Modular and Divergent Synthesis of 2,*N*3-Disubstituted 4-Quinazolinones Facilitated by Regioselective *N*-Alkylation

Kelly E. Kim,* Jason R. Comber, Alexander J. Pursel, Grant C. Hobby, Carter J. McCormick, Matthew F. Fisher, Kyle Marasa, and Benjamin Perry

Sciences and Mathematics Division, School of Interdisciplinary Arts and Sciences, University of Washington Tacoma, 1900 Commerce Street, Tacoma, WA 98402 (USA)

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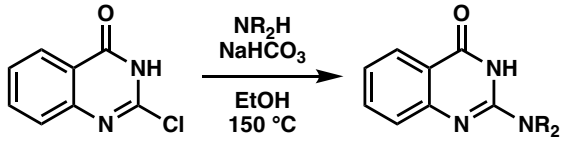
Materials and Methods

Unless noted in the specific procedure, reactions were performed in flame-dried or oven-dried glassware under nitrogen atmosphere. Dried and deoxygenated solvents (Fisher Scientific) were prepared by passage through columns of activated aluminum before use.[1] Commercial reagents (Sigma Aldrich, Combi-Blocks, or Alfa Aesar) were used as received. 2-chloroquinazolin-4(3*H*)-one (**4**) was prepared according to literature procedure[2] and matched previously reported characterization data.[3] Brine is defined as a saturated aqueous solution of sodium chloride. Reactions requiring external heat were modulated to the specified temperatures using a Fisherbrand Isotemp hot plate equipped with a glass thermometer. Reaction progress was monitored by thin-layer chromatography (TLC). TLC was performed using E. Merck silica gel 60 F254 precoated plates (0.25 mm) and visualized by UV fluorescence quenching, potassium permanganate, or *p*-anisaldehyde staining. SiliCycle P60 Academic Silica gel (particle size 0.040–0.063 mm) was used for flash chromatography.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVance spectrometer equipped with a BrukerPABBI-ATMA 1H {X-BB} multinuclear probe (300 MHz and 75 MHz, respectively) or a Bruker AVANCE DRX series spectrometer equipped with a triple resonance TBI probe (500 MHz and 126 MHz, respectively), and are reported in terms of chemical shift relative to residual $CHCl_3$ (δ 7.26 and δ 77.16 ppm, respectively). Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm) (multiplicity, coupling constant (Hz), integration). Abbreviations are used as follows: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = complex multiplet. Infrared (IR) spectra were recorded using thin film samples on a Perkin Elmer Spectrum 100 spectrometer equipped with a diamond/ZnSe UATR and are reported in frequency of absorption (cm^{-1}). High-resolution mass spectra (HRMS) were obtained using an Agilent 1290 Infinity UPLC coupled to an Agilent 6530 quadrupole time-of-flight high resolution mass spectrometry (qTOF-HRMS) under ESI+ ionization at the *m/z* range of 100–1700. Melting point data were measured with a Vernier Melt Station Melting Temperature Sensor equipped with a Vernier LabQuest3 temperature console or an Electrothermal Mel-Temp apparatus equipped with a Fluke 51 II thermometer.

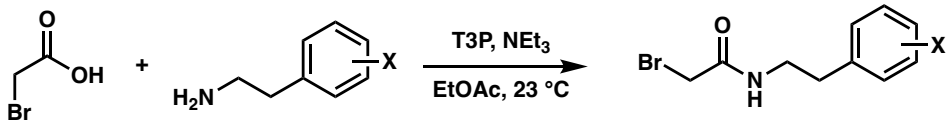
General Experimental Procedures



4 NR_2H = piperidine *2a* (84% yield)
pyrrolidine *2b* (97% yield)
NEt_2H *2c* (93% yield)

General Procedure A. *Preparation of aminated quinzolinones **2a–c**.*

To a suspension of quinazolinone **4** (100 mg, 0.55 mmol, 1.0 equiv) and sodium bicarbonate (231 mg, 2.75 mmol, 5.0 equiv) in ethanol (2 mL) at 23 °C was added amine (1.66 mmol, 3.0 equiv). The flask was sealed (via Kontes valve) and the resulting mixture was stirred and heated in an oil bath to 150 °C. After 6 hours, the reaction mixture was filtered over Celite and concentrated. The crude residue was purified by silica gel column chromatography (30% ethyl acetate in hexanes) to afford the aminated quinazolinone as a white solid.



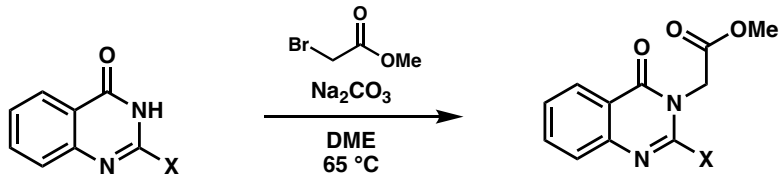
6 *7*

X = *p*-Cl, *m*-Cl, *o*-Cl, *p*-OMe, H

X = *p*-Cl *3a* (75% yield)
m-Cl *3b* (85% yield)
o-Cl *3c* (72% yield)
p-OMe *3d* (66% yield)
H *3e* (69% yield)

General Procedure B. *Preparation of Bromoamides **3a–e**.*

To a solution of bromoacetic acid (4.46 g, 32.1 mmol, 1.0 equiv) in ethyl acetate (40 mL) at 0 °C was added 1-propanephosphonic acid cyclic anhydride (50 wt % in ethyl acetate, 30.7 mL, 48.2 mmol, 1.5 equiv). While stirring the resulting mixture, a solution of substituted phenethylamine (32.1 mmol, 1.0 equiv) and triethylamine (8.96 mL, 64.3 mmol, 2.0 equiv) in ethyl acetate (20 mL) was added, and the reaction was allowed to warm up gradually overnight. The reaction mixture was washed with H_2O (2 x 30 mL), and brine (30 mL), and the organic layer was dried over Na_2SO_4. After filtration and concentration, the crude material was purified by silica gel column chromatography (25% → 30% → 50% ethyl acetate in hexanes).



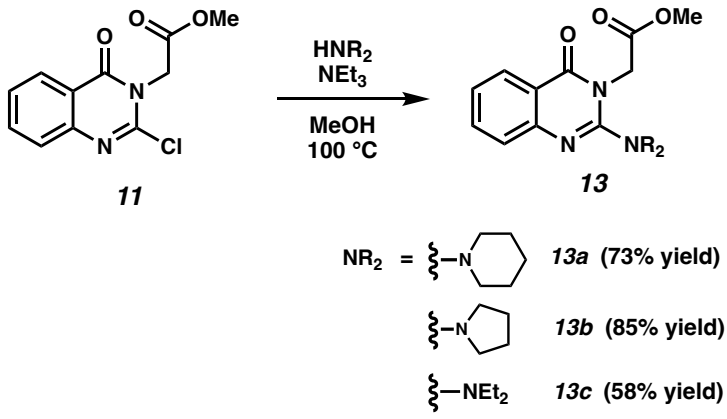
4 **X = Cl** ***11*** **X = Cl**

14 **X = H** ***16*** **X = H**

15 **X = CH_3** ***17*** **X = CH_3**

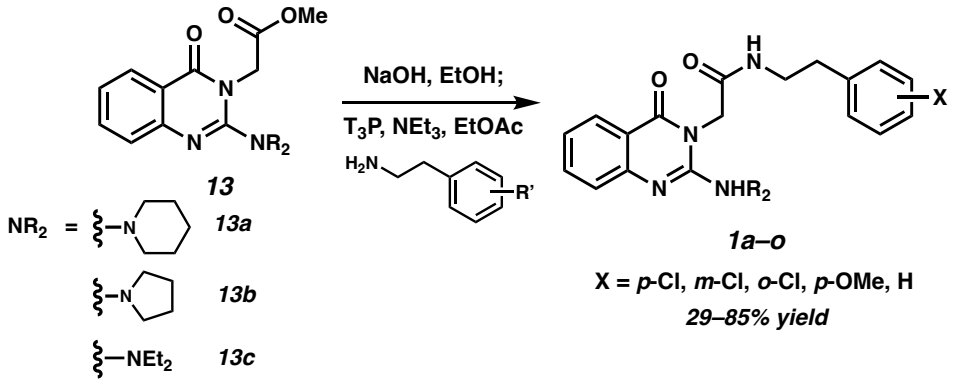
General Procedure C. *Alkylation of 4-Quinazolinones using Na_2CO_3 in DME.*

To a solution of quinazolinone **4**, **14**, or **15** (0.30 mmol, 1.0 equiv) in DME (3.0 mL) was added sodium carbonate (0.315 mmol, 1.05 equiv) at 23 °C. After stirring for 10 minutes, methyl bromoacetate (0.057 mL, 0.60 mmol, 2.0 equiv) was added dropwise, and the reaction flask was sealed and heated in an oil bath to 65 °C. After 1.5 hours, the reaction was removed from heat and poured into brine (10 mL) after 25 minutes, then extracted with ethyl acetate (6 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo to afford *N*-alkylated product **11**, **16**, or **17**.



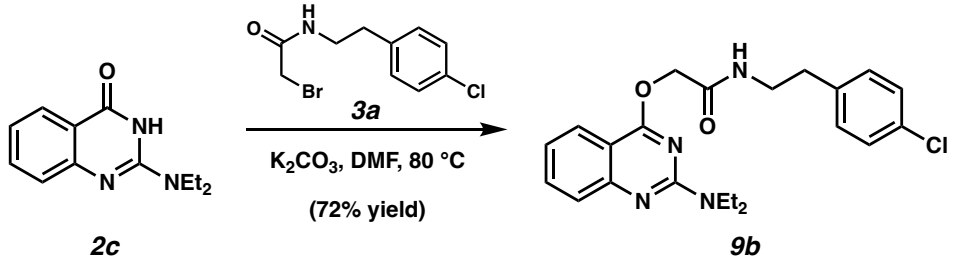
General Procedure D. *Preparation of 2-Amino-N3-Alkyl Quinazolinones **13a–c**.*

To a solution of methyl ester **11** (200 mg, 0.792 mmol, 1.0 equiv) in methanol (8.0 mL) at 23 °C was added amine **5a**, **5b**, or **5c** (3.96 mmol, 5.0 equiv) and triethylamine (0.55 mL, 3.16 mmol, 5.0 equiv) sequentially. The flask was sealed (via Kontes valve) and the resulting mixture was stirred and heated in an oil bath to 100 °C. After 2 hours, the reaction mixture was removed from heat, filtered over Celite, and concentrated. The crude residue was purified by silica gel column chromatography (20% to 25% to 50% ethyl acetate in hexanes) to afford aminoquinazolinone **13a**, **13b**, or **13c** as a white solid.



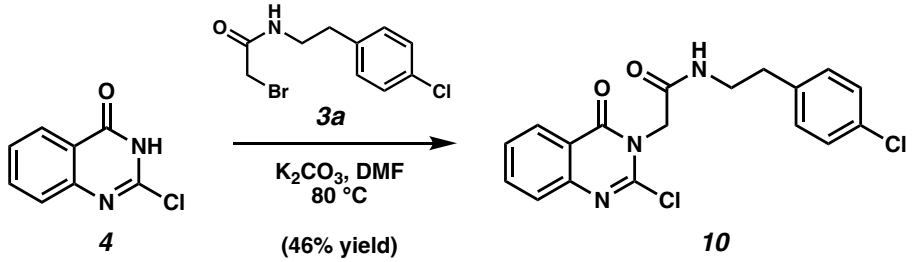
General Procedure E. *Hydrolysis–Amidation to Prepare Target Derivatives **1a–o**.*
To a solution of aminoquinazolinone **13** (0.05 mmol, 1.0 equiv) in ethanol (5 mL) was added a 1N solution of NaOH (0.3 mL, 0.3 mmol, 5.0 equiv). The resulting mixture was stirred vigorously at 23 °C. When TLC analysis showed complete conversion of **13** (typically after 24 hours), the reaction flask was cooled to 0 °C in an ice bath, and 0.1M HCl was added dropwise to adjust the pH to 4–5. The solvents were then removed under reduced pressure at 37 °C, and the resulting white solid was diluted with ethyl acetate (3 mL) and cooled to 0 °C. To this suspension was added 1-propanephosphonic acid cyclic anhydride (50 wt % in ethyl acetate, 0.05 mL, 0.16 mmol, 3.0 equiv). While stirring the resulting mixture, a solution of substituted phenethylamine (0.11 mmol, 2.0 equiv) and triethylamine (0.03 mL, 0.22 mmol, 4.0 equiv) in ethyl acetate (2 mL) was added, and the reaction was allowed to warm up gradually overnight. The reaction mixture was washed with H_2O (2 x 10 mL), and brine (10 mL), and the organic layer was dried over Na_2SO_4. After filtration and concentration, the crude material was purified by silica gel column chromatography (ethyl acetate/hexanes).

Quinazolinone Alkylation Procedures



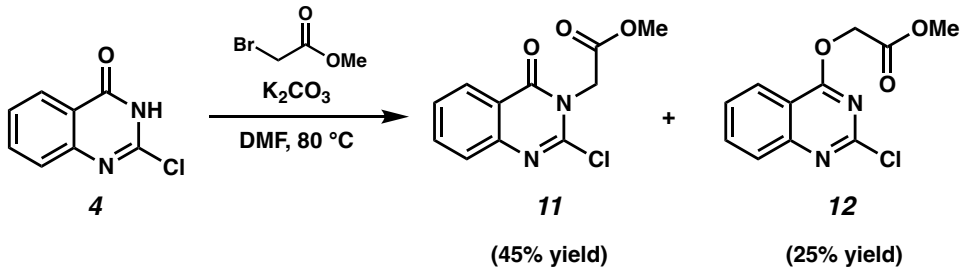
*Synthesis of Undesired O-alkylation product **9b**.*

A flame-dried Kontes-valve flask was charged with aminated quinazolinone **2c** (50 mg, 0.23 mmol, 1.0 equiv), bromoamide **3a** (76 mg, 0.28 mmol, 1.2 equiv), and potassium carbonate (40 mg, 0.292 mmol, 1.27 equiv) and diluted with DMF (5 mL). The flask was sealed and heated in an oil bath to 80 °C while the contents were stirred. After 2 hours, a pink color was observed, and after 22 hours, the reaction mixture had turned orange. At this time, the reaction was removed from heat and diluted with water (3 mL), then extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to afford a red-orange oil. Purification by silica gel column chromatography (20% ethyl acetate in hexanes) afforded the *O*-alkylation product **9b** as a white solid (69 mg, 72% yield).



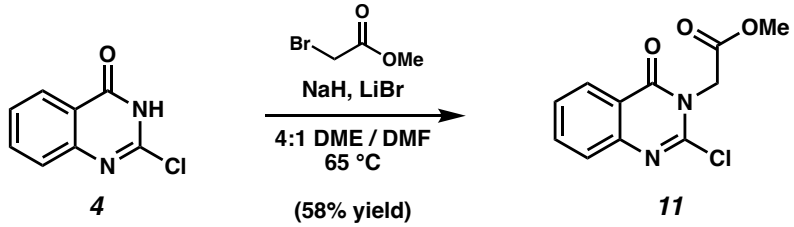
*Synthesis of 2-Chloro N3-Alkylamido N-Alkylation Product **10**.*

A flame-dried Kontes-valve flask was charged with aminated quinazolinone **2c** (50 mg, 0.23 mmol, 1.0 equiv), bromoamide **3a** (76 mg, 0.28 mmol, 1.2 equiv), and potassium carbonate (40 mg, 0.292 mmol, 1.27 equiv) and diluted with DMF (5 mL). The flask was sealed and heated in an oil bath to 80 °C while the contents were stirred. After 21 hours, the reaction had a bright orange color and was removed from heat and diluted with water (3 mL), then extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to afford a red-orange oil. Purification by silica gel column chromatography (20% to 30% ethyl acetate in hexanes) afforded the *N*-alkylation product **10** as a white solid (40 mg, 46% yield).



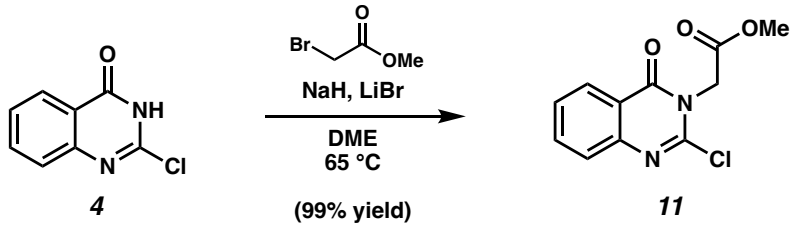
*Alkylation of **4** using Methyl Bromoacetate in DMF.*

A flame-dried Kontes-valve flask was charged with quinazolinone **4** (200 mg, 1.11 mmol, 1.0 equiv), methyl bromoacetate (0.13 mL, 1.41 mmol, 1.27 equiv), and potassium carbonate (184 mg, 1.33 mmol, 1.2 equiv) and diluted with DMF (17 mL). The flask was sealed and heated in an oil bath to 50 °C while the contents were stirred. After 20 hours, the reaction was removed from heat and diluted with water (3 mL), then extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (10% to 20% ethyl acetate in hexanes) to furnish *N*-alkylation product **11** as a white solid (125 mg, 45% yield) and *O*-alkylation product **12** as a white solid (70 mg, 25% yield).



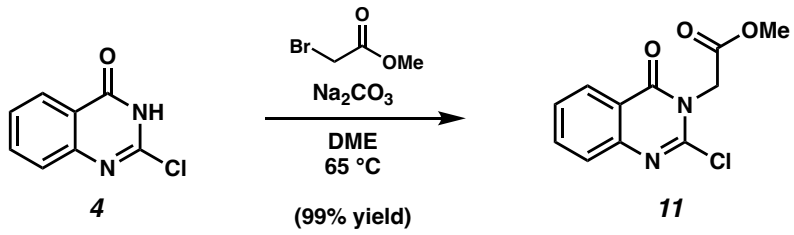
*Alkylation of **4** using Methyl Bromoacetate in 4:1 DME/DMF.*

To a solution of quinazolinone **4** (54 mg, 0.30 mmol, 1.0 equiv) in DME (2.4 mL) and DMF (0.6 mL) was added sodium hydride (60% dispersion in mineral oil, 13 mg, 0.315 mmol, 1.05 equiv) at 0 °C. After stirring for 10 minutes, lithium bromide (52 mg, 0.60 mmol, 2.0 equiv) was added, and the resulting mixture was stirred at 23 °C for 15 minutes. After this, methyl bromoacetate (0.057 mL, 0.60 mmol, 2.0 equiv) was added dropwise, and the reaction flask was sealed and heated in an oil bath to 65 °C. After 6.5 hours, the reaction was removed from heat and poured into brine (10 mL) after 25 minutes, then extracted with ethyl acetate (6 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford a white solid. The crude material was purified by silica gel column chromatography (10% ethyl acetate in hexanes) to furnish *N*-alkylation product **11** as a white solid (44 mg, 58% yield).



*Alkylation of **4** using Methyl Bromoacetate in DME.*

To a solution of quinazolinone **4** (54 mg, 0.30 mmol, 1.0 equiv) in DME (3.0 mL) was added sodium hydride (60% dispersion in mineral oil, 13 mg, 0.315 mmol, 1.05 equiv) at 0 °C. After stirring for 10 minutes, lithium bromide (52 mg, 0.60 mmol, 2.0 equiv) was added, and the resulting mixture was stirred at 23 °C for 15 minutes. After this, methyl bromoacetate (0.057 mL, 0.60 mmol, 2.0 equiv) was added dropwise, and the reaction flask was sealed and heated in an oil bath to 65 °C. After 1.5 hours, the reaction was removed from heat and poured into brine (10 mL) after 25 minutes, then extracted with ethyl acetate (6 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford **11** as a white solid that required no further purification (75 mg, 99% yield).

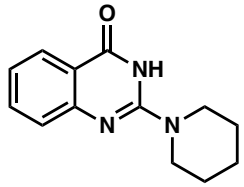


4 **(99% yield)** ***11***

*Alkylation of **4** using Methyl Bromoacetate in DME with Na_2CO_3 as the Base.*

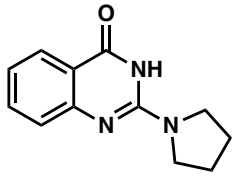
To a solution of quinazolinone **4** (1.00 g, 5.54 mmol, 1.0 equiv) in DME (24 mL) was added sodium carbonate (619 mg, 5.82 mmol, 1.05 equiv) at 23 °C. After stirring for 10 minutes, methyl bromoacetate (1.05 mL, 11.1 mmol, 2.0 equiv) was added dropwise, and the reaction flask was sealed and heated in an oil bath to 65 °C. After 2 hours, the reaction was removed from heat and poured into brine (20 mL), then extracted with ethyl acetate (6 x 20 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford **11** as a white solid that required no further purification (1.39 g, 99% yield).

Characterization Data for Aminated Quinazolinones 2a–c.



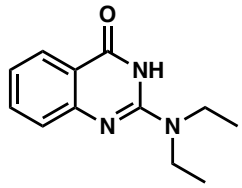
2a

2-Piperidylquinazolin-4-(3*H*)-one 2a. White solid, 106 mg, 84% yield. R*f* = 0.50 (50% hexanes in ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.57 (t, *J* = 7.4 Hz, 1H), 7.37 (d, *J* = 8.2 Hz, 1H), 7.12 (t, *J* = 7.5 Hz, 1H), 3.80–3.71 (m, 4H), 1.76–1.68 (m, 6H);^{13}C NMR ($CDCl_3$, 101 MHz) δ 165.7, 151.7, 150.5, 134.8, 126.3, 125.2, 122.2, 116.6, 46.4, 25.8, 24.6; IR (Neat Film) 2932, 1665, 1602, 1326, 1279, 762 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{13}H_{16}N_3O$ $[M+H]^+$: 230.1293, found 230.1301.



2b

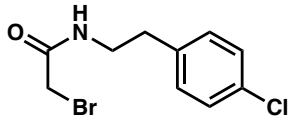
2-Pyrrolidinylquinazolin-4-(3*H*)-one 2b. White solid, 115 mg, 97% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.42 (br s, 1H), 8.07 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.58 (ddd, *J* = 8.6, 7.1, 1.7 Hz, 1H), 7.38 (d, *J* = 8.3 Hz, 1H), 7.12 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H), 3.66–3.53 (m, 4H), 2.11–2.00 (m, 4H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 164.7, 152.1, 148.9, 134.9, 126.5, 125.0, 121.8, 116.6, 46.8, 25.6; IR (Neat Film) 2974, 1674, 1605, 1513, 1476, 1441, 1404, 1318, 763 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{12}H_{14}N_3O$ $[M+H]^+$: 216.1137, found 216.1143.



2c

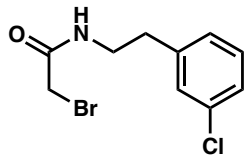
2-Triethylaminoquinazolin-4-(3*H*)-one 2c. White solid, 111 mg, 93% yield; mp 177.6–179.0 °C (from EtOAc/hex). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.07 (br s, 1H), 8.04 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.56 (ddd, *J* = 8.4, 7.0, 1.6 Hz, 1H), 7.35 (d, *J* = 8.1 Hz, 1H), 7.09 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H), 3.69 (q, *J* = 7.1 Hz, 4H), 1.29 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 165.5, 152.2, 149.5, 134.7 126.3, 125.2, 121.7, 116.4, 42.3, 13.6; IR (Neat Film) 3058, 2984, 2935, 1691, 1674, 1612, 1599, 1583, 1437, 1327, 1287, 761 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{12}H_{16}N_3O$ $[M+H]^+$: 218.1293, found 218.1300.

Characterization Data for Bromoamides 3a–e.



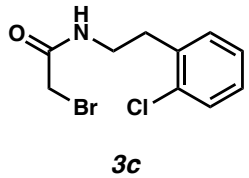
3a

Bromoamide 3a. White solid, 6.70 g, 75% yield; mp 93.9–95.1 °C (from EtOAc/hex). R*f* = 0.44 (50% hexanes in ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33–7.27 (m, 2H), 7.18–7.09 (m, 2H), 6.48 (s, 1H), 3.85 (s, 2H), 3.53 (q, *J* = 6.8 Hz, 2H), 2.82 (t, *J* = 7.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 165.5, 136.9, 132.8, 130.3, 129.0, 41.3, 35.0, 29.3; IR (Neat Film) 3244, 3078, 2942, 1640, 1565, 1493, 1435, 1328, 1201,1090, 924, 807, 654 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{10}H_{12}BrClNO$ $[M+H]^+$: 275.9791, found 275.9785.



3b

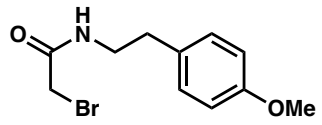
Bromoamide 3b. White solid, 8.43 g, 95% yield; mp 78.9–79.5 °C (from EtOAc/hex). R*f* = 0.53 (50% hexanes in ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (d, *J* = 7.8 Hz, 1H), 7.26–7.21 (m, 2H), 7.11 (dt, *J* = 7.2, 1.8 Hz, 1H), 6.55 (s, 1H), 3.88 (s, 2H), 3.56 (q, *J* = 6.7 Hz, 2H), 2.85 (t, *J* = 7.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 165.5, 140.5, 134.7, 130.1, 129.1, 127.1, 127.1, 41.2, 35.3, 29.3; IR (Neat Film) 3286, 3082, 1652, 1555, 1476, 1430, 1312, 1210, 1130, 1080, 783, 685 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{10}H_{12}BrClNO$ $[M+H]^+$: 275.9791, found 275.9787.



3c

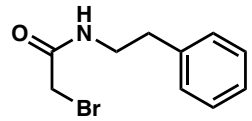
Bromoamide 3c. White solid, 6.39 g, 72% yield; mp 79.4–79.9 °C (from EtOAc/hex). R*f* = 0.68 (50% hexanes in ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–7.36 (m, 1H), 7.25–7.17 (m, 3H), 6.52 (s, 1H), 3.85 (s, 2H), 3.58 (q, *J* = 6.8 Hz, 2H), 3.00 (t, *J* = 7.0 Hz,

2H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 165.6, 136.2, 134.3, 131.2, 129.9, 128.4, 127.2, 40.0, 33.2, 29.3; IR (Neat Film) 3256, 3070, 2945, 1636, 1557, 1474, 1442, 1362, 1320, 1210, 749, 727 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{10}H_{12}BrClNO$ $[M+H]^+$: 275.9791, found 275.9786.



3d

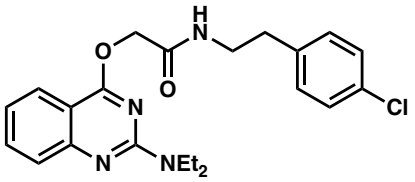
Bromoamide 3d. White solid, 5.90 g, 66% yield; mp 79.9–80.7 °C (from EtOAc/hex). R*f* = 0.48 (50% hexanes in ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.10 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 6.62 (s, 1H), 3.80 (s, 2H), 3.77 (s, 3H), 3.48 (q, *J* = 6.9 Hz, 2H), 2.76 (t, *J* = 7.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 165.5, 158.4, 130.4, 129.8, 114.2, 55.3, 41.6, 34.5, 29.2; IR (Neat Film) 3084, 3089, 2941, 1650, 1613, 1561, 1514, 1462, 1422, 1325, 1302, 823 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{11}H_{15}BrNO_2$ $[M+H]^+$: 272.0286, found 272.0284.



3e

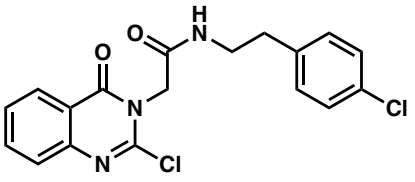
Bromoamide 3e. White solid, 6.02 g, 69% yield; mp 78.0–78.5 °C (from EtOAc/hex). R*f* = 0.57 (50% hexanes in ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30–7.25 (m, 2H), 7.22–7.18 (m, 1H), 7.17–7.15 (m, 2H), 6.58 (s, 1H), 3.77 (s, 2H), 3.49 (q, *J* = 7.0 Hz, 2H), 2.80 (q, *J* = 6.9 Hz, 2H);^{13}C NMR ($CDCl_3$, 126 MHz) δ 165.5, 138.4, 128.8, 128.8, 126.8, 41.4, 35.4, 29.3; IR (Neat Film) 3277, 3085, 2934, 1651, 1554, 1497, 1455, 1365, 1314, 1213, 749, 699 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{10}H_{13}BrNO$ $[M+H]^+$: 242.0181, found 242.0176.

Characterization Data for Alkylation Products 9–12 and 16–17.



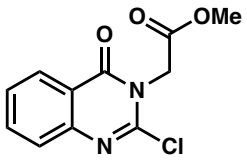
9b

***O*-Alkylated Product 9b.** White solid, 69 mg, 72% yield; mp 149.4–150.5 °C (from EtOAc/hex). R*f* = 0.43 (50% ethyl acetate in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66–7.58 (m, 2H), 7.51 (d, *J* = 8.5 Hz, 1H), 7.18–7.08 (m, 3H), 7.01 (dd, *J* = 8.3, 1.7 Hz, 2H), 6.28 (s, 1H), 4.96 (s, 2H), 3.68 (q, *J* = 7.2 Hz, 4H), 3.59 (q, *J* = 6.5 Hz, 2H), 2.78 (t, *J* = 6.6 Hz, 2H), 1.20 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.0, 165.1, 157.6, 154.7, 136.9, 134.0, 132.7, 130.1, 129.0, 125.4, 122.9, 121.4, 110.2, 64.8, 42.1, 39.9, 35.1, 13.5; IR (Neat Film) 2930, 1660, 1630, 1592, 1560, 1528, 1490, 1437, 1375, 1267, 1158, 1092, 763 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{22}H_{26}ClN_4O_2$ $[M+H]^+$: 413.1744, found 413.1755.



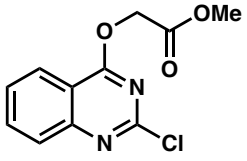
10

2-Chloro-*N*3-Alkylamido *N*-Alkylation Product 10. White solid, 40 mg, 33% yield; mp 187.7–189.4 °C (from EtOAc/hex). R*f* = 0.43 (50% ethyl acetate in hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.25 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.74 (ddd, *J* = 8.5, 7.2, 1.6 Hz, 1H), 7.62 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.41 (ddd, *J* = 8.2, 7.1, 1.1 Hz, 1H), 7.30–7.25 (m, 3H), 7.23–7.19 (m, 2H), 4.48 (s, 2H), 4.09–4.01 (m, 2H), 3.14–3.03 (m, 2H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 167.6, 159.1, 148.9, 148.5, 135.9, 135.1, 132.9, 130.4, 128.9. 127.0, 126.7, 125.7, 119.8, 46.8, 40.8, 32.9; IR (Neat Film) 1749, 1688, 1639, 1608, 1475, 1448, 1369, 1133, 1092, 818, 772, 693 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{18}H_{15}ClN_3O_2$ $[M–Cl]^+$: 340.0853, found 340.0854.



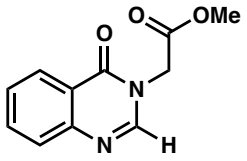
11

2-Chloro-*N*3-Alkylated Product 11. White solid, 125 mg, 45% yield; mp 99.9–101.7 °C (from EtOAc/hex); R*f* = 0.68 (50% ethyl acetate in hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.23 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.78 (ddd, *J* = 7.9, 7.1, 1.1 Hz, 1H), 7.64 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.51 (ddd, *J* = 8.2, 7.2, 1.0 Hz, 1H), 5.06 (s, 2H), 3.81 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 167.4, 161.7, 146.6, 143.9, 135.5, 127.9, 127.6, 127.1, 120.0, 53.0, 47.0; IR (Neat Film) 3231, 1635, 1603, 1555, 1503, 1472, 1460, 1357, 1280, 1194, 907, 732 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{11}H_{10}ClN_2O_3$ $[M+H]^+$: 253.0380, found 253.0377.



12

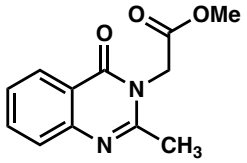
2-Chloro *O*-Alkylated Product 12. White solid, 70 mg, 25% yield; dec pt 152–153 °C (no melting); R*f* = 0.75 (50% ethyl acetate in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (dd, *J* = 8.2, 2.5 Hz, 1H), 7.93–7.81 (m, 2H), 7.60 (ddd, *J* = 8.2, 5.1, 3.1 Hz, 1H), 5.18 (s, 2H), 3.82 (s, 3H).^{13}C NMR ($CDCl_3$, 126 MHz) δ 167.8, 167.0, 155.4, 152.5, 135.0, 127.6, 127.1, 123.9, 114.5, 63.4, 52.5; IR (Neat Film) 2955, 1759, 1618, 1560, 1494, 1435, 1414, 1397, 1215, 1161, 1189, 1108, 1030, 922, 768, 588 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{11}H_{10}ClN_2O_3$ $[M+H]^+$: 253.0380, found 253.0371.



16

***N*-Alkylated Quinazolinone Product 16.** White solid, 44 mg, 99% yield; mp 153.1–154.5 °C; R*f* = 0.24 (50% ethyl acetate in hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.99 (s, 1H), 7.77 (ddd, *J* = 8.4, 7.0, 1.6 Hz, 1H), 7.72 (dd, *J* = 8.2, 1.3

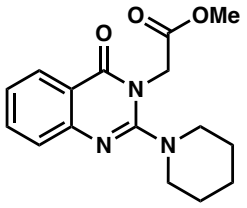
Hz, 1H), 7.51 (ddd, *J* = 8.2, 6.9, 1.3 Hz, 1H), 4.72 (s, 2H), 3.79 (s, 3H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 167.9, 161.1, 148.2, 146.2, 134.7, 127.8, 127.7, 126.9, 122.0, 53.0, 47.4; IR (Neat Film) 3001, 2959, 1751, 1671, 1614, 1475, 1365, 1222, 787, 775 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{11}H_{11}N_2O_3$ $[M+H]^+$: 219.0770, found 219.0777.



17

***N*-Alkylated Quinazolinone Product 17.** White solid, 47 mg, 67% yield; mp 114.8–116.6 °C; R*f* = 0.25 (50% ethyl acetate in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.75 (ddd, *J* = 8.5, 7.1, 1.6 Hz, 1H), 7.64 (dd, *J* = 8.5, 1.1 Hz, 1H), 7.46 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 4.89 (s, 2H), 3.81 (s, 3H), 2.56 (s, 3H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.2, 162.0, 153.6, 147.4, 134.7, 127.0, 126.9, 126.8, 120.1, 52.9, 45.3, 23.2; IR (Neat Film) 2957, 1749, 1677, 1603, 1438, 1389, 1371, 1213, 1185, 1001, 775, 705 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{12}H_{13}N_2O_3$ $[M+H]^+$: 233.0926, found 233.0933.

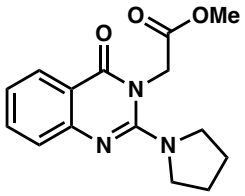
Characterization Data for 2-Amino-*N*3-Alkylated 4-Quinazolinones 13a–c.



13a

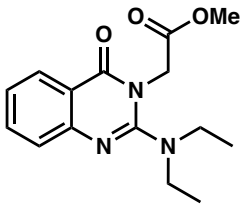
***N*3-Alkylated 2-Piperidinylquinazolinone 13a.** White solid; 173 mg, 73% yield; mp 86.0–87.5 °C (from EtOAc/hex). R*f* = 0.72 (50% ethyl acetate in hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.65 (ddd, *J* = 7.7, 7.1, 1.6 Hz, 1H), 7.53 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.32 (ddd, *J* = 7.5, 7.1, 1.2 Hz, 1H), 4.80 (s, 2H), 3.77 (s, 3H), 3.11–3.05 (m, 4H), 1.70–1.63 (m, 4H), 1.63–1.56 (m, 2H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.9, 163.7, 155.6, 147.7, 134.5, 127.1, 126.5, 125.4, 119.4, 52.6, 51.5, 45.9, 25.5, 24.2; IR (Neat Film) 2938, 2351, 1751, 1680, 1587, 1567, 1472, 1439, 1383, 1333, 1207, 1183,

1102, 986, 858, 771, 710 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{16}H_{20}N_3O_3$ $[M+H]^+$: 302.1505, found 302.1513.



13b

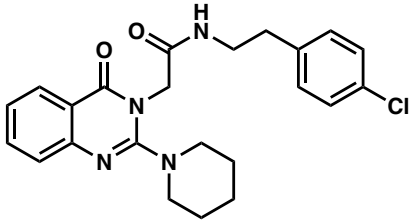
***N*3-Alkylated 2-Pyrrolidinylquinazolinone 13b.** White solid; 194 mg, 85% yield; mp 152.7–153.6 °C (from EtOAc/hex). R*f* = 0.77 (50% ethyl acetate in hexanes); ^1H NMR (500 MHz, $CDCl_3$) δ 8.14 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.63 (ddd, *J* = 8.4, 7.1, 1.6 Hz, 1H), 7.45 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.24 (dd, *J* = 7.6, 1.1 Hz, 1H), 4.77 (s, 2H), 3.80 (s, 3H), 3.52–3.47 (m, 4H), 1.96–1.92 (m, 4H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 169.1, 164.2, 153.6, 148.4, 134.7, 127.0, 125.8, 124.0, 118.2, 52.7, 50.6, 47.0, 25.7; IR (Neat Film) 2955, 2878, 1752, 1677, 1611, 1557, 1473, 1213, 1183, 1007, 769, 739, 709 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{15}H_{18}N_3O_3$ $[M+H]^+$: 288.1348, found 288.1356.



13c

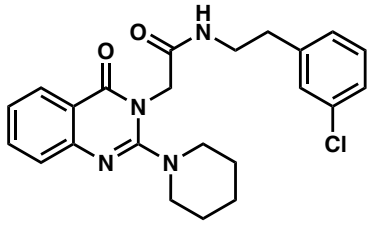
***N*3-Alkylated 2-Triethylaminoquinazolinone 13c.** Colorless oil, 31 mg, 58% yield. R*f* = 0.83 (50% ethyl acetate in hexanes); ^1H NMR (300 MHz, $CDCl_3$) δ 8.17 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.65 (ddd, *J* = 8.5, 7.1, 1.6 Hz, 1H), 7.53 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.32 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H), 4.83 (s, 2H), 3.75 (s, 3H), 3.16 (q, *J* = 7.1 Hz, 4H), 1.11 (t, *J* = 7.1 Hz, 6H).^{13}C NMR ($CDCl_3$, 126 MHz) δ 169.0, 164.0, 154.5, 147.6, 134.6, 127.1, 126.6, 125.4, 119.4, 52.6, 46.0, 45.7, 12.7; IR (Neat Film) 2959, 2931, 2874, 1755, 1720, 1587, 1473, 1378, 1268, 1210, 1117, 1019, 989, 875, 871, 731, 711 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{15}H_{20}N_3O_3$ $[M+H]^+$: 290.1505, found 290.1511.

Characterization Data for 2-Amino-*N*3-Alkylamido-4-Quinazolinones 1a–o.



1a

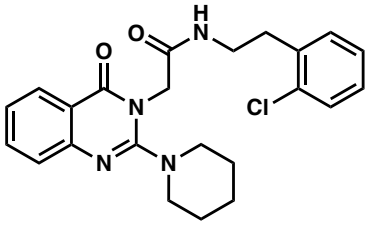
Target Derivative 1a. White solid, 26 mg, 86% yield from 21 mg (0.070 mmol) of **13a**; mp 177.2–180.1 °C (from EtOAc/hex). R*f* = 0.45 (50% hexanes in ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.69 (ddd, *J* = 8.5, 7.1, 1.6 Hz, 1H), 7.55 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.36 (ddd, *J* = 8.1, 7.1, 1.1 Hz, 1H), 7.21–7.15 (m, 2H), 7.12–7.06 (m, 2H), 6.47 (s, 1H), 4.65 (s, 2H), 3.53 (td, *J* = 7.0, 5.9 Hz, 2H), 3.13 – 3.08 (m, 4H), 2.79 (t, *J* = 7.0 Hz, 2H), 1.74–1.67 (m, 4H), 1.66–1.59 (m, 2H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.1, 164.9, 155.8, 147.9, 137.2, 134.9, 132.5, 130.2, 128.8, 126.9, 126.5, 125.4, 119.1, 51.7, 49.5, 40.8, 35.1, 25.7, 24.3; IR (Neat Film) 2959, 2929, 2873, 2960, 1719, 1462, 1408, 1380, 1265, 1247, 1115, 1101, 1019, 874, 729 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{23}H_{26}ClN_4O_2$ $[M+H]^+$: 425.1744, found 425.1756.



1b

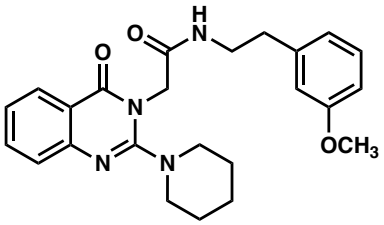
Target Derivative 1b. White solid, 28 mg, 71% yield from 28 mg (0.091 mmol) of **13a**; mp 179.5–181.2 °C (from EtOAc/hex). R*f* = 0.44 (50% hexanes in ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.67 (t, *J* = 7.5 Hz, 1H), 7.53 (d, *J* = 8.1 Hz, 1H), 7.33 (t, *J* = 7.5 Hz, 1H), 7.18–7.09 (m, 3H), 7.04 (dd, *J* = 5.2, 2.6 Hz, 1H), 6.62 (t, *J* = 5.9 Hz, 1H), 4.65 (s, 2H), 3.53 (q, *J* = 6.7 Hz, 2H), 3.14–3.08 (m, 4H), 2.79 (t, *J* = 7.0 Hz, 2H), 1.73–1.66 (m, 4H), 1.65–1.57 (m, 2H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ

168.2, 164.9, 155.8, 147.8, 140.9, 134.8, 134.4, 129.9, 129.0, 127.1, 127.0, 126.8, 126.5, 125.3, 119.1, 51.7, 49.5, 40.7, 35.4, 25.7, 24.3; IR (Neat Film) 1749, 1688, 1639, 1609, 1562, 1475, 1448, 1369, 1133, 818, 772, 693 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{23}H_{26}ClN_4O_2$ $[M+H]^+$: 425.1744, found 425.1754.



1c

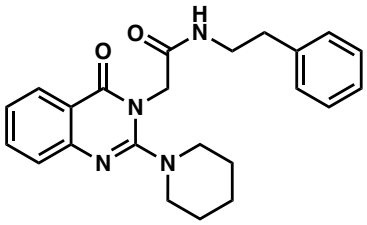
Target Derivative 1c. White solid, 27 mg, 67% yield from 29 mg (0.095 mmol) of **13a**; mp 161.3–166.1 °C (from EtOAc/hex). R*f* = 0.85 (50% hexanes in ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (d, *J* = 8.0 Hz, 1H), 7.67 (t, *J* = 7.8 Hz, 1H), 7.53 (d, *J* = 8.3 Hz, 1H), 7.33 (t, *J* = 7.6 Hz, 1H), 7.20–6.98 (m, 4H), 6.70 (br s, 1H), 4.66 (s, 2H), 3.52 (q, *J* = 6.7 Hz, 2H), 3.15–3.07 (m, 4H), 2.79 (t, *J* = 7.1 Hz, 2H), 1.73–1.65 (m, 4H), 1.64–1.57 (m, 2H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.2, 164.9, 155.8, 147.8, 140.9, 134.8, 134.4, 129.9, 129.0, 127.1, 126.9, 126.8, 126.5, 125.3, 119.0, 51.7, 49.5, 40.7, 35.3, 25.6, 24.2; IR (Neat Film) 3296, 2933, 2853, 1651, 1611, 1566, 1473, 1452, 1373, 1336, 1232, 1207, 1156, 1101, 1027, 912, 860, 770, 686 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{23}H_{26}ClN_4O_2$ $[M+H]^+$: 425.1744, found 425.1753.



1d

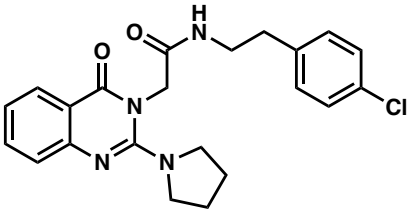
Target Derivative 1d. White solid, 17 mg, 43% yield from 28 mg (0.093 mmol) of **13a**; mp 141.7–143.5 °C (from EtOAc/hex). R*f* = 0.44 (50% hexanes in ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.68 (ddd, *J* = 8.4, 7.0, 1.6 Hz, 1H), 7.54 (d, *J* = 8.1 Hz, 1H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.11 (t, *J* = 7.8 Hz, 1H), 6.78–6.67 (m,

3H), 6.47 (t, J = 5.8 Hz, 1H), 4.65 (s, 2H), 3.74 (s, 3H), 3.55 (q, J = 6.7 Hz, 2H), 3.18–3.03 (m, 4H), 2.80 (t, J = 7.0 Hz, 2H), 1.73–1.66 (m, 4H), 1.64–1.58 (m, 2H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.0, 164.8, 159.9, 155.9, 147.8, 140.4, 134.8, 129.7, 127.0, 126.5, 125.3, 121.1, 119.1, 114.5, 112.1, 55.2, 51.7, 49.5, 40.8, 35.7, 25.7, 24.3; IR (Neat Film) 3301, 3082, 2933, 2853, 1659, 1610, 1584, 1567, 1489, 1473, 1454, 1374, 1259, 1235, 1154, 1036, 983, 858, 771, 696 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{24}H_{29}ClN_4O_3$ $[M+H]^+$: 421.2240, found 421.2245.



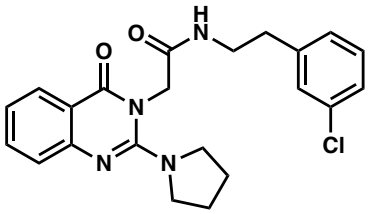
1e

Target Derivative 1e. White solid, 8 mg, 29% yield from 21 mg (0.071 mmol) of **13a**; mp 174.3–175.5 °C (from EtOAc/hex). Rf = 0.41 (50% hexanes in ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (dd, J = 8.0, 1.6 Hz, 1H), 7.69 (ddd, J = 8.4, 7.0, 1.6 Hz, 1H), 7.54 (d, J = 8.1 Hz, 1H), 7.35 (ddd, 8.2, 7.3, 1.2 Hz, 1H), 7.23–7.19 (m, 2H), 7.18 – 7.13 (m, 3H), 6.41 (t, J = 6.7 Hz, 1H), 4.65 (s, 2H), 3.56 (q, J = 6.7 Hz, 2H), 3.14–3.07 (m, 4H), 2.82 (t, J = 7.0 Hz, 2H), 1.73–1.67 (m, 4H), 1.65–1.58 (m, 2H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.0, 164.9, 155.9, 147.9, 138.8, 134.8, 128.9, 128.7, 127.0, 126.6, 126.5, 125.4, 119.1, 51.7, 49.5, 40.9, 35.7, 25.7, 24.3; IR (Neat Film) 3304, 2930, 2852, 1660, 1611, 1584, 1567, 1473, 1454, 1374, 1235, 1209, 1103, 1029, 982, 769, 699 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{23}H_{27}N_4O_2$ $[M+H]^+$: 391.2134, found 391.2146.



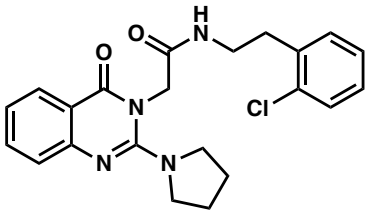
1f

Target Derivative 1f. White solid, 17 mg, 70% yield from 17 mg (0.058 mmol) of **13b**; mp 198.6–199.8 °C (from EtOAc/hex). R*f* = 0.38 (50% hexanes in ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.65 (ddd, *J* = 8.5, 7.0, 1.6 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 1H), 7.28–7.23 (m, 1H), 7.21–7.15 (m, 2H), 7.12–7.07 (m, 2H), 6.63 (s, 1H), 4.58 (s, 2H), 3.55 (q, *J* = 6.7 Hz, 2H), 3.49–3.44 (m, 4H), 2.80 (t, *J* = 7.0 Hz, 2H), 1.93–1.87 (m, 4H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.6, 165.7, 153.5, 148.7, 137.2, 135.1, 132.5, 130.3, 128.8, 126.9, 125.7, 123.9, 117.6, 51.0, 50.9, 40.7, 35.1, 25.8; IR (Neat Film) 3866, 3296, 2922, 2240, 1647, 1612, 1553, 1492, 1472, 1400, 1355, 1247, 1187, 1088, 1016, 989, 807, 763, 724 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{22}H_{24}ClN_4O_2$ $[M+H]^+$: 411.1588, found 411.1591.



