Quantum yields

The relative quantum yields of tolans were obtained by comparison with the known quantum yields of 4-methyl-7-aminocoumarin. In the comparative method, the quantum yields are calculated using the slope of the line determined from the plot of the absorbance against the integrated fluorescence intensities as shown in Table 1. Using this method, the quantum yields were calculated using the equation: $Q = Q_r(m/m_r)(n/n_r)$, where m is the slope of the line obtained from the plot of the integrated fluorescence intensity vs. absorbance; n is the refractive index of the solvent and the subscript r refers to Coumarin-120 of known quantum yield. The value of (n/n_r) is unity if the measurements of the unknown compounds are conducted in the same solvent used for the reference compounds.

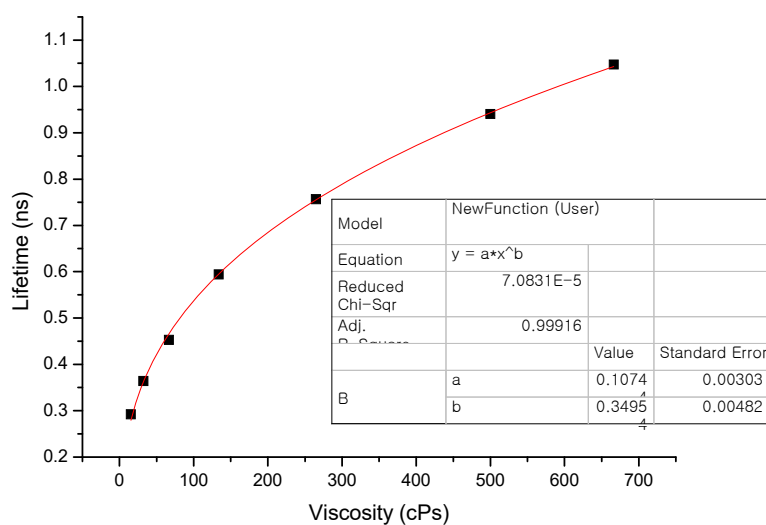


Figure S20. Plot of fluorescence lifetime (τ) of **5d** vs viscosity (η) that fits to the equation of $\tau = z k_0 \eta^\alpha$ where k_0 is the radiative rate constant, and z and α are constants.

Properties of 5d as a viscosity sensor

Determination of Viscosity and Fluorescence Lifetime

Viscosity was measured with a DV-1 viscometer at 25 °C. Each solution of **5d** with a specific viscosity were prepared by adding 1 mL of **5d** (0.05 mM in MeOH) to 9 mL of solvent mixture (methanol-glycerol systems) to obtain the final concentration of **5d** (5 µM). These solutions were shaken for 0.5 min. After standing for 2 hours at 25 °C to eliminate air bubbles, the solutions were measured in a UV spectrophotometer and a fluorescence spectrophotometer. A Fluo Time 200 lifetime fluorometer was used to obtain the fluorescence lifetimes of compounds, with the excitation wavelength at 375 nm and detection at 441 nm.

Cell Incubation

HeLa cells were cultured in DEME (Invitrogen) supplemented with 10% FCS (Invitrogen). One day before imaging, cells were seeded into 24-well flat-bottomed plates. The next day, the cells were incubated with 5.0 µM of **5d** for 0.5 h at 37 °C under 5% CO₂ and washed with phosphate-buffered saline (PBS) three times. Cells were fixed for 10 minutes at room temperature with 4% paraformaldehyde in PBS followed by 2-3 washes with PBS to remove excess formaldehyde.

Experimental details for fluorescence lifetime measurement

Probe molecule immersed cells as well as control cells were imaged using a confocal microscope (TCS SP8, Leica). Fluorescence lifetime imaging (FLIM) study was performed using an inverted-type scanning confocal microscope (MicroTime-200, Picoquant, Germany) with a 40× (air) objective (UPLSAPO, Olympus). The lifetime measurements were performed at the Korea Basic Science Institute (KBSI), Daegu Center, Korea. Single-mode pulsed diode lasers (375 nm with a pulse width of ~30 ps) were used as excitation sources. A dichroic mirror (Z375RDC, AHF), a longpass filter (HQ405lp, AHF), a 75 µm pinhole, a band-pass filter, and an avalanche photodiode detector (PDM series, MPD) were used to collect emissions from the cells. Time-correlated single-photon counting (TCSPC) technique was used to count fluorescence photons. TRPL images consisted of 200×200 pixels were recorded using the time-tagged time-resolved (TTTR) data acquisition method. Exponential fittings for the obtained fluorescence decays with a temporal resolution of 8 ps were performed using Symphotime-64 software (Ver. 2.2) by the bi-exponential decay model; $I(t) = \sum A_i e^{-t/\tau_i}$, where $I(t)$ is the time-dependent PL intensity, A is the amplitude, τ is the PL lifetime, and i is 2.

NMR Spectra of tolan derivatives

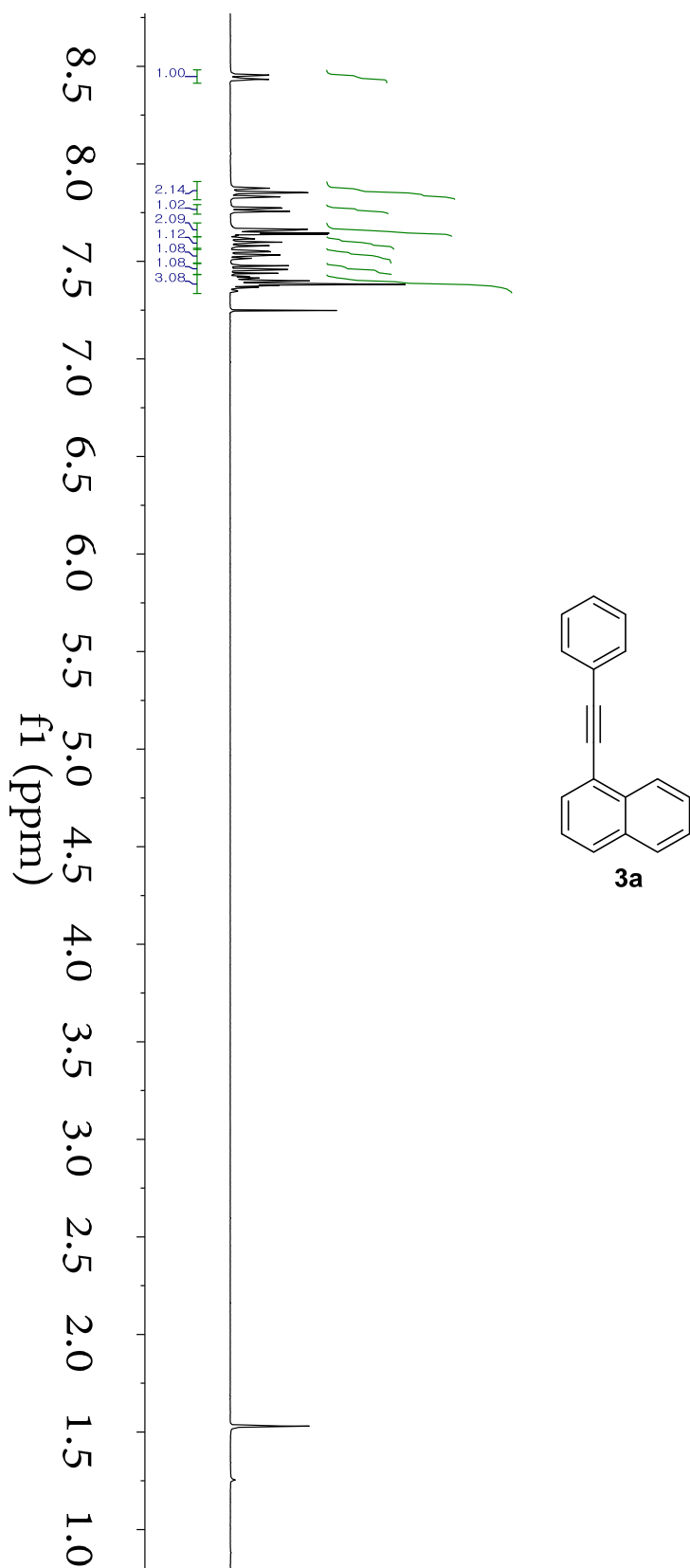


Figure S21. ^1H NMR spectrum of **3a** recorded in CDCl_3 at 25°C (400 MHz)

