Supporting Information

An Efficient Total Synthesis of Ammosamide B

P. V. Narasimha Reddy, Biplab Banerjee, and Mark Cushman*

Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmaceutical Sciences, and the Purdue University Center for Cancer Research, Purdue University, West Lafayette, Indiana 47907

Compound 16

Compound 17

Ammosamide B (2)

Experimental Section

General Procedures. Melting points were determined in capillary tubes using a Mel-Temp apparatus and are not corrected. Infrared spectra were obtained as films on KBr salt plates except where otherwise specified, using a Perkin-Elmer Spectrum One FT-IR spectrometer, and are baseline corrected. ^{1}H NMR spectra were obtained with $CDCl_3$ at 300 or 500 MHz, using Bruker ARX300 or Bruker Avance 500 (TXI 5 mm probe) spectrometers (residual chloroform referenced to 7.25 ppm) or DMSO-d_6 (residual DMSO referenced to 2.49 ppm and residual water in DMSO-d_6 appearing at 3.33 ppm). ^{13}C NMR spectra were recorded with $CDCl_3$ at 75 MHz or 125 MHz, using Bruker ARX300 or Bruker Avance 500 (TXI 5 mm probe) spectrometers (residual chloroform referenced to 77.0 ppm) or DMSO-d_6 (residual DMSO referenced to 39.5 ppm). Mass spectral analyses were performed at the Purdue University Campus-Wide Mass Spectrometry Center. ESIMS was performed using a FinniganMAT LCQ Classic mass spectrometer system. EI/CIMS was performed using a Hewlett-Packard Engine or GCQ FinniganMAT mass spectrometer system. Analytical thin-layer chromatography was carried out on Baker-flex silica gel IB2-F plastic-backed TLC plates. Preparative thin-layer chromatography was performed on Analtech silica gel 1500 μm glass plates. Compounds were visualized with both short- and long-wavelength UV light. Silica gel flash chromatography was accomplished using 230-400 mesh silica gel. All yields reported refer to yields of isolated compounds. Unless otherwise stated, chemicals and solvents were of reagent grade and used as obtained from commercial sources without further purification.

1,3-Diamino-4,6-dinitrobenzene (9). Ammonia was bubbled slowly into a well-stirred, clear yellow solution of 1,3-dichloro-4,6-dinitrobenzene (**8**, 3.0 g, 12.6 mmol) in ethylene glycol (20 mL) at 140 ^{o}C. The color of the solution changed within 30 min from orange to deep red. One hour after the start of the reaction an orange, crystalline precipitate started to form. Heating was continued for an additional 2 h as a slow steam of ammonia gas was bubbled through the reaction mixture. After cooling the reaction mixture, the product was filtered washed with boiling water and boiling ethanol, and dried to afford the orange-brown product **9** (2.2 g, 88%): mp 298 °C (lit[11] mp 300 $^{\circ}$C).

Di-*tert*-butyl (4,6-dinitro-1,3-phenylene)bis(*tert*-butoxycarbonylcarbamate) (10). Compound **9** (2.1 g, 10.6 mmol) was dissolved in anhydrous DMF (12 mL). DMAP (0.646 g,

5.3 mmol) and *tert*-butyl dicarbonate (11.5 g, 53.0 mmol) were added and the reaction mixture stirred at room temperature for 12 h. The reaction mixture was poured into water and extracted with diethyl ether (2 x 50 mL), the combined ether layer was dried over Na_2SO_4, concentrated and purified by silica gel flash column chromatography (hexane-EtOAc 9.5:0.5) to afford the product **10** (5.07 g, 80%) as a light yellow solid along with some of the corresponding di nitro tri-Boc side product (0.528 g, 10%).

Compound (10): mp 133-135 °C. IR (KBr) 2982, 1811, 1731, 1615, 1596, 1539, 1151, 1100, 847, 675, 576 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.72 (s, 1 H), 7.26 (s, 1 H), 1.35 (s, 36 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 148.7, 143.7, 137.3, 134.5, 121.8, 84.8, 27.5; ESIMS (*m/z,* relative intensity) 621 (MNa^+, 100), 521 (93), 429 (26), 365 (12); HRMS calcd for $C_{26}H_{38}N_4O_{12}Na$, 621.2384, found 621.2394.

Tri-Boc Side Product [*tert*-butyl tert-butoxycarbonyl(5-((tert-butoxycarbonyl)amino)-2,4-dinitrophenyl)carbamate]: mp 154-15 °C. IR (KBr) 3326, 2983, 1733, 1702, 1594, 1369, 1239, 1146, 960, 836, 767, 636, 545 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 9.96 (s, 1 H), 9.05 (s, 1 H), 8.73 (s, 1 H), 1.54 (s, 9 H), 1.42 (s, 18 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.0, 149.6, 140.1, 139.7, 137.7, 132.7, 124.0, 122.5, 84.7, 83.5, 28.0, 27.7; ESIMS (*m/z,* relative intensity) 521 (MNa^+, 100), 429 (11), 365 (8); HRMS Calcd for $C_{21}H_{30}N_4O_{10}$ 521.1860, found 521.1855.

Di-*tert*-butyl (4,6-dinitro-1,3-phenylene)dicarbamate (11). Tetracarbamate **10** (5 g, 8.36 mmol) was dissolved in CH_2Cl_2 (25 mL) and TFA (3.81 g, 33.4 mmol) was added dropwise at 0 °C. The reaction mixture stirred at the same temperature for 4 h. The solvent was removed on a rotary evaporator, and the residue was washed with saturated aqueous $NaHCO_3$ (2 x 25 mL), extracted with EtOAc, concentrated and purified by silica gel flash column chromatography (hexane-EtOAc 9.5:0.5) to provide the product as light yellow solid (2.9 g, 90%): mp 177-179 °C. IR (KBr) 3344, 1746, 1583, 1456, 1338, 1140, 837, 744, 616 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 10.07 (s, 1 H), 9.80 (s, 1 H), 9.21 (s, 1 H), 1.55 (s, 18 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.0, 141.7, 128.3, 126.1, 107.8, 83.1, 28.0; ESIMS (*m/z,* relative intensity) 421 (MNa^+, 100), 366 (7), 365 (38) 309 (13); HRMS Calcd for $C_{16}H_{22}N_4O_8Na$ 421.1335, found 421.1333.

Di-*tert*-butyl (4,6-diamino-1,3-phenylene)dicarbamate (12). Dinitro compound **11** (2.8 g, 7.03 mmol) was subjected to catalytic hydrogenation over 10% Pd/C (50 mg) at 30 psi in EtOAc (40 mL) for 12 h to yield compound **12** (2 g, 85%) as a light brown solid: mp 170-172 °C. IR (KBr) 3213, 2984, 1703, 1629, 1539, 1367, 1239, 1163, 881, 820, 705, 592 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.96 (s, 1 H), 6.10 (s, 1 H), 6.02 (br s, 1 H), 3.67 (br s 4 H), 1.47 (s, 18 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.5, 140.6, 115.2, 80.2, 28.3; ESIMS (*m/z,* relative intensity) 361 (MNa^+, 100), 338 (23), 301 (21), 183 (34), 227 (19); HRMS Calcd for $C_{16}H_{26}N_4O_4Na$ 361.1852, found 361.1858.

Methyl 6,8-Bis(*tert*-butoxycarbonylamino)-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinoline-4-carboxylate (14). A solution of (*E*)-dimethyl 4-oxopent-2-enedioate (**13**, 0.980 g, 5.28 mmol) in chloroform (20 mL) was added to a solution of diamine **12** (1.5 g, 4.4 mmol) in chloroform (10 mL) and the reaction mixture was stirred for 30 min. A catalytic amount of PTSA (0.084 g, 0.44 mmol) and $Cu(OAc)_2$ (0.080 g, 0.44 mmol) were added and the solution was heated at reflux for 8 h. The reaction mixture was washed 3 times with $NaHCO_3$ (15 mL). The organic layer was separated and dried over Na_2SO_4 and purified by silica gel column chromatography using hexane-EtOAc 7:3 to produce the product **14** (1.0 g, 50%) as a reddish solid: mp 145-147 °C. IR (KBr) 3232, 2977, 1734, 1704, 1638, 1528, 1244, 1161, 1062, 743, 553 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 9.44 (s, 1 H), 8.67 (s, 1 H), 8.35 (s, 1 H), 7.92 (s, 1 H), 7.00 (s, 1 H), 4.07 (s, 3 H), 1.55 (s, 9 H), 1.51 (s, 9 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.6, 165.1, 152.5, 146.9, 135.1, 132.8, 123.5, 121.0, 119.6, 115.6, 110.7, 82.7, 81.5, 53.2, 28.2, 28.08; ESIMS (*m/z,* relative intensity) 459 (MH^+, 100); HRMS calcd for $C_{22}H_{26}N_4O_7$ 459.1880, found 459.1884.

Methyl 6,8-Bis(*tert*-butoxycarbonylamino)-7-chloro-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinoline-4-carboxylate (15). Pyrroloquinoline **14** (0.7 g, 1.52 mmol) was subjected to chlorination with NCS (0.264 g, 1.98 mmol) in anhydrous DMF (4 mL) at 60 ˚C for 30 min. The reaction mixture poured into water extracted with EtOAc (3 x 30 mL). The organic layer was separated and dried over Na_2SO_4, concentrated, and purified by silica gel column chromatography using hexane-EtOAc 7:3 to provide the product **15** (0.563 g, 75%) as a yellow solid: mp 178-180 ˚C. IR (KBr) 3412, 2981, 1734, 1690, 1495, 1273, 1157, 1074, 760, 663 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 9.47 (s, 1 H), 8.64 (s, 1 H), 7.47 (s, 1 H), 7.10 (s, 1 H), 4.06 (s, 3

H), 1.55 (s, 9 H), 1.52 (s, 9 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.8, 165.0, 152.6, 152.3, 149.4, 138.5, 135.1, 129.1, 127.2, 122.1, 121.8, 119.3, 117.9, 83.5, 81.5, 53.2, 28.1, 28.09; **:** ESIMS (*m/z,* relative intensity) 515 (MNa^+, 100), 517 (MNa^+, 33 chlorine isotope), 459 (8). HRMS calcd for $C_{22}H_{25}ClN_4O_7Na$ 515.1309, found 515.1312.