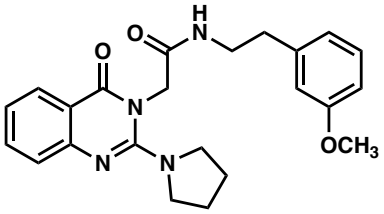
1g

Target Derivative 1g. White solid, 19 mg, 76% yield from 17 mg (0.060 mmol) of **13b**; mp 185.8–187.9 °C (from EtOAc/hex). R*f* = 0.38 (50% hexanes in ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.63 (ddd, *J* = 8.6, 7.2, 1.6 Hz, 1H), 7.43 (d, *J* = 8.2 Hz, 1H), 7.26–7.22 (m, 1H), 7.20–7.11 (m, 3H), 7.07–7.03 (m, 1H), 6.67 (t, *J* = 6.9 Hz, 1H), 4.58 (s, 2H), 3.56 (q, *J* = 6.7 Hz, 2H), 3.51–3.45 (m, 4H), 2.81 (t, *J* = 7.0 Hz, 2H), 1.96–1.83 (m, 4H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.7, 165.8, 153.6, 148.7, 140.8, 135.1, 134.5, 129.9, 129.0, 127.1, 127.0, 126.9, 125.7, 123.9, 117.7, 51.1, 50.9, 40.7, 35.5, 25.8; IR (Neat Film) 3525, 3310, 2923, 1643, 1611, 1551, 1471, 1432, 1398, 1355, 1251, 1187, 1082, 990, 981, 825, 788, 731, 699, 683 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{22}H_{24}ClN_4O_2$ $[M+H]^+$: 411.1588 found 411.1595.



1h

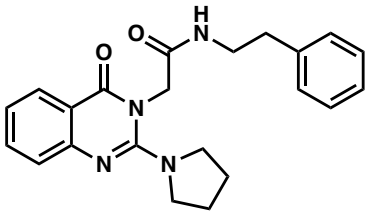
Target Derivative 1h. White solid, 15 mg, 77% yield from 13 mg (0.046 mmol) of **13b**; mp 192.7–193.4 °C (from EtOAc/hex). R*f* = 0.44 (50% hexanes in ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.64 (ddd, *J* = 8.5, 7.1, 1.6 Hz, 1H), 7.43 (d, *J* = 8.2 Hz, 1H), 7.30–7.26 (m, 1H), 7.25–7.20 (m, 2H), 7.14–7.08 (m, 2H), 6.64 (t, *J* = 6.1 Hz, 1H), 4.59 (s, 2H), 3.60 (q, *J* = 6.7 Hz, 2H), 3.50–3.44 (m, 4H), 2.97 (t, *J* = 7.0 Hz, 2H), 1.95–1.83 (m, 4H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.6, 165.6, 153.6, 148.7, 136.4, 135.0, 134.3, 131.2, 129.7, 128.2, 127.1, 127.0, 125.7, 123.9, 117.7, 50.9, 50.9, 39.5, 33.3, 25.8; IR (Neat Film) 3296, 3069, 2953, 2876, 1659, 1611, 1555, 1474, 1425, 1396, 1246, 1137, 1132, 1024, 920, 868, 765, 704 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{22}H_{24}ClN_4O_2$ $[M+H]^+$: 411.1588, found 411.1594.



1i

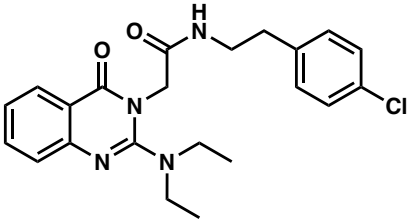
Target Derivative 1i. White solid, 24 mg, 43% yield from 40 mg (0.14 mmol) of **13b**; mp 171.2–173.0 °C (from EtOAc/hex). R*f* = 0.79 (50% hexanes in ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.63 (ddd, *J* = 8.5, 7.0, 1.6 Hz, 1H), 7.42 (d, *J* = 8.2 Hz, 1H), 7.23 (ddd, *J* = 8.0, 7.1, 1.1 Hz, 1H), 7.11 (t, *J* = 7.7 Hz, 1H), 6.71 (ddd, *J* = 12.8, 10.3, 5.0 Hz, 3H), 6.60 (t, *J* = 5.8 Hz, 1H), 4.57 (s, 2H), 3.73 (s, 3H), 3.56 (q, *J* = 6.8 Hz, 2H), 3.50–3.44 (m, 4H), 2.80 (t, *J* = 7.0 Hz, 2H), 1.92–1.86 (m, 4H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.5, 165.6, 159.9, 153.6, 148.7, 140.3, 135.0, 129.6, 127.0, 125.7, 123.8, 121.1, 117.7, 114.4, 112.1, 55.2, 50.9, 50.8, 40.8, 35.7, 25.8; IR (Neat Film) 3522, 3312, 3094, 3062, 2968, 2936, 2882, 1669, 1646, 1610, 1545, 1491, 1469, 1399,

1364, 1353, 1286, 1261, 1188, 1164, 1043, 989, 909, 867, 765, 711, 692 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{23}H_{27}N_4O_3$ $[M+H]^+$: 407.2083, found 407.2094.



1j

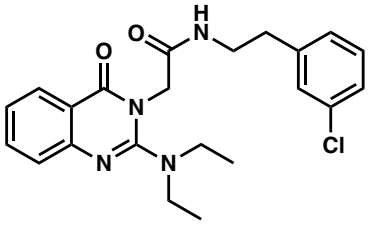
Target Derivative 1j. White solid, 10 mg, 67% yield from 11 mg (0.040 mmol) of **13b**; mp 198.7–200.3 °C (from EtOAc/hex). R*f* = 0.44 (50% hexanes in ethyl acetate); ^1H NMR (500 MHz, $CDCl_3$) δ 8.12 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.64 (ddd, *J* = 8.5, 7.0, 1.6 Hz, 1H), 7.45 (d, *J* = 8.2 Hz, 1H), 7.25–7.19 (m, 3H), 7.18–7.14 (m, 3H), 6.58 (s, 1H), 4.58 (s, 2H), 3.57 (q, *J* = 6.8 Hz, 2H), 3.50–3.45 (m, 4H), 2.83 (t, *J* = 7.0 Hz, 2H), 1.93–1.85 (m, 4H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.4, 165.5, 153.6, 148.7, 138.7, 135.0, 128.8, 128.7, 126.9, 126.6, 125.7, 123.8, 117.7, 50.8, 41.7, 40.9, 35.7, 25.8; IR (Neat Film) 3294, 2919, 2850, 1656, 1611, 1555, 1473, 1395, 1353, 1259, 1187, 987, 764, 700 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{22}H_{25}N_4O_2$ $[M+H]^+$: 377.1978, found 377.1990.



1k

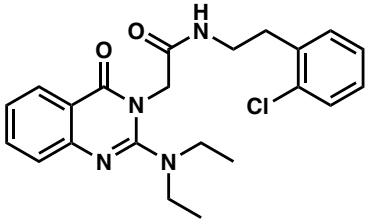
Target Derivative 1k. White solid, 21 mg, 67% yield from 22 mg (0.075 mmol) of **13c**; mp 186.2–186.6 °C (from EtOAc/hex). R*f* = 0.49 (50% hexanes in ethyl acetate); ^1H NMR (500 MHz, $CDCl_3$) δ 8.15 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.69 (ddd, *J* = 8.4, 7.0, 1.6 Hz, 1H), 7.55 (d, *J* = 8.2 Hz, 1H), 7.35 (t, *J* = 7.5 Hz, 1H), 7.16 (d, *J* = 8.4 Hz, 2H), 7.08 (d, *J* = 8.2 Hz, 2H), 6.41 (t, *J* = 5.8 Hz, 1H), 4.66 (s, 2H), 3.51 (q, *J* = 6.7 Hz, 2H), 3.19 (q, *J* = 7.0 Hz, 4H), 2.78 (t, *J* = 7.0 Hz, 2H), 1.11 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.1, 165.0, 154.5, 147.7, 137.2, 134.8, 132.5, 130.2, 128.8, 126.9, 126.5, 125.4, 119.0,

49.2, 45.6, 40.8, 35.1, 12.7; IR (Neat Film) 3307, 2931, 1684, 1654, 1582, 1563, 1472, 1418, 1377, 1333, 1237, 1188, 1164, 1087, 1014, 986, 815, 771, 706 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{22}H_{26}ClN_4O_2$ $[M+H]^+$: 413.1744, found 413.1753.



1l

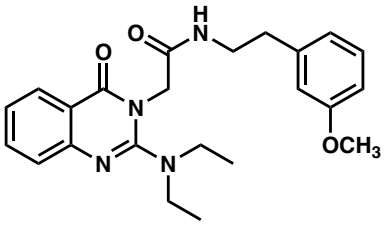
Target Derivative 1l. White solid, 16 mg, 36% yield from 32 mg (0.11 mmol) of **13c**; mp 163.7–165.6 °C (from EtOAc/hex). R*f* = 0.63 (50% hexanes in ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.68 (ddd, *J* = 8.5, 7.1, 1.6 Hz, 1H), 7.54 (d, *J* = 8.1 Hz, 1H), 7.34 (t, *J* = 7.4 Hz, 1H), 7.20 – 7.09 (m, 3H), 7.06–7.02 (m, 1H), 6.46 (t, *J* = 5.5 Hz, 1H), 4.67 (s, 2H), 3.53 (q, *J* = 6.8 Hz, 2H), 3.20 (q, *J* = 7.1 Hz, 4H), 2.80 (t, *J* = 7.0 Hz, 2H), 1.12 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.1, 165.0, 154.5, 147.7, 140.9, 134.8, 134.5, 129.9, 129.0, 127.1, 126.9, 126.9, 126.5, 125.3, 119.0, 49.3, 45.6, 40.7, 35.4, 12.7; IR (Neat Film) 3301, 3071, 2971, 2933, 2872, 1660, 1611, 1565, 1474, 1428, 1373, 1337, 1241, 1169, 1082, 983, 770, 687 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{22}H_{26}ClN_4O_2$ $[M+H]^+$: 413.1744, found 413.1751.



1m

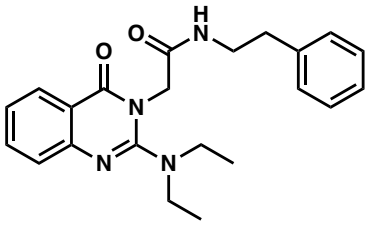
Target Derivative 1m. White solid, 13 mg, 40% yield from 23 mg (0.081 mmol) of **13c**; mp 146.0–152.8 °C (from EtOAc/hex). R*f* = 0.71 (50% hexanes in ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.69 (ddd, *J* = 8.5, 7.1, 1.6 Hz, 1H), 7.55 (d, *J* = 8.1 Hz, 1H), 7.35 (ddd, *J* = 7.5, 7.1, 1.1 Hz, 1H), 7.28 (dd, *J* = 7.7, 1.6 Hz, 1H),

7.20 (dd, J = 7.2, 2.1 Hz, 1H), 7.15–7.03 (m, 2H), 6.36 (t, J = 5.9 Hz, 1H), 4.67 (s, 2H), 3.58 (q, J = 6.7 Hz, 2H), 3.19 (q, J = 7.1 Hz, 4H), 2.96 (t, J = 7.0 Hz, 2H), 1.11 (t, J = 7.1 Hz, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 168.1, 165.0, 154.6, 147.7, 136.5, 134.8, 134.2, 131.2, 129.7, 128.2, 127.1, 127.0 126.5, 125.3, 119.1, 49.3, 45.6, 39.5, 33.3, 12.7; IR (Neat Film) 3301, 3070, 2970, 2929, 2872, 1661, 1611, 1583, 1566, 1474, 1375, 1338, 1242, 1188, 1170, 1058, 1024, 983, 770, 752 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{22}H_{26}ClN_4O_2$ $[M+H]^+$: 413.1744, found 413.1751.



1n

Target Derivative 1n. White solid, 25 mg, 73% yield from 24 mg (0.082 mmol) of **13c**; mp 125.4–126.3 °C (from EtOAc/hex). R*f* = 0.49 (50% hexanes in ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (dd, J = 8.0, 1.6 Hz, 1H), 7.68 (ddd, J = 8.5, 7.1, 1.6 Hz, 1H), 7.54 (d, J = 8.1 Hz, 1H), 7.34 (t, J = 7.4 Hz, 1H), 7.10 (t, J = 8.1 Hz, 1H), 6.76–6.66 (m, 3H), 6.36 (t, J = 5.9 Hz, 1H), 4.66 (s, 2H), 3.74 (s, 3H), 3.55 (q, J = 6.7 Hz, 2H), 3.20 (q, J = 7.1 Hz, 4H), 2.79 (t, J = 7.0 Hz, 2H), 1.11 (t, J = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 167.9, 164.9, 159.9, 154.6, 147.7, 140.3, 134.7, 129.7, 126.9, 126.4, 125.2, 121.1, 119.0, 114.4, 112.1, 55.2, 49.2, 45.5, 40.8, 35.7, 12.7; IR (Neat Film) 3302, 3073, 2970, 2934, 2873, 2836, 1658, 1610, 1582, 1564, 1473, 1374, 1337, 1259, 1248, 1188, 1168, 1061, 1039, 983, 771, 696 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{23}H_{29}N_4O_3$ $[M+H]^+$: 409.2240, found 409.2252.



1o

Target Derivative 1o. White solid, 18 mg, 64% yield from 22 mg (0.075 mmol) of **13c**; mp 137.5–139.4 °C (from EtOAc/hex). R*f* = 0.61 (50% hexanes in ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.68 (ddd, *J* = 8.5, 7.0, 1.6 Hz, 1H), 7.54 (d, *J* = 8.2 Hz, 1H), 7.34 (t, *J* = 7.5 Hz, 1H), 7.22–7.17 (m, 2H), 7.17–7.12 (m, 3H), 6.35 (t, *J* = 6.0 Hz, 1H), 4.66 (s, 2H), 3.55 (q, *J* = 6.7 Hz, 2H), 3.19 (q, *J* = 7.0 Hz, 4H), 2.81 (t, *J* = 7.0 Hz, 2H), 1.11 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 167.9, 164.9, 154.6, 147.7, 138.7, 134.7, 128.8, 128.7, 127.0, 126.6, 126.5, 125.3, 119.1, 49.2, 45.6, 40.9, 35.7, 12.7; IR (Neat Film) 3300, 3065, 2971, 2933, 2872, 1656, 1610, 1580, 1563, 1473, 1385, 1336, 1240, 1187, 1169, 1087, 982, 770, 749, 699 cm^{-1}; HRMS (ESI+) *m/z* calc'd for $C_{22}H_{27}N_4O_2$ $[M+H]^+$: 379.2134, found 379.2145.

Notes and References

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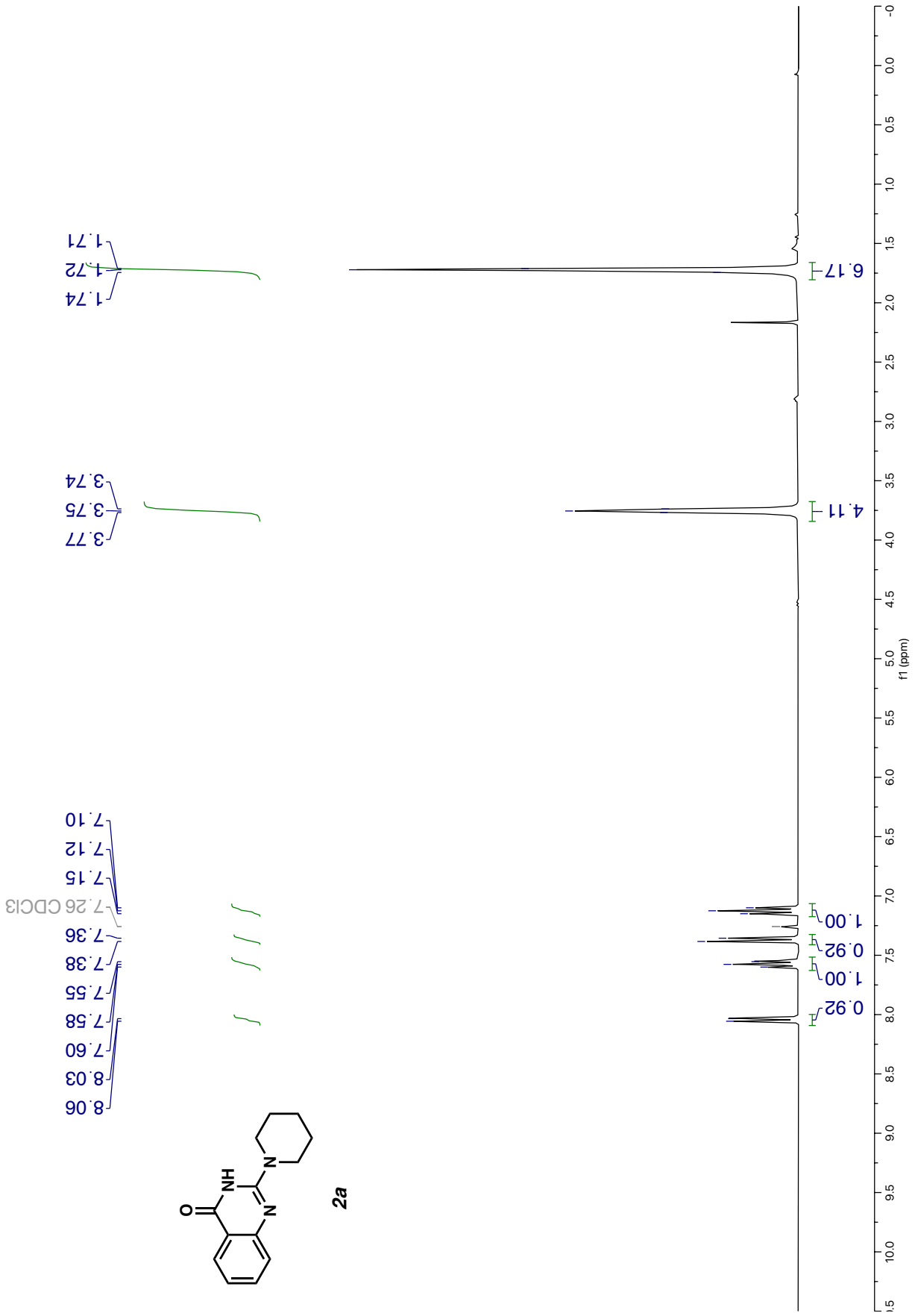
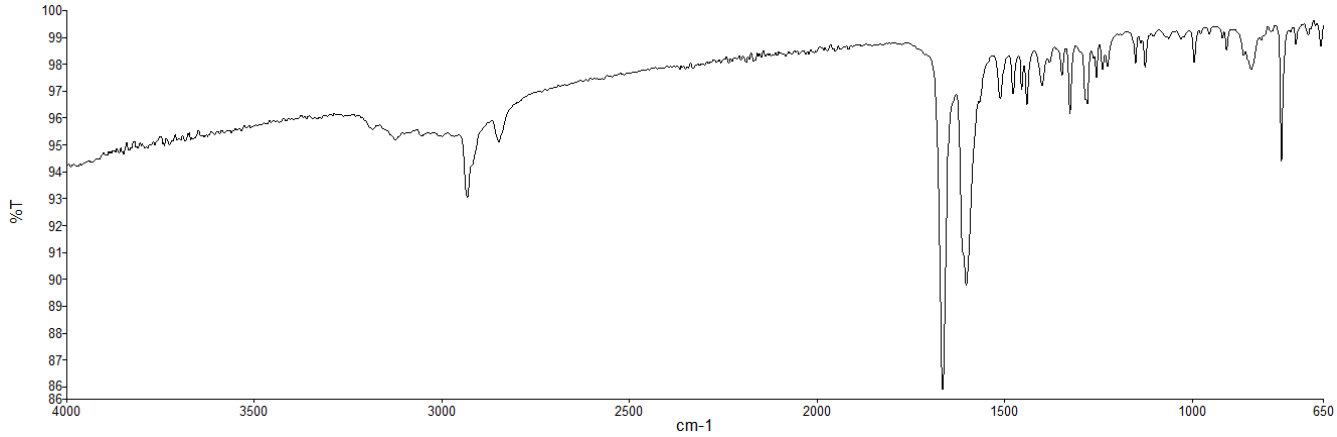
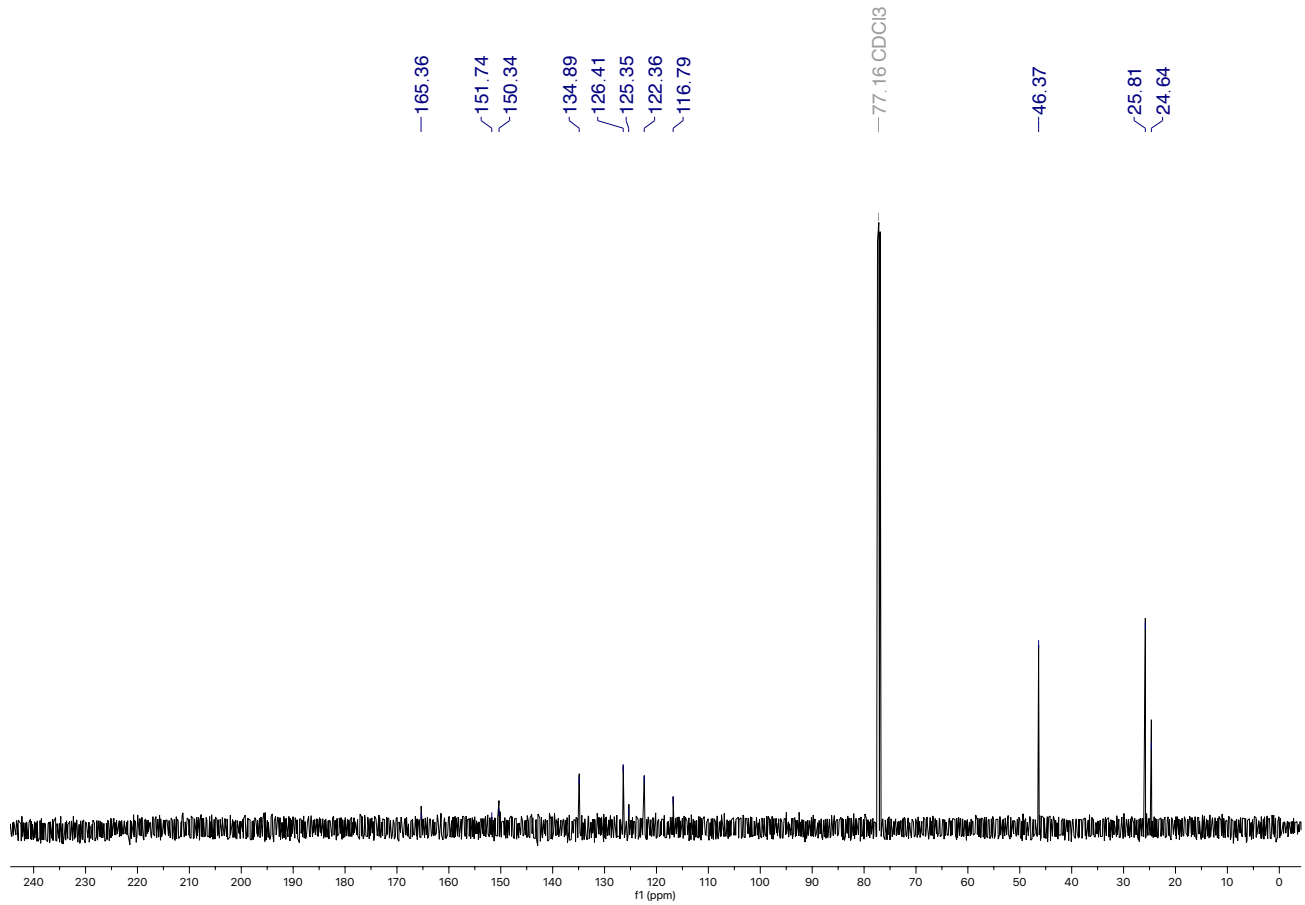


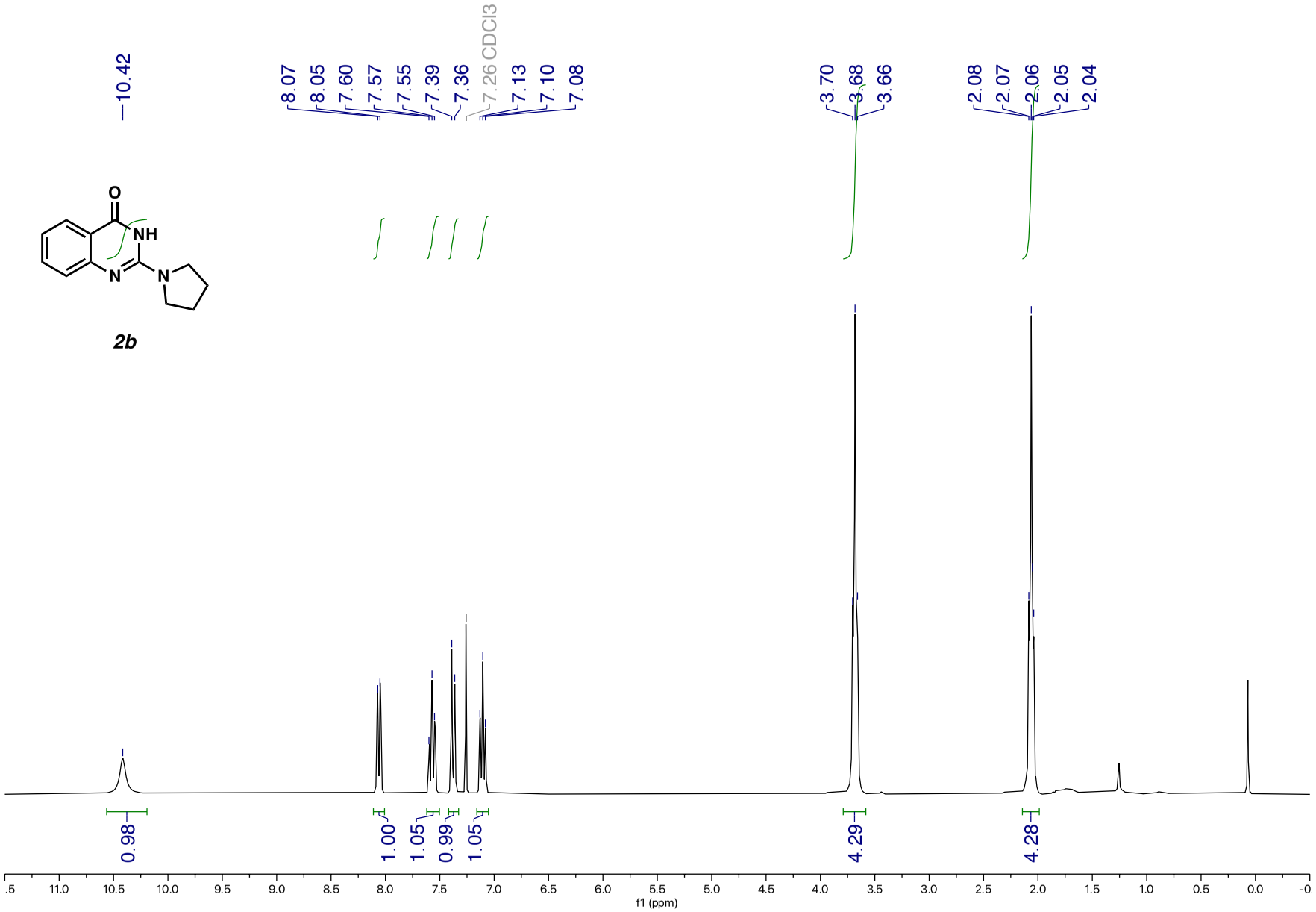
^{1}H NMR (300 MHz, $CDCl_3$) of compound **2a**.



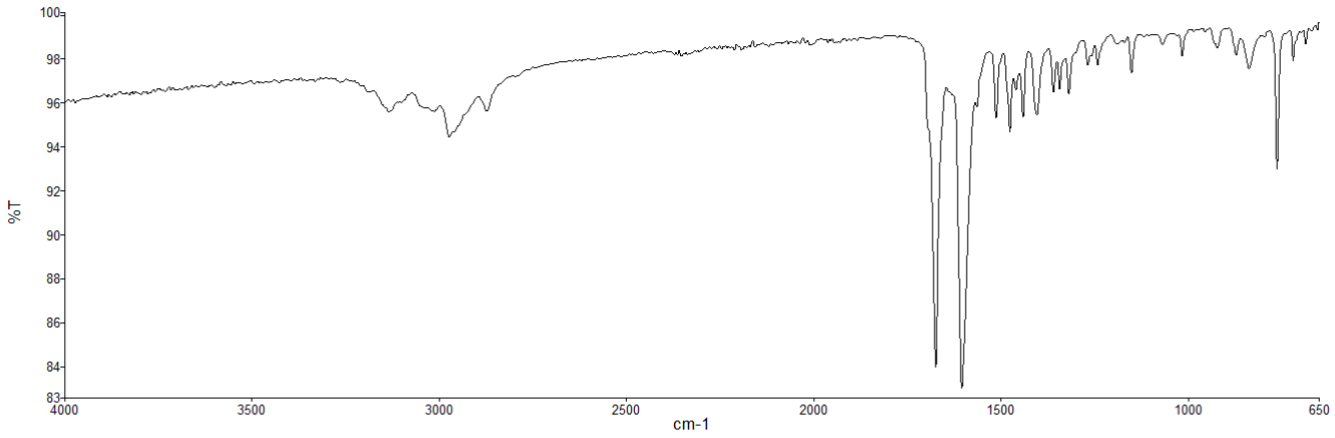
Infrared spectrum (Thin Film) of compound **2a**.



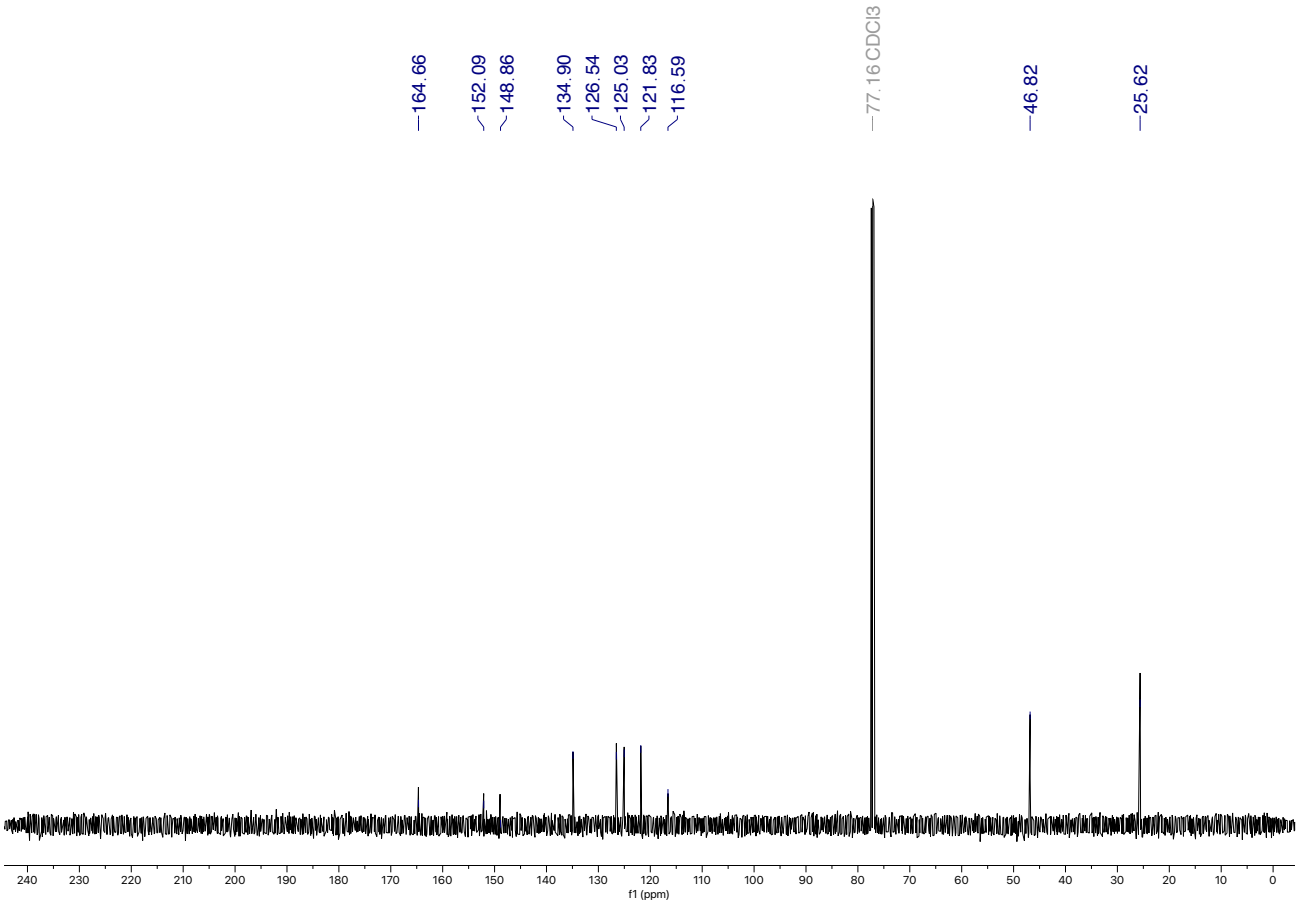
^{13}C NMR (126 MHz, $CDCl_3$) of compound **2a**.



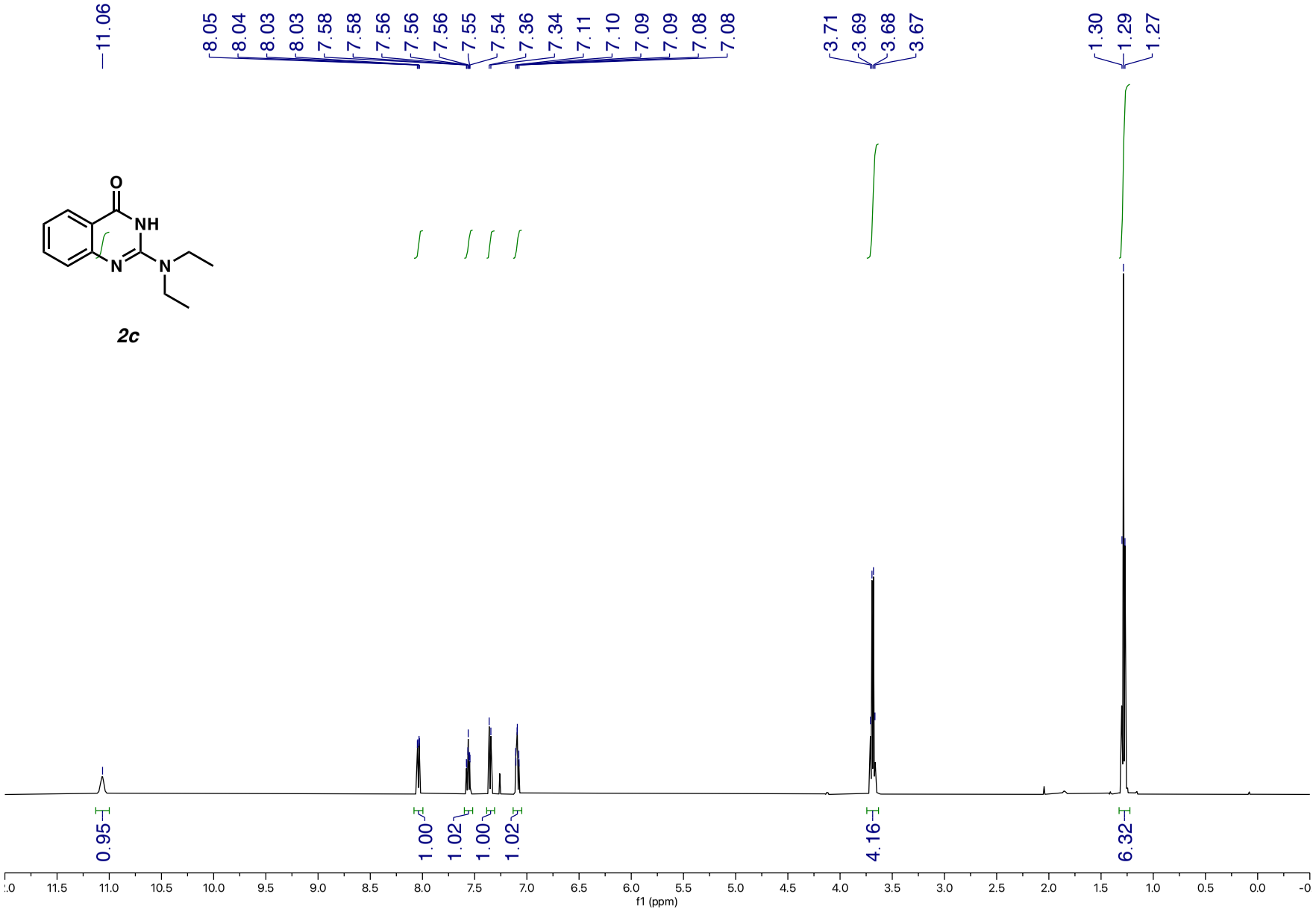
^{1}H NMR (300 MHz, $CDCl_3$) of compound **2b**.



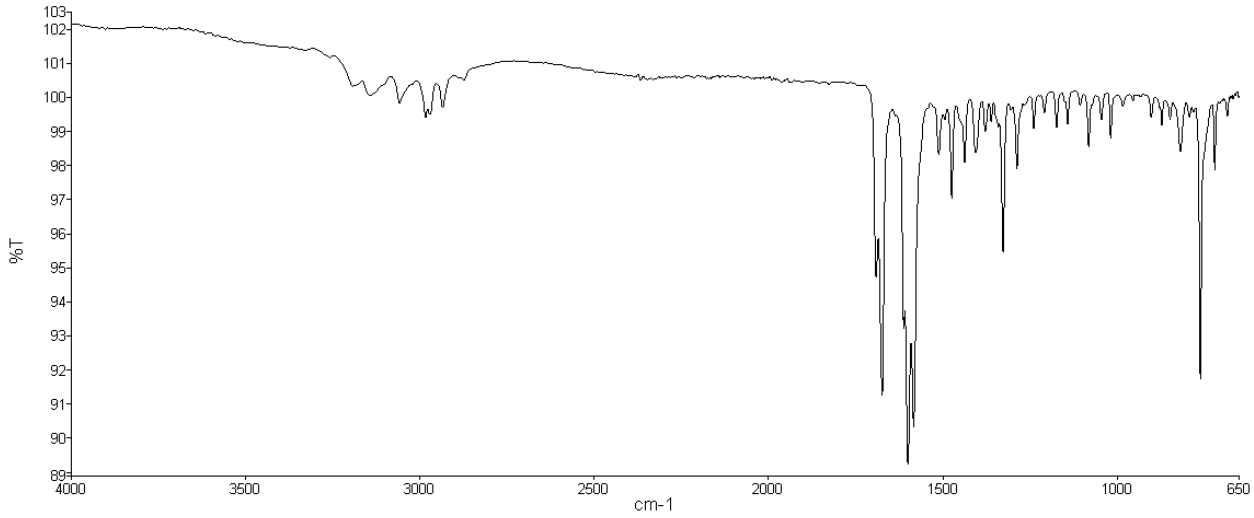
Infrared spectrum (Thin Film) of compound **2b**.



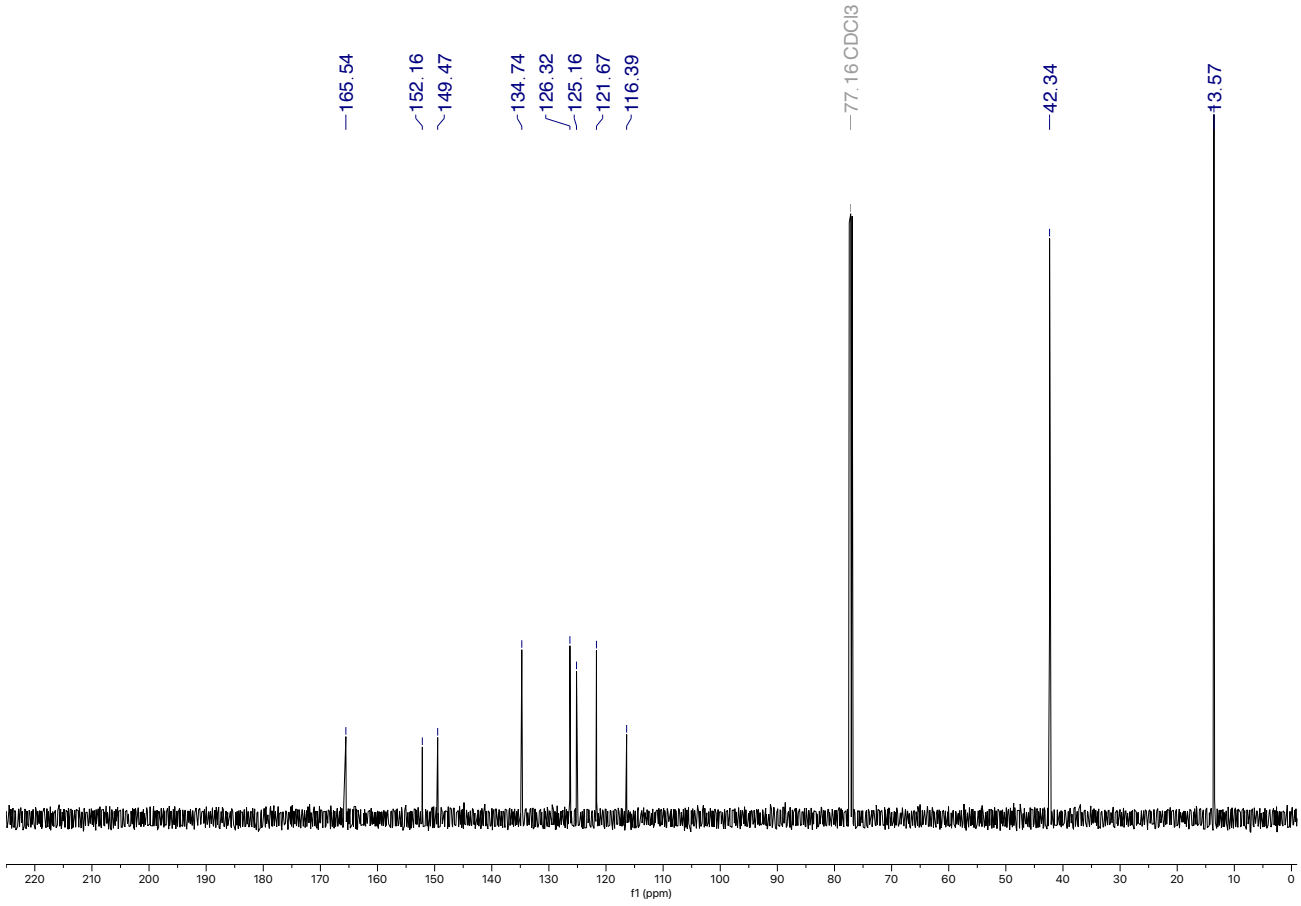
^{13}C NMR (126 MHz, $CDCl_3$) of compound **2b**.



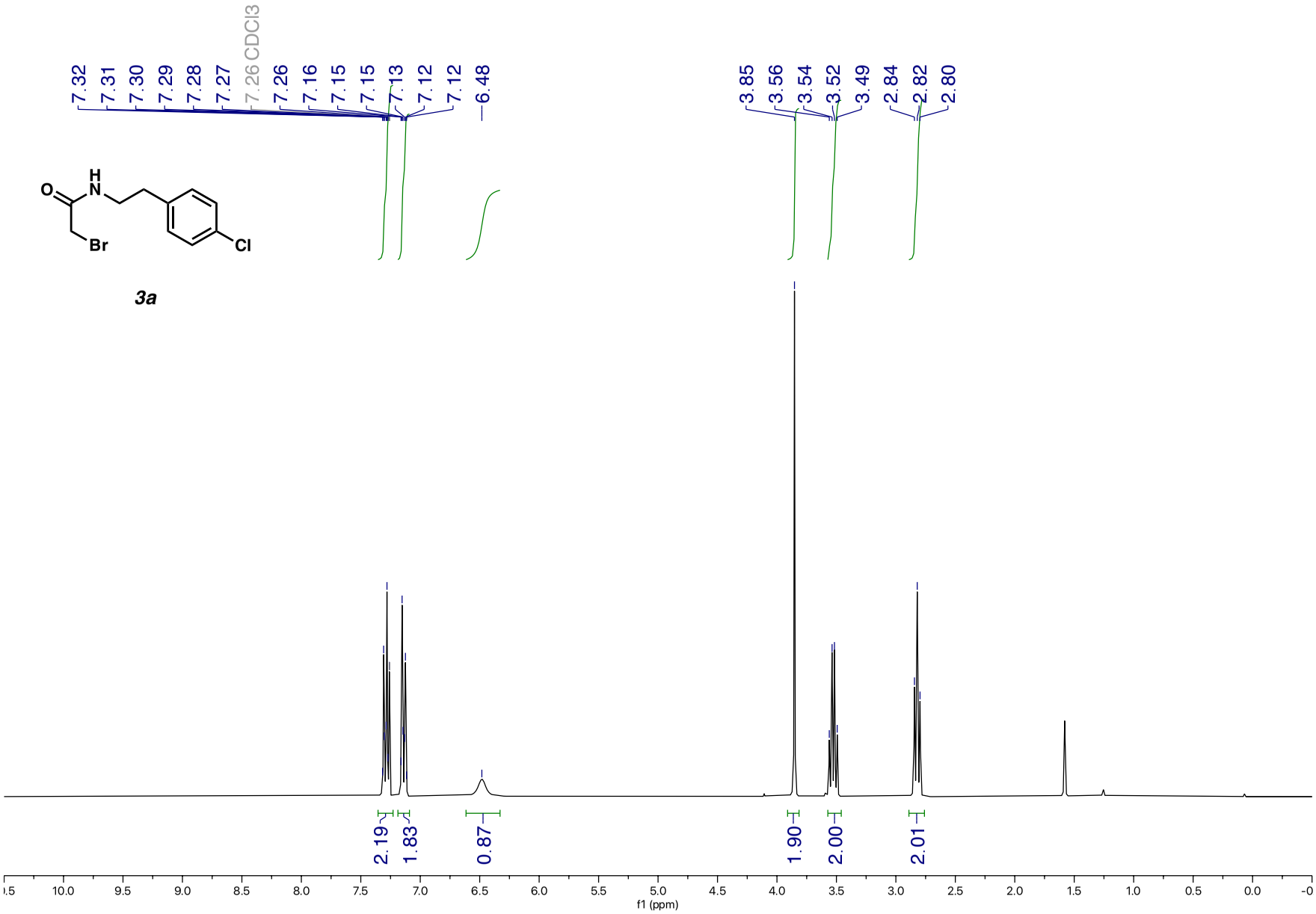
^{1}H NMR (500 MHz, $CDCl_3$) of compound **2c**.



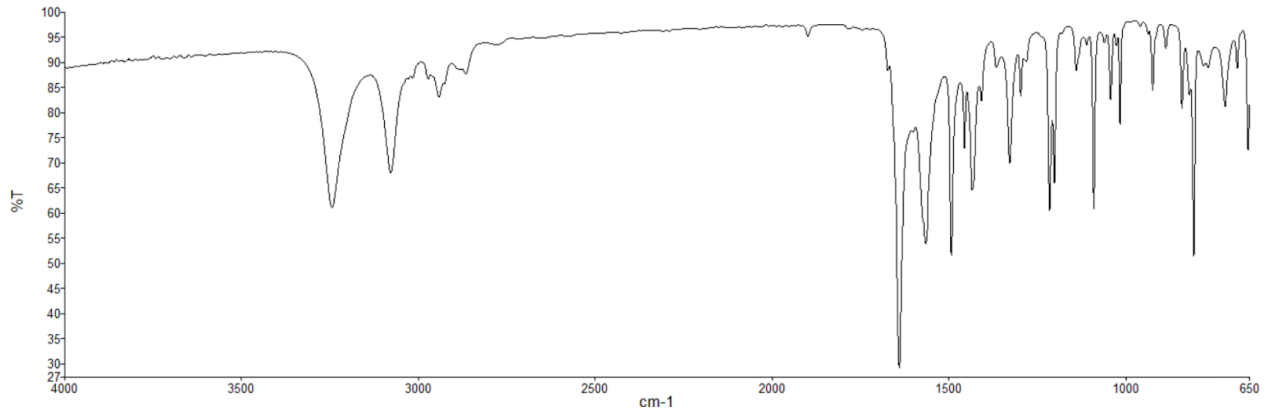
Infrared spectrum (Thin Film) of compound **2c**.