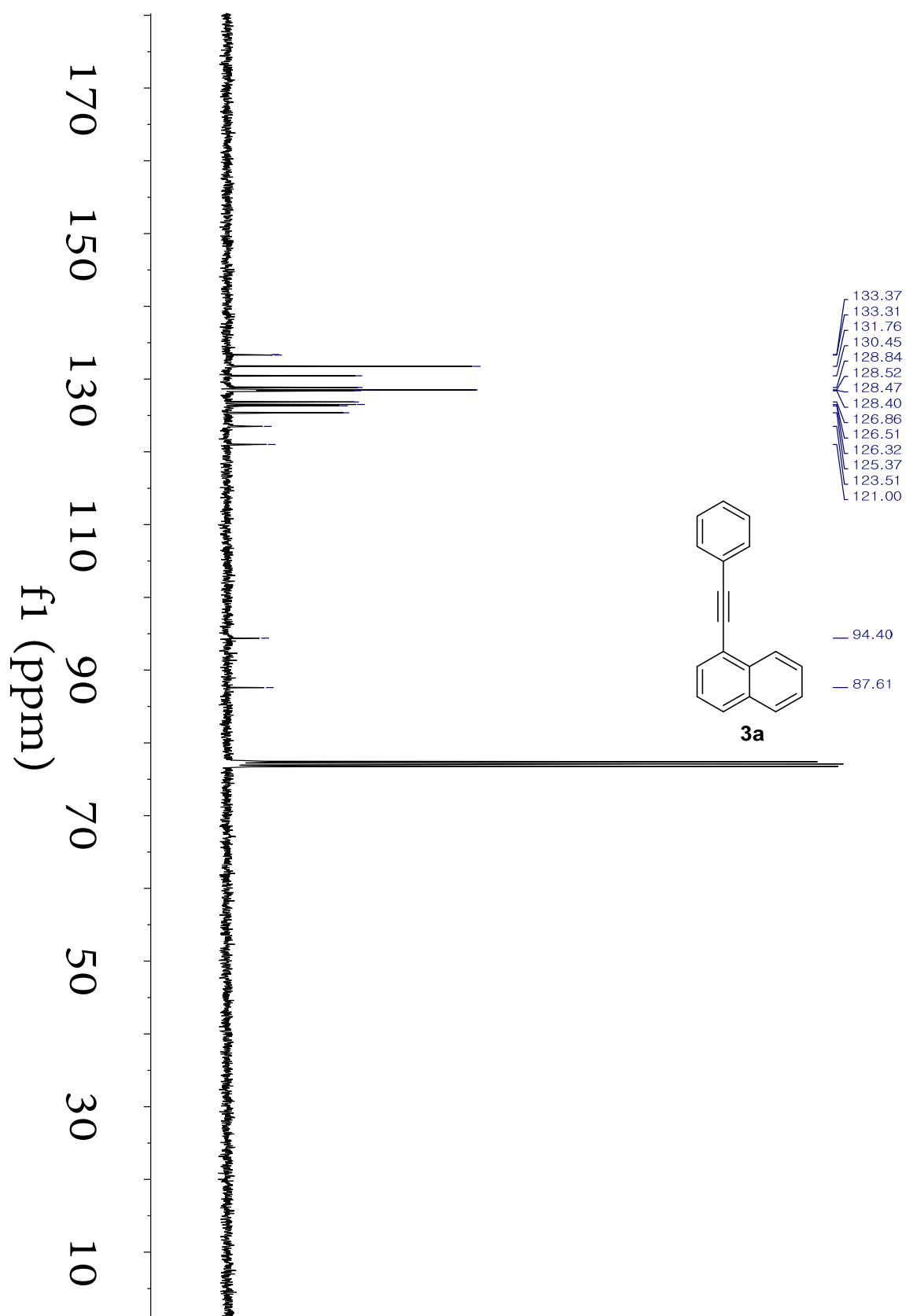


Figure S22. ¹³C NMR spectrum of **3a** recorded in CDCl₃ at 25°C (400 MHz)

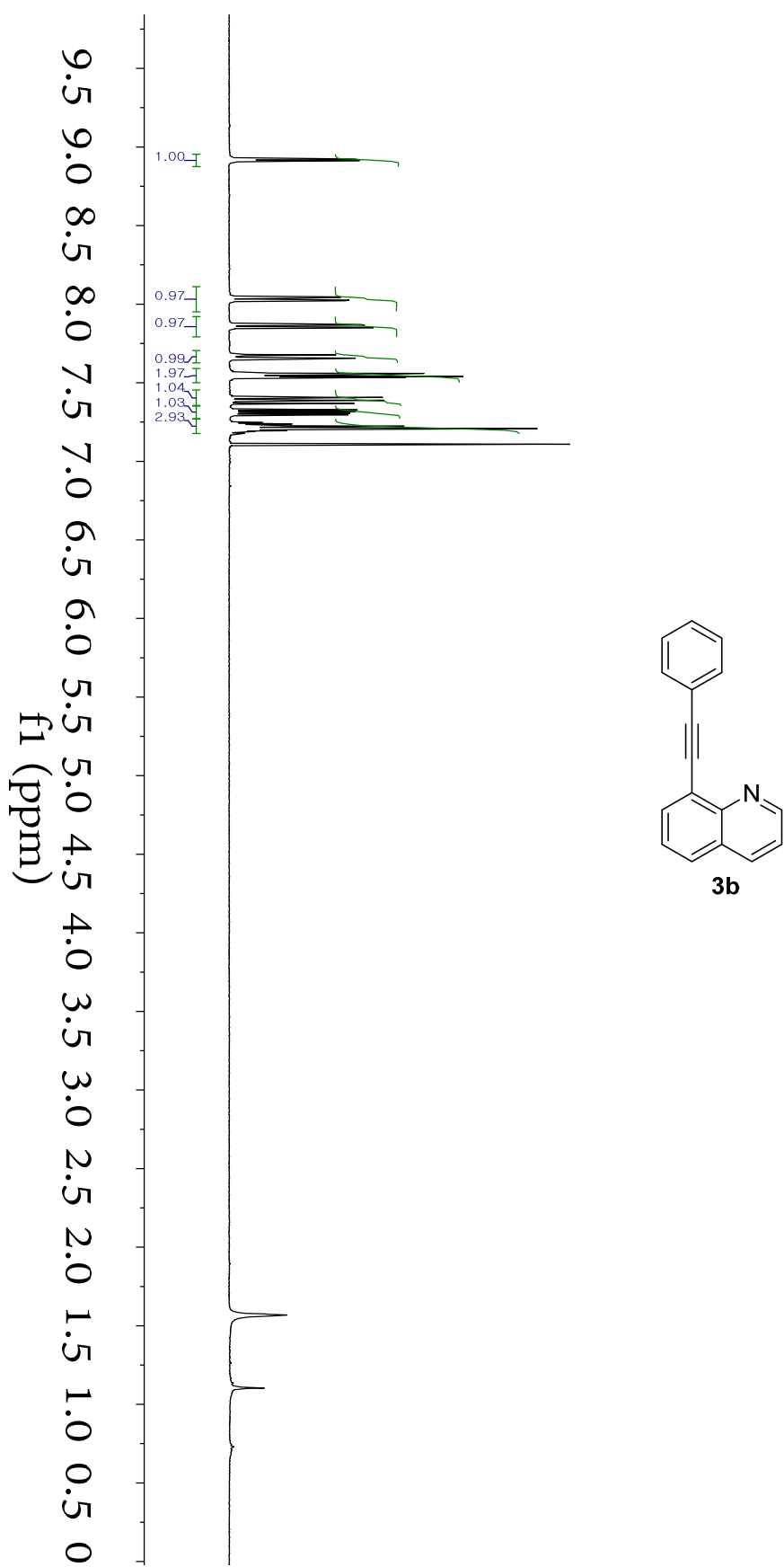


Figure S23. ^1H NMR spectrum of **3b** recorded in CDCl_3 at 25°C (400 MHz)

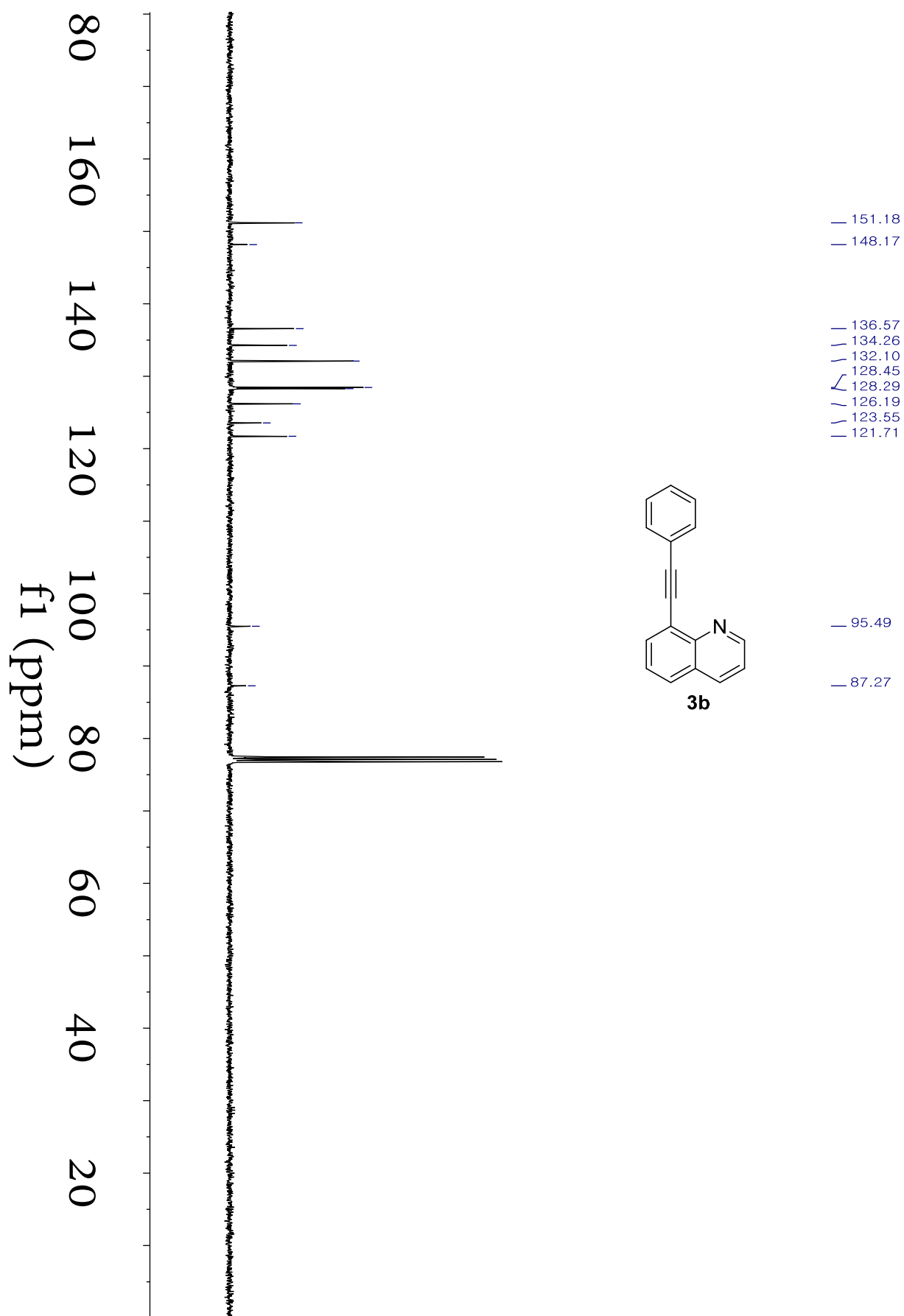


Figure S24. ^{13}C NMR spectrum of **3b** recorded in CDCl_3 at 25°C (400 MHz)