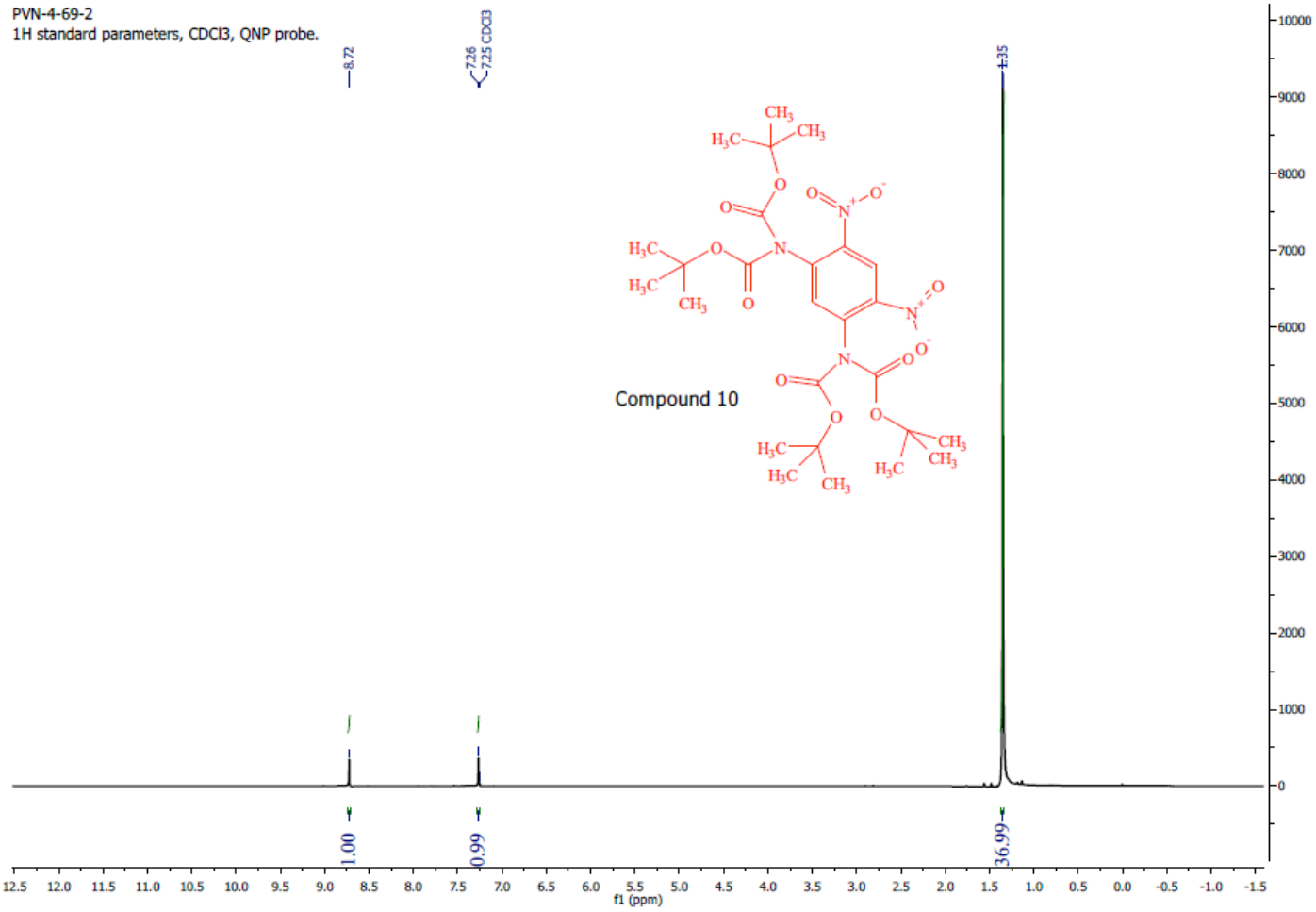
Methyl 6,8-Diamino-7-chloro-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinoline-4-carboxylate (16). Trifluoroacetic acid (0.926 g, 8.13 mmol) was added to a solution of compound **15** (0.4 g, 0.81 mmol) in CH_2Cl_2 (8 mL) and the mixture was stirred at room temperature for 6 h. The reaction mixture was concentrated, and washed with aq. $NaHCO_3$ and extracted with EtOAc (5 x 40 mL). The combined organic layer was dried over Na_2SO_4, concentrated, and purified by basic alumina column chromatography using $CHCl_3$-MeOH 9.2:0.8 to generate the product **16** (0.142 g, 65%) as a purple solid: mp 220-222 °C. IR (KBr) 3341, 3217, 2954, 1711, 1648, 1452, 1265, 1170, 662, 547 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.18 (s, 1 H), 8.33 (s, 1 H), 6.36 (s, 2 H), 6.09 (s, 2 H), 3.93 (s, 3 H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 165.4, 164.7, 141.4, 140.0, 132.2, 132.1, 131.3, 119.9, 118.7, 104.7, 104.6, 52.4; ESIMS (*m/z,* relative intensity) 291 [$(M\text{-}H^+)^-$, 54], 293 [$(M\text{-}H^+)^-$, 18 chlorine isotope], 317 (100). HRMS calcd for $C_{12}H_9ClN_4O_3Na$ 315.0465, found 315.0471.

Methyl 6,8-Diamino-7-chloro-1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-*de*]quinoline-4-carboxylate (17). NaH (10 mg, 0.20 mmol) followed by CH_3I (28 mg, 0.2 mmol) were added to a stirred solution of **16** (0.050 g, 0.171 mmol) in DMF (2 mL). The mixture was stirred at room temperature for 30 min, quenched with saturated aqueous NH_4Cl, and extracted with EtOAc (4 x 30 mL). The combined organic layer was dried over Na_2SO_4, concentrated, and purified by silica gel column chromatography using $CHCl_3$-MeOH 9.4:0.6 to furnish the product **17** (0.036 g, 70%) as a purple solid: IR (KBr) 3450, 3365, 3252, 2947, 2346, 1642, 1610, 1494, 1301, 825, 736, 674, 487 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.36 (s, 1 H), 6.34 (s, 2 H), 6.17 (s, 2 H), 3.95 (s, 3 H), 3.59 (s, 3 H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 165.2, 163.6, 142.6, 140.3, 133.1, 131.9, 130.2, 119.0, 118.5.0, 106.5, 105.4, 52.5, 28.6; EIMS (*m/z,* relative intensity) 329 (MNa^+ , 21) 635 (2M+Na, 100), 637 (2M+Na, 34 chlorine isotope).

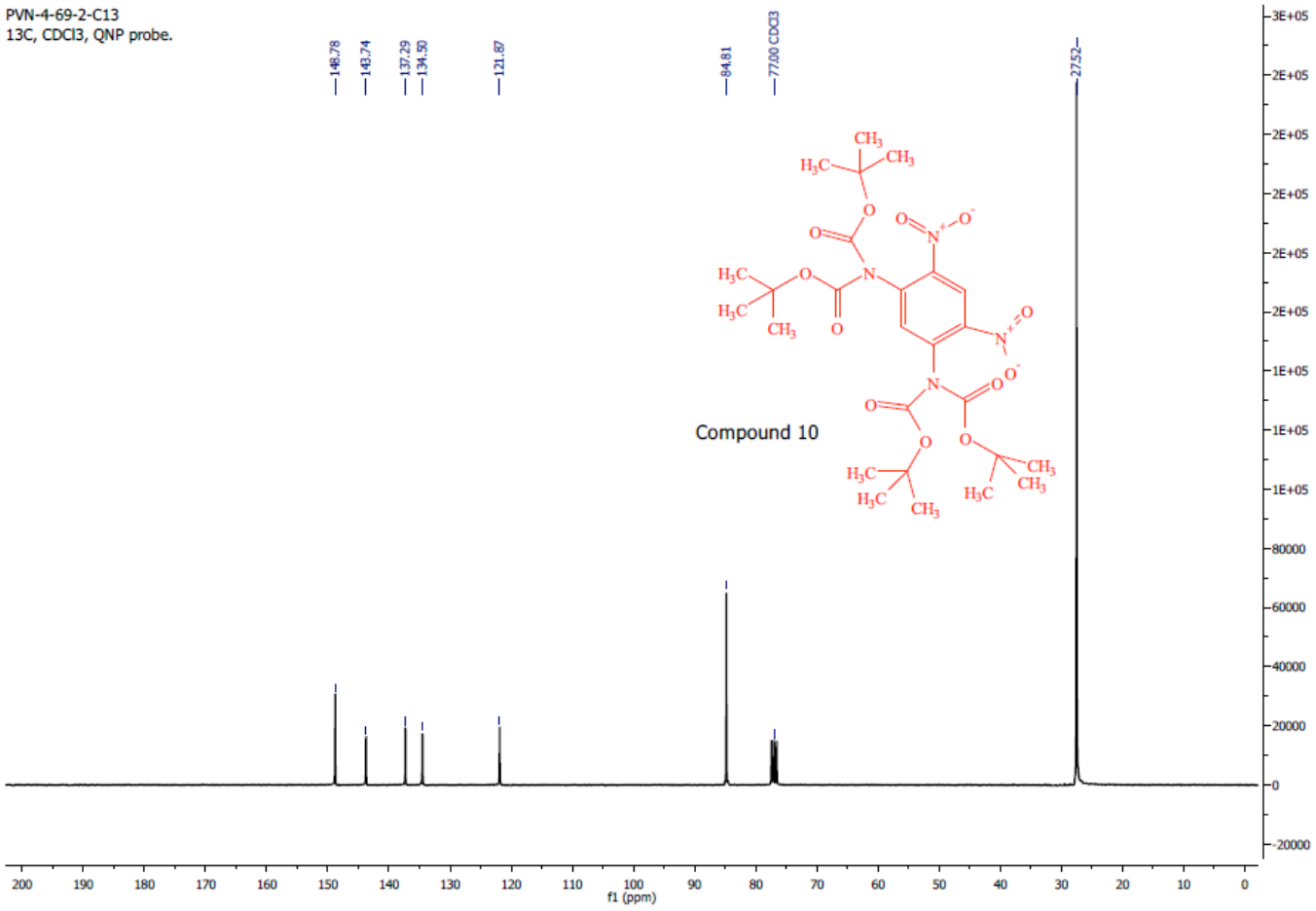
6,8-Diamino-7-chloro-1-methyl-2-oxo-1,2-dihydropyrrolo[4,3,2-de]quinoline-4-carboxamide (Ammosamide B, 2). Compound **17** (0.015 g, 0.04 mmol) was dissolved in THF (10 mL) and a 30% NH_4OH solution (1 mL) was added. The reaction mixture stirred at room

