Supporting Information

Quinazolin-4-piperidine sulfamides are specific inhibitors of human NPP1 and prevent pathologic mineralization of valve interstitial cells

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General methods, synthetic procedures, ^{1}H NMR spectra for known compounds and full characterization of all new compounds

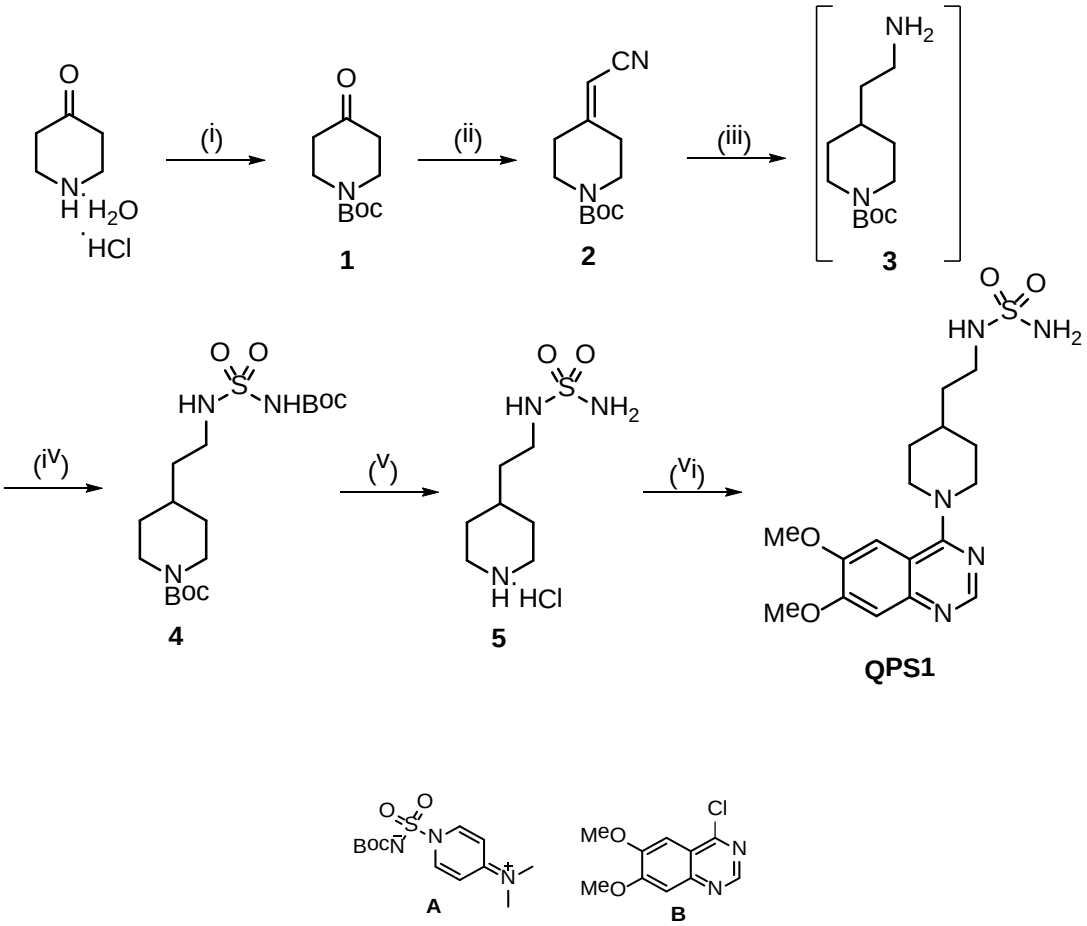
Table of contents

General information

The following includes experimental procedures and spectroscopic information for the new compounds prepared. Thin-layer chromatography (TLC) analysis of reaction mixtures was performed using Silicyle silica gel 60 Å F254 TLC plates. Flash column chromatography was carried out on Silicycle Silica Gel 60 Å, 230 × 400 mesh. Nuclear magnetic resonance (NMR) spectra were recorded using Agilent DD2 500. ^{1}H and ^{13}C chemical shifts are reported in ppm downfield of tetramethylsilane and referenced to tetramethylsilane (δ = 0 ppm) or residual chloroform peak (δ = 7.26 ppm), methanol peak (δ = 3.31 ppm) or dimethyl sulfoxide peak (δ = 2.50 ppm). Coupling constants (*J*) are measured in hertz (Hz). Multiplicities are reported using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad resonance. High-resolution mass spectra were obtained on a LC/MS-TOF Agilent 6210 using electrospray ionization (ESI). Infrared spectra were recorded using a Thermo Scientific Nicolet 380 FTIR spectrometer. Melting points were recorded on a Stanford Research Systems OptiMelt capillary melting point apparatus and are uncorrected. All the synthesized compounds (**1-10**, **QPS1-2**) have purity >95% (estimated by ^{1}H NMR).

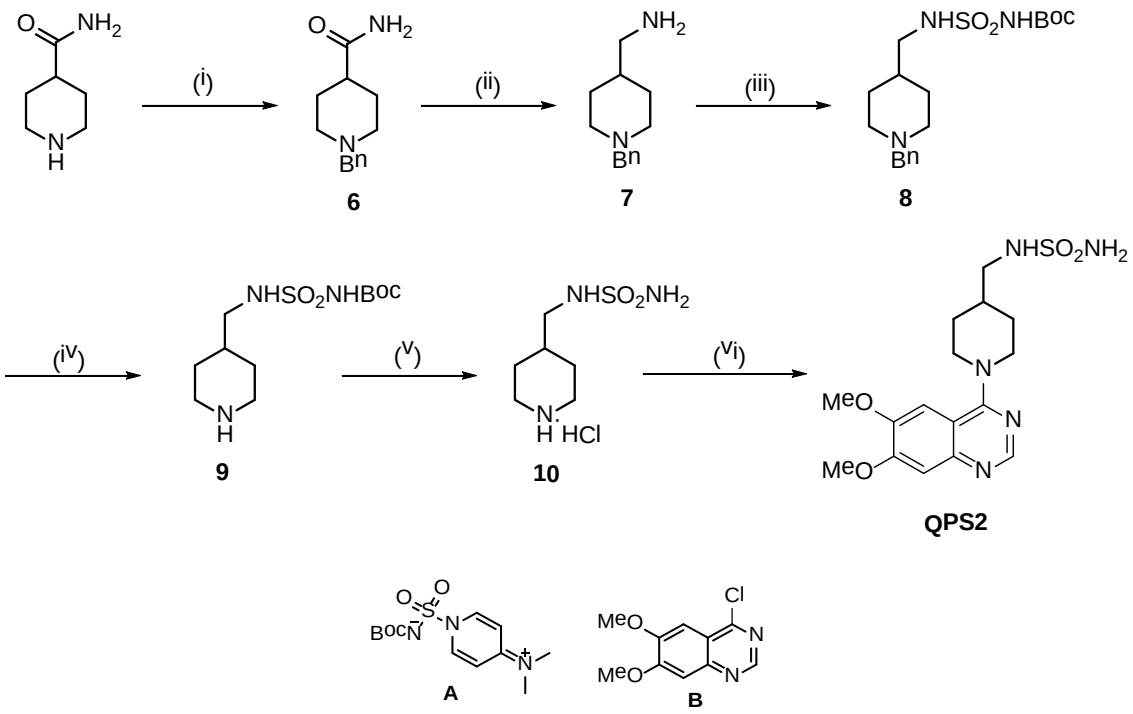
Synthetic schemes

Scheme 1. Synthesis of QPS1[a]



[a] Conditions : (i) NaOH, Boc_2O, THF/H_2O, 16 h (93%); (ii) diethyl (cyanomethyl)phosphonate, LiBr, Et_3N, THF, 16 h (96%); (iii) H_2, Ni/Ra, LiOH·H_2O, Pd/C, dioxane/H_2O; (iv) **A,** DIPEA, CH_2Cl_2 (61%); (v) 4M HCl/dioxane, 2 h, quant.; (vi) **B**, K_2CO_3, CH_3CN, 90 °C (59%).

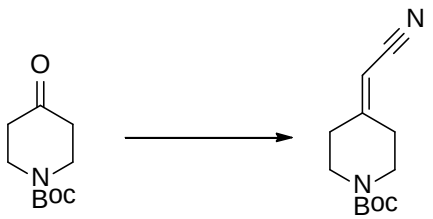
Scheme 2. Synthesis of QPS2[a]



[a] Conditions: (i) BnBr, K_2CO_3, EtOH, 16 h (78%); (ii) $LiAlH_4$, THF, 6 h, 70 °C (81%); (iii) **A,** DIPEA, CH_2Cl_2 (77%); (iv); H_2, Pd/C, $PdCl_2$, MeOH, 71% (v) 4M HCl/dioxane, 2 h, quant.; (vi) **B**, K_2CO_3, *i*PrOH, 90 °C (78%).

Materials and methods

Synthetic route for QPS1

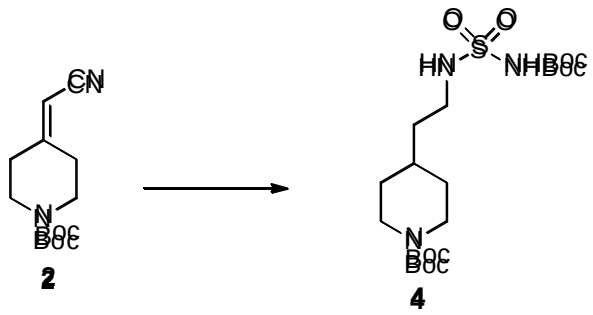


1 **2**

***tert*-Butyl 4-(cyanomethylene)piperidine-1-carboxylate (2)**

Diethyl (cyanomethyl)phosphonate (4.56 mL, 28.2 mmol) and triethylamine (7.56 mL, 54.2 mmol) were added to a stirred solution of LiBr (2.82 g, 32.5 mmol) in THF (120 mL). *tert*-Butyl 4-oxopiperidine-1-carboxylate (**1**)[1] was added after 5 min and the mixture was stirred at ambient temperature overnight. If the reaction wasn't complete, LiBr was added. When the reaction was finished (TLC analysis), solvent was evaporated under vacuum and the mixture was dissolved in AcOEt. The organic layer was washed with $NaHCO_3$ and water. The aqueous layer was extracted with AcOEt and the organic layers were combined, dried over $MgSO_4$ and concentrated. Then the residue was chromatographed on silica gel eluting using 8/2 hexanes/AcOEt to give 5.78 g (96%) of *tert*-butyl 4-(cyanomethylene)piperidine-1-carboxylate (**2**) as a white solid. Mp: 115-118 °C; IR (ATR, ZnSe) = 3012, 2936, 2216, 1677, 1426, 1360, 1324, 827, 785 cm^{-1}; ^1H NMR ($CDCl_3$): δ (ppm) 5.20 (s, 1H), 3.51 (dt, *J* = 11.8 Hz, *J* = 5.7 Hz, 4H), 2.56 (t, *J* = 5.6 Hz, 2H), 2.33 (t, *J* = 5.3 Hz, 2H), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$): δ (ppm) 163.4, 154.2, 116.1, 94.3, 80.2, 44.1 (2C), 34.9, 32.5, 28.3; HRMS-ESI calculated for $C_{12}H_{18}N_2NaO_2$ $[M+Na]^+$ 245.1260 found 245.1258.