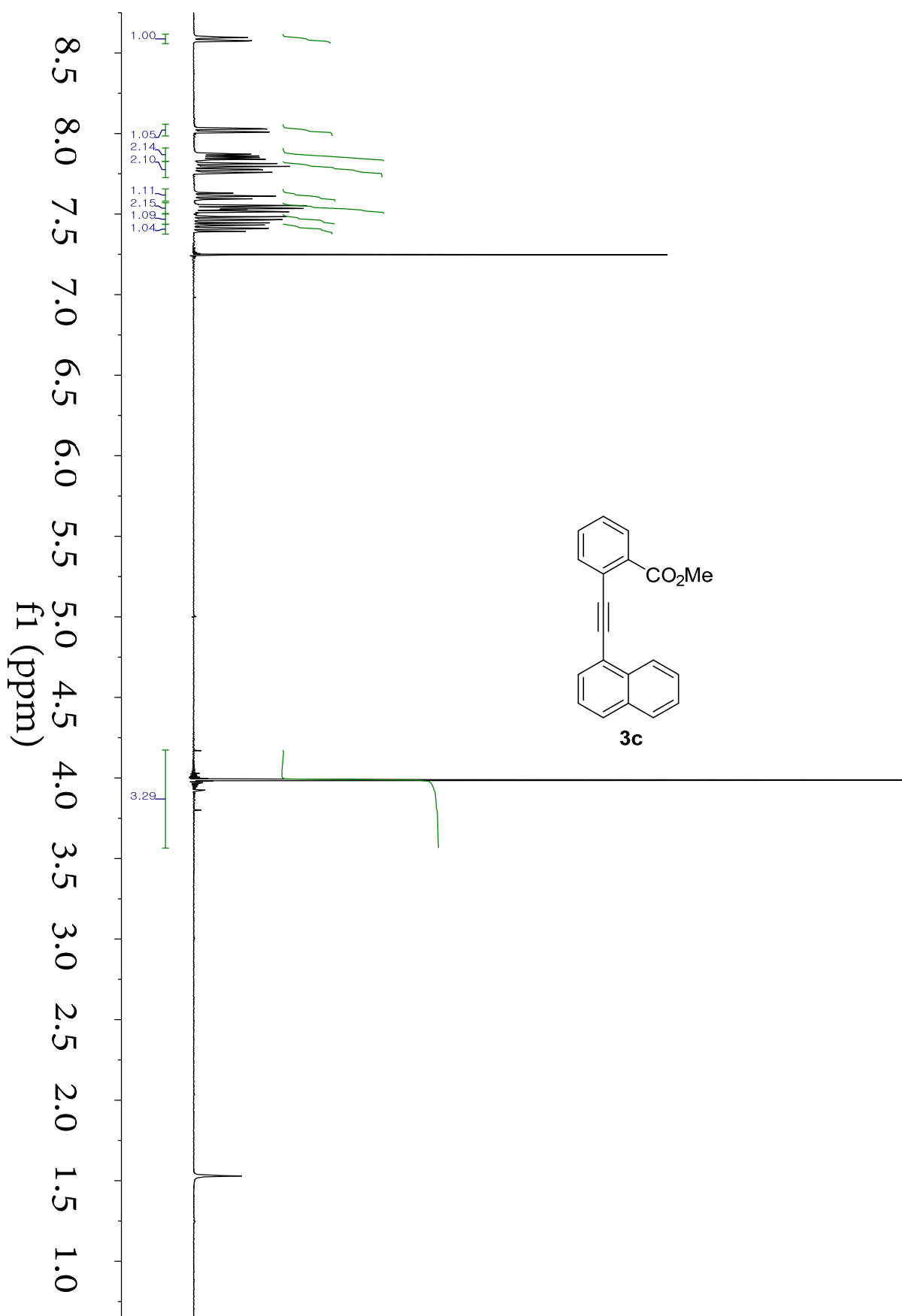


Figure S25. ¹H NMR spectrum of **3c** recorded in CDCl₃ at 25°C (400 MHz)

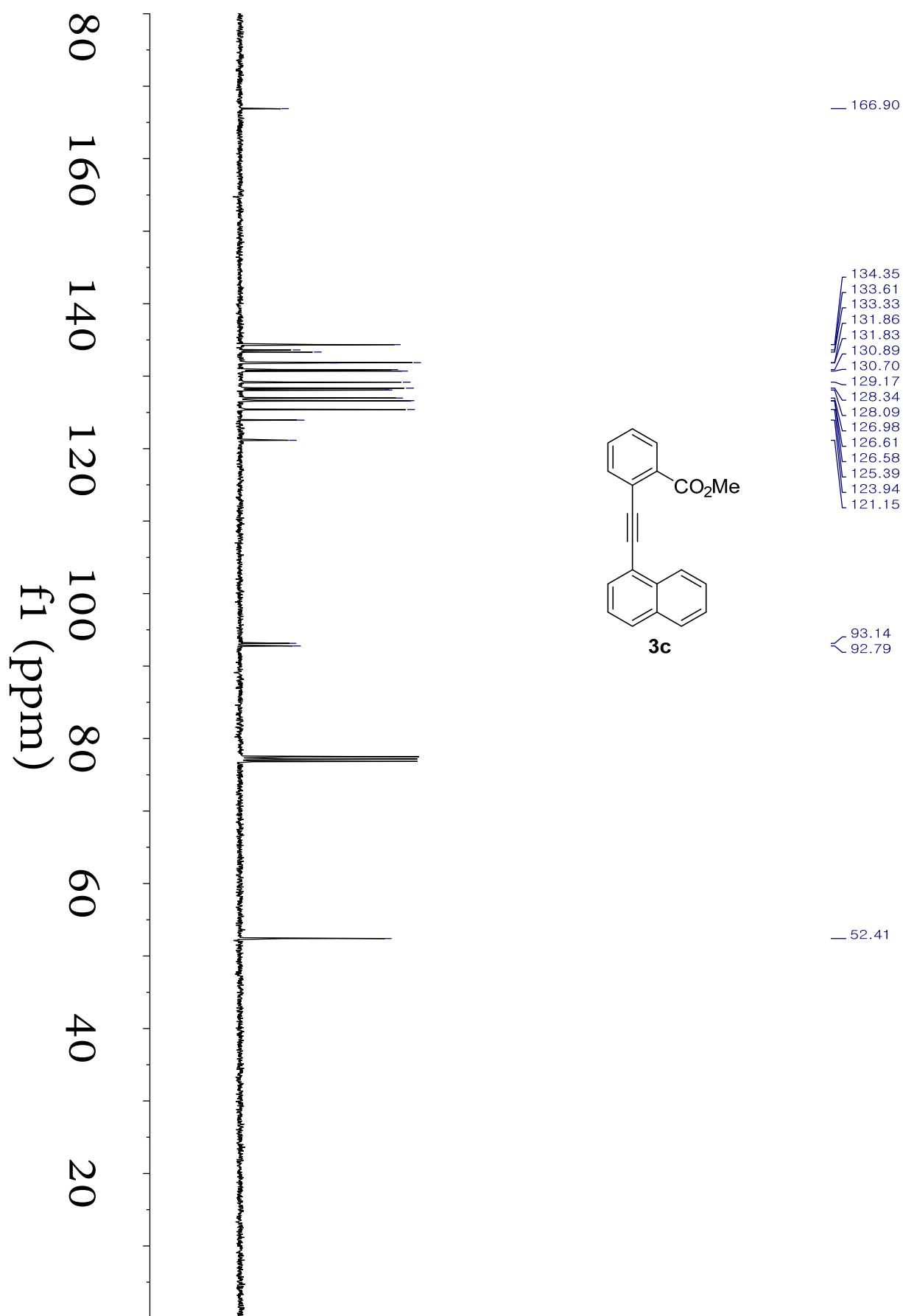


Figure S26. ^{13}C NMR spectrum of **3c** recorded in CDCl_3 at 25°C (400 MHz)

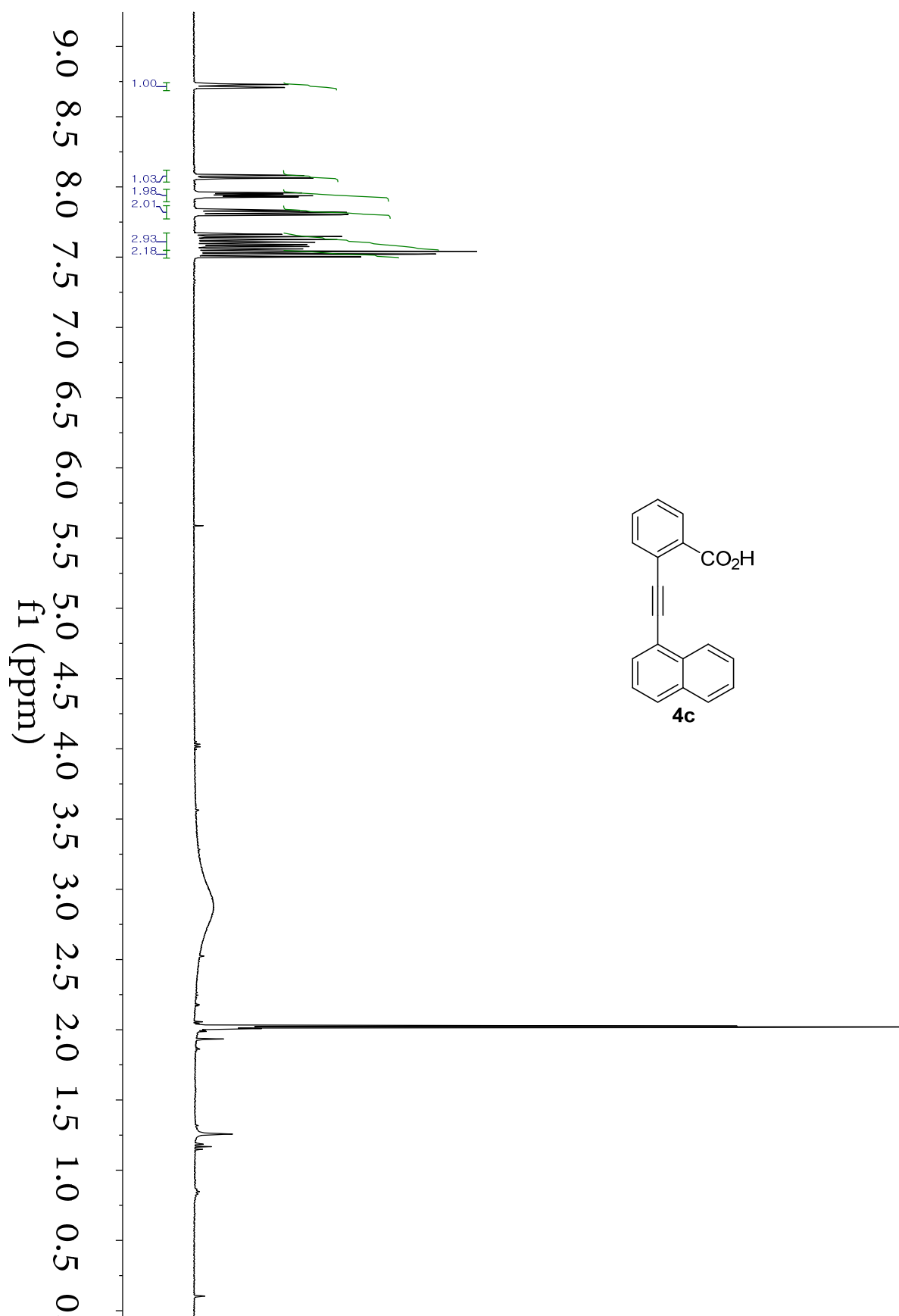


Figure S27. ¹H NMR spectrum of **4c** recorded in acetone-*d*₆ at 25°C (400 MHz)