temperature for 24 h, by which time all of the ester had been converted to amide. THF was removed on a rotary evaporator, and the product was purified by silica gel column chromatography using $CHCl_3$-MeOH 9.0:1.0 to yield the product **2** (0.010 g, 80%) as a purple solid: IR (KBr) 3365, 3177, 2929, 2820, 1661, 1393, 1153, 86, 587, 466 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.88 (s, 1 H), 8.33 (s, 1 H), 7.63 (s, 1 H), 6.70 (s, 1 H), 6.15 (s, 1 H), 3.58 (s, 3 H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 166.1, 163.9, 144.7, 140.5, 132.4, 130.8, 130.6, 119.0, 115.3, 106.2, 104.5, 28.6 ESIMS (*m/z,* relative intensity) 330 (MK^+, 63), 330 (MK^+, 21 chlorine isotope) 314 (MNa^+, 100), 316 (MNa^+, 31 chlorine isotope), 332 (38); HRMS Calcd for $C_{12}H_{10}ClN_5O_2Na$ 314.0421, found 314.0424.

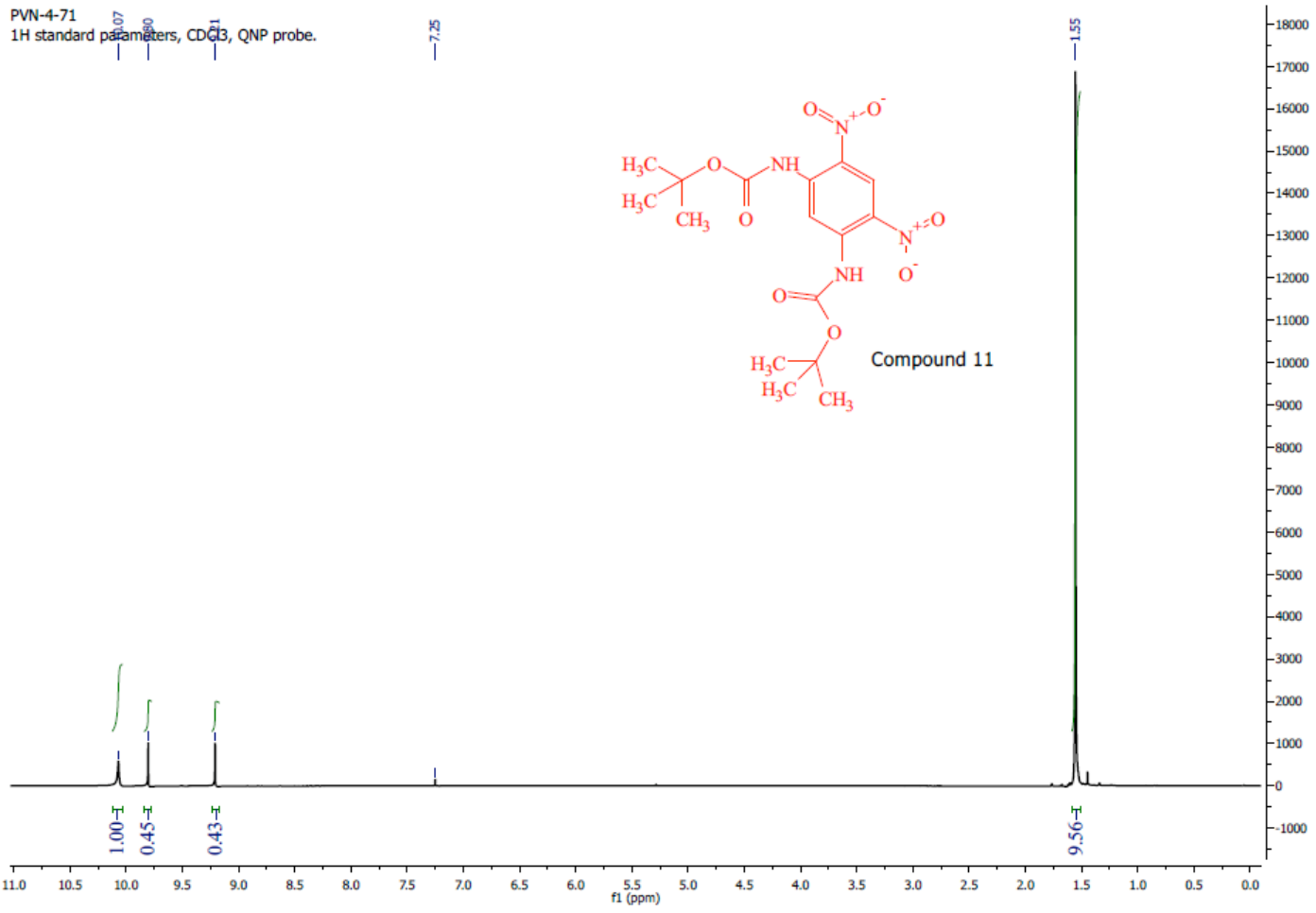
NMR Spectra



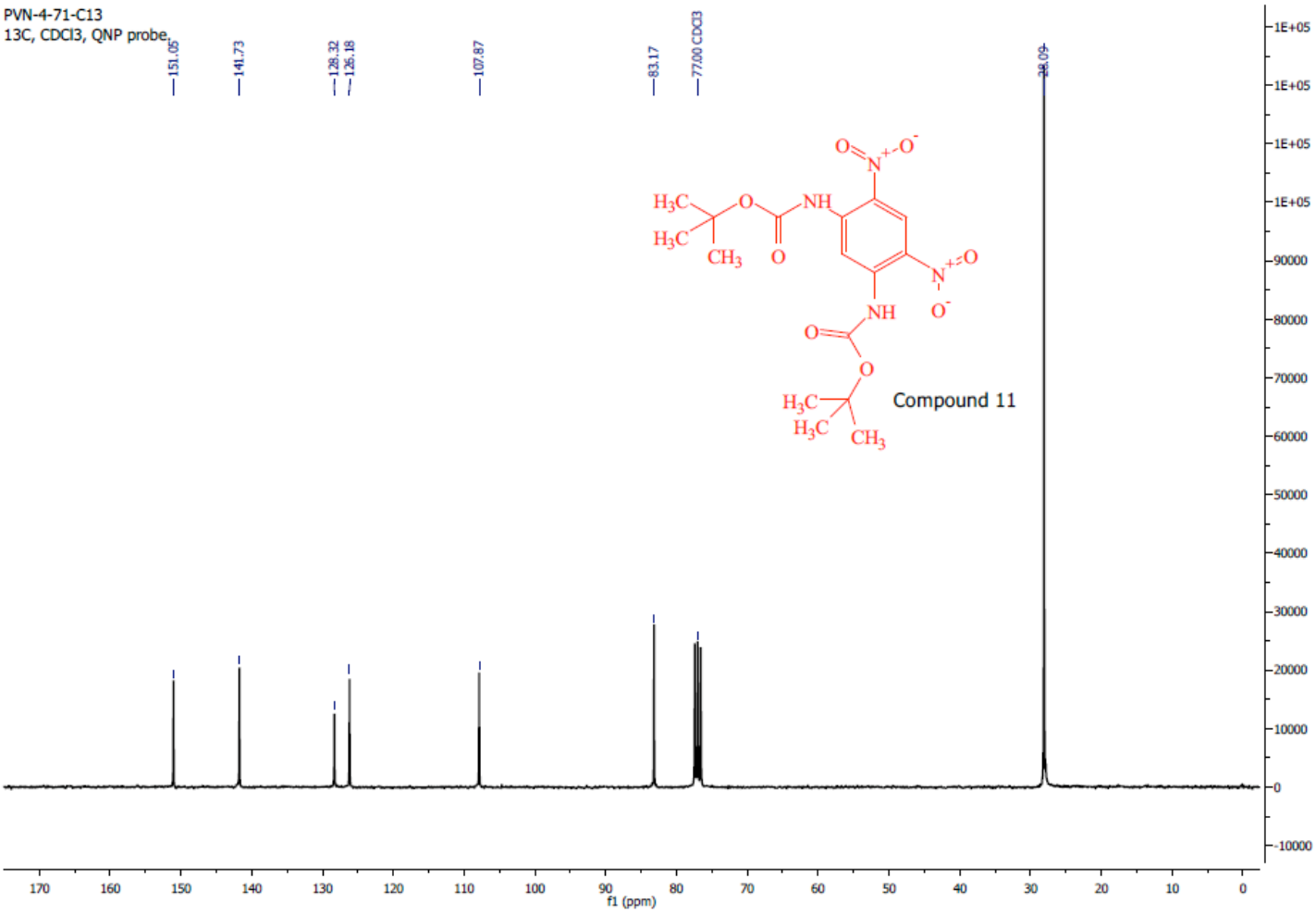
^{1}H NMR (300 MHz, $CDCl_3$) of Compound 10



