Electronic Supplementary Information

Application of tobramycin benzyl ether as an antibiotic adjuvant capable of sensitizing multidrug-resistant Gram-negative bacteria to rifampicin

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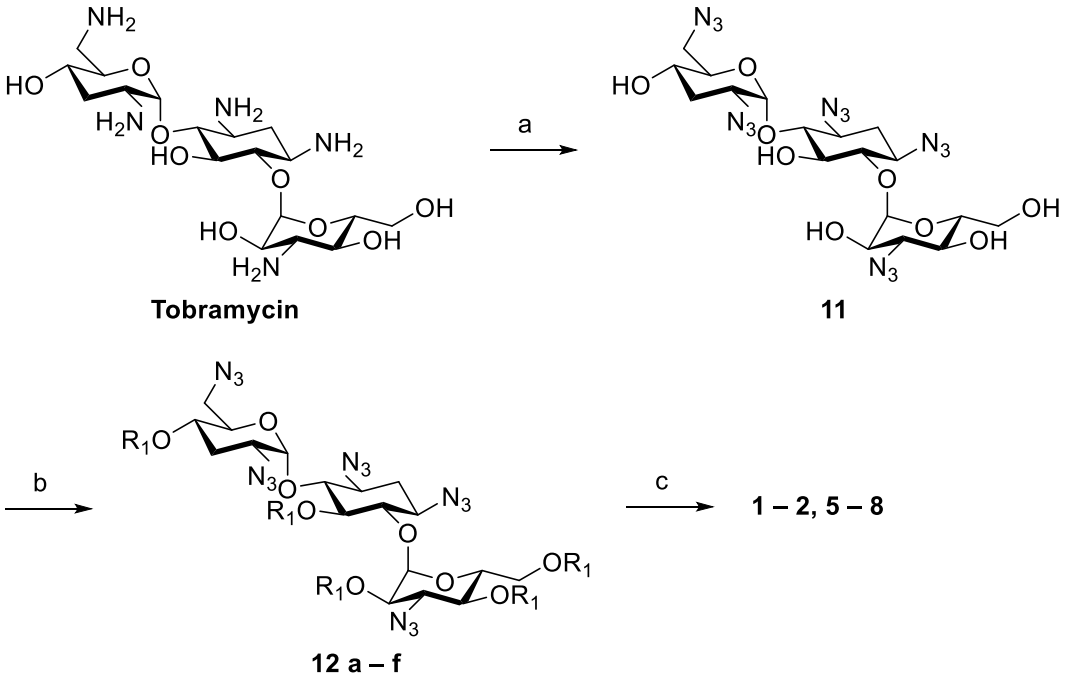
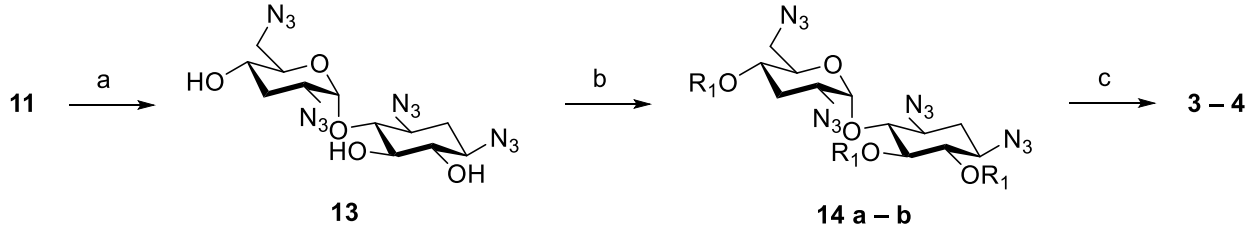
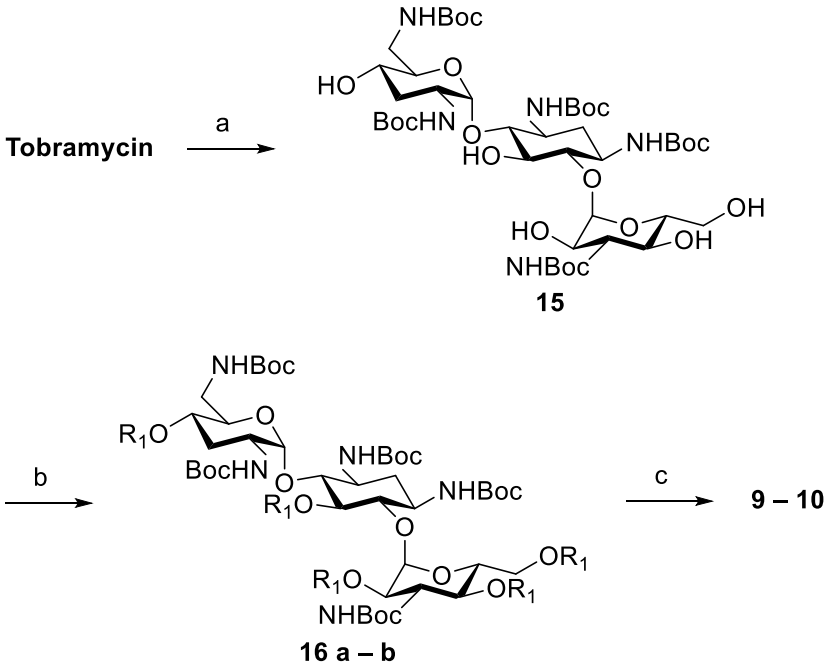


Scheme S1. Synthesis of tobramycin ether derivatives. Reagents and conditions: a) TfN_3, $ZnCl_2$, Et_3N, $H_2O/MeOH/CH_2Cl_2$, (95%); b) NaH, DMF, alkyl bromide or iodide, TBAI, (70 – 90%); c) 1.0 M PMe_3 in THF, H_2O/THF (1:8), 0.1 N NaOH, (80 – 97%).



Scheme S2. Synthesis of nebramine ether derivatives. Reagents and conditions: a) 10% HCl in MeOH, 70 °C, 24h, (87%); b) NaH, DMF, alkyl bromide or iodide, TBAI, (73 – 74%); c) 1.0 M PMe_3 in THF, H_2O/THF (1:8), 0.1 N NaOH, (82 – 98%).



Scheme S3. Synthesis of tobramycin carbamate derivatives. Reagents and conditions: a) Boc_2O, Et_3N, H_2O/MeOH, 55 °C, (39 – 46%); b) Pyridine, aryl isocyanate; c) TFA, 0 °C, 3 min (92 – 95%).

Chemistry

All reagents and solvents were purchased from commercially accessible vendors such as Sigma Aldrich, AK Scientific and Fisher Scientific, and used without further purification. The reaction progress was monitored by thin layer chromatography (TLC) on 0.25 mm silica gel 60 F254 plates from Merck and visualized by staining in ninhydrin in butanol and/or 10% H_2SO_4 in EtOH solutions. The compounds were purified by normal- and reverse-phase flash chromatography using SiliaFlash P60 (40 – 63 μm) and C18 (17%C) (40 – 63 μM) silica gels from Silicycle, or through the Biotage Selekt Flash instrument with Biotage Sfär columns. The chemical structures of all intermediates and final products were characterized by nuclear magnetic resonance (NMR) spectroscopy (^{1}H, ^{13}C, COSY, HSQC and HMBC) on Bruker AMX-400 and AMX-500 spectrometers. ^{13}C NMR spectra were fully decoupled. Chemical shifts were reported in parts per million (ppm) using deuterated solvents $CDCl_3$, MeOD-d_4, and D_2O as internal standards. Matrix-assisted laser desorption ionization mass spectrometer (MALDI-MS) analyses were performed in positive ion mode with 2,5-dihydrobenzoic acid as matrix on Bruker Daltonics Ultraflextreme MALDI – time of flight (TOF)/TOF and electrospray ionization (ESI) mass spectrometer. Compounds **1** – **10**, **11**, **12a** – **f**, **15** and **16a** – **b** were synthesized based on reported protocols.

Synthetic procedure for the preparation of 1,3,2',6',3"-pentaazido tobramycin (11)[1]

Sodium azide (NaN_3) (2 g, 32.11 mmol, 6 equiv/amine) was dissolved in water (5 mL, 0.4g/mL of NaN_3) and stirred at 0 °C, followed by the addition of dichloromethane (DCM) (5 mL). After 5 min, while vigorously stirring the reaction mixture, trifluoromethanesulfonic anhydride was added dropwise. The reaction was stirred at 0 °C for 2 h. A solution of saturated sodium bicarbonate was then added dropwise until CO_2 evolution ceased. The reaction solution was extracted twice using DCM (6.25 mL). The collected DCM layer corresponded to 0.6 M of triflyl azide (based on 50% conversion) and used in the subsequent step.

Tobramycin was first dissolved in 1 mL of water. The volume of water was increased, followed by the addition of methanol and DCM to achieve a final ratio of 3:10:3 water/methanol/DCM. The solution was stirred vigorously at ambient temperature. Zinc (II) chloride (7.2 mg, 0.53 mmol, 0.01 equiv/amine) was then added, followed by triethylamine (2.33 mL, 160.5 mmol, 3

equiv/amine). Afterwards, the freshly prepared triflyl azide solution was added to the reaction mixture and continuously stirred for 3 – 3.5 h. The reaction progress was monitored by TLC (1:9 MeOH/DCM). After completion, the reaction was concentrated under reduced pressure at 10 – 15 °C and the resulting residue was purified using flash chromatography to give the desired product **11** (0.610 g, 95%) as a white solid. ^{1}H NMR (400 MHz, MeOD-d_4) δ 5.60 (d, J = 3.5 Hz, 1H), 5.25 (d, J = 3.7 Hz, 1H), 4.12 – 4.08 (m, 1H), 4.06 – 4.02 (m, 1H), 3.78 (dd, J = 11.8, 2.4 Hz, 1H), 3.71 – 3.64 (m, 4H), 3.61 – 3.51 (m, 3H), 3.50 – 3.40 (m, 3H), 3.38 – 3.33 (m, 2H), 3.22 (dt, J = 13.0, 4.1 Hz, 1H), 2.39 (dt, J = 12.5, 4.2 Hz, 1H), 2.15 (dt, J = 11.4, 4.5 Hz, 1H), 2.05 – 1.97 (m, 1H), 1.62 – 1.53 (m, 1H). MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{18}H_{27}N_{15}O_9Na^+$: 620.2008; measured: 620.2030.

General procedure A for the preparation of 1,3,2′,6′,3″-penta-azido 5,4′,2″,4″,6″-penta-*O*-benzyl/alkyl tobramycin (12a – f)[1]

Sodium hydride (25 equiv, 60% dispersion in mineral oil) was added to dry *N,N*-dimethylformamide (DMF) (3 mL) under inert atmosphere and stirred at 0 °C. Penta-azidotobramycin **11** (1 equiv in dry DMF) was then added dropwise. After 5 min, the alkyl halide (10 equiv) was added, followed by tetrabutylammonium iodide (1 equiv). The reaction mixture was warmed to ambient temperature and stirred for 16 h. The reaction progress was monitored using TLC (2:8 ethyl acetate/hexanes). After completion, the reaction mixture was concentrated under reduced pressure. The residue was diluted with ethyl acetate (100 mL), washed thrice with ice-cold water (50 mL) and brine solution (50 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude azido compound was purified using flash chromatography with an elution gradient of 8 – 10% ethyl acetate in hexanes (v/v) to obtain the desired product.

1,3,2′,6′,3″-penta-azido 5,4′,2″,4″,6″-penta-*O*-benzyl tobramycin (12a)[1]

The synthesis followed general procedure A using penta-azidotobramycin **11** (0.040 g, 0.067 mmol) and benzyl bromide (0.114 g, 0.669 mmol) to afford compound **12a** (0.063 g, 90%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.16 (m, 16H), 7.16 – 7.10 (m, 4H), 7.05 – 6.95

(m, 3H), 6.93 – 6.88 (m, 2H), 5.57 (d, *J* = 3.7 Hz, 1H), 5.37 (d, *J* = 3.6 Hz, 1H), 4.84 (s, 2H), 4.77 – 4.61 (m, 2H), 4.56 (dd, *J* = 16.5, 11.2 Hz, 2H), 4.39 (dd, *J* = 11.8, 4.4 Hz, 2H), 4.25 – 4.10 (m, 3H), 3.76 – 3.55 (m, 4H), 3.53 – 3.43 (m, 3H), 3.43 – 3.24 (m, 5H), 3.20 (dd, *J* = 11.0, 1.9 Hz, 1H), 3.04 (dd, *J* = 10.9, 2.7 Hz, 1H), 2.98 – 2.89 (m, 1H), 2.37 – 2.21 (m, 2H), 2.01 – 1.87 (m, 1H), 1.64 – 1.51 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.92, 137.64, 137.48, 137.39, 137.33, 128.55, 128.54, 128.43, 128.34, 128.21, 128.14, 128.10, 128.06, 127.89, 127.85, 127.76, 127.52, 127.18, 126.22, 96.35, 95.83, 83.29, 77.84, 77.48, 77.24, 77.14, 75.95, 74.87, 74.51, 73.52, 73.09, 71.86, 71.00, 70.84, 70.09, 67.73, 65.40, 60.22, 59.46, 56.22, 51.23, 31.95, 27.81. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{53}H_{57}N_{15}O_9Na^+$: 1070.4361; measured: 1070.4364.

1,3,2′,6′,3″-penta-azido 5,4′,2″,4″,6″-penta-*O*-isopentyl tobramycin (12b)

The synthesis followed general procedure A using penta-azidotobramycin **11** (0.040 g, 0.067 mmol) and isopentyl iodide (0.132 g, 0.669 mmol) to afford compound **12b** (0.044 g, 70%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.63 (d, *J* = 3.6 Hz, 1H), 5.44 (d, *J* = 3.6 Hz, 1H), 4.11 – 4.03 (m, 1H), 3.86 – 3.13 (m, 24H), 3.18 – 3.01 (m, 1H), 2.39 – 2.22 (m, 2H), 1.98 – 1.83 (m, 1H), 1.39 (d, *J* = 1.9 Hz, 16H), 0.86 – 0.78 (m, 30H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 96.54, 95.11, 83.31, 78.72, 77.23, 76.94, 76.28, 76.16, 74.14, 72.64, 71.29, 71.04, 70.06, 70.04, 69.93, 68.81, 67.30, 65.43, 60.78, 59.52, 56.31, 51.30, 39.28, 38.67, 38.59, 38.45, 38.42, 32.22, 27.75, 25.57, 25.07, 24.92, 24.87, 24.67, 22.88, 22.76, 22.69, 22.65, 22.60, 22.56, 22.53, 22.48, 22.47. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{43}H_{77}N_{15}O_9Na^+$: 970.5926; measured: 970.5924.

1,3,2′,6′,3″-penta-azido 5,4′,2″,4″,6″-penta-*O*-(4-chloro)benzyl tobramycin (12c)[1]

The synthesis followed general procedure A using penta-azidotobramycin **11** (0.100 g, 0.167 mmol) and chlorobenzyl chloride (0.269 g, 1.67 mmol) to afford compound **12c** (0.148 g, 72.54%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 – 7.14 (m, 12H), 7.07 – 7.03 (m, 4H,), 6.95 (d, *J* = 8.1 Hz, 2H), 6.76 (d, *J* = 8.0 Hz, 2H), 5.58 (d, *J* = 3.6 Hz, 1H) 5.27 (d, *J* = 3.6 Hz, 1H), 4.80 (d, *J* = 4.9 Hz, 2H), 4.72 – 4.69 (m, 1H), 4.61 – 4.58 (m, 1H), 4.55 – 4.48 (m, 2H), 4.38 – 4.33 (m, 2H), 4.15 – 4.10 (m, 2H), 4.05 – 4.03 (m, 1H), 2.32 (dt, *J* = 13.4, 4.5 Hz, 1H), 2.24 (dt, *J* = 11.6, 4.5 Hz, 1H), 1.96 – 1.88 (m, 1H), 1.60 – 1.53 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 136.00, 135.95, 135.88,

135.86, 135.73, 133.94, 133.84, 133.79, 133.58, 132.89, 129.45, 129.40, 129.08, 129.04, 128.71, 128.64, 128.49, 128.35, 127.31, 96.40, 95.57, 83.26, 77.69, 77.55, 77.19, 75.86, 74.07, 73.81, 72.75, 72.24, 72.01, 71.03, 70.05, 69.86, 67.57, 65.24, 60.27, 59.38, 56.16, 51.17, 31.96, 27.75. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{53}H_{52}Cl_5N_{15}O_9Na^+$: 1240.2407; measured: 1240.2439.

1,3,2′,6′,3″-penta-azido 5,4′,2″,4″,6″-penta-*O*-(4-*tert*-butyl)benzyl tobramycin (12d)

The synthesis followed general procedure A using penta-azidotobramycin **11** (0.100 g, 0.167 mmol) and *tert*-butyl benzyl bromide (0.379 g, 1.67 mmol) to afford compound **12d** (0.178 g, 80%) as an off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 – 7.27 (m, 5H), 7.25 – 7.20 (m, 4H), 7.17 – 7.14 (m, 5H), 7.08 (d, J = 7.9 Hz, 2H), 7.04 (d, J = 8.0 Hz, 2H), 7.01 (d, J = 8.0 Hz, 2H), 5.53 (d, J = 3.6 Hz, 1H), 5.42 (d, J = 3.6 Hz, 1H), 4.81 – 4.74 (m, 2H), 4.71 – 4.64 (m, 2H), 4.58 – 4.54 (m, 2H), 4.37 – 4.34 (m, 2H), 4.21 (d, J = 10.7 Hz, 1H), 4.14 – 4.10 (m, 2H), 3.83 – 3.75 (m, 2H), 3.59 (dt, J = 29.8, 9.3 Hz, 2H), 3.48 – 3.31 (m, 8H), 3.22 – 3.11 (m, 2H), 2.98 (dt, J = 13.0, 4.1 Hz, 1H), 2.28 (dt, J = 12.8, 4.4 Hz, 2H), 2.02 – 1.91 (m, 1H), 1.50 – 1.40 (m, 1H), 1.29 – 1.00 (m, 27H), 1.19 (s, 9H), 1.13 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 150.06, 149.97, 149.63, 149.43, 149.17, 134.02, 133.69, 133.40, 133.14, 126.97, 126.87, 126.74, 126.58, 126.03, 124.43, 124.22, 124.11, 124.05, 95.22, 95.12, 82.34, 77.37, 76.37, 76.23, 75.92, 74.81, 73.87, 73.31, 72.23, 71.91, 70.68, 69.92, 69.54, 69.22, 66.67, 64.45, 59.06, 58.50, 55.12, 50.20, 33.55, 33.48, 33.46, 33.37, 30.90, 30.47, 30.40, 30.34, 30.31, 30.29, 26.72. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{73}H_{97}N_{15}O_9Na^+$: 1350.7486; measured: 1350.7467.

1,3,2′,6′,3″-penta-azido 5,4′,2″,4″,6″-penta-*O*-biphenyl tobramycin (12e)[1]

The synthesis followed general procedure A using penta-azidotobramycin **11** (0.100 g, 0.167 mmol) and 4-bromobiphenyl (0.413 g, 1.67 mmol) to afford compound **12e** (0.182 g, 76%) as an off-white solid. ^{1}H NMR (500 MHz, CDCl3) δ 7.70 – 7.64 (m, 8H), 7.59 (d, J = 7.8 Hz, 2H), 7.52 – 7.38 (m, 26H), 7.34 – 7.27 (m, 7H), 6.98 (d, J = 7.7 Hz, 2H), 5.83 (d, J = 3.6 Hz, 1H), 5.58 (d, J = 3.6 Hz, 1H), 5.09 – 5.02 (m, 2H), 4.96 (d, J = 11.9 Hz, 1H), 4.88 (d, J = 11.8 Hz, 1H), 4.78 (d, J = 11.0 Hz, 1H), 4.74 (d, J = 11.6 Hz, 1H), 4.67 (d, J = 12.2 Hz, 1H), 4.54 (d, J = 11.6 Hz, 1H), 4.36 (d, J = 12.2

Hz, 1H), 4.32 – 4.29 (m, 2H), 4.00 – 3.96 (m, 2H), 3.85 – 3.79 (m, 2H), 3.68 – 3.61 (m, 3H), 3.58 – 3.49 (m, 5H), 3.46 – 3.44 (m, 1H), 3.37 – 3.34 (m, 1H), 3.10 (dt, *J* = 13.1, 4.1 Hz, 1H), 2.48 – 2.41 (m, 2H), 2.16 – 2.09 (m, 1H), 1.78 – 1.70 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 141.09, 141.07, 140.86, 140.75, 140.72, 140.68, 140.59, 140.53, 140.17, 139.89, 136.87, 136.53, 136.49, 136.45, 128.89, 128.87, 128.84, 128.79, 128.75, 128.73, 128.41, 128.02, 127.47, 127.43, 127.37, 127.35, 127.28, 127.19, 127.17, 127.11, 127.01, 126.94, 126.89, 126.83, 96.42, 95.83, 83.47, 77.78, 77.52, 77.36, 77.21, 76.11, 74.90, 74.34, 73.33, 72.84, 71.97, 71.10, 70.58, 70.08, 67.59, 65.54, 60.33, 59.55, 56.25, 51.30, 32.02, 27.88. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{83}H_{77}N_{15}O_9Na^+$: 1450.5921; measured: 1450.5947.

1,3,2′,6′,3″-penta-azido 5,4′,2″,4″,6″-penta-*O*-(3,5-dichloro)benzyl tobramycin (12f)

The synthesis followed general procedure A using penta-azidotobramycin **11** (0.070 g, 0.117 mmol) and 3,4-dichlorobenzyl bromide (0.281 g, 1.17 mmol) to afford compound **12f** (0.118 g, 72%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.3 Hz, 1H), 7.31 (dd, *J* = 13.3, 2.1 Hz, 2H), 7.26 – 7.16 (m, 7H), 7.13 – 7.11 (m, 2H), 7.06 (dd, *J* = 8.4, 2.1 Hz, 1H), 6.95 (d, *J* = 2.1 Hz, 1H), 6.74 (d, *J* = 8.3 Hz, 1H), 5.69 (d, *J* = 3.6 Hz, 1H), 5.16 (d, *J* = 3.6 Hz, 1H), 4.93 (d, *J* = 14.1 Hz, 1H), 4.85 – 4.81 (m, 2H), 4.70 (d, *J* = 12.8 Hz, 1H), 4.62 – 4.58 (m, 2H), 4.49 – 4.41 (m, 2H), 4.31 (d, *J* = 13.1 Hz, 1H), 4.25 (d, *J* = 12.1 Hz, 1H), 4.15 – 4.12 (m, 1H), 3.70 (t, *J* = 10.0 Hz, 1H), 3.65 – 3.62 (m, 2H), 3.57 – 3.47 (m, 4H), 3.43 – 3.31 (m, 5H), 3.24 (t, *J* = 9.8 Hz, 1H), 3.16 – 3.14 (m, 1H), 2.92 (dt, *J* = 13.1, 4.1 Hz, 1H), 2.37 – 2.33 (m, 1H), 2.31 – 2.27 (m, 1H), 1.94 – 1.87 (m, 1H), 1.64 – 1.56 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.37, 134.29, 134.28, 134.03, 134.00, 133.93, 133.87, 133.76, 133.73, 133.55, 133.54, 133.27, 133.04, 131.64, 130.27, 130.17, 130.05, 129.95, 129.27, 129.19, 129.14, 129.05, 128.73, 127.33, 127.23, 127.10, 127.00, 126.82, 126.51, 96.70, 95.15, 83.34, 78.28, 77.54, 77.24, 75.53, 72.43, 71.82, 71.00, 70.73, 69.94, 69.84, 69.20, 68.33, 67.35, 65.29, 60.11, 59.19, 56.29, 51.14, 31.80, 29.69, 27.67. MALDI-TOF-MS m/z $[M+H]^+$ calculated for $C_{53}H_{48}Cl_{10}N_{15}O_9^+$: 1388.0645; measured: 1388.0666.

Synthetic procedure for the preparation of 1,3,2′,6′-tetra-azidonebramine (13)

Penta-azidotobramycin **11** (0.500 g, 0.837 mmol) was dissolved in 10% HCl in MeOH and heated at 70 °C for 24 h. After reaction completion, the solvent was evaporated. The crude residue was purified using flash chromatography and eluted using 2% MeOH in DCM (v/v) to afford tetra-azidonebramine **13** (0.180 g, 87%) as an off-white solid. ^{1}H NMR (500 MHz, MeOD-d_4) δ 5.58 (d, J = 3.5 Hz, 1H), 4.09 – 4.05 (m, 1H), 3.59 – 3.54 (m, 1H), 3.51 – 3.44 (m, 4H), 3.43 – 3.37 (m, 2H), 3.27 – 3.24 (m, 1H), 3.19 (dt, J = 12.9, 4.1 Hz, 1H), 2.28 – 2.21 (m, 1H), 2.14 (dt, J = 11.4, 4.6 Hz, 1H), 2.03 – 1.96 (m, 1H), 1.46 – 1.38 (m, 1H). ^{13}C NMR (125 MHz, MeOD-d_4) δ 96.91, 79.13, 76.63, 76.56, 72.62, 65.17, 60.36, 59.64, 56.45, 51.11, 31.82, 30.87. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{12}H_{18}N_{12}O_5Na^+$: 433.1415; measured: 433.1450.

1,3,2′,6′-tetra-azido-5,6,4′-tri-*O* benzyl nebramine (14a)

The synthesis followed general procedure A using tetra-azidonebramine **13** (0.065 g, 0.158 mmol), benzyl bromide (0.188 g, 0.950 mmol) and NaH (15 equiv) to afford compound **14a** (0.079 g, 74%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.29 (m, 15H), 5.59 (d, J = 3.6 Hz, 1H), 5.03 (d, J = 10.6 Hz, 1H), 4.93 – 4.82 (m, 3H), 4.69 (d, J = 11.5 Hz, 1H), 4.51 (d, J = 11.6 Hz, 1H), 4.28 – 4.25 (m, 1H), 3.71 (t, J = 9.5 Hz, 1H), 3.62 (t, J = 9.2 Hz, 1H), 3.59 – 3.41 (m, 6H), 3.09 (dt, J = 13.0, 4.1 Hz, 1H), 2.40 (dt, J = 11.4, 4.5 Hz, 1H), 2.33 (dt, J = 13.3, 4.5 Hz, 1H), 2.13 – 2.06 (m, 1H), 1.56 – 1.48 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.92, 137.59, 137.41, 128.56, 128.51, 128.47, 128.10, 128.06, 128.02, 127.90, 127.69, 127.33, 96.75, 84.75, 84.53, 77.31, 75.90, 75.10, 72.03, 70.97, 70.89, 60.36, 59.64, 56.17, 51.24, 32.34, 27.82. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{33}H_{36}N_{12}O_5Na^+$: 703.2824; measured: 703.2452.

1,3,2′,6′-tetra-azido-5,6,4′-tri-*O* isopentyl nebramine (14b)

The synthesis followed general procedure A using tetra-azidonebramine **13** (0.065 g, 0.158 mmol), isopentyl iodide (0.162 g, 0.950 mmol) and NaH (15 equiv) to afford compound **14b** (0.072g, 73%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.51 (d, J = 3.6 Hz, 1H), 4.14 – 4.11 (m, 1H), 3.93 – 3.88 (m, 1H), 3.82 – 3.71 (m, 3H), 3.67 – 3.62 (m, 1H), 3.53 – 3.48 (m, 2H), 3.44 (dd, J = 13.1, 4.6 Hz, 1H), 3.39 – 3.26 (m, 5H), 3.13 – 3.07 (m, 2H), 2.38 – 2.34 (m, 1H), 2.24 – 2.20

(m, 1H), 2.00 – 1.93 (m, 1H), 1.72 – 1.35 (m, 10H), 0.92 – 0.89 (m, 18H). ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 96.74, 85.11, 84.69, 72.68, 72.57, 72.48, 70.97, 67.27, 60.14, 59.64, 56.33, 51.26, 39.34, 39.09, 38.65, 32.28, 27.80, 25.28, 25.06, 24.89, 22.87, 22.76, 22.75, 22.57, 22.54, 22.45. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{27}H_{48}N_{12}O_5Na^+$: 643.3763; measured: 643.2077.

General procedure B for the preparation of polycarbamoyl tobramycin (16a – b)[2]

N-Boc-protected tobramycin **15** was dissolved in dry pyridine and stirred at ambient temperature under inert atmosphere. Subsequently, aryl isocyanate (12.5 equiv) was added and continuously stirred for 36 – 48 h. After reaction completion, the mixture was concentrated under reduced pressure. The resulting residue was purified using flash chromatography and eluted using 30% ethyl acetate in hexanes (v/v) to obtain the desired products.