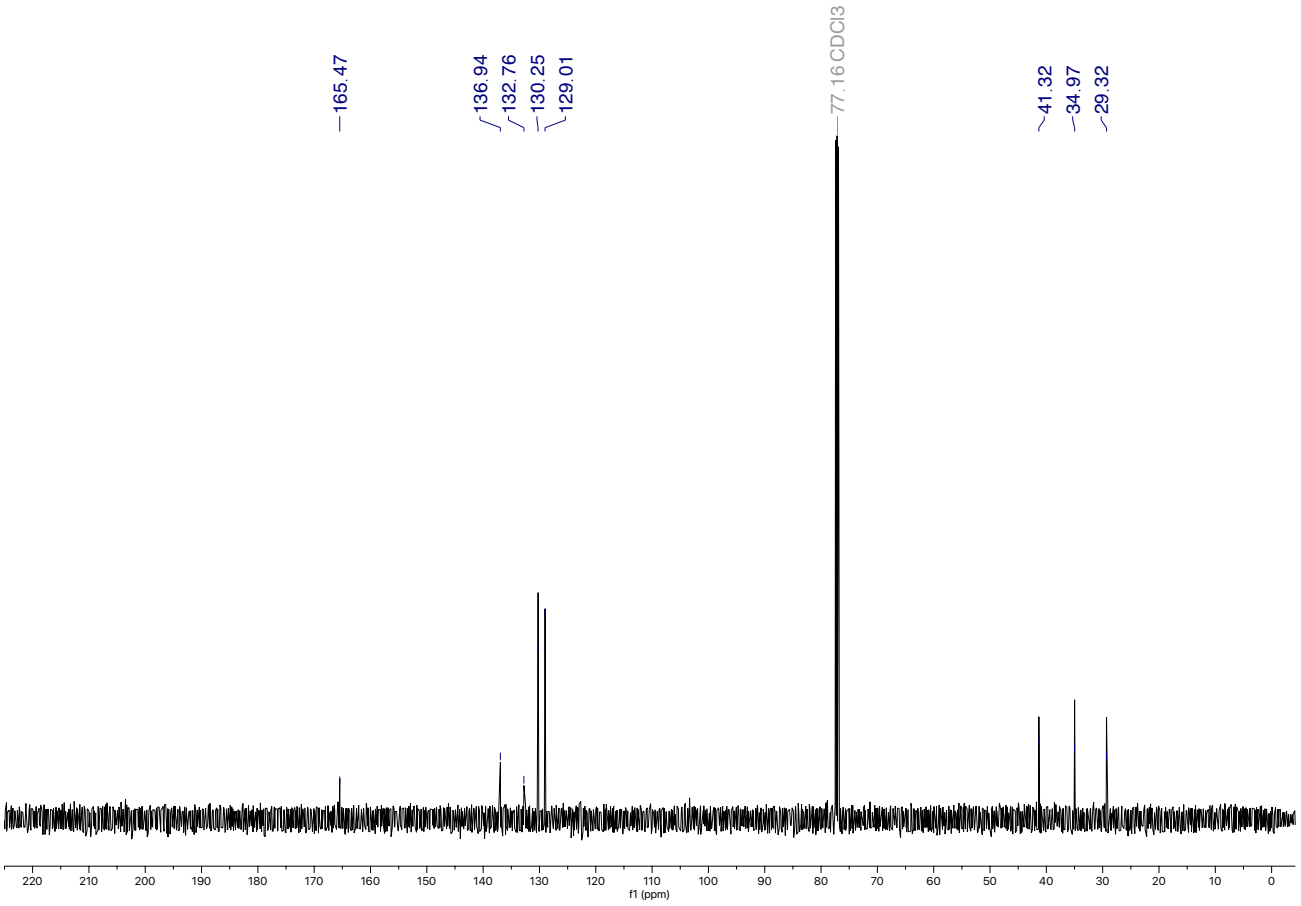
^{13}C NMR (126 MHz, $CDCl_3$) of compound **2c**.



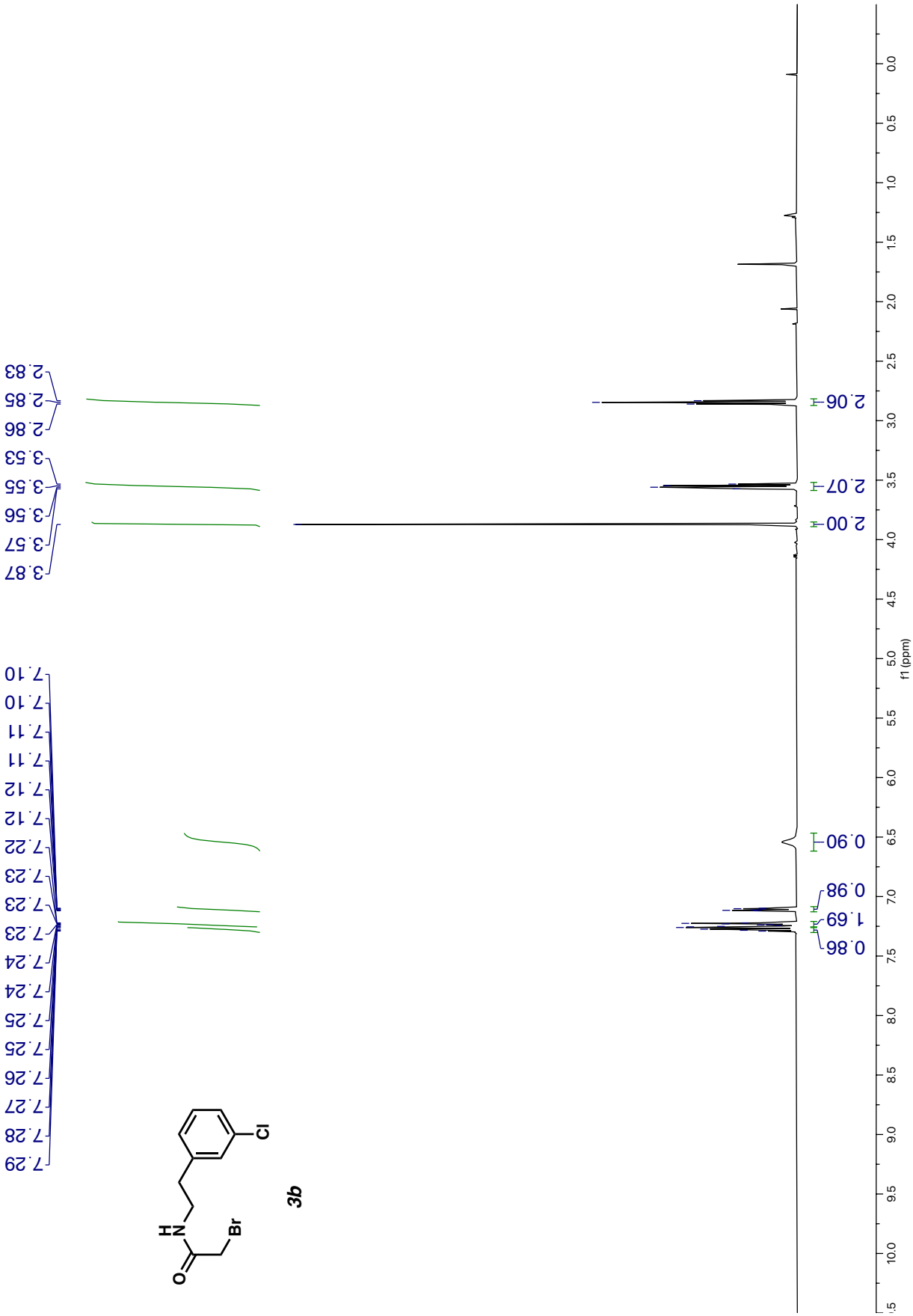
^{1}H NMR (300 MHz, $CDCl_3$) of compound **3a**.



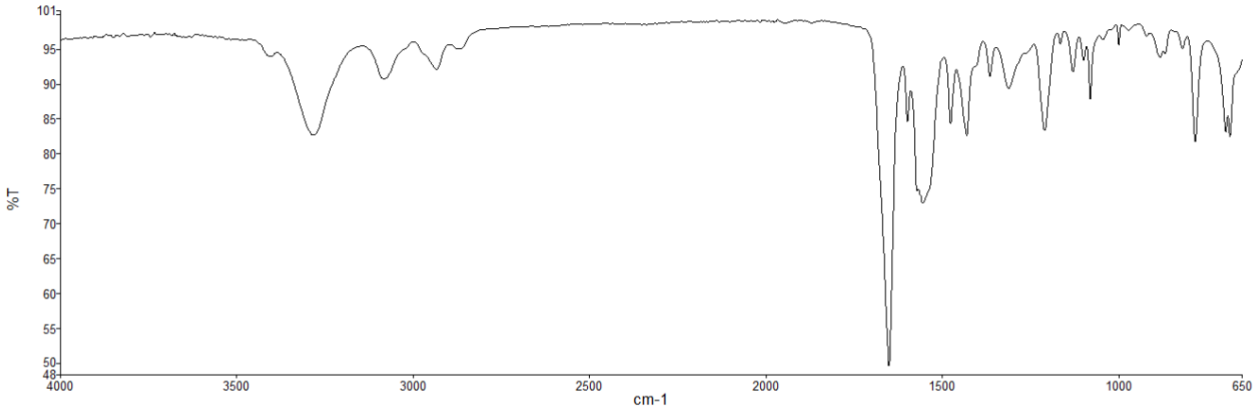
Infrared spectrum (Thin Film) of compound **3a**.



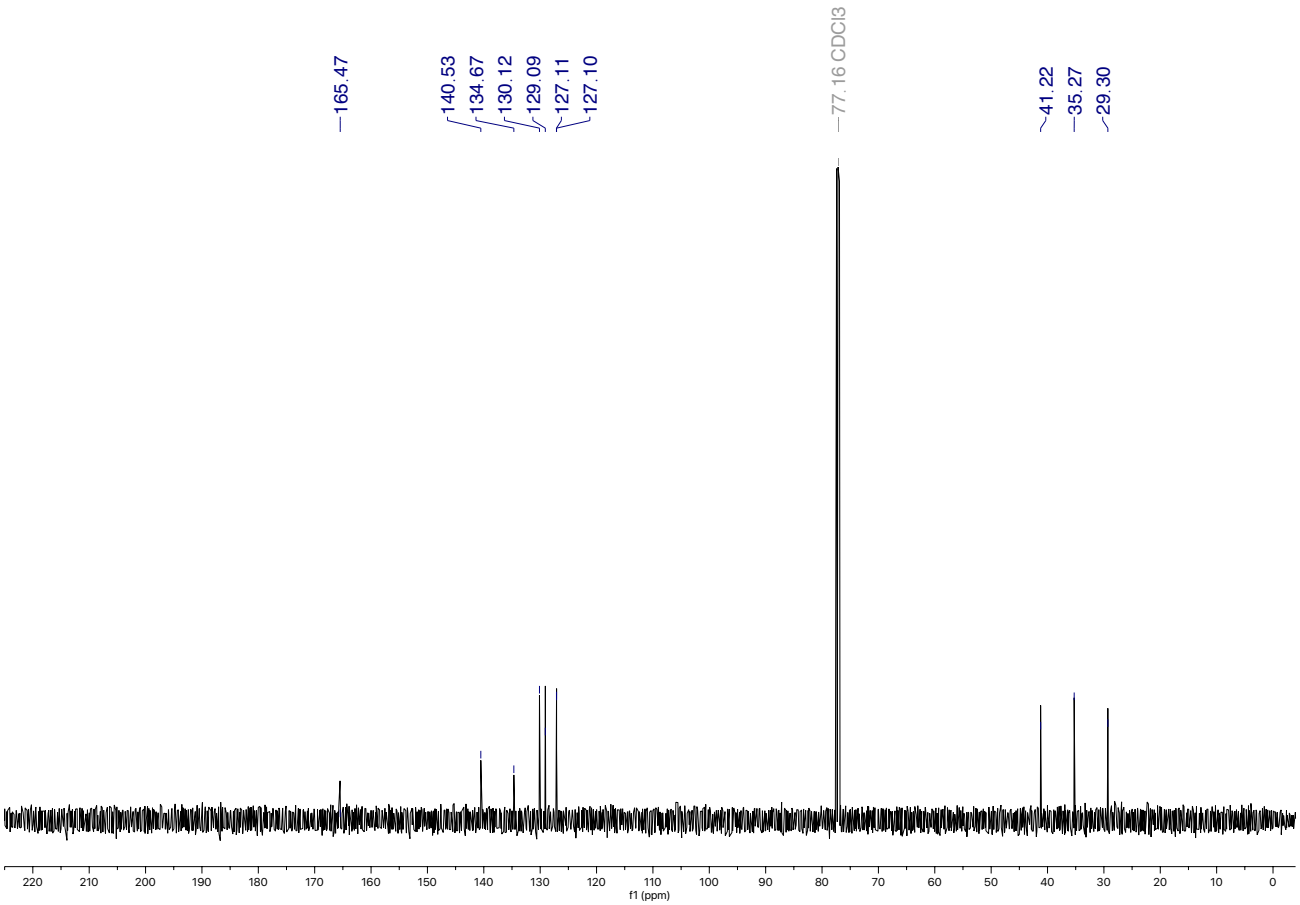
^{13}C NMR (126 MHz, $CDCl_3$) of compound **3a**.



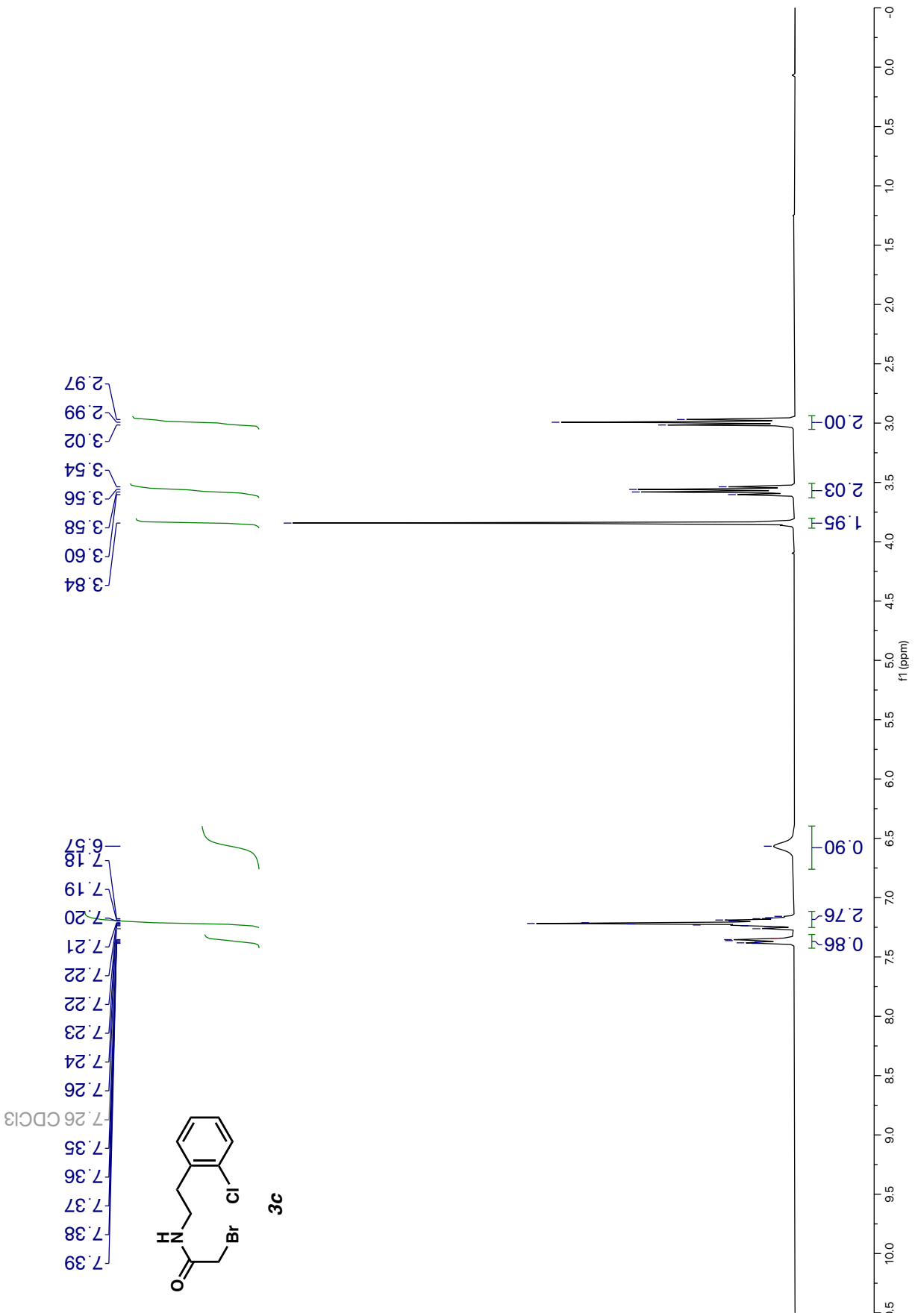
^{1}H NMR (500 MHz, $CDCl_3$) of compound **3b**.



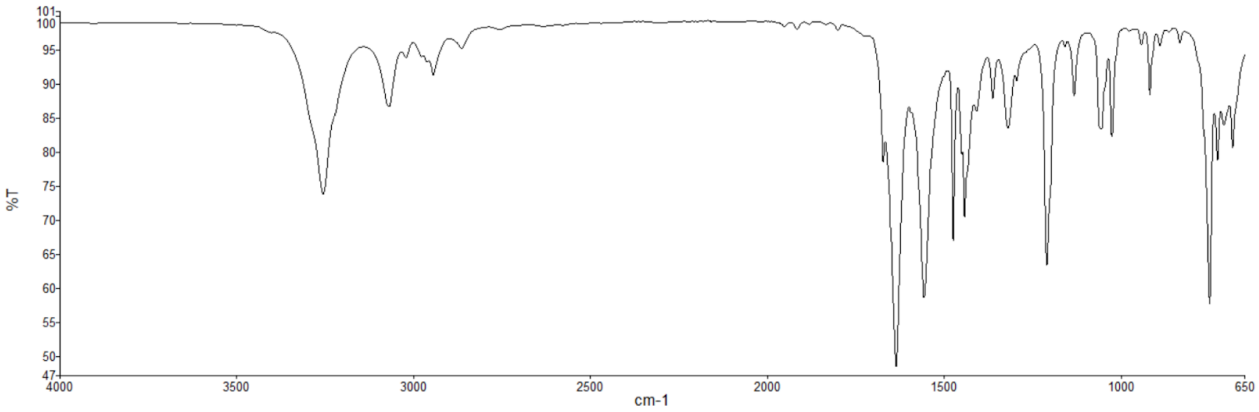
Infrared spectrum (Thin Film) of compound **3b**.



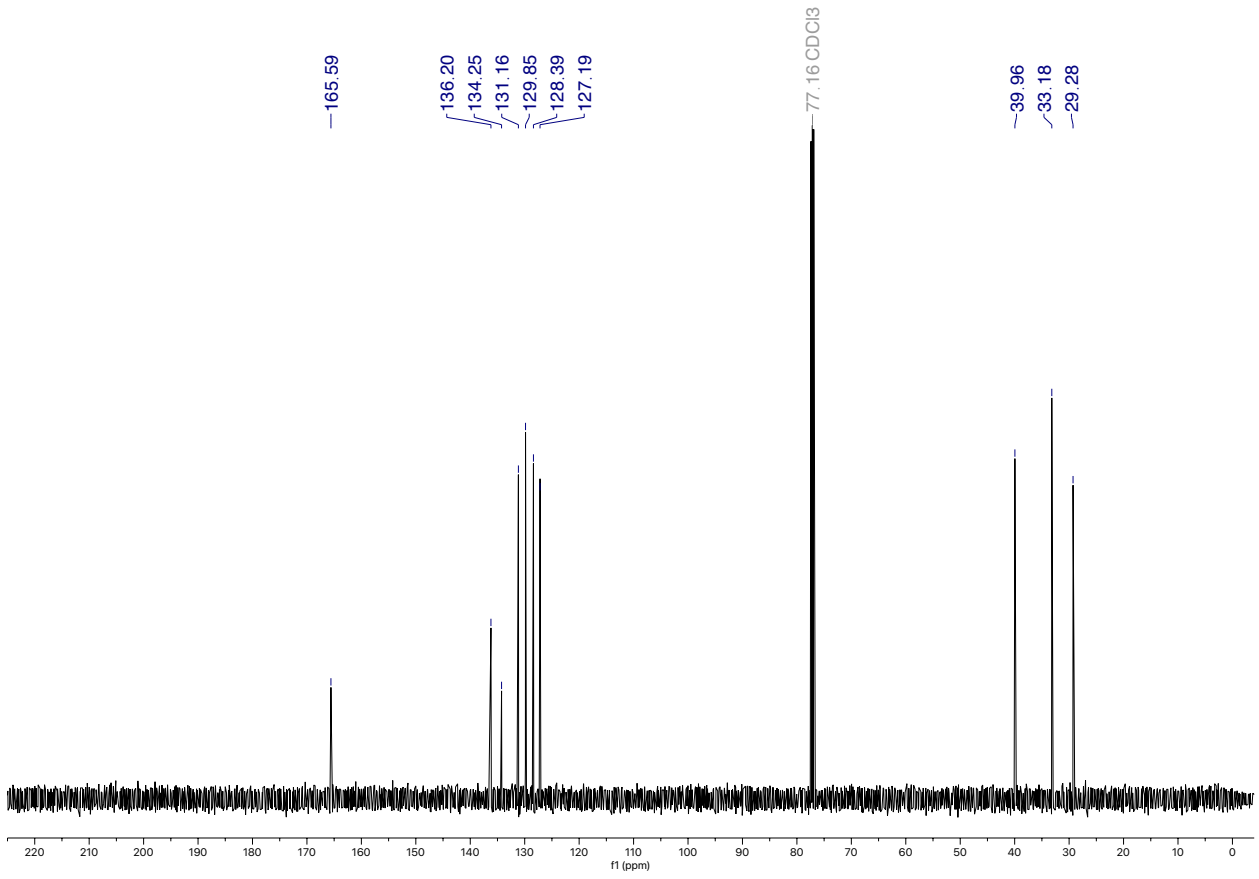
^{13}C NMR (126 MHz, $CDCl_3$) of compound **3b**.



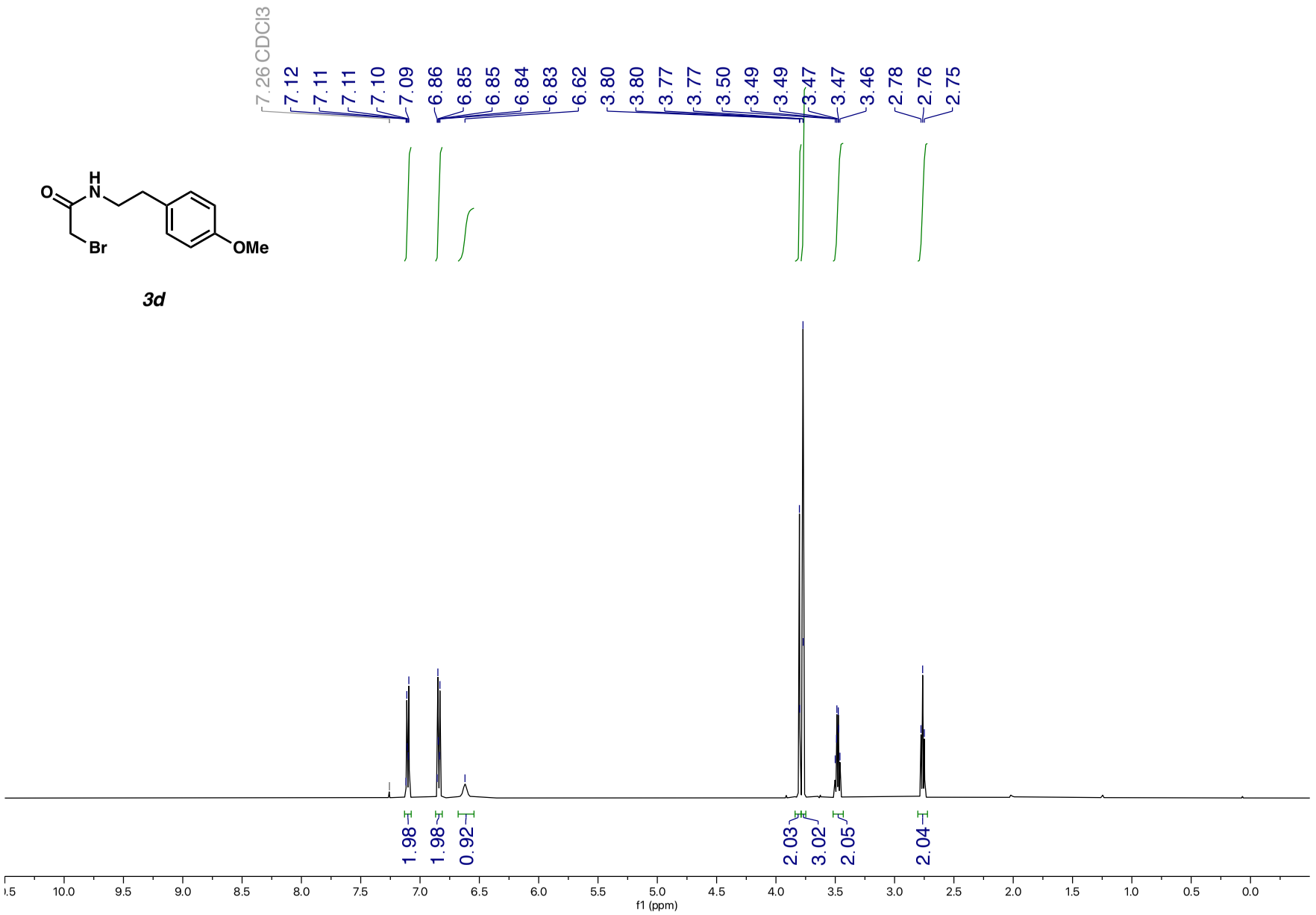
^{1}H NMR (300 MHz, $CDCl_3$) of compound **3c**.



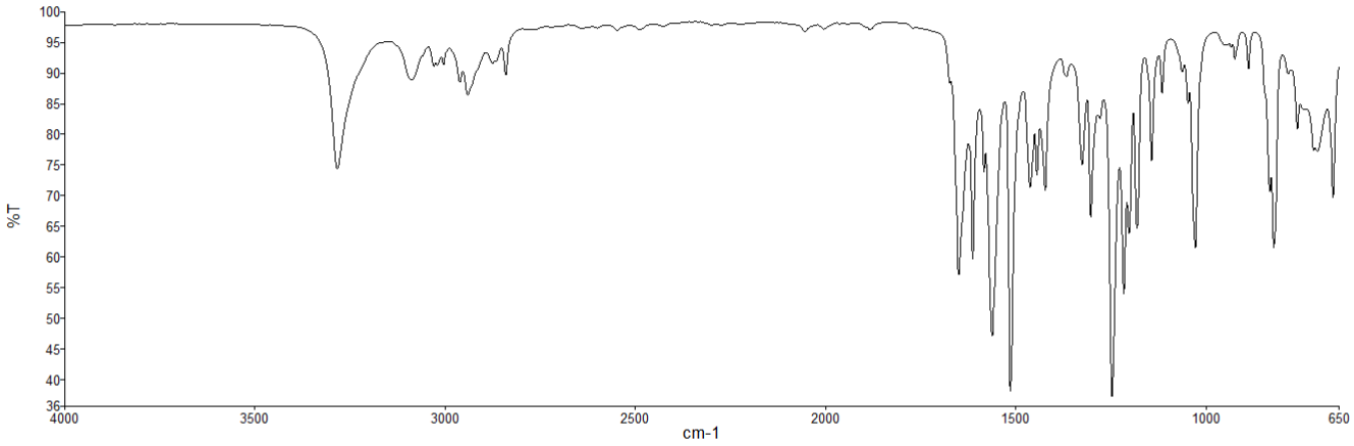
Infrared spectrum (Thin Film) of compound **3c**.



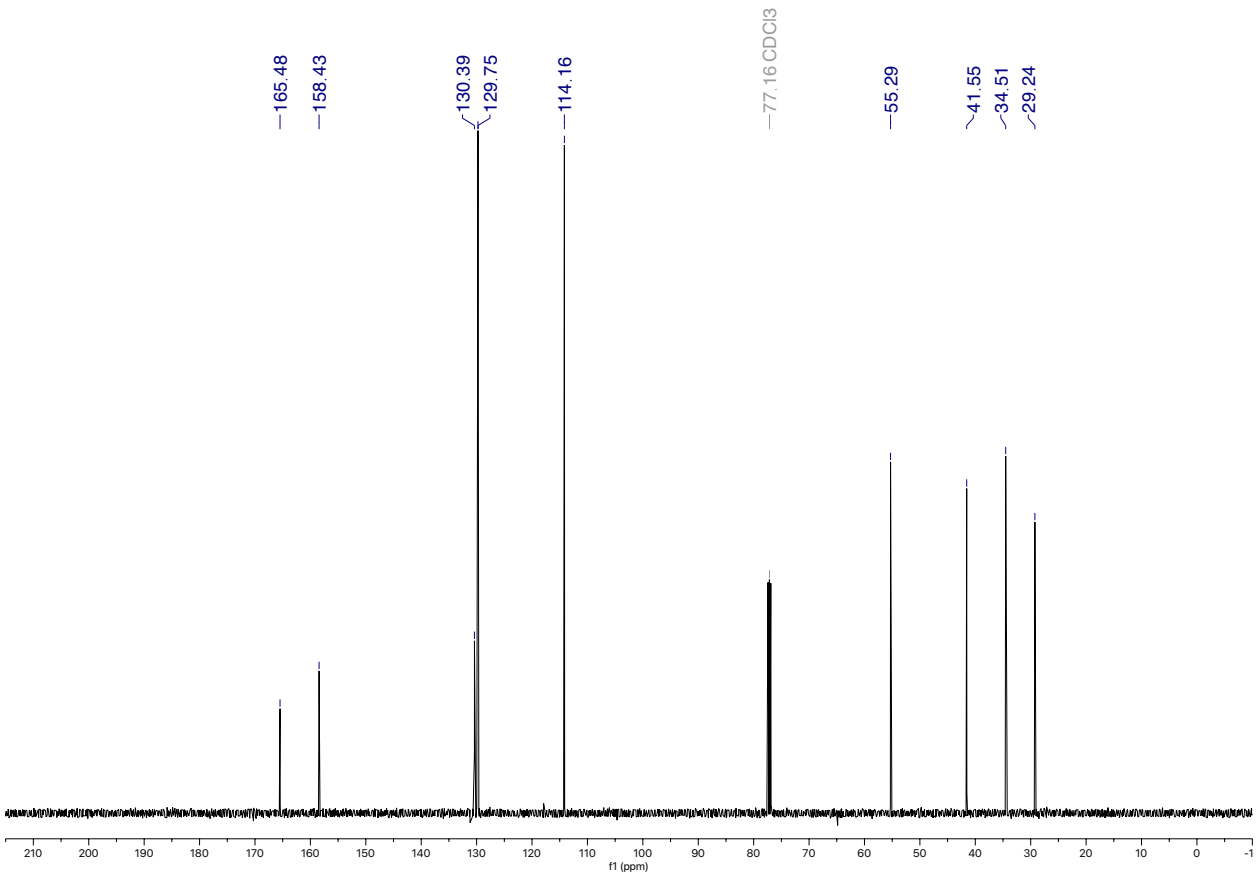
^{13}C NMR (126 MHz, $CDCl_3$) of compound **3c**.



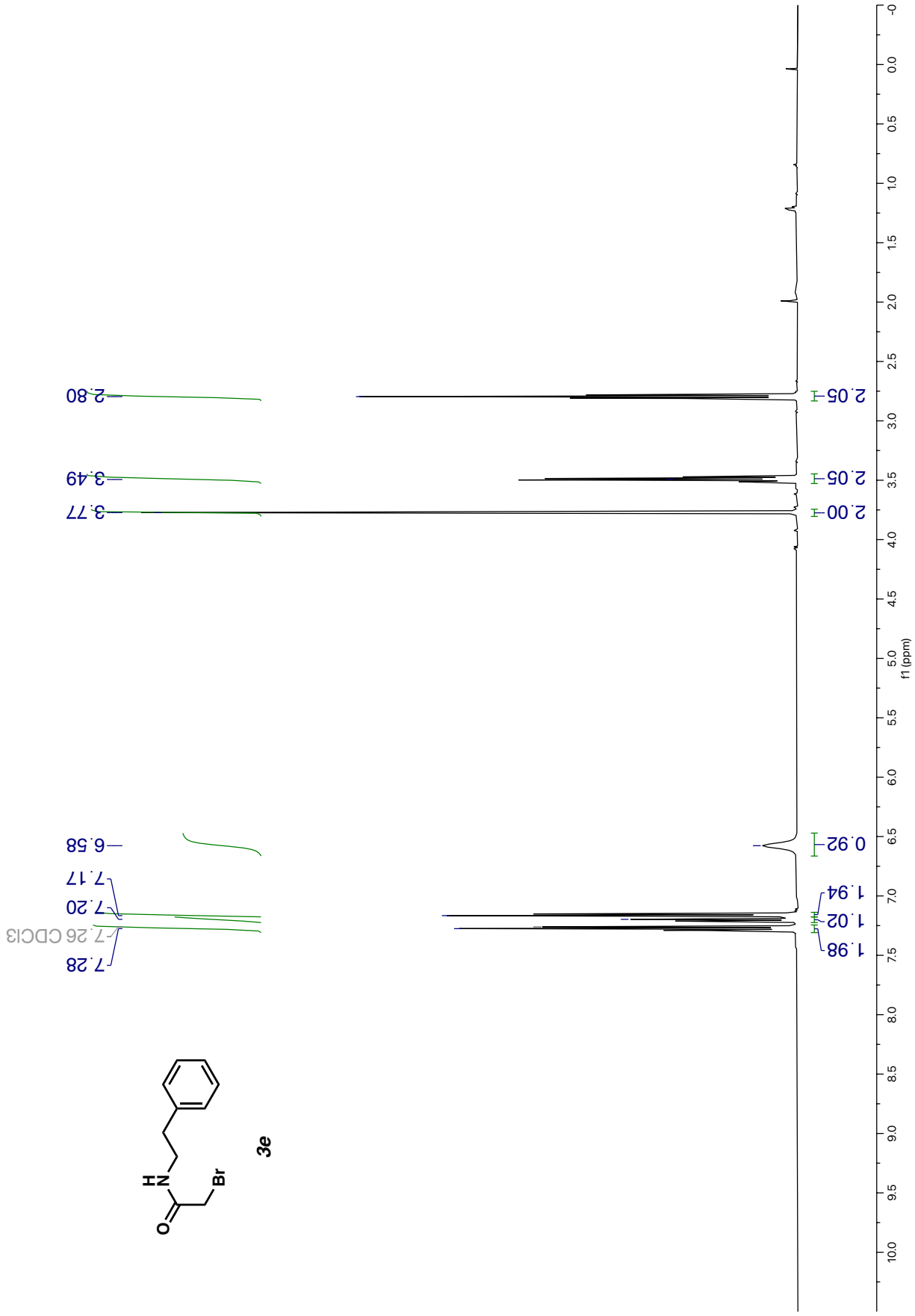
^{1}H NMR (500 MHz, $CDCl_3$) of compound **3d**.



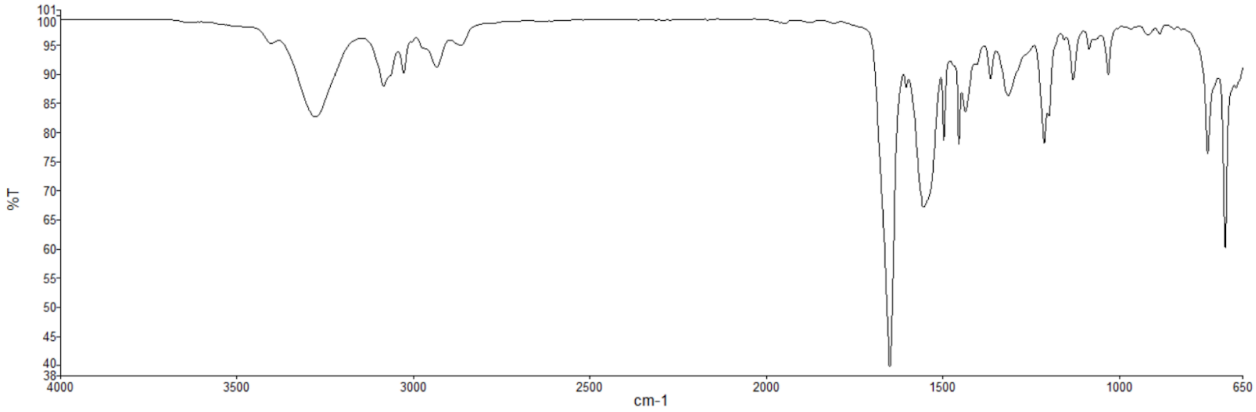
Infrared spectrum (Thin Film) of compound **3d**.



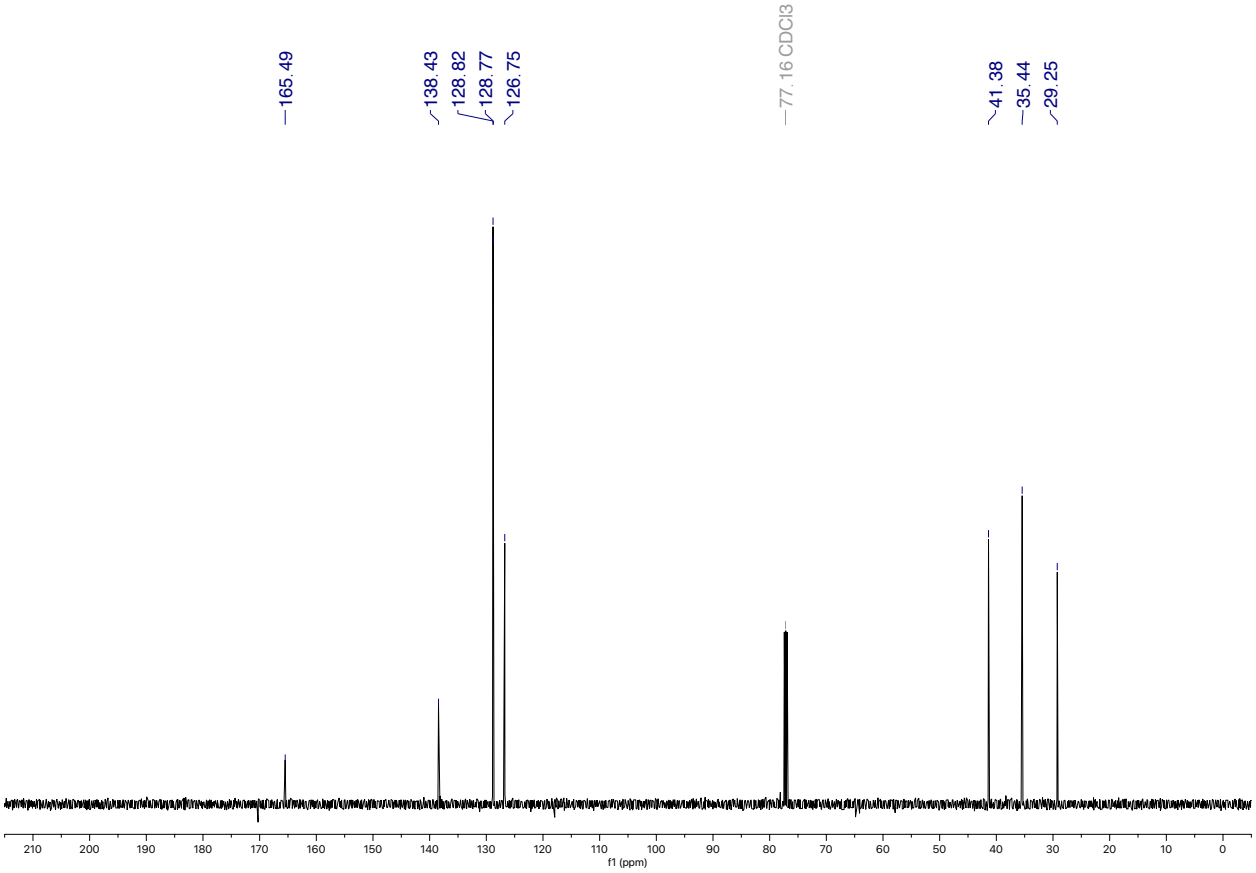
^{13}C NMR (126 MHz, $CDCl_3$) of compound **3d**.



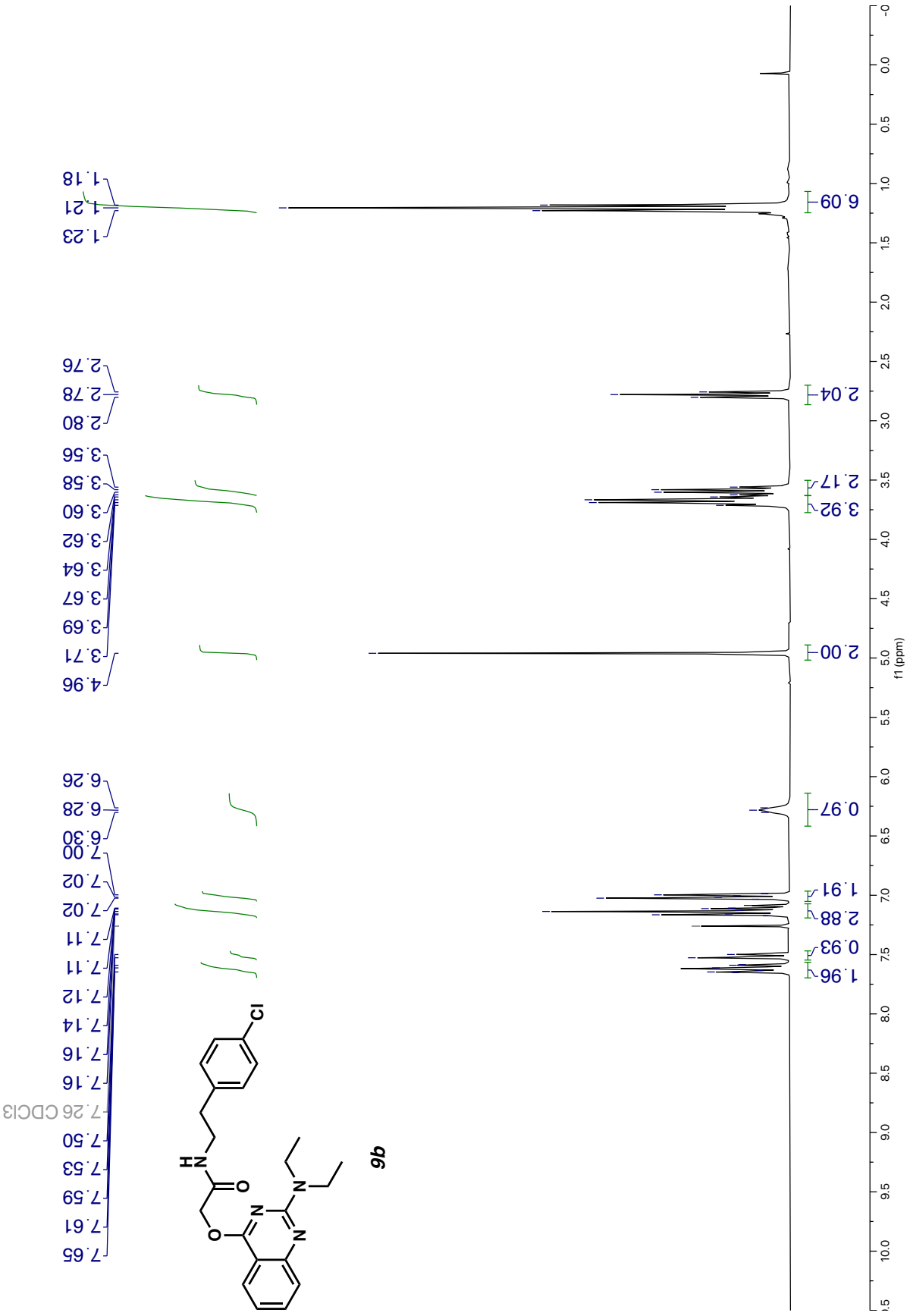
^{1}H NMR (500 MHz, $CDCl_3$) of compound **3e**.



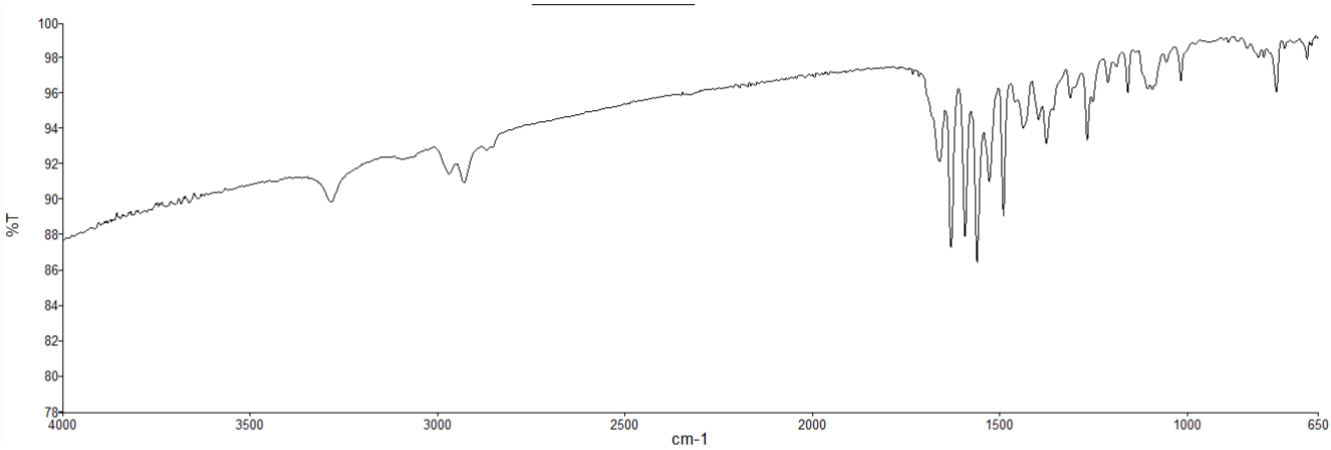
Infrared spectrum (Thin Film) of compound **3e**.



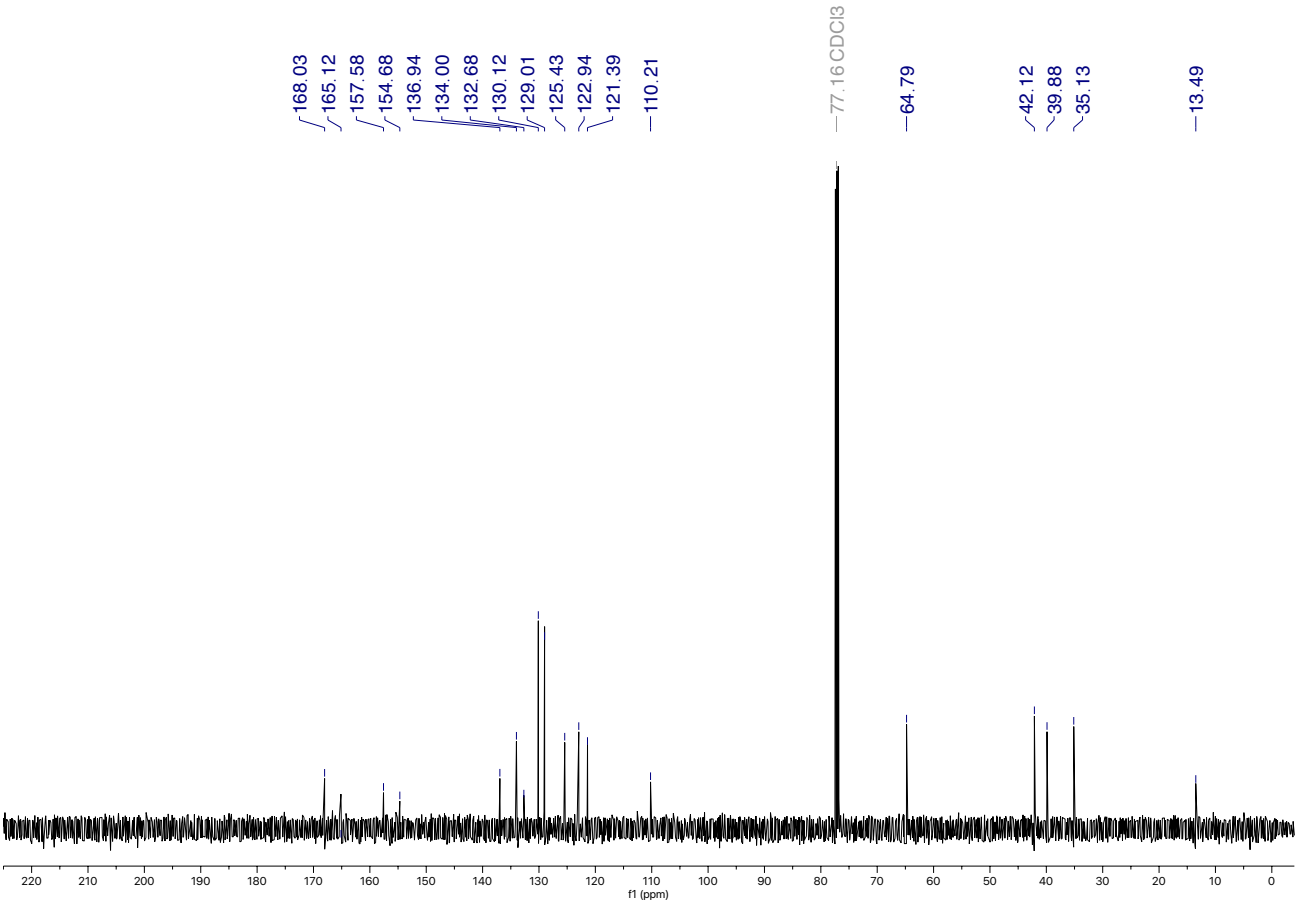
^{13}C NMR (126 MHz, $CDCl_3$) of compound **3e**.