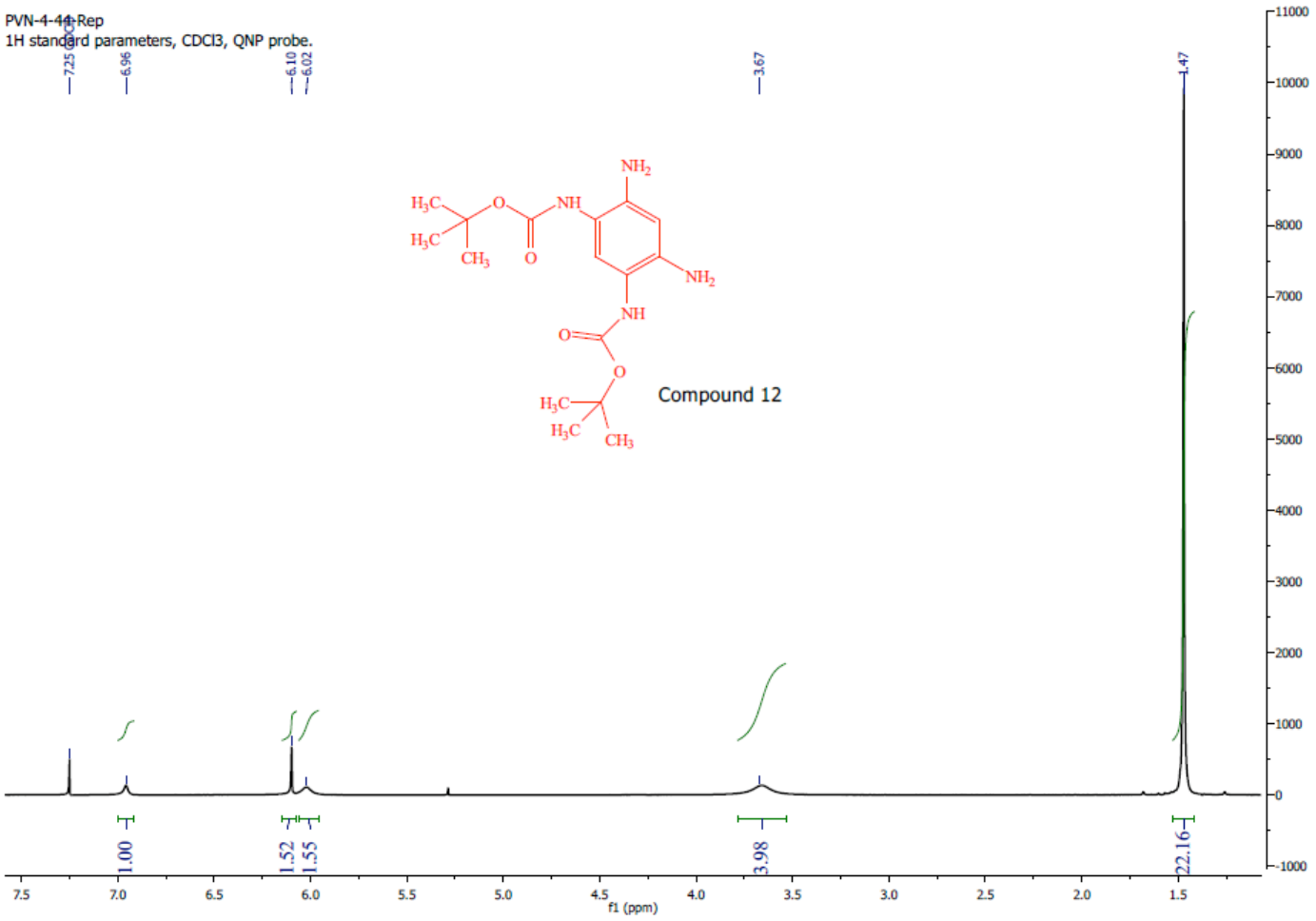
^{13}C NMR (75 MHz, $CDCl_3$) of Compound 10



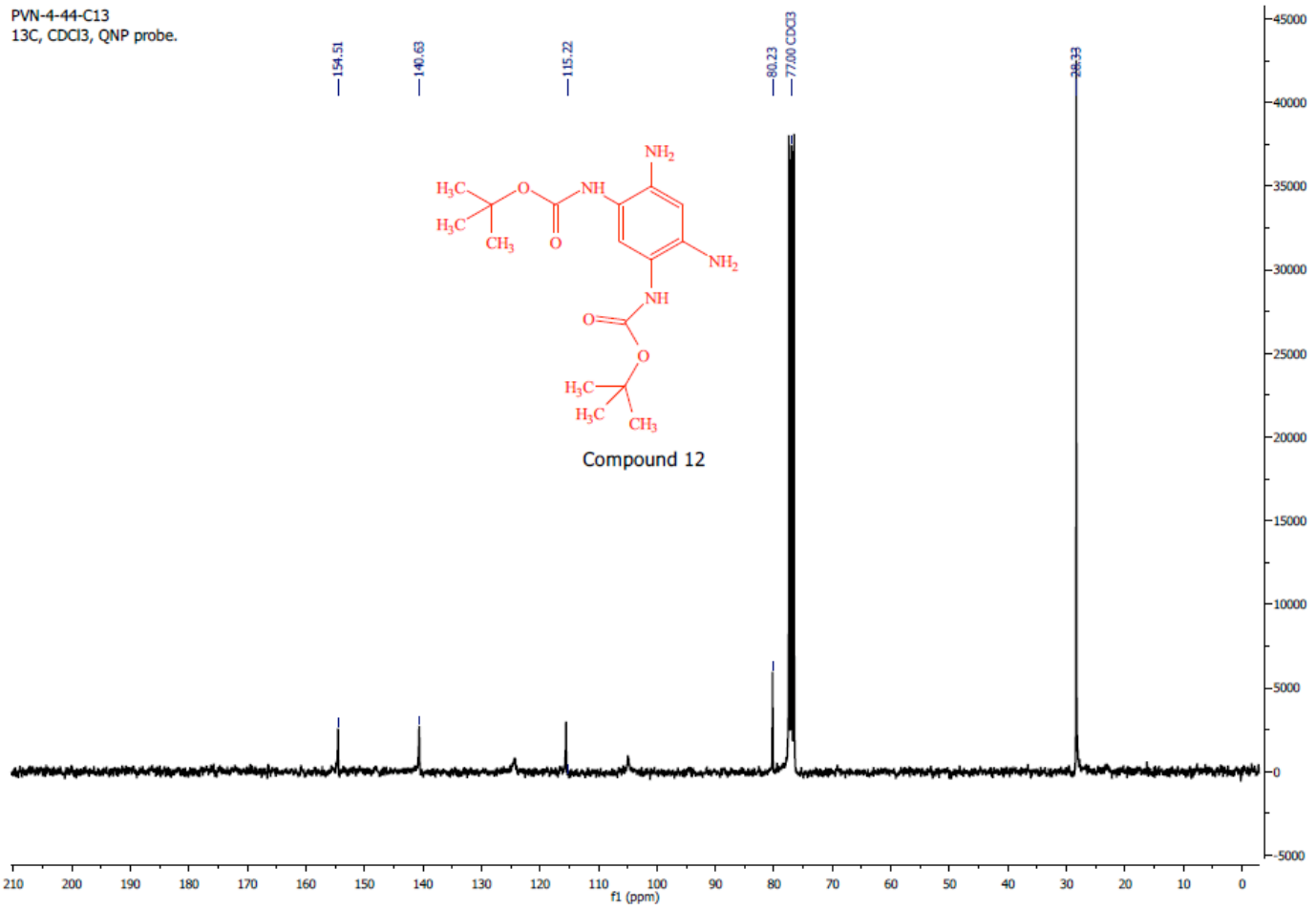
^{1}H NMR (300 MHz, $CDCl_3$) of Compound 11