1,3,2′,6′,3″-penta-*N*-Boc 5,4′,2″,4″,6″-penta-phenylcarbamoyl tobramycin (16a)

The synthesis followed general procedure B using *N*-Boc-protected tobramycin **15** (0.100 g, 0.103 mmol) and phenyl isocyanate (0.154 g, 1.29 mmol) to afford compound **16a** (0.074 g, 46%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) *δ* 7.44 (d, *J* = 7.9 Hz, 2H), 7.38 (d, *J* = 7.9 Hz, 2H), 7.32 (ddd, *J* = 8.4, 2.8, 1.3 Hz, 4H), 7.28 – 7.13 (m, 13H), 7.00 (h, *J* = 5.4, 3.6 Hz, 5H), 6.91 (t, *J* = 7.4 Hz, 1H), 5.24 (s, 1H), 4.94 (d, *J* = 8.1 Hz, 2H), 4.82 (d, *J* = 9.5 Hz, 2H), 4.43 (s, 1H), 4.05 – 3.96 (m, 5H), 3.85 – 3.82 (m, 1H), 3.69 (s, 0H), 3.61 (s, 1H), 3.55 – 3.53 (m, 1H), 3.46 (s, 1H), 3.27 – 3.18 (m, 2H), 2.16 – 2.08 (m, 1H), 1.81 (s, 1H), 1.73 (s, 1H), 1.60 – 1.53 (m, 1H), 1.35 – 1.02 (m, 45H). ^{13}C NMR (125 MHz, DMSO-d_6) *δ* 155.53, 154.88, 153.75, 153.30, 153.27, 153.12, 152.74, 139.58, 139.32, 139.19, 138.99, 128.99, 128.91, 128.87, 123.16, 123.22, 123.00, 122.89, 122.80, 119.96, 119.59, 119.30, 119.26, 119.20, 96.89, 95.92, 78.96, 78.80, 78.38, 70.66, 70.10, 69.71, 68.99, 68.42, 62.59,40.85, 40.68, 40.52, 30.87, 28.80, 28.76, 28.52, 28.50, 28.39. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{78}H_{102}N_{10}O_{24}Na^+$: 1585.6961; measured: 1585.6943.

1,3,2′,6′,3″-penta-*N*-Boc 5,4′,2″,4″,6″-penta-(4-chlorophenyl)carbamoyl tobramycin (16b)

The synthesis followed general procedure B using *N*-Boc-protected tobramycin **15** (0.100 g, 0.103 mmol) and 4-chlorophenyl isocyanate (0.198 g, 1.29 mmol) to afford compound **16b** (0.070 g,

39%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.88 – 9.69 (m, 3H, NH carbamate), 9.55 – 9.41 (m, 2H, NH carbamate), 7.54 – 7.21 (m, 20H, C_6H_4Cl), 7.11 – 7.00 (m, 2H, NH Boc), 6.76 – 6.54 (m, 3H, NH Boc), 5.20 – 4.91 (m, 4H), 4.81 – 4.78 (m, 1H), 4.58 – 4.54 (m, 1H), 4.13 – 3.62 (m, 9H), 3.21 – 3.19 (m, 1H), 2.15 (s, 1H), 1.86 – 1.75 (m, 2H), 1.49 – 1.13 (m, 48H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 156.27, 155.95, 155.79, 155.50, 154.77, 153.44, 153.12, 152.97, 152.50, 148.16, 138.84, 138.64, 138.41, 138.37, 137.65, 129.23, 129.09, 129.00, 128.97, 128.94, 128.91, 128.84, 126.99, 126.77, 126.53, 126.38, 126.28, 124.67, 121.15, 120.74, 120.30, 120.20, 119.16, 97.30, 96.03, 79.64, 78.59, 78.34, 78.22, 76.42, 70.34, 69.63, 69.07, 68.54, 68.02, 61.92, 53.72, 52.02, 50.95, 49.21, 48.98, 34.10, 30.72, 28.72, 28.47, 28.22, 28.08. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{78}H_{97}Cl_5N_{10}O_{24}Na^+$: 1755.5018; measured: 1755.5027.

General procedure C for the preparation of 1,3,2′,6′,3″-penta-amino 5,4′,2″,4″,6″-penta-*O*-benzyl/alkyl tobramycin and 1,3,2′,6′-tetra-amino 5,6,4′-tri-*O*-benzyl/alkyl nebramine (1–8)[1]

The azido compound (1.0 equiv) was dissolved in tetrahydrofuran (THF) (4 mL) and water (0.5 mL) and stirred at ambient temperature. A solution of 1 M trimethylphosphine (PMe_3) in THF (10.0 equiv) was then added, followed by an 0.1 N aqueous solution of sodium hydroxide (0.2 mL). The reaction progress was monitored by TLC (28% ammonium hydroxide (NH_4OH) in 1:9 MeOH/DCM). After 24 h, the reaction mixture was concentrated under reduced pressure. The crude residue was purified using flash chromatography and eluted with 28% (10% NH_4OH in MeOH)/DCM to give the desired product. The amino compounds were dissolved in 3 M HCl in MeOH (0.5 mL), stirred for 1 min and the solvent was evaporated under reduced pressure to obtain the products as HCl salts.

1,3,2′,6′,3″-penta-amino 5,4′,2″,4″,6″-penta-*O*-benzyl tobramycin (1)[1]

The synthesis followed general procedure C using penta-azido **12a** to afford compound **1** as a white solid. HCl salt: ^{1}H NMR (500 MHz, D_2O) δ 7.56 – 7.40 (m, 18H, benzyl), 7.31 – 7.30 (m, 3H, benzyl), 7.24 – 7.18 (m, 4H, benzyl), 5.32 (dd, 1H, H-1″), 5.17 (dd, 1H, H-1′), 5.05 – 5.02 (m, 1H, CH_2 benzylic), 4.87 (m, 2H, CH_2 benzylic), 4.70 – 4.63 (m, 3H, CH_2 benzylic), 4.57 – 4.54 (m, 1H, H-5′), 4.44 – 4.42 (m, 1H, CH_2 benzylic), 4.31 – 4.25 (m, 2H, H-4, CH_2 benzylic), 4.14 – 4.11 (m, 1H,

CH_2 benzylic), 4.00 – 3.93 (m, 3H, H-5, H-6, H-4″), 3.81 – 3.80 (m, 1H, H-2″), 3.79 – 3.74 (m, 2H, H-3″, H-5″), 3.71 – 3.69, 3.68 (m, 1H, H-4′), 3.65 – 3.52 (m, 3H, H-1, H-3, H-6″), 3.33, – 3.23 (m, 3H, H-2′, H-6′, H-6″), 3.13 – 3.10 (m, 1H, H-6′), 2.53 – 2.49 (m, 1H, H-2), 2.30 – 2.27 (m, 1H, H-3′), 2.05 – 1.93 (m, 2H, H-2, H-3′). ^{13}C NMR (126 MHz, D_2O) δ 137.42, 137.00, 136.41, 136.25, 135.55, 129.53, 129.46, 129.27, 129.15, 128.95, 128.92, 128.89, 128.86, 128.74, 128.57, 128.56, 128.53, 128.33, 126.82, 98.32, 92.97, 81.88, 81.56, 78.09, 75.37, 75.14, 74.69, 74.48, 73.82, 73.19, 73.15, 71.66, 71.32, 70.03, 66.72, 52.81, 49.68, 48.32, 46.82, 37.93, 27.39, 25.72. MALDI-TOF-MS m/z $[M+K]^+$ calculated for $C_{53}H_{67}N_5O_9K^+$: 956.4576; measured: 956.4537.

1,3,2′,6′,3″-penta-amino 5,4′,2″,4″,6″-penta-*O*-isopentyl tobramycin (2)

The synthesis followed general procedure C using penta-azido **12a** to afford compound **2** as a white solid. HCl salt: ^{1}H NMR (500 MHz, D_2O) *δ* 5.36 – 5.35 (m, 1H, H-1″), 5.34 – 5.33 (m, 1H, H-1′), 4.53 – 4.51 (d, 1H, H-5′), 4.22 – 4.18 (m, 1H, H-4), 3.96 – 3.46 (m, 23H, OCH_2 isopentyl, H-1, H-3, H-5, H-6, H-2′, H-4′, H-6′, H-2″, H-3″, H-4″, H-6″), 3.30 – 3.26 (m, 1H, H-6′), 2.58 – 2.53 (m, 1H, H-2), 2.40 – 2.30 (m, 2H, H-3′), 2.10 – 2.02 (m, 1H, H-2), 1.72 – 1.46 (m, 15H, CH_2 isopentyl, CH isopentyl), 0.96 – 090 (m, 30H, CH_3 isopentyl). ^{13}C NMR (126 MHz, D_2O) δ 98.32, 93.03, 81.95, 81.14, 77.84, 76.85, 74.54, 73.48, 72.36, 71.72, 71.55, 70.73, 70.28, 68.42, 67.88, 53.11, 49.67, 48.30, 47.33, 38.33, 38.16, 38.06, 37.85, 37.70, 37.56, 27.45, 25.81, 25.06, 24.61, 24.57, 24.50, 22.53, 22.19, 22.13, 21.96, 21.94, 21.84, 21.82, 21.77. MALDI-TOF-MS m/z $[M+H]^+$ calculated for $C_{43}H_{88}N_5O_9^+$: 818.6582; measured: 818.6563.

1,3,2′,6′-tetra-amino 5,6,4′-tri-*O*-benzyl nebramine (3)

The synthesis followed general procedure C using compound **14a** (0.070g, 0.102 mmol) and PMe_3 solution (0.822 ml, 0.822 mmol) to afford compound **3** (0.058g, 98%) as a colorless oil. Free amine: ^{1}H NMR (500 MHz, MeOD-d_4) *δ* 7.41 – 7.38 (m, 8H, benzyl), 7.37 – 7.29 (m, 7H, benzyl), 5.16 (d, *J* = 3.2 Hz, 1H, H-1′), 5.08 (d, *J* = 11.9 Hz, 1H, CH_2 benzylic), 4.94 (d, *J* = 11.1 Hz, 1H, CH_2 benzylic), 4.88 (d, *J* = 12.3 Hz, 1H, CH_2 benzylic), 4.79 (d, *J* = 11.1 Hz, 1H, CH_2 benzylic), 4.71 (d, *J* = 11.6 Hz, 1H, CH_2 benzylic), 4.52 (d, *J* = 11.5 Hz, 1H, CH_2 benzylic), 3. ′81 – 3.77 (m, 1H, H-5′), 3.64 (t, *J* = 9.3 Hz, 1H, H-4), 3.54 (t, *J* = 9.3 Hz, 1H, H-4′), 3.42 – 3.39 (m, 1H, H-5), 3.37 – 3.34 (m, 1H,

H-6), 3.06 (dd, *J* = 13.4, 3.2 Hz, 1H, H-6'), 2.95 – 2.85 (m, 2H, H-1, H-3), 2.80 – 2.74 (m, 2H, H-2', H-6'), 2.20 (dt, *J* = 11.8, 4.4 Hz, 1H, H-3), 2.07 (dt, *J* = 13.0, 4.3 Hz, 1H, H-2), 1.65 – 1.58 (m, 1H, H-3'), 1.36 – 1.29 (m, 1H, H-2). ^{13}C NMR (125 MHz, MeOD-*d*$_4$) *δ* 138.36, 138.25, 128.12, 128.04, 127.63, 127.44, 127.37, 127.15, 126.54, 99.19, 87.10, 84.61, 84.17, 75.04, 74.19, 73.63, 72.37, 70.06, 51.22, 50.67, 49.68, 42.25, 36.08, 31.79. MALDI-TOF-MS m/z: $[M+Na]^+$ calculated for $C_{33}H_{44}N_4O_5Na^+$ 599.3204; measured: 599.3204. HCl salt: ^{1}H NMR (500 MHz, MeOD-*d*$_4$) *δ* 7.42 (s, 2H), 7.39 – 7.25 (m, 13H), 5.43 (s, 1H), 4.91 (q, *J* = 9.4, 7.1 Hz, 4H), 4.65 (dd, *J* = 20.5, 8.6 Hz, 2H), 4.53 (d, *J* = 12.4 Hz, 2H), 4.04 – 4.00 (m, 1H), 3.86 – 3.82 (m, 1H), 3.71 – 3.52 (m, 3H), 3.26 – 3.17 (m, 3H), 2.55 – 2.53 (m, 1H), 2.28 – 2.24 (m, 2H), 2.15 – 2.13 (m, 1H). ^{13}C NMR (125 MHz, MeOD-*d*$_4$) *δ* 139.15, 138.73, 138.63, 129.79, 129.58, 129.41, 129.26, 129.11, 129.08, 128.88, 128.77, 128.63, 93.99, 84.74, 81.82, 77.61, 76.31, 76.16, 74.12, 72.62, 72.50, 50.65, 50.36, 40.39, 29.28, 27.98. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{33}H_{44}N_4O_5Na^+$: 599.3204; measured: 599.3237.

1,3,2',6'-tetra-amino 5,6,4'-tri-*O*-isopentyl nebramine (4)

The synthesis followed general procedure C using tetra-azido nebramine **14b** (0.070g, 0.113 mmol) and PMe_3 solution (0.902 ml, 0.902 mmol) to afford compound **4** (0.082g, 82%) as a light-yellow oil. Free amine: ^{1}H NMR (500 MHz, MeOD-*d*$_4$) *δ* 5.12 (d, *J* = 3.2 Hz, 1H, H-1'), 3.99 – 3.93 (m, 2H, *O*-CH_2 isopentyl), 3.83 – 3.72 (m, 4H, *O*-CH_2 isopentyl), 3.51 – 3.47 (m, 1H, H-5'), 3.41 – 3.38 (m, 1H, H-4), 3.33 – 3.27 (m, 2H, H-5, H-4'), 3.10 (dd, *J* = 13.4, 3.2 Hz, 1H, H-6'), 3.03 (t, *J* = 9.4 Hz, 1H, H-6), 2.92 – 2.74 (m, 4H, H-1, H-3, H-2', H-6'), 2.28 (dt, *J* = 11.8, 4.4 Hz, 1H, H-3'), 2.03 (dt, *J* = 13.0, 4.2 Hz, 1H, H-2), 1.82 – 1.50 (m, 10H, H-3', CH isopentyl, CH_2 isopentyl), 1.31 – 1.23 (m, 1H, H-2), 1.03 – 0.99 (m, 18H, CH_3 isopentyl). ^{13}C NMR (125 MHz, MeOD-*d*$_4$) *δ* 99.39, 86.84, 84.90, 83.96, 74.47, 72.31, 71.74, 71.73, 66.50, 51.33, 50.64, 49.80, 42.32, 39.10, 38.98, 38.62, 35.86, 31.94, 25.24, 24.96, 24.70, 22.00, 21.95, 21.90, 21.61, 21.58, 21.51. MALDI-TOF-MS m/z: $[M+Na]^+$ calculated for $C_{27}H_{56}N_4O_5Na^+$ 539.4143; measured: 539.4143. HCl salt: ^{1}H NMR (500 MHz, MeOD-*d*$_4$) δ 5.48 (s, 1H), 4.38 (s, 2H), 3.91 – 3.25 (m, 13H), 2.48 – 2.15 (m, 4H), 1.73 – 1.25 (m, 10H), 0.92 (s, 18H). ^{13}C NMR (125 MHz, MeOD-*d*$_4$) *δ* 93.83, 84.18, 81.94, 76.82, 73.78, 73.71, 73.43, 73.30, 69.41, 50.73, 50.40, 41.61, 40.60, 39.66, 39.62, 29.11, 28.47, 26.34, 26.31, 25.97,

23.70, 23.32, 23.21, 23.19, 23.12, 23.05. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{27}H_{56}N_4O_5Na^+$: 539.4143; measured: 539.4468.

1,3,2′,6′,3″-penta-amino 5,4′,2″,4″,6″-penta-*O*-(4-chloro)benzyl tobramycin (5)[1]

The synthesis followed general procedure C using compound **12c** (0.080g, 0.066 mmol) to afford compound **5** (0.064g, 90%) as a white solid. Free amine: ^{1}H NMR (500 MHz, MeOD-d_4) δ 7.50 – 7.44 (m, 4H, C_6H_4Cl), 7.40 – 7.29 (m, 12H, C_6H_4Cl), 7.21 (d, J = 8.1 Hz, 2H, C_6H_4Cl), 7.15 (d, J = 8.2 Hz, 2H, C_6H_4Cl), 5.44 (d, J = 3.2 Hz, 1H, H-1″), 5.05 (d, J = 3.2 Hz, 1H, H-1′), 4.98 (d, J = 12.5 Hz, 1H, CH_2 benzylic), 4.83 (d, J = 11.6 Hz, 2H, CH_2 benzylic), 4.74 (d, J = 11.5 Hz, 1H, CH_2 benzyl), 4.67 – 4.62 (m, 2H, CH_2 benzylic), 4.49 (d, J = 11.8 Hz, 1H, CH_2 benzylic), 4.44 (d, J = 11.6 Hz, 1H, CH_2 benzylic), 4.32 (d, J = 12.1 Hz, 1H, CH_2 benzylic), 4.16 (d, J = 12.1 Hz, 1H, CH_2 benzylic), 3.86 – 3.83 (m, 1H, H-5′), 3.82 – 3.79 (m, 1H, H-5″), 3.58 – 3.48 (m, 3H, H-4, H-6, H-5), 3.42 – 3.37 (m, 2H, H-4′, H-4″), 3.46 – 3.32 (m, 2H, H-2″, H-6″), 3.31 – 3.27 (m, 1H, H-3″), 3.19 – 3.16 (m, 1H, H-6″), 3.04 (dd, J = 13.4, 3.2 Hz, 1H, H-6′), 2.95 – 2.90 (m, 1H, H-3), 2.88 – 2.83 (m, 1H, H-1), 2.80 – 2.76 (m, 1H, H-2′), 2.73 (dt, J = 12.0, 3.8 Hz, 1H, H-6′), 2.12 (dt, J = 12.0, 4.3 Hz, 1H, H-3′), 2.01 (dt, J = 13.2, 4.3 Hz, 1H, H-2), 1.59 – 1.50 (m, 1H, H-3′), 1.31 – 1.24 (m, 1H, H-2). ^{13}C NMR (125 MHz, MeOD-d_4) δ 138.69, 138.62, 138.41, 138.05, 137.92, 135.04, 134.50, 134.43, 133.83, 131.14, 130.60, 130.44, 130.24, 129.82, 129.52, 129.48, 129.47, 129.03, 100.33, 99.15, 86.40, 86.25, 84.45, 81.94, 79.69, 75.22, 74.51, 73.92, 73.70, 73.66, 73.52, 72.40, 70.66, 69.67, 54.75, 52.94, 52.36, 51.01, 43.48, 38.02, 33.27. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{53}H_{62}Cl_5N_5O_9Na^+$: 1110.2882; measured: 1110.2825. HCl salt: ^{1}H NMR (500 MHz, D_2O) δ 7.53 – 7.36 (m, 11H), 7.30 – 7.06 (m, 9H), 5.16 – 5.14 (m, 2H), 4.93 – 4.86 (m, 2H), 4.69 – 4.55 (m, 6H), 4.33 – 4.20 (m, 3H), 4.02 – 3.93 (m, 4H), 3.77 – 3.58 (m, 6H), 3.38 – 3.29 (m, 3H), 3.15 – 3.08 (m, 2H), 2.54 – 2.52 (m, 1H), 2.29 – 2.26 (m, 1H), 2.07 – 1.97 (m, 2H). ^{13}C NMR (125 MHz, D_2O) δ 136.11, 135.65, 134.97, 134.89, 134.72, 134.29, 133.77, 133.58, 133.35, 130.90, 130.30, 129.76, 129.63, 129.40, 128.98, 128.78, 128.60, 128.06, 98.11, 93.11, 81.81, 81.15, 78.35, 75.41, 74.63, 74.22, 73.60, 73.37, 73.18, 72.30, 71.52, 70.54, 70.06, 66.58, 52.75, 49.62, 48.34, 46.92, 38.10, 27.39, 25.86.

1,3,2′,6′,3″-penta-amino 5,4′,2″,4″,6″-penta-*O*-(4-*tert*-butyl)benzyl tobramycin (6)

The synthesis followed general procedure C using penta-azido **12d** (0.110 g, 0.086 mmol) and PMe_3 solution (0.864 mL, 0.864 mmol) to afford compound **6** (0.098g, 94%) as an off-white solid. Free amine: ^{1}H NMR (500 MHz, MeOD-d_4) δ 7.49 – 7.47 (m, 2H, C_6H_3-tBu), 7.44 – 7.39 (m, 4H, C_6H_3-tBu), 7.38 – 7.32 (m, 8H, C_6H_3-tBu), 7.31 – 7.29 (m, 2H, C_6H_3-tBu), 7.17 – 7.12 (m, 4H, C_6H_3-tBu), 5.36 (d, *J* = 3.2 Hz, 1H, H-1″), 5.11 (d, *J* = 3.2 Hz, 1H, H-1′), 4.97 (d, *J* = 11.3 Hz, 1H, CH_2 benzylic), 4.86 (d, *J* = 10.0 Hz, 1H, CH_2 benzylic), 4.78 (d, *J* = 11.3 Hz, 1H, CH_2 benzylic), 4.72 (d, *J* = 11.3 Hz, 1H, CH_2 benzylic), 4.64 (d, *J* = 11.5 Hz, 1H, CH_2 benzylic), 4.52 (d, *J* = 11.1 Hz, 1H, CH_2 benzylic), 4.46 (d, *J* = 11.4 Hz, 1H, CH_2 benzylic), 4.41 (d, *J* = 11.1 Hz, 1H, CH_2 benzylic), 4.30 (d, *J* = 11.7 Hz, 1H, CH_2 benzylic), 4.04 (d, *J* = 11.7 Hz, 1H, CH_2 benzylic), 3.86 – 3.80 (m, 2H, H-5′, H-5″), 3.62 – 3.55 (m, 2H, H-4, H-5), 3.50 (t, *J* = 8.7 Hz, 1H, H-4′), 3.44 (t, *J* = 9.7 Hz, 1H, H-4″), 3.41 – 3.38 (m, 1H, H-6), 3.34 – 3.26 (m, 3H, H-2″, H-3″, H-6″), 3.17 (dd, *J* = 11.0, 2.0 Hz, 1H, H-6″), 3.05 (dd, *J* = 13.4, 3.3 Hz, 1H, H-6′), 2.94 (td, *J* = 8.3, 4.2 Hz, 1H, H-3), 2.87 – 2.82 (m, 1H, H-1), 2.82 – 2.76 (m, 1H, H-2′), 2.73 (dt, *J* = 12.0, 3.9 Hz, 1H, H-6′), 2.15 (dt, *J* = 11.9, 4.4 Hz, 1H, H-3′), 2.00 (dt, *J* = 13.1, 4.3 Hz, 1H, H-2), 1.64 – 1.57 (m, 1H, H-3′), 1.36 – 1.31 (m, 46H, H-2, ^{t}Bu). ^{13}C NMR (125 MHz, MeOD-d_4) δ 152.45, 152.00, 151.82, 151.79, 151.27, 136.79, 136.69, 136.54, 136.23, 136.04, 129.49, 129.11, 128.92, 128.82, 127.61, 126.62, 126.33, 126.27, 126.22, 126.20, 99.85, 99.53, 86.78, 85.15, 84.38, 81.76, 79.32, 75.07, 74.87, 74.53, 74.19, 73.32, 72.62, 71.27, 69.35, 54.73, 52.82, 52.15, 50.91, 49.46, 49.29, 43.26, 37.47, 35.45, 35.38, 35.35, 33.02, 31.92, 31.81, 31.79. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{33}H_{107}N_5O_9Na^+$: 1220.7961; measured: 1220.7950. HCl salt: ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.56 (s, 4H), 7.42 – 7.32 (m, 10H), 7.24 (d, *J* = 8.2 Hz, 2H), 7.17 (d, *J* = 8.1 Hz, 2H), 7.04 (d, *J* = 8.0 Hz, 2H), 5.30 (d, *J* = 3.0 Hz, 1H), 5.23 (d, *J* = 2.1 Hz, 1H), 5.06 (d, *J* = 11.4 Hz, 1H), 4.77 – 4.72 (m, 2H), 4.70 – 4.67 (m, 2H), 4.63 (d, *J* = 16.0 Hz, 2H), 4.47 (d, *J* = 11.1 Hz, 1H), 4.18 (d, *J* = 11.7 Hz, 1H), 4.09 (t, *J* = 9.4 Hz, 1H), 4.00 – 3.96 (m, 3H), 3.90 – 3.85 (m, 1H), 3.76 – 3.56 (m, 6H), 3.35 – 3.24 (m, 4H), 3.08 (dd, *J* = 14.0, 2.9 Hz, 1H), 2.57– 2.54 (m, 1H), 2.42– 2.32 (m, 1H), 2.26 (dt, *J* = 15.7, 4.4 Hz, 1H), 1.97 (dt, *J* = 15.5, 3.7 Hz, 1H), 1.57 – 1.54 (m, 1H), 1.33 – 1.25 (m, 45H). ^{13}C NMR (100 MHz, MeOD-d_4) δ 153.35, 152.34, 152.05, 151.93, 151.85, 136.61, 135.89, 135.86, 135.55, 134.35, 130.29, 129.21, 129.07, 128.76, 128.02, 127.24, 126.50, 126.47, 126.23, 126.20, 99.24, 94.50, 83.54, 82.42, 79.37, 76.71, 75.65, 75.59,

74.97, 74.35, 73.73, 72.31, 71.82, 68.80, 54.44, 51.09, 49.93, 48.49, 39.48, 35.61, 35.41, 35.40, 35.36, 35.33, 31.84, 31.80, 31.78, 31.76, 31.74, 28.73, 27.76.

1,3,2′,6′,3″-penta-amino 5,4′,2″,4″,6″-penta-*O*-biphenyl tobramycin (7)[1]

The synthesis followed general procedure C using penta-azido **12e** (0.100 g, 0.070 mmol) and PMe_3 solution (0.700 mL, 0.70 mmol) to afford compound **7** (0.073g, 80%) as an off-white solid. Free amine: ^{1}H NMR (500 MHz, MeOD-d_4) δ 7.64 (d, J = 8.2 Hz, 2H, biphenyl), 7.60 (d, J = 8.1 Hz, 2H, biphenyl), 7.55 (d, J = 8.1 Hz, 4H, biphenyl), 7.51 (d, J = 8.2 Hz, 2H, biphenyl), 7.45 – 7.41 (m, 4H, biphenyl), 7.40 – 7.23 (m, 27H, biphenyl), 7.11 (d, J = 8.1 Hz, 2H, biphenyl), 7.03 (d, J = 8.2 Hz, 2H, biphenyl), 5.40 (d, J = 3.2 Hz, 1H, H-1″), 4.99 (d, J = 3.2 Hz, 1H, H-1′), 4.96 (d, J = 12.4 Hz, 1H, CH_2 benzylic), 4.79 – 4.75 (d, J = 11.5 Hz, 4H, CH_2 benzylic), 4.61 – 4.57 (m, 2H, CH_2 benzylic), 4.46 (d, J = 11.8 Hz, 1H, CH_2 benzylic), 4.38 – 4.33 (m, 2H, CH_2 benzylic), 4.09 (d, J = 12.1 Hz, 1H, CH_2 benzylic), 3.87 – 3.83 (m, 1H, H-5′), 3.77 – 3.73 (m, 1H, H-5″), 3.52 – 3.44 (m, 3H, H-4, H-6, H-5), 3.41 – 3.36 (m, 2H, H-2″, H-6″), 3.29 – 3.27 (m, 2H, H-4″, H-6″), 3.15 (dd, J = 10.9, 1.9 Hz, 1H, H-3″), 2.97 (dd, J = 13.5, 3.3 Hz, 1H, H-3), 2.86 – 2.77 (m, 2H, H-1, H-6′), 2.76 – 2.70 (m, 1H, H-2′), 2.62 (dt, J = 12.0, 3.7 Hz, 1H, H-6′), 2.03 (dt, J = 12.0, 4.4 Hz, 1H, H-3′), 1.93 (dt, J = 13.1, 4.3 Hz, 1H, H-2), 1.54 – 1.47 (m, 1H, H-3′), 1.27 – 1.17 (m, 1H, H-2). ^{13}C NMR (125 MHz, MeOD-d_4) δ 141.00, 140.51, 140.49, 140.46, 140.41, 140.36, 140.34, 140.31, 140.06, 139.63, 137.48, 137.46, 137.25, 136.74, 136.61, 128.86, 128.52, 128.49, 128.47, 128.41, 128.07, 127.76, 127.13, 127.00, 126.95, 126.91, 126.90, 126.57, 126.52, 126.49, 126.48, 126.46, 126.44, 126.41, 126.37, 99.05, 97.79, 85.11, 84.59, 82.93, 80.38, 78.26, 73.72, 73.61, 73.07, 73.04, 72.69, 72.52, 70.89, 69.86, 67.98, 53.48, 51.72, 51.02, 49.62, 42.12, 36.67, 31.87. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{83}H_{87}N_5O_9Na^+$: 1320.6396; measured: 1320.6375. HCl salt: ^{1}H NMR (500 MHz, MeOD-d_4) δ 7.74 – 7.07 (m, 45H), 5.36 (s, 1H), 5.20 – 5.11 (m, 3H), 4.72 – 4.54 (m, 7H), 4.25 – 3.92 (m, 6H), 3.78 – 3.54 (m, 6H), 3.21 – 3.01 (m, 4H), 2.52 – 2.28 (m, 3H), 1.92 – 1.89 (m, 1H), 1.28 – 1.24 (s, 1H). ^{13}C NMR (125 MHz, MeOD-d_4) δ 143.11, 142.30, 141.95, 141.84, 141.76, 141.71, 141.68, 141.56, 138.68, 137.89, 137.80, 137.62, 136.42, 131.25, 130.00, 129.96, 129.90, 129.86, 129.83, 129.79, 129.62, 129.30, 128.84, 128.75, 128.55, 128.50, 128.42, 128.35, 128.12, 128.09, 128.05,

127.88, 127.85, 127.83, 99.06, 94.62, 83.73, 82.20, 79.50, 76.93, 75.36, 74.92, 74.51, 74.13, 73.60, 72.20, 72.10, 68.94, 54.56, 51.23, 50.00, 39.63, 29.29, 27.89.