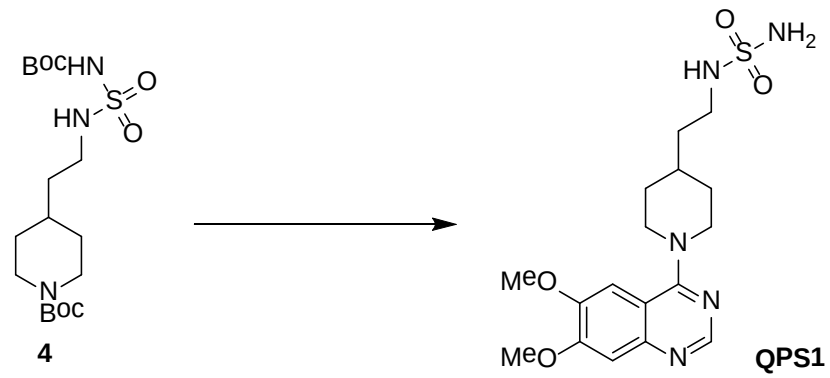
[1] Wang, Z.; Miller, E. J.; Scalia, S. J. *Org. Lett*. **2011**, 13, 6540-6543.



***tert*-Butyl 4-(cyanomethylene)piperidine-1-carboxylate (4)**

Acrylonitrile **2** (700 mg, 3.15 mmol) was dissolved in a mixture of dioxane (15 mL) and water (5 mL). Raney-Nickel (699 mg, 5.95 mmol) as a 50% suspension in water and 10% palladium of charcoal (210 mg, 0.197 mmol) were added with lithium hydroxide monohydrate (285 mg, 6.80 mmol), and the mixture was stirred under hydrogen atmosphere at ambient temperature overnight. The catalyst was filtered on celite, the solvents were removed under vacuum and the residue was used directly in the next step without further purification. The crude product, sulfamoylating agent **A**[2] (949 mg, 3.15 mmol) and DIPEA (0.82 mL, 4.73 mmol) were stirred 16 h, at room temperature in 60 mL of dichloromethane. Then the mixture was washed with NH_4Cl (2 ×) and brine. The organic layers were combined, dried over $MgSO_4$, concentrated, then the residue was chromatographed on silica gel, eluting using 7/3 hexanes/AcOEt to give *tert*-butyl 4-(cyanomethylene)piperidine-1-carboxylate **4** as a white solid (778 mg, 61% over 2 steps). Mp: 145-151 °C; IR (ATR, ZnSe) = 3307, 1722, 1659, 1478, 1211, 930, 785, 727 cm^{-1}; ^1H NMR ($CDCl_3$): δ (ppm) 7.46 (s, 1H, NH), 5.17 (t, *J* = 6.1 Hz, 1H, NH), 4.09 (br s, 2H), 3.13-3.09 (m, 2H), 2.68 (br s, 2H), 1.66 (d, *J* = 12.8 Hz, 2H), 1.54-1.48 (m, 12H), 1.46 (s, 9H), 1.14-1.06 (m, 2H); ^{13}C NMR ($CDCl_3$): δ (ppm) 154.8, 150.3, 83.8, 79.4, 43.8, 41.2, 35. 6, 33.1, 31.7, 28. 5, 28.0; HRMS-ESI calcd for $C_{17}H_{33}N_3NaO_6S$ $[M+Na]^+$ 430.1982 found 430.1982.

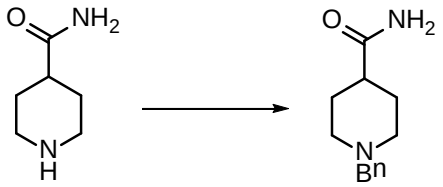
[2] Winum, J-Y.; Toupet, L.; Barragan, V.; Dewynter, G.; Montero, J-L. *Org. Lett.* **2001**, *3*, 2241-2243.



2-(1-(6,7-Dimethoxyquinazolin-4-yl)piperidin-4-yl)ethyl sulfamide (QPS1)

In a round-bottomed flask, 11.9 mL of HCl in dioxane (4 M) was added on sulfamide **4** (324 mg, 0.80 mmol). After 2 h, Boc deprotection was completed and solvent was evaporated under vacuum to give **5** in quantitative yield. The piperidine salt was dissolved in acetonitrile (12 mL) and stirred overnight at 90 °C. The solvent was removed under vacuum and the product was purified by flash column chromatography, eluting using 9/1 CH_2Cl_2/MeOH to give the desired product **QPS1** as a beige solid (170 mg, 59%). Mp: 156-160 °C; IR (ATR, ZnSe) = 3311, 2855, 1577, 1455, 1376, 1263, 861, 794 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ (ppm) 8.51 (s, 1H), 7.20 (s, 1H), 7.10 (s, 1H), 6.50 (s, 2H), 4.14 (d, *J* = 13.1 Hz, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 3.02 (t, *J* = 11.8 Hz, 2H), 2.96-2.94 (m, 2H), 1.81 (d, *J* = 10.9 Hz, 2H), 1.74–1.65 (m, 1H), 1.51-1.47 (m, 2H), 1.41-1.32 (m, 2H); ^{13}C NMR (MeOD-d_4): δ (ppm) 165.2, 156.5, 153.4, 150.1, 149.4, 112.3, 107.0, 105.0, 56.6, 56.5, 51.3, 41. 6, 37.2, 34.8, 33.2; HRMS-ESI calcd for $C_{17}H_{26}N_5O_4S$ $[M+H]^+$ 396.1700 found 396.1710.

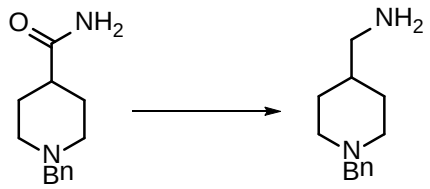
Synthetic route for QPS2



6

1-Benzylpiperidine-4-carboxamide (6)

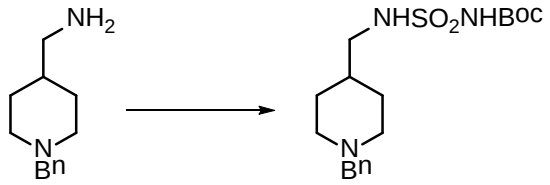
To a stirred suspension of isonipectamide (5.0 g, 39 mmol) and K_2CO_3 (10.78 g, 78.02 mmol) in EtOH (210 mL) was added benzylbromide (5.10 mL, 42.90 mmol) and the mixture was heated under reflux overnight, cooled to room temperature and filtered. The filtrate was evaporated under vacuum and H_2O was added. The aqueous layer was extracted with dichloromethane (× 3), the organic layers combined and dried over $MgSO_4$ and filtrated. The solvent was evaporated under vacuum to give 1-benzylpiperidine-4-carboxamide (**6**) as a yellowish solid (6.66 g, 78%). Mp: 156-159 °C; IR (ATR, ZnSe) = 3329, 3151, 2922, 1627, 1494, 1432, 1390, 1148, 1129, 734, 698 cm^{-1}; ^1H NMR ($CDCl_3$): δ (ppm) 7.33-7.31 (m, 4H), 7.28-7.24 (m, 1H), 5.65 (br s, 1H), 5.52 (br s, 1H), 3.51 (s, 2H), 2.96-2.92 (m, 2H), 2.16 (tt, *J* = 11.8, 4.0 Hz, 1H), 2.01 (td, *J* = 11.7, 2.5 Hz, 2H), 1.89-1.84 (m, 2H), 1.80-1.71 (m, 2H); ^{13}C NMR ($CDCl_3$): δ (ppm) 178.2, 138.1, 129.1, 128.2, 127.0, 63.1, 53.0, 42.7, 28.8; HRMS-ESI calcd for $C_{13}H_{19}N_2O$ $[M+H]^+$ 219.1492 found 219.1493.



6 **7**

(1-Benzylpiperidin-4-yl)methanamine (7)

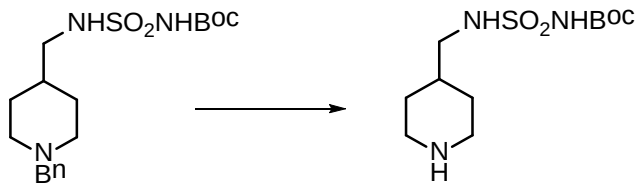
A suspension of **6** (2.0 g, 9.2 mmol) in dry THF (24 mL) was added slowly to a solution of $LiAlH_4$ (0.52 g, 13.7 mmol) in dry THF (30 mL). The mixture was stirred under reflux and argon atmosphere for 6 h. After cooling, water was added at 0 °C, the precipitate was filtered and washed with Et_2O. The filtrate was extracted with Et_2O (× 2), dried over $MgSO_4$ and the solvent was evaporated under vacuum to give (1-benzylpiperidin-4-yl)methanamine (**7**) as an orange solid (1.52 g, 81%). Mp: 86-90 °C; IR (ATR, ZnSe) = 3344, 3024, 2933, 1579, 1476, 1230, 1029, 740, 698 cm^{-1}; ^1H NMR ($CDCl_3$): δ (ppm) 7.31-7.26 (m, 5H), 3.49 (s, 2H), 2.90 (d, *J* = 11.8 Hz, 2H), 2.57 (d, *J* = 5.9 Hz, 2H), 1.94 (t, *J* = 11.2 Hz, 2H), 1.70-1.67 (m, 2H), 1.29-1.21 (m, 3H); ^{13}C NMR ($CDCl_3$): δ (ppm) 138.6, 129.2, 128.1, 126.9, 63.5, 53. 7, 48.2, 39.4, 30.0; HRMS-ESI calcd for $C_{13}H_{21}N_2$ $[M+H]^+$ 205.1699 found 205.1699.