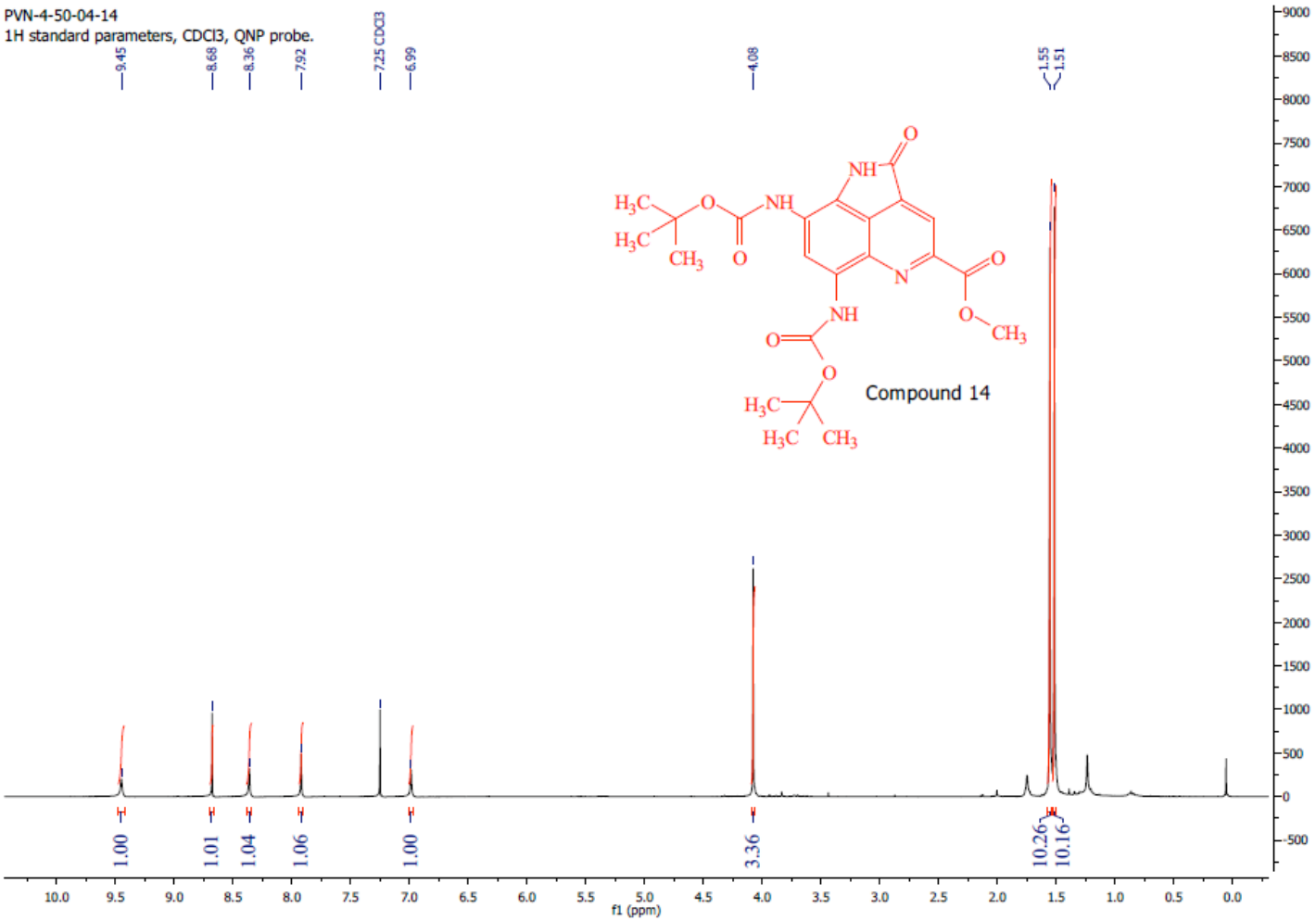
^{13}C NMR (75 MHz, $CDCl_3$) of Compound 11



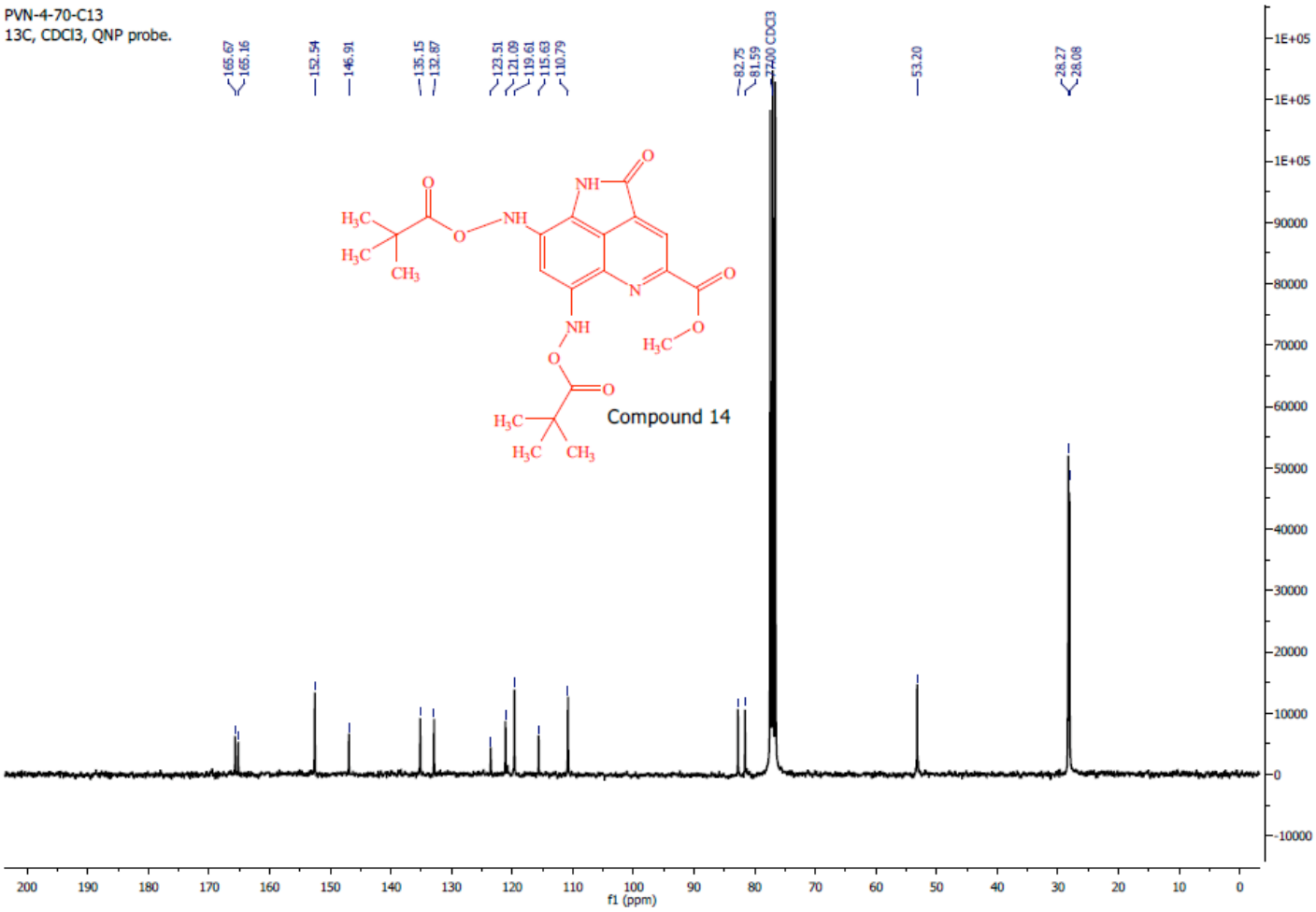
^{1}H NMR (300 M Hz, CDCl3) of Compound 12



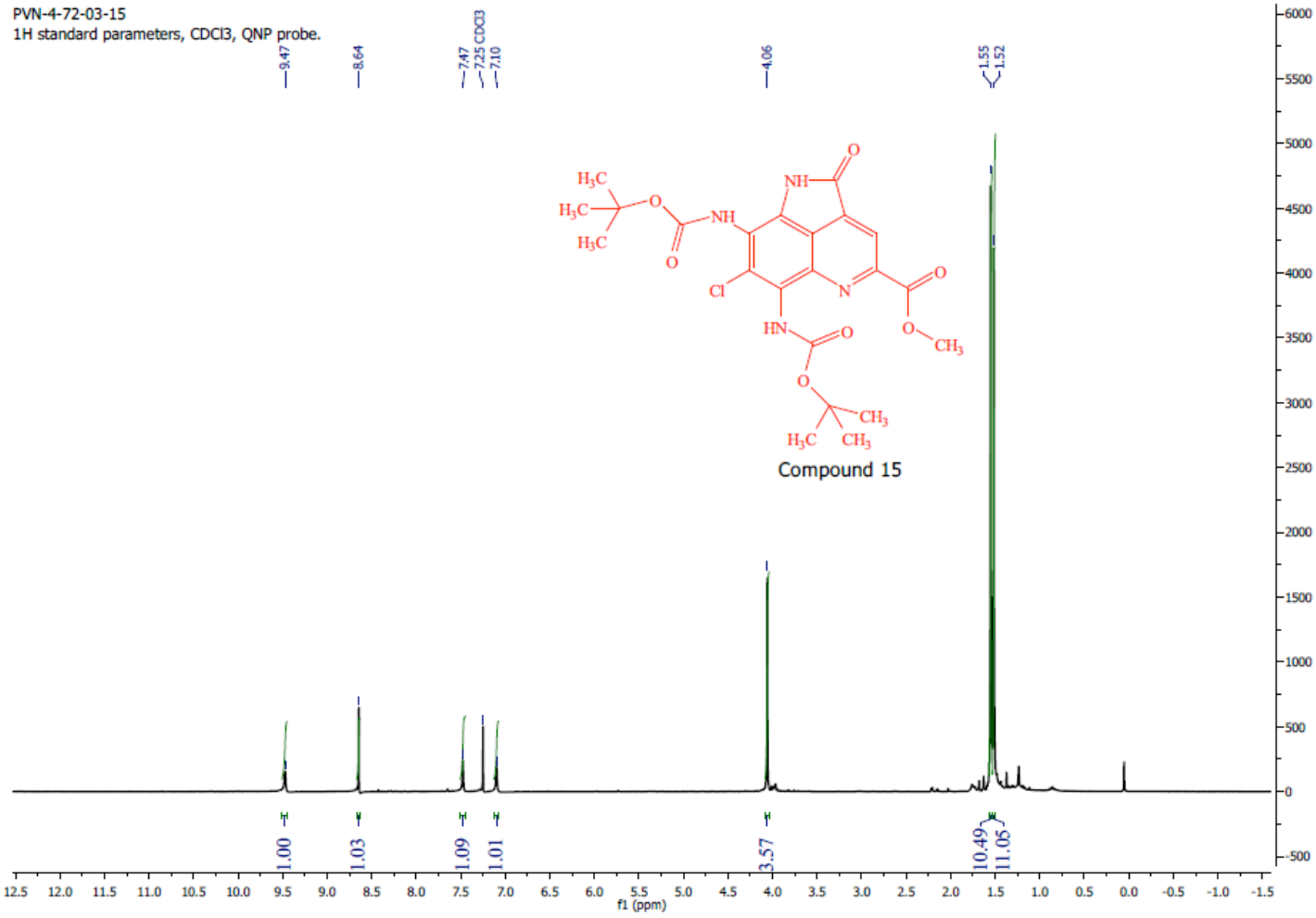
^{13}C NMR (75 MHz, $CDCl_3$) of Compound 12