1,3,2′,6′,3″-penta-amino 5,4′,2″,4″,6″-penta-*O*-(3,5-dichloro)benzyl tobramycin (8)

The synthesis followed general procedure C using penta-azido **12f** (0.100g, 0.072 mmol) and PMe_3 solution (0.720 ml, 0.72 mmol) to afford compound **8** (0.088g, 97%) as a white solid. Free amine: ^{1}H NMR (500 MHz, MeOD-d_4) δ 7.55 (d, *J* = 8.3 Hz, 1H, $C_6H_3Cl_2$), 7.51 (d, *J* = 8.3 Hz, 1H, $C_6H_3Cl_2$), 7.47 (d, *J* = 2.1 Hz, 1H, $C_6H_3Cl_2$), 7.40 – 7.38 (m, 2H, $C_6H_3Cl_2$), 7.35 – 7.33 (m, 2H, $C_6H_3Cl_2$), 7.31 – 7.30 (m, 1H, $C_6H_3Cl_2$), 7.27 – 7.20 (m, 5H, $C_6H_3Cl_2$), 7.17 (dd, *J* = 8.3, 2.1 Hz, 1H, $C_6H_3Cl_2$) 7.14 – 7.12 (m, 1H, $C_6H_3Cl_2$), 5.49 (d, *J* = 3.2 Hz, 1H, H-1″), 4.92 (d, *J* = 3.2 Hz, 1H, H-1′), 4.89 – 4.86 (m, 2H, CH_2 benzylic), 4.75 (d, *J* = 12.1 Hz, 2H, CH_2 benzylic), 4.68 (d, *J* = 12.6 Hz, 1H, CH_2 benzylic), 4.63 (d, *J* = 12.2 Hz, 1H, CH_2 benzylic), 4.47 – 4.43 (m, 2H, CH_2 benzylic), 4.32 (d, *J* = 12.9 Hz, 1H, CH_2 benzylic), 4.15 (d, *J* = 12.9 Hz, 1H, CH_2 benzylic), 3.74 – 3.69 (m, 2H, H-5′ H-5″), 3.60 – 3.56 (m, 1H, H-4), 3.50 – 3.46 (m, 2H, H-5, H-6), 3.40 – 3.33 (m, 2H, H-4′, H-2″), 3.28 – 3.23 (m, 3H, H-3″, H-4″, H-6″), 3.15 (dd, *J* = 11.1, 1.9 Hz, 1H, H-6″), 2.94 (dd, *J* = 13.5, 3.2 Hz, 1H, H-6′), 2.87 – 2.76 (m, 2H, H-1, H-3), 2.71 – 2.67 (m, 1H, H-2′), 2.63 (dt, *J* = 12.2, 3.7 Hz, 1H, H-6′), 2.05 (dt, *J* = 11.8, 4.3 Hz, 1H, H-3′), 1.92 (dt, *J* = 13.1, 4.2 Hz, 1H, H-2), 1.48 – 1.31 (m, 1H, H-3′), 1.24 – 1.16 (m, 1H, H-2). ^{13}C NMR (125 MHz, MeOD-d_4) δ 136.48, 136.18, 136.13, 135.82, 135.71, 135.59, 135.51, 135.26, 135.14, 135.09, 134.99, 134.86, 134.37, 134.32, 132.78, 132.62, 132.06, 131.78, 131.34, 130.41, 130.09, 130.05, 129.95, 129.75, 129.53, 128.70, 128.42, 128.35, 128.31, 128.21, 100.74, 98.93, 87.09, 86.01, 84.55, 82.10, 79.74, 75.51, 74.17, 72.31, 71.86, 71.56, 71.04, 70.83, 70.15, 68.07, 54.84, 53.06, 52.52, 51.02, 43.70, 38.42, 33.25. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{53}H_{57}Cl_{10}N_5O_9Na^+$: 1280.0934; measured: 1280.0972. HCl salt: ^{1}H NMR (500 MHz, D_2O) *δ* 7.74 (d, *J* = 8.3 Hz, 1H), 7.62 – 7.59 (m, 2H), 7.52 – 7.44 (m, 5H), 7.41 – 7.38 (m, 2H), 7.32 (s, 1H), 7.25 – 7.20 (m, 2H), 7.10 (s, 2H), 5.27 (s, 1H), 5.23 (s, 1H), 5.13 – 5.05 (m, 3H), 4.99 – 4.93 (m, 2H), 4.78 – 4.71 (m, 2H), 4.63 – 4.57 (m, 2H), 4.50 – 4.46 (m, 1H), 4.22 – 4.17 (m, 3H), 4.10 – 3.93 (m, 4H), 3.87 – 3.77 (m, 3H), 3.66 (s, 1H), 3.54 – 3.52 (m, 1H), 3.45 – 3.37 (m, 1H), 3.31 – 3.23 (m, 3H), 2.70 (s, 1H), 2.32 – 2.25 (m, 1H), 2.13 (s, 2H). ^{13}C NMR (125 MHz, D_2O) *δ* 135.49, 134.81, 134.53, 134.36, 134.28, 134.12, 133.81, 133.48, 133.16, 132.96, 132.83, 132.05, 131.66, 131.22,

130.58, 130.44, 129.79, 129.25, 129.01, 128.87, 128.22, 127.78, 127.58, 127.42, 127.06, 97.93, 93.24, 82.23, 80.79, 78.49, 75.51, 74.02, 73.79, 72.07, 71.16, 70.37, 70.19, 69.66, 68.10, 67.79, 52.75, 49.40, 48.33, 47.31, 38.77, 27.52, 26.96, 26.67.

General procedure D for the preparation of 1,3,2',6',3"-penta-amino 5,4',2",4",6"-penta-phenylcarbamoyl tobramycin (9–10)[2]

TFA (1.5 mL) cooled to 0 °C was added to Boc-protected polycarbamoyl tobramycin and allowed to react for 3 min. The reaction was concentrated under reduced pressure. Afterwards, 2% MeOH in ether was added to the resulting residue, stirred for 1 minute and the solvent decanted. The crude product was purified using reverse-phase flash chromatography eluted with 100% deionized water.

1,3,2',6',3"-penta-amino 5,4',2",4",6"-penta-phenylcarbamoyl tobramycin (9)

The synthesis followed general procedure D using Boc-protected polycarbamoyl tobramycin **16a** to afford compound **9** (95%) as a white solid. ^{1}H NMR (500 MHz, MeOD-*d*$_4$) δ 7.52 – 7.51 (2H, m, benzyl), 7.46 –7.40 (6H, m, benzyl), 7.32 – 7.17 (12H, m, phenyl), 7.09 – 7.06 (1H, m, phenyl), 7.04 – 6.97 (3H, m, phenyl), 6.88 – 6.85 (1H, m, phenyl), 5.43 – 5.41 (2H, m, H-1', H-1"), 5.31 – 5.27 (1H, m, H-5), 5.15 – 5.11 (1H, m, H-4"), 5.07 – 5.04 (1H, m, H-2"), 4.85 (1H, m, H-4'), 4.43 – 4.37 (3H, m, H, H-4, H-5', H-6"), 4.27 – 4.18 (3H, m, H-6, H-3", H-5"), 4.11 – 4.08 (1H, m, H-6"), 3.79 – 3.73 (1H, m, H-1), 3.64 – 3.58 (1H, m, H-3), 3.56 – 3.51 (1H, m, H-6'), 3.37 – 3.34 (1H, m, H-2'), 3.23 – 3.20 (1H, m, H-6'), 2.66, 2.65 – 2.61 (1H, m, H-2), 2.36 – 2.26 (1H, m, H-3'), 2.23 – 2.18 (1H, m, H-2). ^{13}C NMR (126 MHz, MeOD-*d*$_4$) δ 162.14, 161.86, 153.62, 153.53, 152.80, 152.34, 152.13, 138.44, 138.12, 137.87, 137.63, 128.73, 128.45, 128.42, 128.35, 128.21, 123.56, 123.38, 123.17, 123.06, 122.59, 118.96, 118.87, 118.72, 118.35, 97.73, 92.99, 80.43, 77.47, 75.25, 74.35, 70.35, 69.24, 66.92, 65.41, 60.97, 51.20, 48.99, 48.41, 48.04, 46.67, 37.76, 27.63, 26.26. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{53}H_{62}N_{10}O_{14}Na^+$: 1085.4345; measured: 1085.4339.

1,3,2′,6′,3″-penta-amino 5,4′,2″,4″,6″-penta-(4-chloro)phenylcarbamoyl tobramycin (10)

The synthesis followed general procedure D using Boc-protected polycarbamoyl tobramycin **16b** to afford compound **10** (92%) as a white solid. ^{1}H NMR (500 MHz, MeOD-*d*$_4$) δ 7.53 – 7.50 (2H, m, benzyl), 7.48 – 7.43 (4H, m, phenyl), 7.40 – 7.38 (2H, m, benzyl), 7.32 – 7.29 (2H, m, phenyl), 7.28 – 7.25 (2H, m, phenyl), 7.22 – 7.17 (6H, m, phenyl), 7.16 – 7.13 (2H, m, phenyl), 5.40 – 5.38 (2H, m, H-1′, H-1″), 5.31 – 5.28 (1H, m, H-5), 5.14 – 5.10 (1H, m, H-4″), 5.06 – 5.03 (1H, dd, H-2″), 4.90 – 4.88 (1H, m, H-4′), 4.43 – 4.38 (2H, m, H, H-4, H-5′), 4.34 – 4.31 (1H, m, H-6″), 4.27 – 4.19 (3H, m, H-6, H-3″, H-5″), 4.09 – 4.06 (1H, m, H-6″), 3.80 – 3.75 (1H, m, H-1), 3.65 – 3.59 (1H, m, H-3), 3.56 – 3.51 (1H, m, H-6′), 3.34 – 3.33 (1H, m, H-2′), 3.22 – 3.19 (1H, m, H-6′), 2.67 – 2.63 (1H, m, H-2), 2.36 – 2.21 (2H, m, H-2, H-3′). ^{13}C NMR (126 MHz, MeOD-*d*$_4$) δ 161.96, 161.68, 161.40, 161.11, 153.34, 152.65, 152.23, 151.97, 137.28, 137.09, 137.02, 136.78, 136.58, 128.65, 128.41, 128.36, 128.31, 128.28, 128.21, 127.89, 127.44, 120.21, 120.14, 119.83, 119.60, 117.73, 115.41, 97.61, 92.94, 80.22, 77.23, 75.16, 70.31, 69.03, 66.89, 65.46, 60.97, 51.12, 48.87, 48.37, 48.20, 48.03, 47.86, 46.58, 38.30, 38.13, 37.97, 37.80, 37.63, 27.36, 26.14. MALDI-TOF-MS m/z $[M+Na]^+$ calculated for $C_{53}H_{57}Cl_5N_{10}O_{14}Na^+$: 1255.2396; measured: 1255.2353.

Table S1. Checkerboard studies of rifampicin and compounds **1** – **10** against *P. aeruginosa* PAO1.

Compound	Rifampicin MIC (μg/mL)	Rifampicin MIC combo (μg/mL)	Adjuvant MIC	MIC adjuvant combo (μg/mL)	Fold potentiation	FIC index
1	32	4	64	8	8	0.25
2	32	2	16	8	16	0.5625
3	16	4	>128	8	4	0.25<*x*<0.3125
4	16	4	>128	8	4	0.25<*x*<0.3125
5	32	32	>128	8	1	1<*x*<1.0625
6	32	32	>128	8	1	1<*x*<1.0625
7	32	32	>128	8	1	1<*x*<1.0625
8	32	32	>128	8	1	1<*x*<1.0625
9	16	16	64	8	1	1.125
10	16	16	64	8	1	1.125

Synergistic combinations are highlighted.

Table S2. Checkerboard studies of rifampicin and compounds **1** – **10** against *E. coli* ATCC 25922.

Compound	Rifampicin MIC (μg/mL)	Rifampicin MIC combo (μg/mL)	Adjuvant MIC	MIC adjuvant combo (μg/mL)	Fold potentiation	FIC index
1	16	0.0078125	32	8	2048	0.250488
2	16	16	4	1	1	1.25
3	16	0.03125	>128	8	512	0.001953 <*x*<0.064453
4	8	0.015625	>128	8	512	0.001953 <*x*<0.064453
5	16	16	>128	8	1	1<*x*<1.0625
6	16	16	>128	8	1	1<*x*<1.0625
7	16	16	>128	8	1	1<*x*<1.0625
8	16	16	>128	8	1	1<*x*<1.0625
9	8	0.0078125	32	8	1024	0.250977
10	8	0.5	32	8	16	0.3125

Synergistic combinations are highlighted.

Table S3. Checkerboard studies of rifampicin and compounds **1** – **10** against *A. baumannii* ATCC 17978.

Compound	Rifampicin MIC (μg/mL)	Rifampicin MIC combo (μg/mL)	Adjuvant MIC	MIC adjuvant combo (μg/mL)	Fold potentiation	FIC index
1	8	0.00390625	64	8	2048	0.125488
2	8	0.00390625	16	4	2048	0.250488
3	4	1	>128	8	4	0.25<*x*<0.3125
4	4	0.5	>128	8	8	0.125 <*x*<0.1875
5	4	4	>128	8	1	1<*x*<1.0625
6	4	4	>128	8	1	1<*x*<1.0625
7	4	4	>128	8	1	1<*x*<1.0625
8	4	4	>128	4	1	1<*x*<1.03125
9	4	0.03125	>128	8	128	0.007813 <*x*<0.070313
10	4	0.00390625	32	8	1024	0.250977

Synergistic combinations are highlighted.

Table S4. Checkerboard studies of novobiocin and compound **1** against Gram-negative bacteria.

Strain	Novobiocin MIC (μg/mL)	Novobiocin MIC combo (μg/mL)	Adjuvant MIC	MIC adjuvant combo (μg/mL)	Fold potentiation	FIC index
***P. aeruginosa* PAO1**	>1024	>1024	128	8	1	0.0625 <*x*<1.0625
***E. coli* ATCC 25922**	256	0.125	32	8	2048	0.250488
***A. baumannii* ATCC 17978**	32	0.5	64	8	64	0.140625

Synergistic combinations are highlighted.

Table S5. Checkerboard studies of vancomycin and compound **1** against Gram-negative bacteria.

Strain	Vancomycin MIC (μg/mL)	Vancomycin MIC combo (μg/mL)	Adjuvant MIC	MIC adjuvant combo (μg/mL)	Fold potentiation	FIC index
***P. aeruginosa* PAO1**	>256	>256	128	8	1	0.0625 <*x*<1.0625
***E. coli ATCC* 25922**	256	64	128	8	4	0.3125
***A. baumannii* ATCC 17978**	256	32	128	8	8	0.1875

Synergistic combinations are highlighted.

Table S6. Checkerboard studies of minocycline and compound **1** against Gram-negative bacteria.

Strain	Minocycline MIC (μg/mL)	Minocycline MIC combo (μg/mL)	Adjuvant MIC	MIC adjuvant combo (μg/mL)	Fold potentiation	FIC index
***P. aeruginosa* PAO1**	32	0.5	128	8	64	0.078125
***E. coli ATCC* 25922**	1	0.125	32	8	8	0.375
***A. baumannii* ATCC 17978**	0.125	0.0625	64	8	2	0.625

Synergistic combinations are highlighted.

Table S7. Checkerboard studies of doxycycline and compound **1** against Gram-negative bacteria.

Strain	Doxycycline MIC (μg/mL)	Doxycycline MIC combo (μg/mL)	Adjuvant MIC	MIC adjuvant combo (μg/mL)	Fold potentiation	FIC index
***P. aeruginosa* PAO1**	64	16	128	8	4	0.3125
***E. coli ATCC* 25922**	1	0.125	128	8	8	0.1875
***A. baumannii* ATCC 17978**	0.25	0.125	128	8	2	0.5625

Synergistic combinations are highlighted.

Table S8. Susceptibility profiles of MDR/XDR *P. aeruginosa* isolates

Stock number	PTZ	A/C	AZT	FOX	CFZ	CTR	CPM	CAZ	CTX	IMI	MER	DOR	ETP	CIP	LEV	MOX	TOB	GEN	AMK	TGC	ERC	OMC	CAM	CST
P259-969	64	>32	64	>32	>128	>64	>64	512	2048	32	1024	>1024	>32	>16	256	>16	256	>32	>64	32	8	64	1024	0.5
P262-101856	64	>32	32	>32	>128	64	32	16	128	32	32	16	>32	>16	64	>16	1024	>32	>64	32	8	64	2048	1
P264-104354	256	>32	64	>32	>128	>64	32	128	2048	32	64	16	>32	>16	64	>16	128	>32	8	32	8	64	4096	1
114228	ND	ND	32	ND	ND	ND	ND	8	128	ND	8	8	ND	ND	ND	ND	2	ND	ND	ND	16	128	ND	4
101243	128	>32	>128	>32	>128	>64	64	>128	ND	16	16	16	>32	1	ND	8	128	>32	>64	ND	ND	ND	1	1024

Table S9. Susceptibility profiles of MDR/XDR *A. baumannii* isolates

Stock number	PTZ	AZT	FOX	CFZ	CPM	CAZ	CTX	C/T	IMI	MER	CIP	LEV	MOX	TOB	GEN	AMK	TGC	MIN	DOX	ERC	OMC	CAM	CST
AB027	512	1024	ND	>128	>128	2048	>256	>16	32	16	>16	8	8	ND	32	>64	4	0.25	4	0.5	1	128	0.25
AB031	4	64	ND	>128	4	32	16	>16	0.25	1	0.25	0.25	0.125	ND	<0.5	2	8	0.25	0.5	0.25	2	128	0.25
110193	ND	64	ND	ND	ND	32	ND	ND	ND	ND	≤1	≤1	≤1	ND	ND	ND	ND	1	ND	ND	ND	128	0.5
92247	<1	128	32	128	4	16	ND	2	ND	4	≤0.063	ND	ND	ND	ND	<1	0.25	0.125	ND	ND	ND	ND	4
LAC-4	ND	128	ND	ND	ND	>128	1	8	<1	<1	>4	2	ND	>4	>4	4	<4	4	<4	0.063	1	32	0.125

Table S10. Susceptibility profiles of MDR/XDR *E. coli* isolates

Stock number	PTZ	A/C	AZT	FOX	CFZ	CPM	CAZ	C/T	IMI	MER	ETP	CIP	LEV	MOX	TOB	GEN	AMK	TGC	ERC	OMC	CAM	CST
94393	≤1	4	≤0.13	4	1	≤0.25	≤0.25	0.25	0.25	≤0.03	≤0.03	0.5	1	1	≤0.5	≤0.5	2	0.25	0.5	4	4	4
94474	16	>32	≤0.13	16	4	≤0.25	0.5	0.5	0.25	≤0.03	≤0.03	>16	32	16	32	16	2	1	1	16	4	16
107115	>512	>32	>64	>32	>128	>64	>32	>16	8	32	>32	>16	32	16	8	>32	2	0.25	0.125	4	512	0.125

PTZ: piperacillin-tazobactam, A/C: amoxicillin-clavulanic acid, AZT: aztreonam, FOX: cefoxitin, CFZ: cefazolin, CTP: ceftobiprole, CTR: ceftriaxone, CPM: cefepime, CTX: cefotaxime, CAZ: ceftazidime, C/T: ceftolozane-tazobactam, IMI: imipenem, MER: meropenem, DOR: doripenem, ETP: ertapenem, CIP: ciprofloxacin, LEV: levofloxacin, MOX: moxifloxacin, TOB: tobramycin, GEN: gentamicin, AMK: amikacin, TGC: tigecycline, MIN: minocycline, DOX: doxycycline, ERC: eravacycline, OMC: omadacycline, CAM: chloramphenicol, CST: colistin, ND: not determined.

Table S11. Checkerboard studies of rifampicin and compounds **1** against Gram-negative bacteria in the presence of 20 mM Mg^{2+}.

Strain	Rifampicin MIC (μg/mL)	Rifampicin MIC combo (μg/mL)	Adjuvant MIC	MIC adjuvant combo (μg/mL)	Fold potentiation	FIC index
***P. aeruginosa* PAO1**	16	16	128	8	1	1.0625
***E. coli ATCC* 25922**	16	8	128	8	2	0.5625
***A. baumannii* ATCC 17978**	4	0.25	128	8	16	0.125

Synergistic combinations are highlighted.

Table S12. Checkerboard studies of rifampicin and compounds **1** against Gram-negative bacteria in the presence of 150 mM Na^{2+}.

Strain	Rifampicin MIC (μg/mL)	Rifampicin MIC combo (μg/mL)	Adjuvant MIC	MIC adjuvant combo (μg/mL)	Fold potentiation	FIC index
***P. aeruginosa* PAO1**	32	32	128	8	1	1.0625
***E. coli ATCC* 25922**	8	0.015625	32	8	512	0.251953
***A. baumannii* ATCC 17978**	2	0.0078125	32	8	256	0.253906

Synergistic combinations are highlighted.

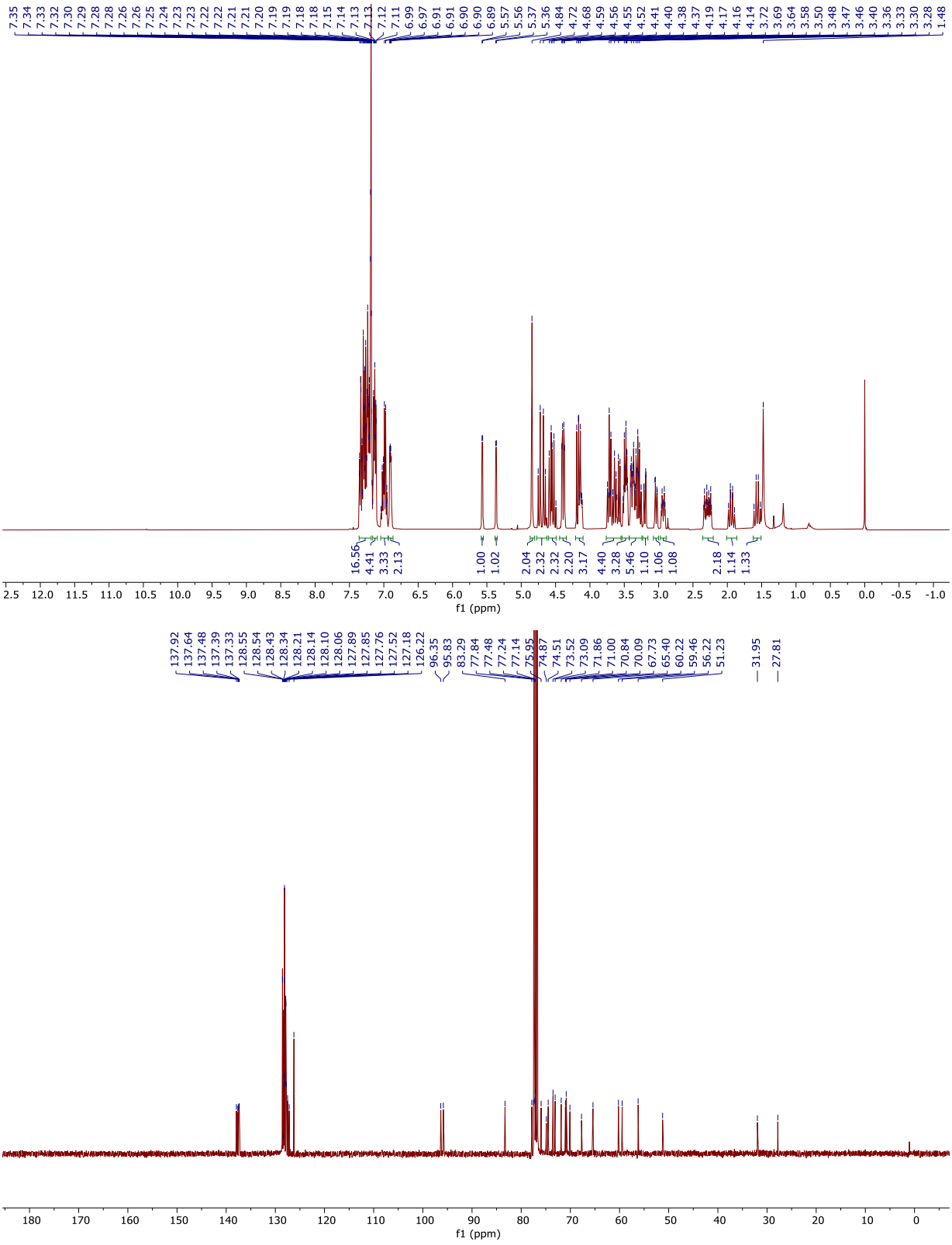
Table S13. Checkerboard studies of rifampicin and PMB against Gram-positive bacteria.

Strain	Rifampicin MIC (μg/mL)	Rifampicin MIC combo (μg/mL)	Adjuvant MIC	MIC adjuvant combo (μg/mL)	Fold potentiation	FIC index
***E. faecium* 114278**	16	16	>128	8	1	1<*x*<1.0625
***E. faecium* 128149**	16	16	>128	8	1	1<*x*<1.0625
MRSA	512	512	>128	8	1	1<*x*<1.0625

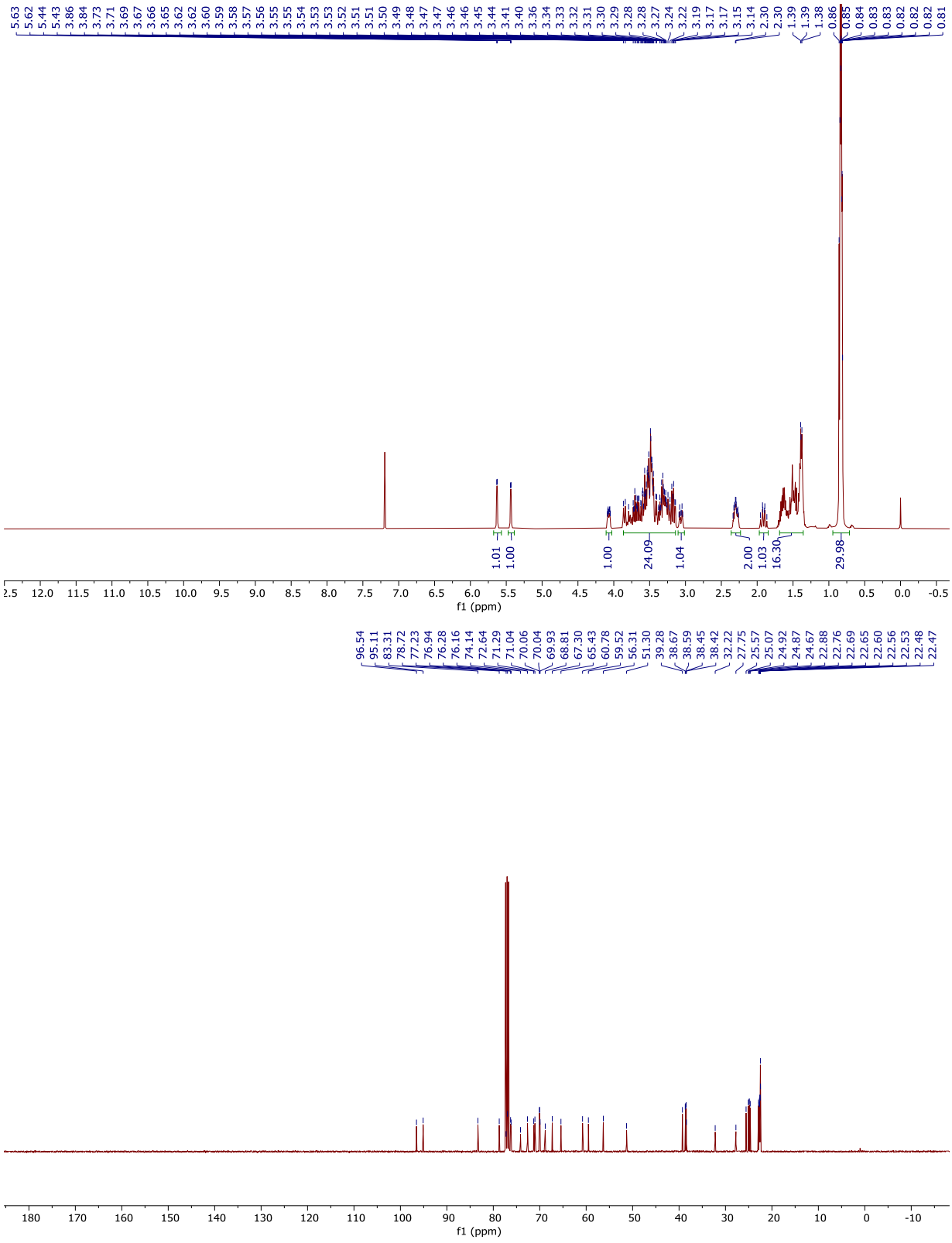
Table S14. Checkerboard studies of rifampicin and compound **1** against Gram-negative bacteria in the presence of FBS.