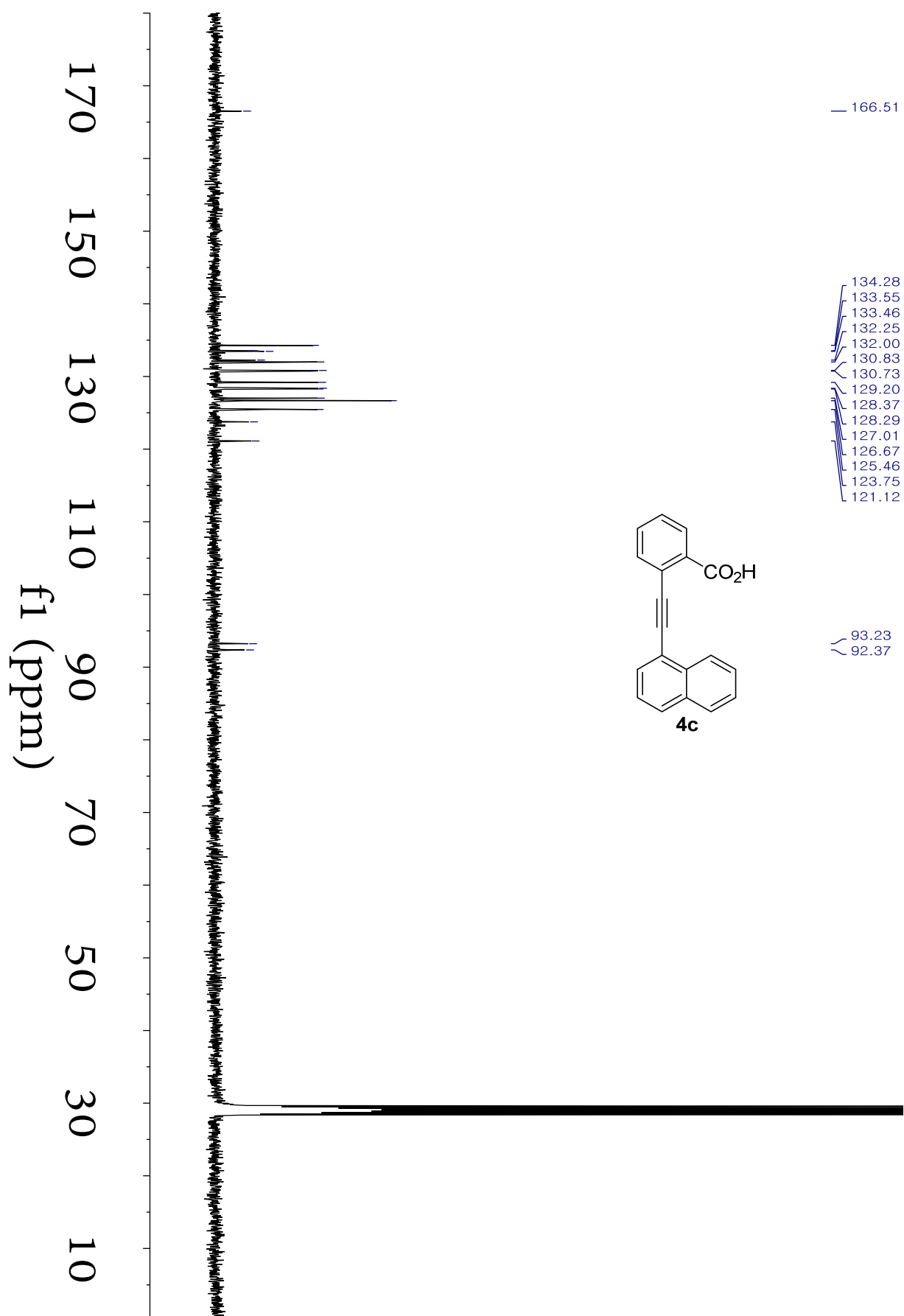


Figure S28. ¹³C NMR spectrum of **4c** recorded in acetone-*d*₆ at 25°C (400 MHz)

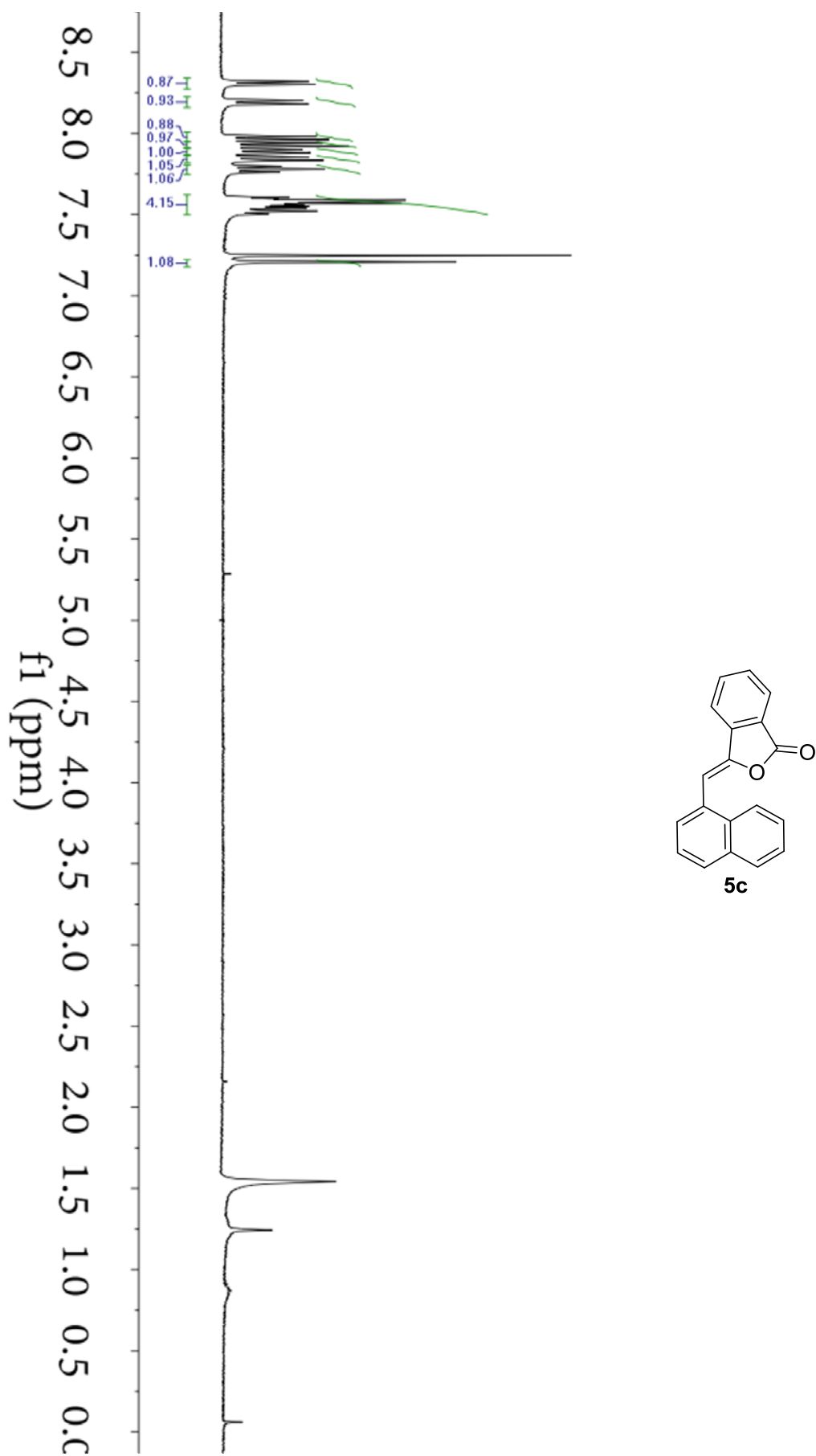


Figure S29. ^1H NMR spectrum of **5c** recorded in CDCl_3 at 25°C (400 MHz)