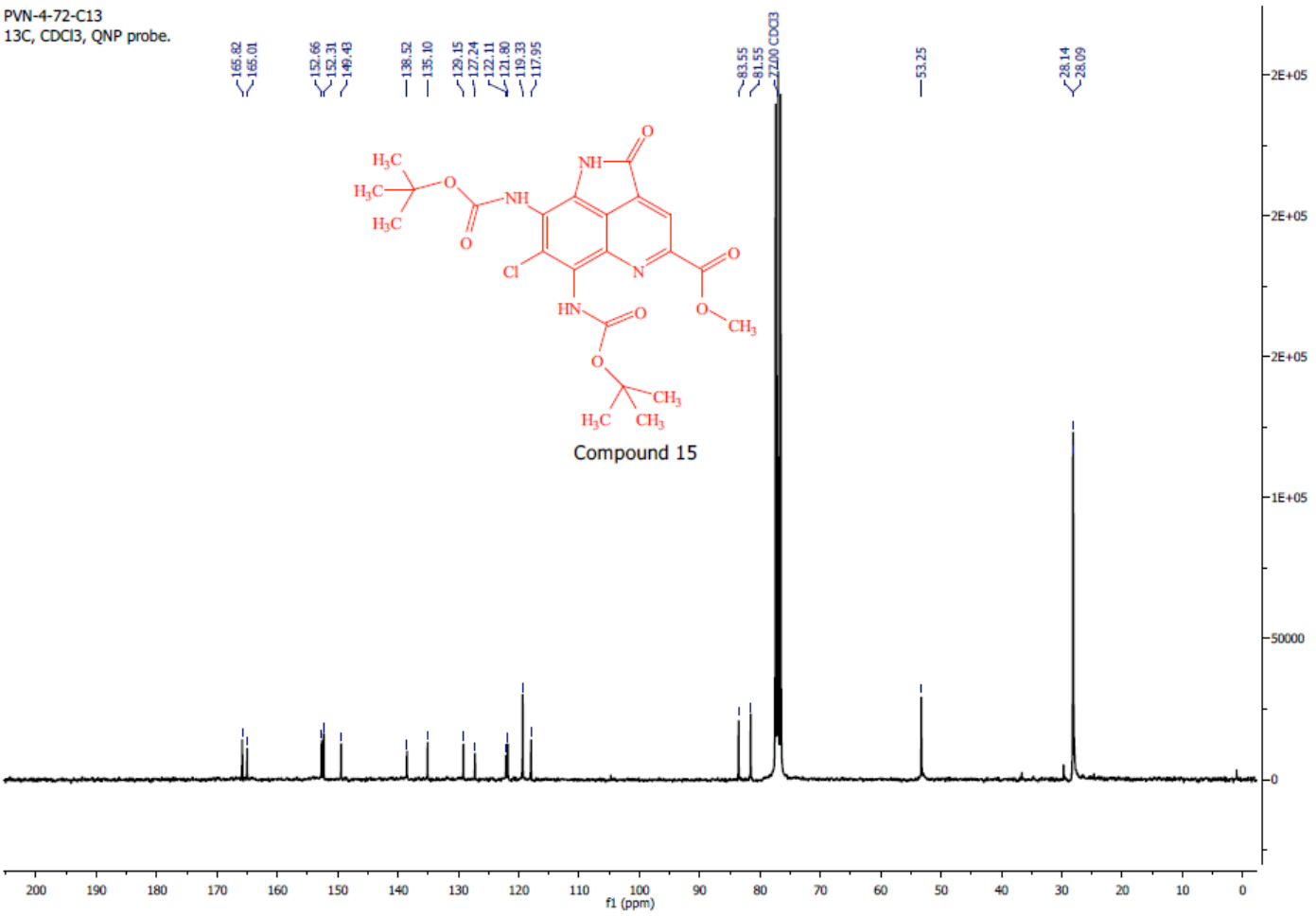
^{1}H NMR (300 MHz, $CDCl_3$) of Compound 14



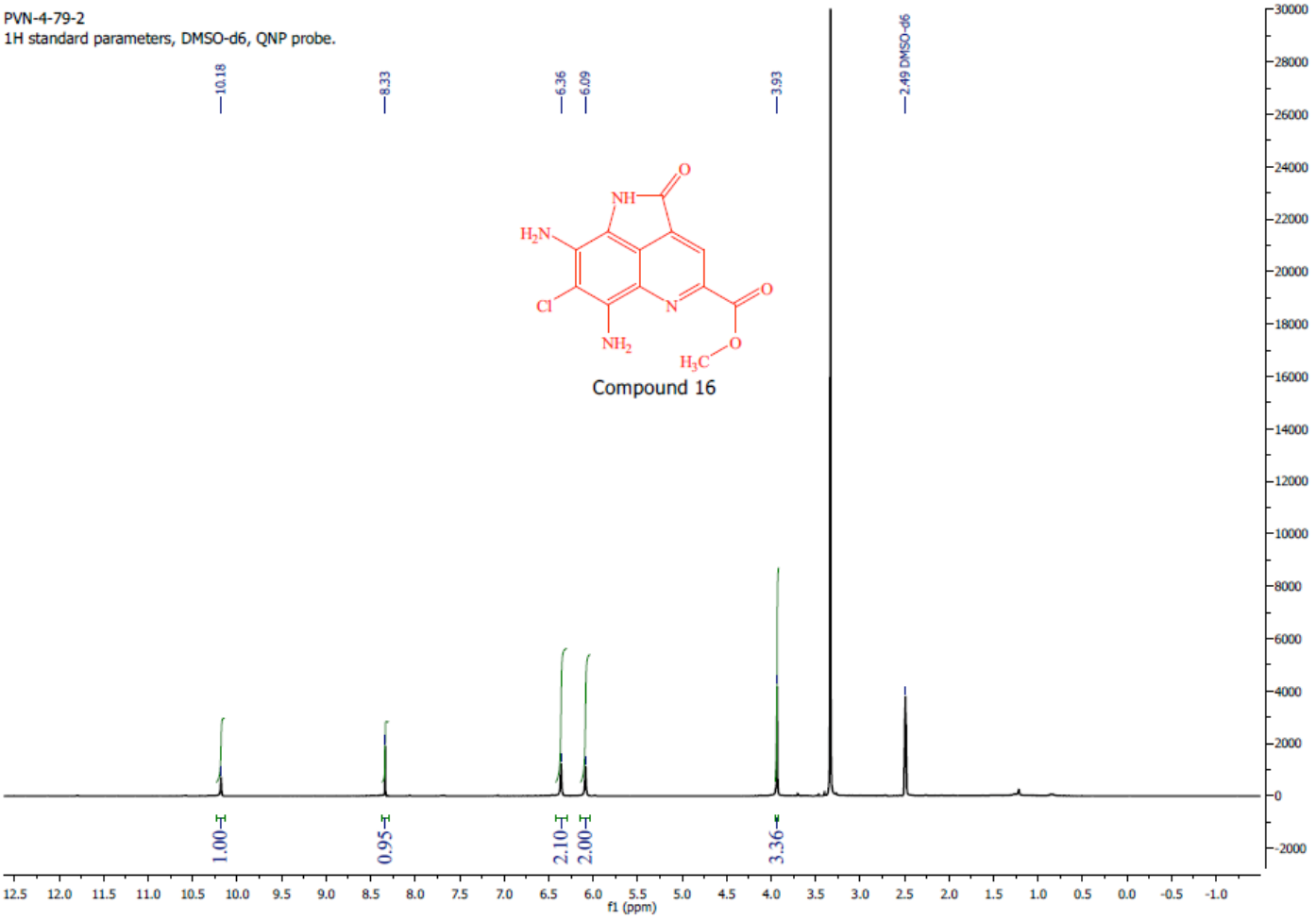
^{13}C NMR (75 MHz, $CDCl_3$) of Compound 14