FBS (%)	Rifampicin MIC (μg/mL)	Rifampicin MIC combo (μg/mL)	Adjuvant MIC	MIC adjuvant combo (μg/mL)	Fold potentiation	FIC index
***P. aeruginosa* PAO1**						
0	32	4	64	8	8	0.25
10	32	32	128	8	1	1.0625
25	ND	ND	ND	ND	N/A	N/A
50	ND	ND	ND	ND	N/A	N/A
***E. coli* ATCC 25922**						
0	16	0.0078125	32	8	2048	0.25<*x*<0.250977
10	8	0.0078125	32	8	1024	0.250977
25	8	0.25	64	16	32	0.28125
50	32	32	64	8	1	1.125
***A. baumannii* ATCC 25922**						
0	8	0.00390625	64	8	2048	0.125<*x*<0.125977
10	16	0.25	128	8	16	0.078125
25	4	1	128	8	4	0.3125
50	8	2	>128	16	4	0.25<*x*<0.375

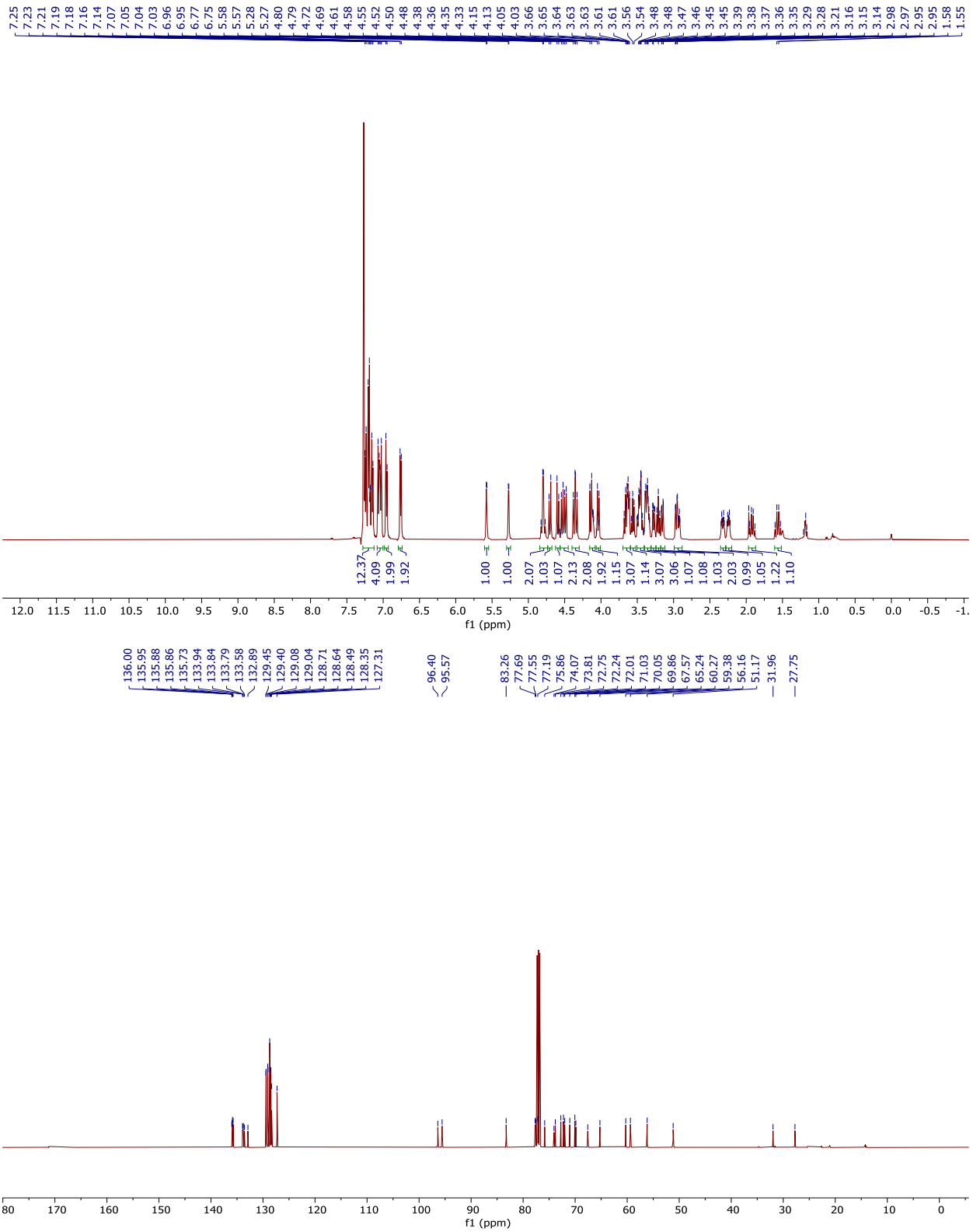
Synergistic combinations are highlighted. ND: not tested, N/A: not applicable.



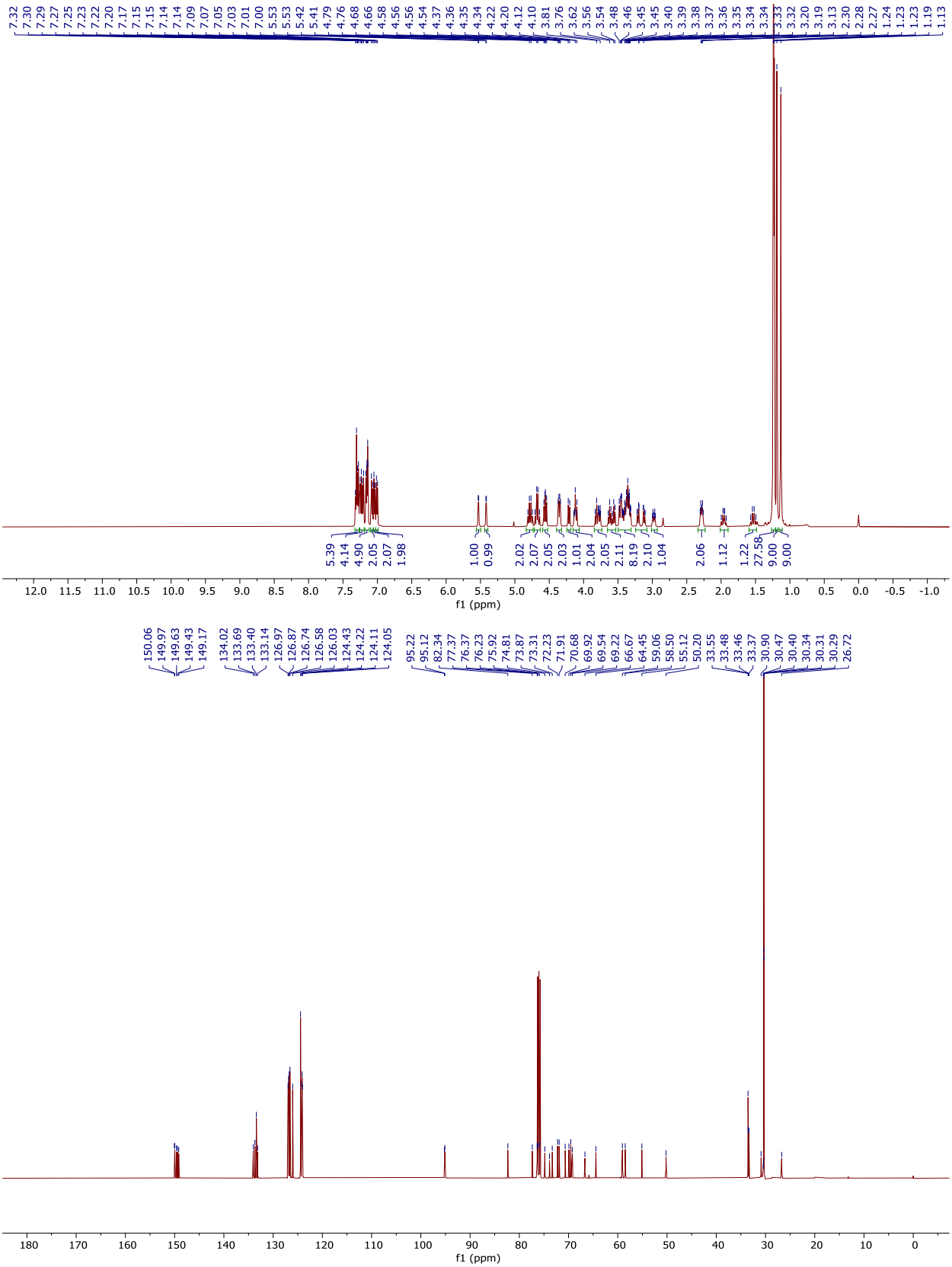
^{1}H and ^{13}C NMR of compound **12a**



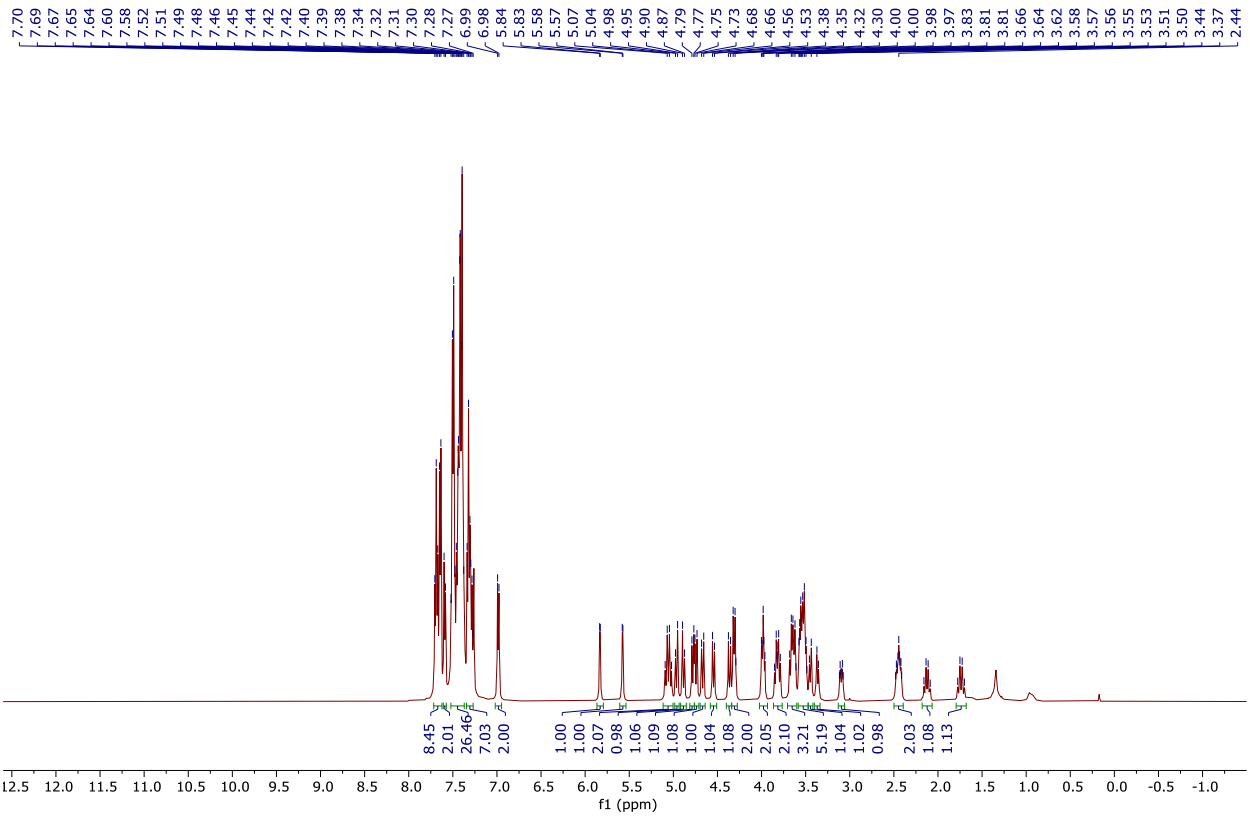
^{1}H and ^{13}C NMR of compound **12b**

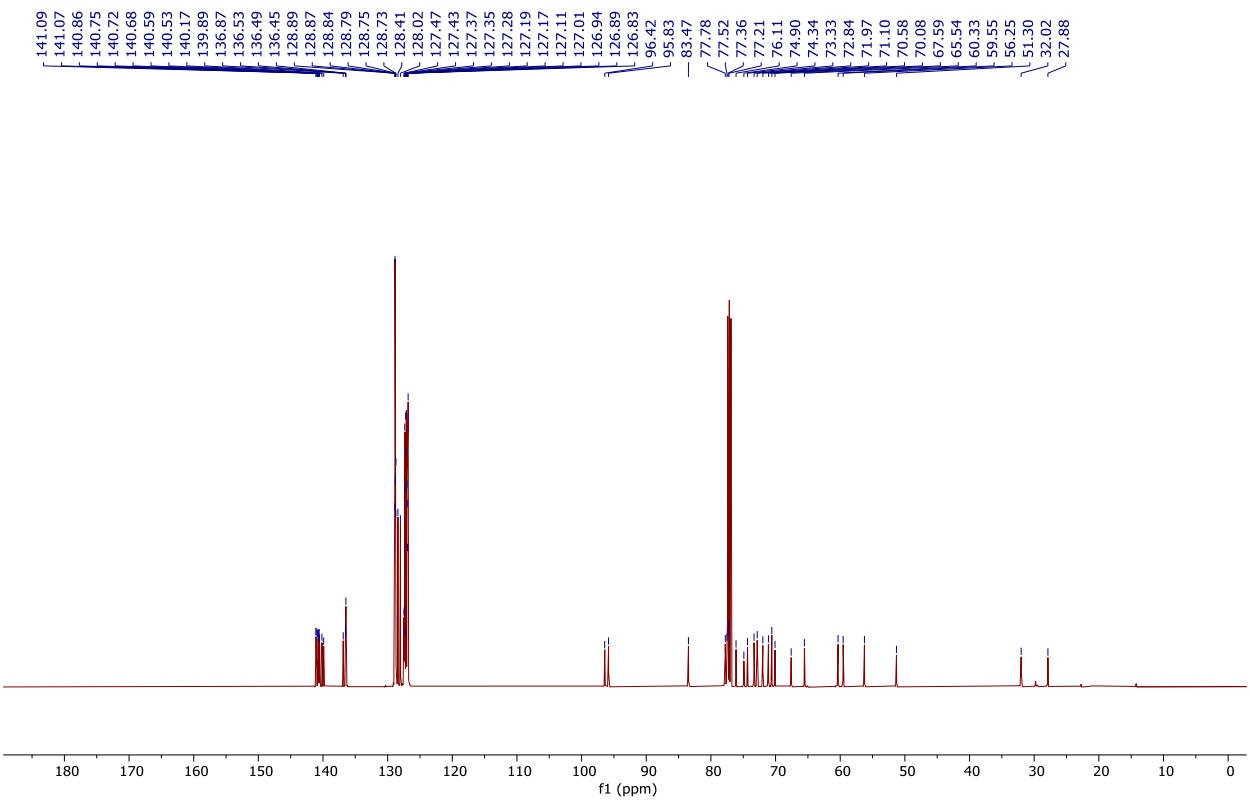


^{1}H and ^{13}C NMR of compound **12c**

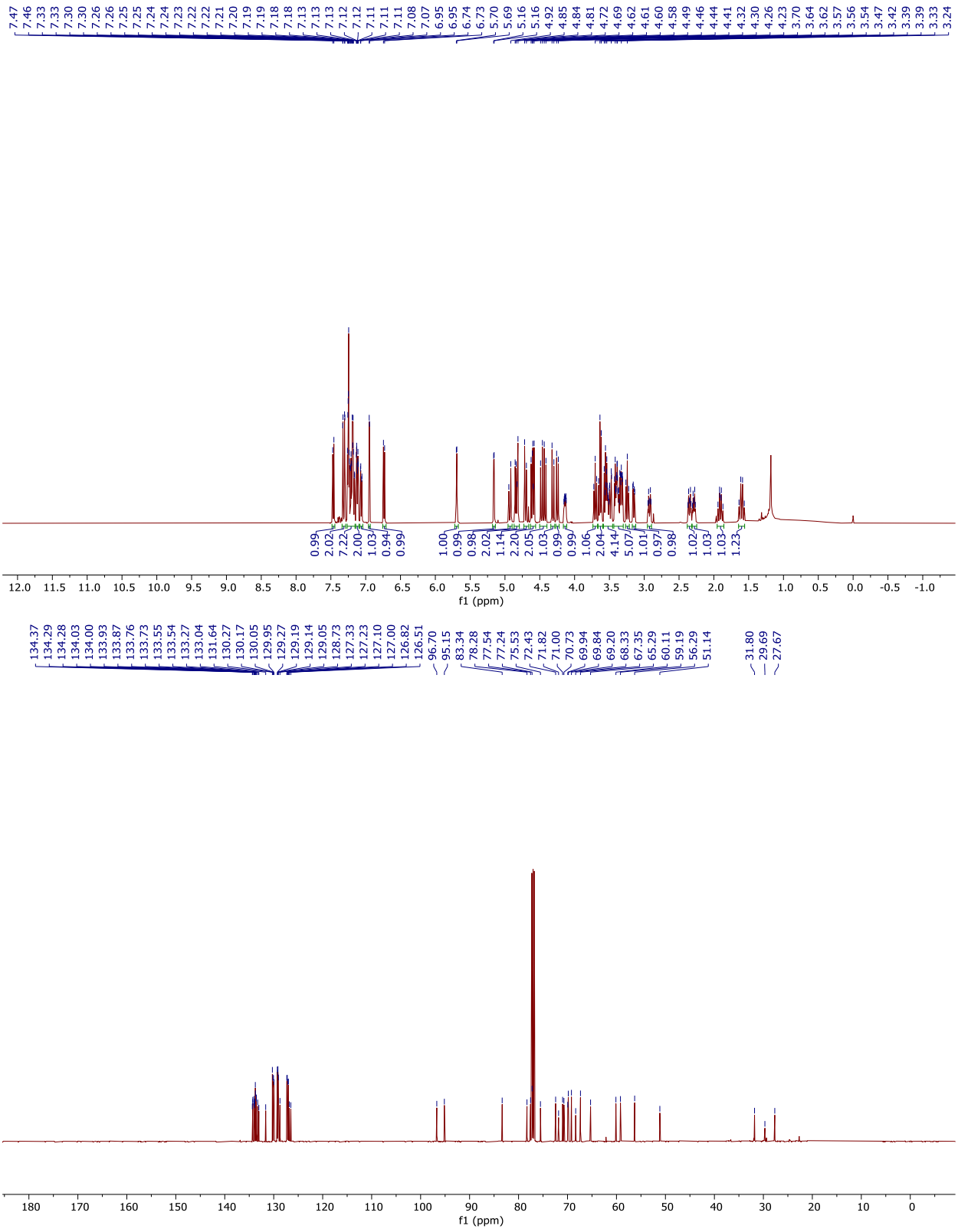


^{1}H and ^{13}C NMR of compound **12d**

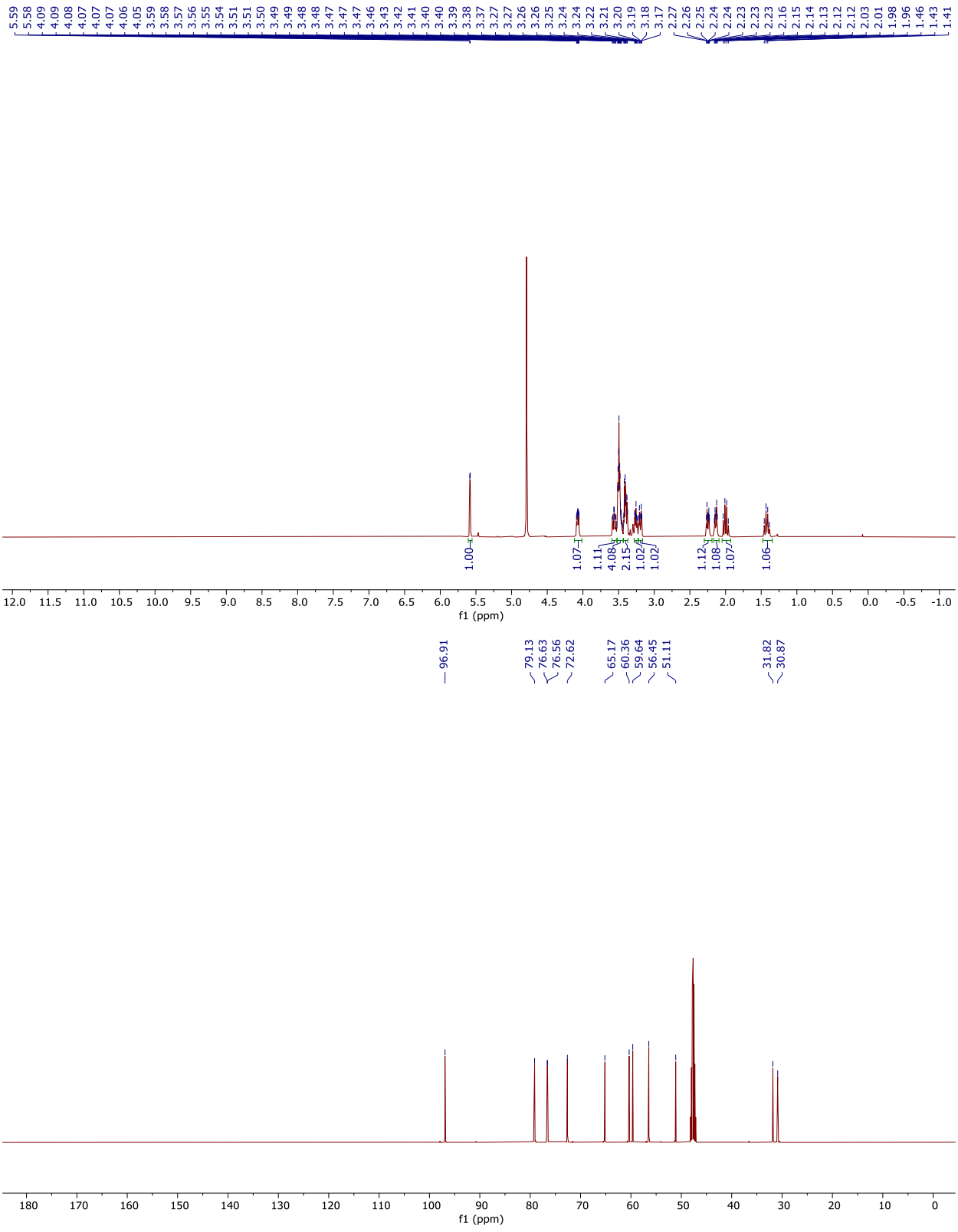




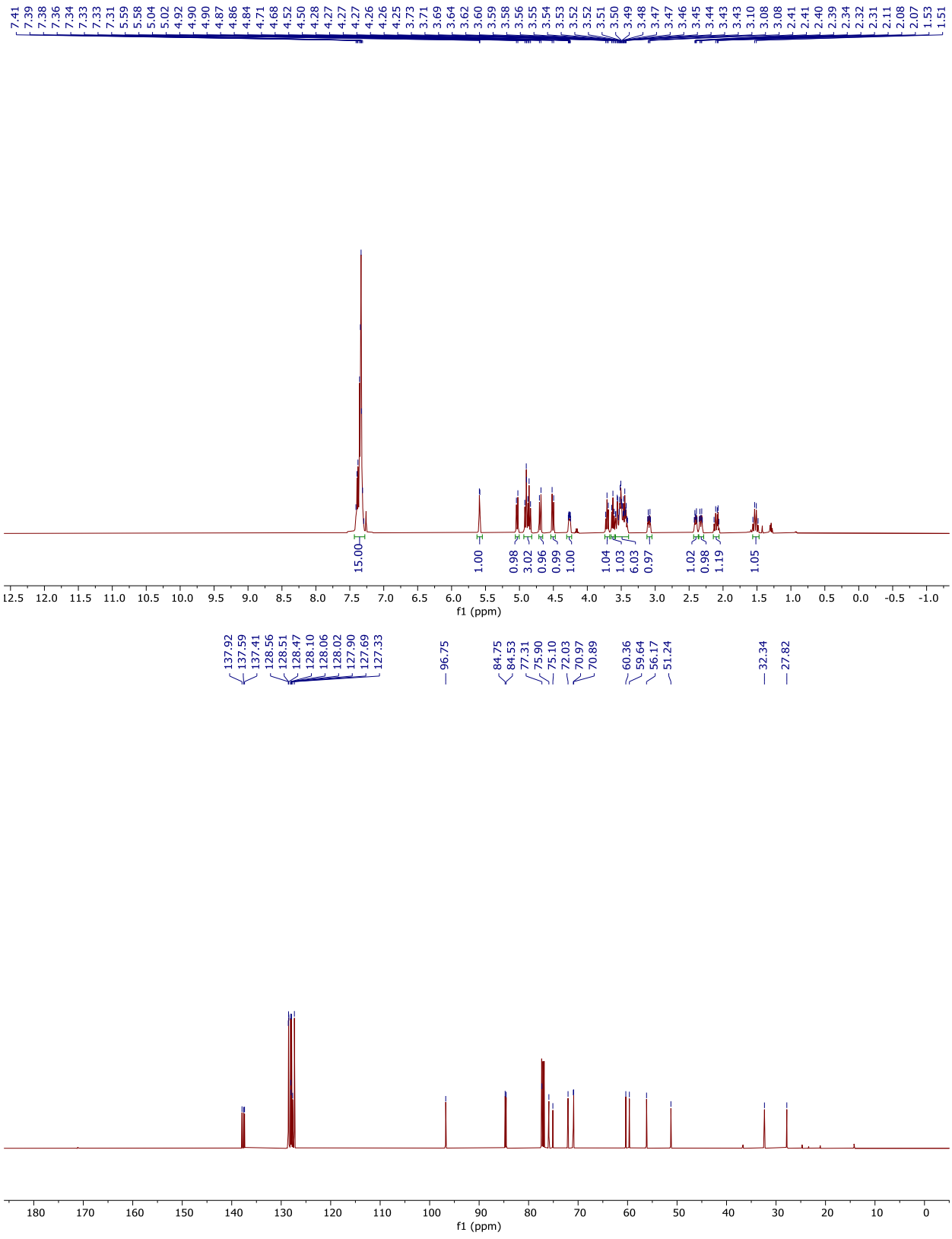
^{1}H and ^{13}C NMR of compound **12e**