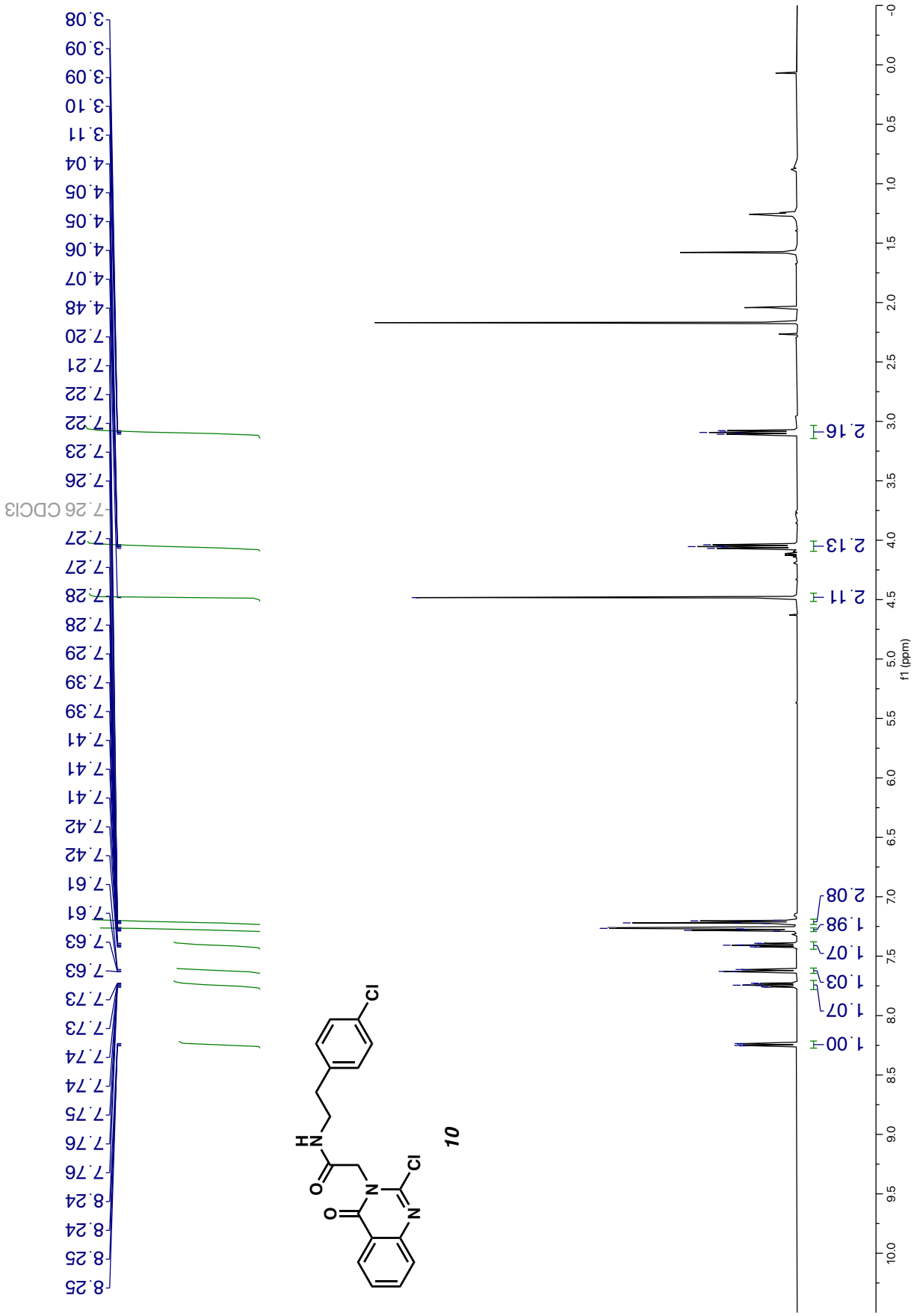
^{1}H NMR (300 MHz, $CDCl_3$) of compound **9b**.



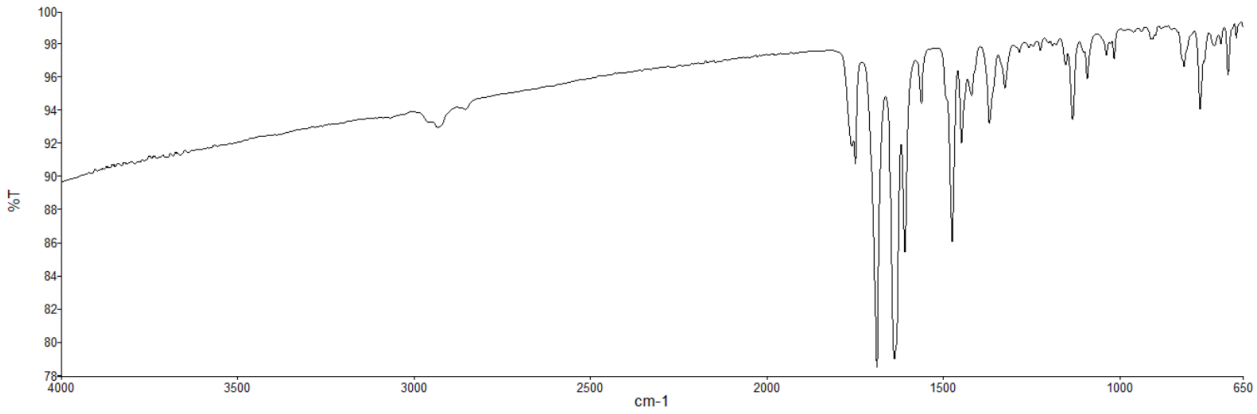
Infrared spectrum (Thin Film) of compound **9b**.



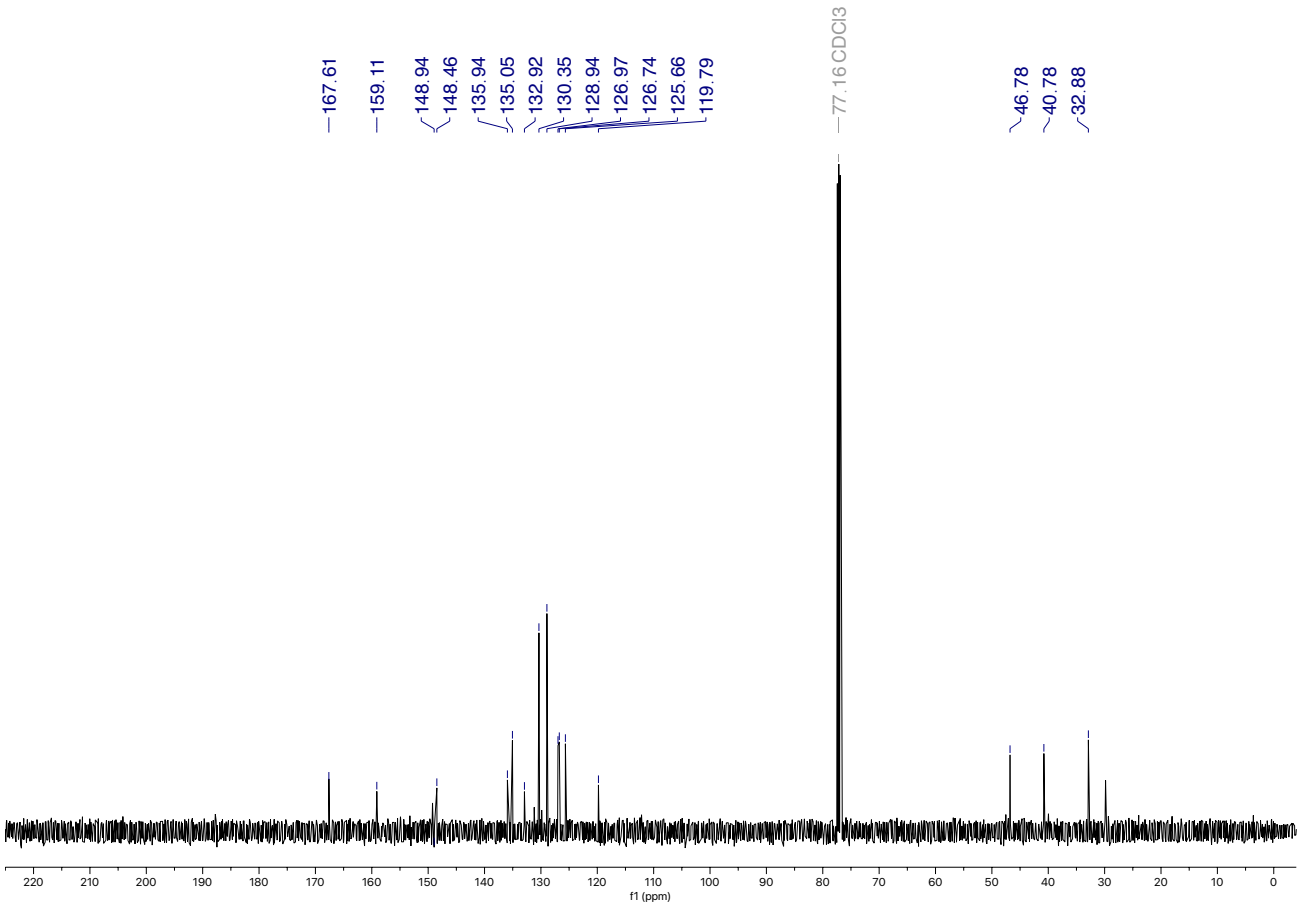
^{13}C NMR (126 MHz, $CDCl_3$) of compound **9b**.



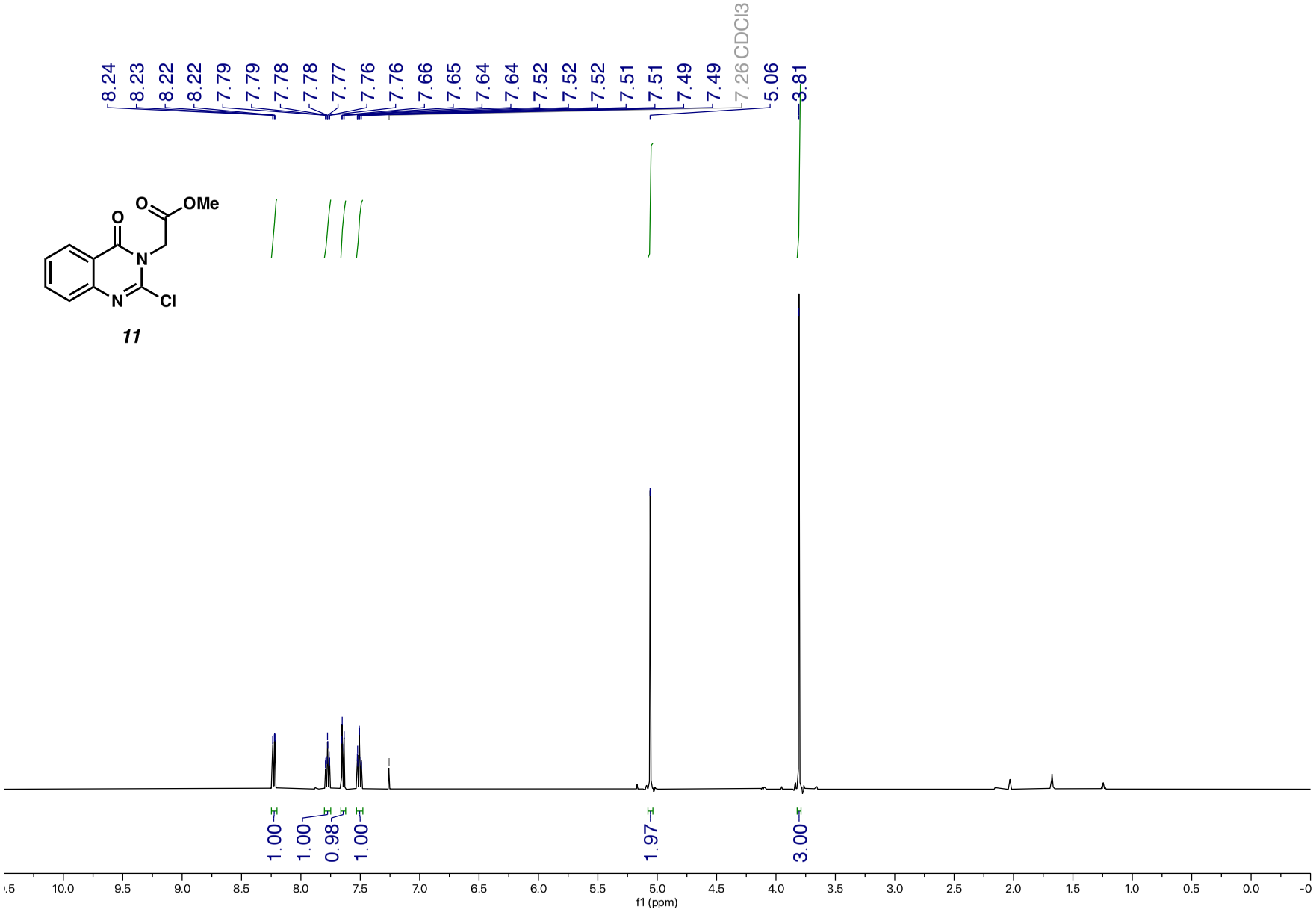
^{1}H NMR (500 MHz, $CDCl_3$) of compound **10**.



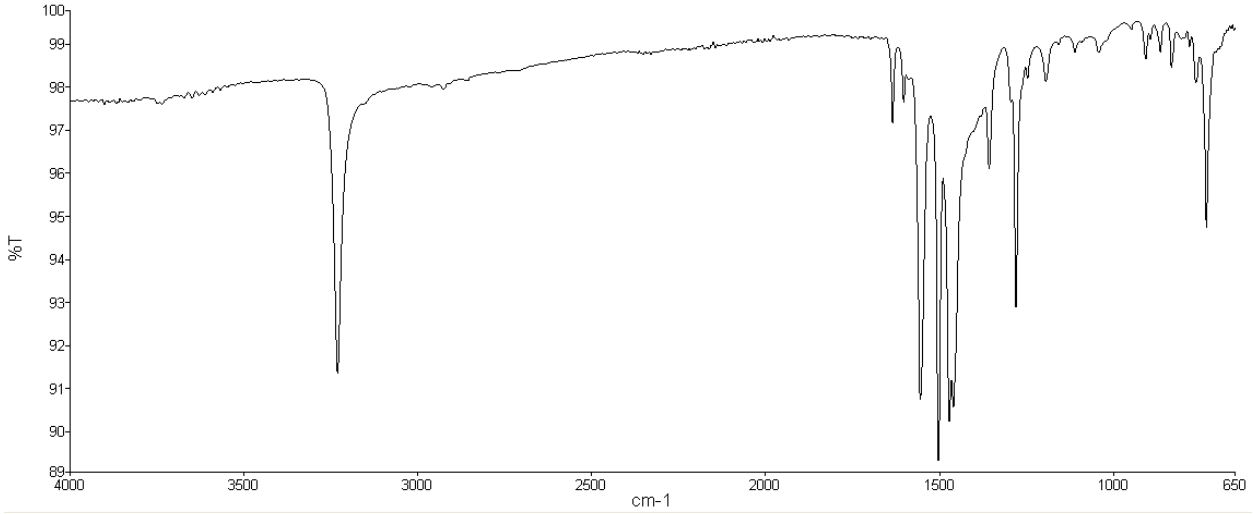
Infrared spectrum (Thin Film) of compound **10**.



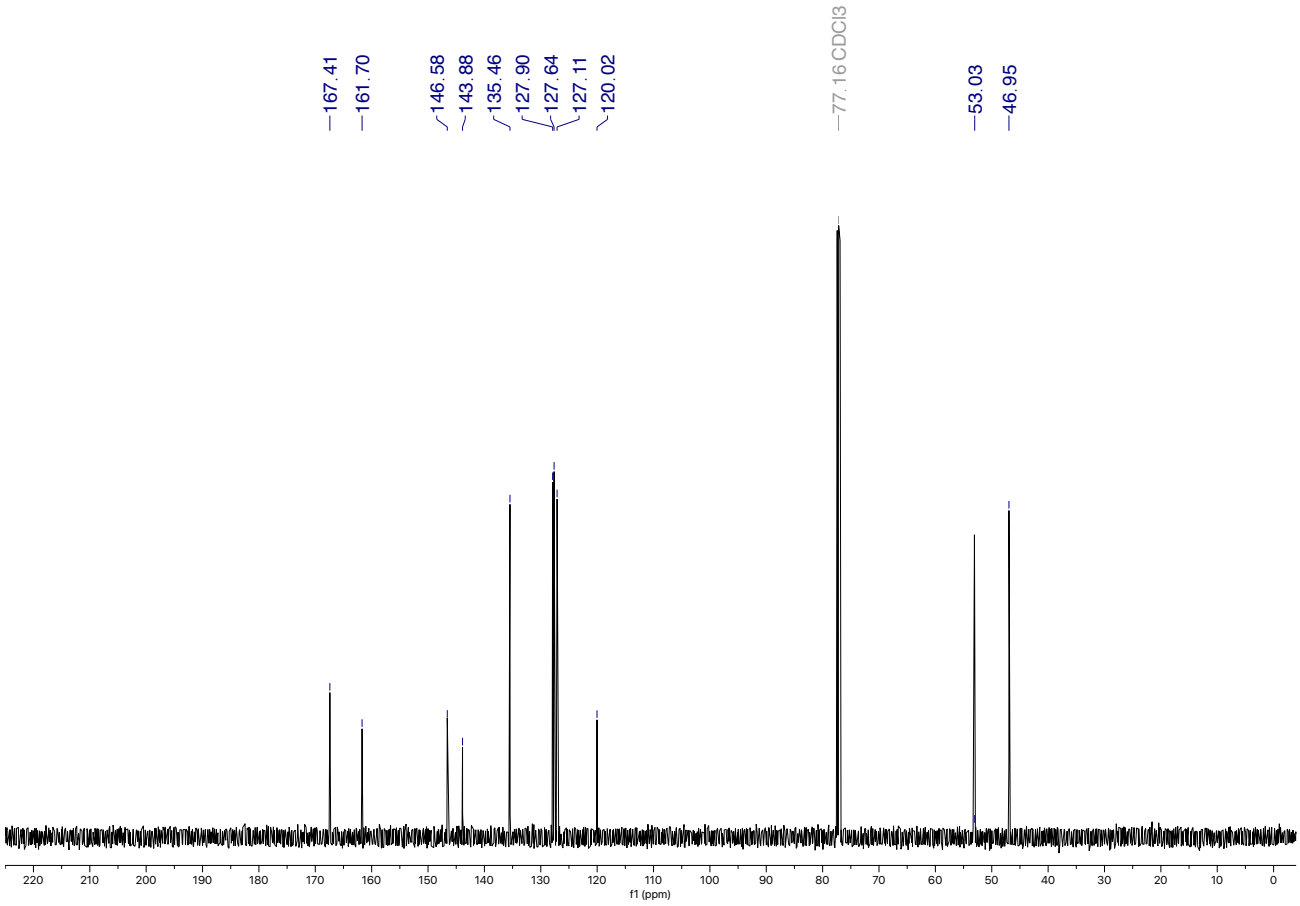
^{13}C NMR (126 MHz, $CDCl_3$) of compound **10**.



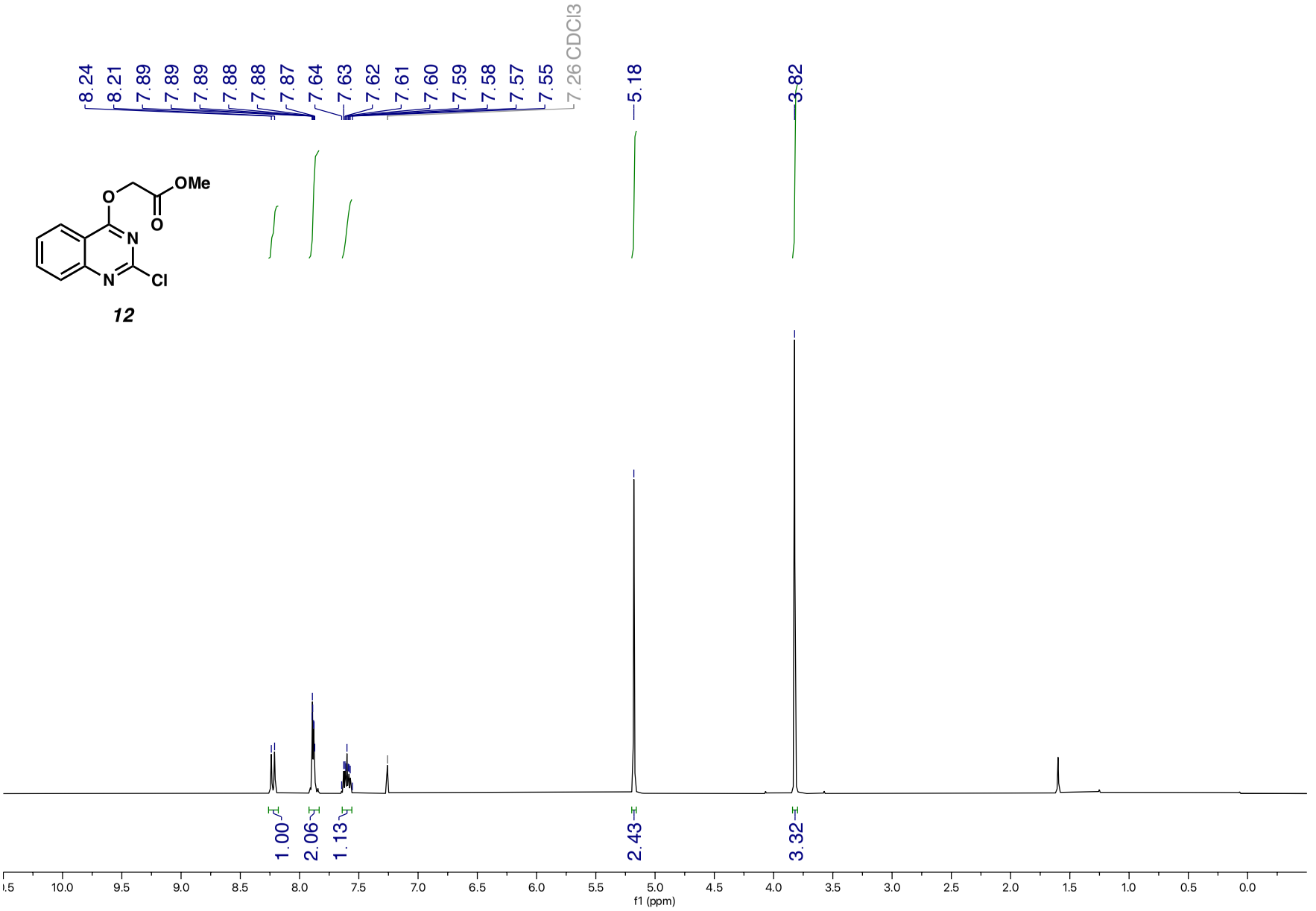
^{1}H NMR (500 MHz, $CDCl_3$) of compound **11**.



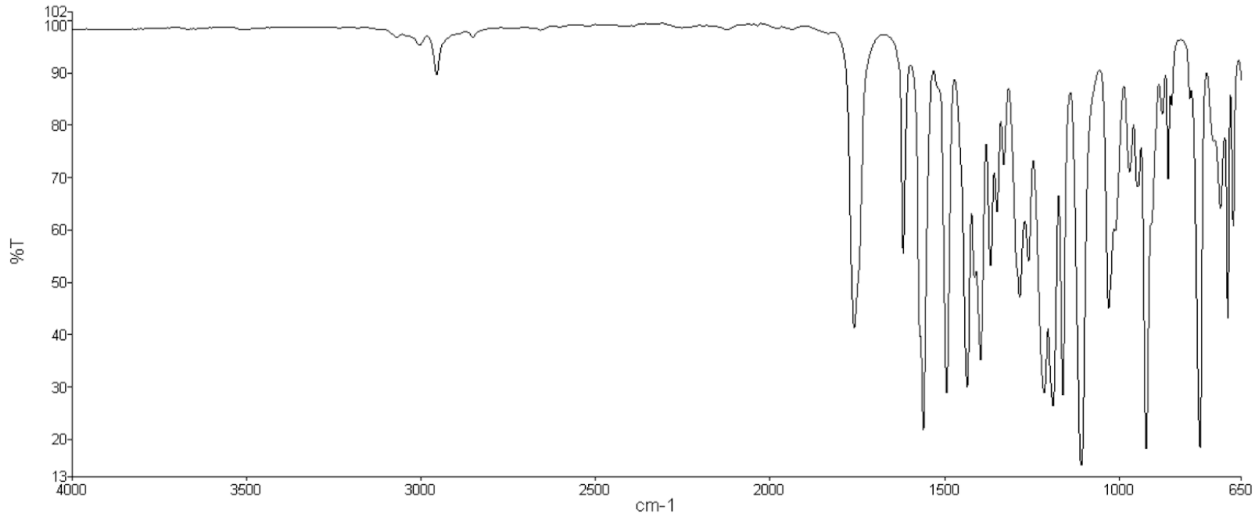
Infrared spectrum (Thin Film) of compound **11**.



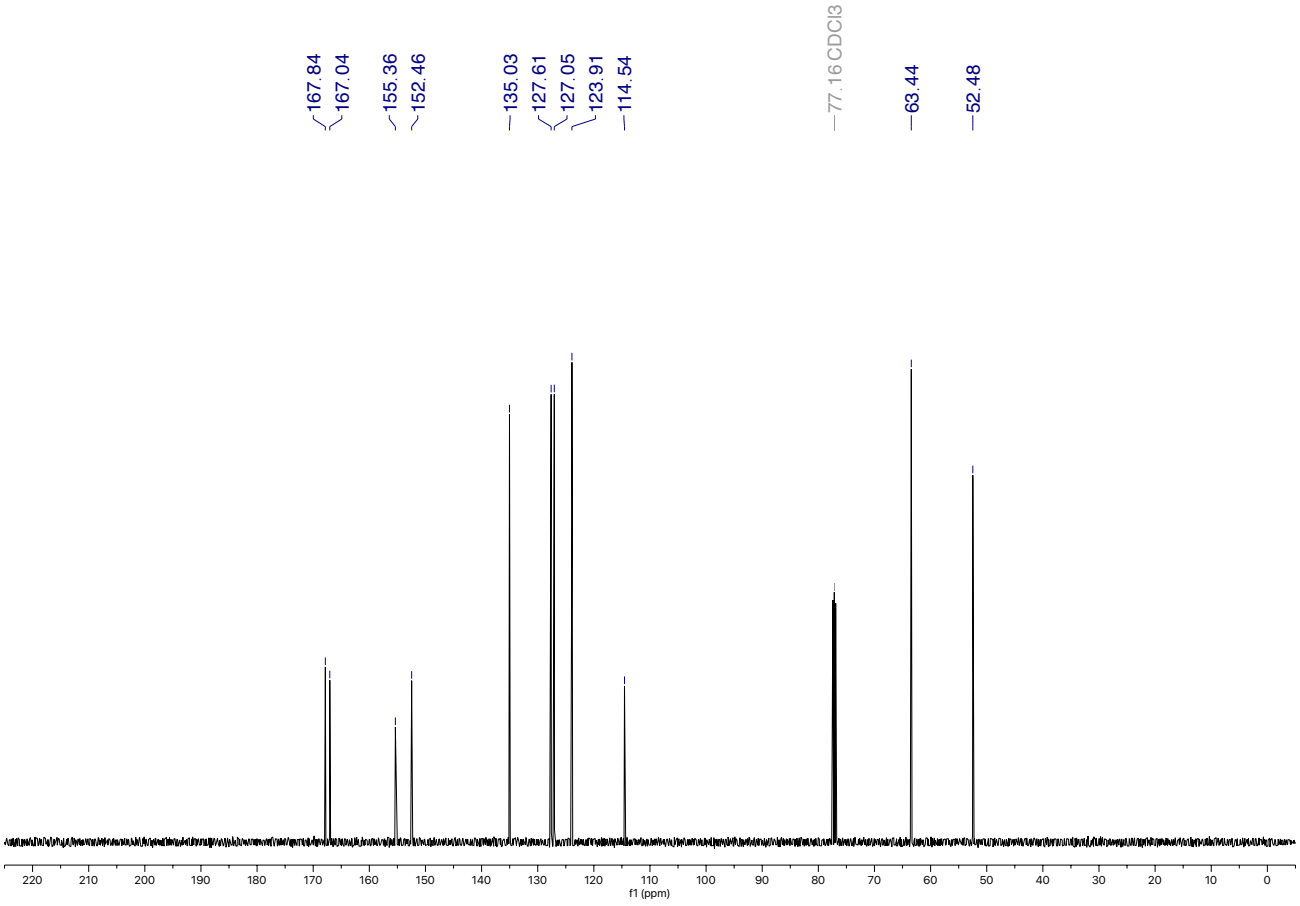
^{13}C NMR (126 MHz, $CDCl_3$) of compound **11**.



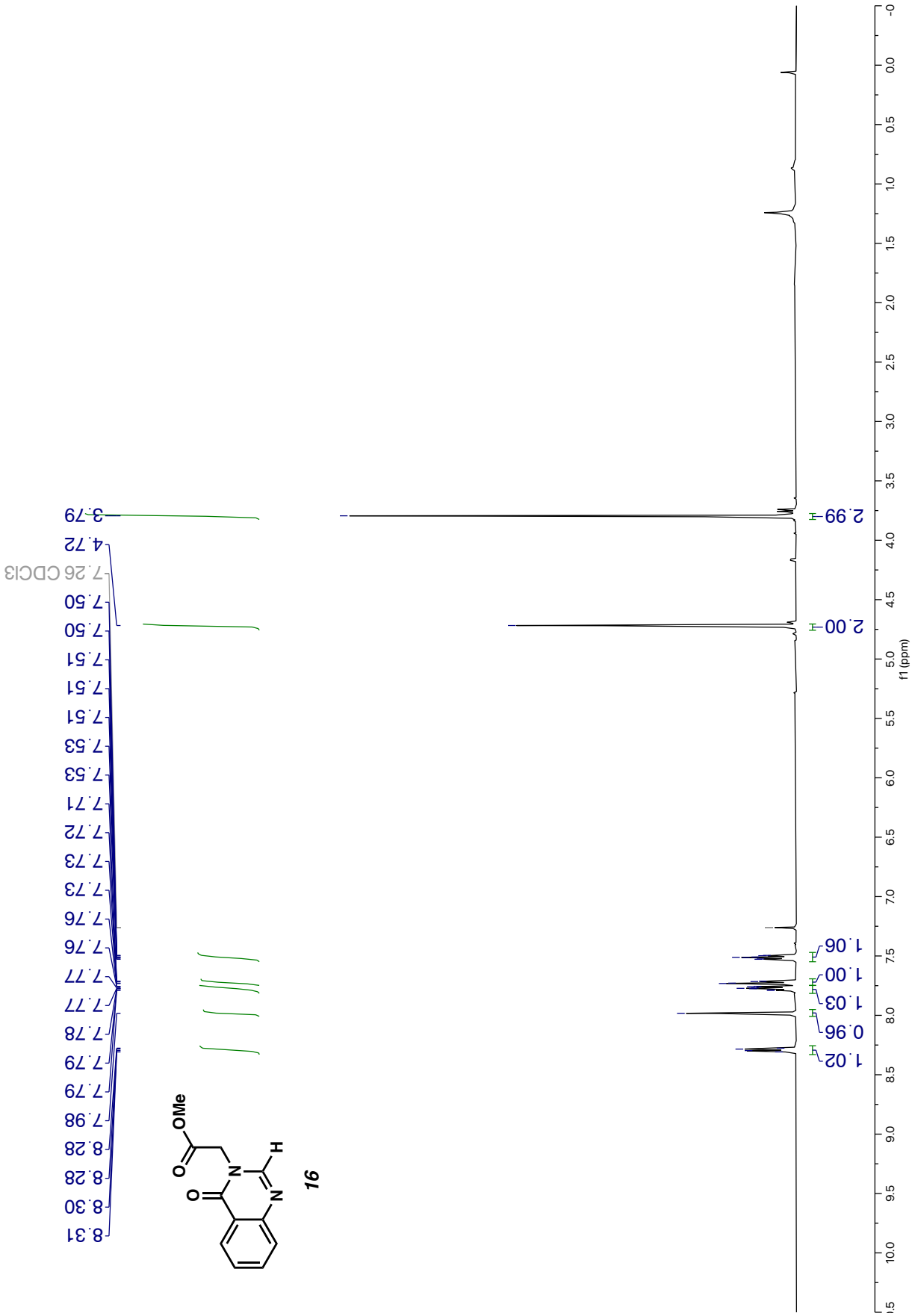
^{1}H NMR (300 MHz, $CDCl_3$) of compound **12**.



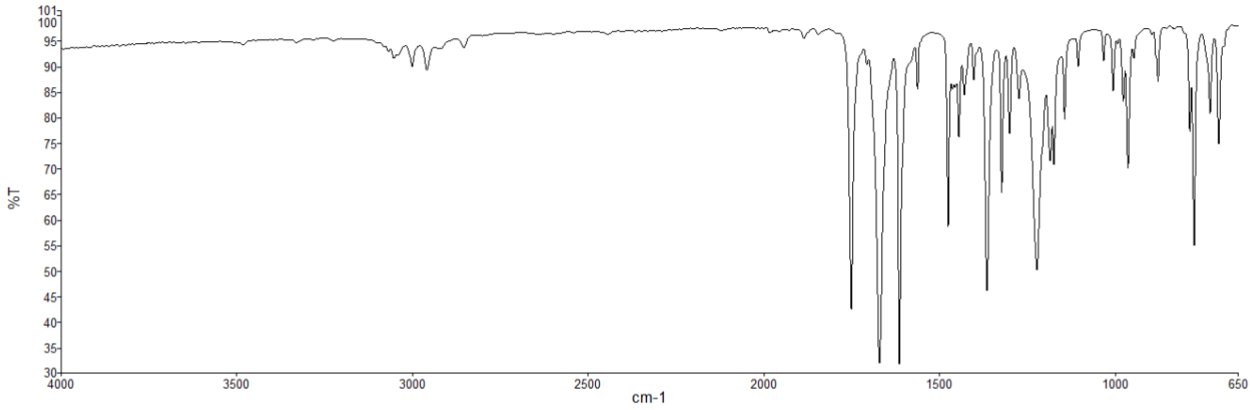
Infrared spectrum (Thin Film) of compound **12**.



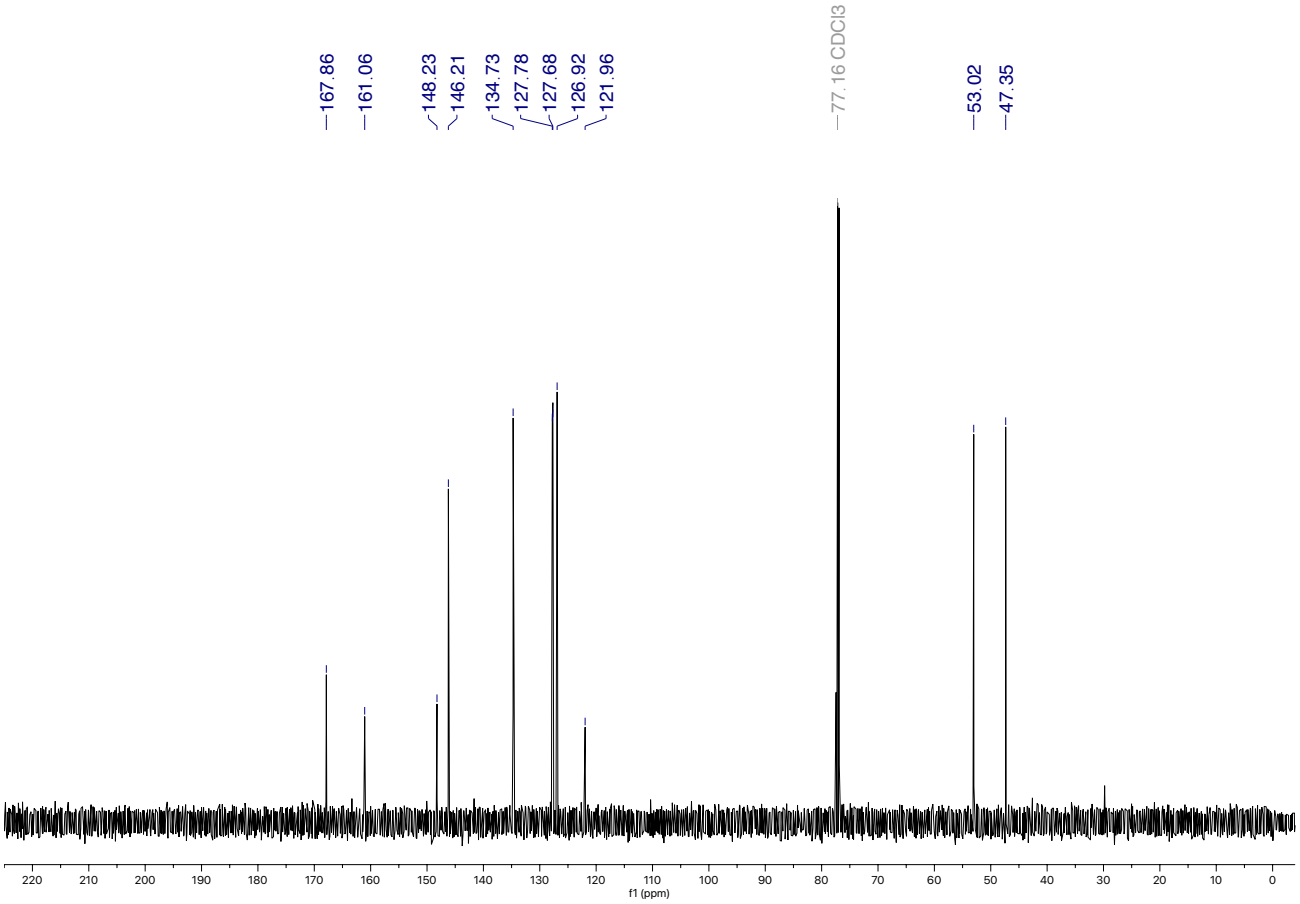
^{13}C NMR (126 MHz, $CDCl_3$) of compound **12**.



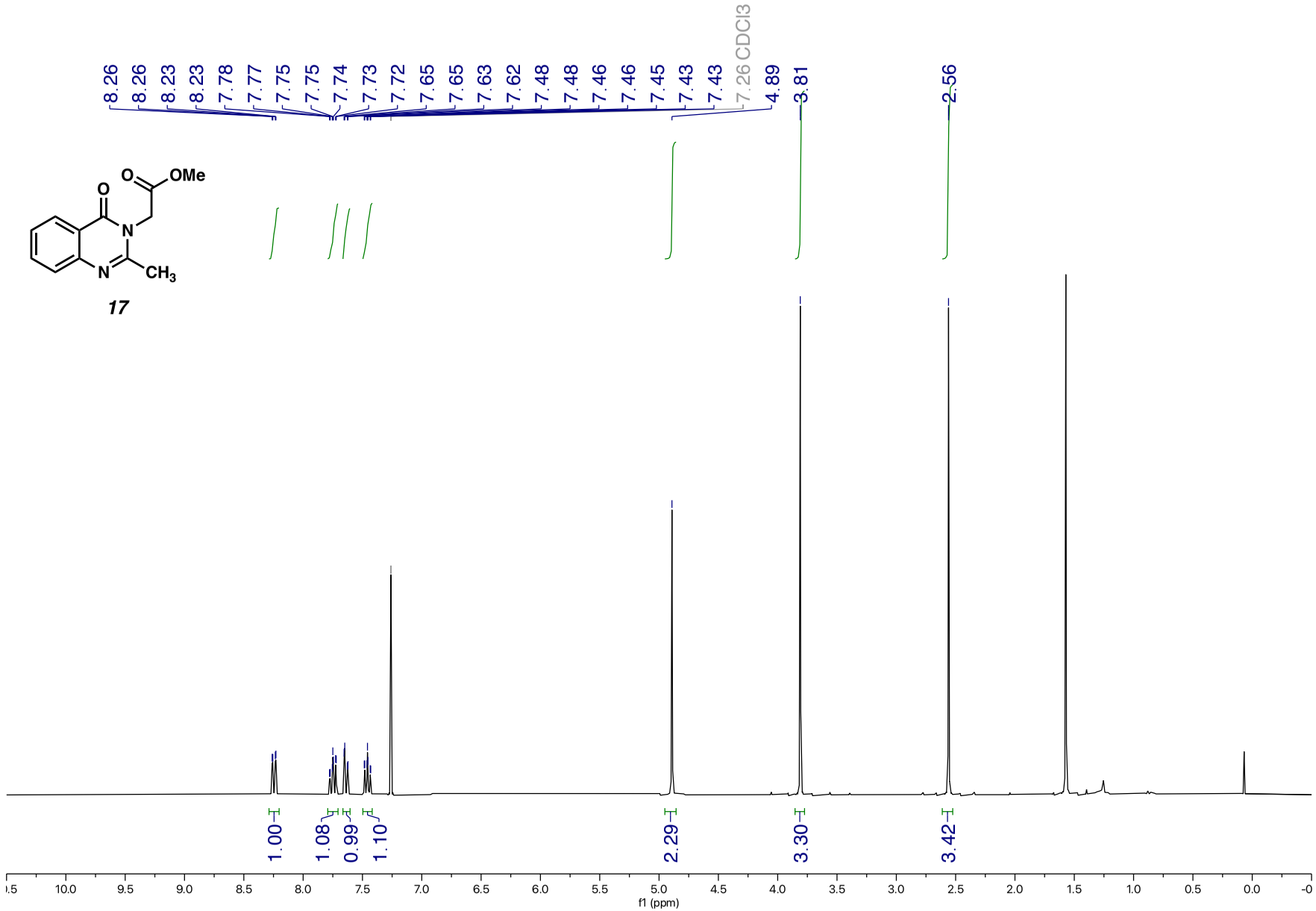
^{1}H NMR (500 MHz, $CDCl_3$) of compound **16**.



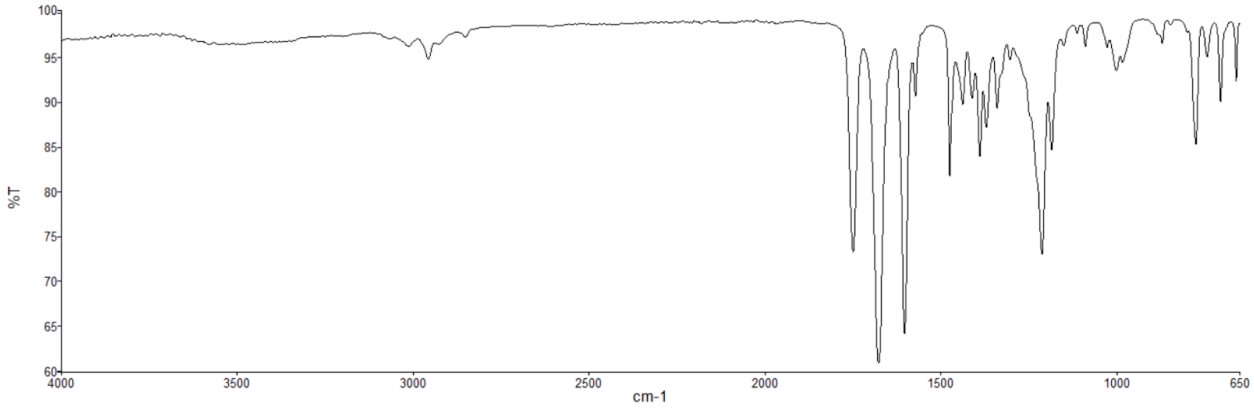
Infrared spectrum (Thin Film) of compound **16**.



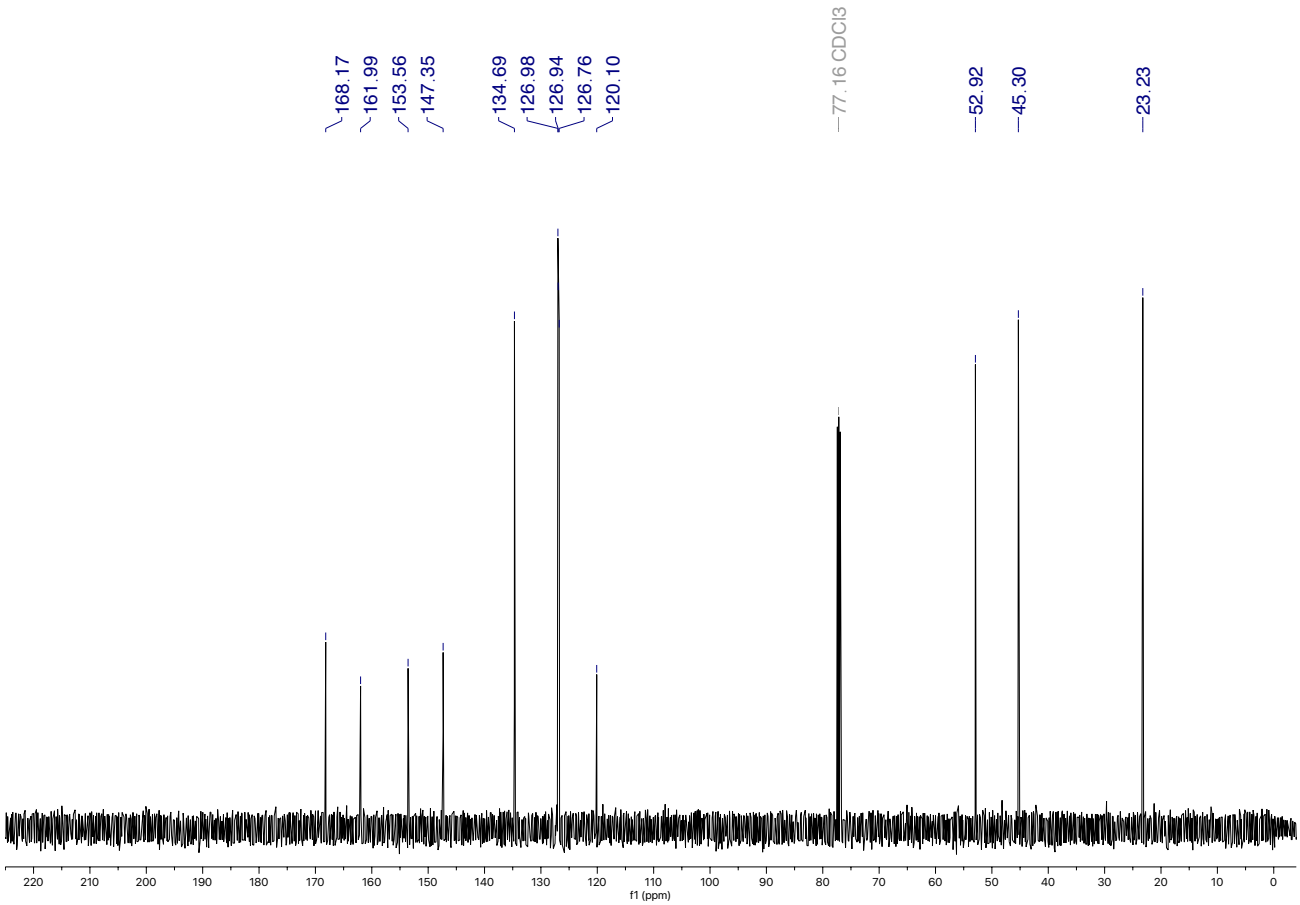
^{13}C NMR (126 MHz, $CDCl_3$) of compound **16**.