7 **8**

***tert*-Butyl *N*-((1-benzylpiperidin-4-yl)methyl)sulfamoylcarbamate (8)**

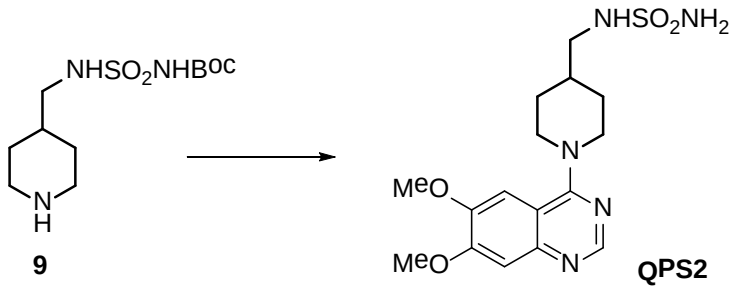
Amine **7** (2.58 g, 12.6 mol), sulfamoylating agent **A**[1] (3.81 g, 12.6 mmol) and DIPEA (3.30 mL, 18.9 mmol) were stirred 16 h, at room temperature in dichloromethane (200 mL). Then the mixture was washed with NH_4Cl (× 2) and brine. The organic layers were combined, dried over $MgSO_4$, concentrated, then the residue was chromatographed on silica gel, eluting using 9/1 CH_2Cl_2/MeOH to give *tert*-butyl *N*-((1-benzylpiperidin-4-yl)methyl)sulfamoylcarbamate (**8**) as a white solid (3.74 g, 77%). Mp: 135 °C (dec.); IR (ATR, ZnSe) = 1644, 1494, 1457, 1294, 1150, 1135, 1088, 843, 770, 746, 696 cm^{-1}; ^1H NMR (DMSO-d_6): δ (ppm) 7.49 (Br s, 1H), 7.49-7.22 (m, 5H), 3.45 (s, 2H), 2.78-2.72 (m, 4H), 1.89 (t, *J* = 11.0 Hz, 2H), 1.64 (d, *J* = 11.9 Hz, 2H), 1.40 (s, 9H), 1.12-1.05 (m, 2H); ^{13}C NMR (MeOD-d_4): δ (ppm) 153.5, 134.9, 129. 8, 128.0, 127. 6, 80.4, 62.1, 52.4, 34.9, 28.4, 27.0; HRMS-ESI calcd for $C_{18}H_{30}N_3O_4S$ $[M+H]^+$ 384.1952 found 384.1962.



8 **9**

***tert*-Butyl *N*-(piperidin-4-ylmethyl)sulfamoylcarbamate (9)**

10 % Pd on activated charcoal (57 mg, 0.54 mmol) and $PdCl_2$ (5 mg, 0.03 mmol) was added to **8** (207 mg, 0.54 mmol) in MeOH (15 mL). The mixture was hydrogenated under H_2 atmosphere overnight. After filtration through a pad of Celite, the filtrate was evaporated to give *tert*-butyl *N*-(piperidin-4-ylmethyl)sulfamoylcarbamate (**9**) (113 mg, 71%) as a white solid. Mp: 150 °C (dec.); IR (ATR, ZnSe) = 2928, 2492, 1652, 1283, 1141, 1087, 979, 910, 851, 795, 747 cm^{-1}; ^1H NMR (DMSO-d_6): δ (ppm) 3.20 (d, *J* = 12.3 Hz, 2H), 2.75 (t, *J* = 11.2 Hz, 2H), 2.62 (d, *J* = 6.6 Hz, 2H), 1.79 (d, *J* = 13.0 Hz, 2H), 1.65 (Br s, 1H), 1.36-1.21 (m, 5H); ^{13}C NMR δ (ppm) 155.1, 78.7, 67.3, 48.4, 43.2, 33.7, 31.7, 28.4, 26.6; HRMS-ESI calcd for $C_{11}H_{24}N_3O_4S$ $[M+H]^+$ 294.1482 found 294.1485.



((1-(6,7-Dimethoxyquinazolin-4-yl)piperidin-4-yl)methyl)sulfamide (QPS2)

In a round-bottomed flask, 3 mL of HCl in dioxane (4 M) was added on sulfamide **9** (118 mg, 0.40 mmol). After 2 h, Boc deprotection was completed and solvent was evaporated under vacuum to give the amine hydrochloride **10** in quantitative yield. The piperidine salt was dissolved in isopropanol (25 mL) and stirred overnight under reflux. The solvent was removed under vacuum and the product was purified by flash column chromatography, eluting using 9/1 CH_2Cl_2/MeOH to give the desired product **QPS2** as a yellowish solid (120 mg, 78%). Mp: 95-100 °C; IR (ATR, ZnSe) = 3269, 2915, 1576, 1504, 1427, 1333, 1245, 1206, 991, 929, 852, 786 cm^{-1}; ^1H NMR (DMSO-d_6): δ (ppm) 8.50 (s, 1H), 7.19 (s, 1H), 7.10 (s, 1H), 6.59 (t, *J* = 6.1 Hz, 1H), 6.49 (s, 2H), 4.14 (d, *J* = 13.3 Hz, 2H), 3.92 (s, 3H), 3.90 (s, 3H), 3.02 (t, *J* = 12.0 Hz, 2H), 2.83 (t, *J* = 6.4 Hz, 2H), 1.86-1.79 (m, 3H), 1.39-1.33 (m, 2H); ^{13}C NMR (DMSO-d_6): δ (ppm) 163.6, 154.5, 152.9, 148.9, 148.4, 111.0, 107. 6, 103.8, 56.3, 56.0, 49.8, 48.5, 36.2, 30.0; HRMS-ESI calcd for $C_{16}H_{24}N_5O_4S$ $[M+H]^+$ 382.1544 found 382.1552.