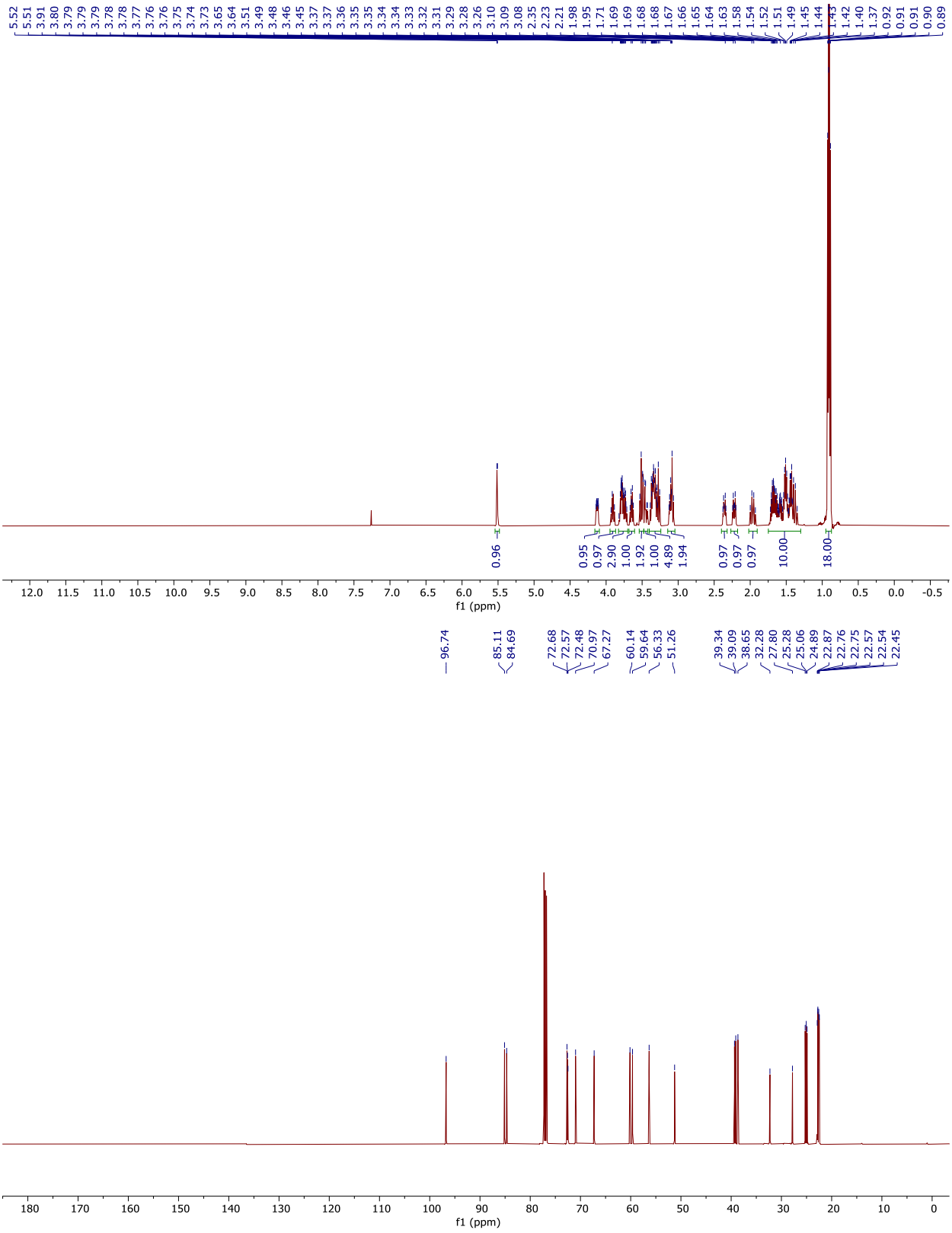
^{1}H and ^{13}C NMR of compound **12f**



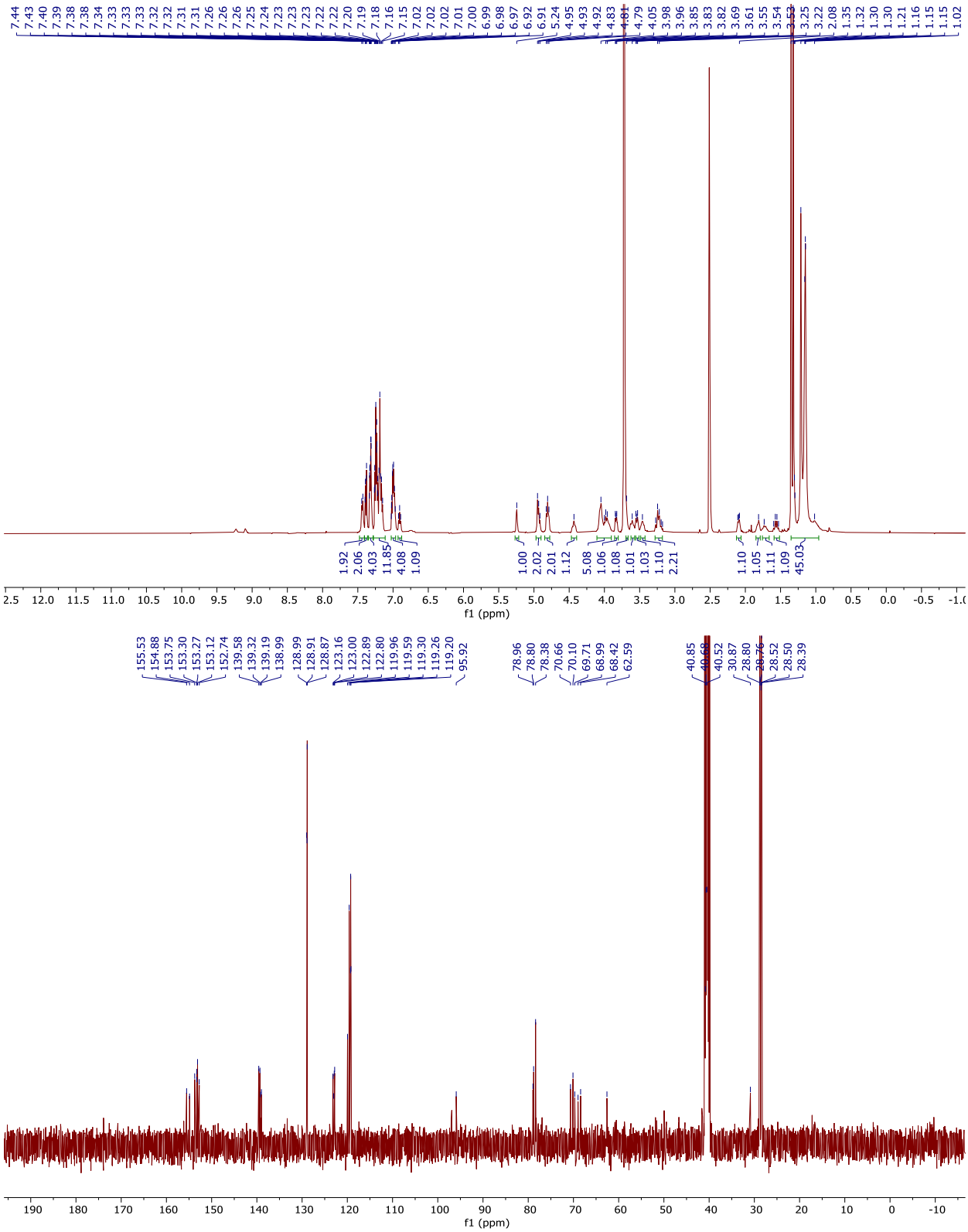
^{1}H and ^{13}C NMR of compound **13**



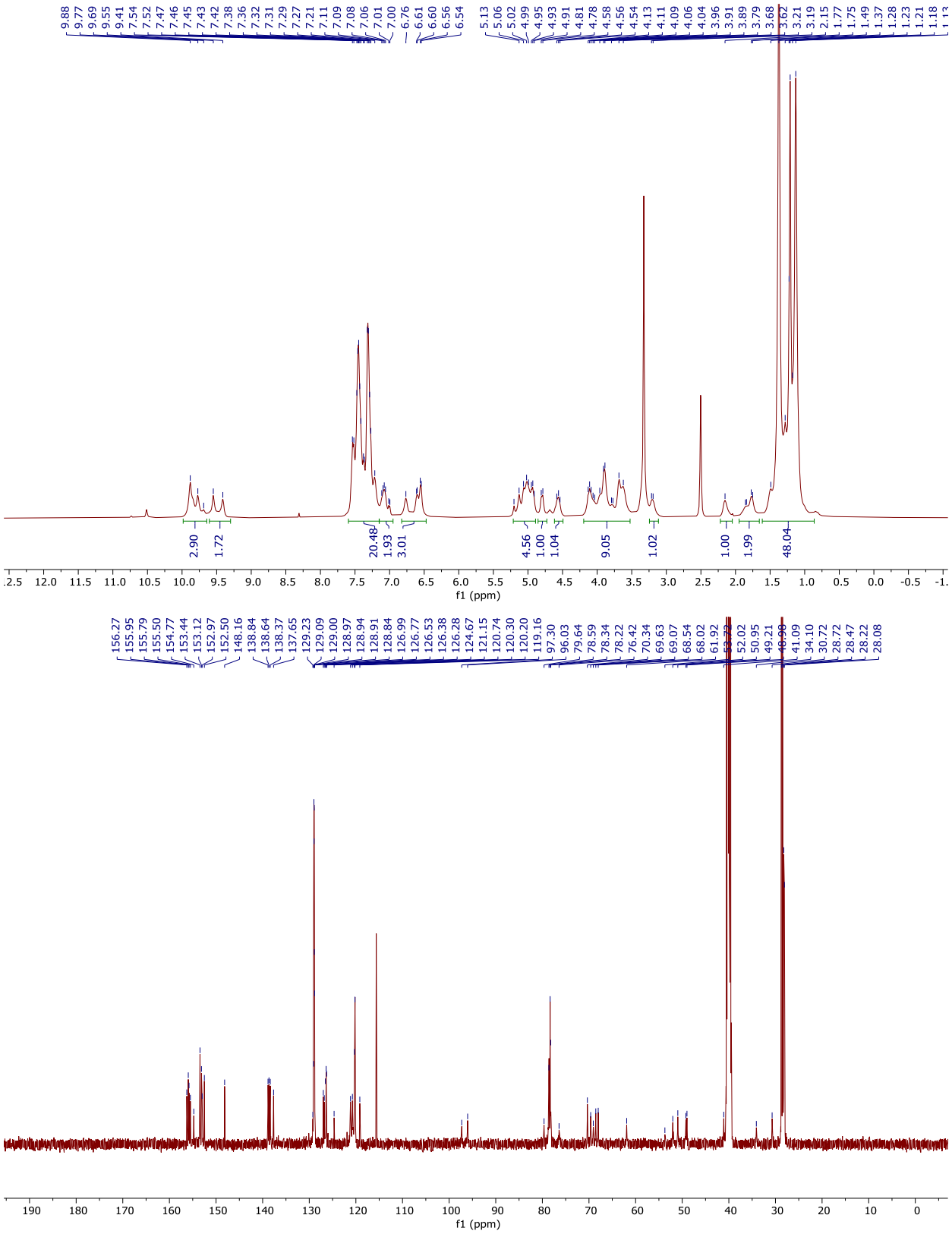
^{1}H and ^{13}C NMR of compound **14a**



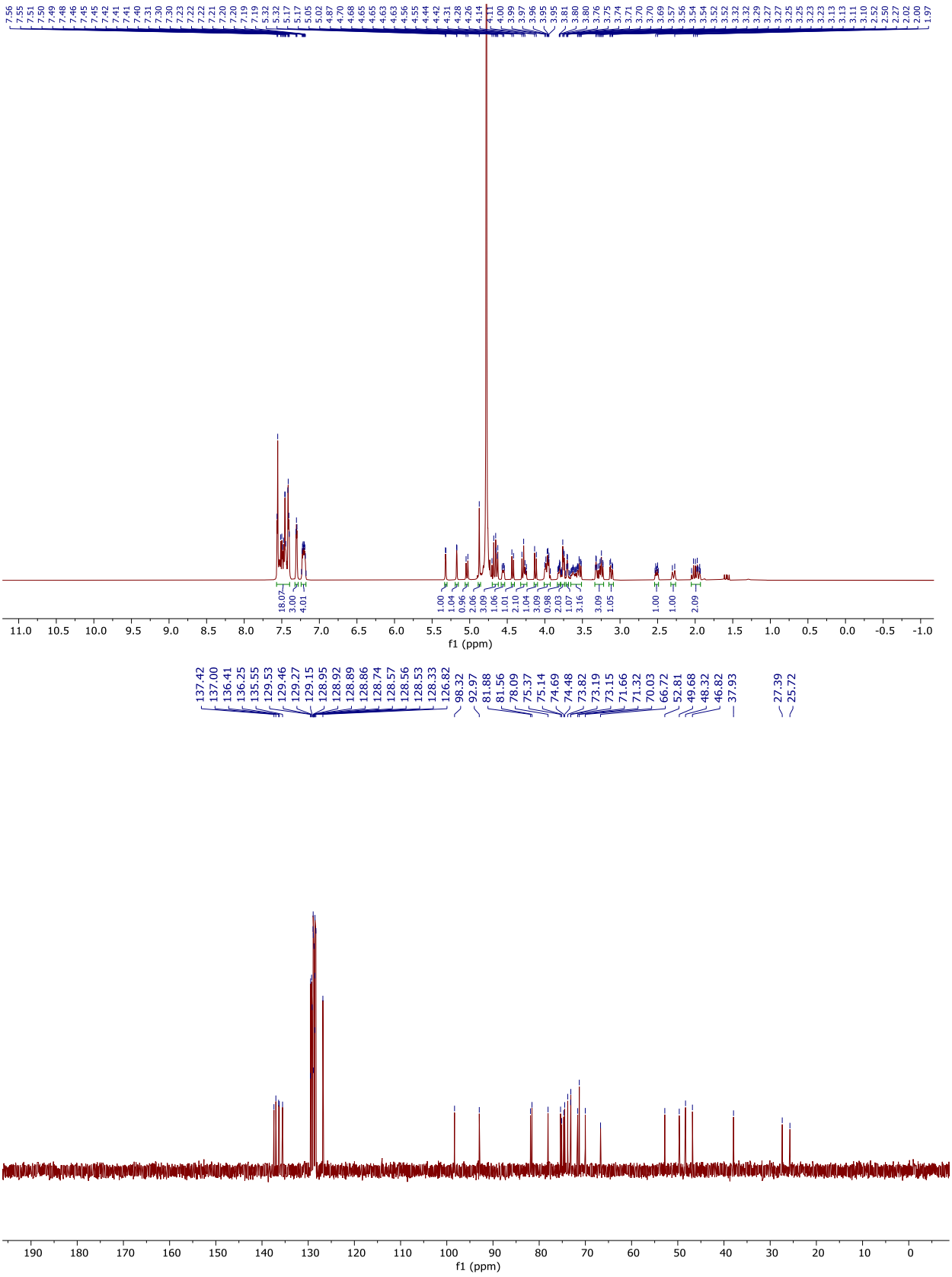
^{1}H and ^{13}C NMR of compound **14b**



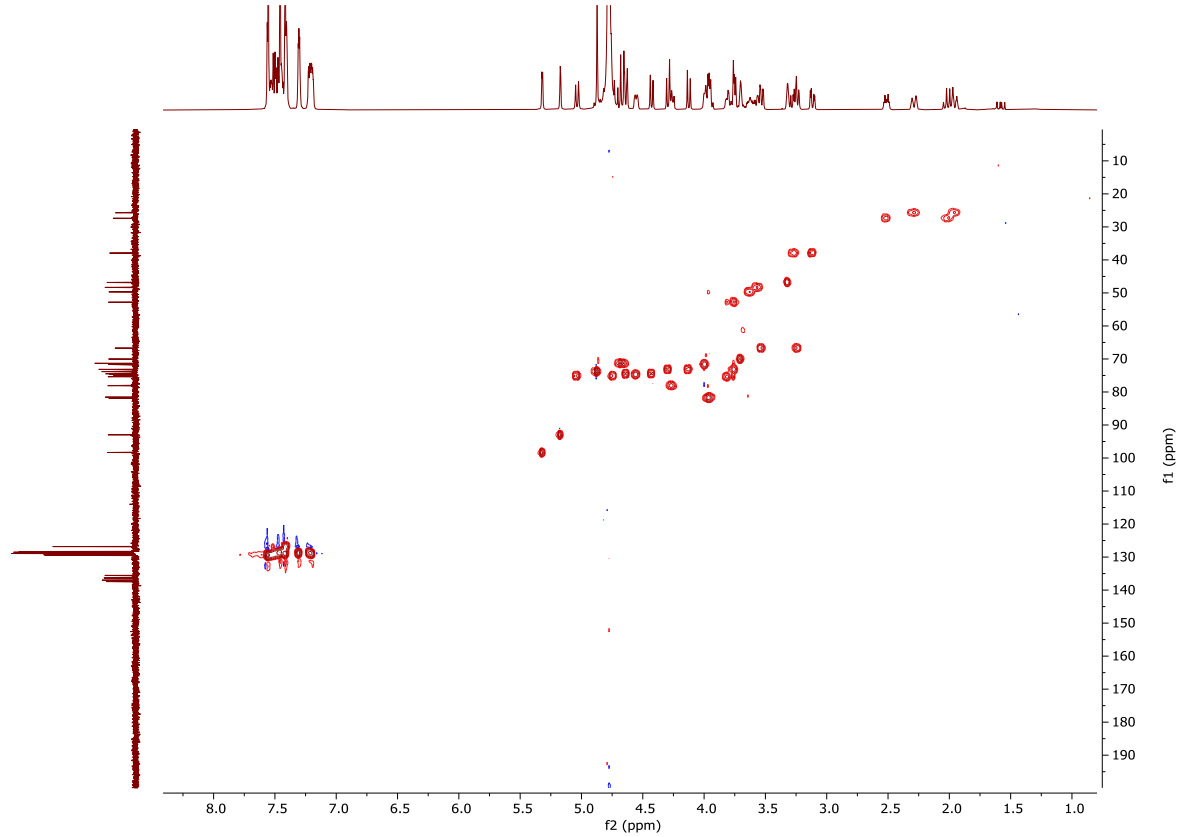
^{1}H and ^{13}C NMR of compound **16a**

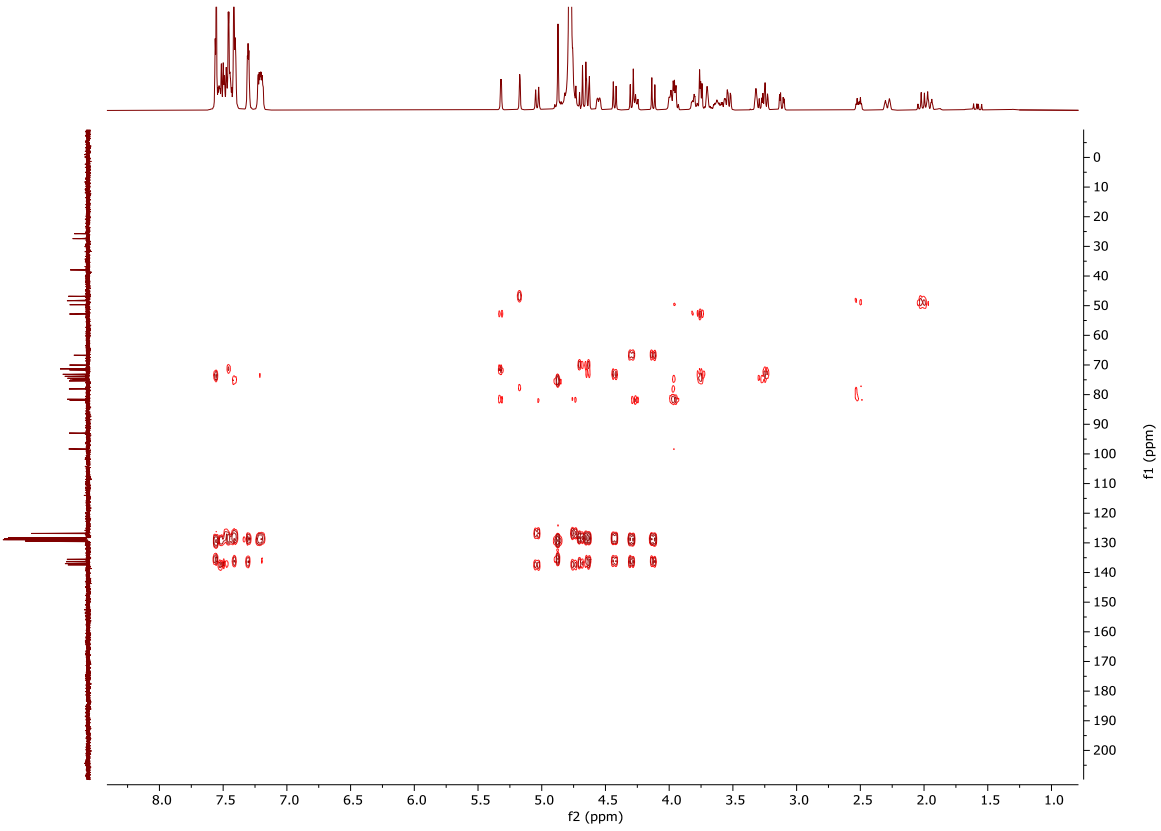


^{1}H and ^{13}C NMR of compound **16b**

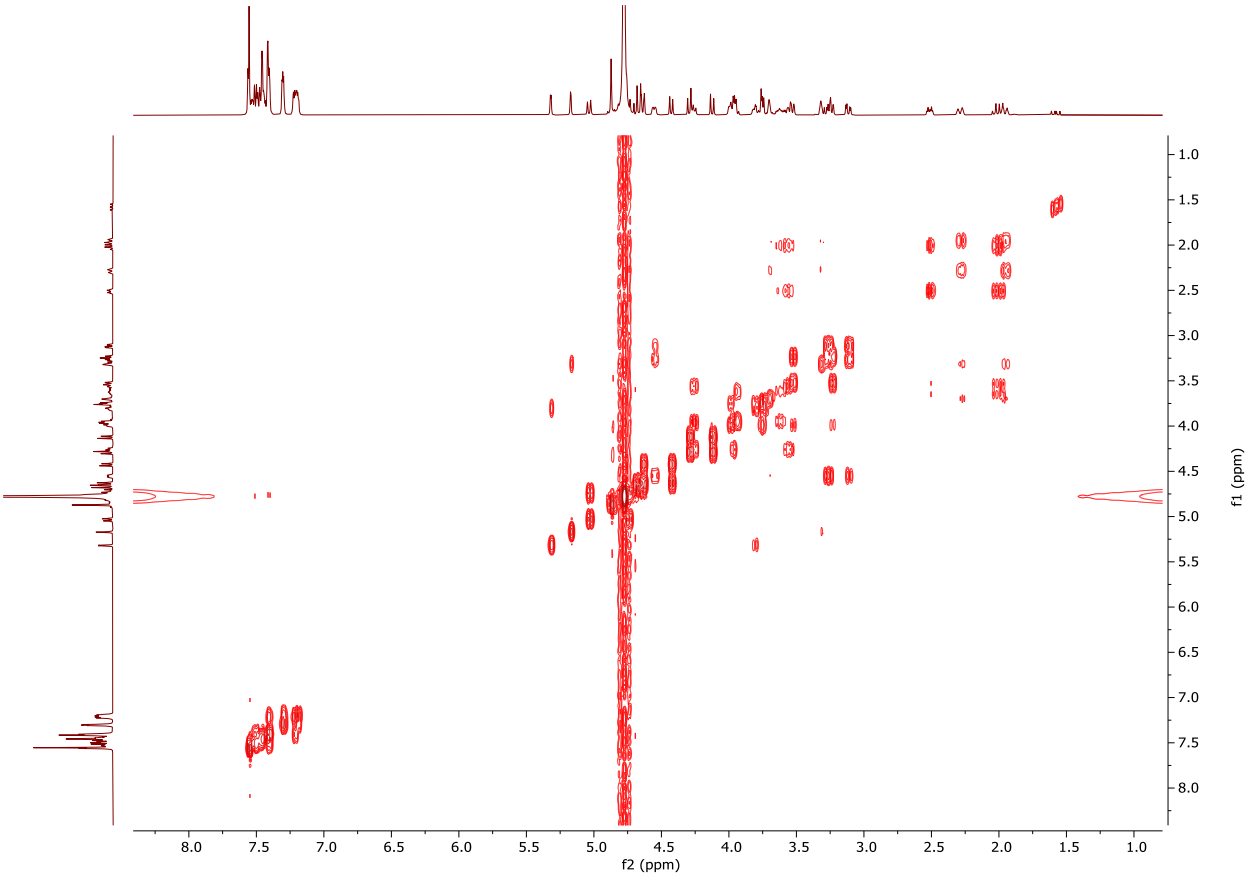


^{1}H and ^{13}C NMR of compound **1** (HCl salt)

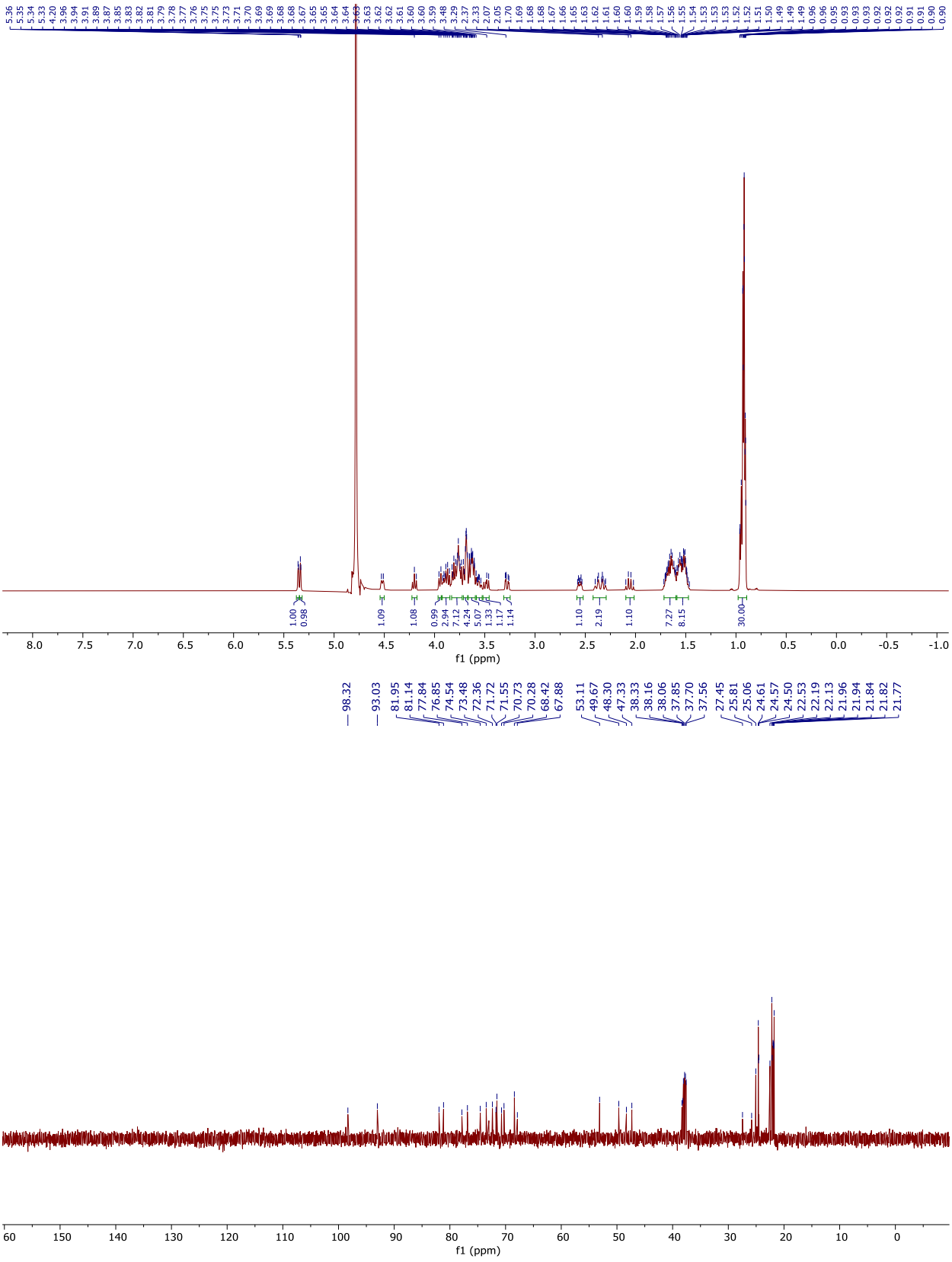




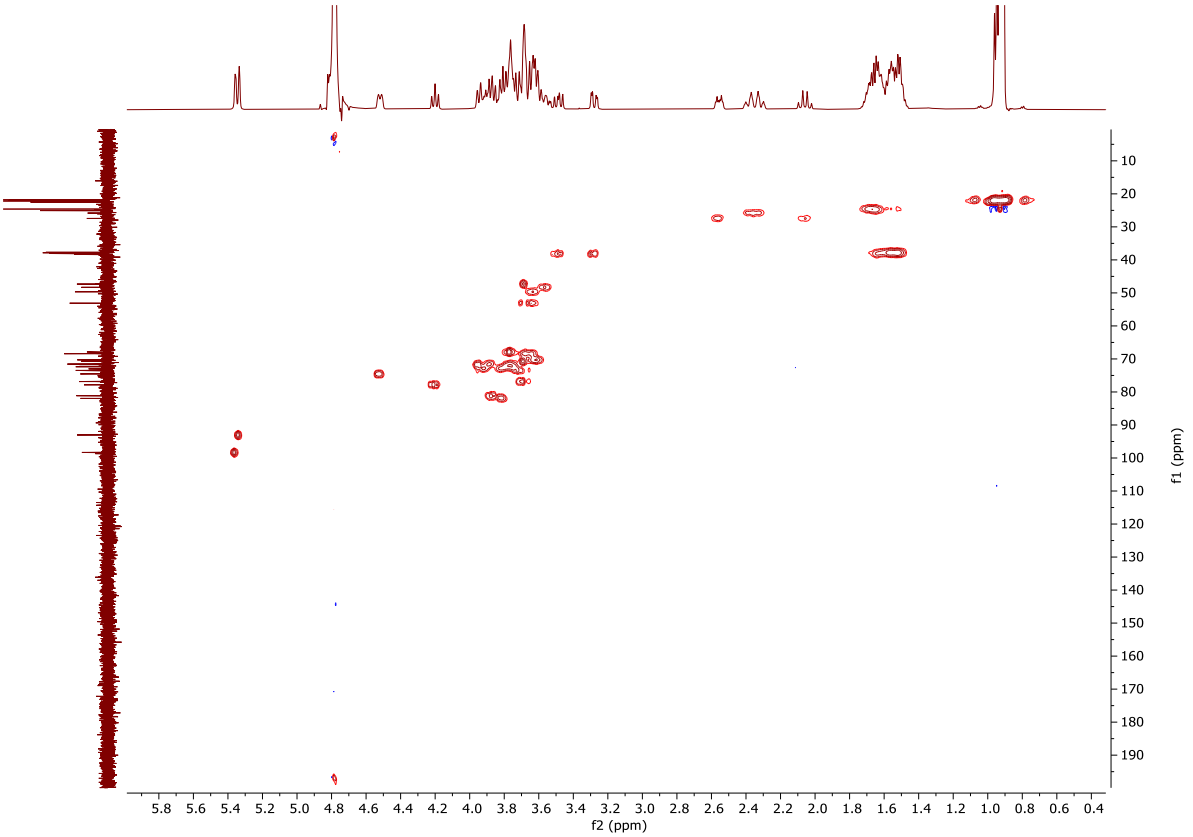
HSQC and HMBC of compound **1** (HCl salt)