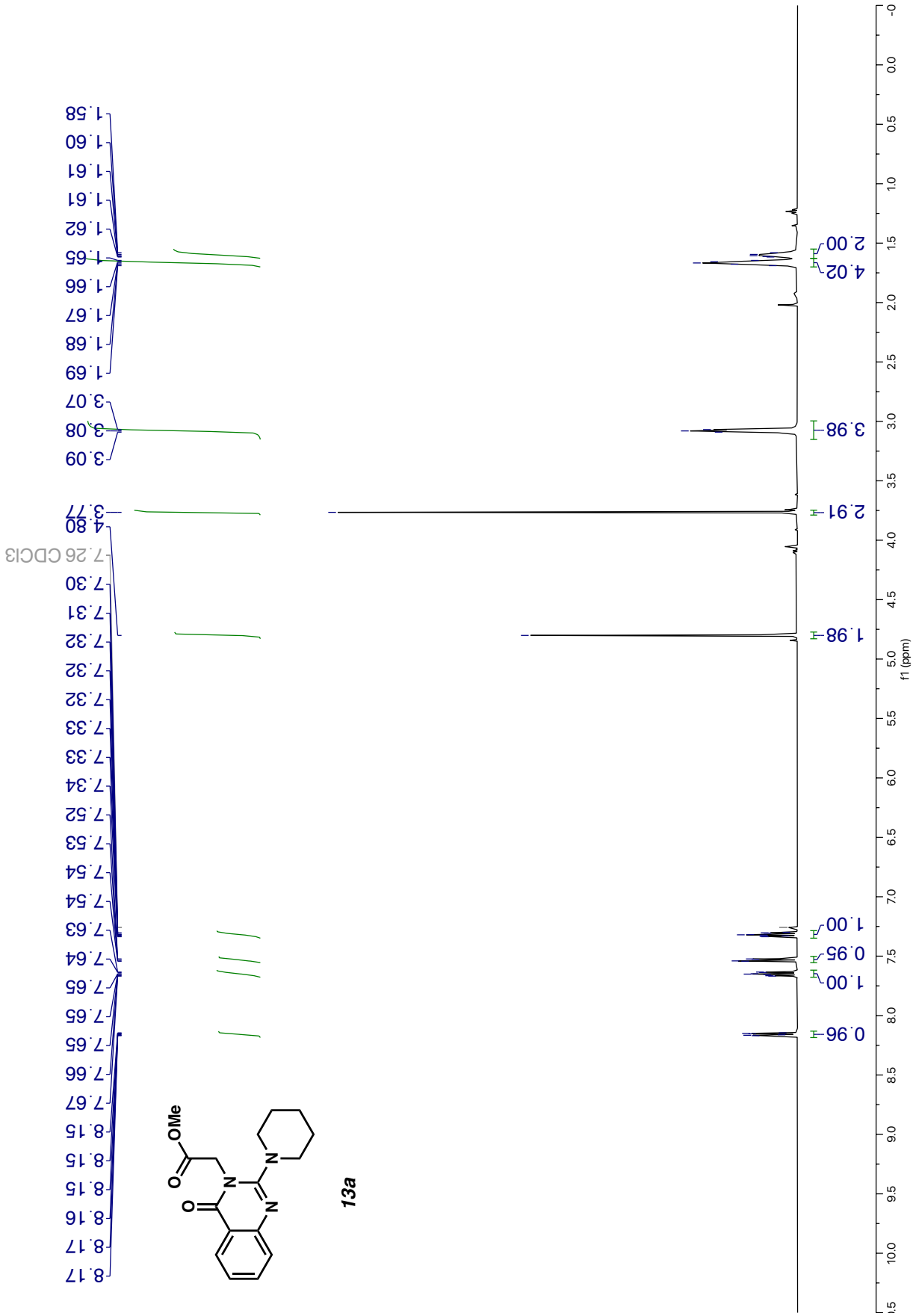
^{1}H NMR (300 MHz, $CDCl_3$) of compound **17**.



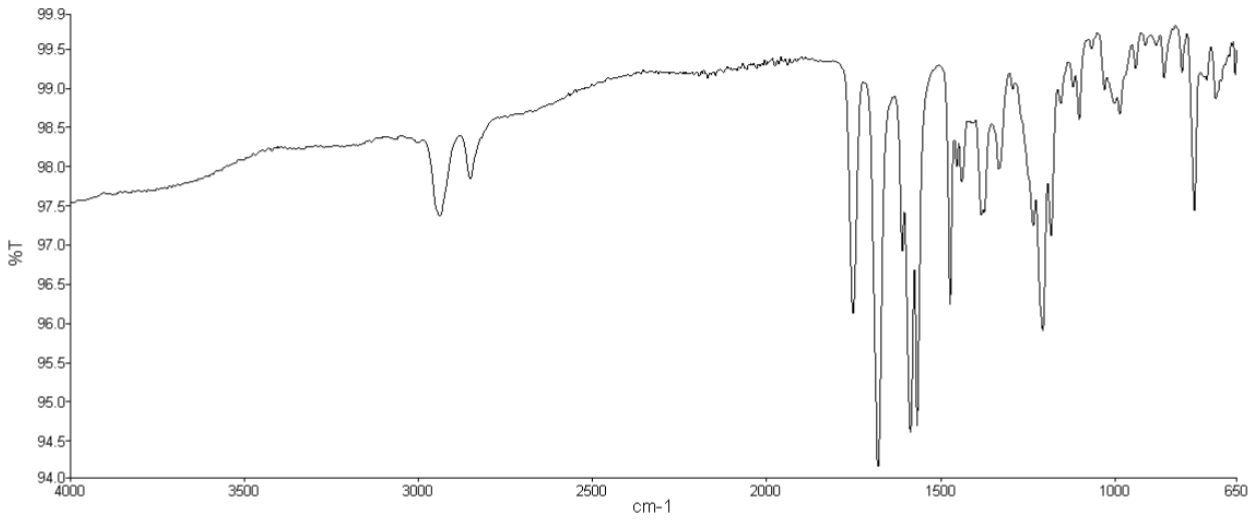
Infrared spectrum (Thin Film) of compound **17**.



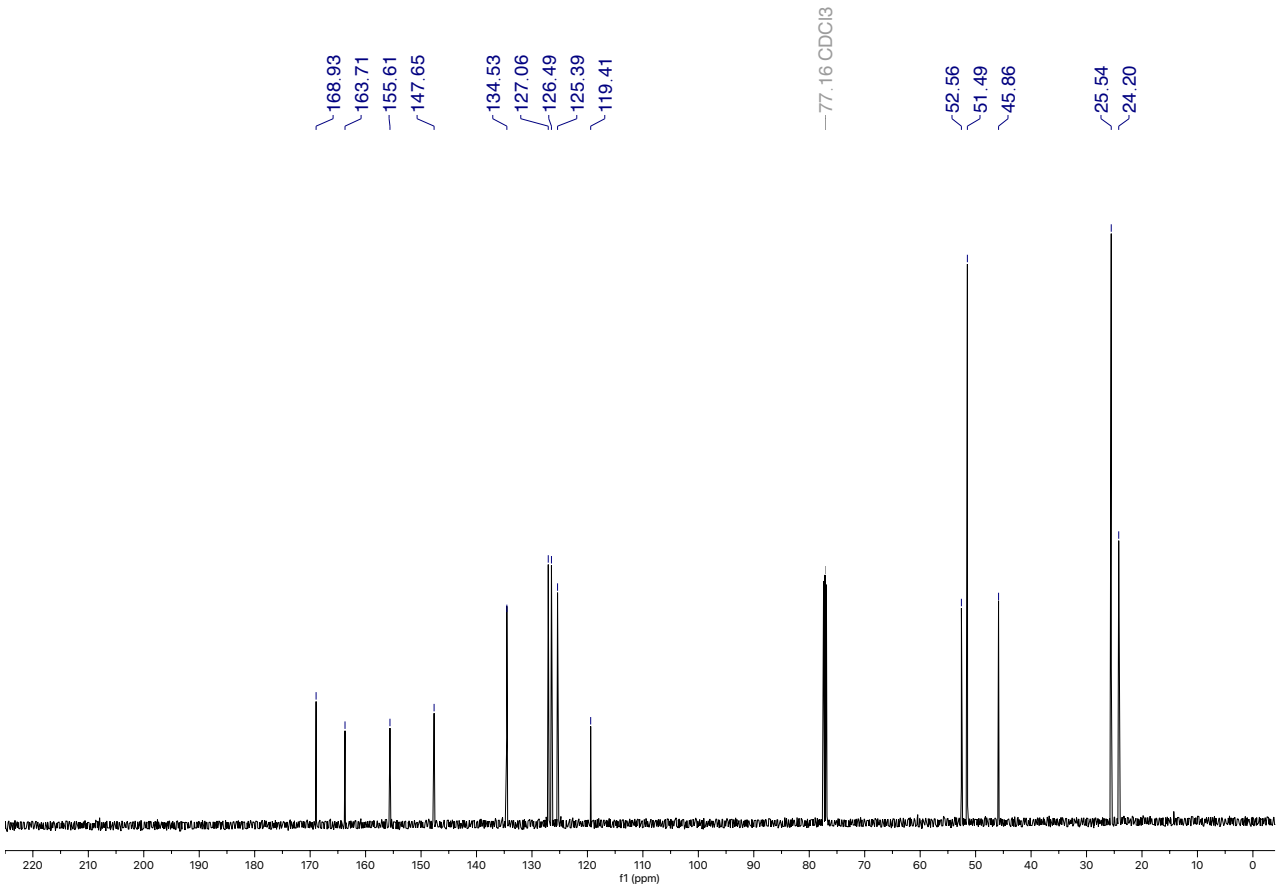
^{13}C NMR (126 MHz, $CDCl_3$) of compound **17**.



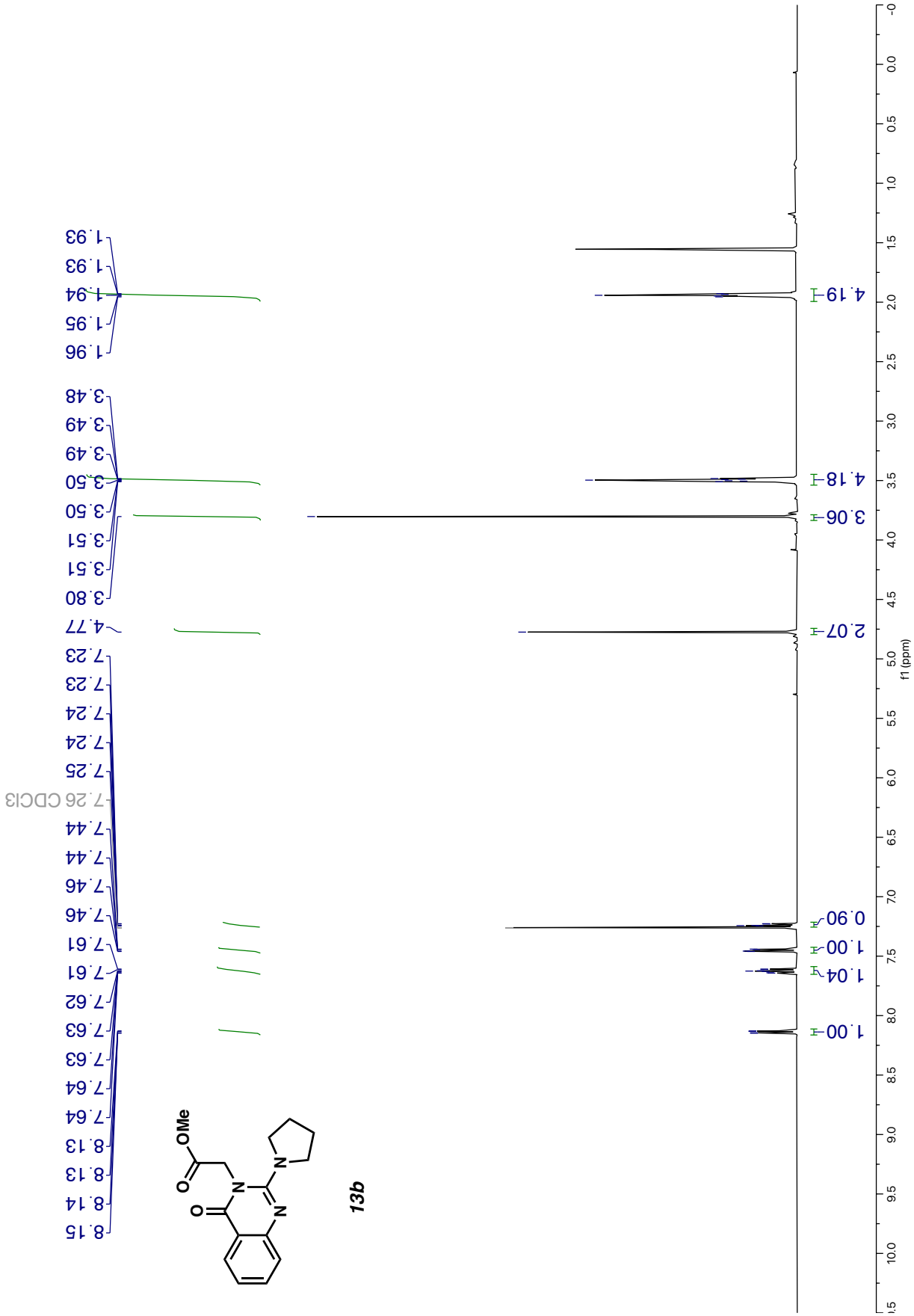
^{1}H NMR (500 MHz, $CDCl_3$) of compound **13a.**



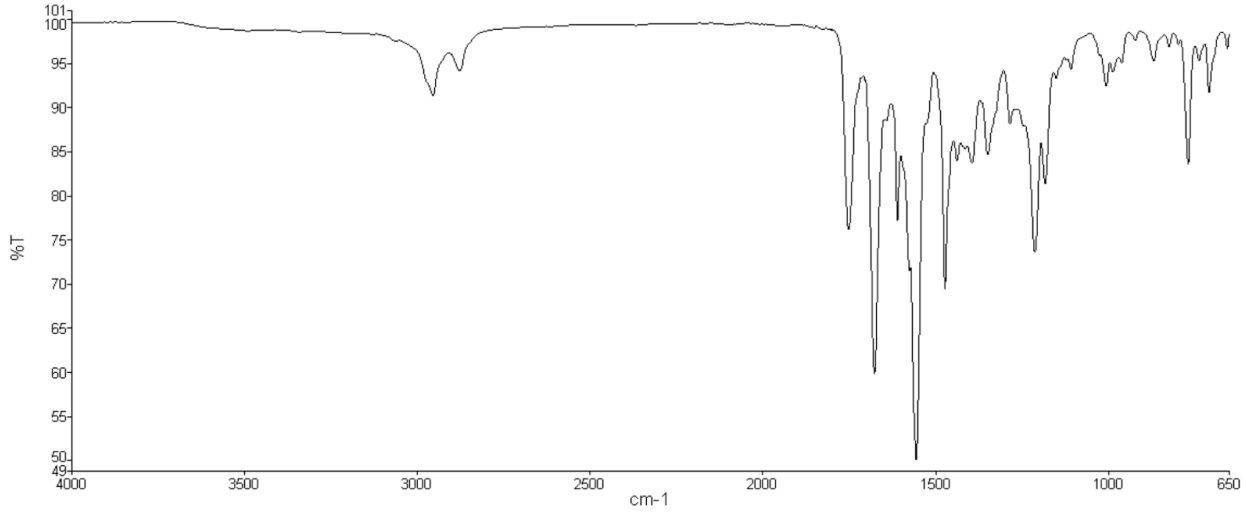
Infrared spectrum (Thin Film) of compound **13a**.



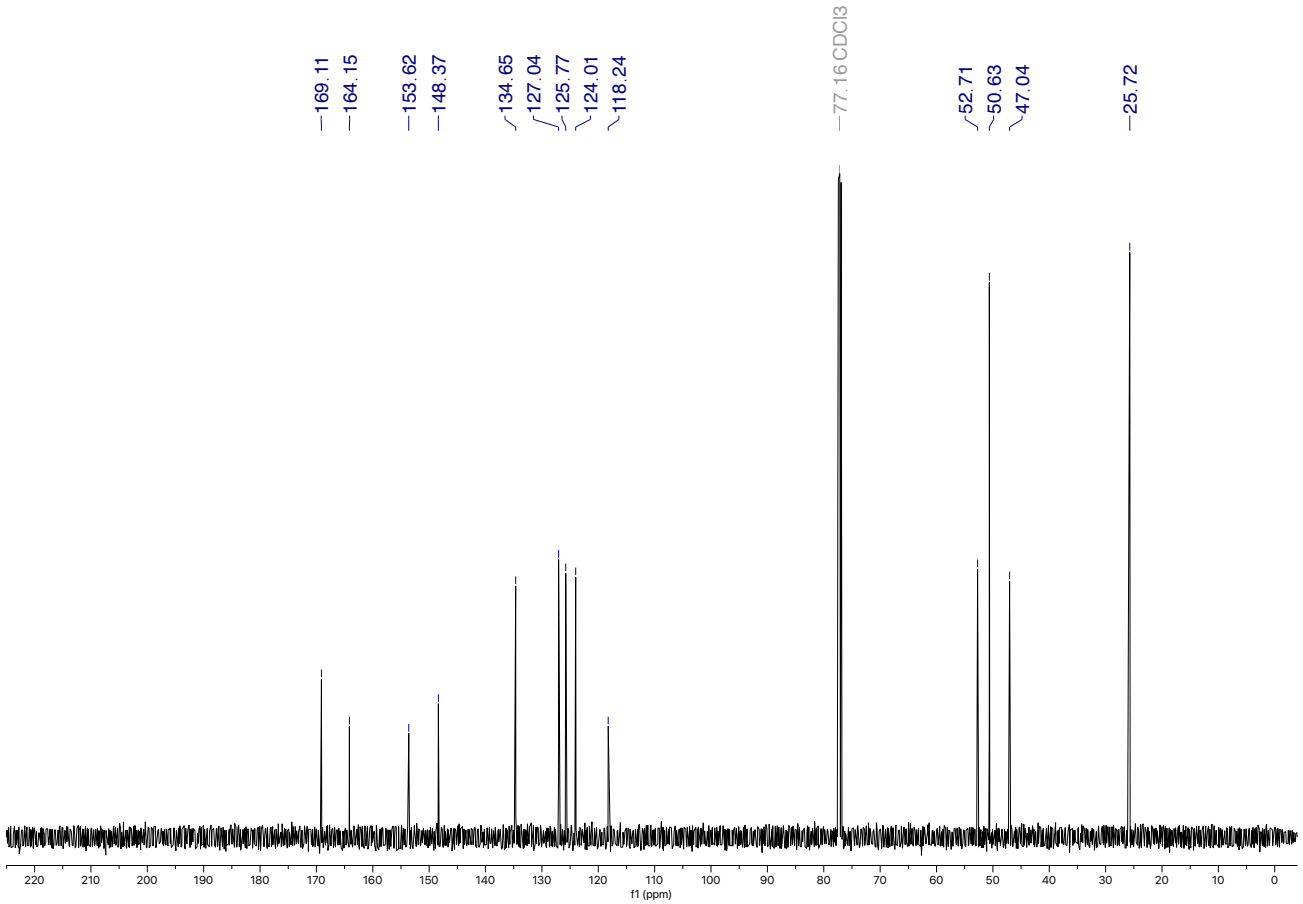
^{13}C NMR (126 MHz, $CDCl_3$) of compound **13a**.



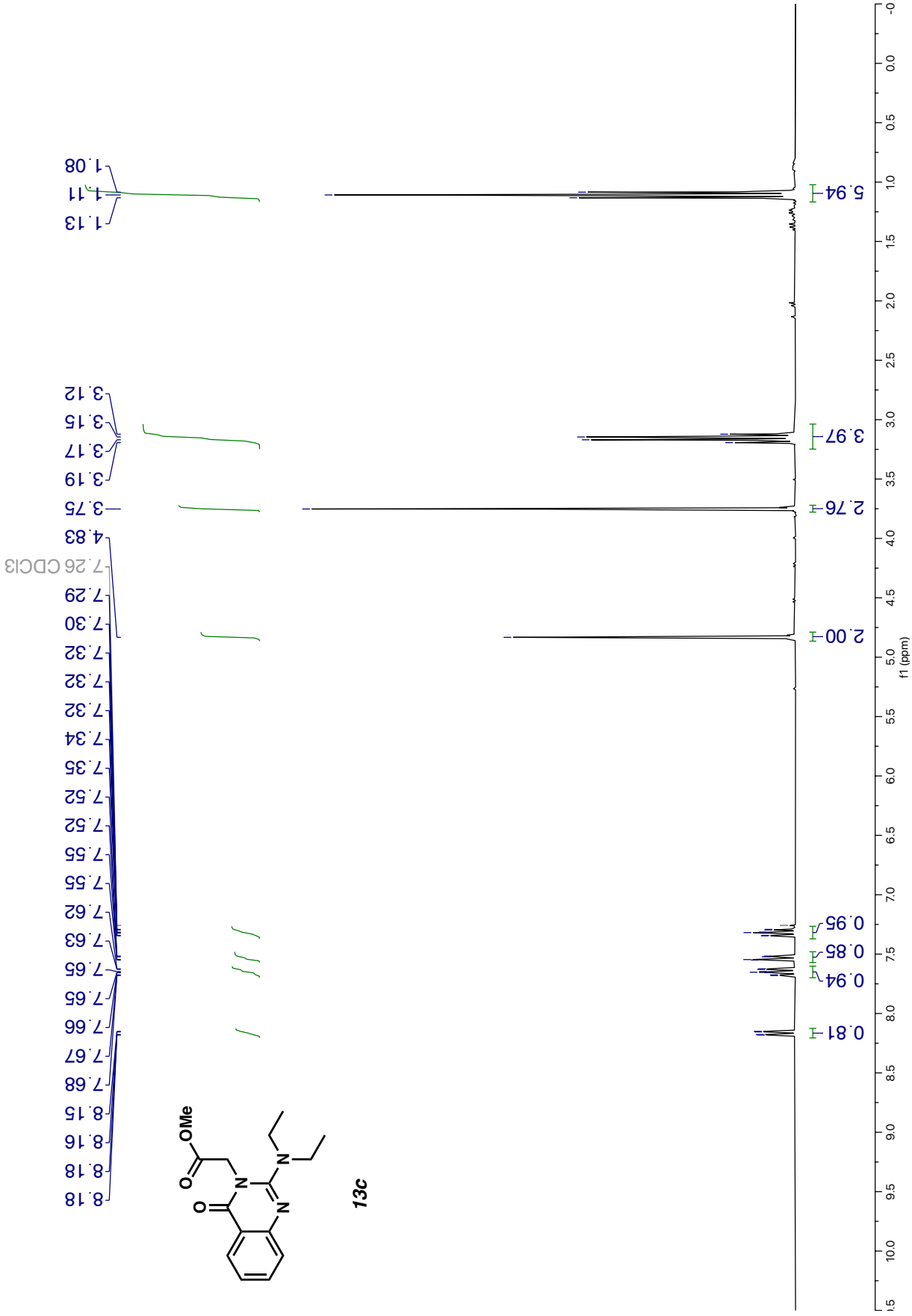
^{1}H NMR (500 MHz, $CDCl_3$) of compound **13b**.



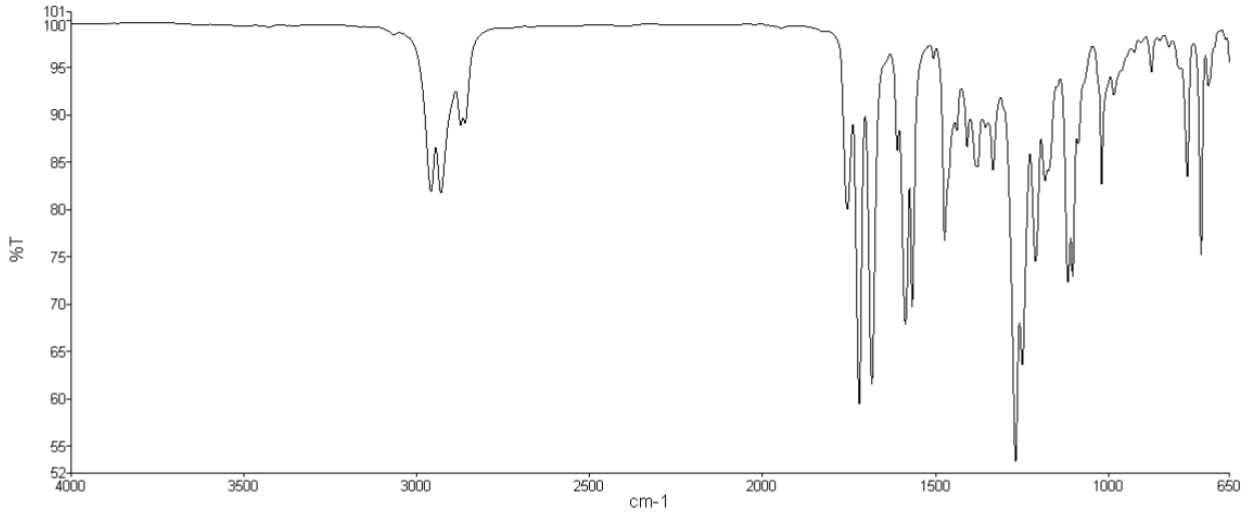
Infrared spectrum (Thin Film) of compound **13b**.



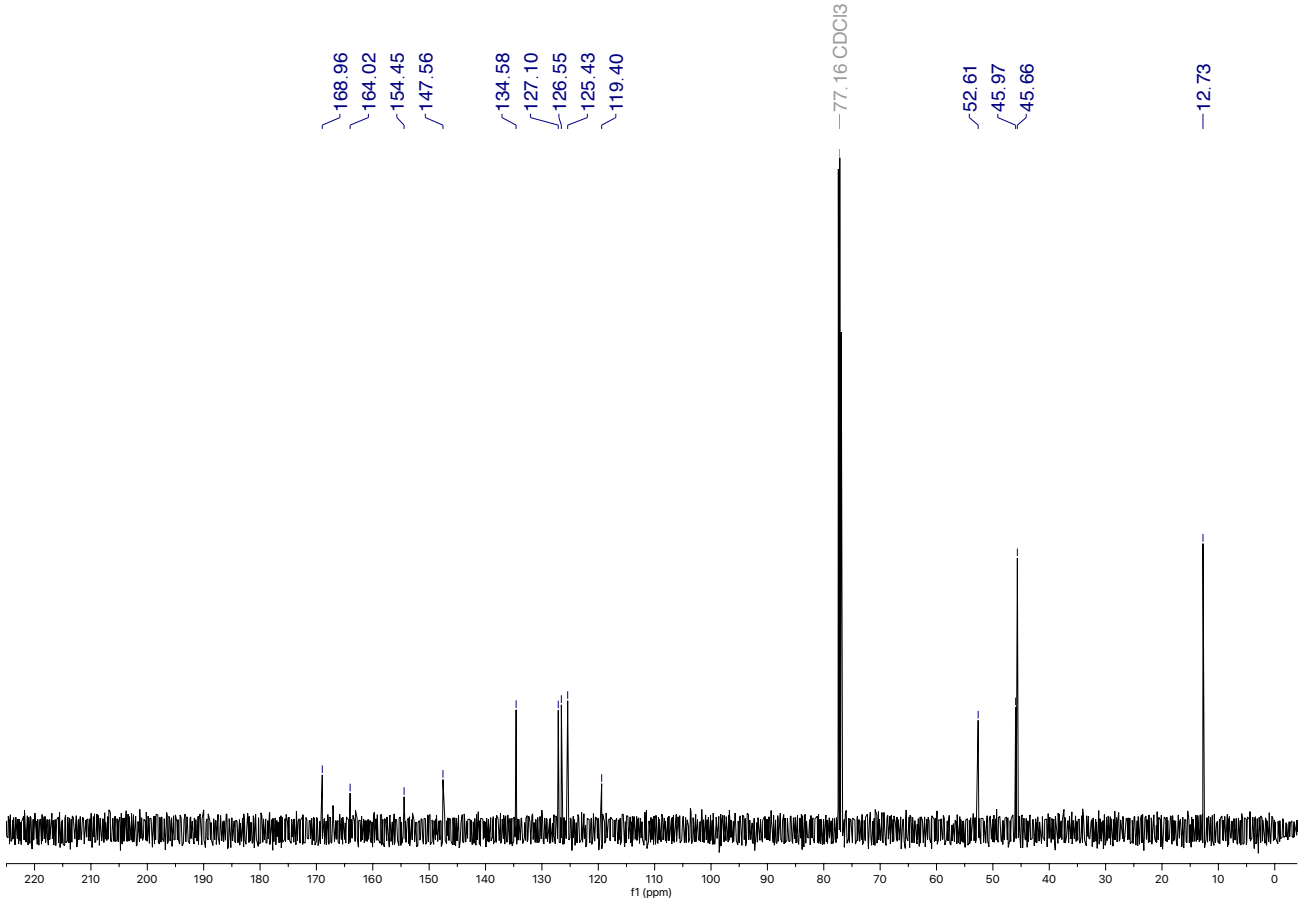
^{13}C NMR (126 MHz, $CDCl_3$) of compound **13b**.



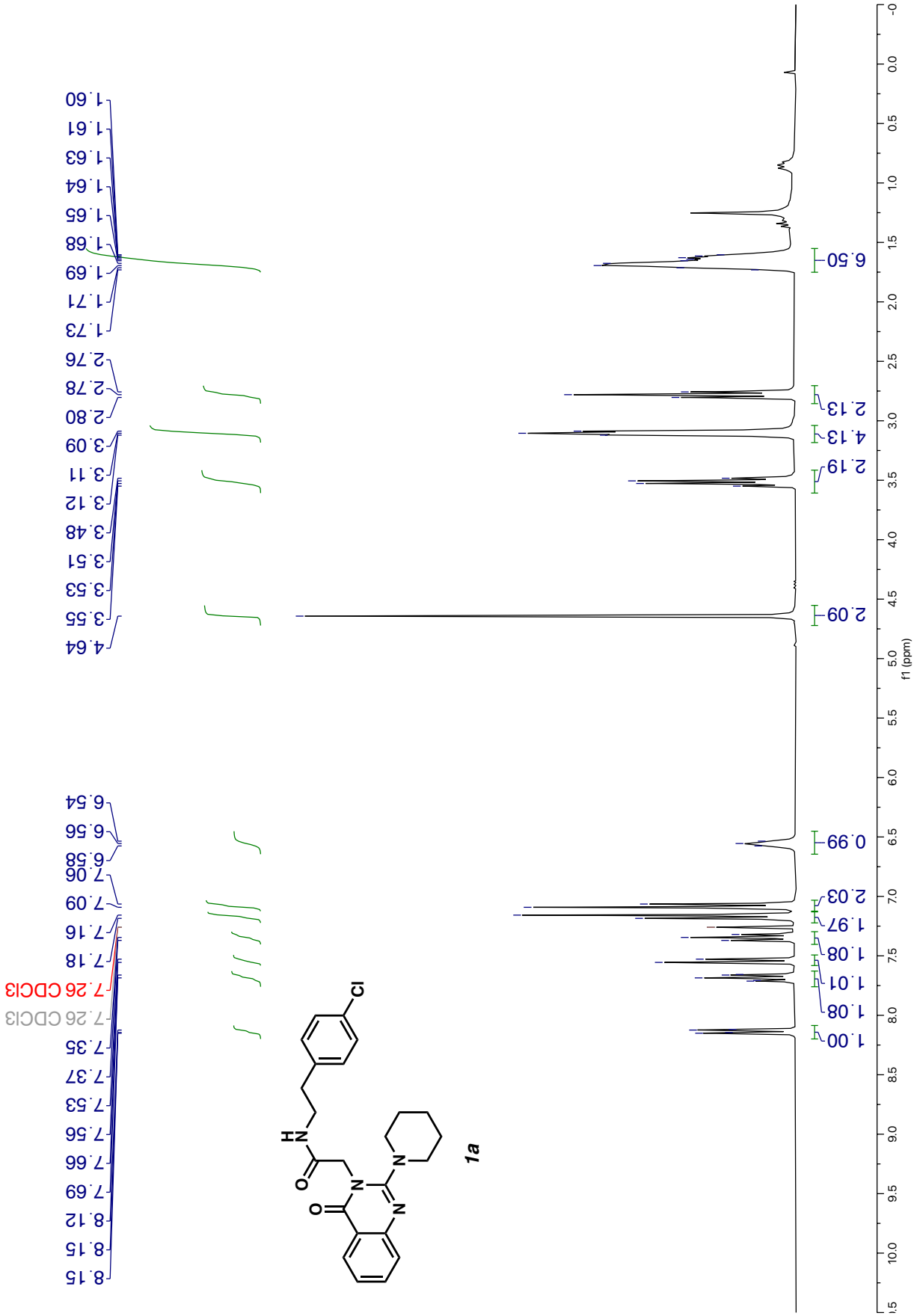
^{1}H NMR (300 MHz, $CDCl_3$) of compound **13c**.



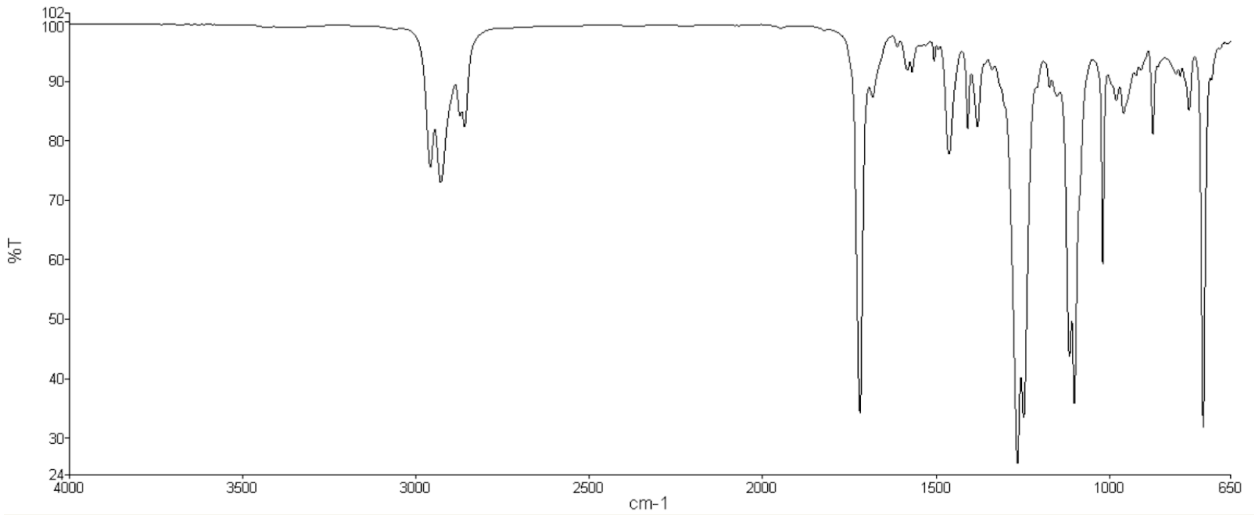
Infrared spectrum (Thin Film) of compound **13c**.



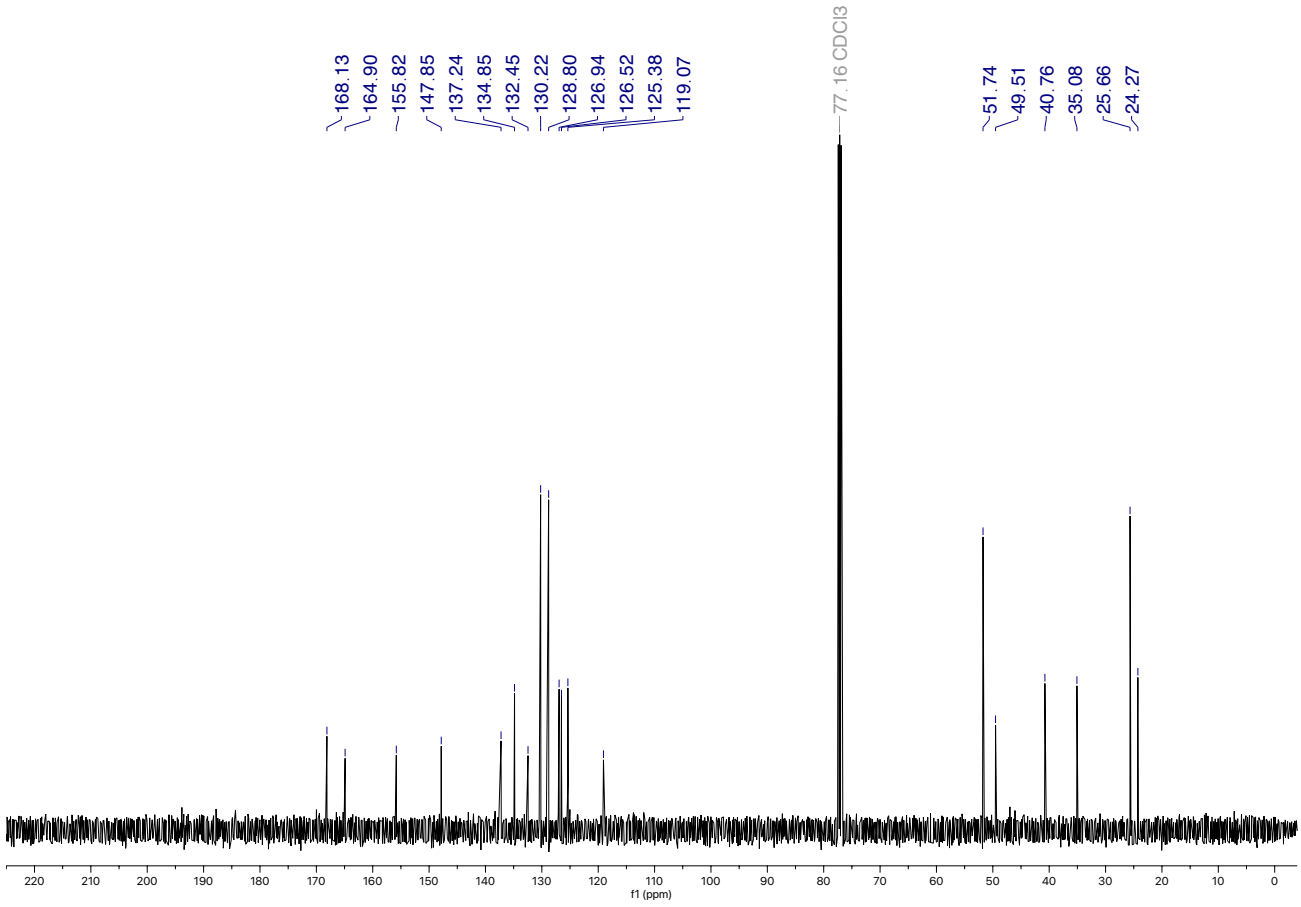
^{13}C NMR (126 MHz, $CDCl_3$) of compound **13c**.



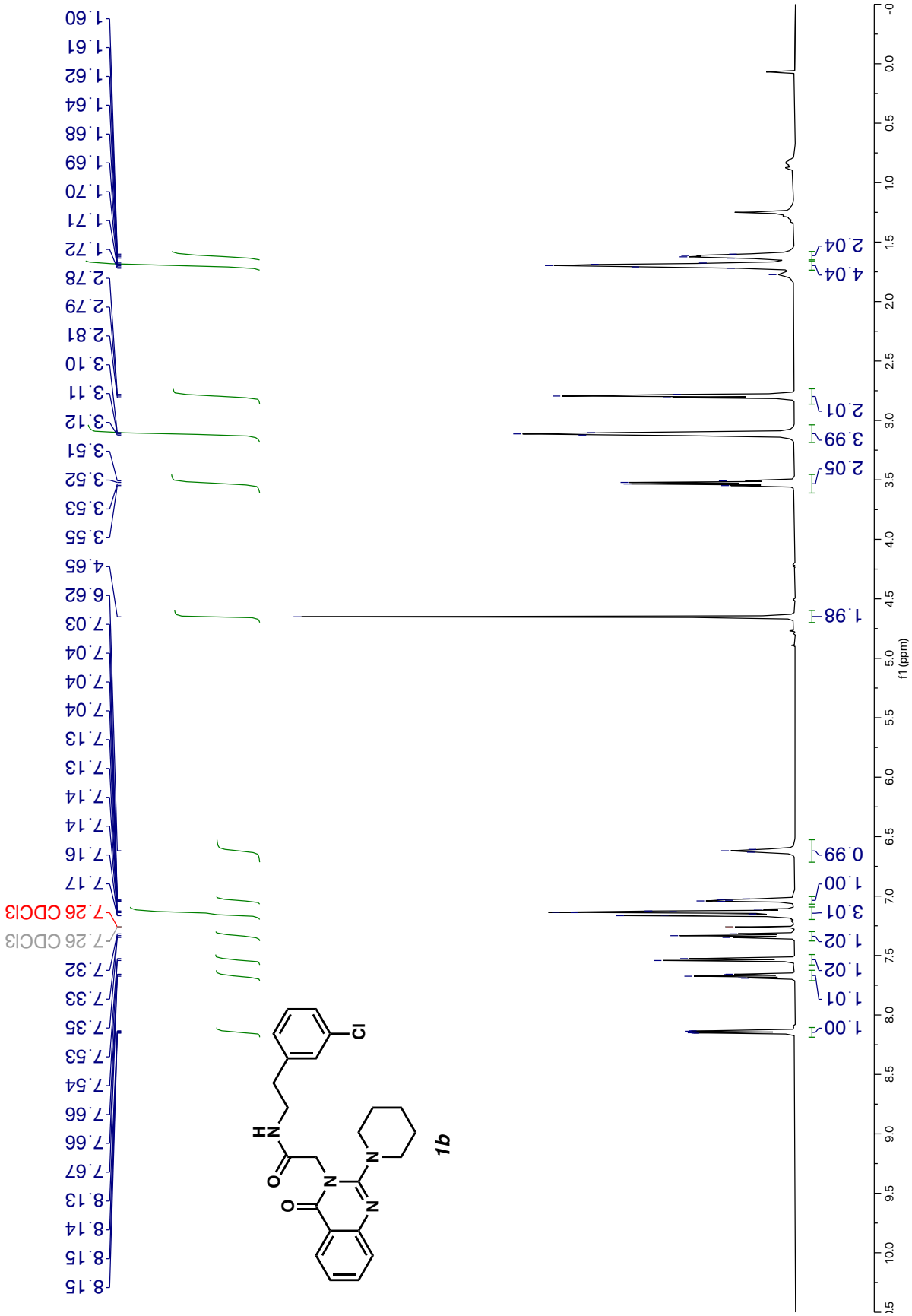
^{1}H NMR (300 MHz, $CDCl_3$) of compound **1a**.



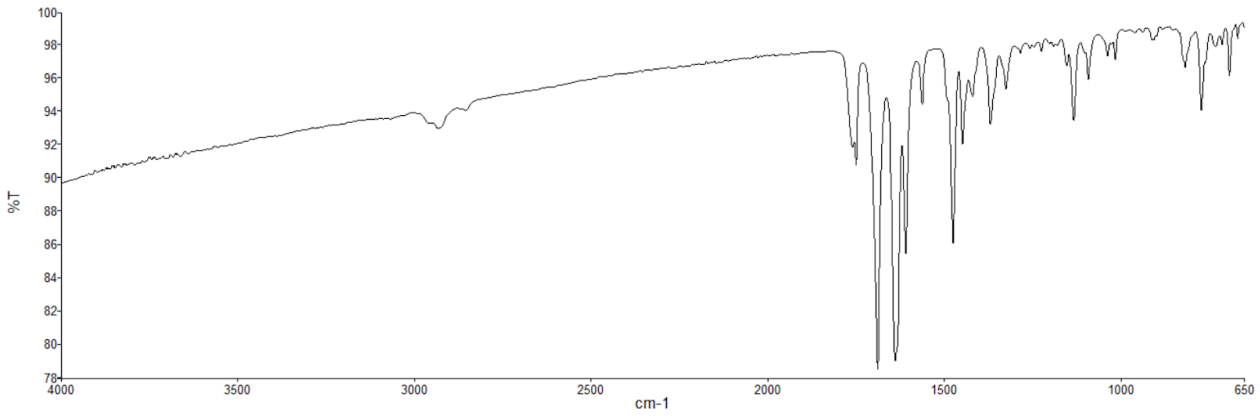
Infrared spectrum (Thin Film) of compound **1a**.



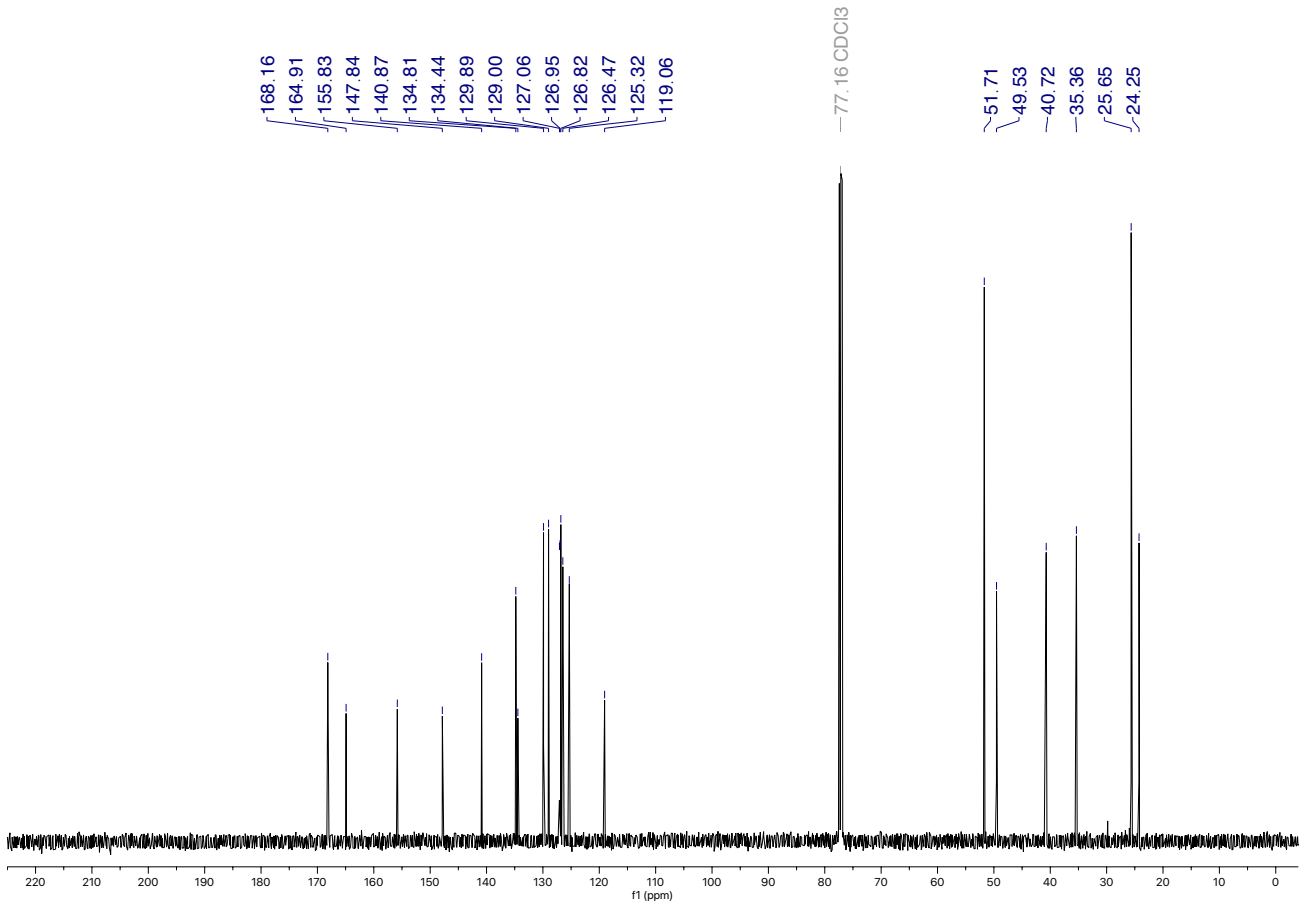
^{13}C NMR (126 MHz, $CDCl_3$) of compound **1a**.