NMR spectra of all the new compounds

Figure S1: **^{1}H NMR spectrum of compound 2**

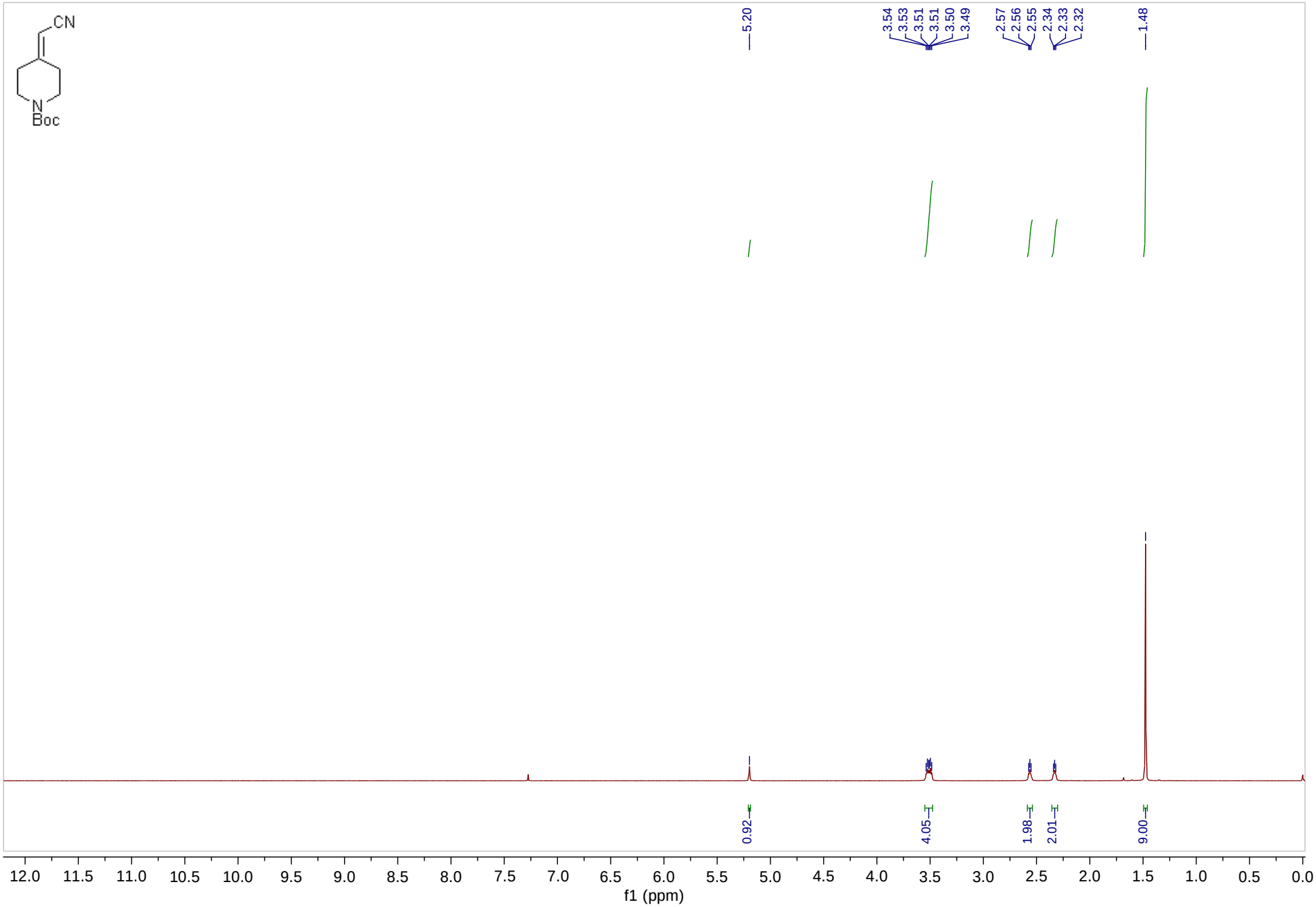


Figure S2: **^{13}C NMR spectrum of compound 2**

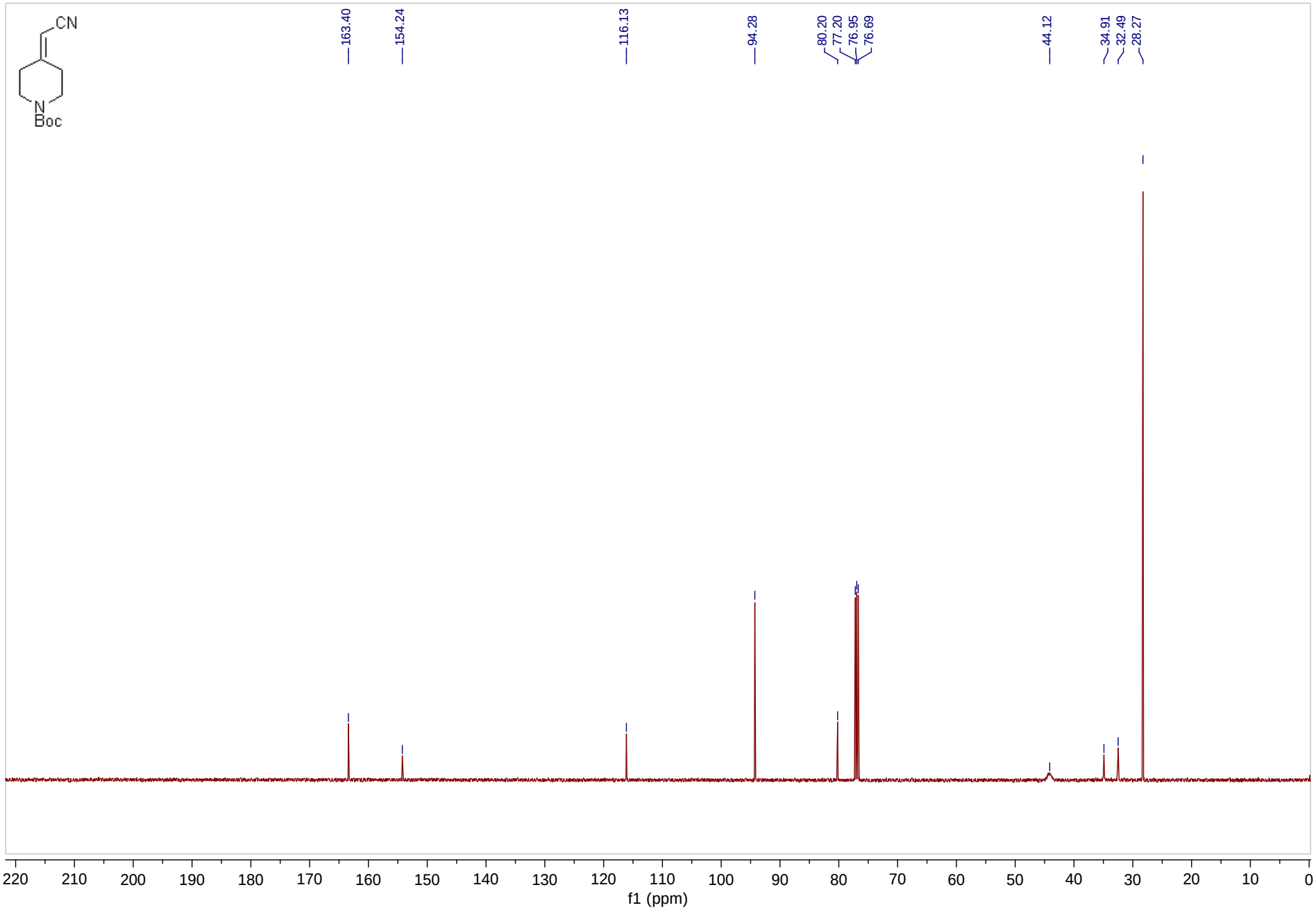


Figure S3: **^{1}H NMR spectrum of compound 4**

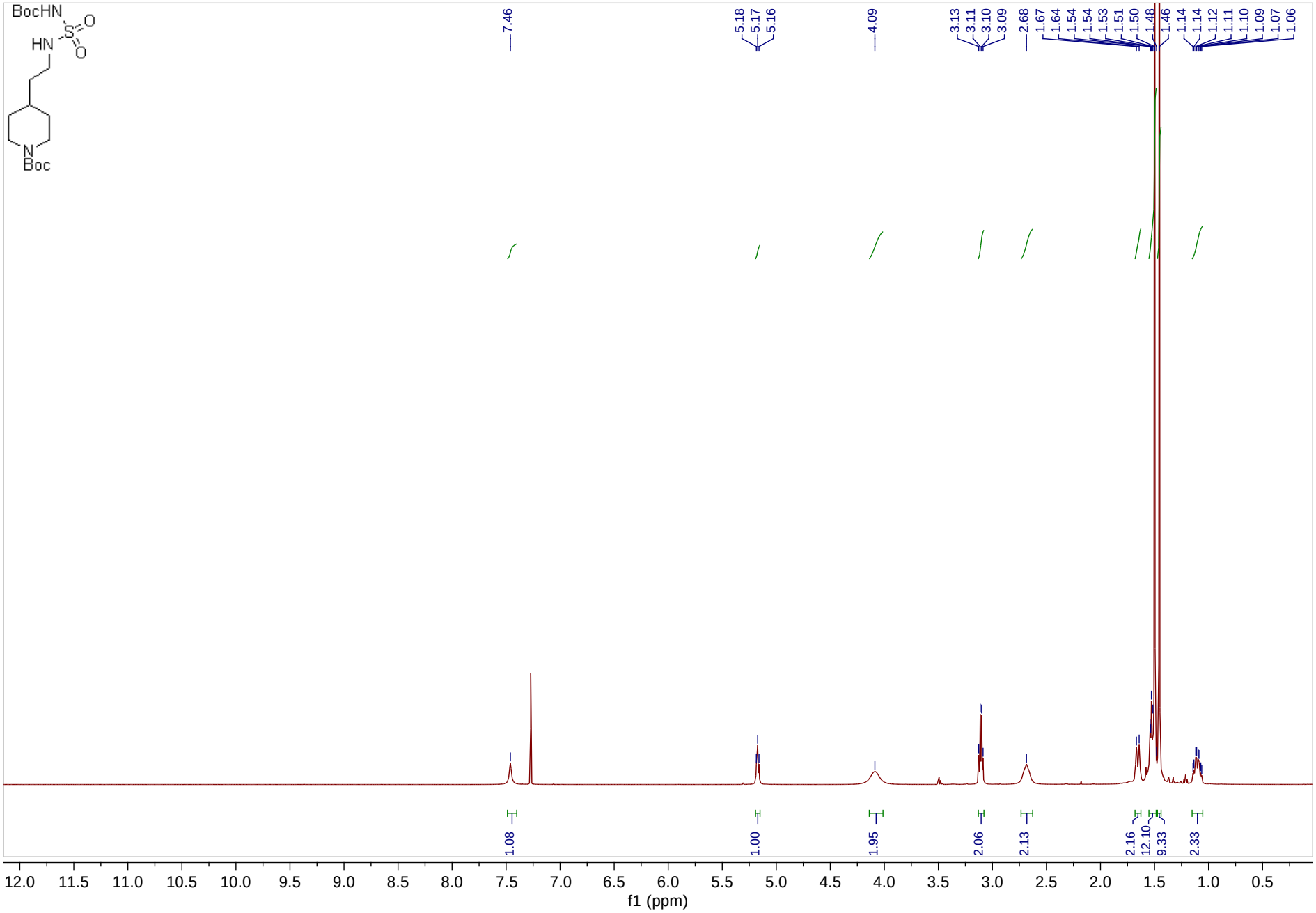