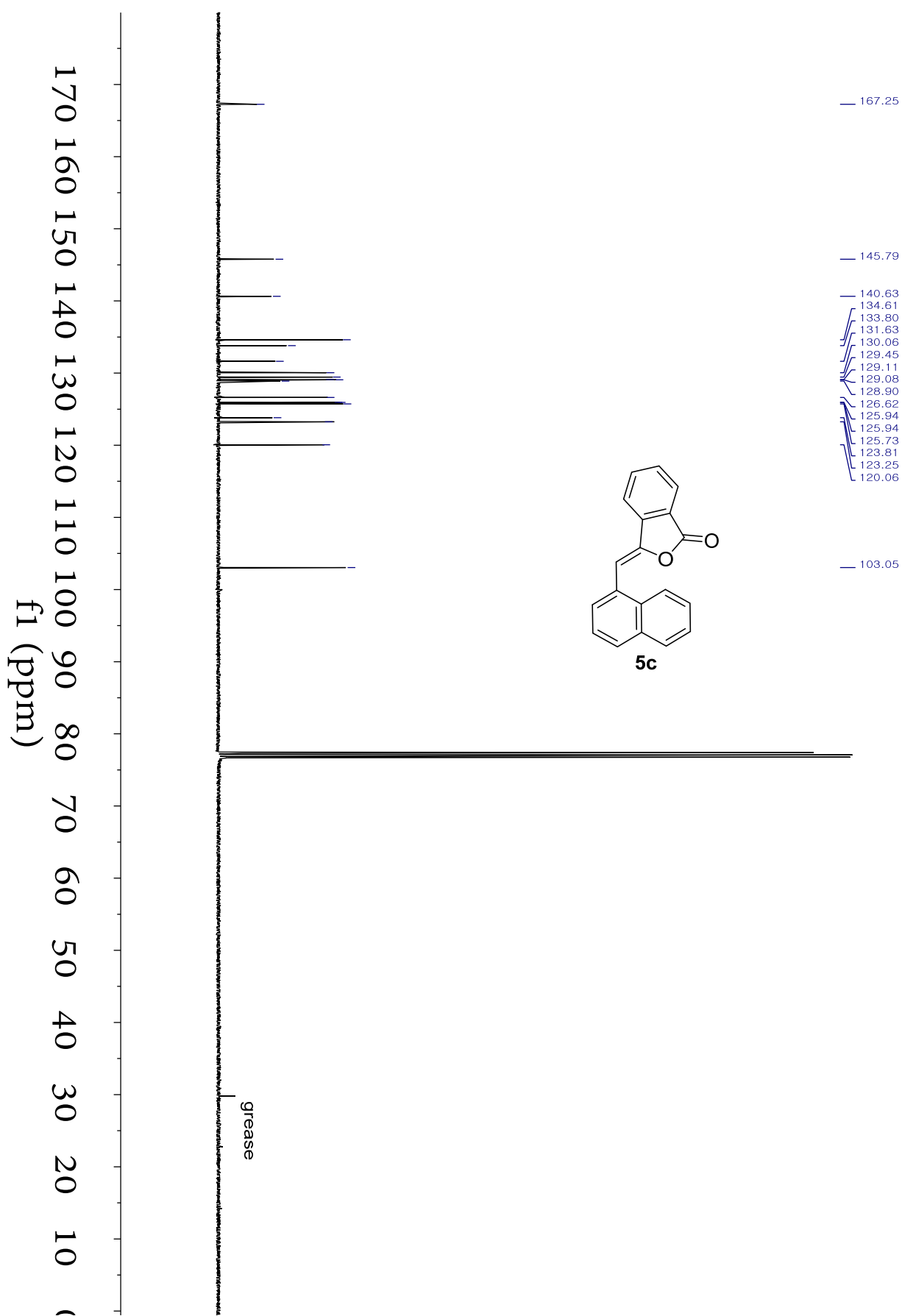


Figure S30. ¹³C NMR spectrum of **5c** recorded in CDCl₃ at 25°C (400 MHz)

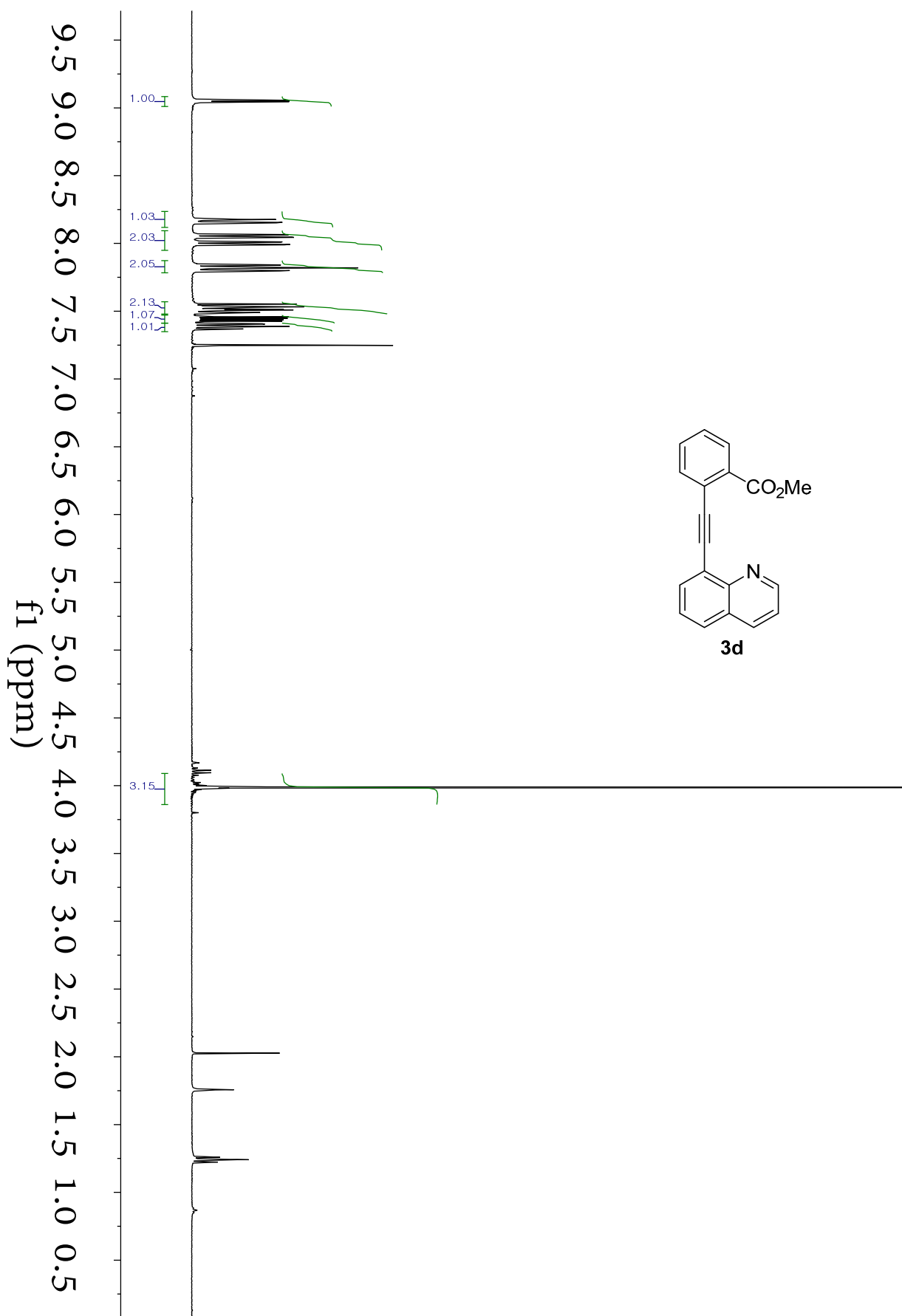


Figure S31. ¹H NMR spectrum of **3d** recorded in CDCl₃ at 25°C (400 MHz)

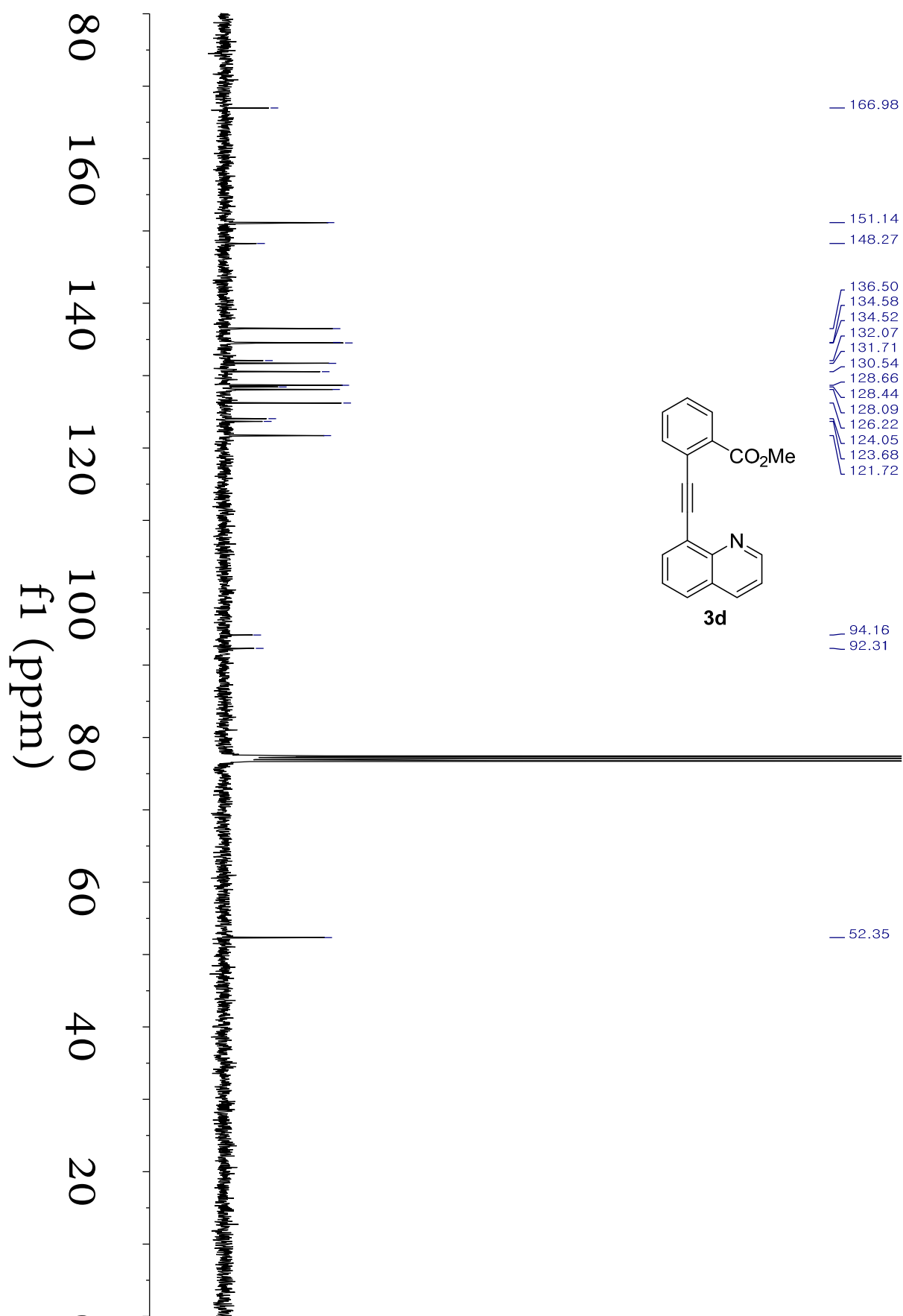


Figure S32. ¹³C NMR spectrum of **3d** recorded in CDCl₃ at 25°C (400 MHz)