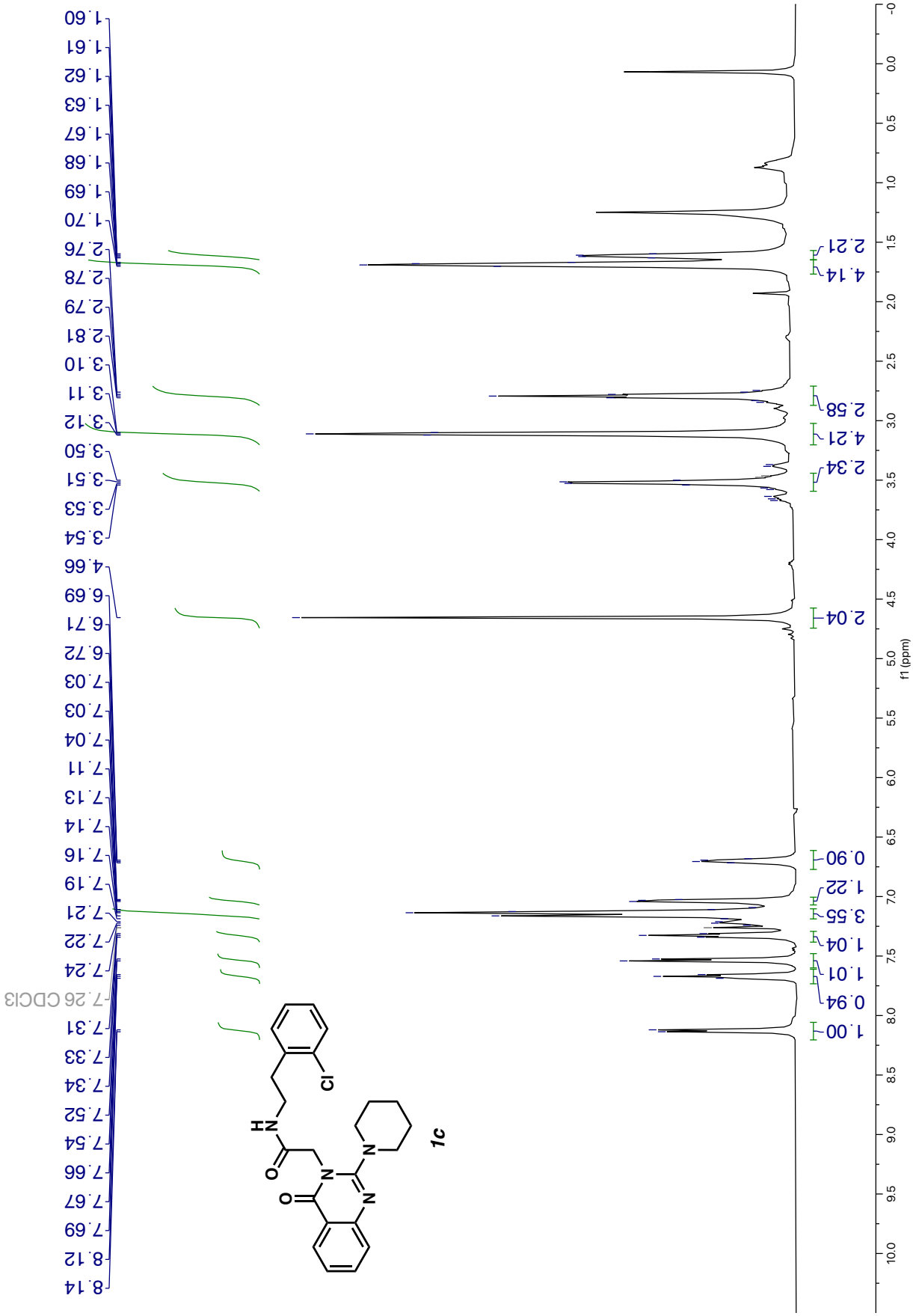
^{1}H NMR (500 MHz, $CDCl_3$) of compound **1b**.



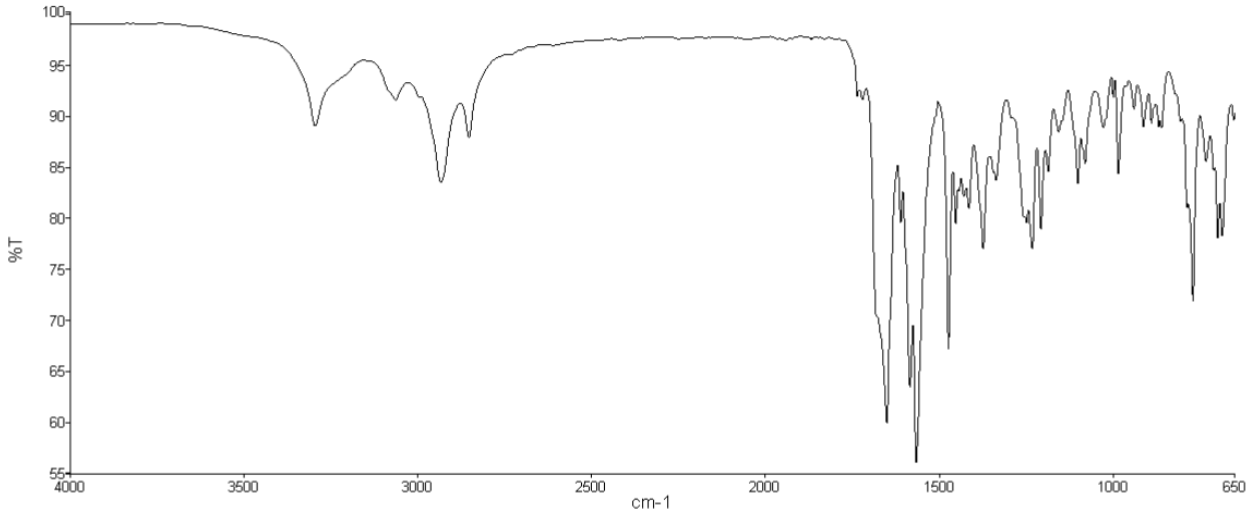
Infrared spectrum (Thin Film) of compound **1b**.



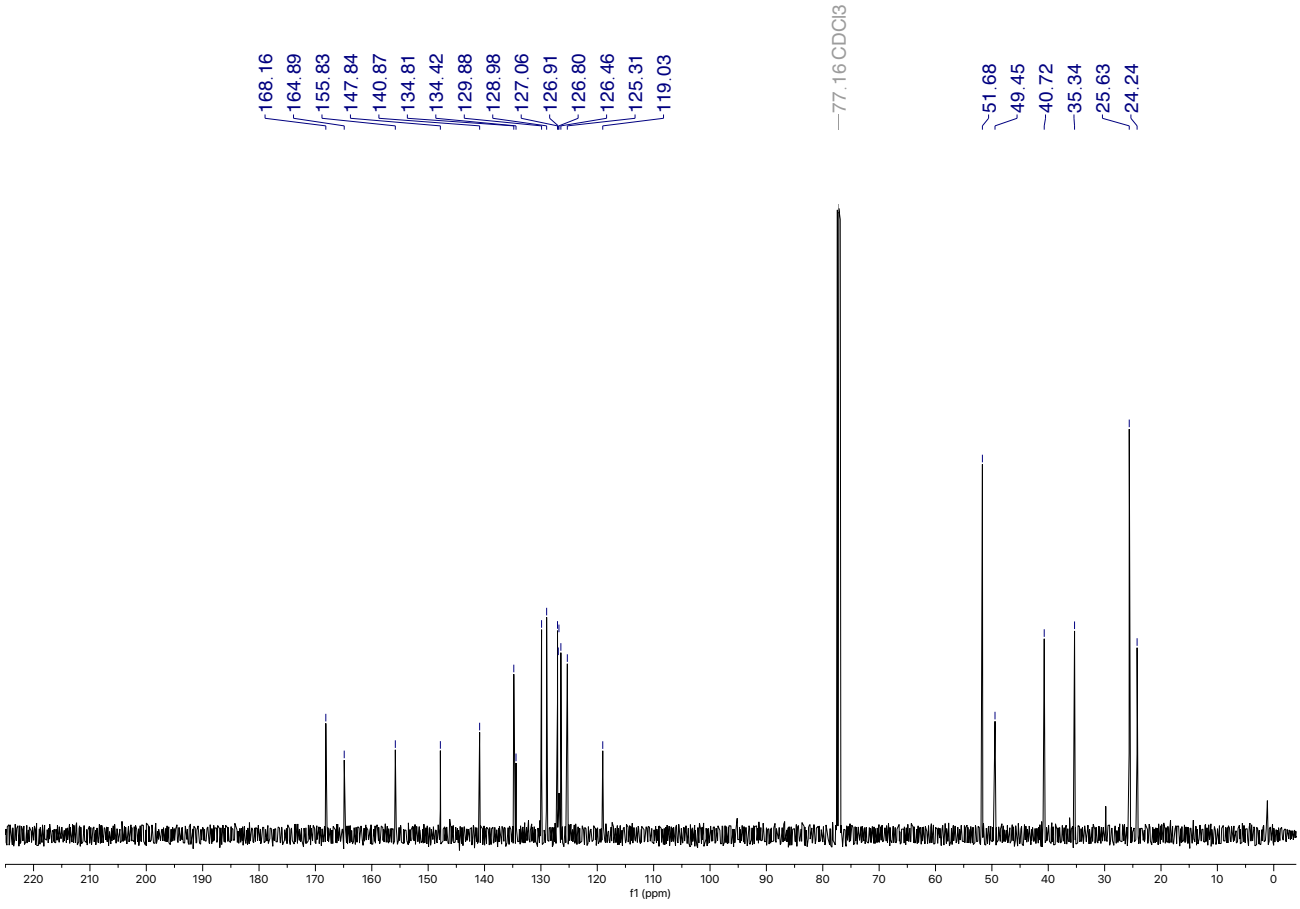
^{13}C NMR (126 MHz, $CDCl_3$) of compound **1b**.



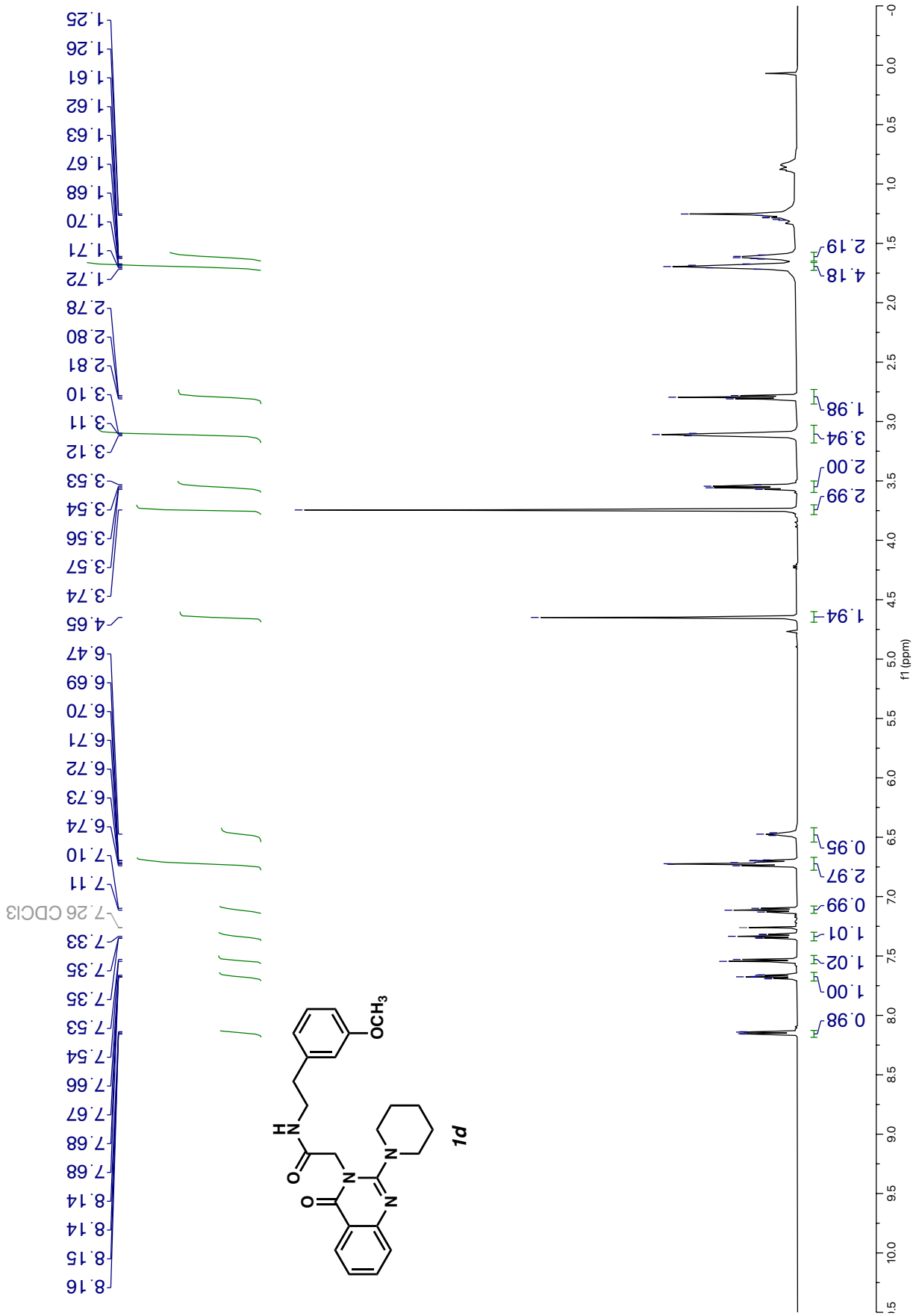
^{1}H NMR (500 MHz, $CDCl_3$) of compound **1c**.



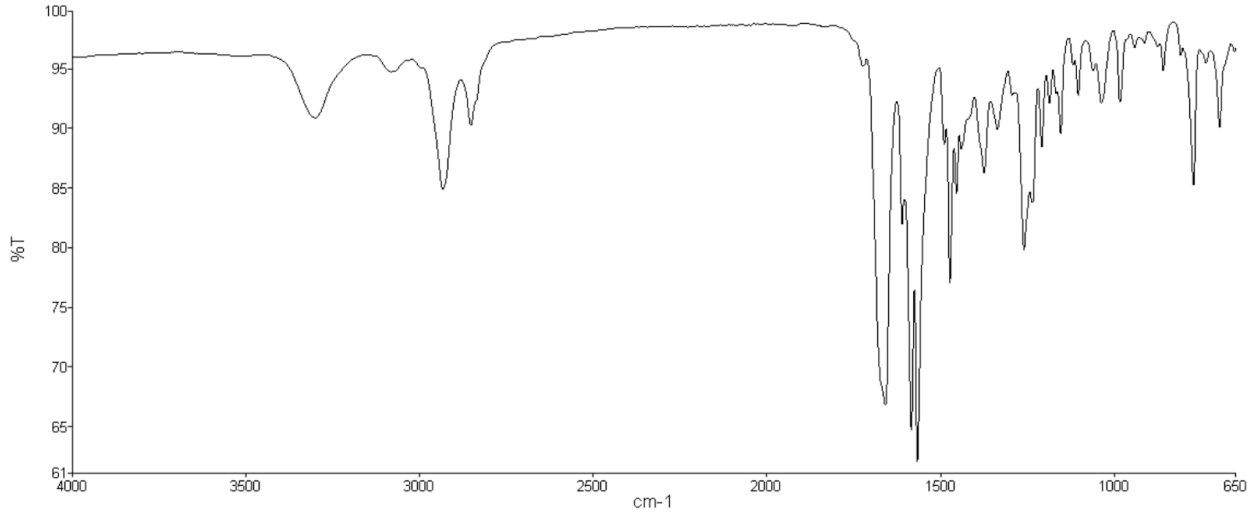
Infrared spectrum (Thin Film) of compound **1c**.



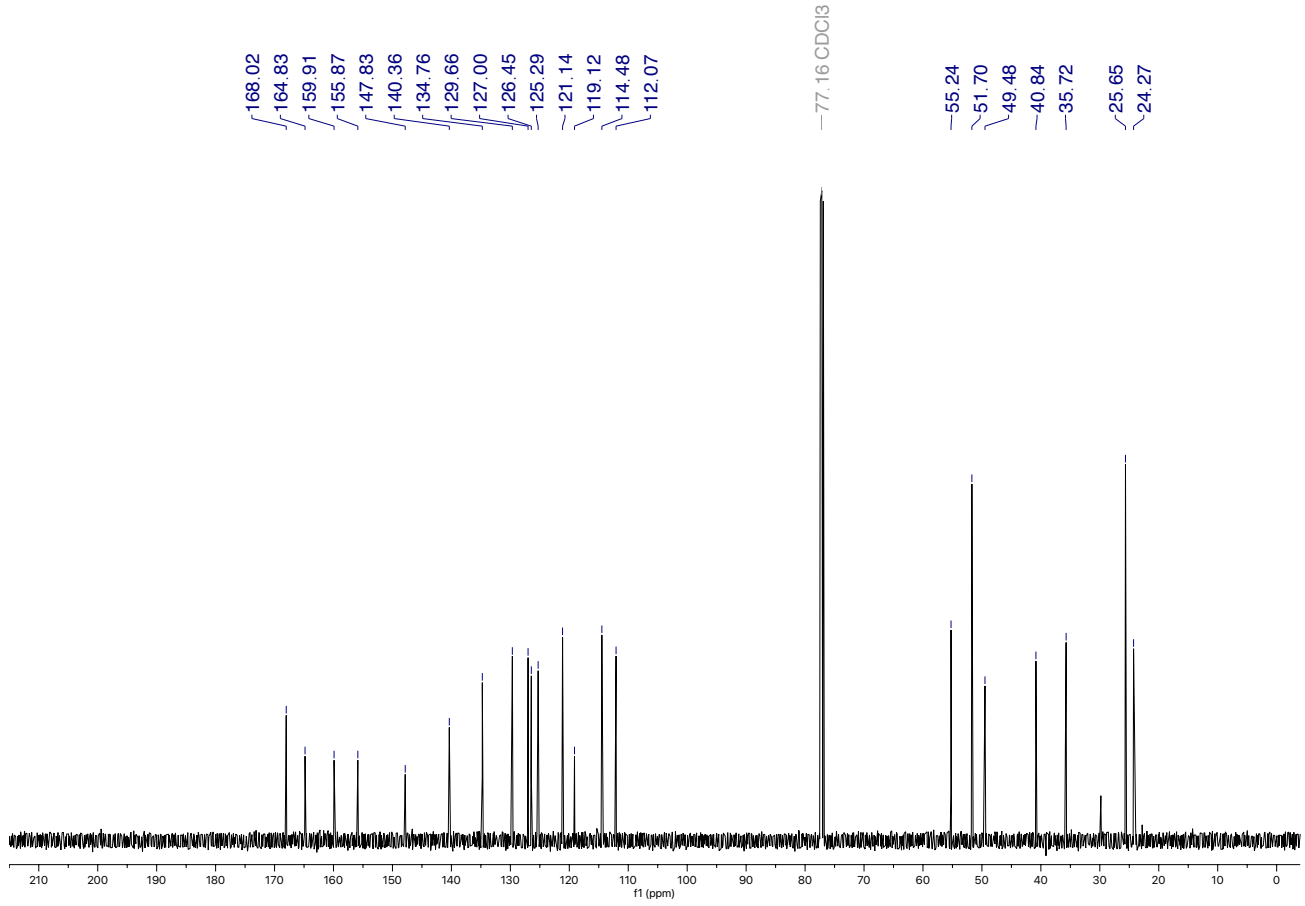
^{13}C NMR (126 MHz, $CDCl_3$) of compound **1c**.



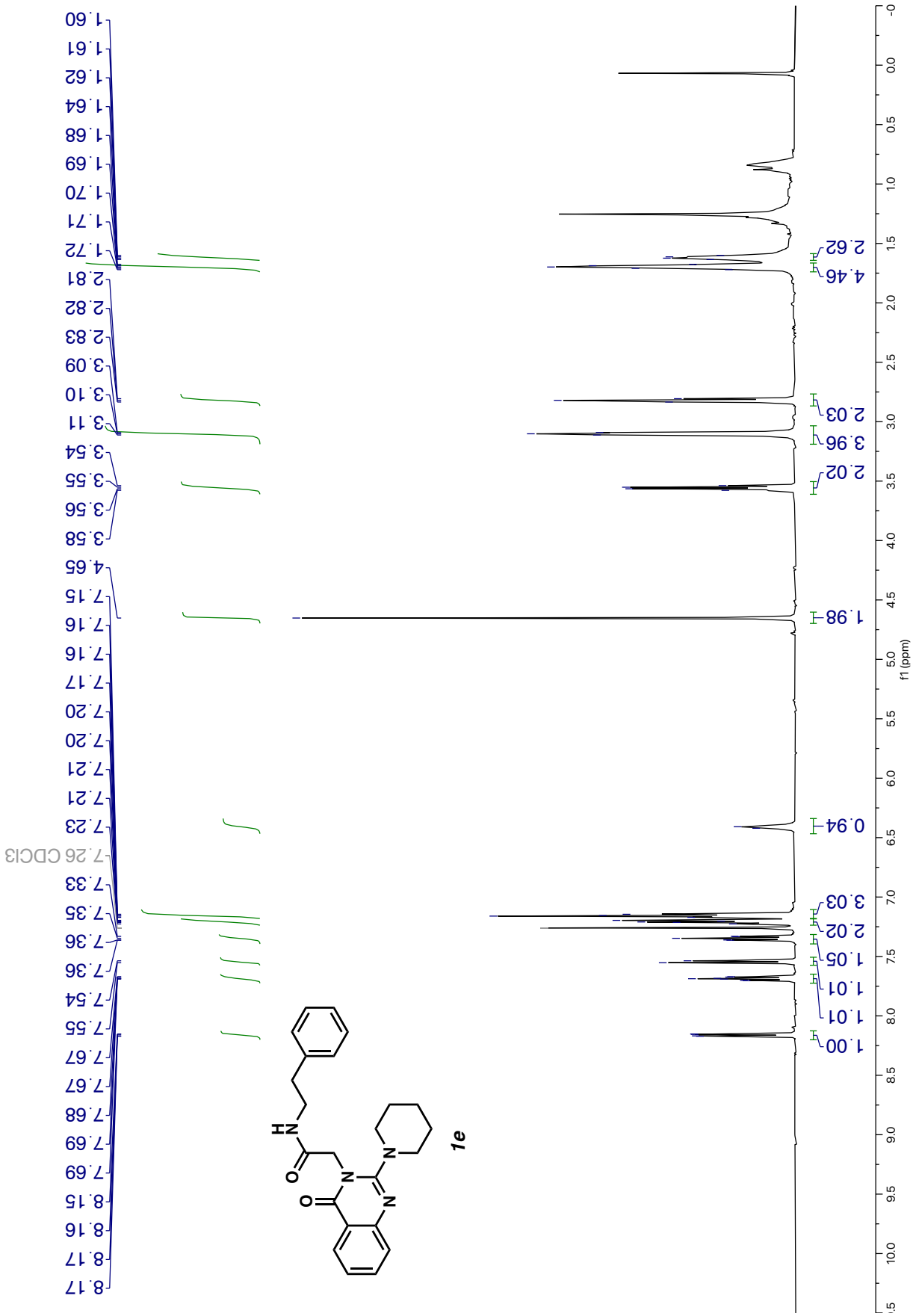
^{1}H NMR (500 MHz, $CDCl_3$) of compound **1d**.



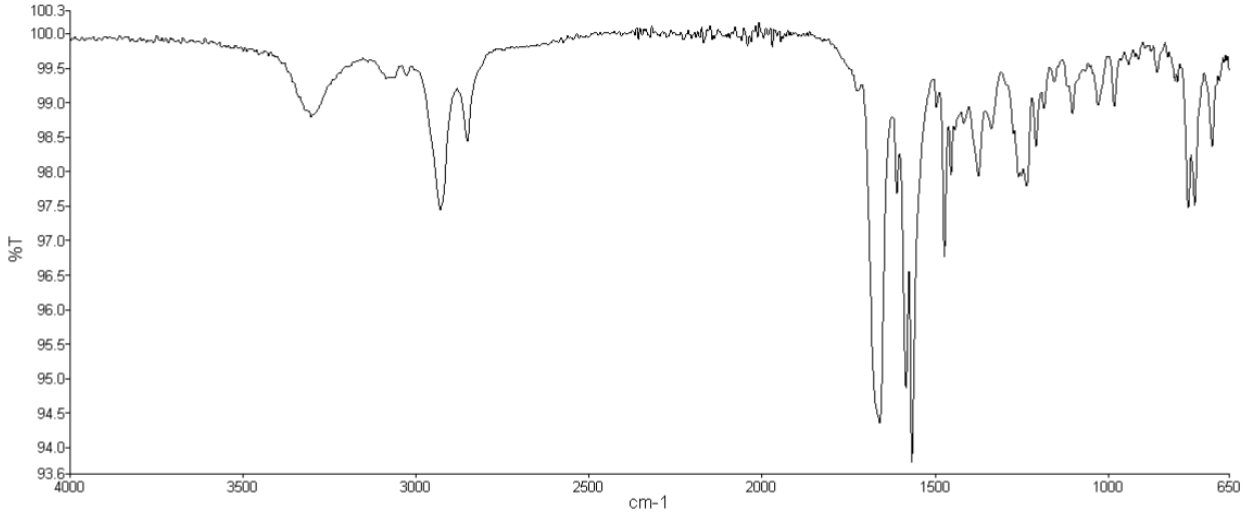
Infrared spectrum (Thin Film) of compound **1d**.



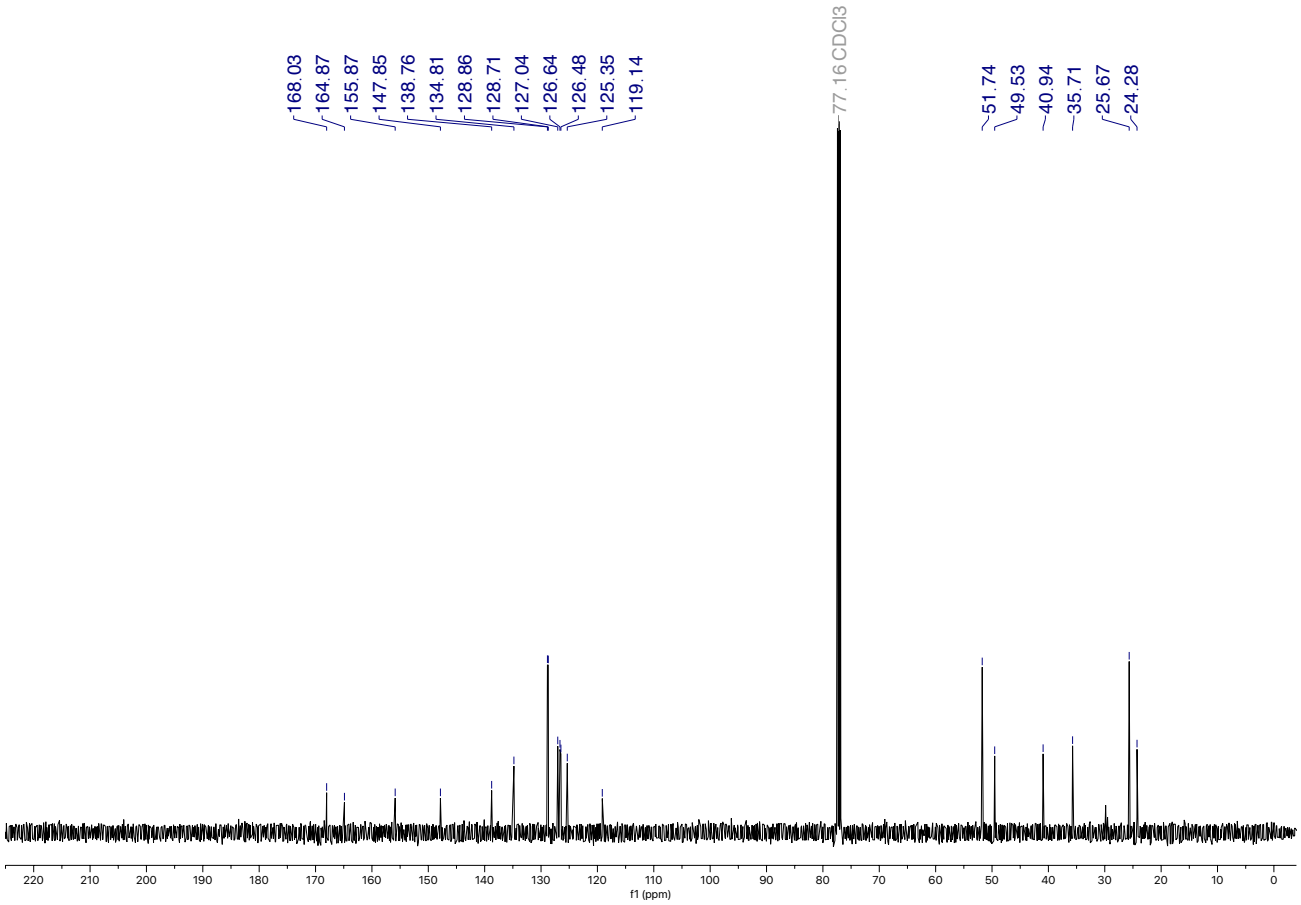
^{13}C NMR (126 MHz, $CDCl_3$) of compound **1d**.



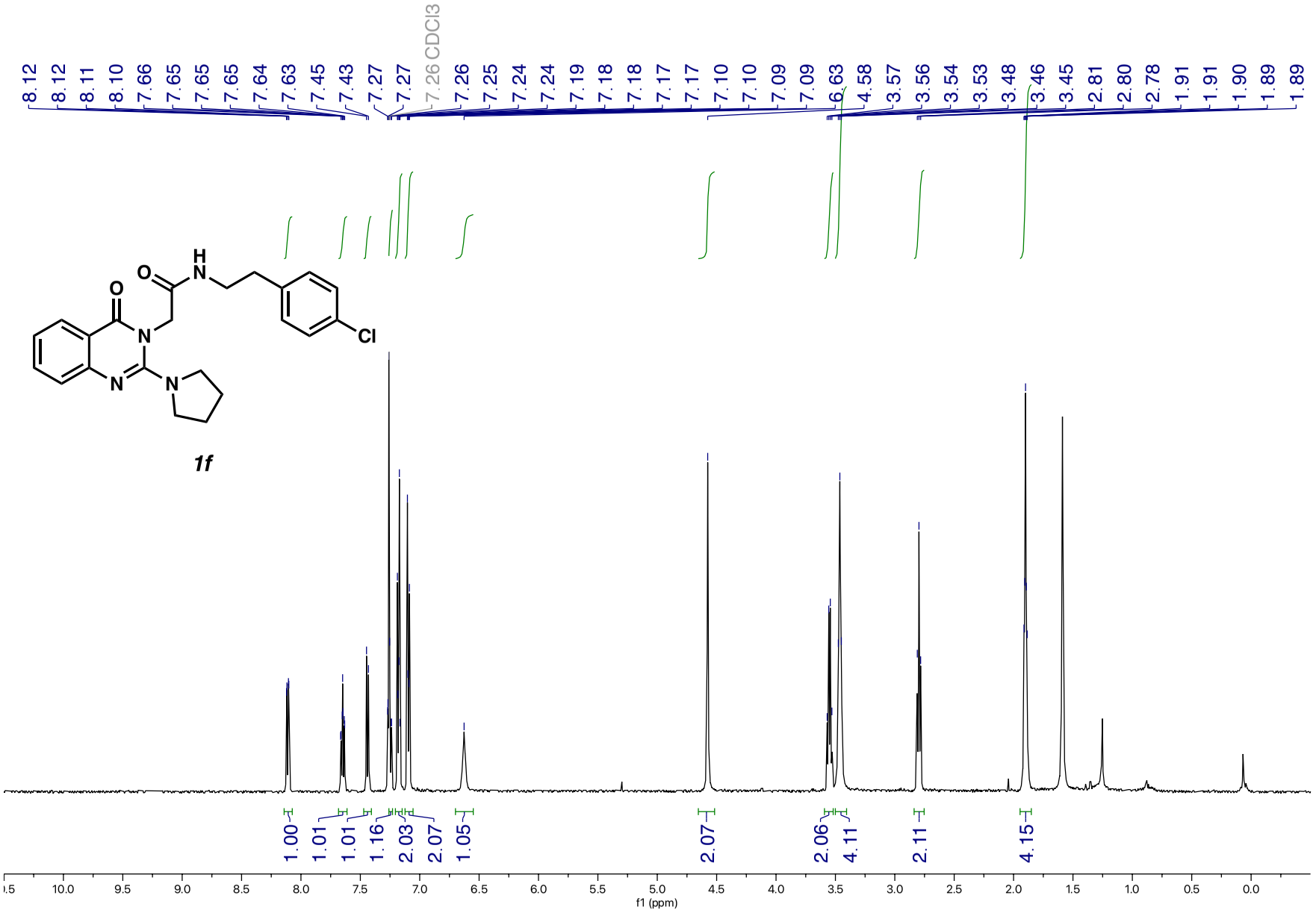
^{1}H NMR (500 MHz, $CDCl_3$) of compound **1e**.



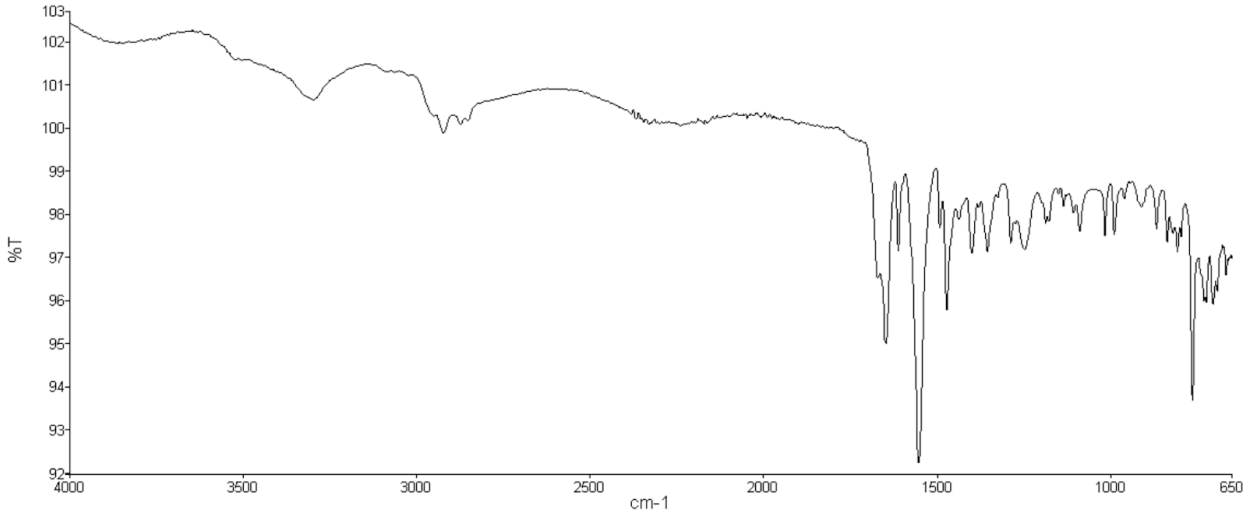
Infrared spectrum (Thin Film) of compound **1e**.



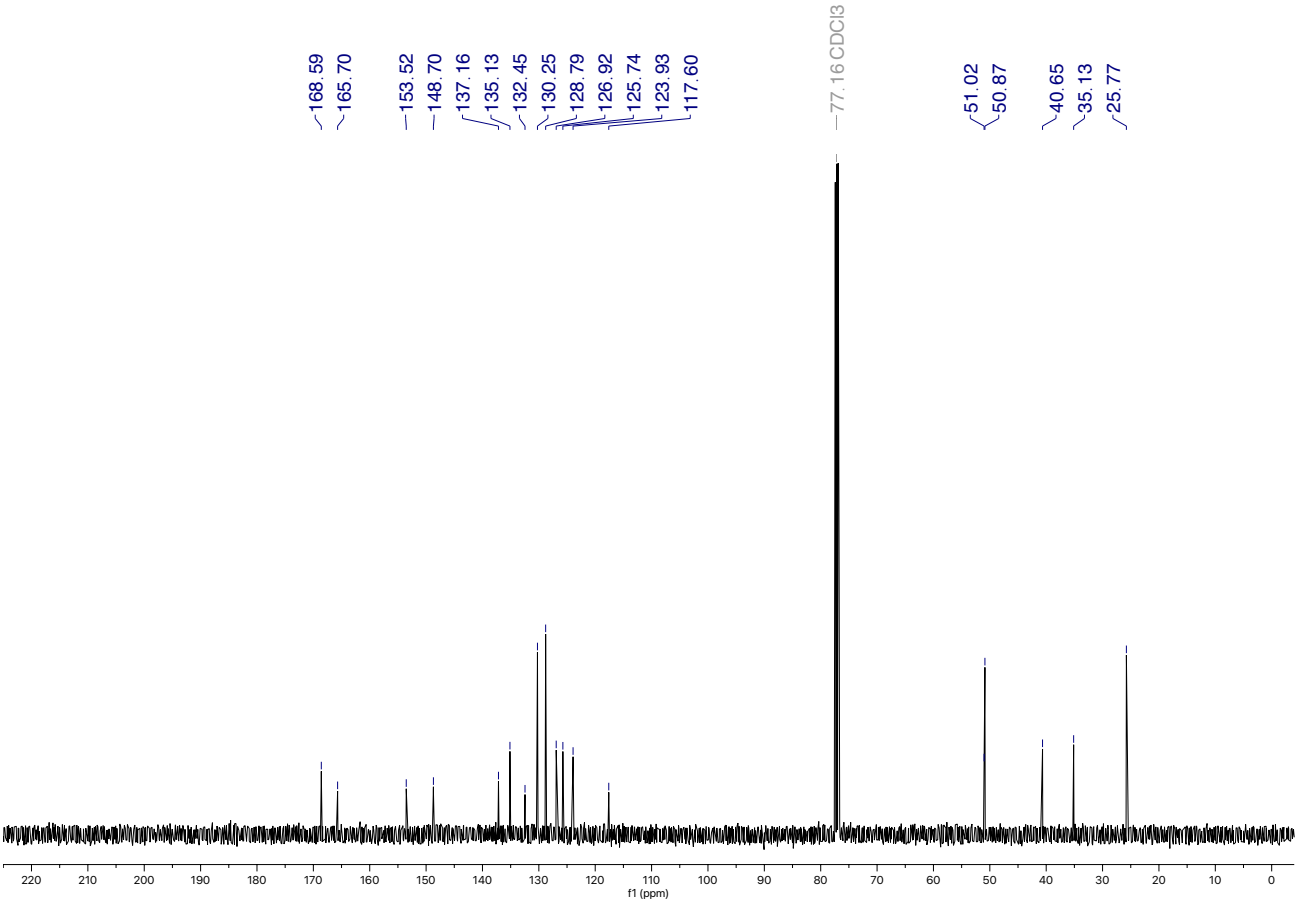
^{13}C NMR (126 MHz, $CDCl_3$) of compound **1e**.



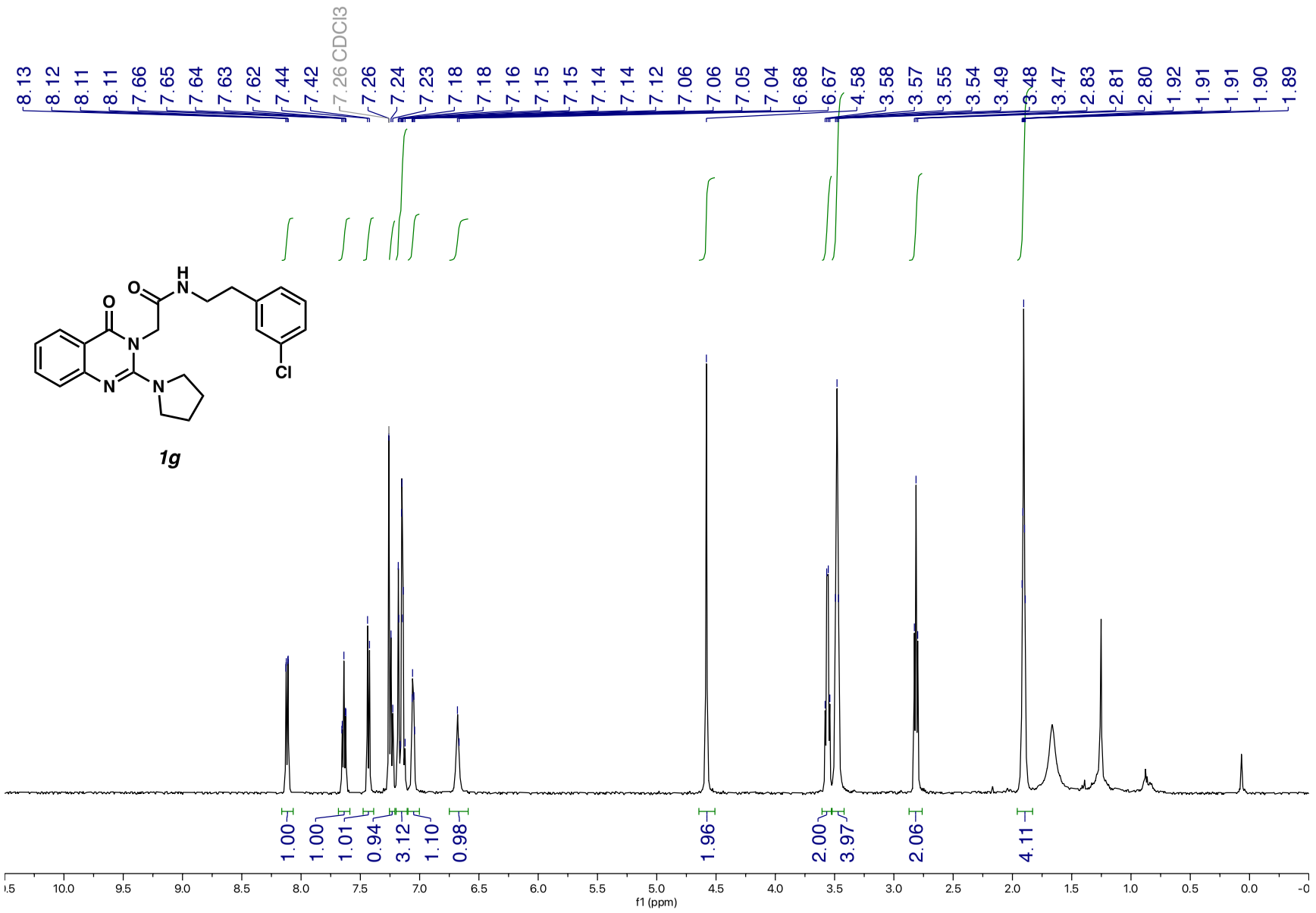
^{1}H NMR (500 MHz, $CDCl_3$) of compound **1f**.



Infrared spectrum (Thin Film) of compound **1f**.

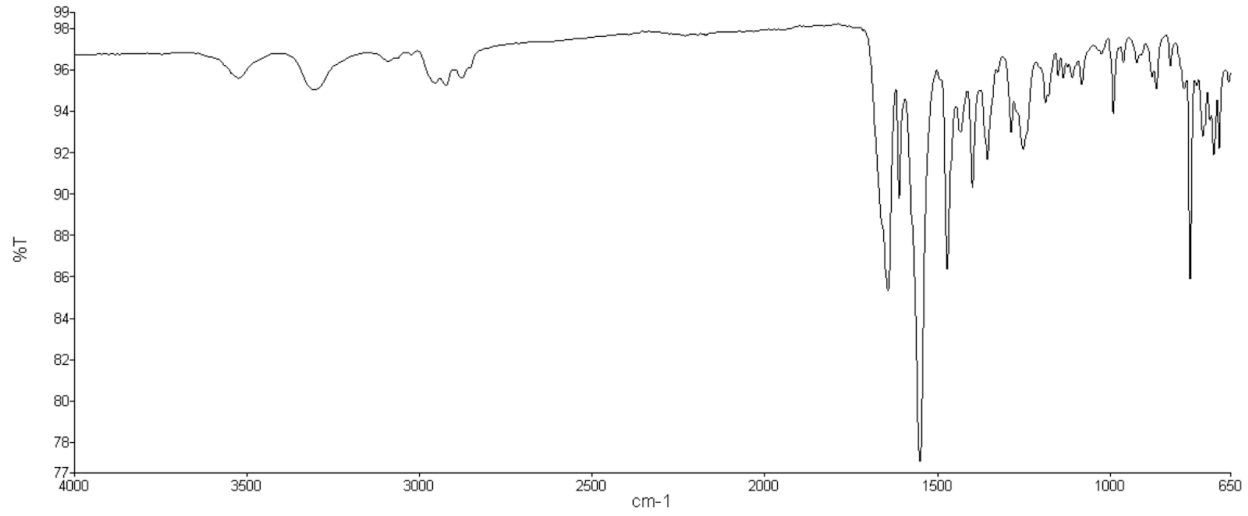


^{13}C NMR (126 MHz, $CDCl_3$) of compound **1f**.

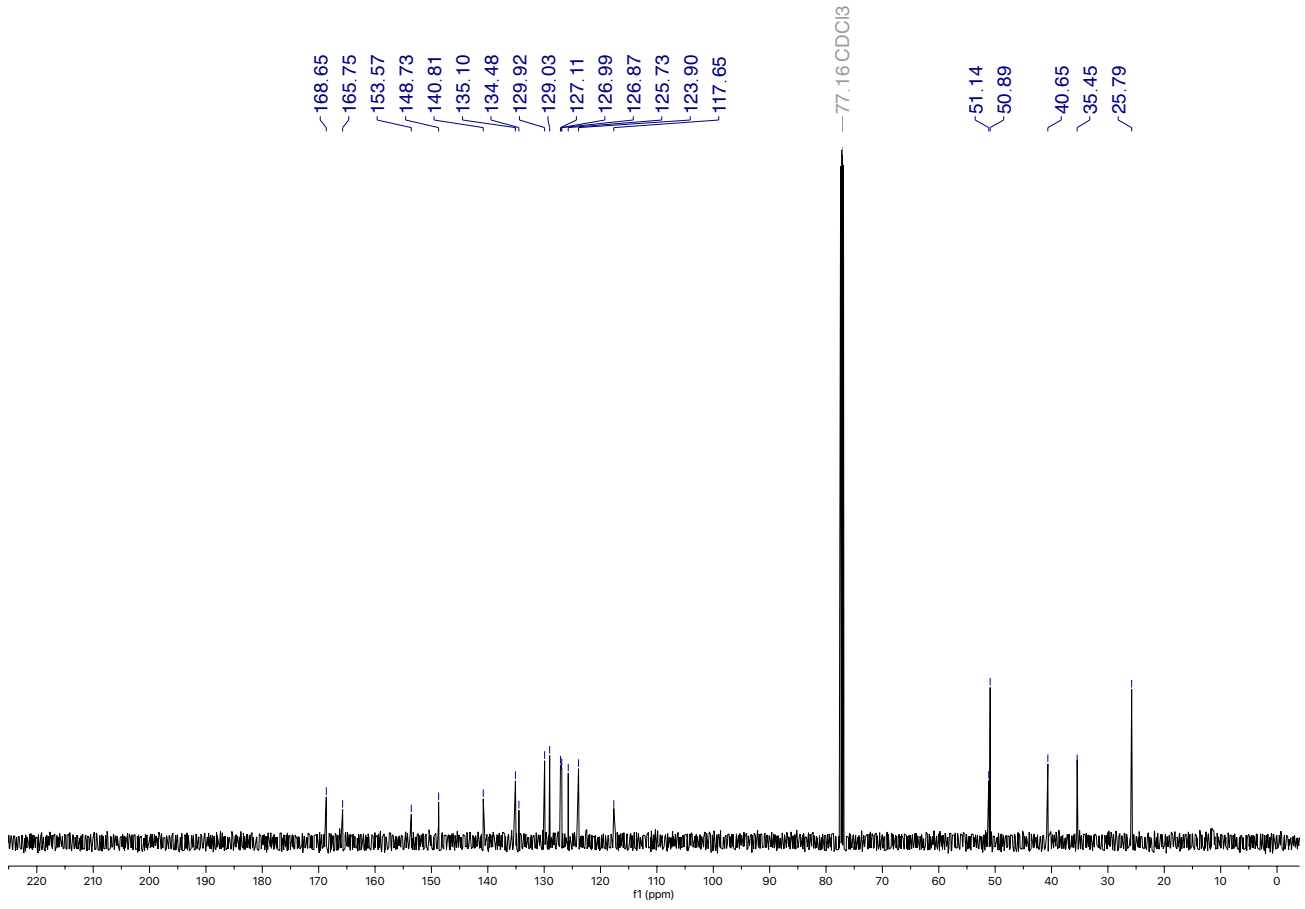


1g

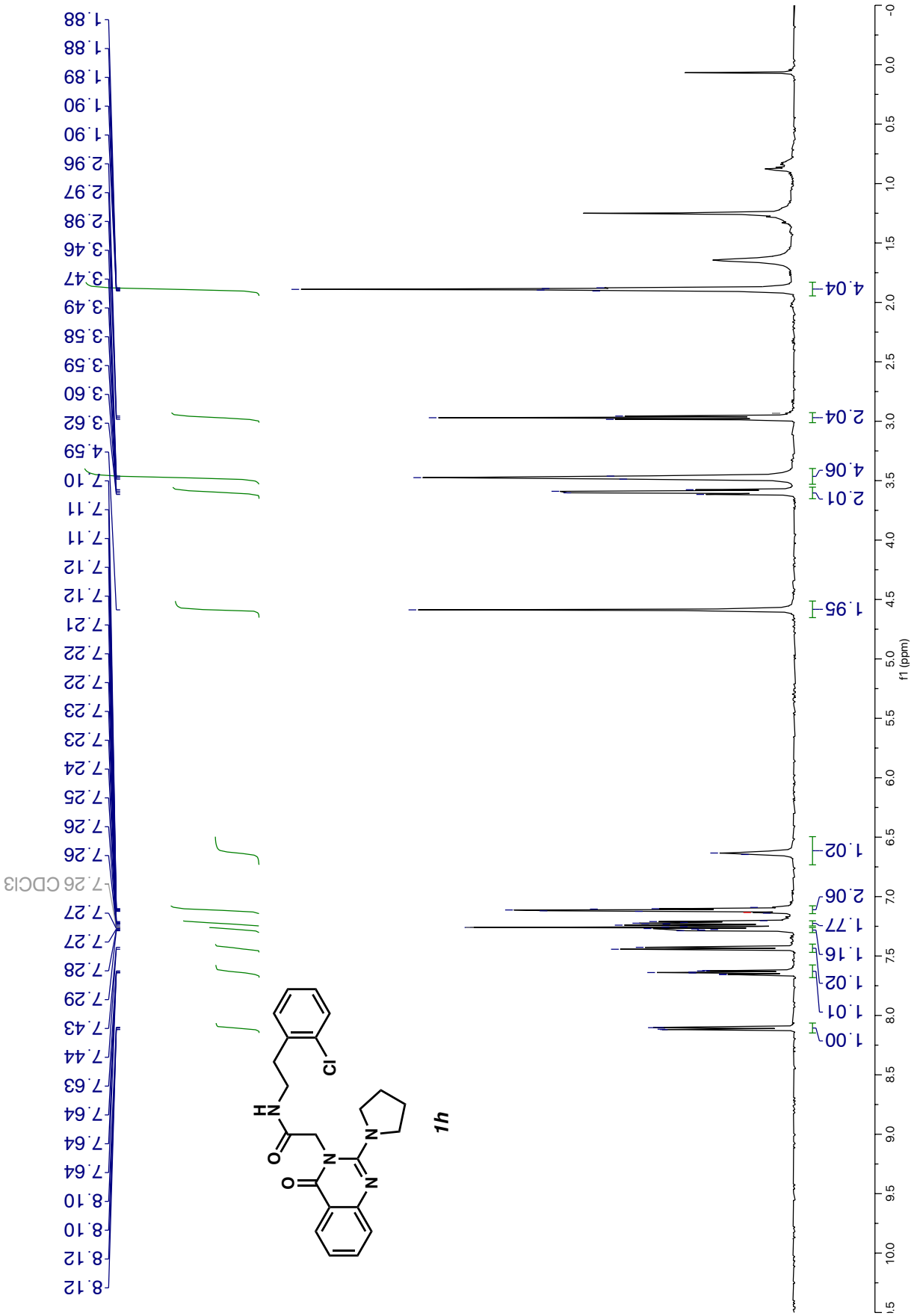
^{1}H NMR (500 MHz, $CDCl_3$) of compound **1g.**



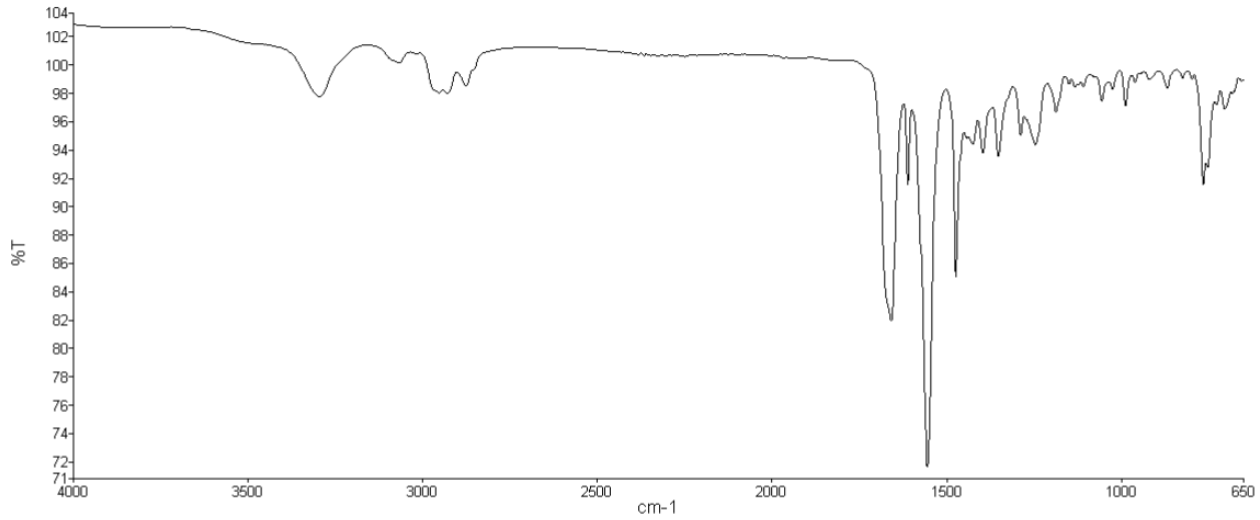
Infrared spectrum (Thin Film) of compound **1g**.