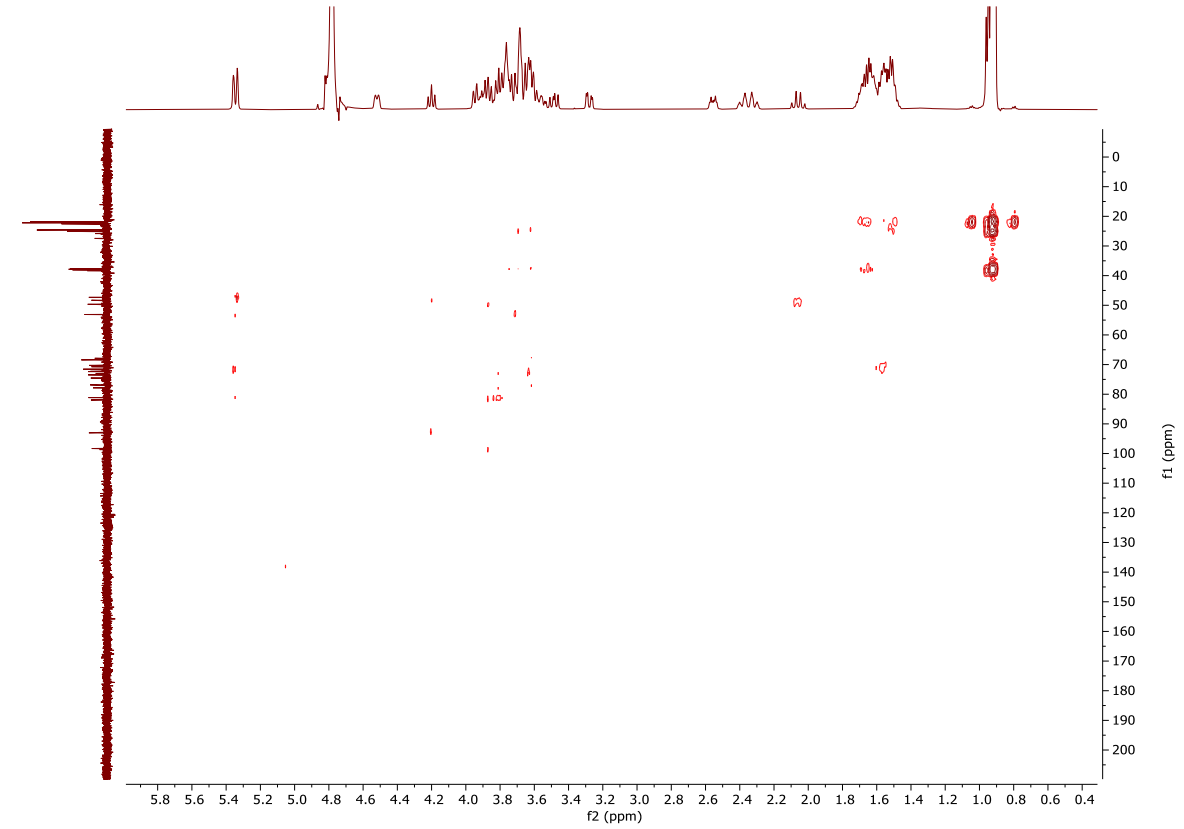


COSY of compound **1** (HCl salt)

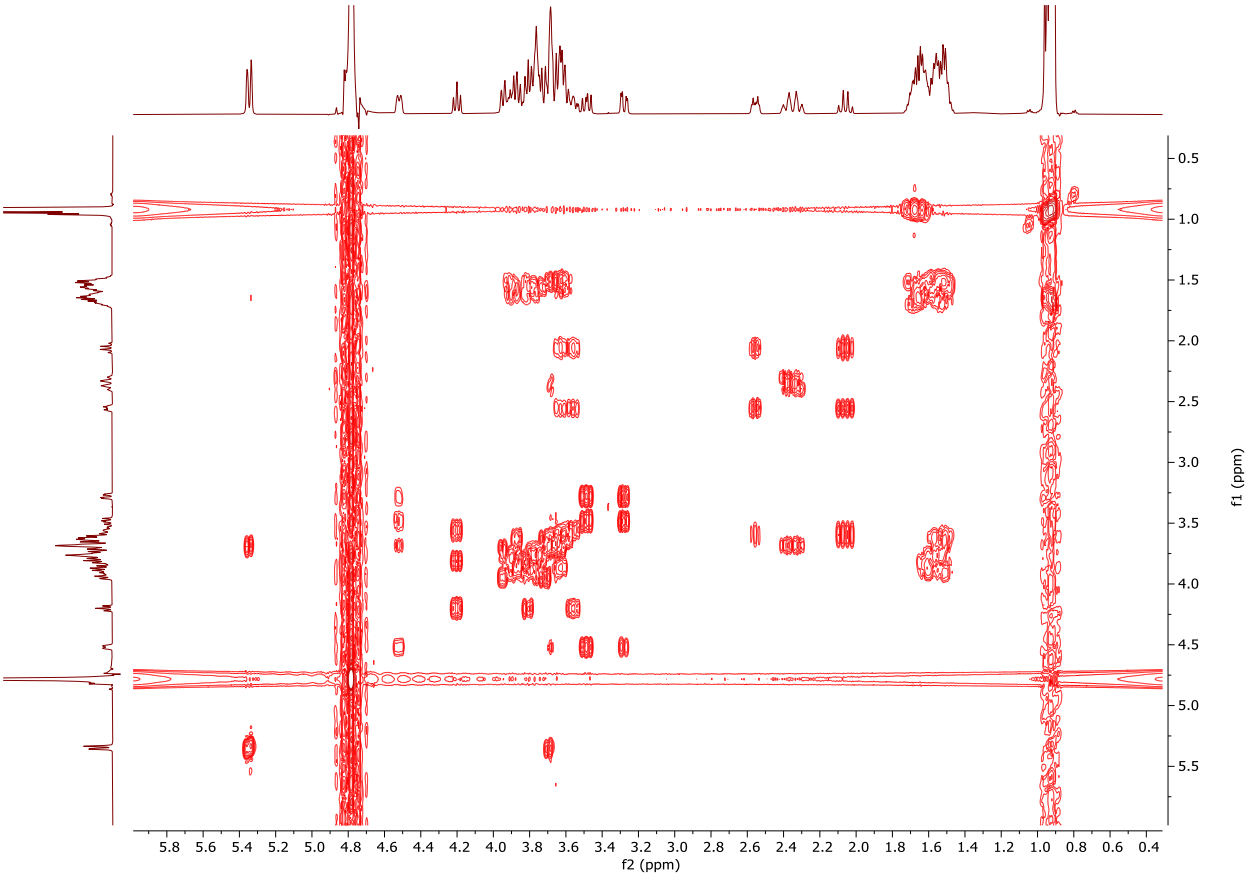


^{1}H and ^{13}C NMR of compound **2** (HCl salt)

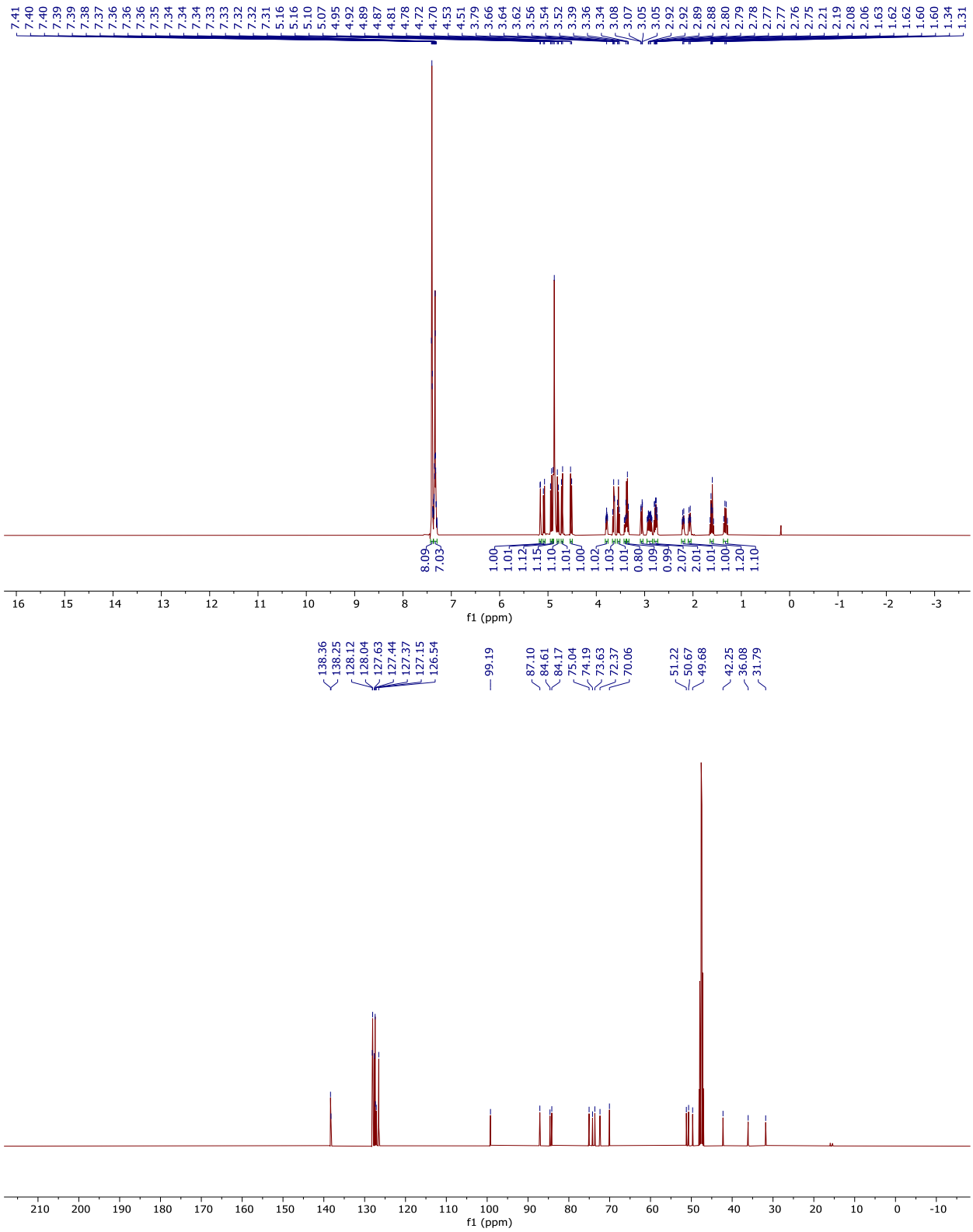




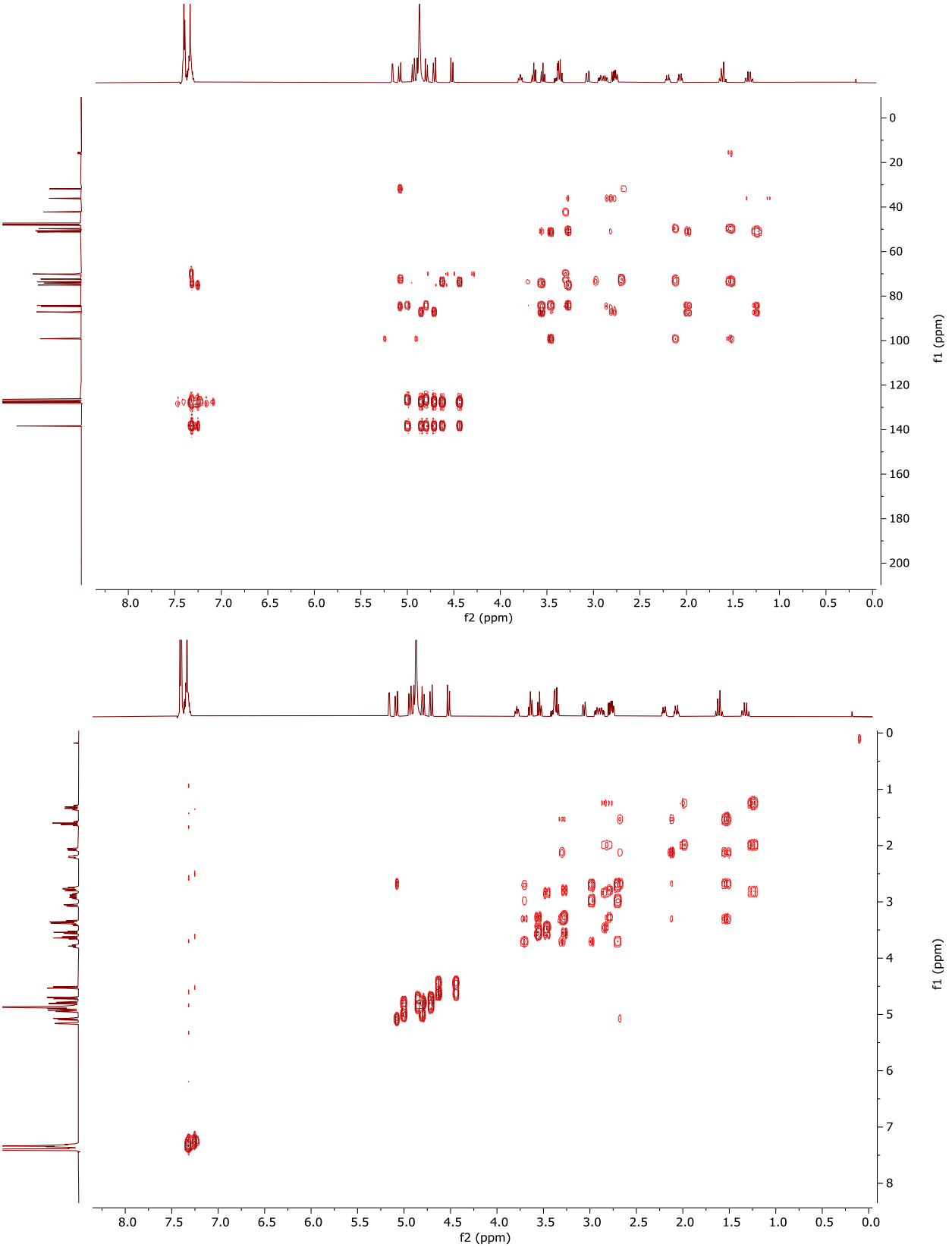
HSQC and HMBC of compound **2** (HCl salt)



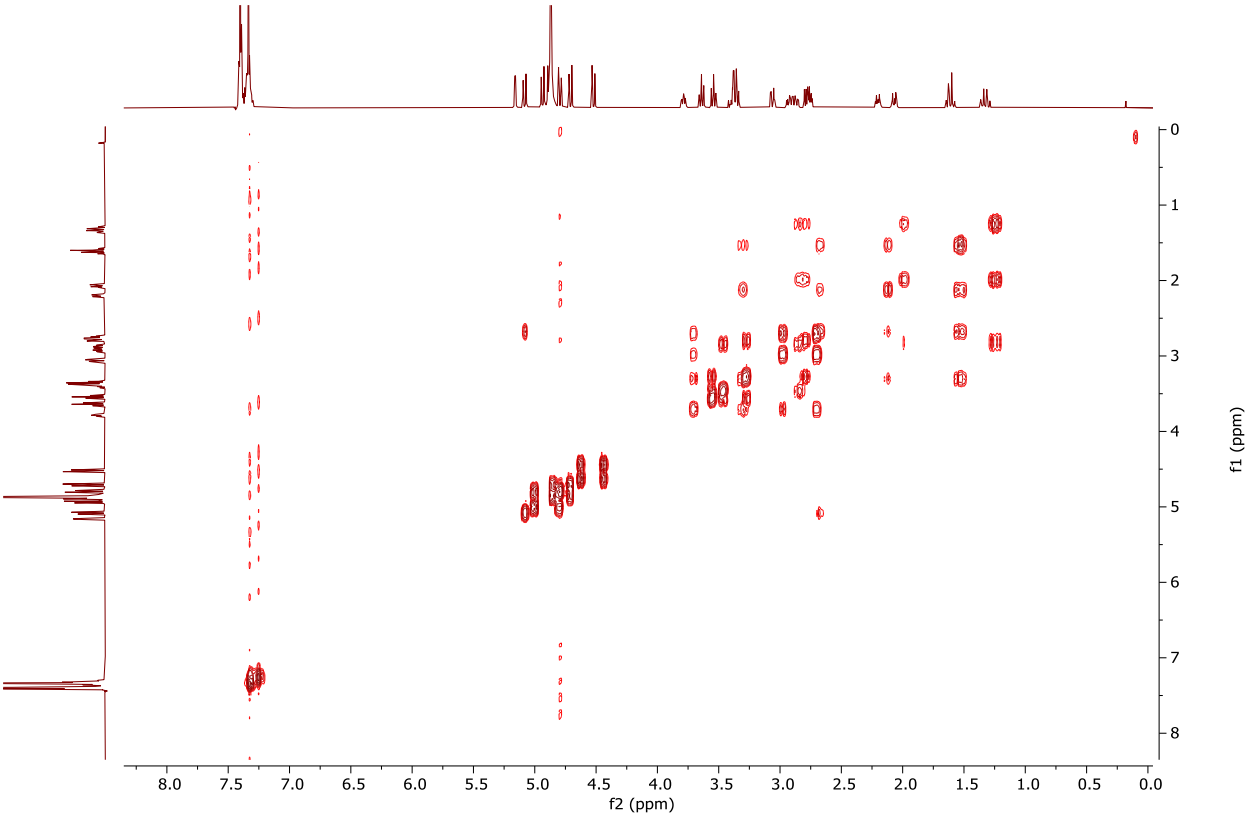
COSY of compound **2** (HCl salt)