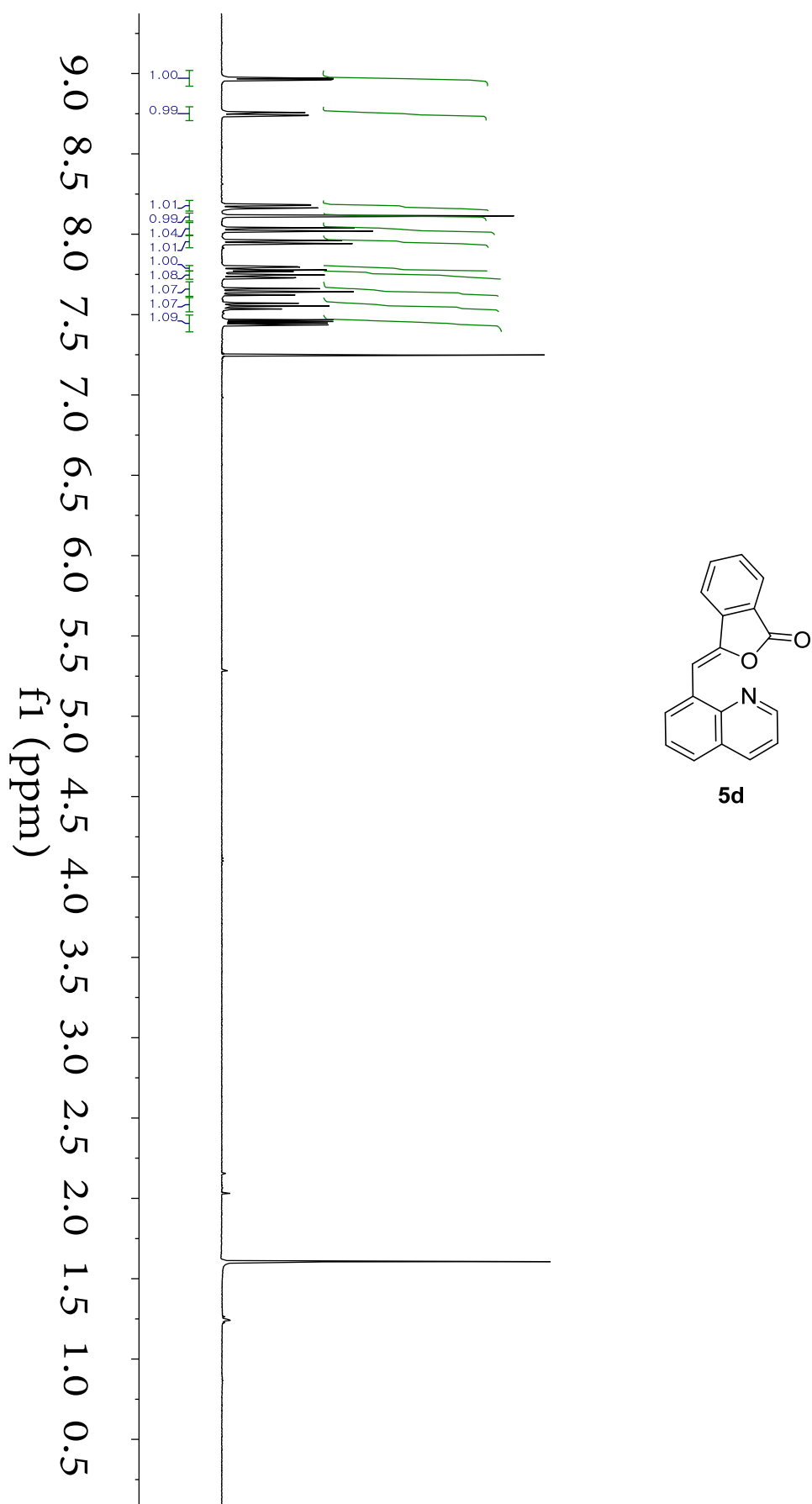


Figure S33. ^1H NMR spectrum of **5d** recorded in CDCl_3 at 25°C (400 MHz)

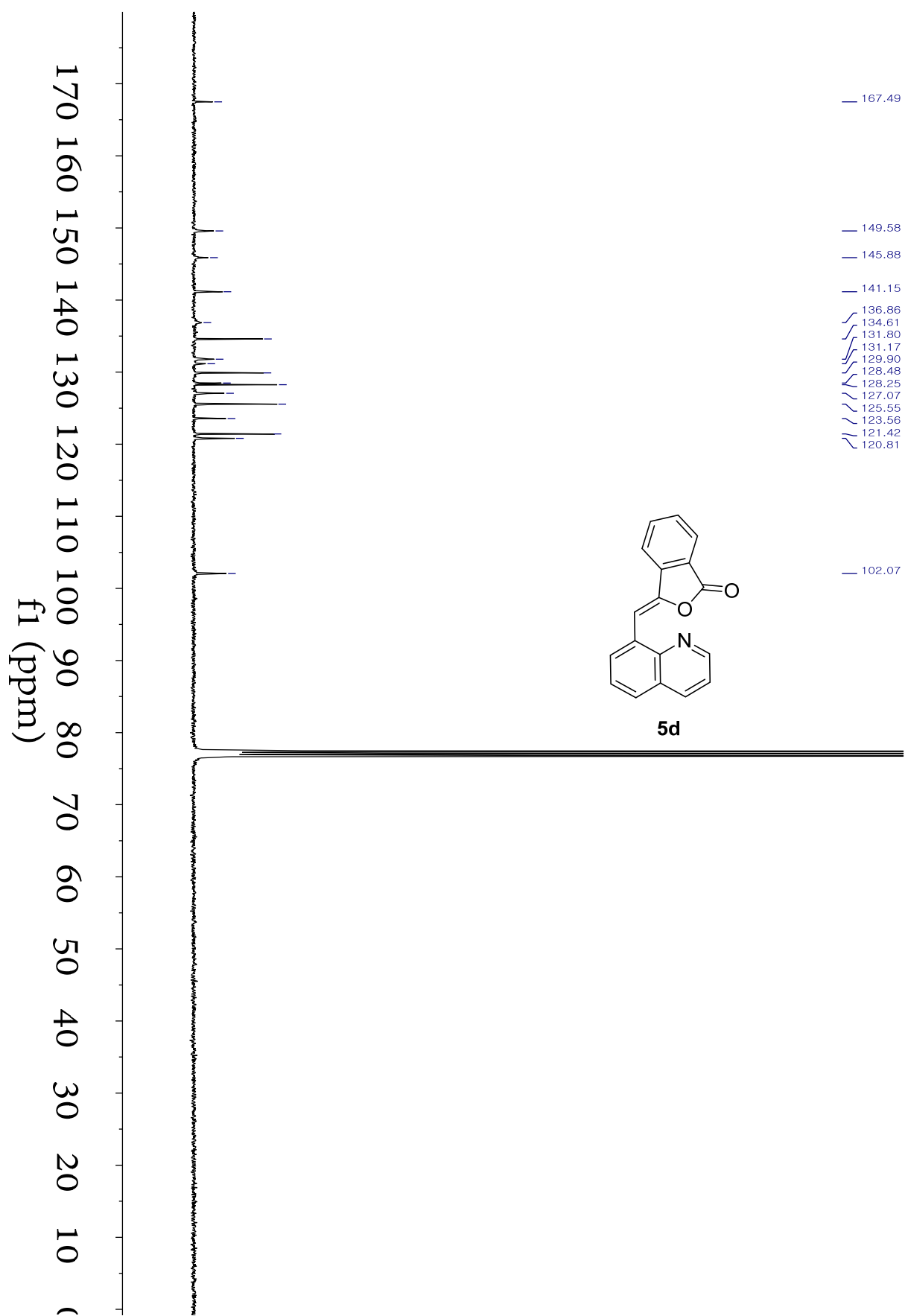


Figure S34. ¹³C NMR spectrum of **5d** recorded in CDCl₃ at 25°C (400 MHz)

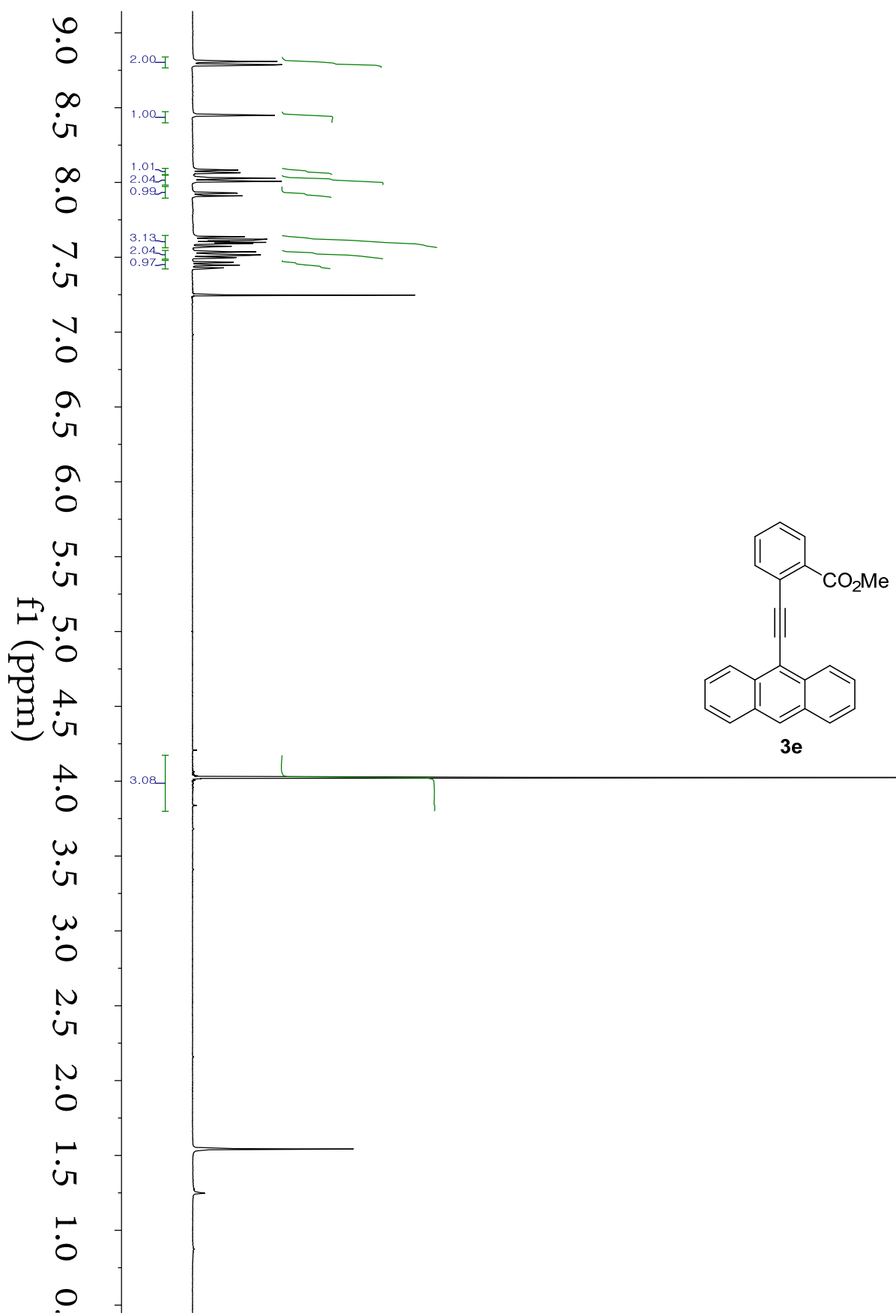


Figure S35. ^1H NMR spectrum of **3e** recorded in CDCl_3 at 25°C (400 MHz)