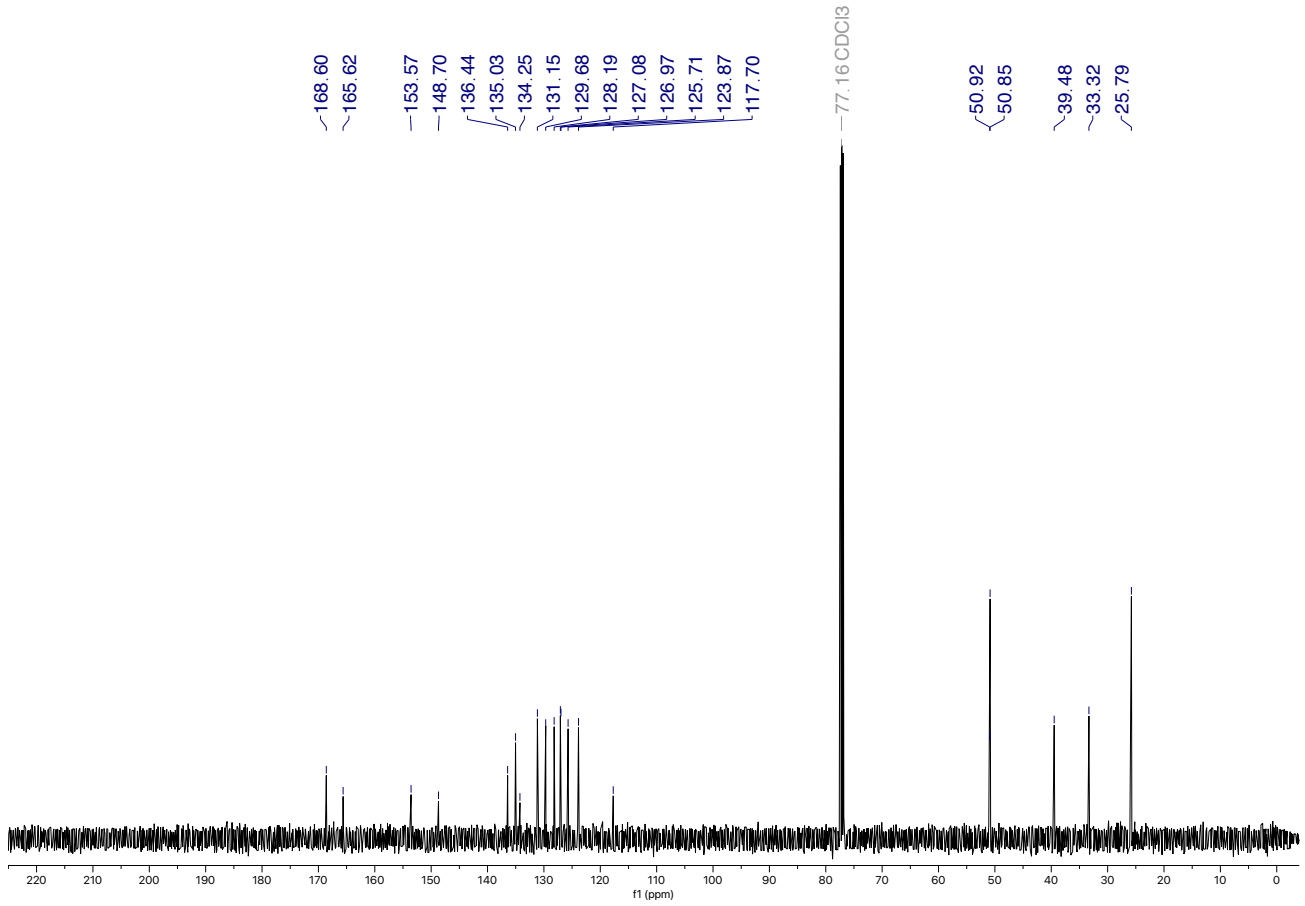
^{13}C NMR (126 MHz, $CDCl_3$) of compound **1g**.



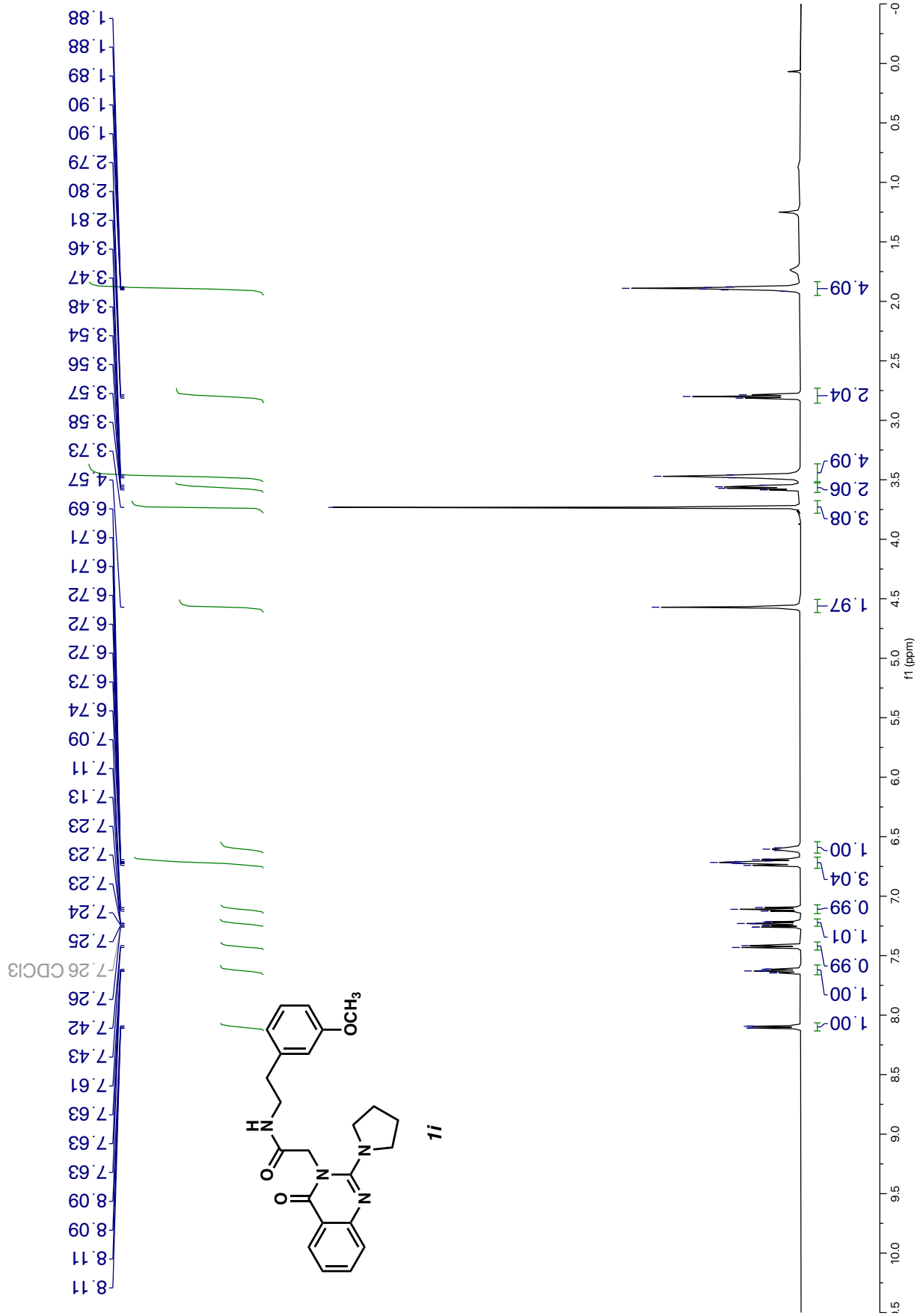
^{1}H NMR (500 MHz, $CDCl_3$) of compound **1h.**



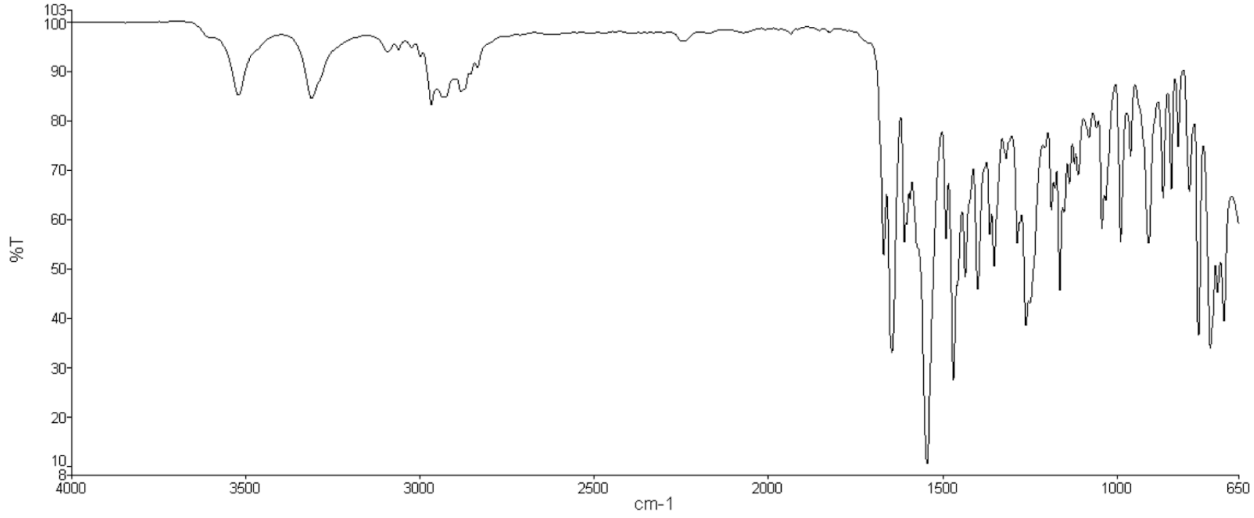
Infrared spectrum (Thin Film) of compound **1h**.



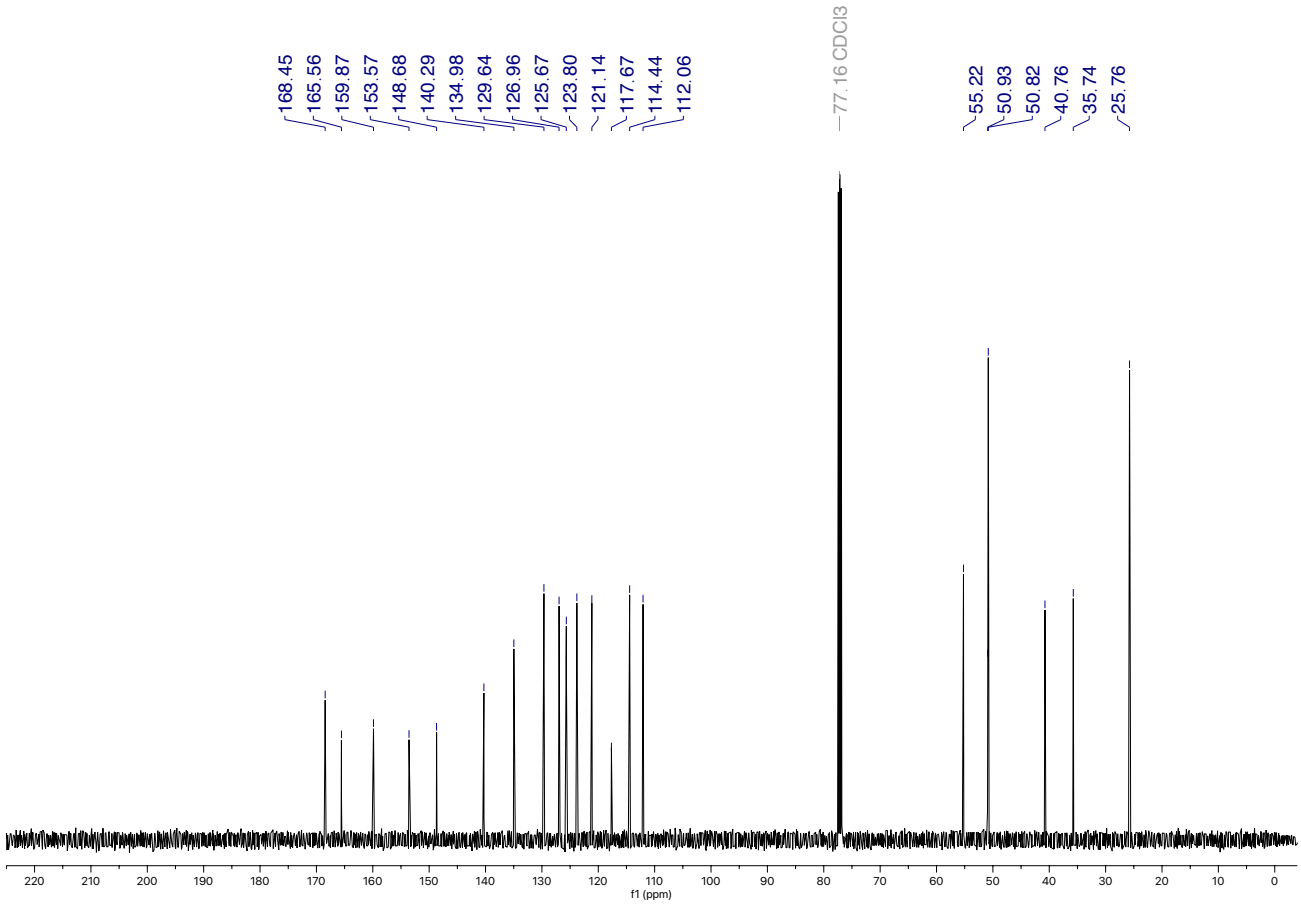
^{13}C NMR (126 MHz, $CDCl_3$) of compound **1h**.



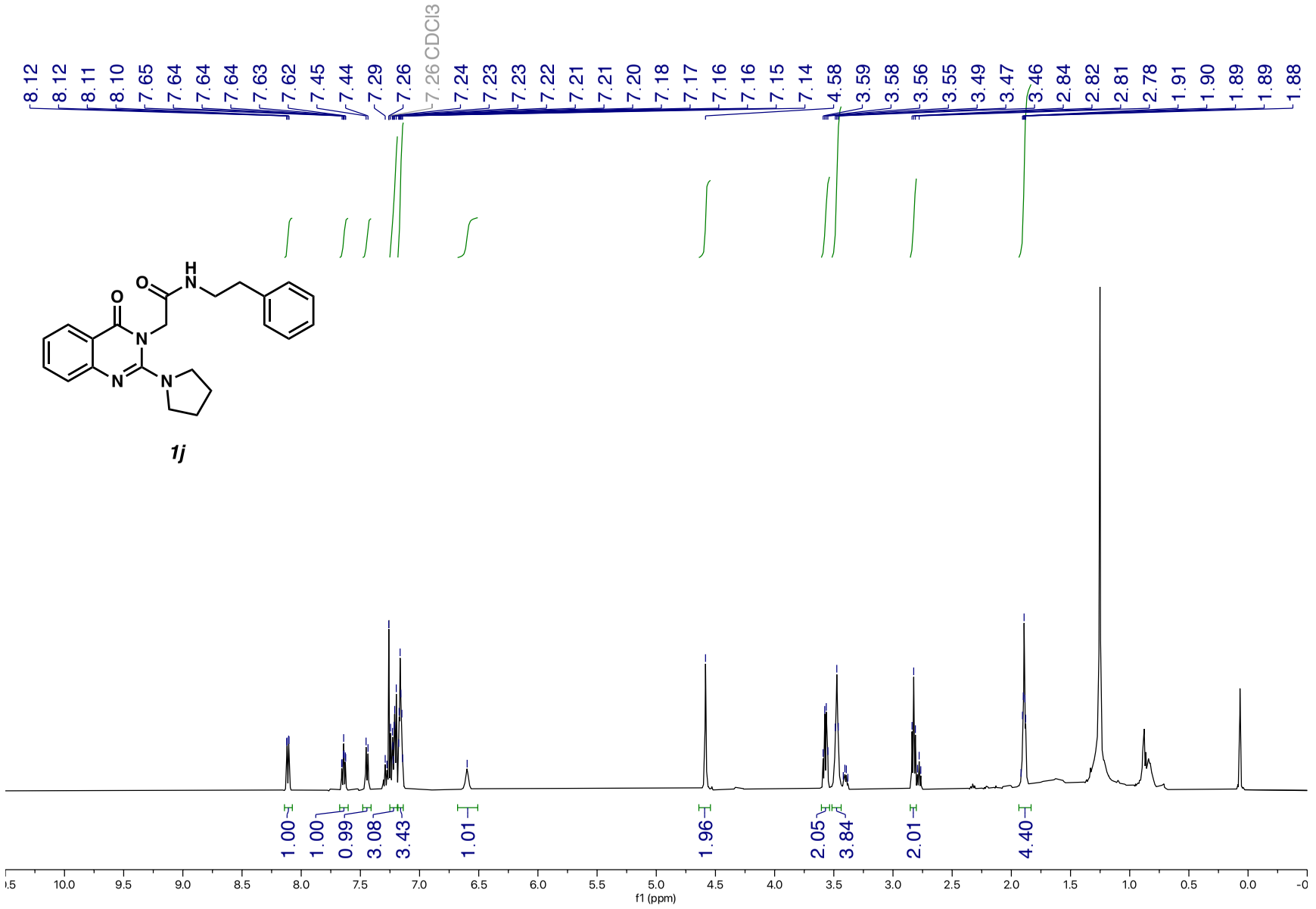
^{1}H NMR (500 MHz, $CDCl_3$) of compound **1i**.



Infrared spectrum (Thin Film) of compound **1i**.

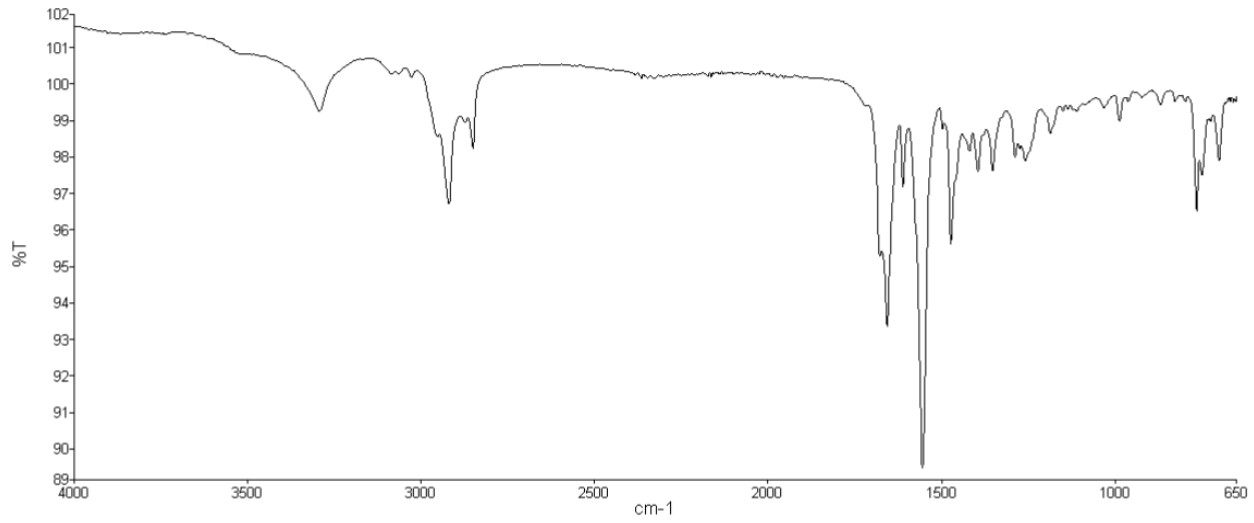


^{13}C NMR (126 MHz, $CDCl_3$) of compound **1i**.

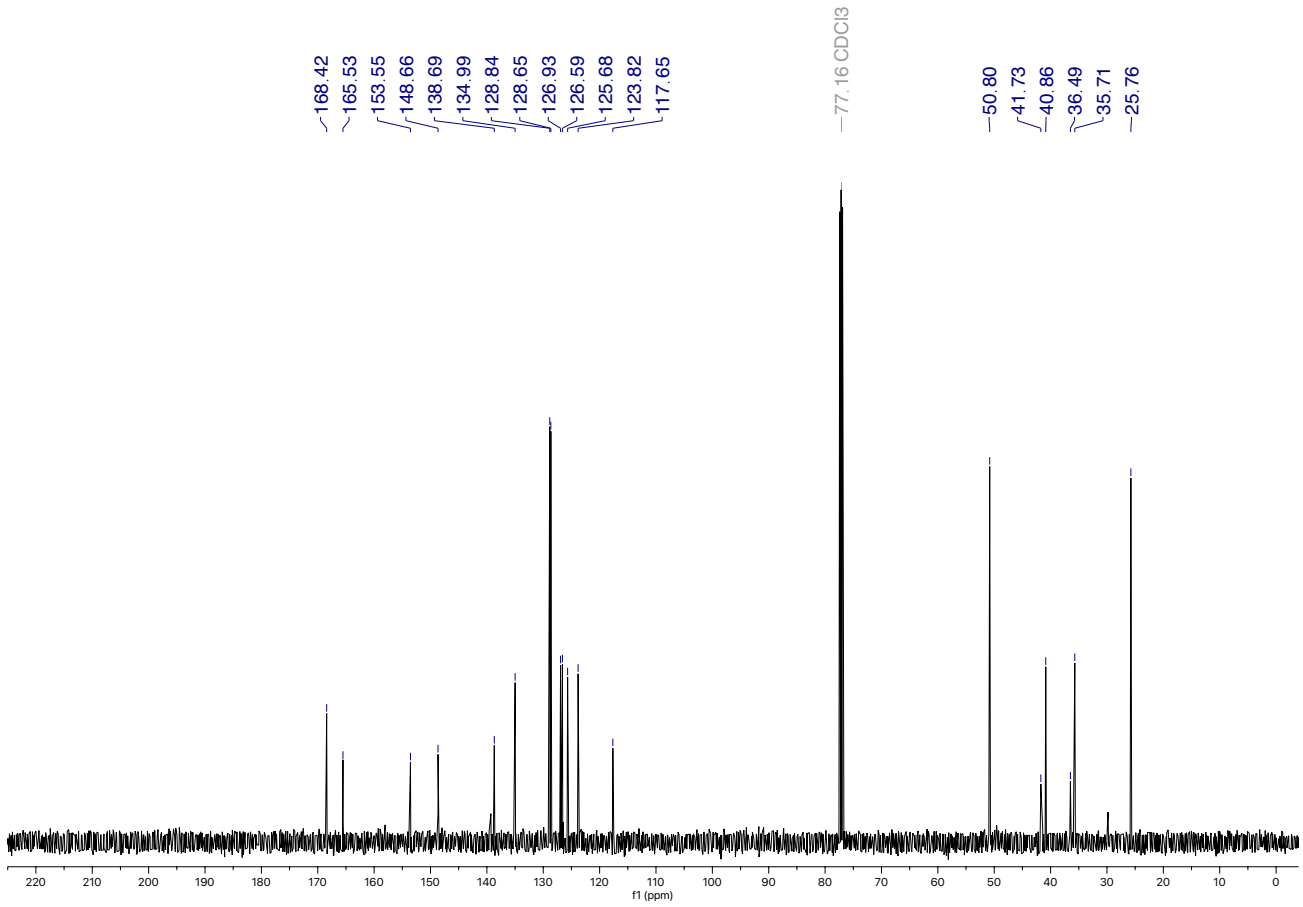


1j

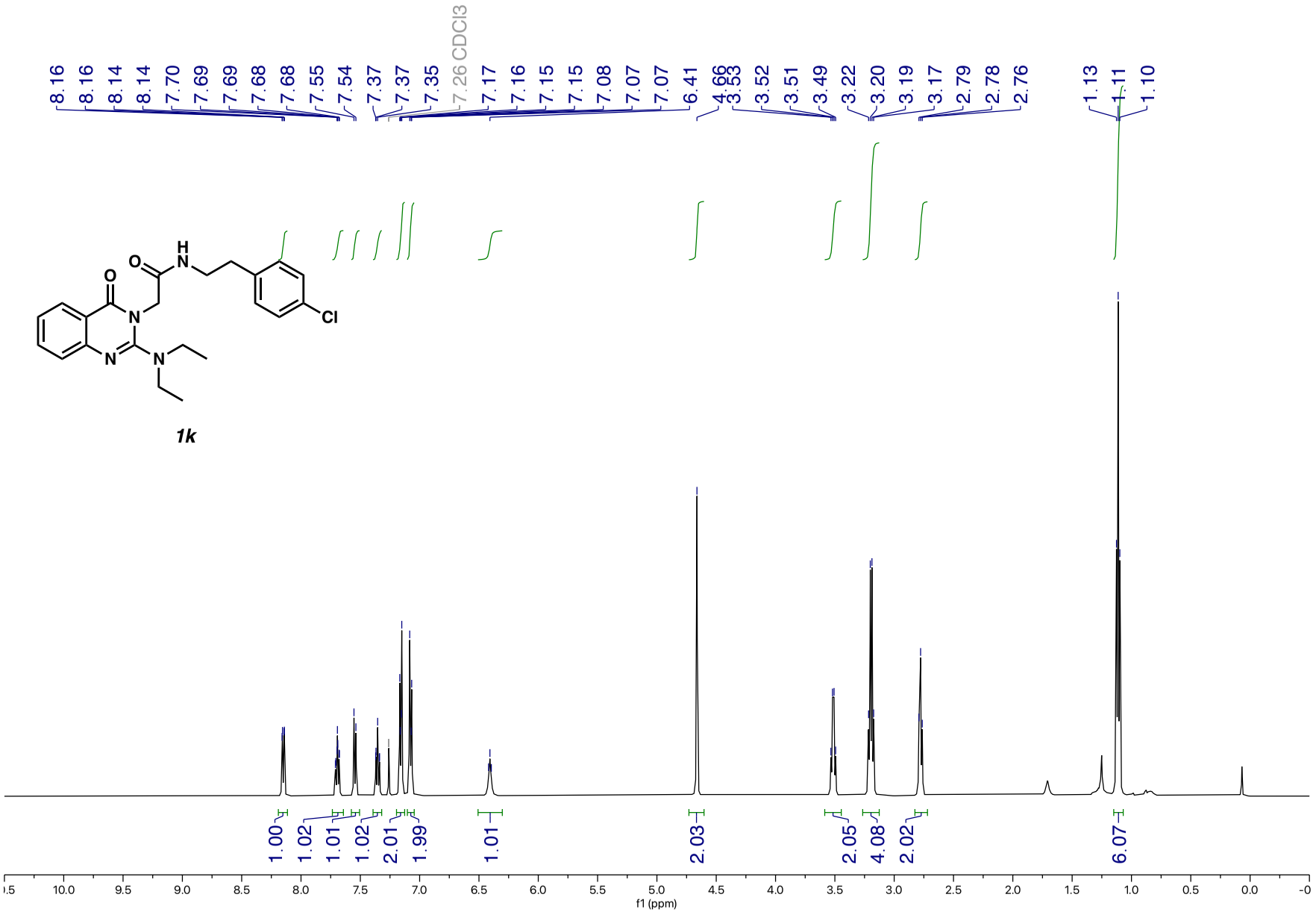
^{1}H NMR (500 MHz, $CDCl_3$) of compound **1j**.



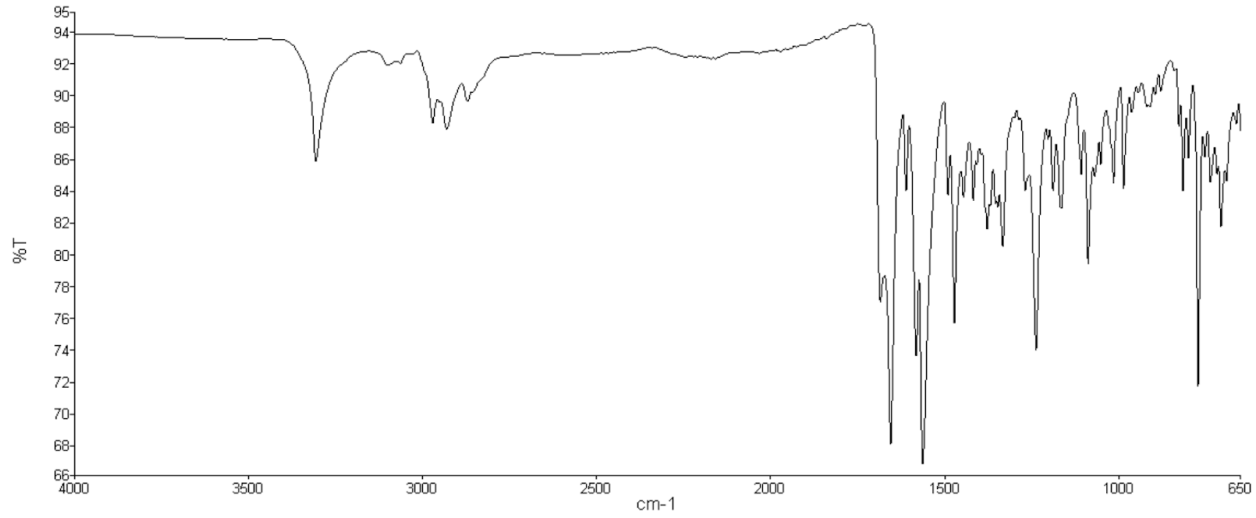
Infrared spectrum (Thin Film) of compound **1j**.



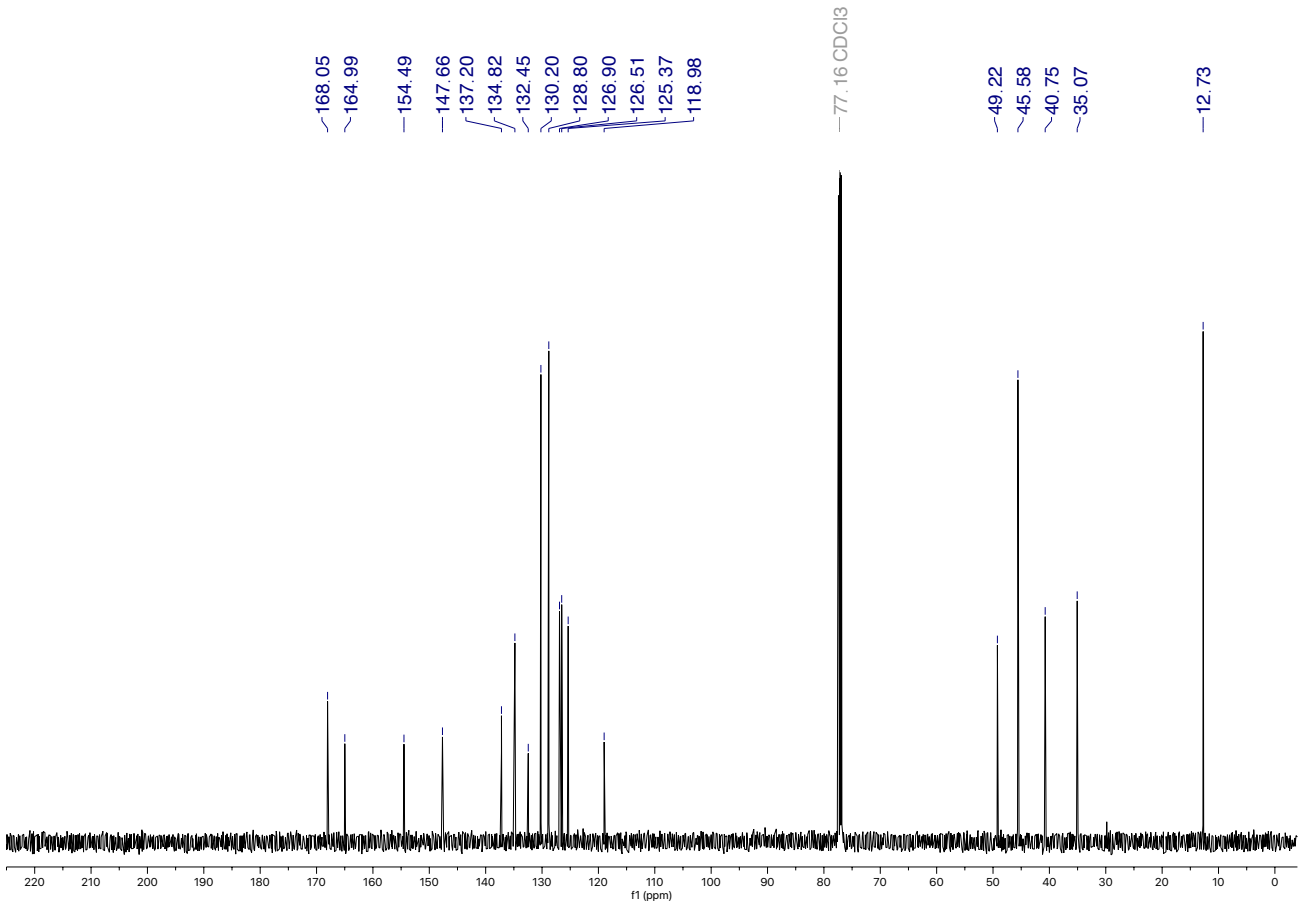
^{13}C NMR (126 MHz, $CDCl_3$) of compound **1j**.



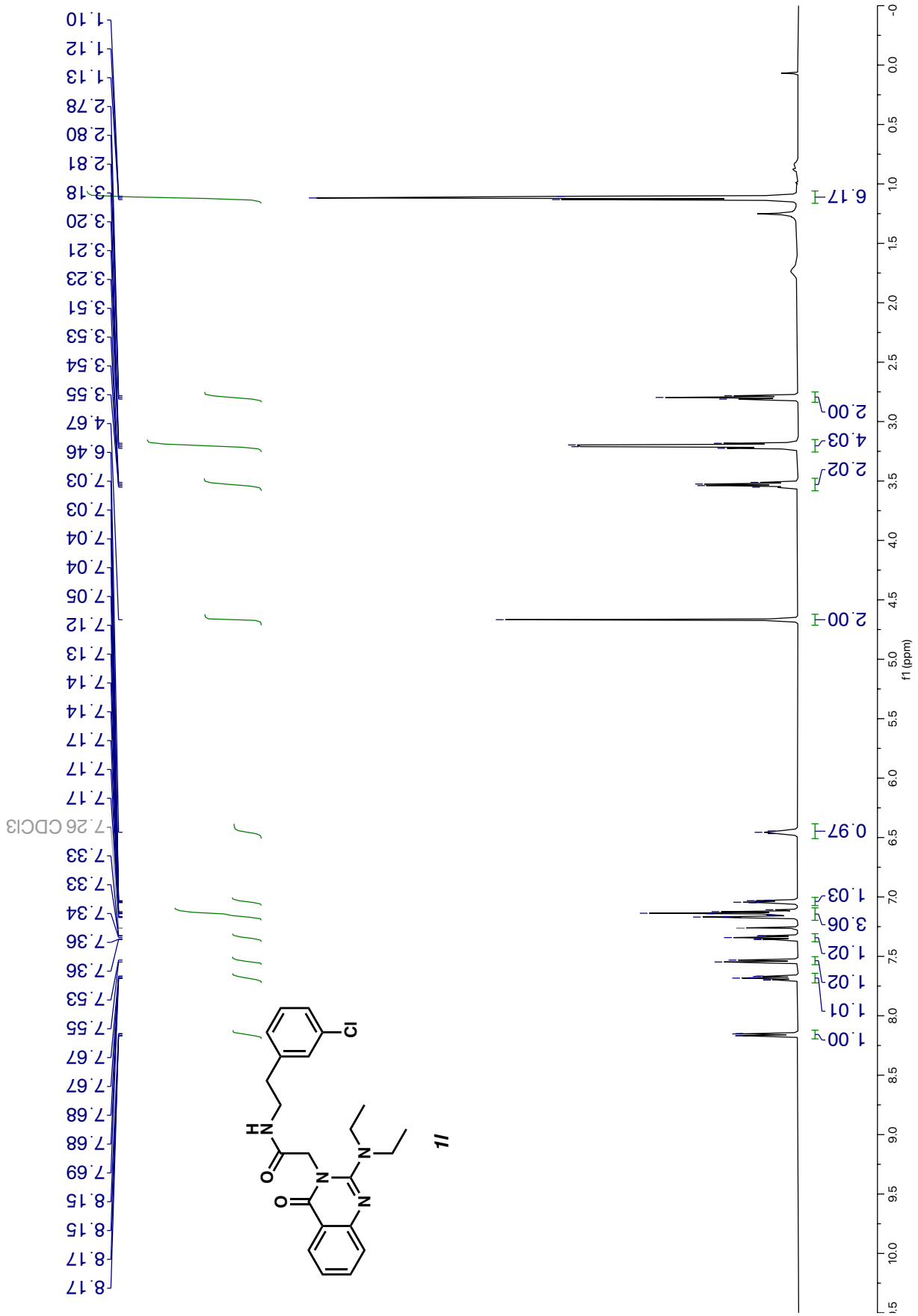
^{1}H NMR (500 MHz, $CDCl_3$) of compound **1k**.



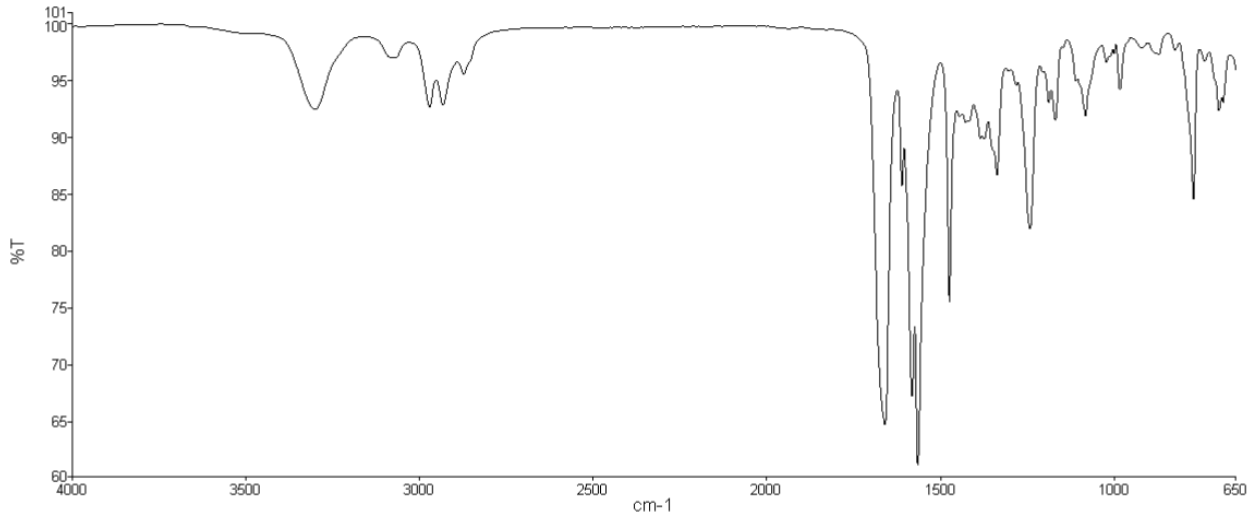
Infrared spectrum (Thin Film) of compound **1k**.



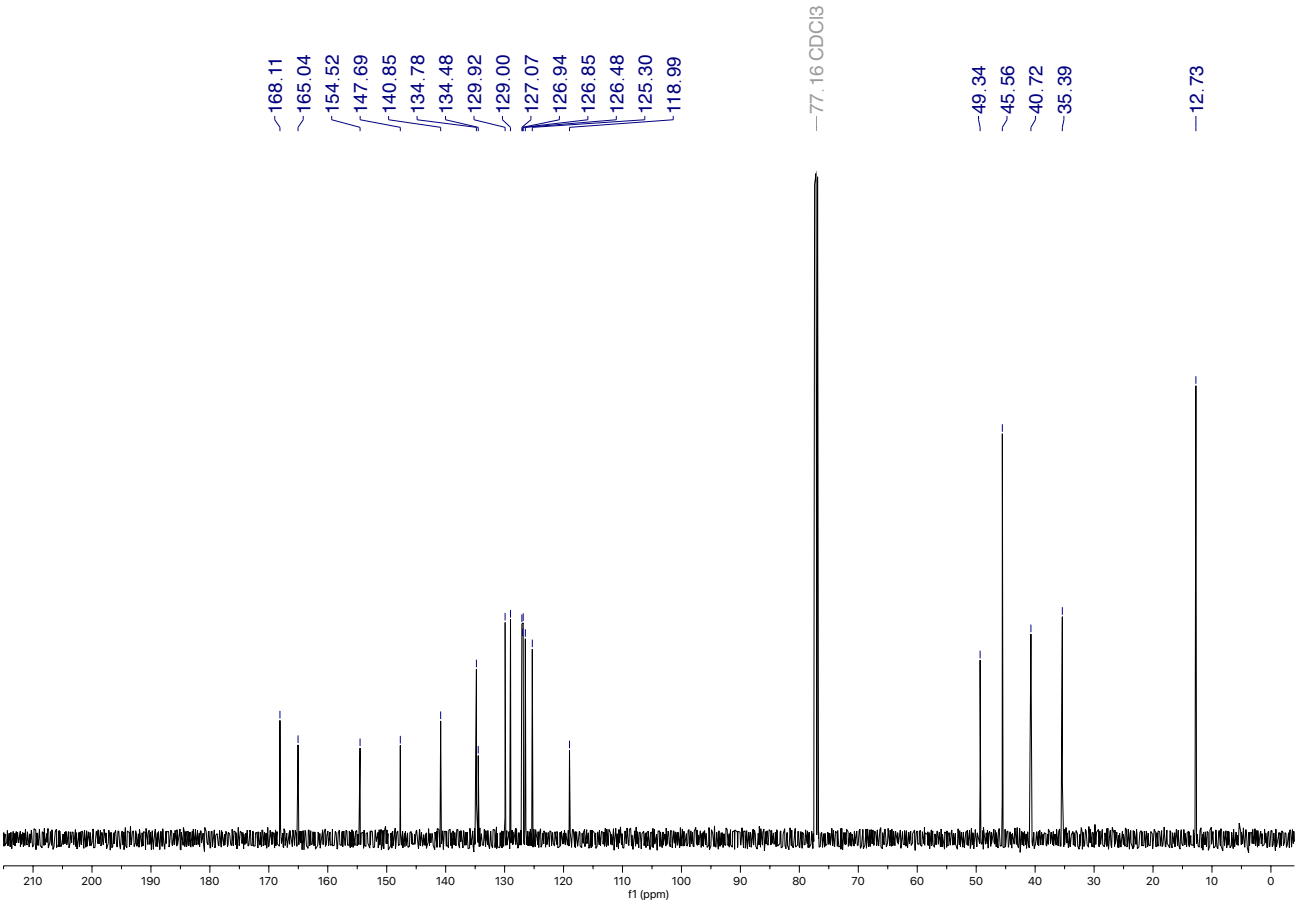
^{13}C NMR (126 MHz, $CDCl_3$) of compound **1k**.