Figure S4: **^{13}C NMR spectrum of compound 4**

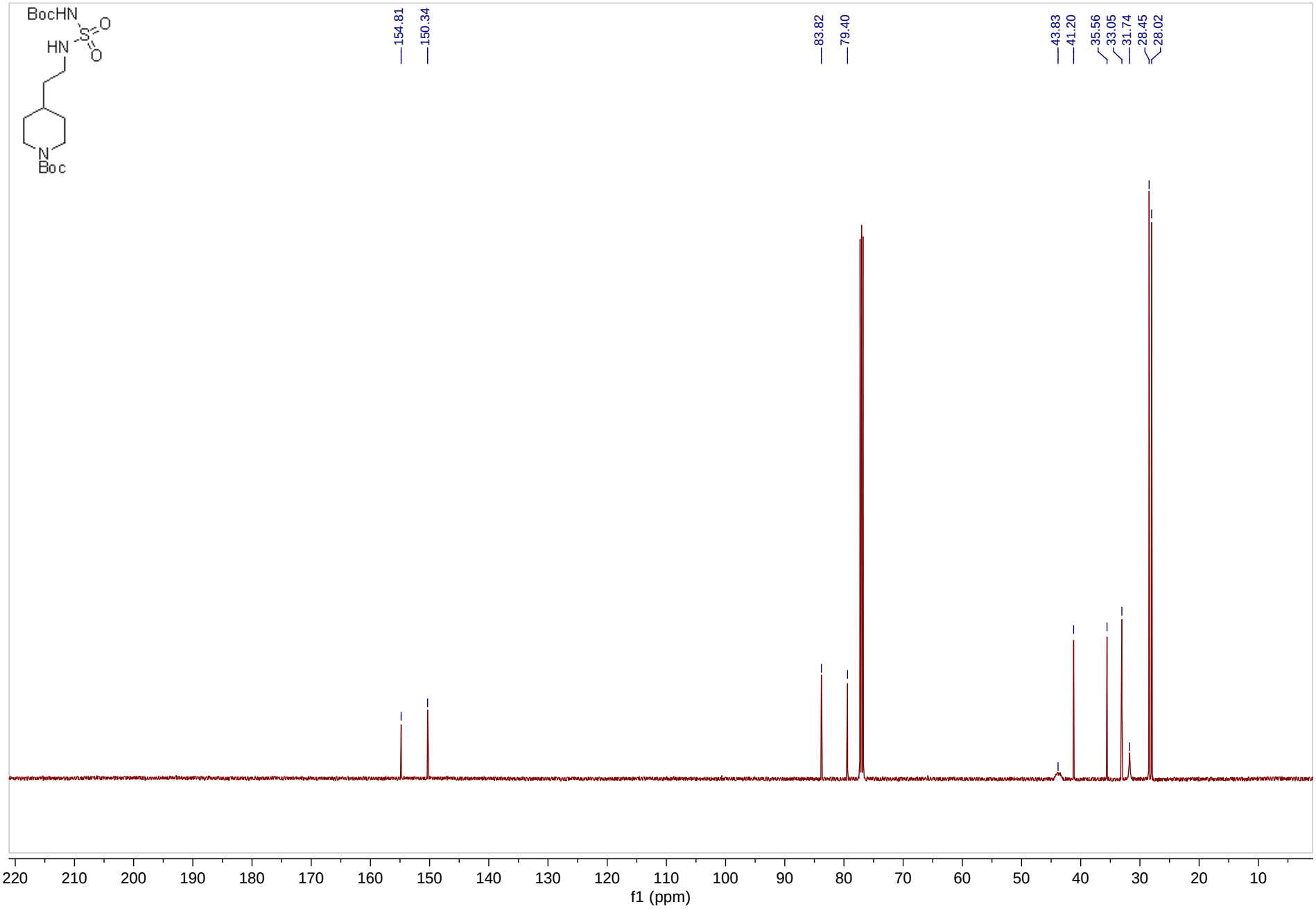


Figure S5: **^{1}H NMR spectrum of compound QPS1**

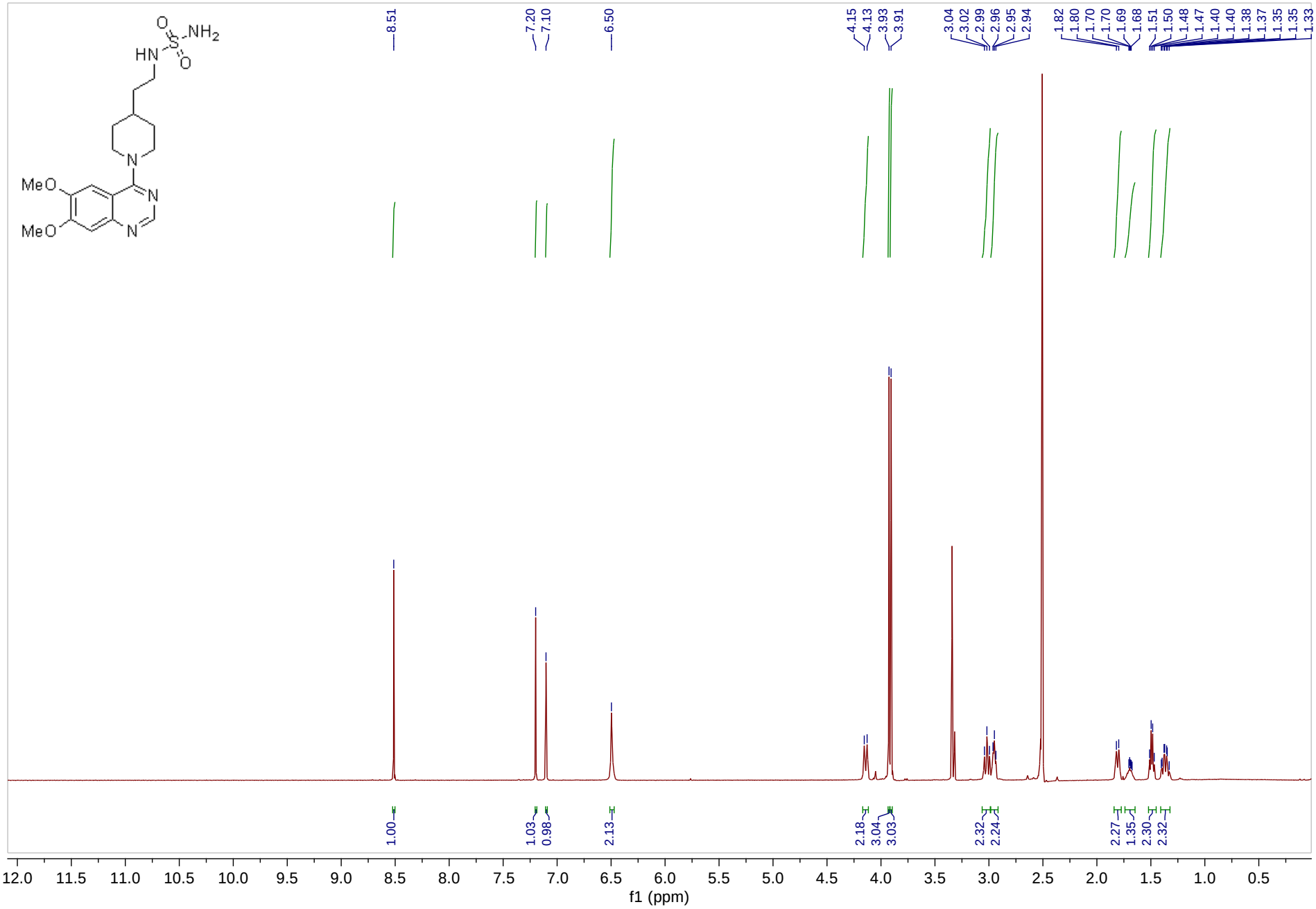


Figure S6: **^{13}C NMR spectrum of compound QPS1**

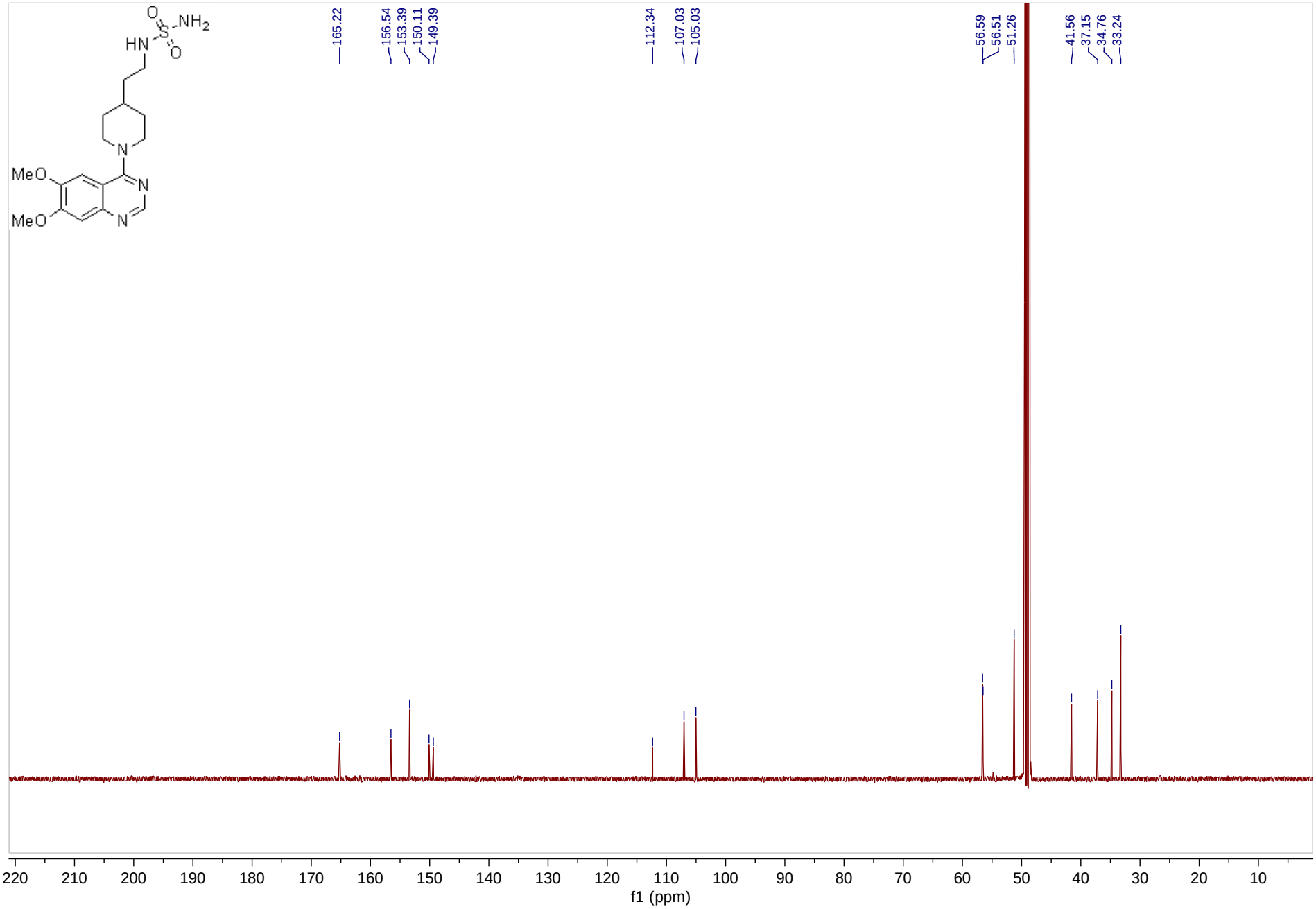