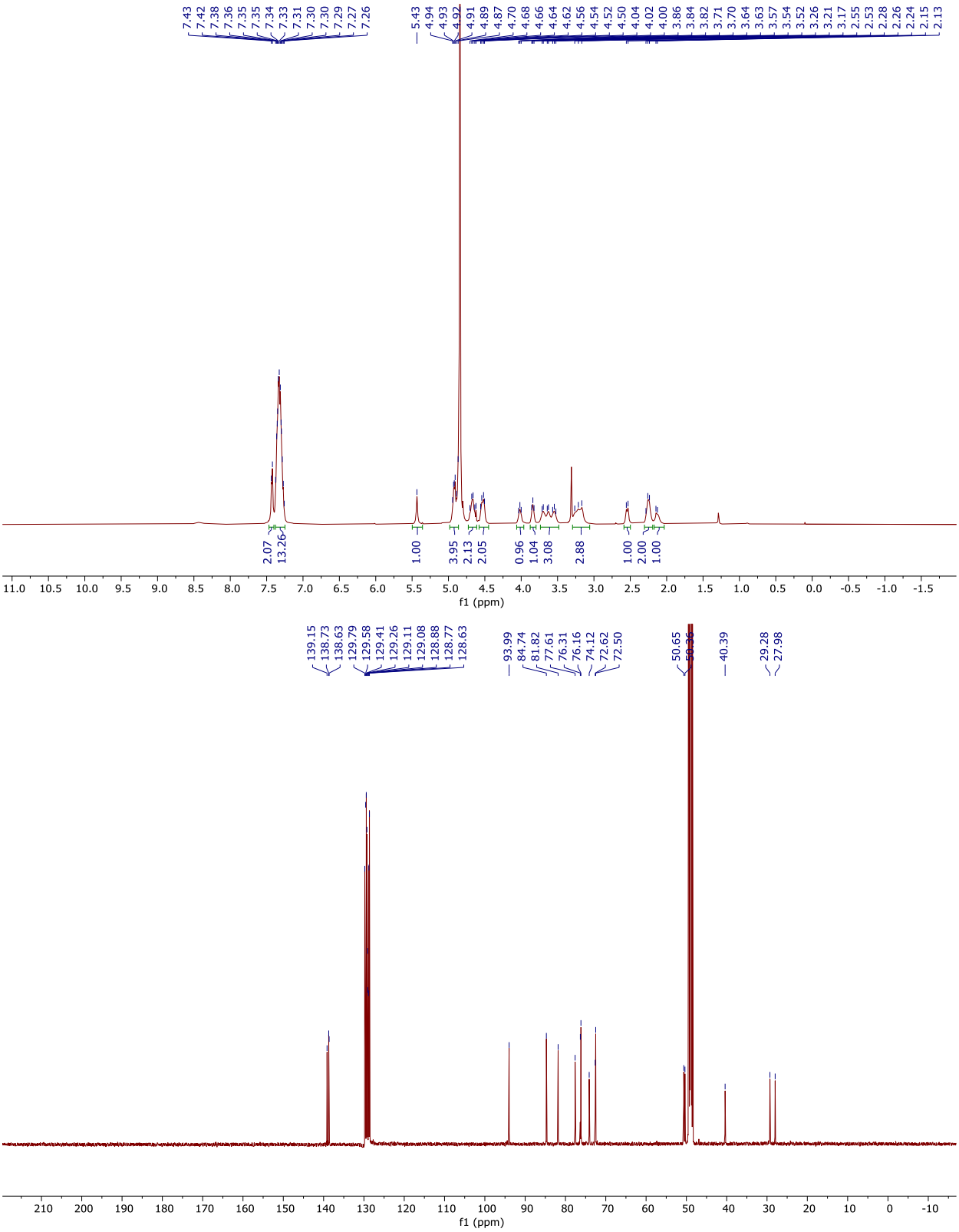
^{1}H and ^{13}C NMR of compound **3**



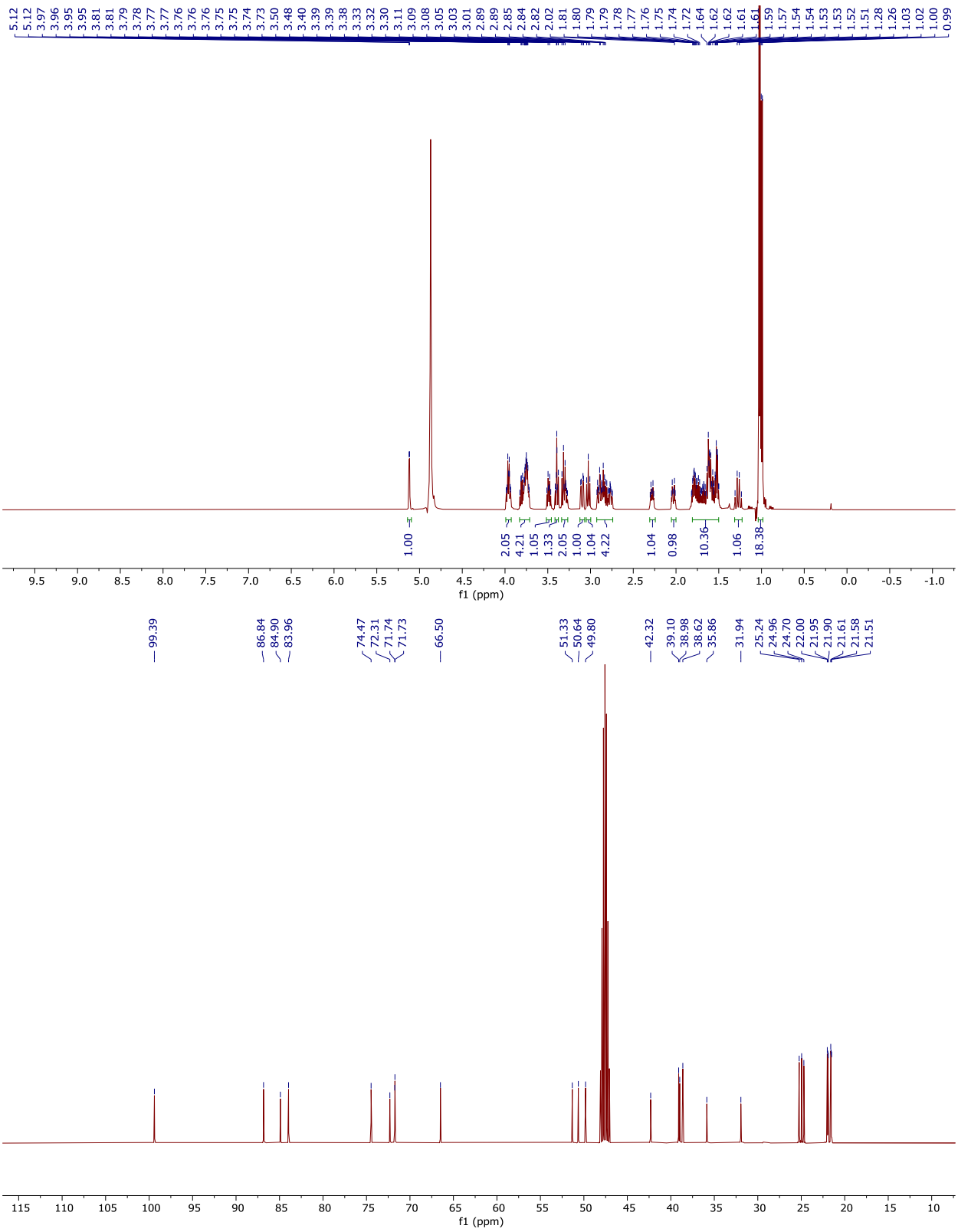
HSQC and HMBC of compound **3**



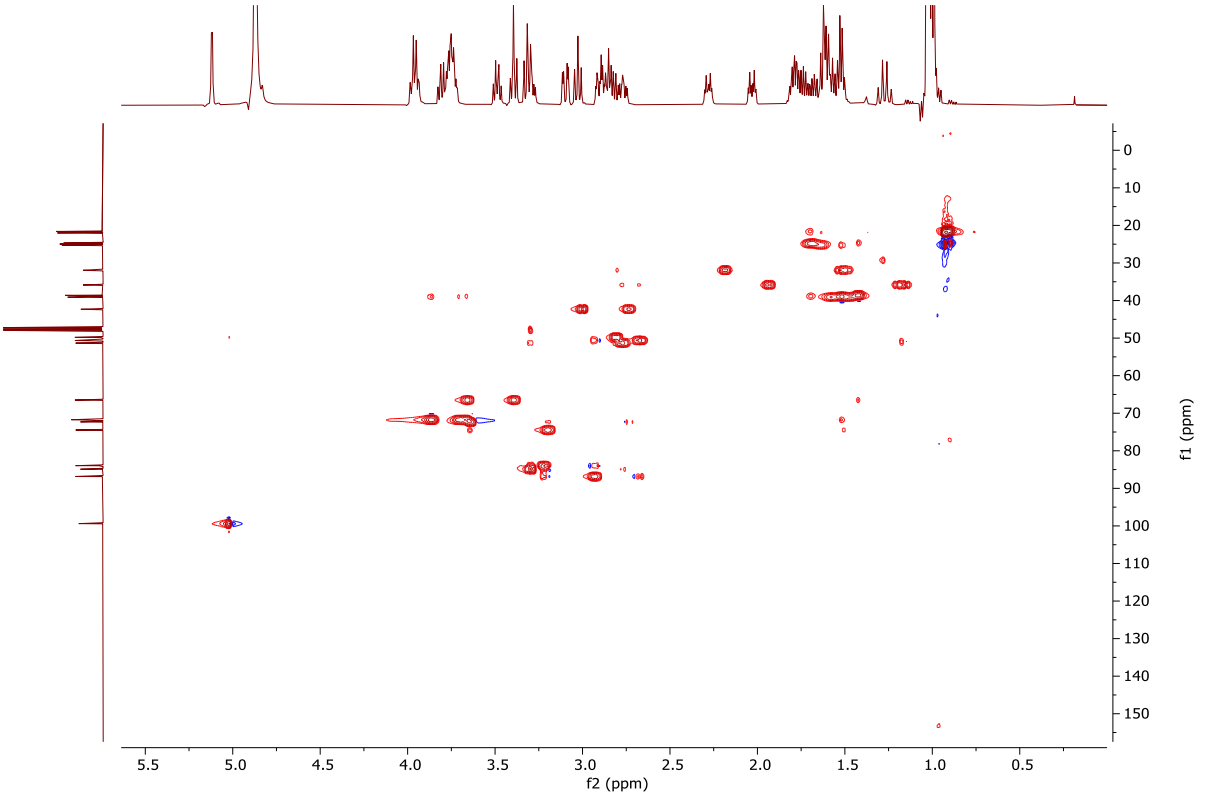
COSY of compound **3**

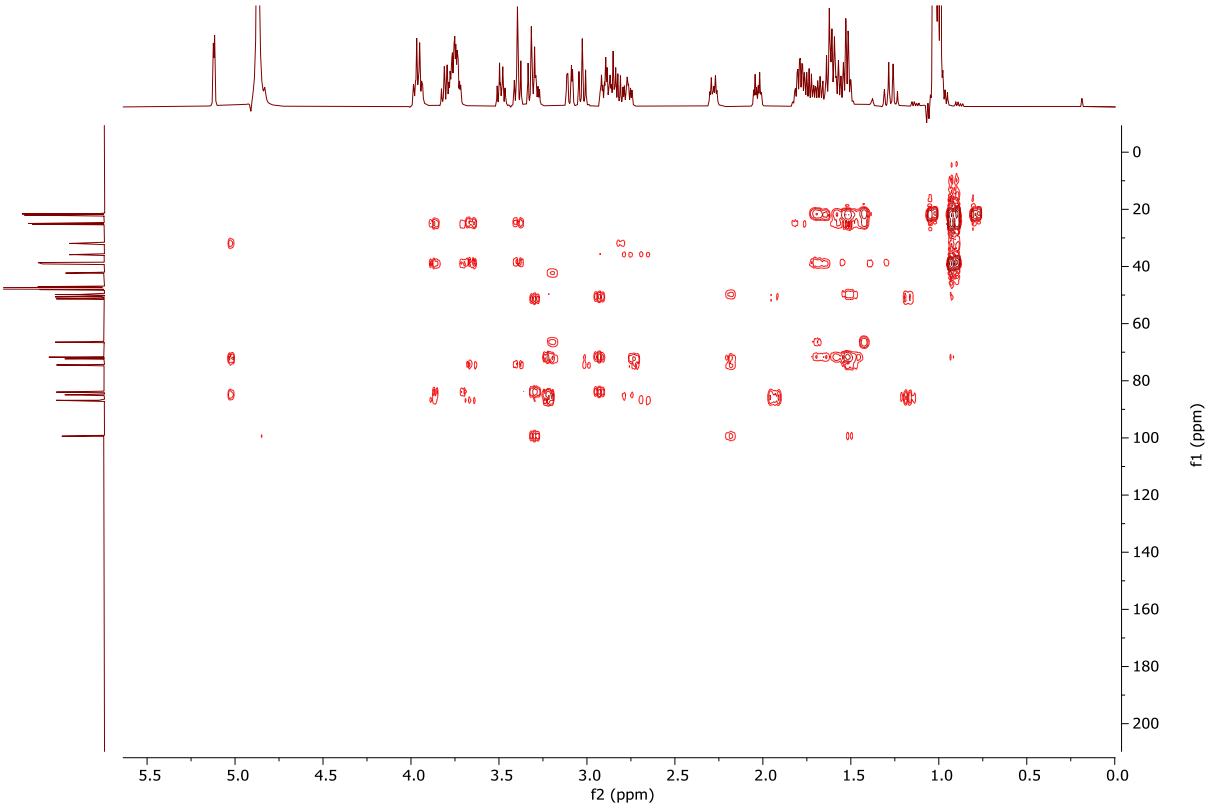


^{1}H and ^{13}C NMR of compound **3** (HCl salt)

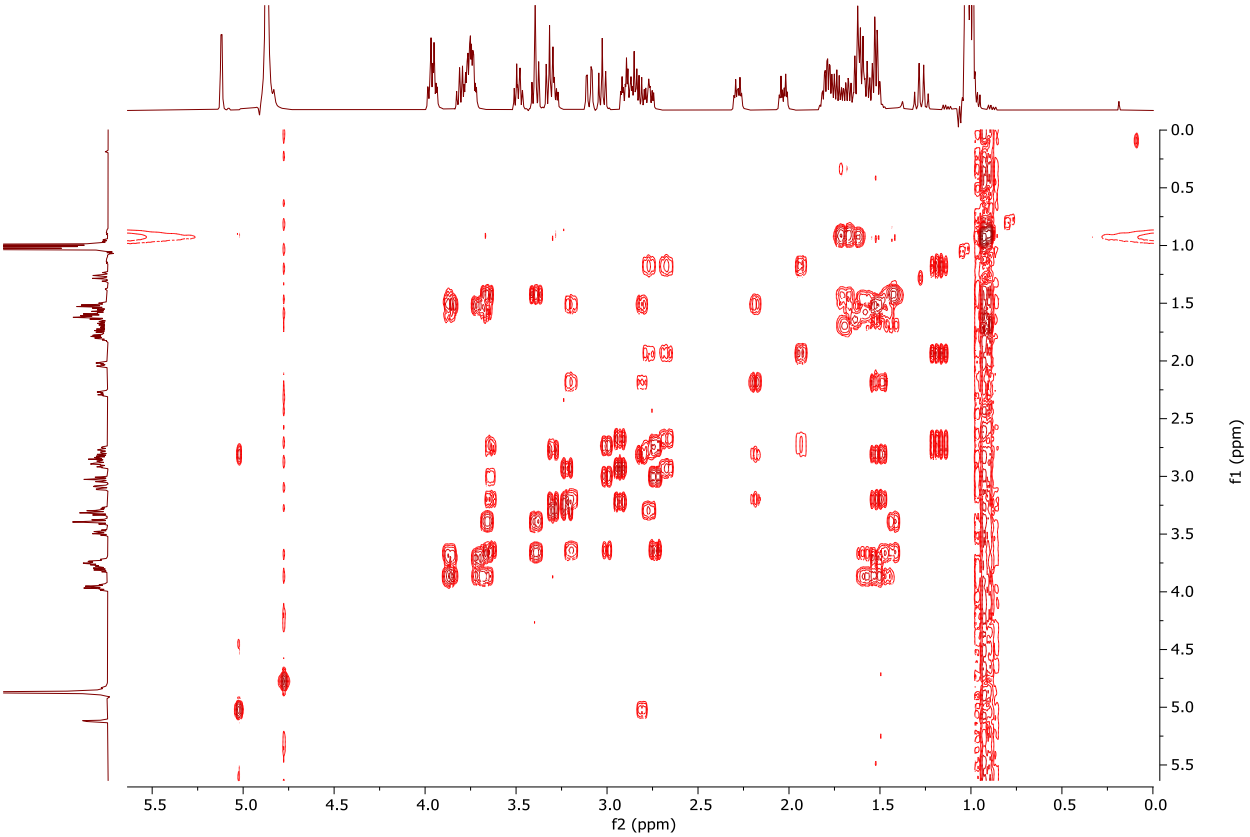


^{1}H and ^{13}C NMR of compound **4**

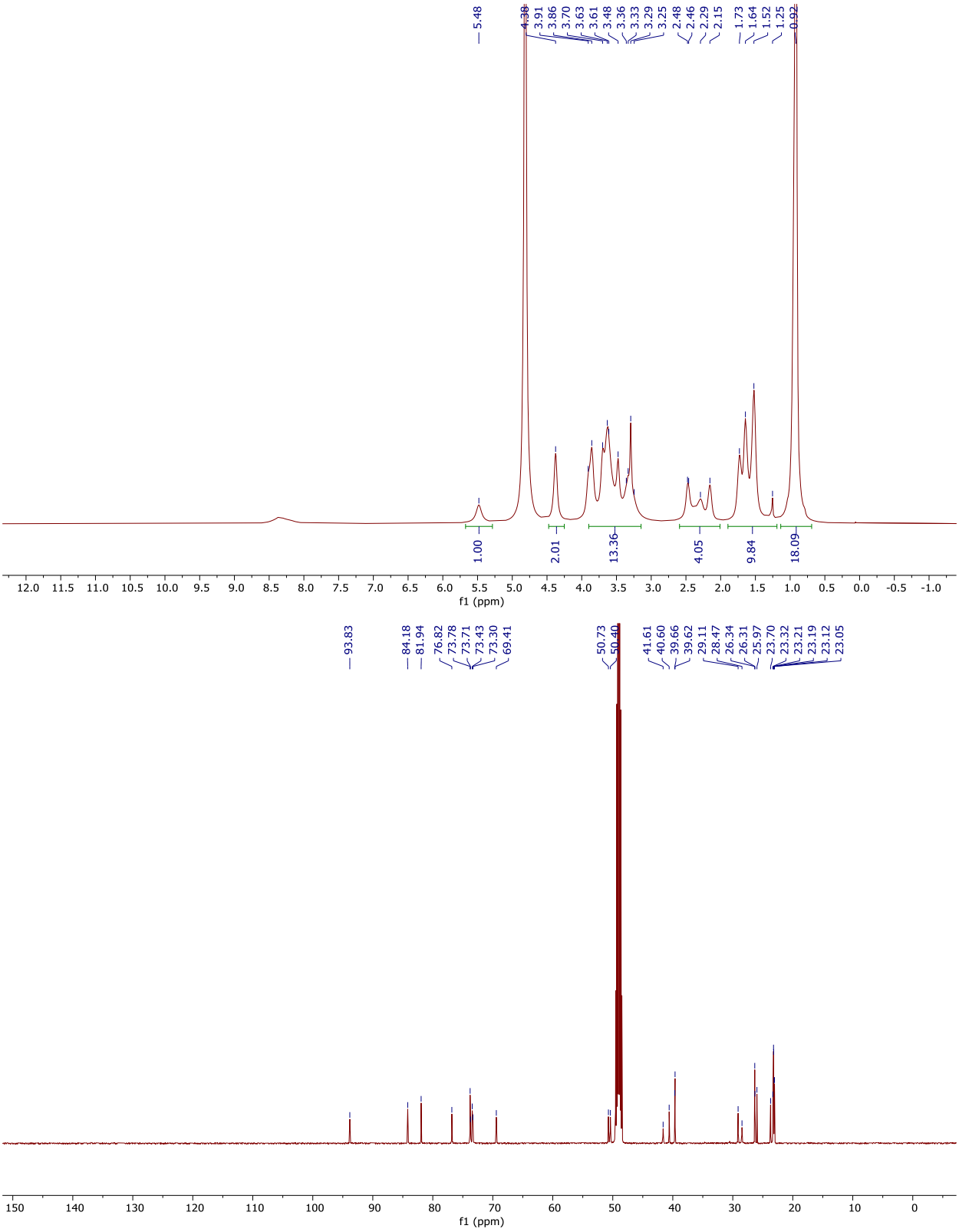




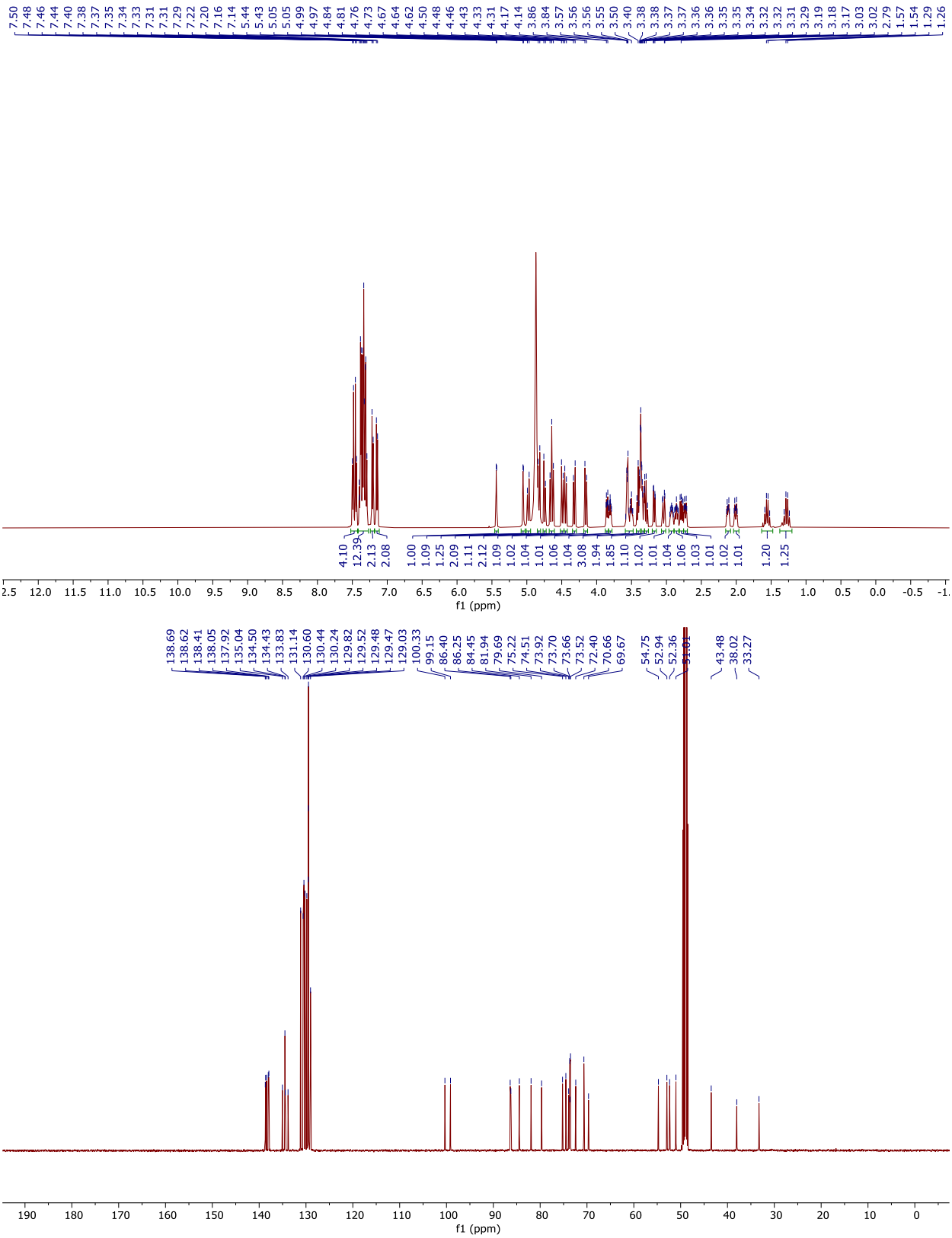
HSQC and HMBC of compound **4**



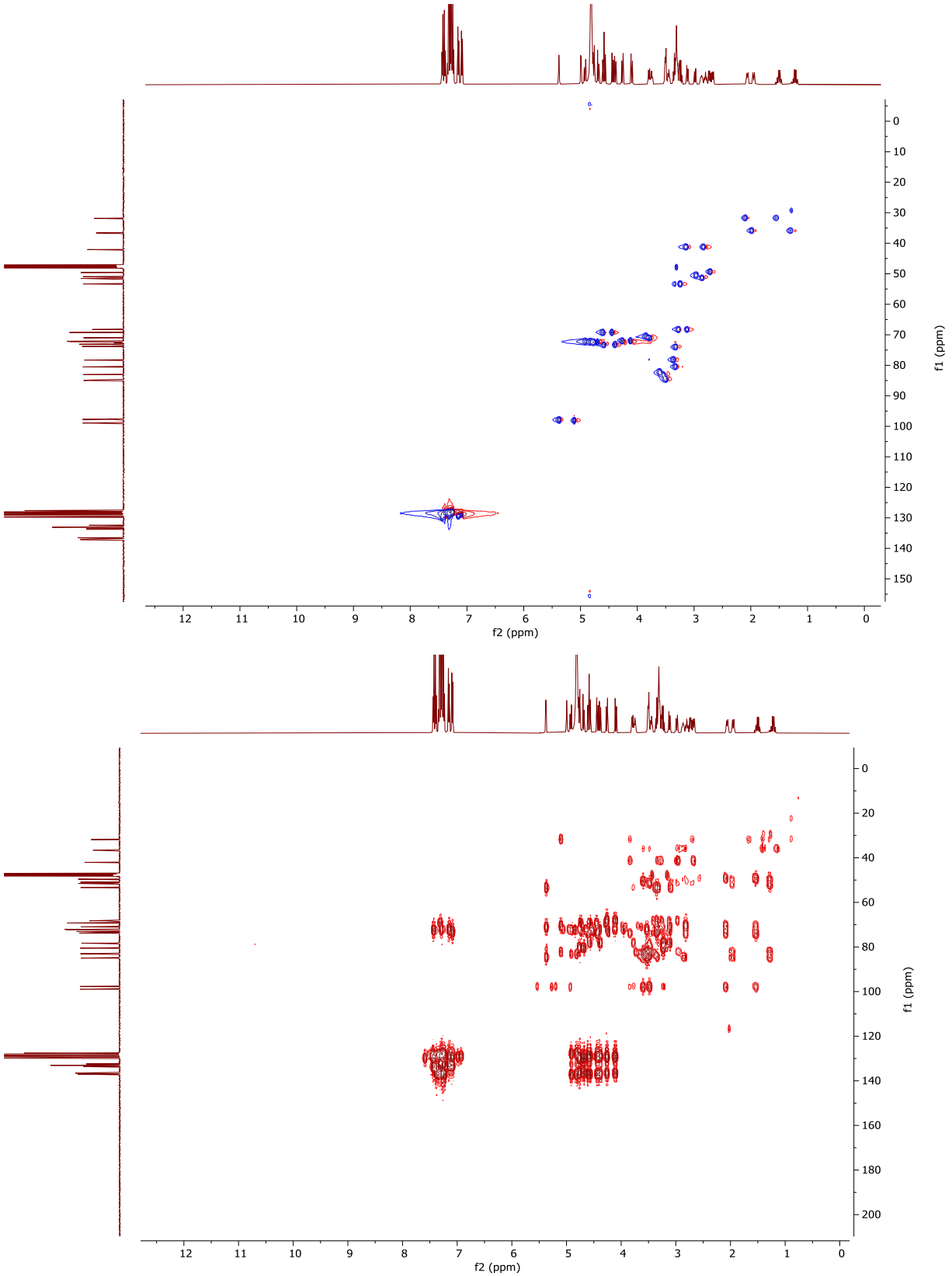
COSY of compound **4**



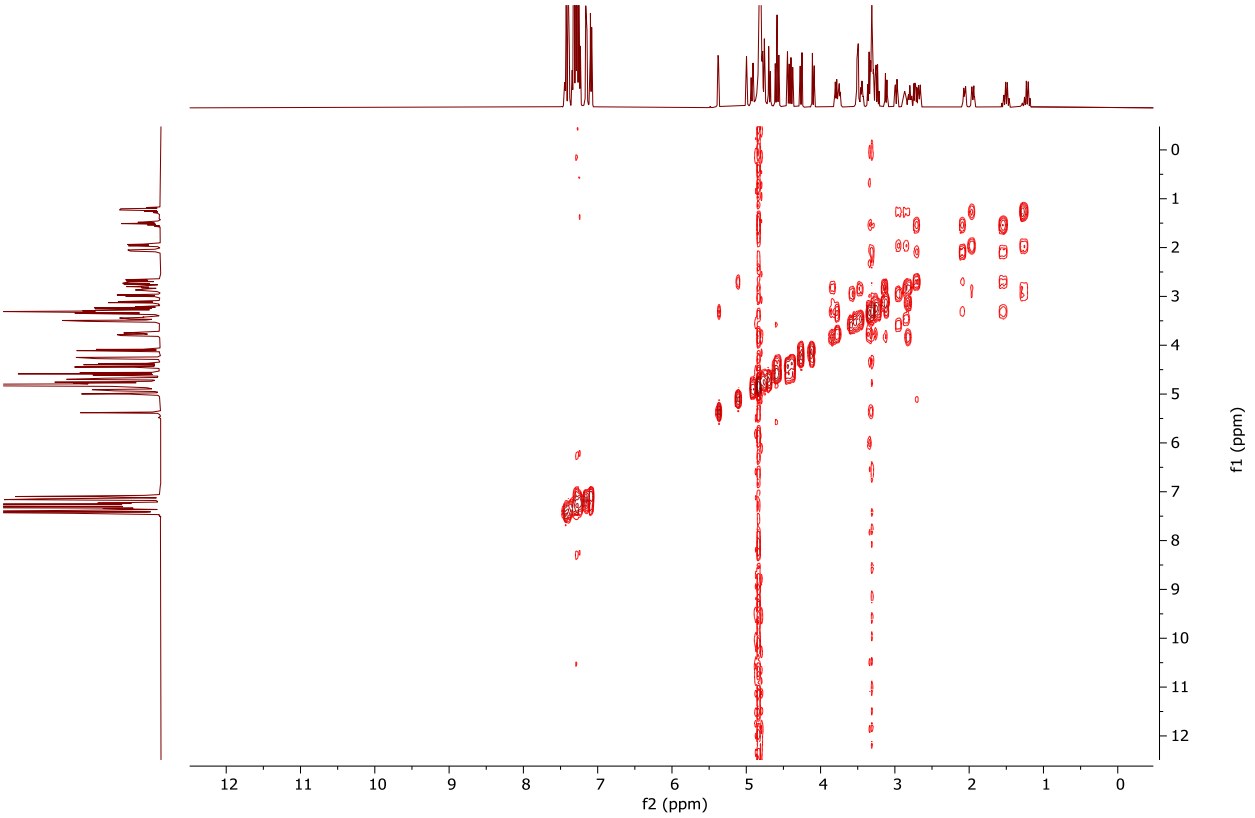
^{1}H and ^{13}C NMR of compound **4** (HCl salt)