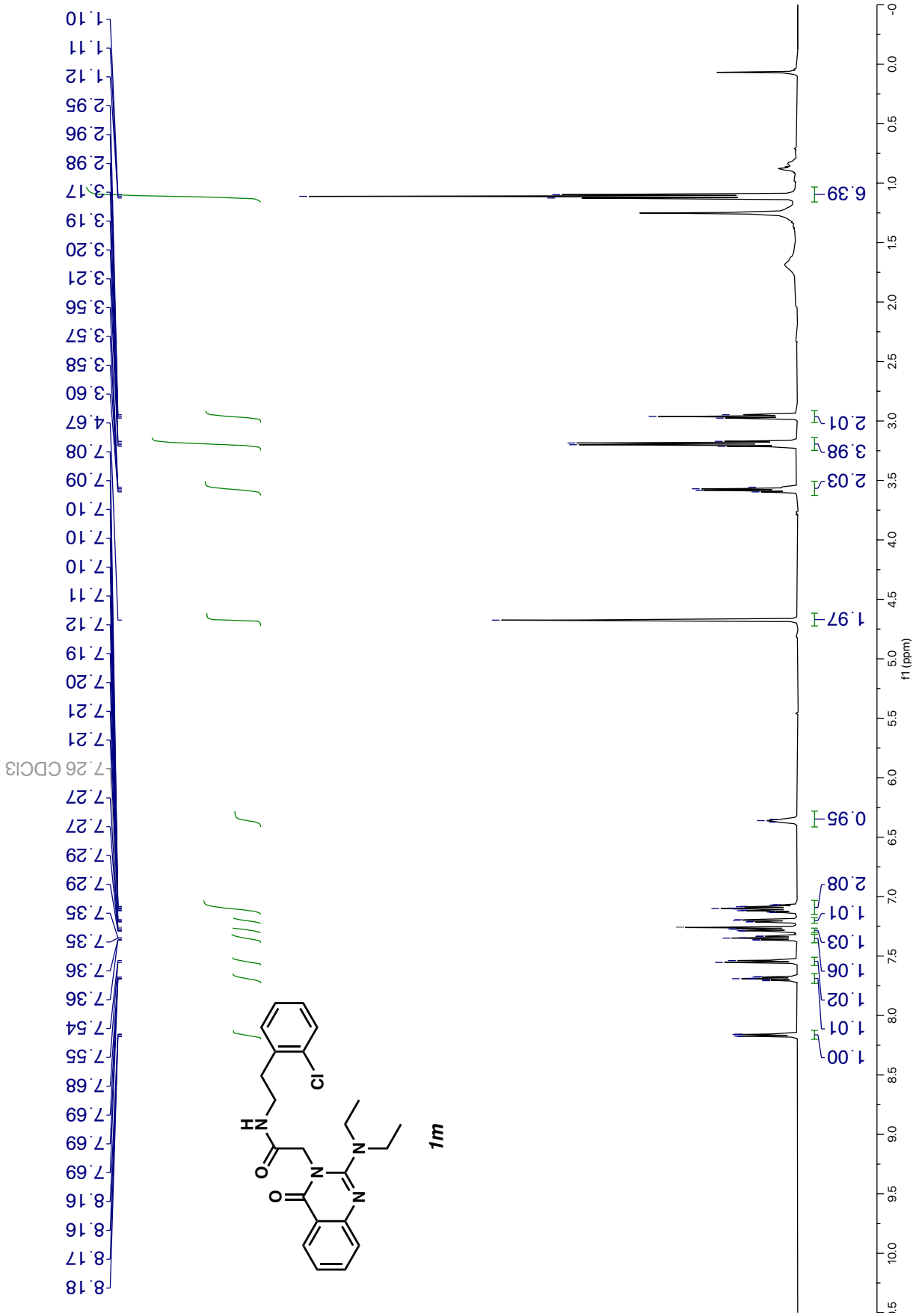
^{1}H NMR (500 MHz, $CDCl_3$) of compound **11**.



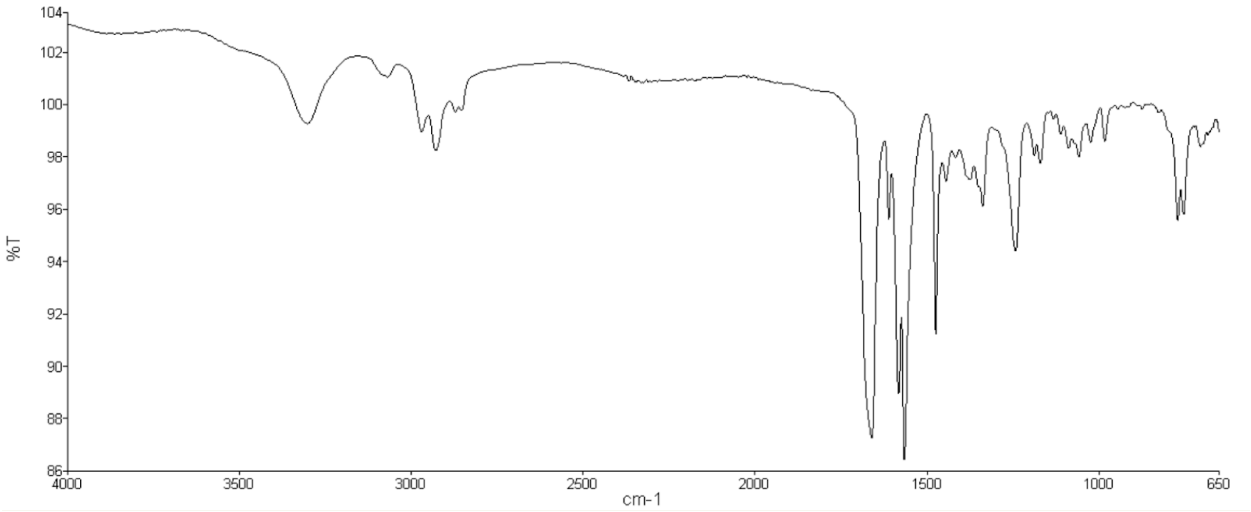
Infrared spectrum (Thin Film) of compound **1l**.



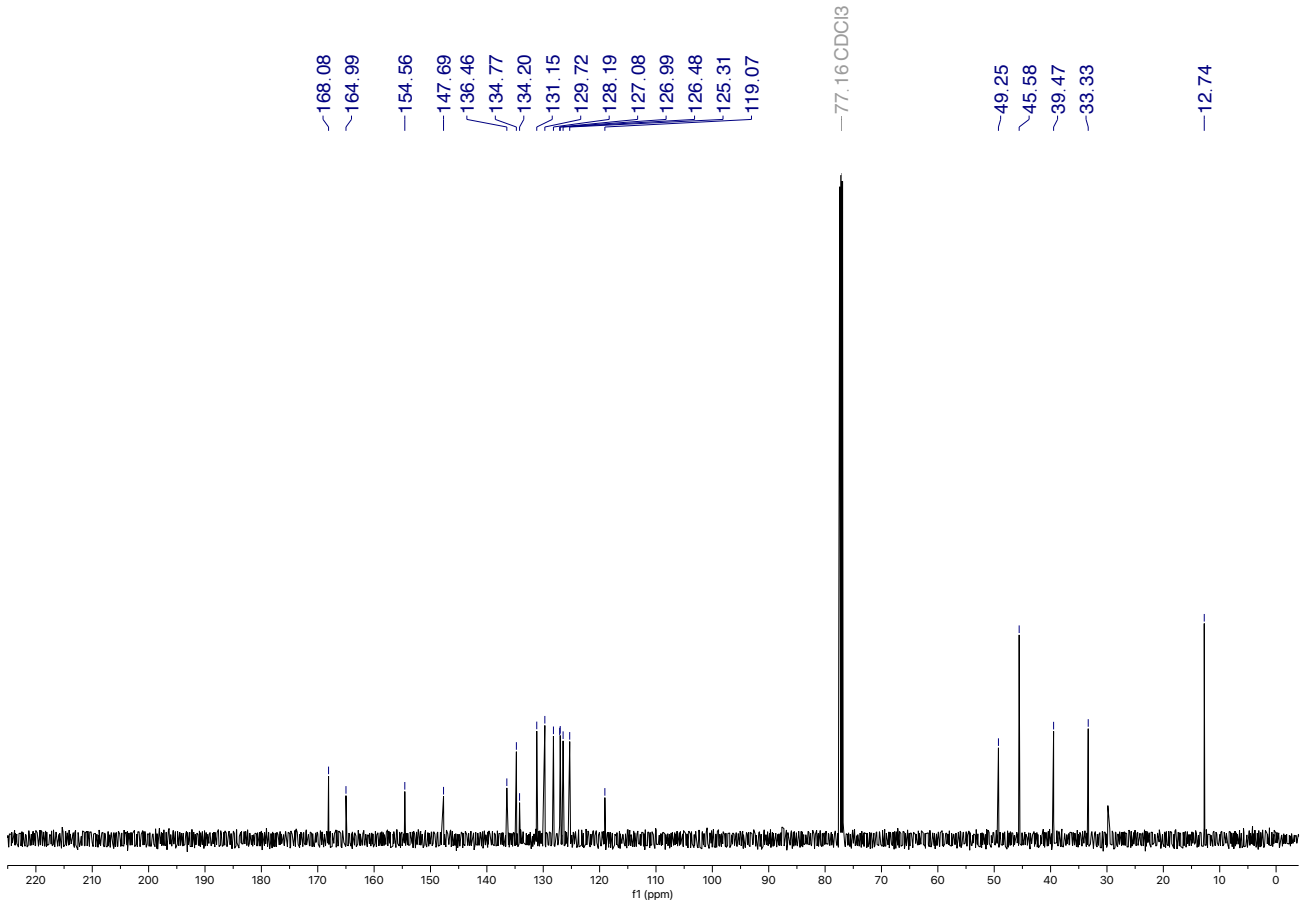
^{13}C NMR (126 MHz, $CDCl_3$) of compound **1l**.



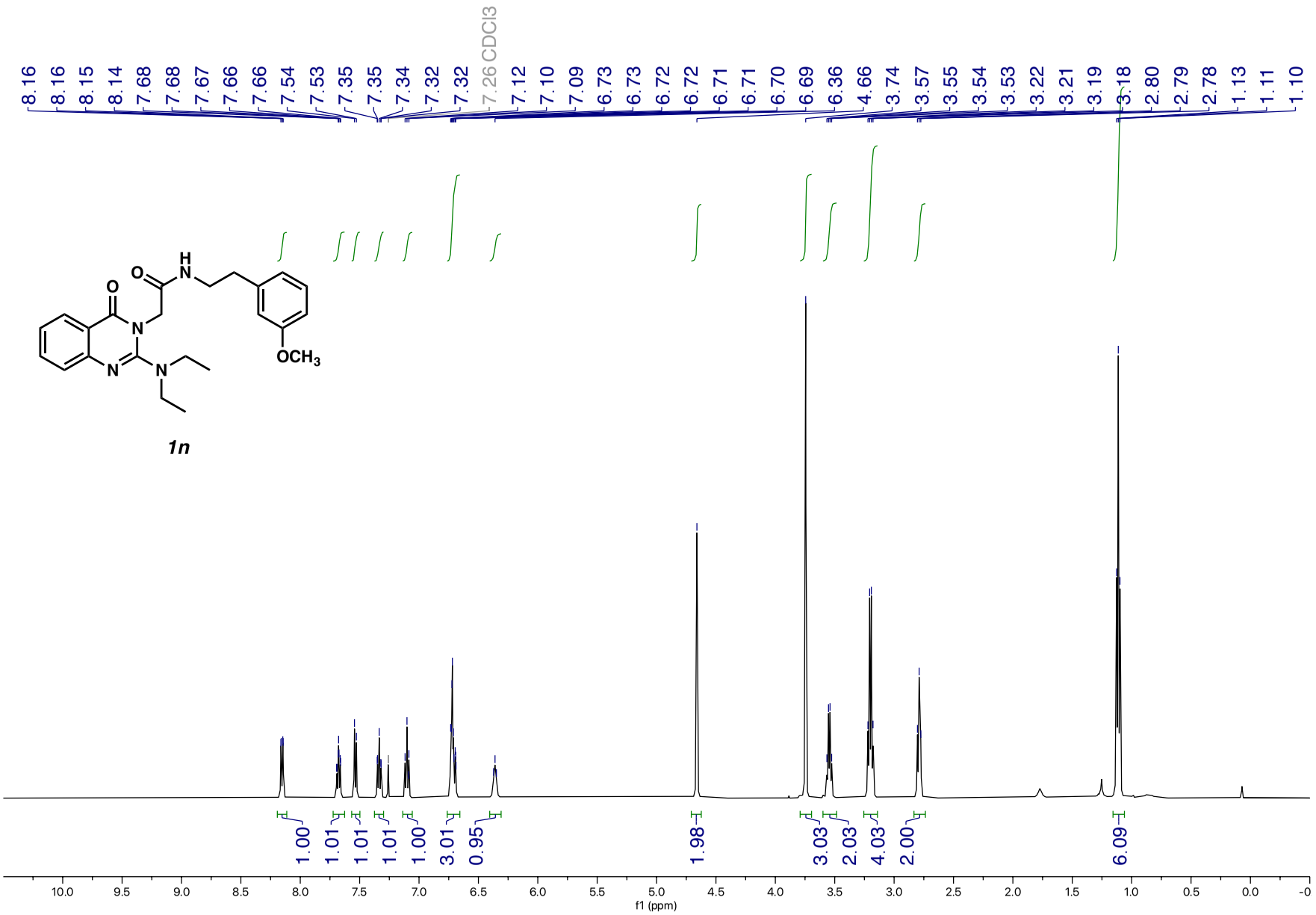
^{1}H NMR (500 MHz, $CDCl_3$) of compound **1m**.



Infrared spectrum (Thin Film) of compound **1m**.

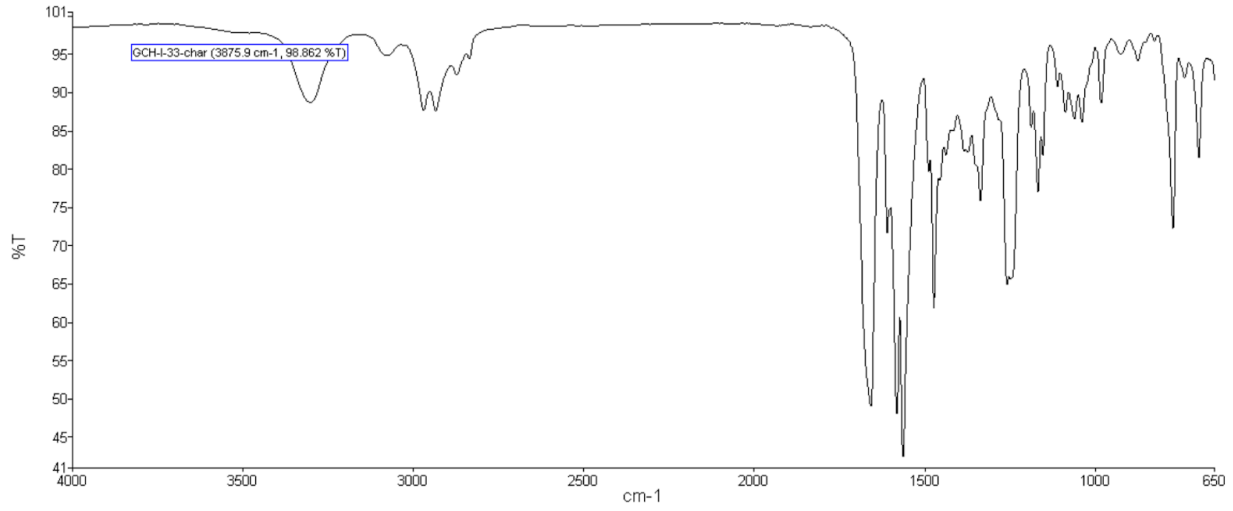


^{13}C NMR (126 MHz, $CDCl_3$) of compound **1m**.

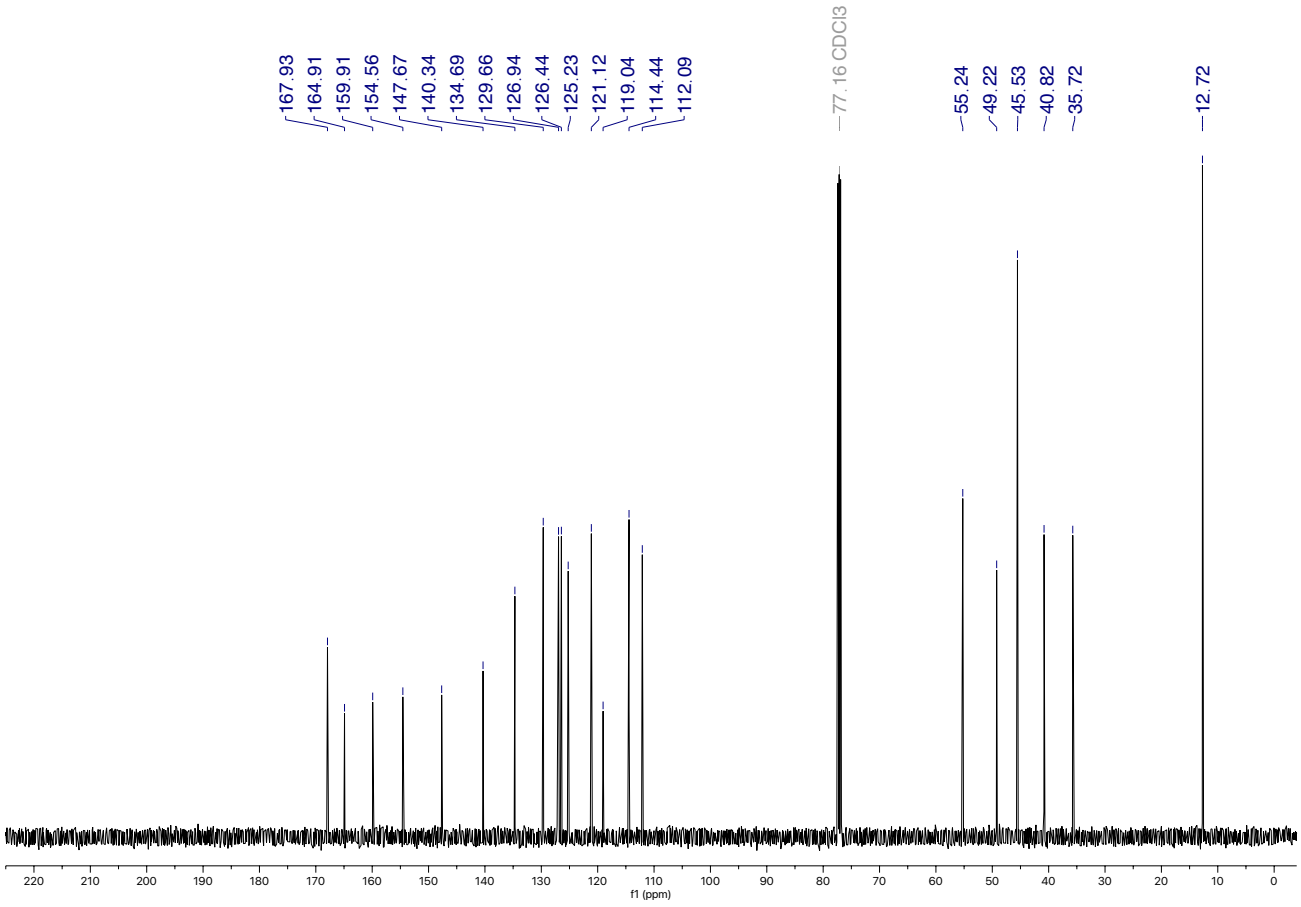


1n

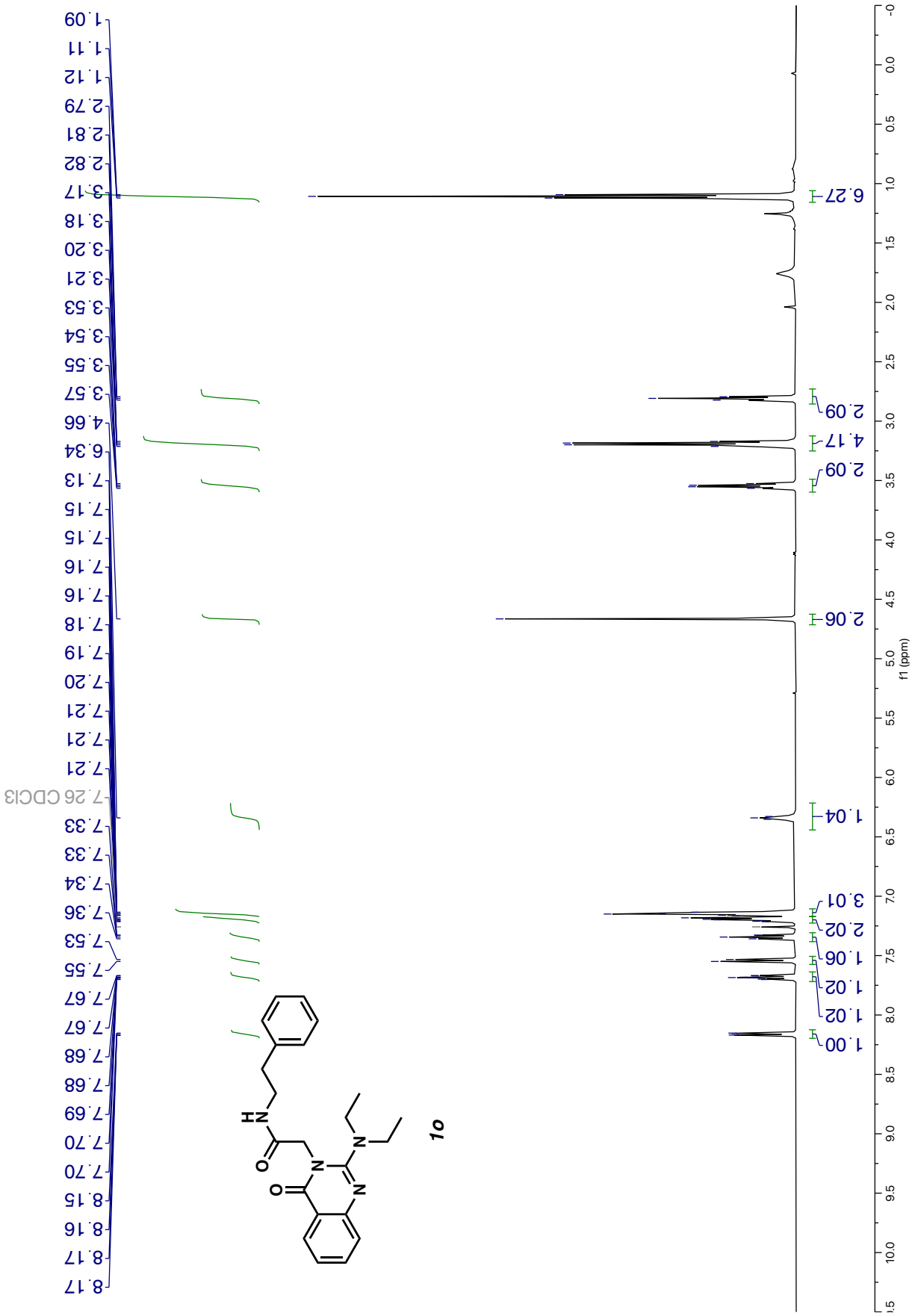
^{1}H NMR (500 MHz, $CDCl_3$) of compound **1n**.



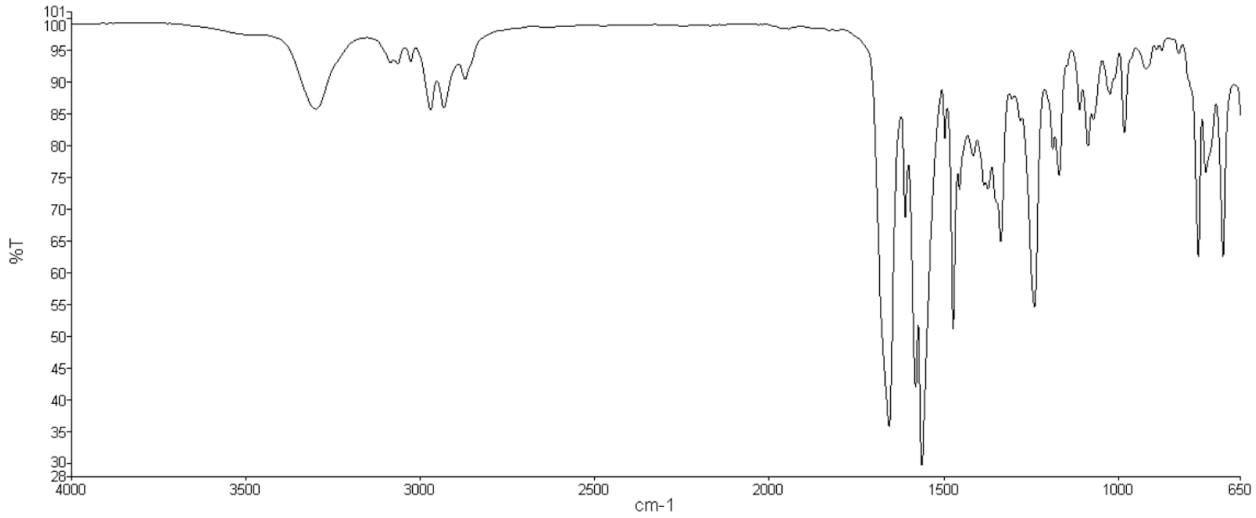
Infrared spectrum (Thin Film) of compound **1n**.



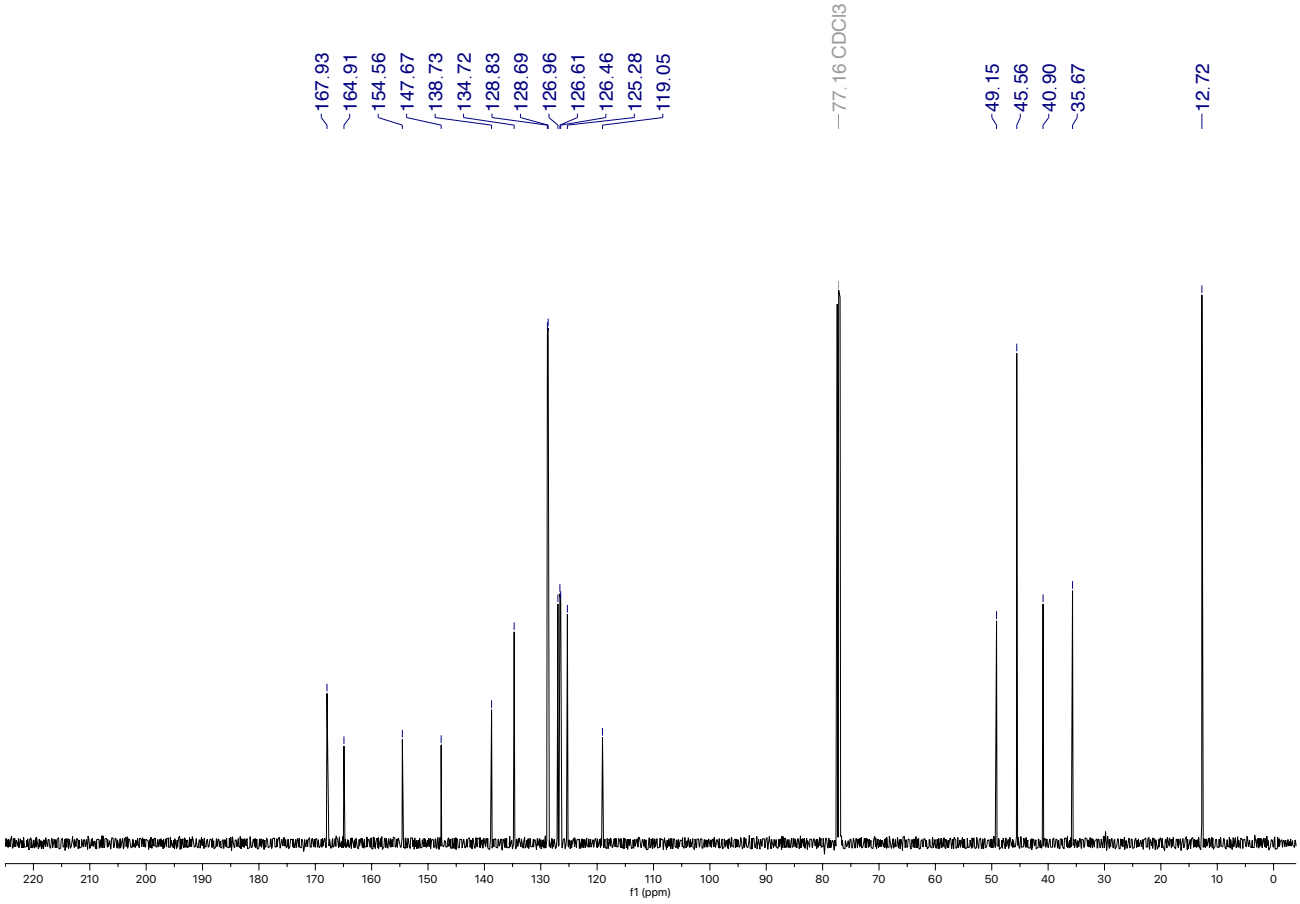
^{13}C NMR (126 MHz, $CDCl_3$) of compound **1n**.



^{1}H NMR (500 MHz, $CDCl_3$) of compound **1o**.

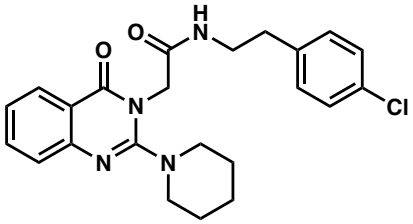
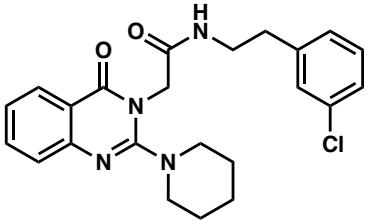
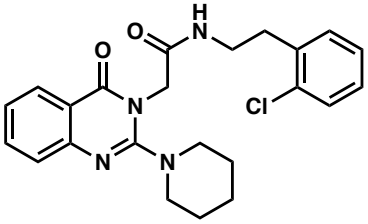
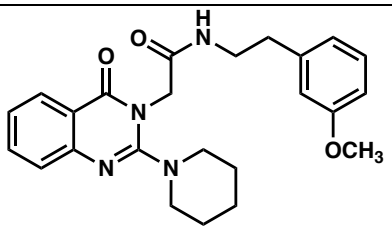
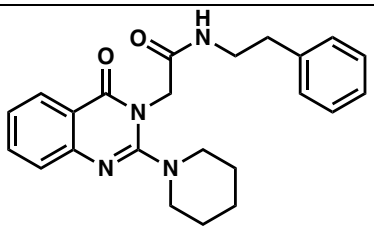


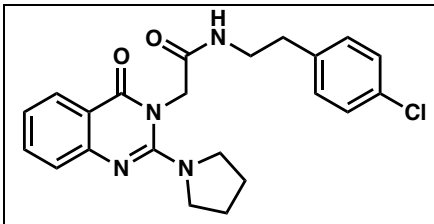
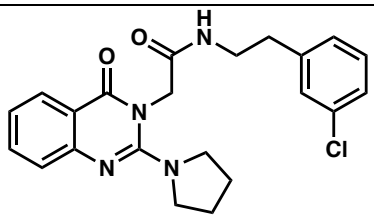
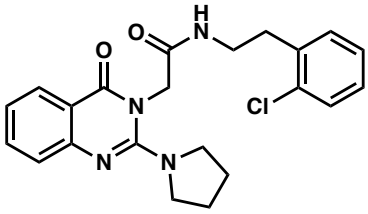
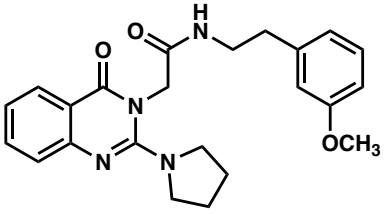
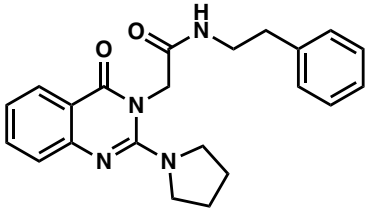
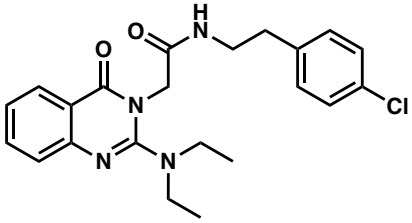
Infrared spectrum (Thin Film) of compound **1o**.

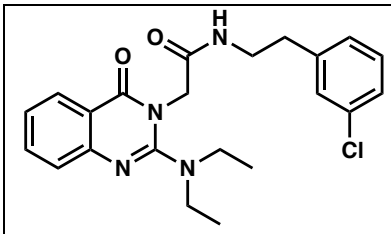
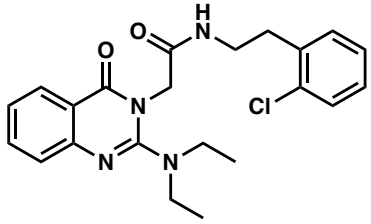
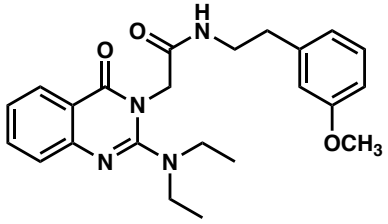
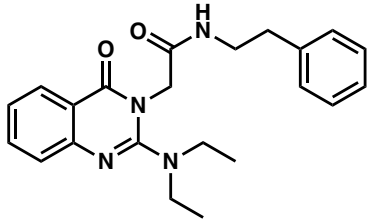


^{13}C NMR (126 MHz, $CDCl_3$) of compound **1o**.

Table S1. Activity of 2,*N*3-Disubstituted Quinazolinones 1a–o Against a Panel of Trypanosomes

Compound	MRC-5 IC_{50} μM	*T.cruzi* IC_{50} μM	*L.infantum* IC_{50} μM	*T.brucei* IC_{50} μM	*T.rhod* IC_{50} μM	PMM IC_{50} μM
 DNDI0003243453 (**1a**)	>64.00	>64.00	>64.00	>64.00	>64.00	>64.00
 DNDI0004041086 (**1b**)	>64.00	>64.00	>64.00	7.51	>64.00	>64.00
 DNDI0004041087 (**1c**)	>64.00	37.97	>64.00	28.04	27.20	>64.00
 DNDI0003975034 (**1d**)	>64.00	>64.00	>64.00	>64.00	>64.00	>64.00
 DNDI0004041088 (**1e**)	>64.00	>64.00	>64.00	>64.00	>64.00	>64.00

 DNDI0004041093 (**1f**)	>64.00	>64.00	38.05	>64.00	29.06	>64.00
 DNDI0004041094 (**1g**)	>64.00	>64.00	>64.00	> 64.00	>64.00	>64.00
 DNDI0004041095 (**1h**)	>64.00	>64.00	>64.00	>64.00	28.47	>64.00
 DNDI0004041096 (**1i**)	>64.00	>64.00	>64.00	>64.00	>64.00	>64.00
 DNDI0004041097 (**1j**)	>64.00	>64.00	>64.00	>64.00	>64.00	>64.00
 DNDI0003243392 (**1k**)	>64.00	>64.00	>64.00	>64.00	>64.00	>64.00

 DNDI0004041089 (**1l**)	>64.00	>64.00	>64.00	>64.00	>64.00	>64.00
 DNDI0004041090 (**1m**)	>64.00	>64.00	>64.00	>64.00	>64.00	>64.00
 DNDI0004041091 (**1n**)	>64.00	>64.00	>64.00	>64.00	>64.00	>64.00
 DNDI0004041092 (**1o**)	>64.00	>64.00	>64.00	>64.00	>64.00	>64.00

Screening procedures for each assay are provided on the following page.

SOP
(abbreviated)

Antwerp University
LMPH
Laboratory of Microbiology, Parasitology and Hygiene

December 2023

Standard procedures
(abbreviated SOP's)

used for the DNDi *in vitro* screening

against

Sleeping sickness

Chagas disease

Leishmaniasis

Cytotoxicity

Prof. Dr. Guy Caljon
Laboratory of Microbiology, Parasitology and Hygiene (LMPH)
University of Antwerp, campus CDE, S7.24
Universiteitsplein 1
B-2610 Wilrijk-Antwerpen
Belgium

Tel: (32)3.265.26.01
Guy.Caljon@uantwerpen.be

Interpretation of results

- Activity is expressed as IC_{50}-values in µM concentrations. For compounds without exact molecular weight, IC_{50}-values are expressed in µg/mL.
- Based on the level of the IC_{50}, semi-quantitative activity scores are given (normalized across the different assays to allow comparison)
- Compounds with score ≥3 may require confirmation testing and further follow-up **if** the observed activity is selective (absence of obvious cytotoxicity, high selectivity index).

In vitro activity of reference compounds

UA	Compund code	Generic name	MW	Screen	IC_{50} (µM)	
R120	UA/ K020	TAMOXIFEN CITRATE SALT	564	MRC-5 SV2	10.2	1.44
R139	TDR/ 42268/1	NICLOSAMIDE	327		7.11	3.71
R126		MILTEFOSIN	408	L.inf	10.07	4.23
R116	UA/ K016	AMPHOTERICIN B	924		1.16	1.25
R126		MILTEFOSIN	408	L.don	6.88	3.64
R116	UA/ K016	AMPHOTERICIN B	924		0.99	0.28
R125	TDR/ 10164/1	BENZNIDAZOL	260	T.cruz	2.89	0.56
R132	TDR/ 10739/1	NIFURTIMOX	287		0.91	0.41
R131	TDR/ 10738/1	SURAMIN	1297	T.b.bruc	0.03	0.01
R152	TDR/ 9957/1	MELARSOPROL	398		0.02	0.004
R131	TDR/ 10738/1	SURAMIN	1297	T.b.rhod	0.04	0.01
R152	TDR/ 9957/1	MELARSOPROL	398		0.005	0.002

IC_{50}-values of reference compounds are based on historical control values.

Only one reference drug is included per assay.

Toxicity	***In vitro* cytotoxicity evaluation on human fibroblasts (MRC-5 cell line)**

Parasite and cell cultures

MRC-5_{SV2}[1] cells are cultured in MEM + Earl's salts-medium, supplemented with L-glutamine, $NaHCO_3$ and 5% inactivated fetal calf serum. All cultures and assays are conducted at 37°C under an atmosphere of 5% $C0_2$. Other cell types (J774, L6, Vero, Hela, *e.a.*) can also be used for determination of cytotoxicity/selectivity.

Compound solutions/dilutions

Compound stock solutions are prepared in 100% DMSO at 20 mM or mg/mL[2]. The compounds are serially pre-diluted (2-fold or 4-fold) in DMSO followed by a further (intermediate) dilution in demineralized water to assure a final in-test DMSO concentration of <1%.

Drug sensitivity assays

Assays are performed in sterile 96-well microtiter plates, each well containing 10 µL of the watery compound dilutions together with 190 µL of MRC-5 $_{SV2}$ inoculum (1.5×10^5 cells/mL). Cell growth is compared to untreated-control wells (100% cell growth) and medium-control wells (0% cell growth). After 3 days incubation, cell viability is assessed fluorimetrically after addition of 50µl resazurin per well[3]. After 4 hours at 37°C, fluorescence is measured (λ_{ex} 550 nm, λ_{em} 590 nm). The results are expressed as % reduction in cell growth/viability compared to control wells and an IC_{50} and an IC_{90} (50% and 90% inhibitory concentrations) are determined.

Primary screen

The MRC-5_{SV2} cell-line is used. The compounds are tested at 5 or 10 concentrations depending upon the number of compounds (4-fold compound dilutions) (64 - 16 - 4 – 1 – 0.25 – 0.0625 – 0.015625 – 0.0039 – 0.000975 and 0.00024 µM or µg/mL). The compound is classified non-toxic when the IC_{50} is higher than 30 µM. Between 30 and 10 µM, the compound is regarded as moderately toxic. When the IC_{50} is lower than 10 µM, the compound is classified as highly toxic. Cytotoxic reference compounds include vinblastine or paclitaxel (IC_{50} <0.01µM), but these are rarely included because of health hazards for laboratory personnel. Other compounds are therefore used: tamoxifen or niclosamide.

Secondary screen

The IC_{50} is determined using an extended dose range (2-fold compound dilutions) still with a highest concentration of 64µM. Other cell lines and primary cells can be included: L6, J774, Hela, Vero and PMM (primary mouse macrophages). Parallel cytotoxicity evaluation is required to assess selective action of test compounds in parasite screens.

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[1] MRC-5_{SV2} cells are diploid human embryonic lung fibroblasts. A SV-40 transformed cell line is now available to obtain unlimited sub-cultivation characteristics.

[2] Concentrations are standard expressed in molar concentrations, except for natural products, drug mixtures and if the molecular weight is not known.

[3] Resazurin stock solution in phosphate buffer: 50µg/mL. Alamar Blue™ can be used as alternative: 5µl of a 1/10 Alamar Blue™ solution is added to each well

Leishmania	***In vitro* drug screening model against *Leishmania donovani* and *Leishmania infantum***

Parasite and cell cultures

Two *Leishmania species* (*L. infantum* MHOM/MA(BE)/67 and *L. donovani* MHOM/ET/67/L82) are used. The strains are maintained in the Golden Hamster (*Mesocricetus auratus*). Amastigotes are collected from the spleen of an infected donor hamster using three centrifugation purification steps (300 rpm, keeping the supernatans, 2,200 rpm, keeping the supernatans and 3,500 rpm, keeping the pellet) and spleen parasite burdens are assessed using the Stauber technique[4]. Primary peritoneal mouse macrophages are used as host cell and are collected 2 days after peritoneal stimulation with a 2% potato starch suspension. All cultures and assays are conducted at 37°C under an atmosphere of 5% $C0_2$.

Compound solutions/dilutions

Compound stock solutions are prepared in 100% DMSO at 20 mM or mg/mL[5]. The compounds are serially pre-diluted (2-fold or 4-fold) in DMSO followed by a further (intermediate) dilution in demineralized water to assure a final in-test DMSO concentration of <1%.

Drug sensitivity assays

Assays are performed in 96-well microtiter plates, each well containing 10 µL of the compound dilutions together with 190 µL of macrophage/parasite inoculum (3×10^4 cells + 4.5×10^5 parasites/well). The inoculum is prepared in RPMI-1640 medium, supplemented with 2 mM L-glutamine, 16.5 mM $NaHCO_3$, and 5% inactivated fetal calf serum. The macrophages are infected after 48 hours. The compounds are added after 2 hours of infection. Parasite multiplication is compared to untreated-infected controls (100% growth) and uninfected controls (0% growth). After 5 days incubation, parasite burdens (mean number of amastigotes/macrophage) are microscopically assessed after staining the cells with a 10% Giemsa solution. The results are expressed as % reduction in parasite burden compared to untreated control wells and an IC_{50} and an IC_{90} (50% and 90% inhibitory concentrations) are calculated.

Primary screen

L. infantum MHOM/MA(BE)/67 strain is used. The compounds are tested at 5 or 10 concentrations depending upon the number of compounds (4-fold compound dilutions) (64 – 16 – 4 – 1 – 0.25 – 0.0625 – 0.015625 – 0.0039 – 0.000975 and 0.00024 µM or µg/mL). Amphotericin B and miltefosin are included as the reference drugs. A test compound is classified as inactive when the IC_{50} is higher than 30 µM. When IC_{50} lies between 30 and 10 µM, the compound is regarded as moderately active. If the IC_{50} is lower than 10 µM, the compound is classified as highly active on the condition that it also demonstrates selective action (absence of cytotoxicity against primary peritoneal macrophages). A final recommendation for activity is given after confirmatory evaluation in a secondary screening.

Secondary screen

L. infantum MHOM/MA(BE)/67 and *L. donovani* MHOM/ET/67/L82 strains are used and the IC_{50}-values are determined using an extended dose range (2-fold compound dilutions). Amphotericin B or miltefosine are included as reference drugs.

[4] Stauber LA. (1966): Characterization of strains of Leishmania donovani. Exp Parasitol. 18: 1-11.
[5] Concentrations are standard expressed in molar concentrations, except for natural products, drug mixtures and if the molecular weight is not known.

Chagas	***In vitro* drug screening model against *Trypanosoma cruzi***

Parasite and cell cultures

Trypanosoma cruzi, Tulahuen CL2, β galactosidase strain (nifurtimox-sensitive) is used[6]. The strain is maintained on MRC-5_{SV2} (human lung fibroblast) cells[7] in MEM medium, supplemented with 2 mM L-glutamine, 16.5 mM $NaHCO_3$, and 5% inactivated fetal calf serum. All cultures and assays are conducted at 37°C under an atmosphere of 5% $C0_2$.

Compound solutions/dilutions

Compound stock solutions are prepared in 100% DMSO at 20 mM or mg/mL[8]. The compounds are serially pre-diluted (2-fold or 4-fold) in DMSO followed by a further (intermediate) dilution in demineralized water to assure a final in-test DMSO concentration of <1%.

Drug sensitivity assays

Assays are performed in sterile 96-well microtiter plates, each well containing 10 µL of the watery compound dilutions together with 190 µL of MRC-5 cell/parasite inoculum (4.10^3 cells/well + 4.10^4 parasites/well). Parasite growth is compared to untreated-infected controls (100% growth) and non-infected controls (0% growth) after 7 days incubation at 37°C and 5% $C0_2$. Parasite burdens are assessed after adding the substrate CPRG (chlorophenolred ß-D-galactopyranoside): 50 µL/well of a stock solution containing 15.2 mg CPRG + 250 µL Nonidet in 100 mL PBS. The change in color is measured spectrophotometrically at 540 nm after 4 hours incubation at 37 °C. The results are expressed as % reduction in parasite burdens compared to control wells and an IC_{50} and IC_{90} (50% and 90% inhibitory concentrations) are calculated.

Primary screen

T. cruzi β-galactosidase strain is used. Compounds are tested at 5 or 10 concentrations depending upon the number of compounds (4-fold compound dilutions) (64 – 16 – 4 – 1 – 0.25 – 0.0625 – 0.015625 – 0.0039 – 0.000975 and 0.00024 µM or µg/mL). Nifurtimox or benznidazole can be included as the reference drugs. The test compound is classified as inactive when the IC_{50} is higher than 30 µM. When IC_{50} lies between 30 and 5 µM, the compound is regarded as being moderate active. When the IC_{50} is lower than 5 µM, the compound is classified as highly active on the condition that it also demonstrates selective action (absence of cytotoxicity). A final recommendation for activity is given after confirmatory evaluation in a secondary screening.

Secondary screen

T. cruzi β-galactosidase strain is used and IC_{50}-values are determined using an extended dose range (2-fold compound dilutions). Nifurtimox or benznidazole is included as reference drugs.

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[6] Buckner FS, Verlinde CL, La Flamme AC, Van Voorhis WC. (1996): Efficient technique for screening drugs for activity against Trypanosoma cruzi using parasites expressing beta-galactosidase. Antimicrob Agents Chemother. 40: 2592-2597.

[7] MRC-5_{SV2} cells are diploid human embryonic lung fibroblasts. A SV-40 transformed cell line is now available to obtain unlimited sub-cultivation characteristics.

[8] Concentrations are standard expressed in molar concentrations, except for natural products, drug mixtures and if the molecular weight is not known.

Sleeping sickness	***In vitro* drug screening model against *Trypanosoma brucei***

Parasite and cell cultures

The *Trypanosoma brucei brucei* Squib 427 strain (suramin-sensitive) or *Trypanosoma brucei rhodesiense* (strain STIB-900) are used for screening. The strains are maintained in Hirumi (HMI-9) medium, supplemented with 10% inactivated fetal calf serum. All cultures and assays are conducted at 37°C under an atmosphere of 5% $C0_2$.

Compound solutions/dilutions

Compound stock solutions are prepared in 100% DMSO at 20 mM or mg/mL[9]. The compounds are serially pre-diluted (2-fold or 4-fold) in DMSO followed by a further (intermediate) dilution in demineralized water to assure a final in-test DMSO concentration of <1%.

Drug sensitivity assays

Assays are performed in sterile 96-well microtiter plates, each well containing 10 µL of the compound dilutions together with 190 µL of the parasite suspension (1.5×10^4 parasites/well - *T. b. brucei* or 4×10^3 parasites/well - *T. b. rhodesiense*). Parasite growth is compared to untreated-infected (100% parasite growth) and uninfected controls (0% growth). After 3 days incubation, parasite growth is assessed fluorimetrically after addition of 50 µL resazurin per well[10]. After 6 hours (*T. b. rhodesiense*) or 24 hours (*T. b. brucei*) at 37°C, fluorescence is measured (λ_{ex} 550 nm, λ_{em} 590 nm). The results are expressed as % reduction in parasite growth/viability compared to control wells and an IC_{50} and IC_{90} (50% and 90% inhibitory concentrations) are calculated.

Primary screen

T. b. brucei Squib 427 strain and *T. b. rhodesiense* STIB-900 strain are used. Compounds are tested at 5 or 10 concentrations depending upon the number of compounds (4-fold compound dilutions) (64 – 16 – 4 – 1 – 0.25 – 0.0625 – 0.015625 – 0.0039 – 0.000975 and 0.00024 µM or µg/mL). Suramin or Melarsoprol are included as the reference drugs. The compound is classified as inactive when the IC_{50} is higher than 5 µM. When IC_{50} lies between 5 and 1 µM, the compound is regarded as being moderate active. When the IC_{50} is lower than 1 µM, the compound is classified as highly active on the condition that it also demonstrates selective action (absence of cytotoxicity). A final recommendation for activity is given after confirmatory evaluation in a secondary screening.

Secondary screen

T. b. brucei and *T. b. rhodesiense* are both included used and IC_{50}-values are determined using an extended dose range (2-fold compound dilutions). Suramin and melarsoprol are included as reference drugs.

[9] Concentrations are standard expressed in molar concentrations, except for natural products, drug mixtures and if the molecular weight is not known.

[10] Resazurin stock solution in phosphate buffer: 50 µg/mL. // Alamar Blue™ can be used as alternative: 5 µL of a 1/10 Alamar Blue™ solution is added to each well