Figure S7: **^{1}H NMR spectrum of compound 6**

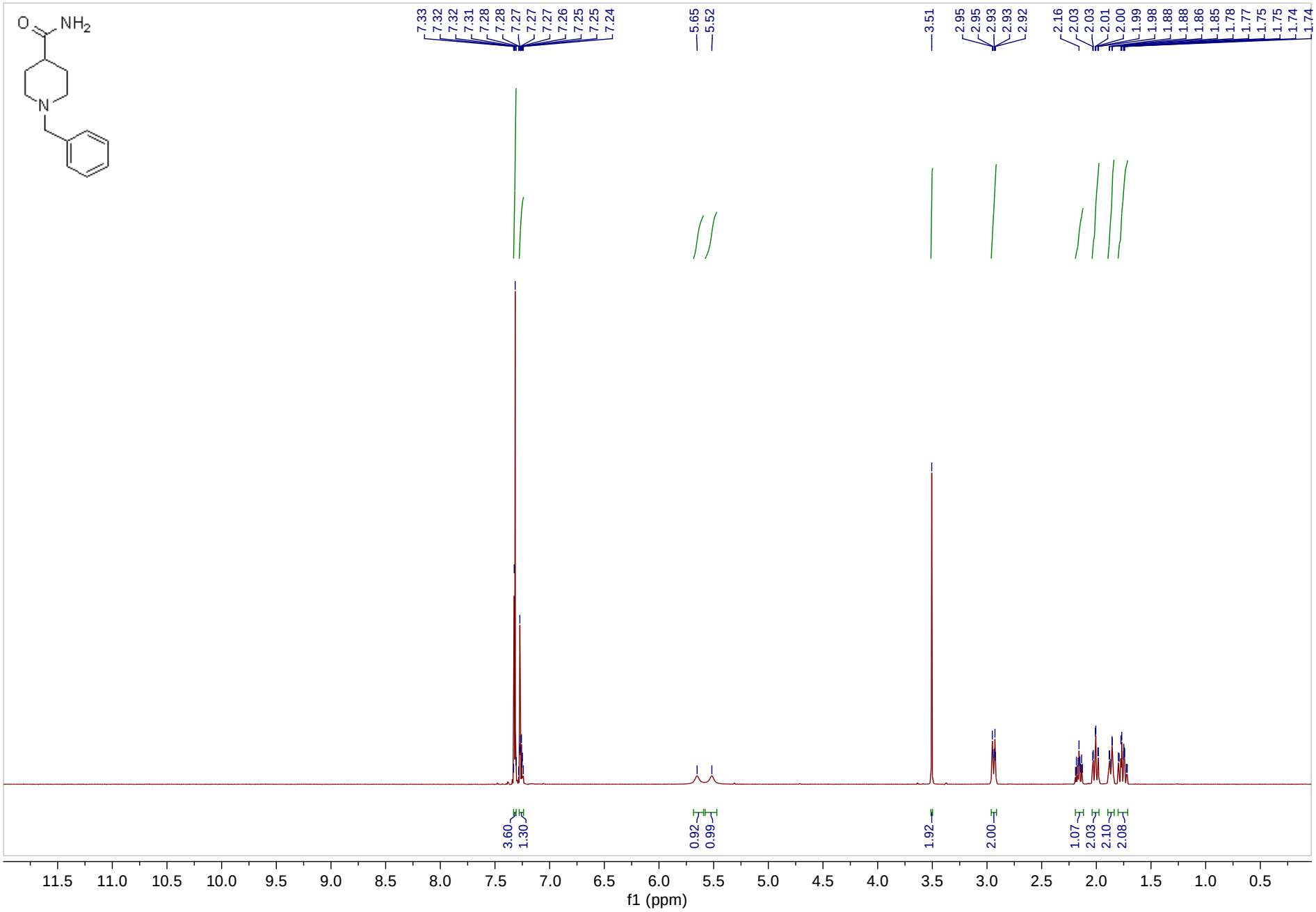


Figure S8: **^{13}C NMR spectrum of compound 6**

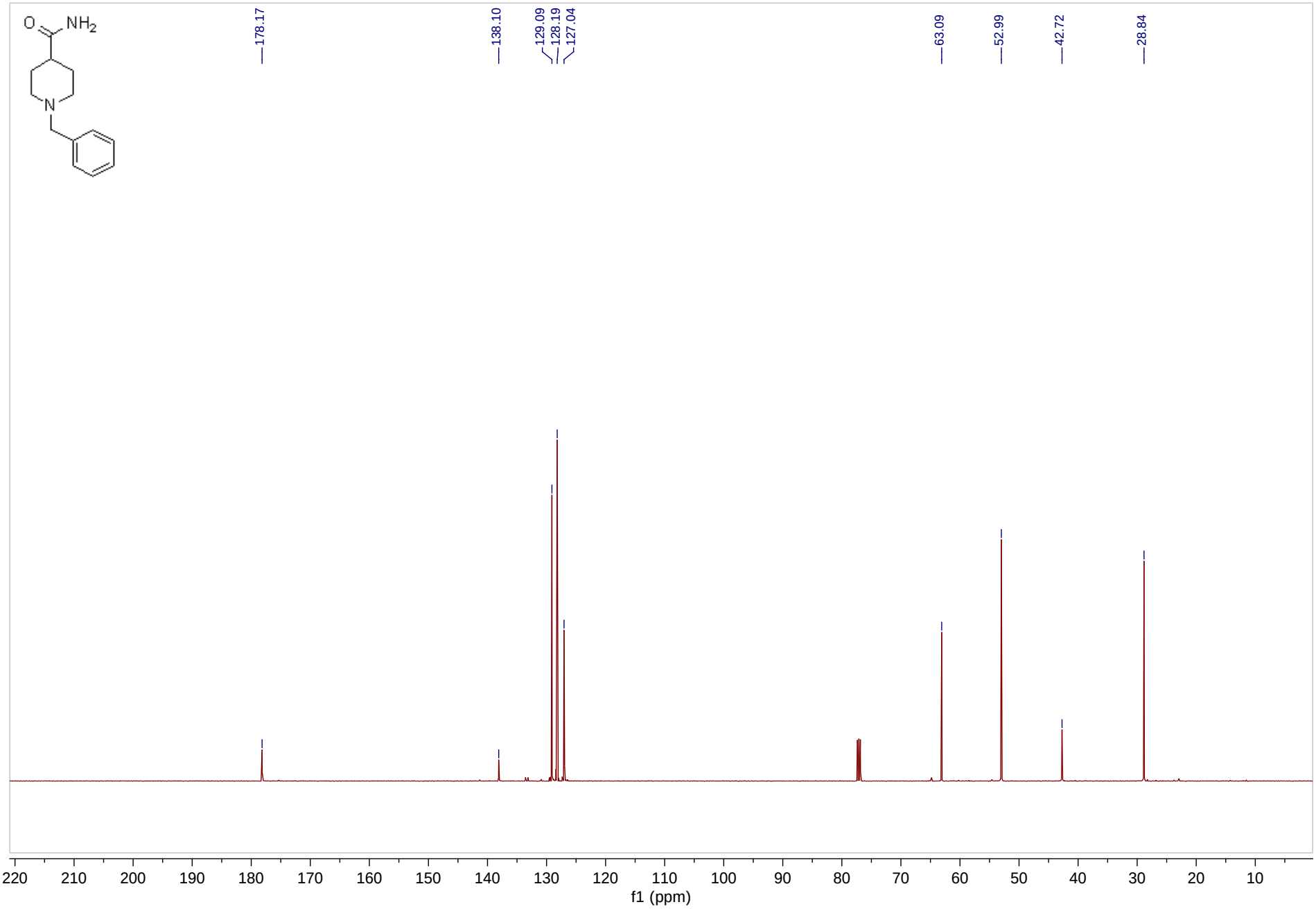


Figure S9: **^{1}H NMR spectrum of compound 7**

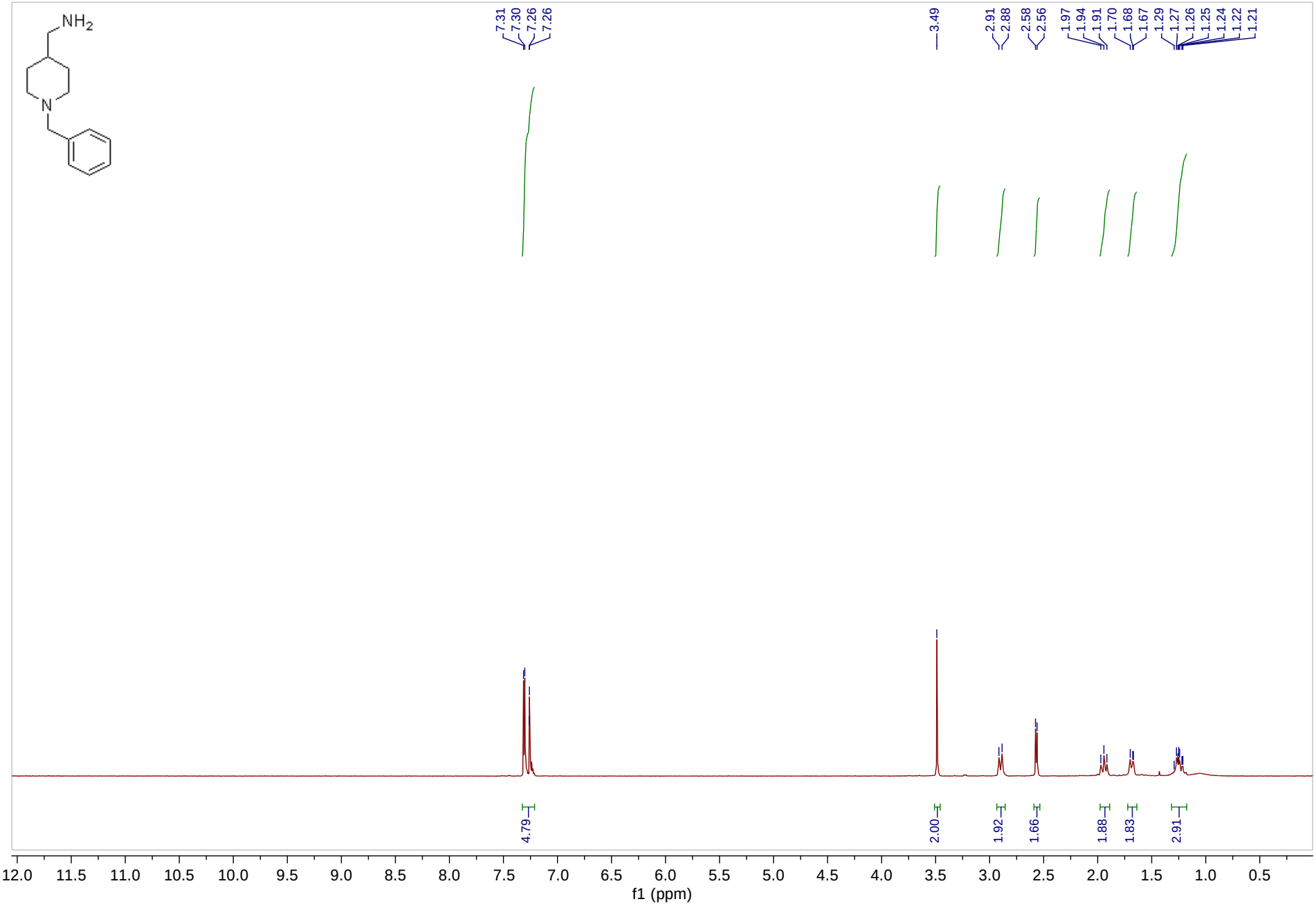