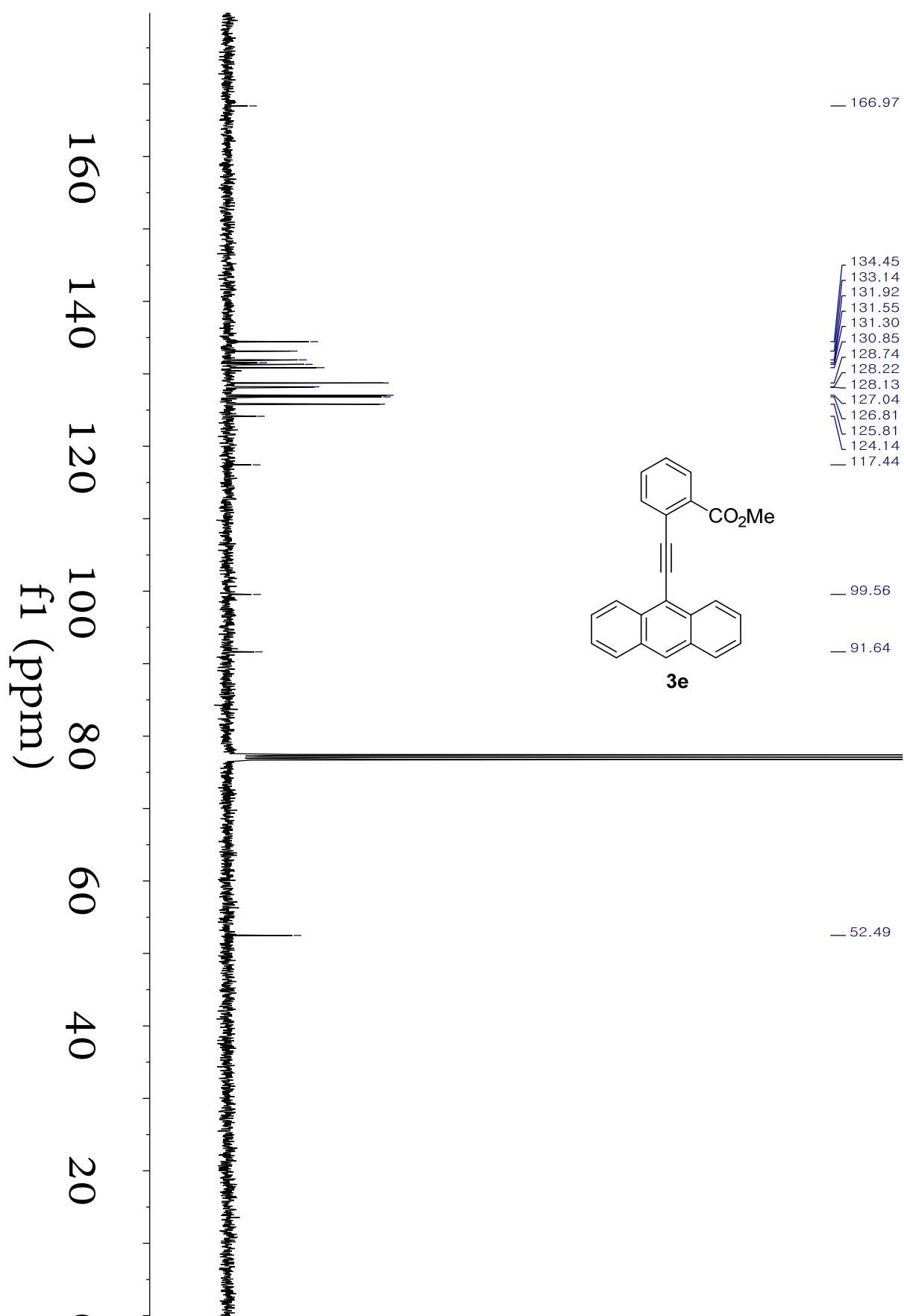


Figure S36. ¹³C NMR spectrum of **3e** recorded in CDCl₃ at 25°C (400 MHz)

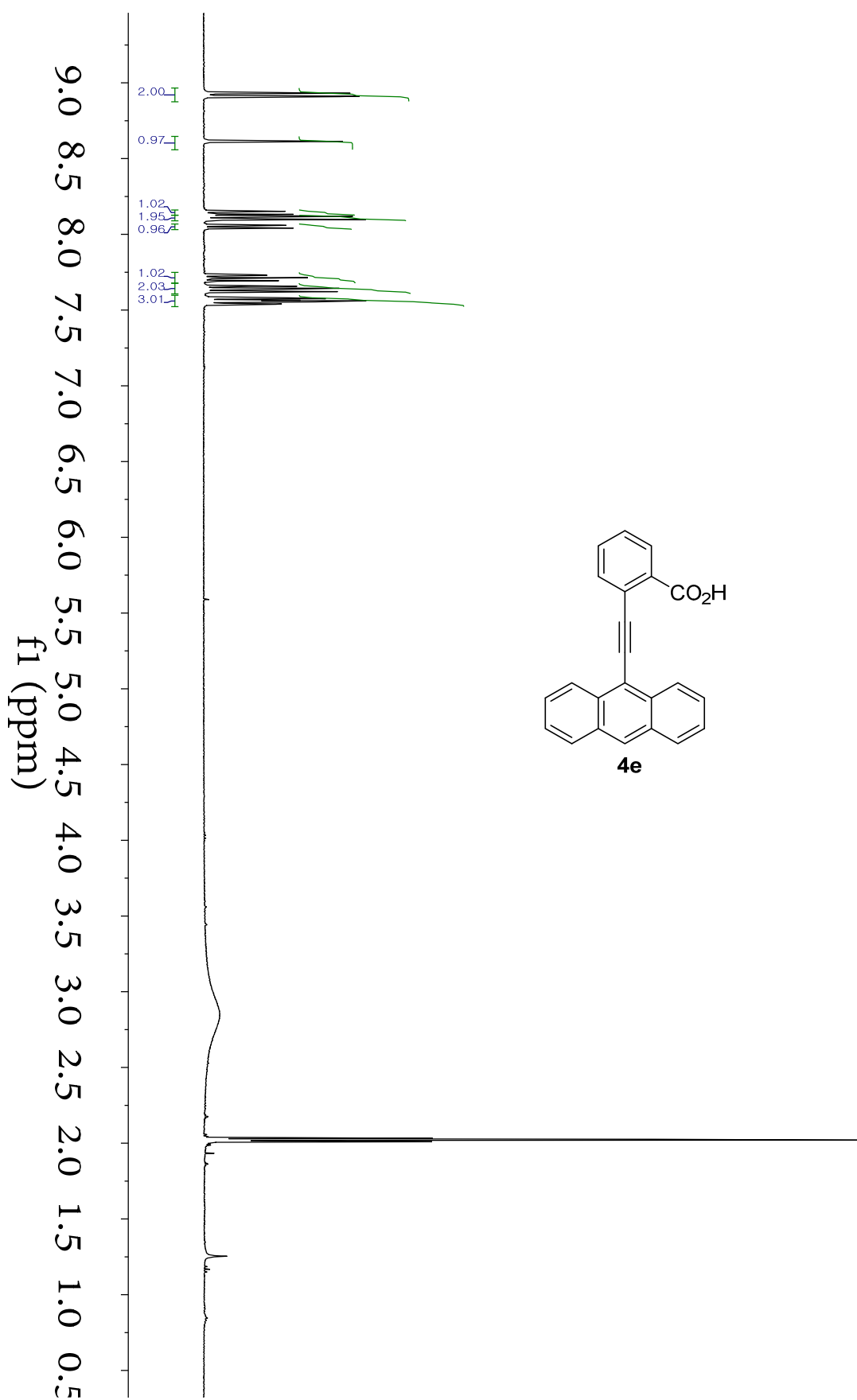


Figure S37. ^1H NMR spectrum of **4e** recorded in $\text{acetone-}d_6$ at 25°C (400 MHz)