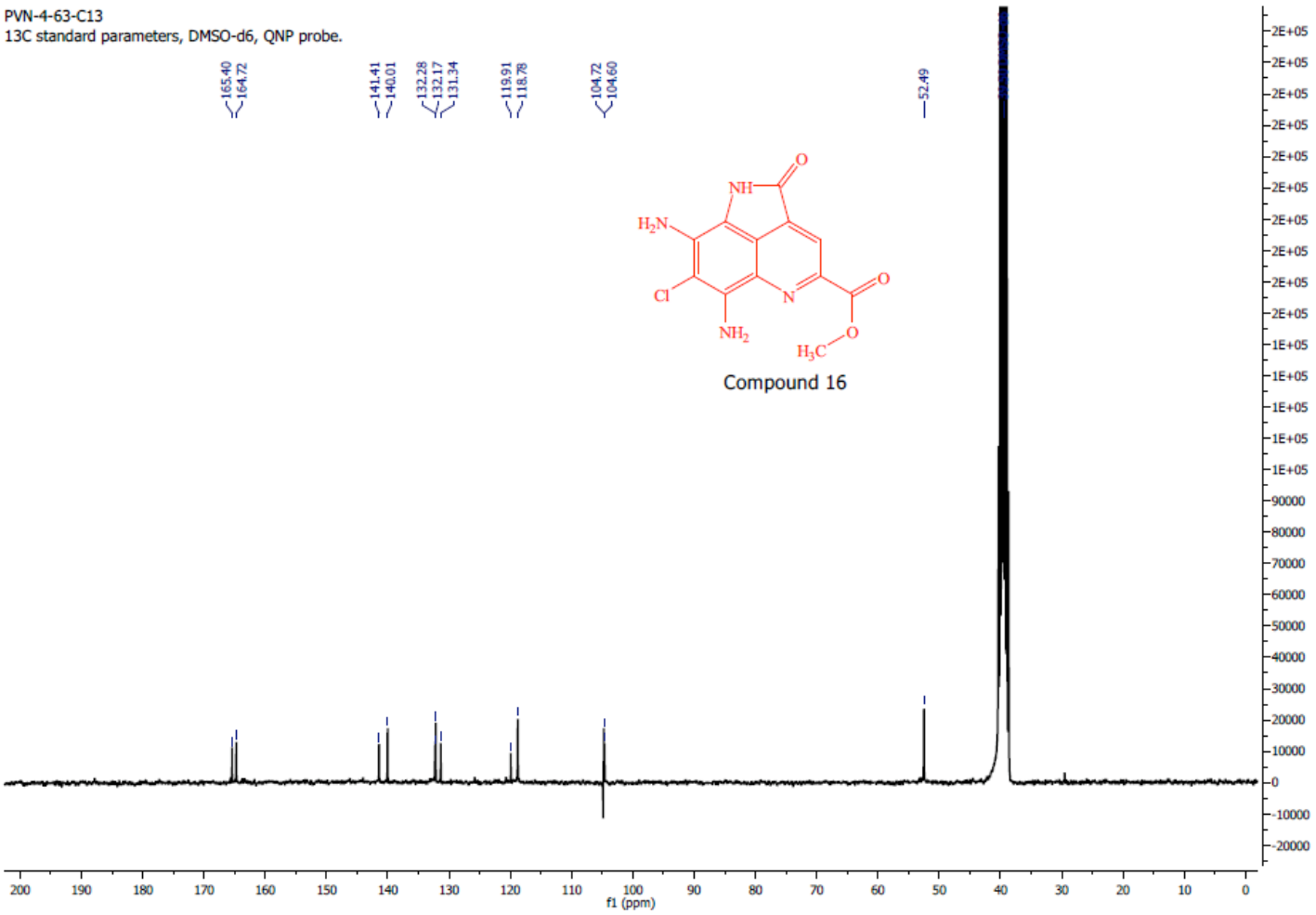
^{1}H NMR (300 MHz, $CDCl_3$) of Compound 15



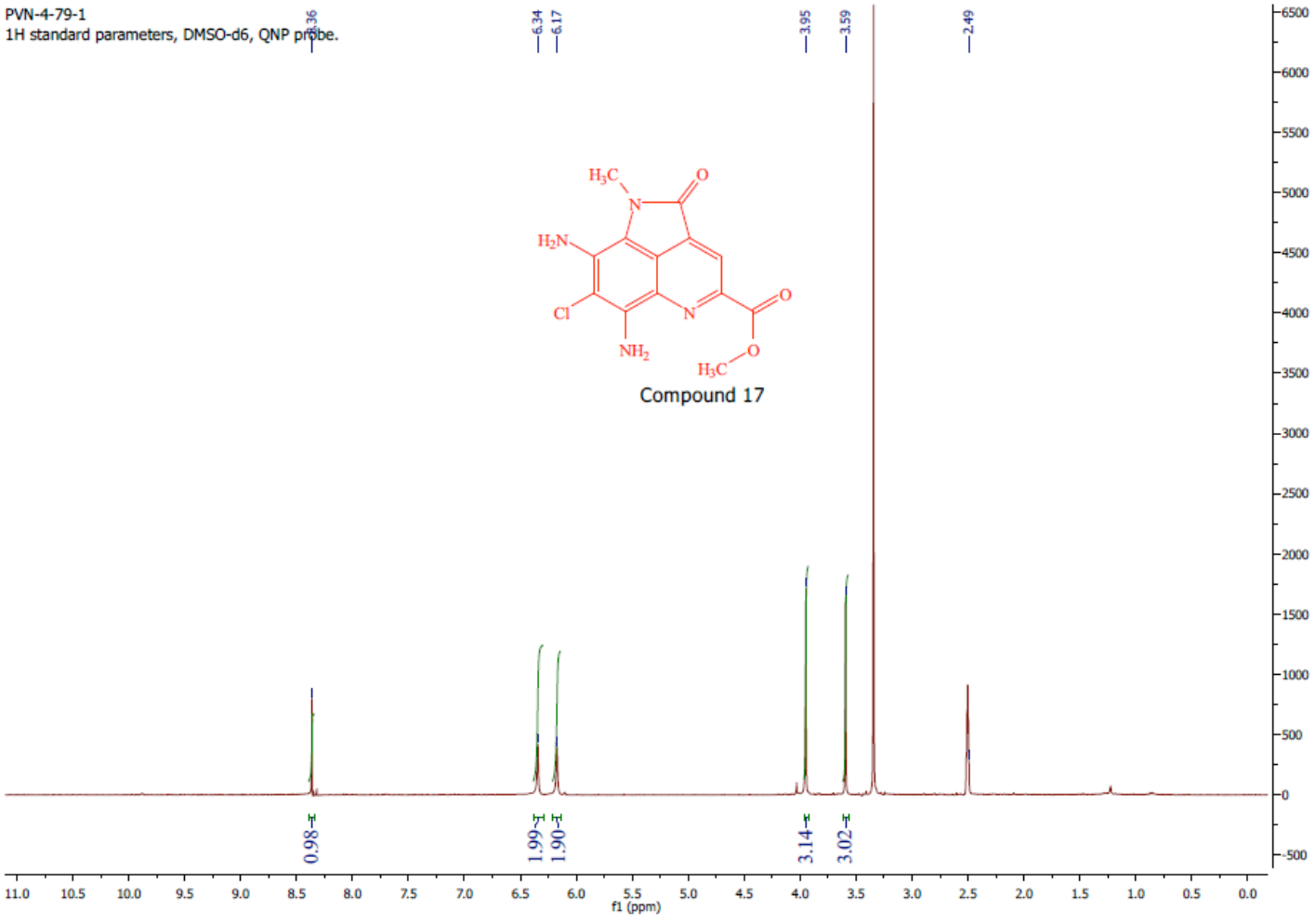
^{13}C NMR (75 MHz, $CDCl_3$) of Compound 15



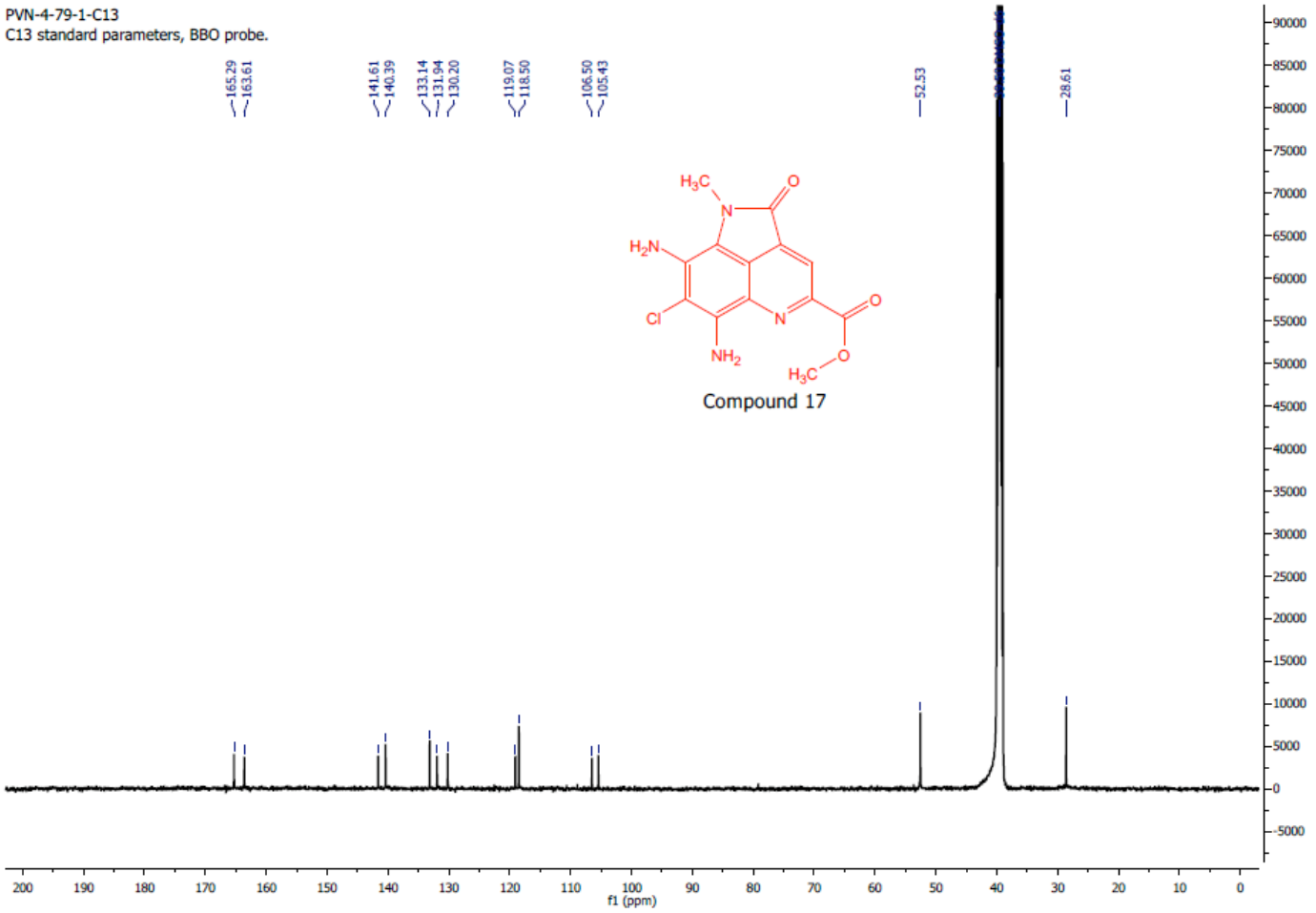
^{1}H NMR (300 MHz, DMSO-*d*6) of Compound 16