Figure S10: **^{13}C NMR spectrum of compound 7**

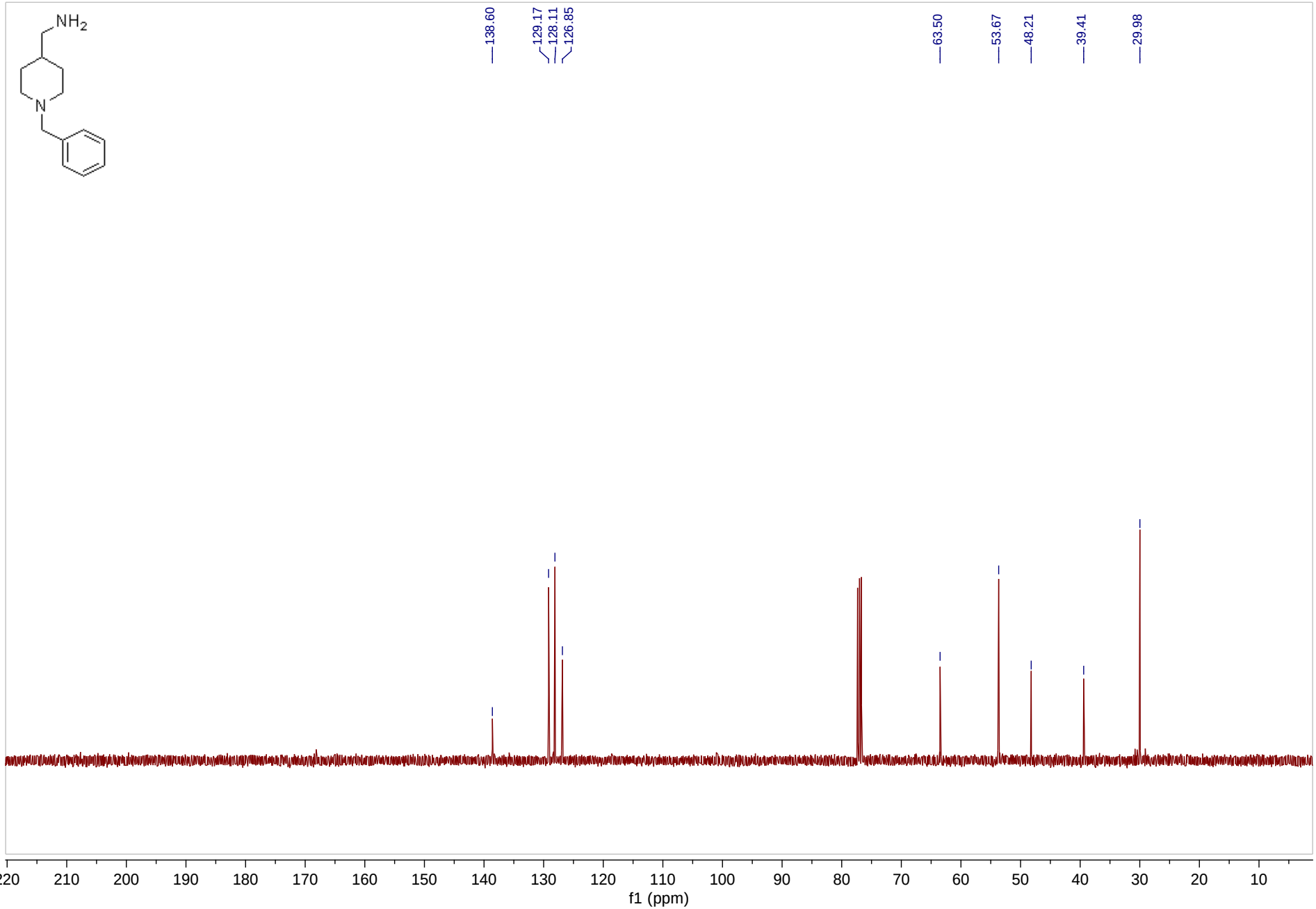


Figure S11: **^{1}H NMR spectrum of compound 8**

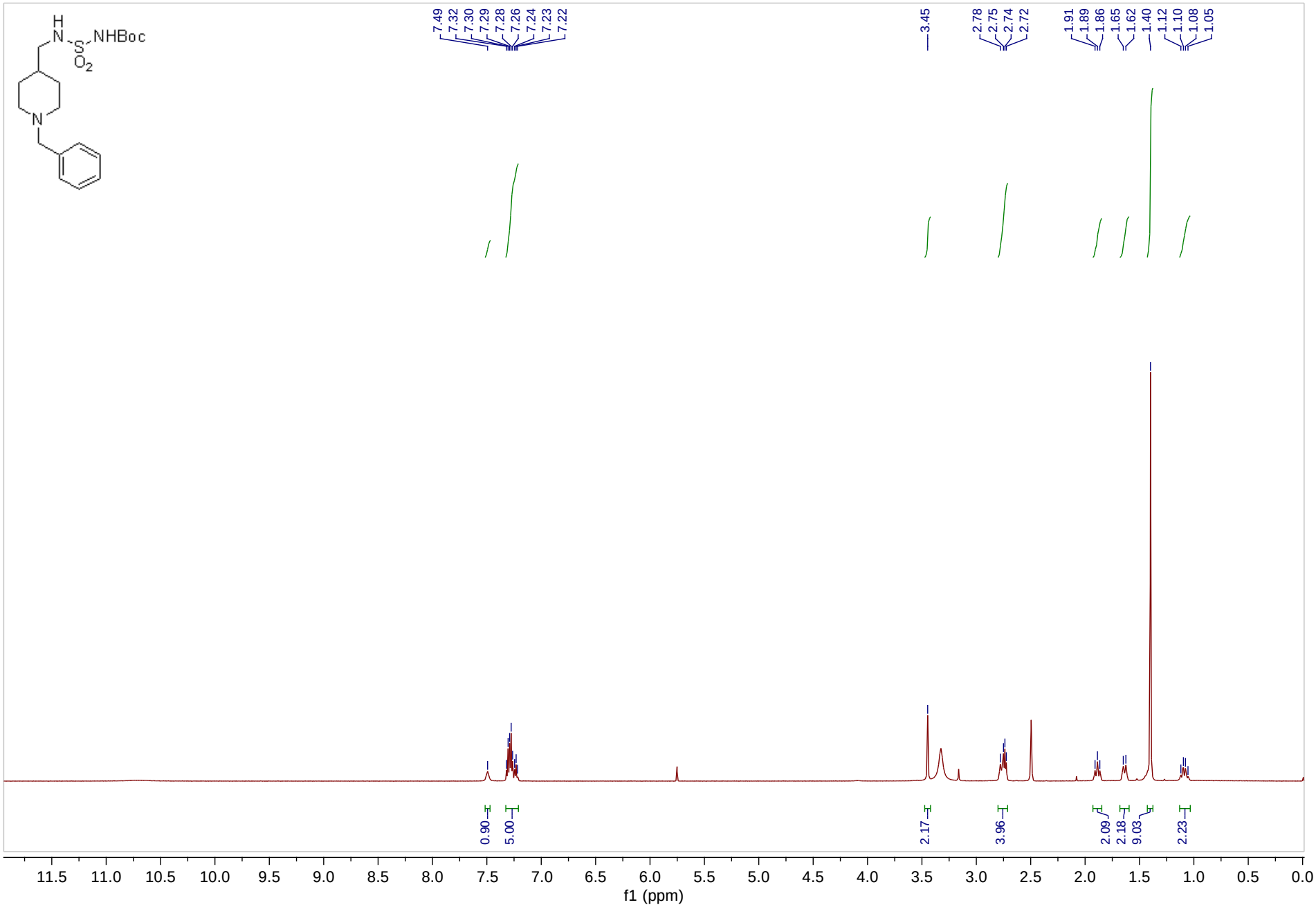


Figure S12: **^{13}C NMR spectrum of compound 8**

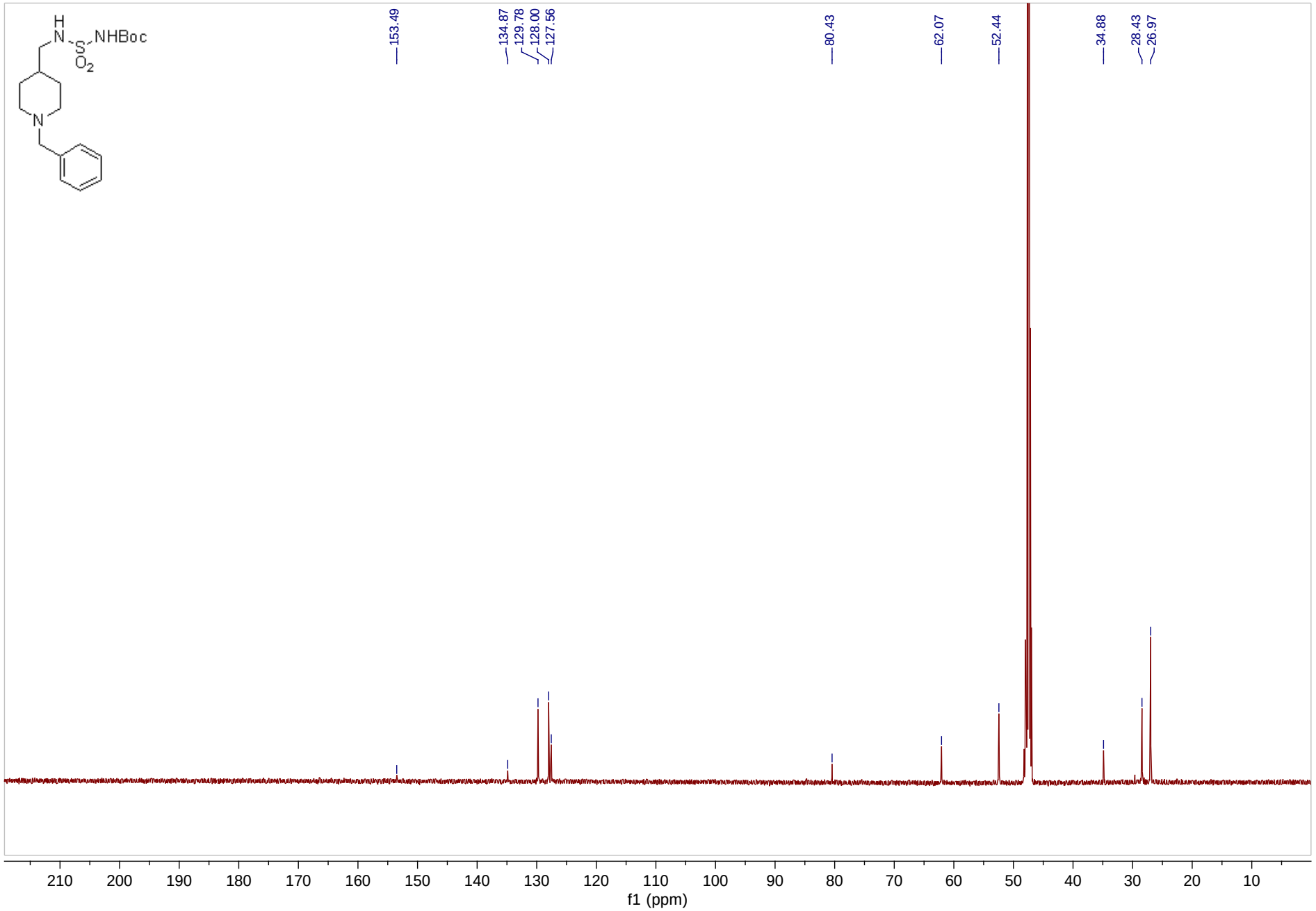


Figure S13: **^{1}H NMR spectrum of compound 9**