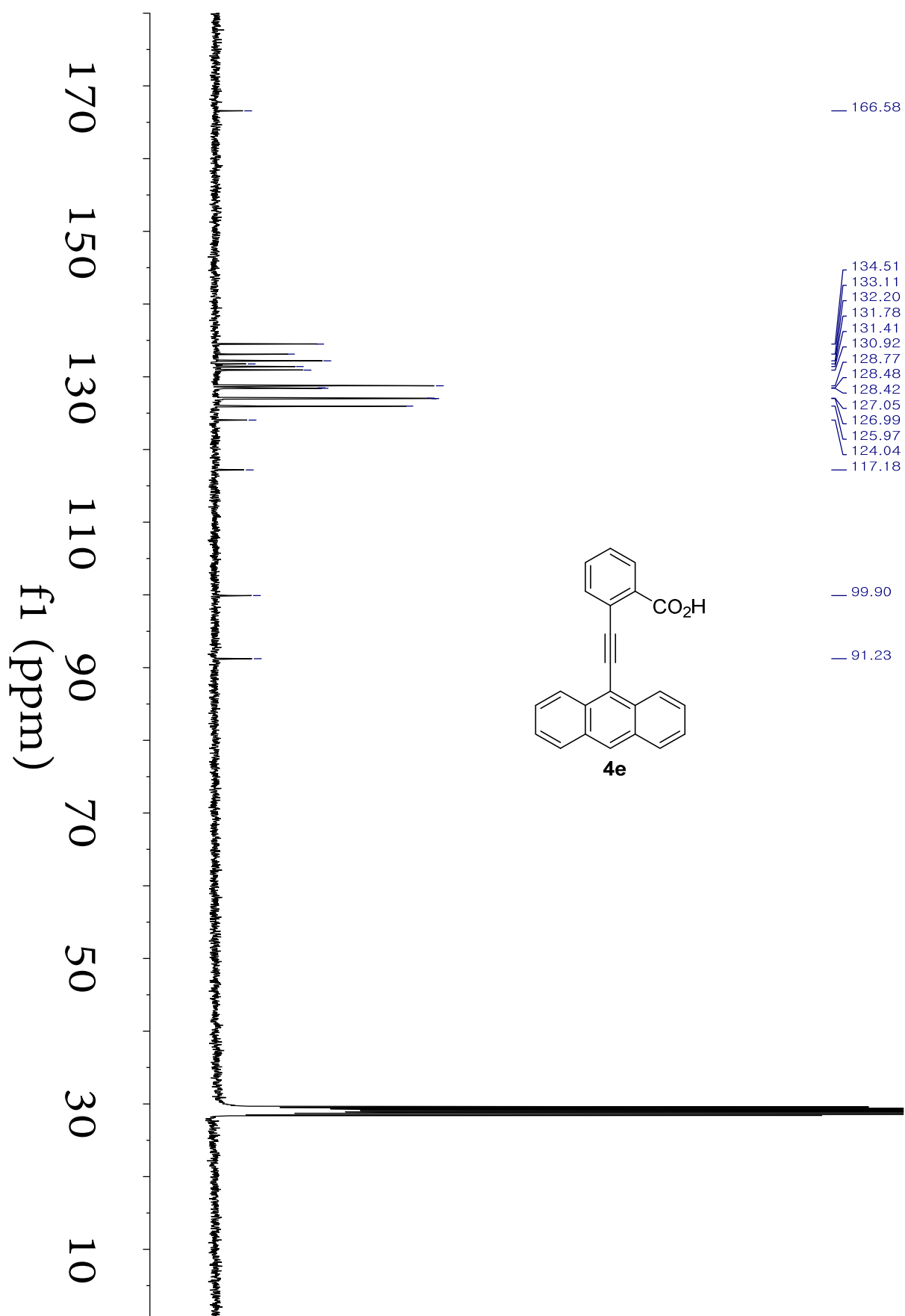


Figure S38. ¹³C NMR spectrum of **4e** recorded in acetone-*d*₆ at 25°C (400 MHz)

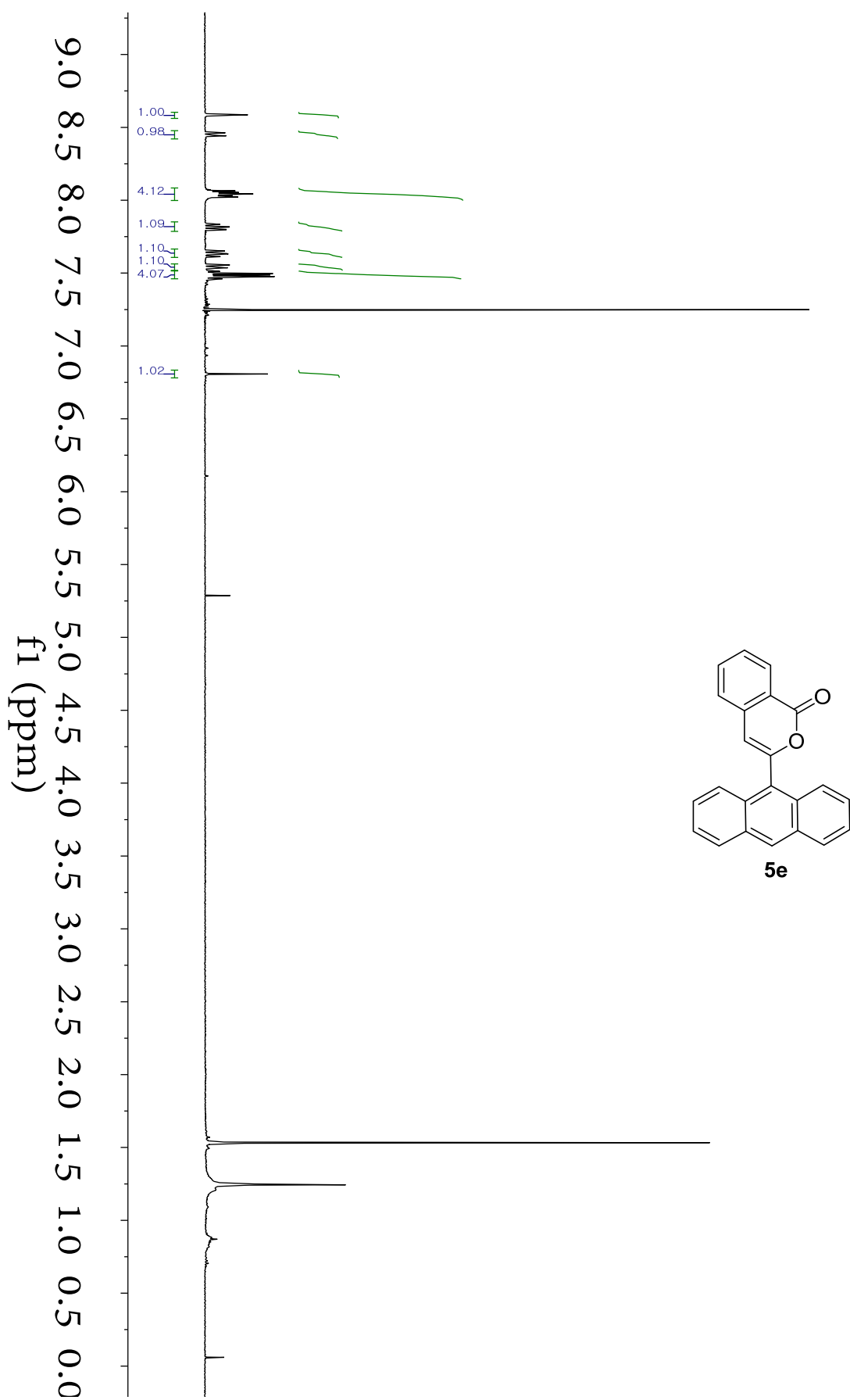


Figure S39. ¹H NMR spectrum of **5e** recorded in CDCl₃ at 25°C (400 MHz)

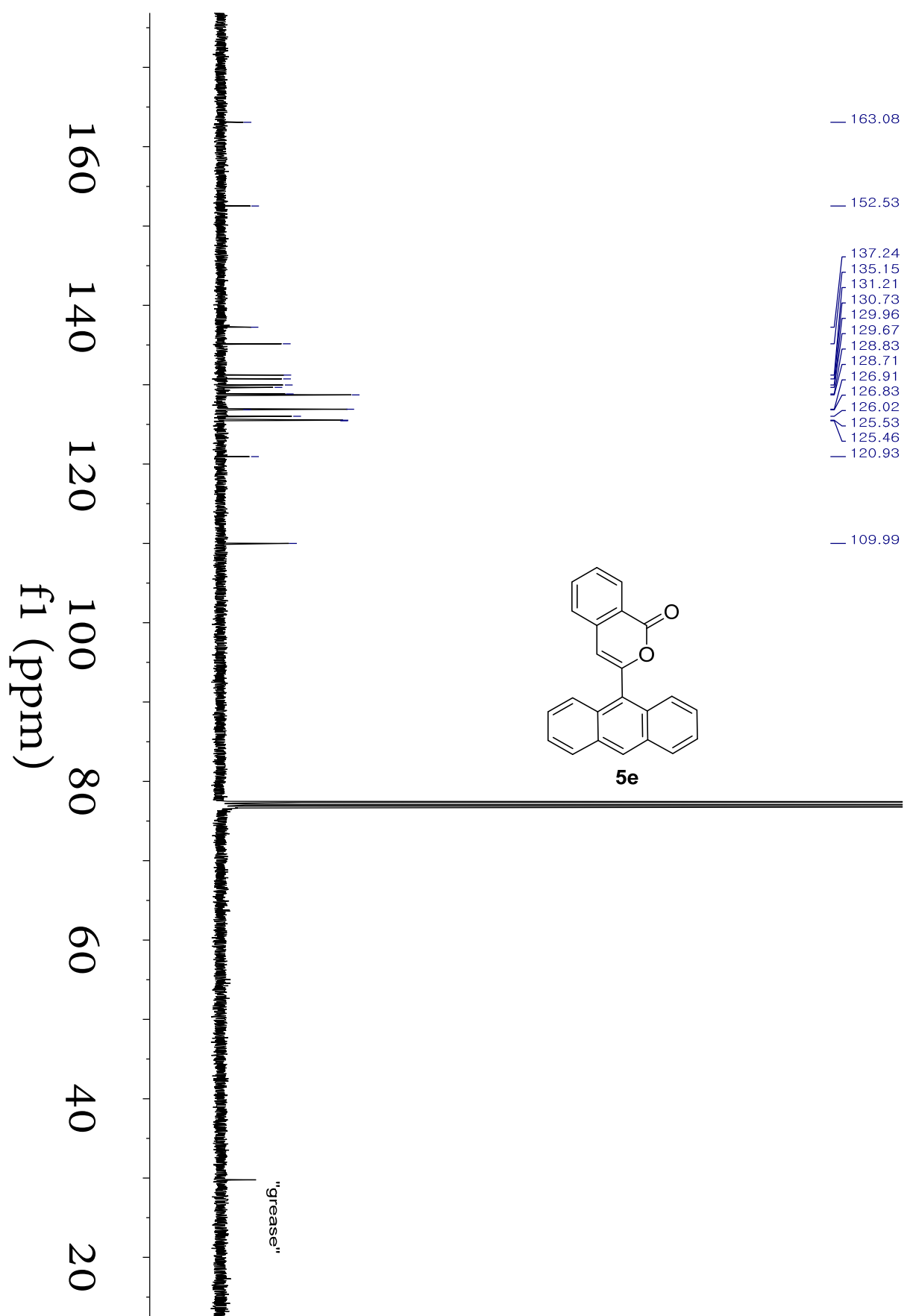


Figure S40. ¹³C NMR spectrum of **5e** recorded in CDCl₃ at 25°C (400 MHz)