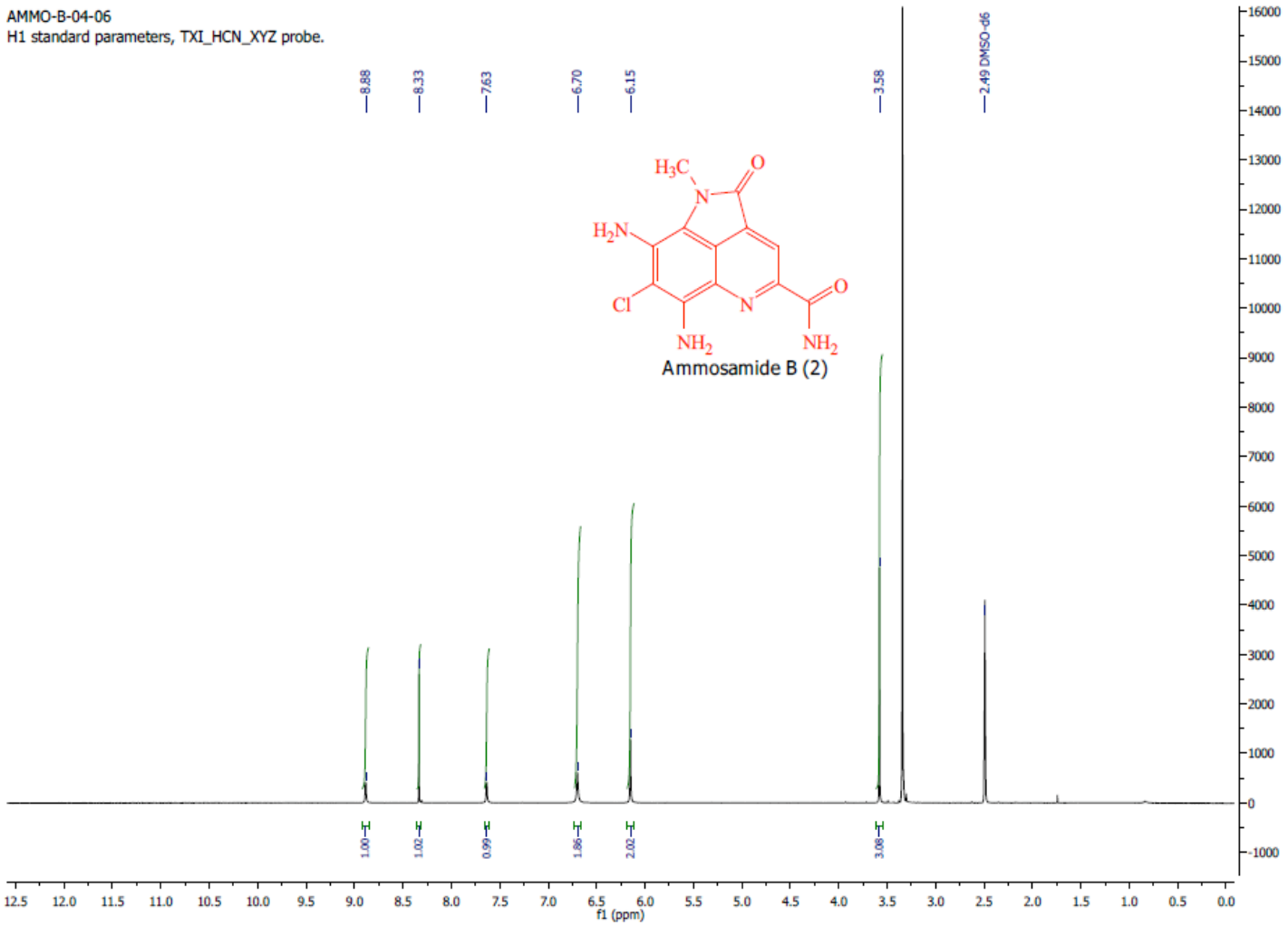
^{13}C NMR (75 MHz, DMSO-*d*6) of Compound 16



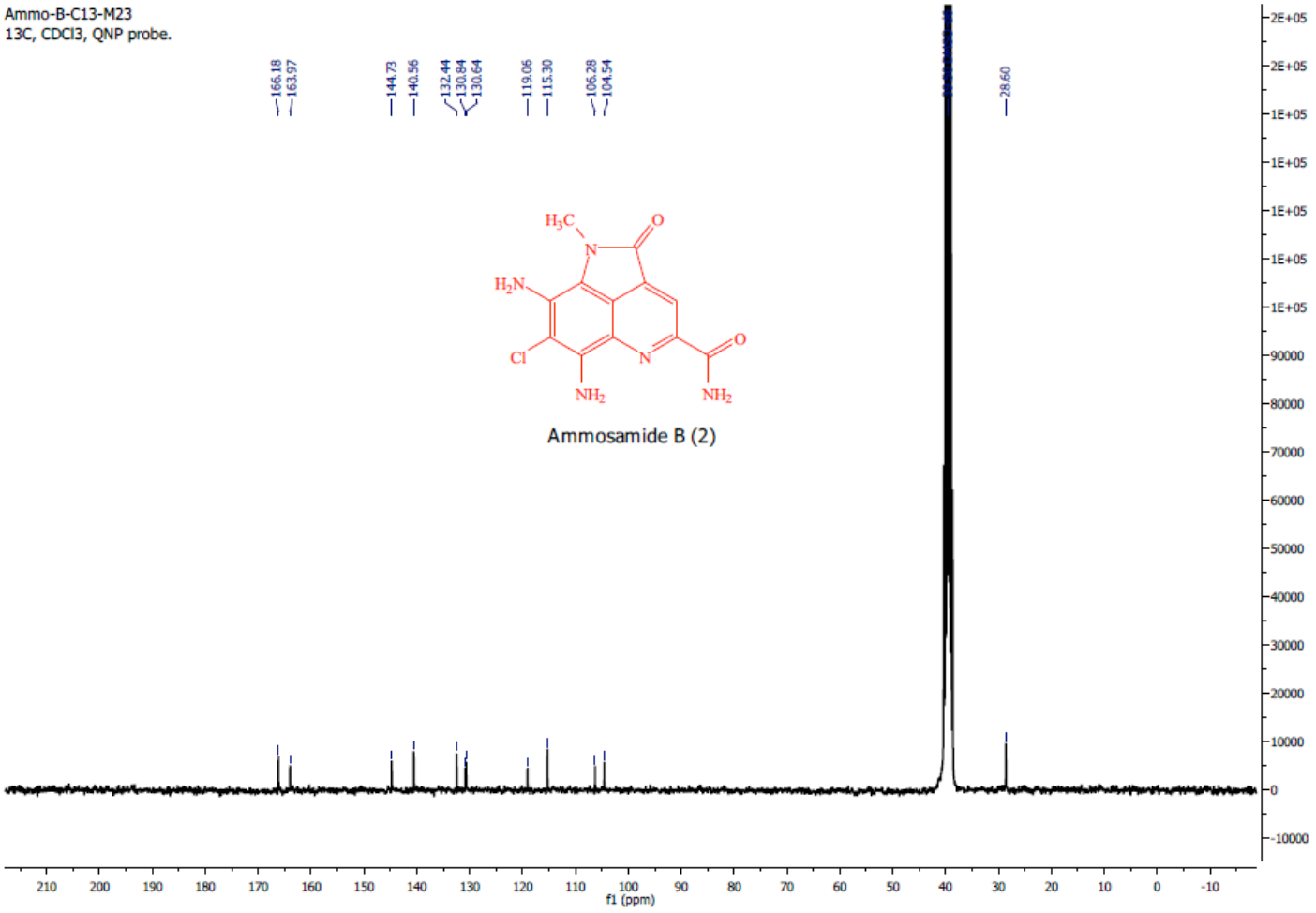
^{1}H NMR (300 MHz, DMSO-*d*6) of Compound 17



^{13}C NMR (125 MHz, DMSO-*d*6) of Compound 17



^{1}H NMR (500 MHz, DMSO-*d*6) of Ammosamide B (2)



^{13}C NMR (75 MHz, DMSO-*d*6) of Ammosamide B (2)