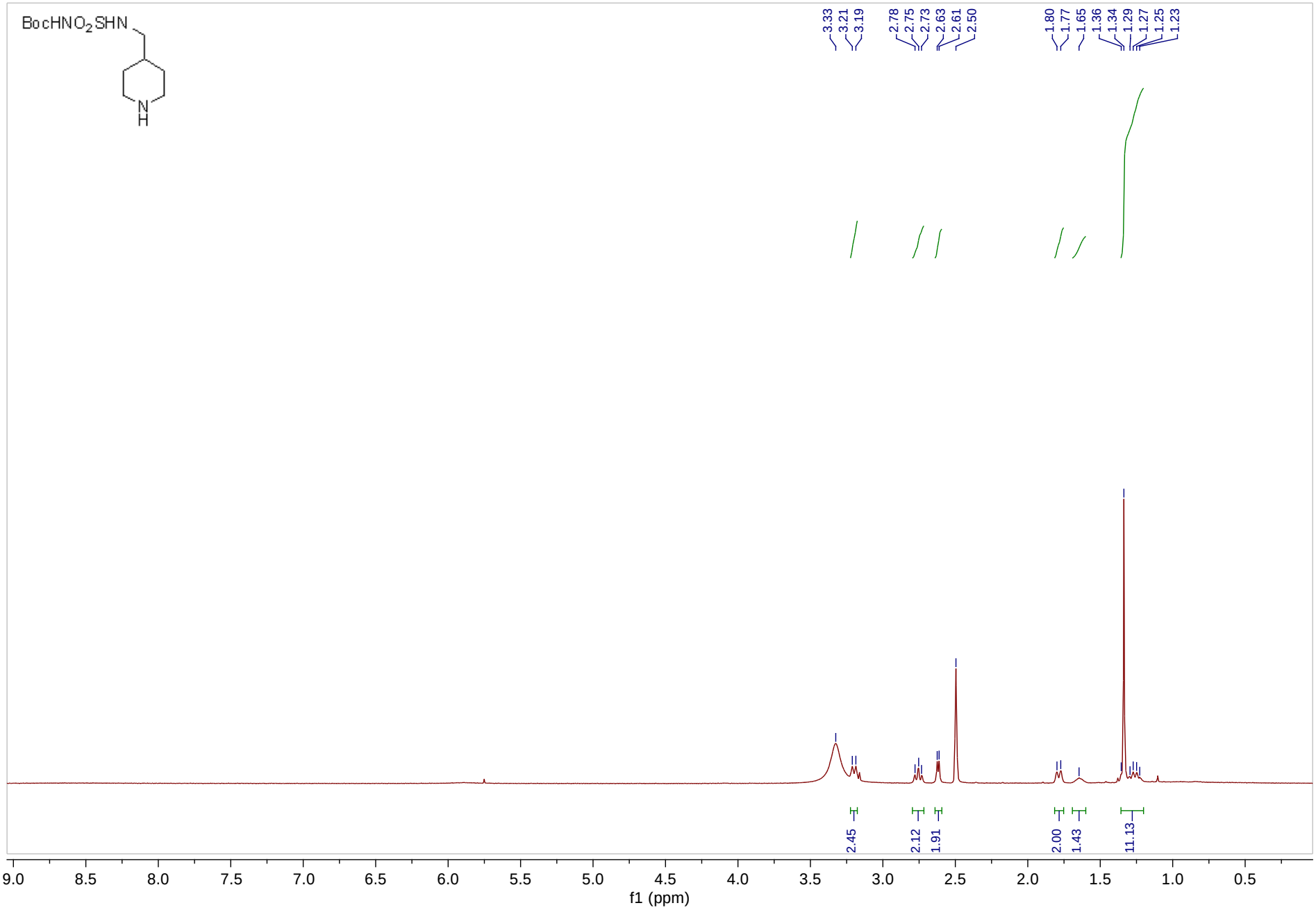


Figure S14: **^{13}C NMR spectrum of compound 9**

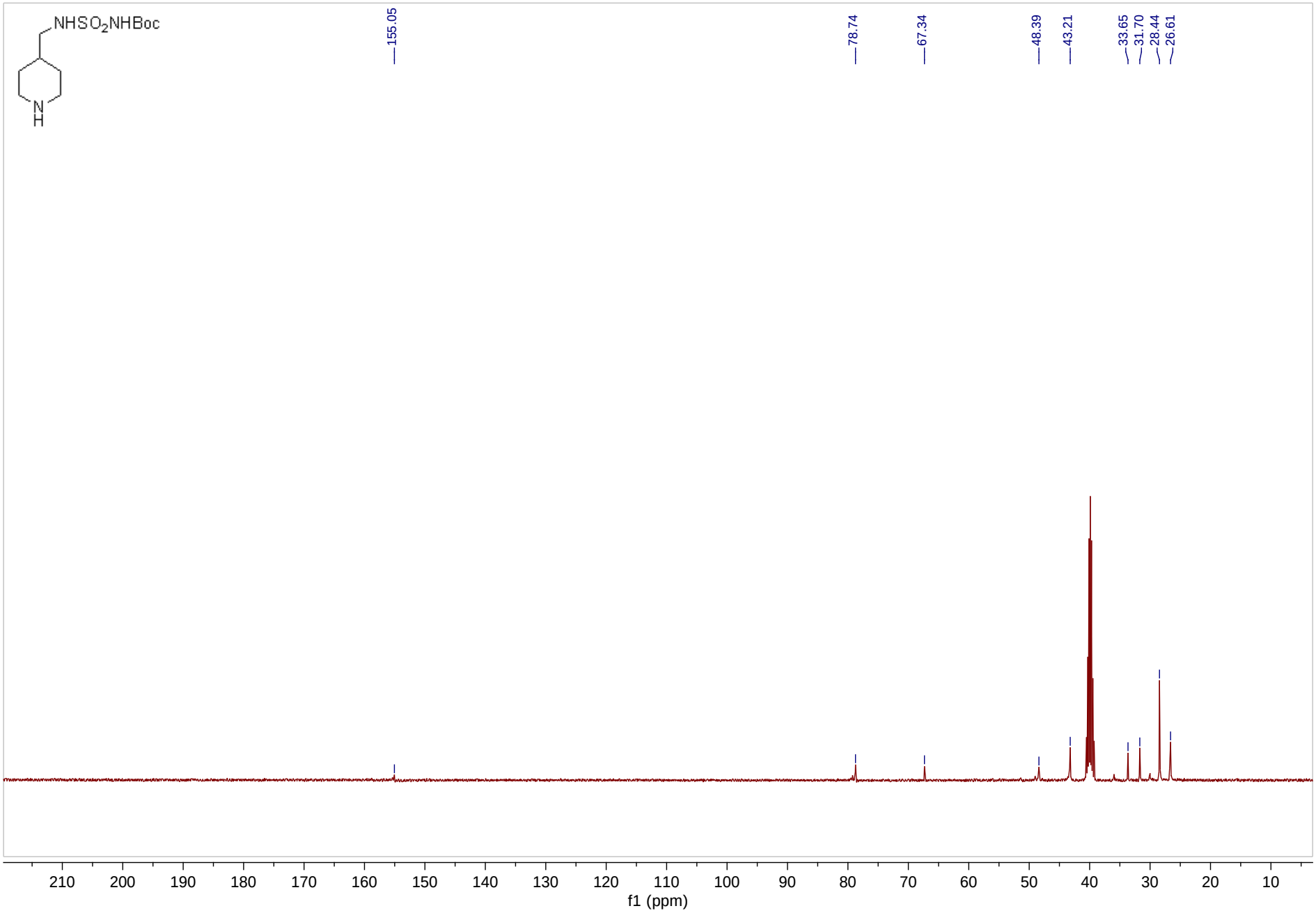


Figure S15: **^{1}H NMR spectrum of compound QPS2**

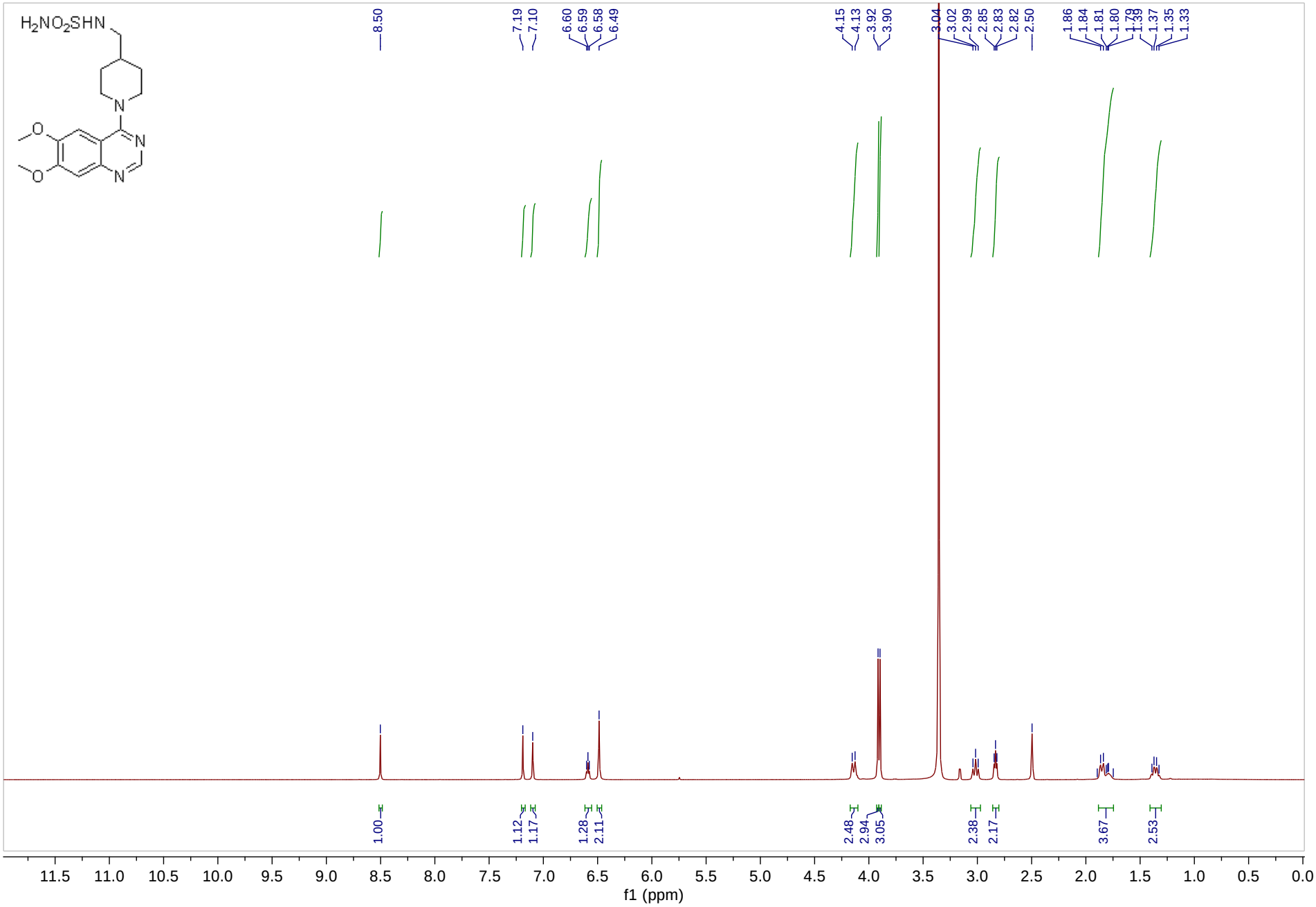


Figure S16: **^{13}C NMR spectrum of compound QPS2**