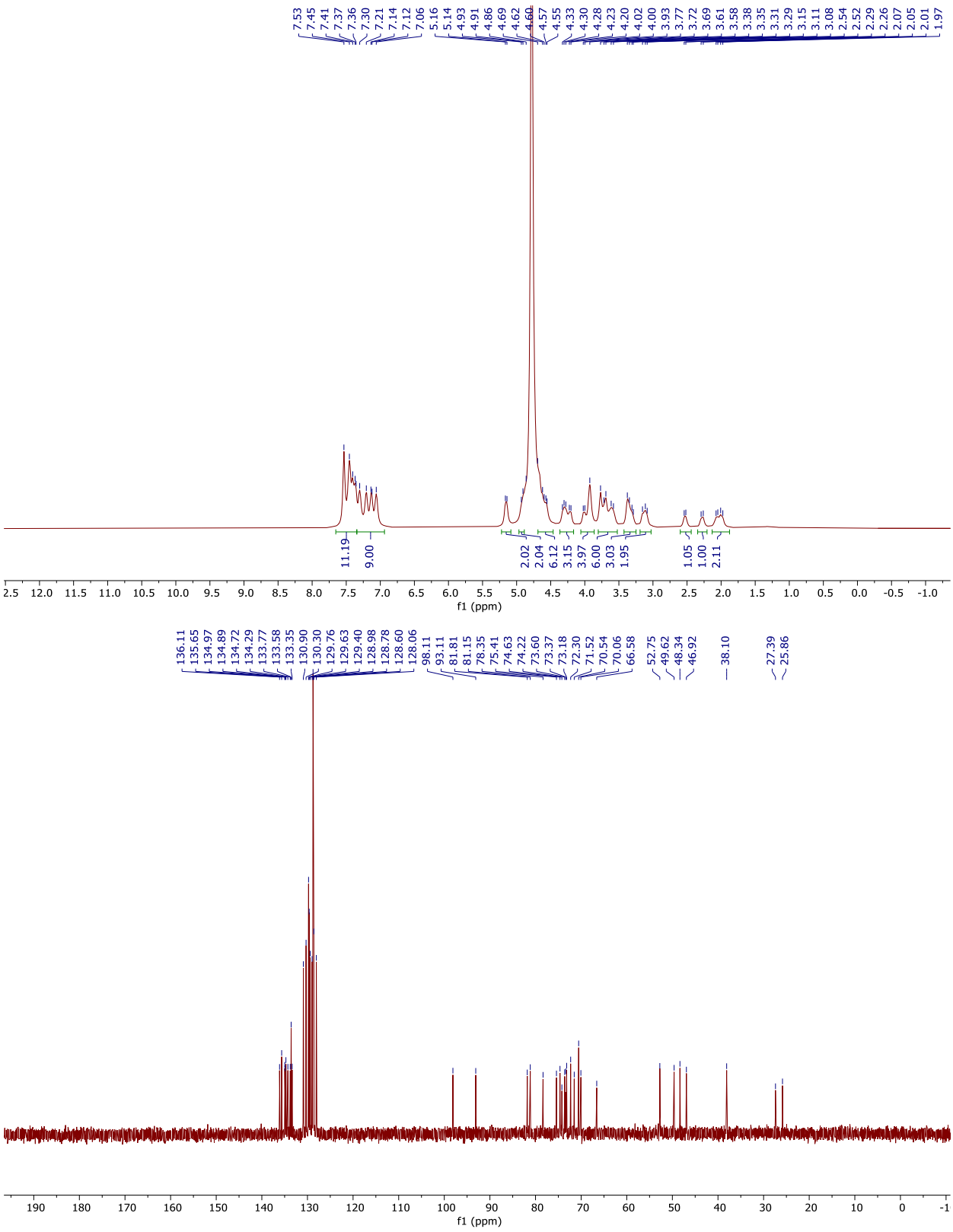
^{1}H and ^{13}C NMR of compound **5**



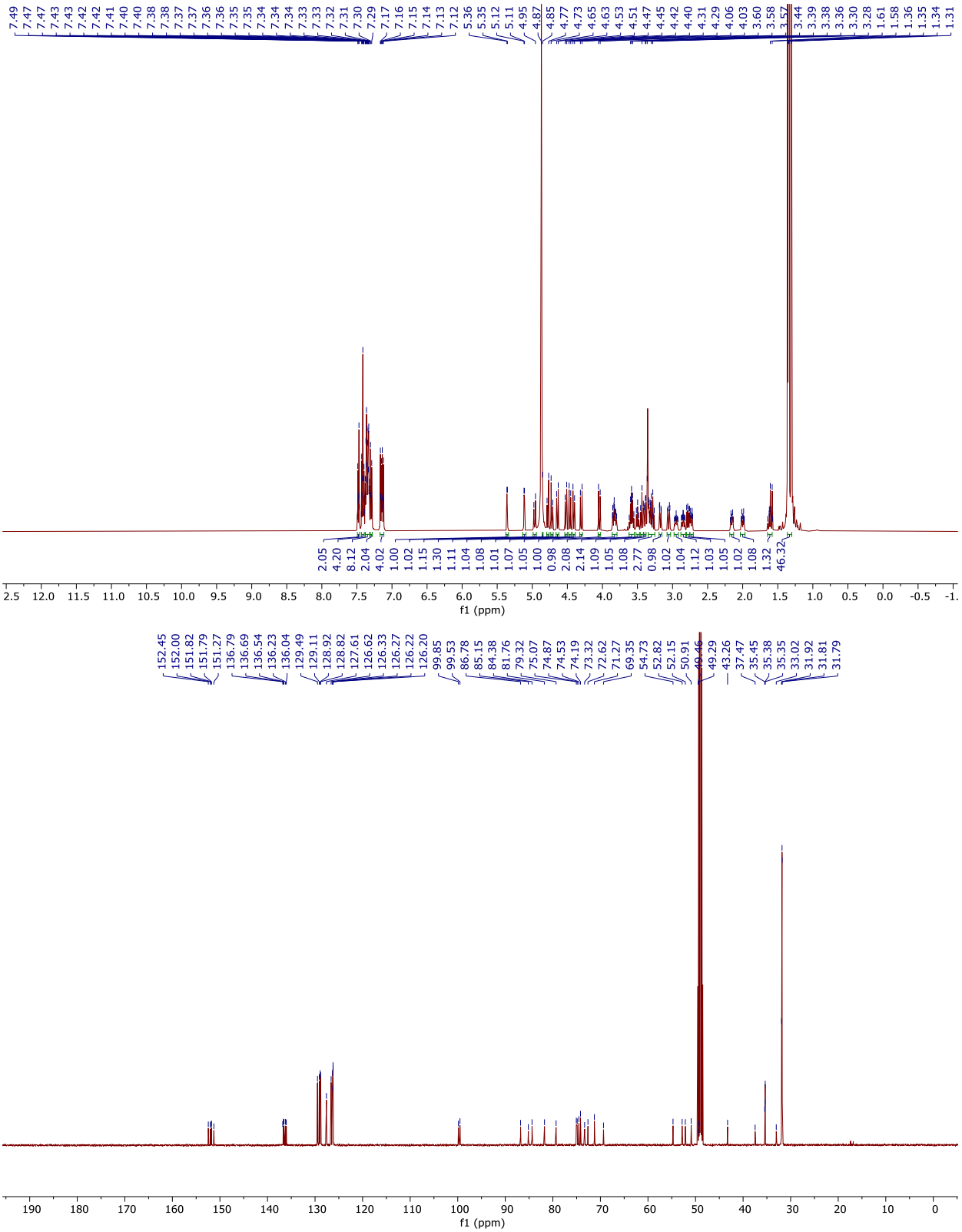
HSQC and HMBC of compound **5**



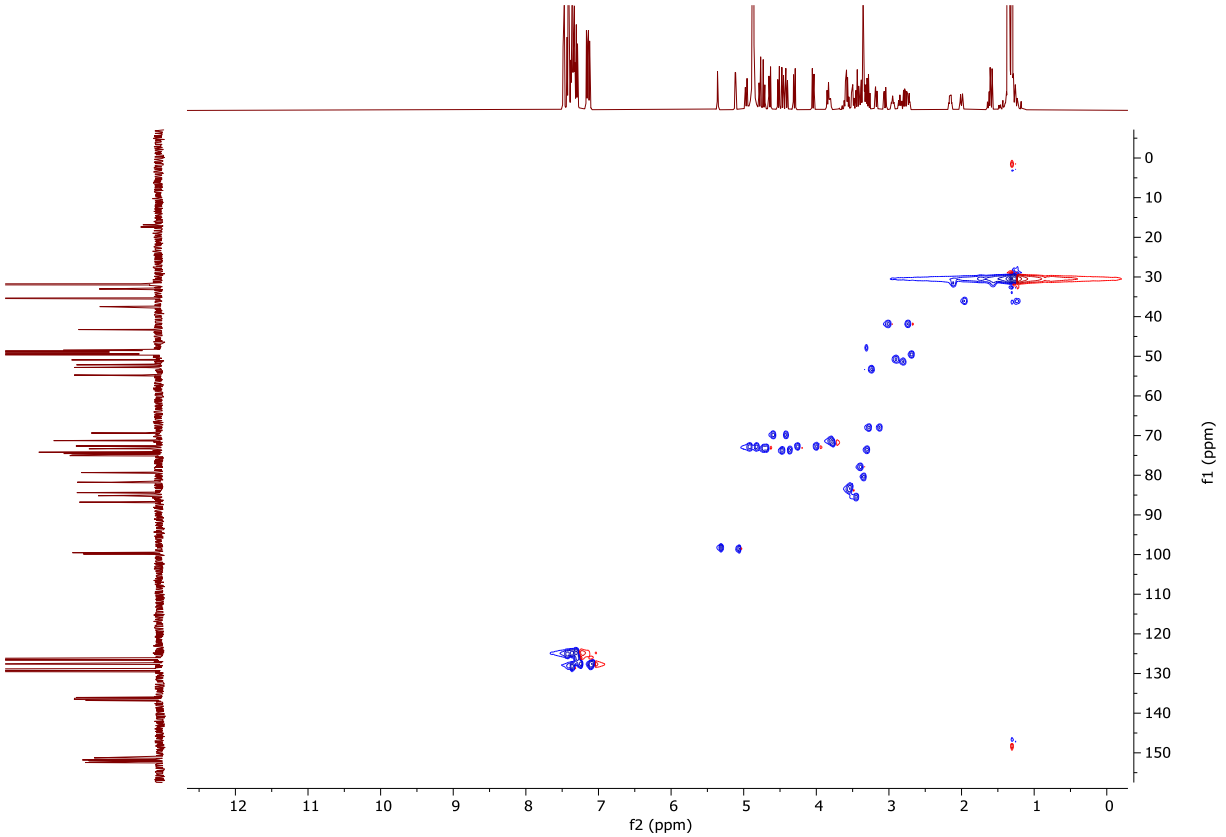
COSY of compound **5**

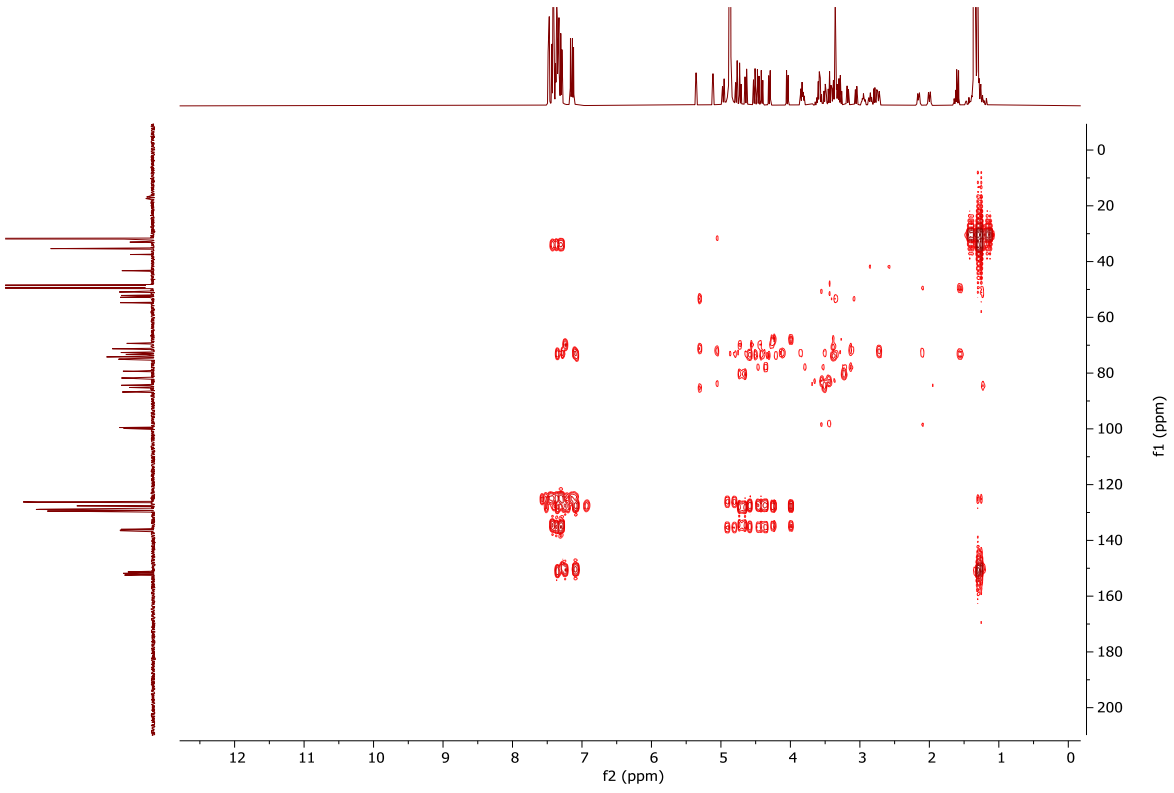


^{1}H and ^{13}C NMR of compound **5** (HCl salt)

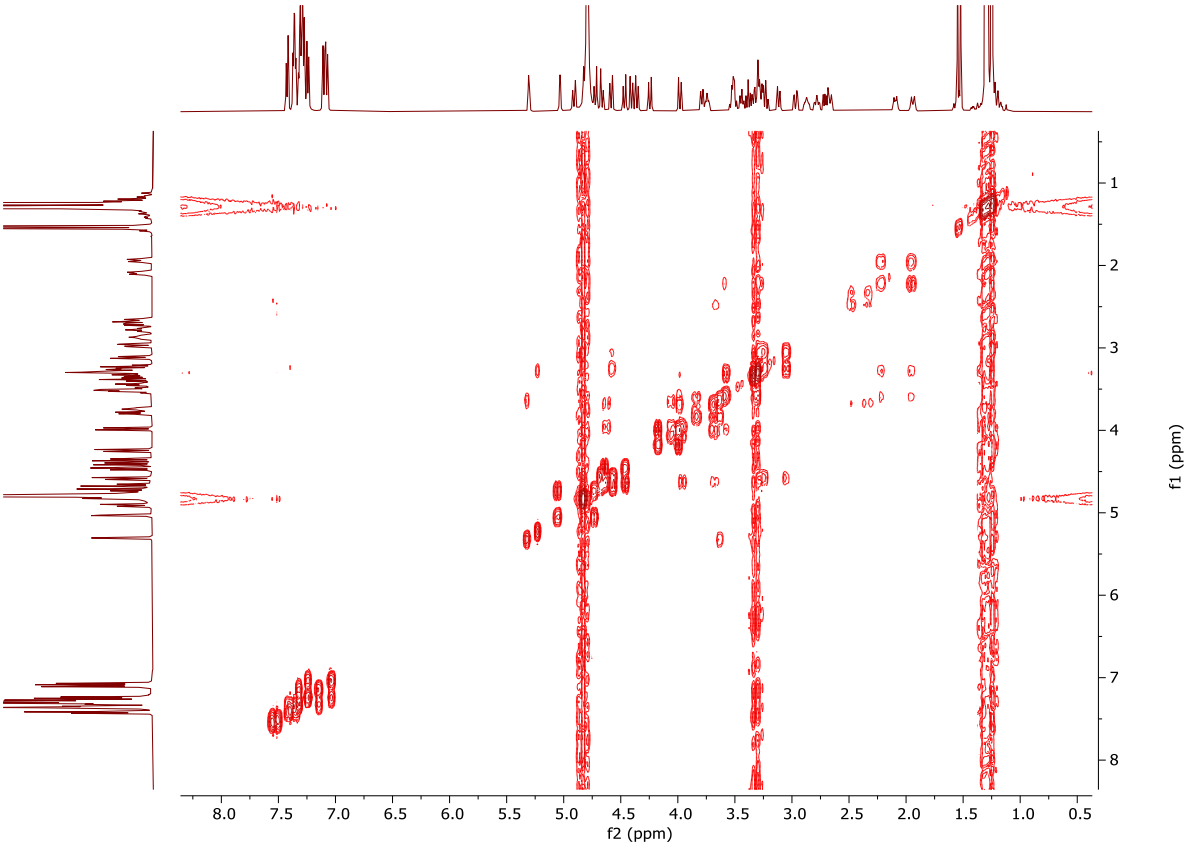


^{1}H and ^{13}C NMR of compound **6**

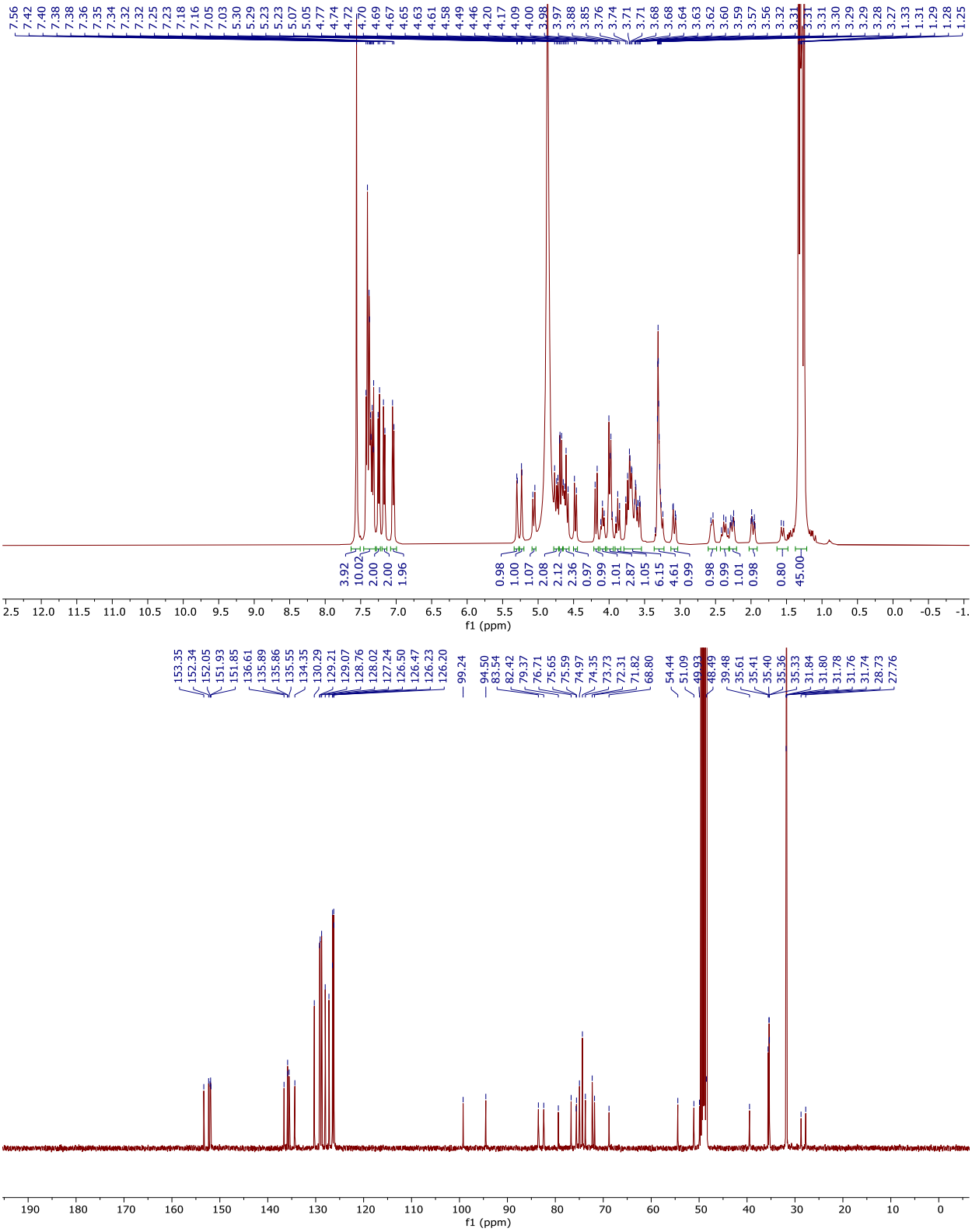




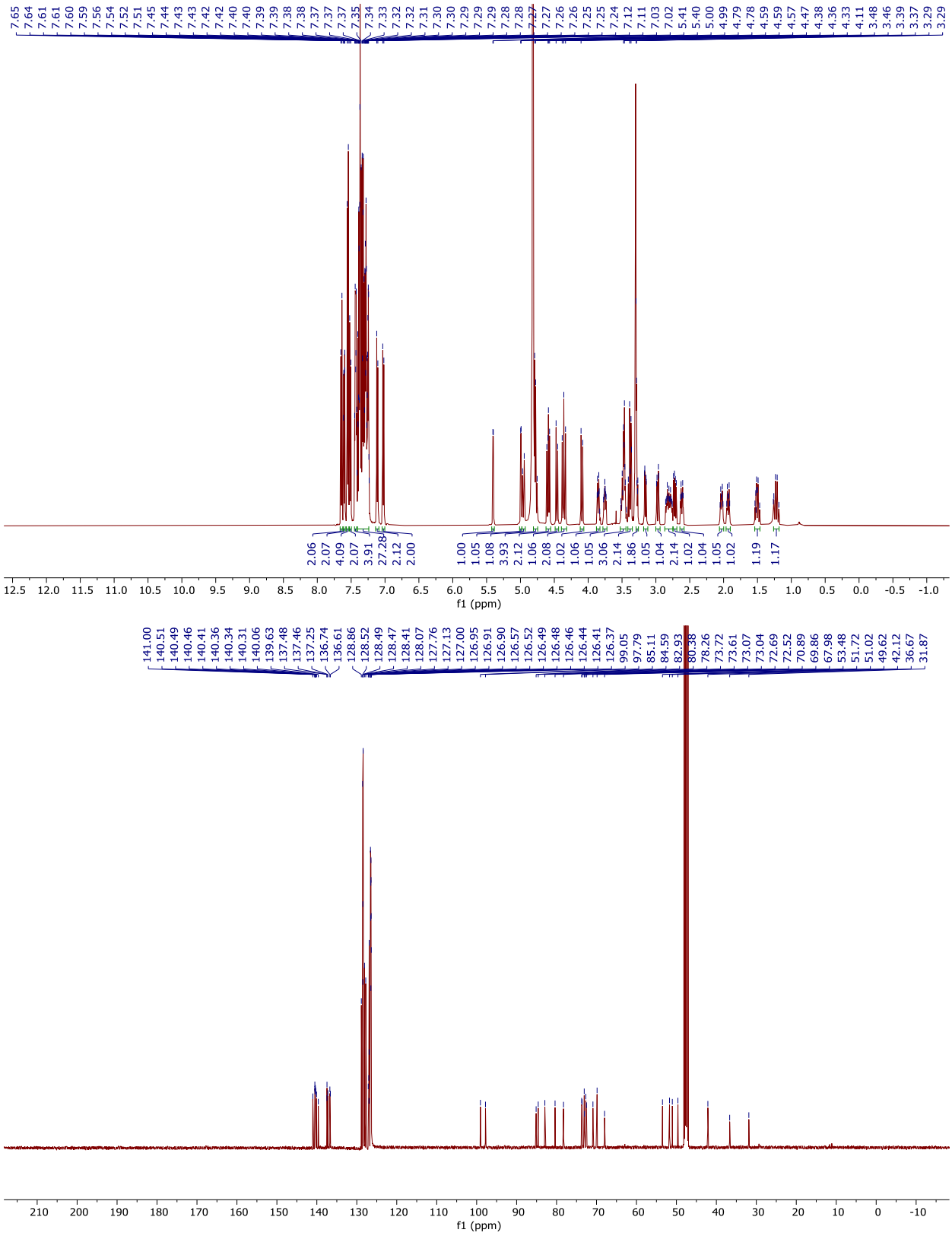
HSQC and HMBC of compound **6**



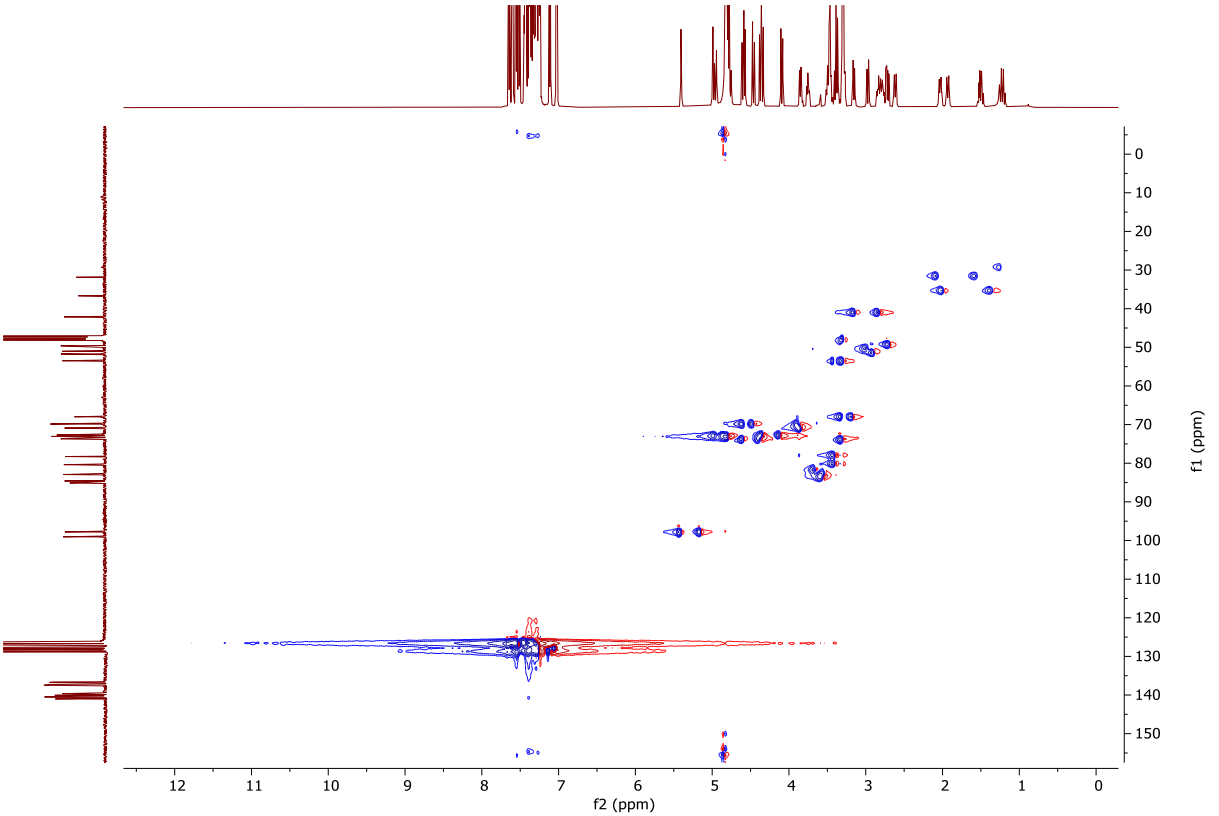
COSY of compound **6**

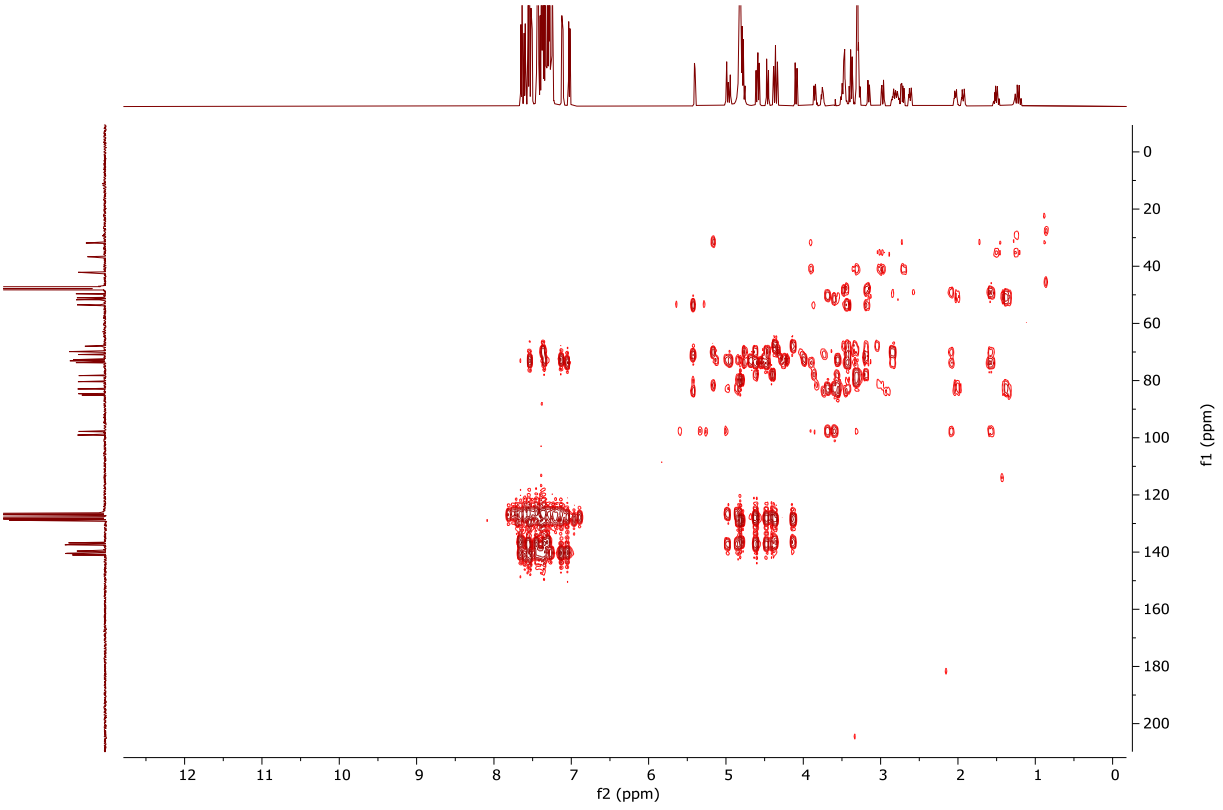


^{1}H and ^{13}C NMR of compound **6** (HCl salt)

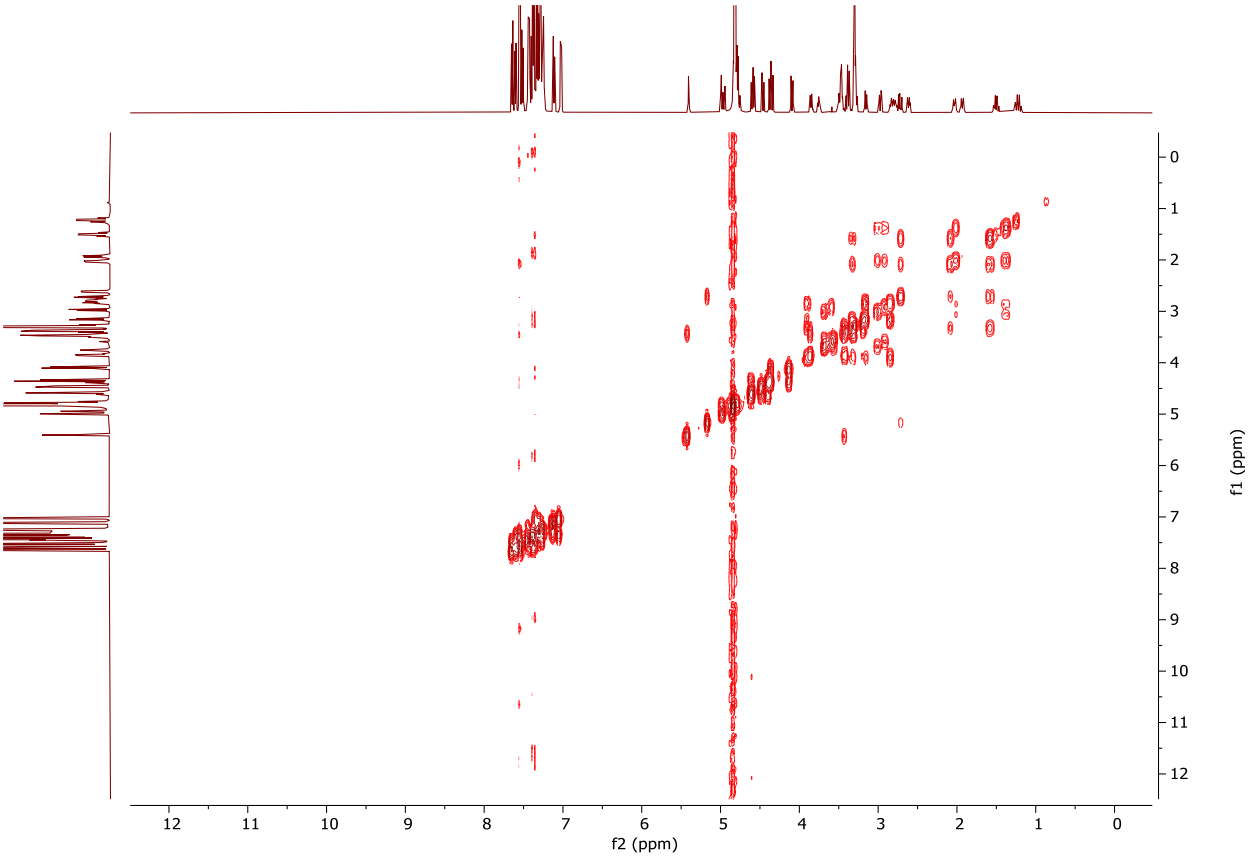


^{1}H and ^{13}C NMR of compound **7**

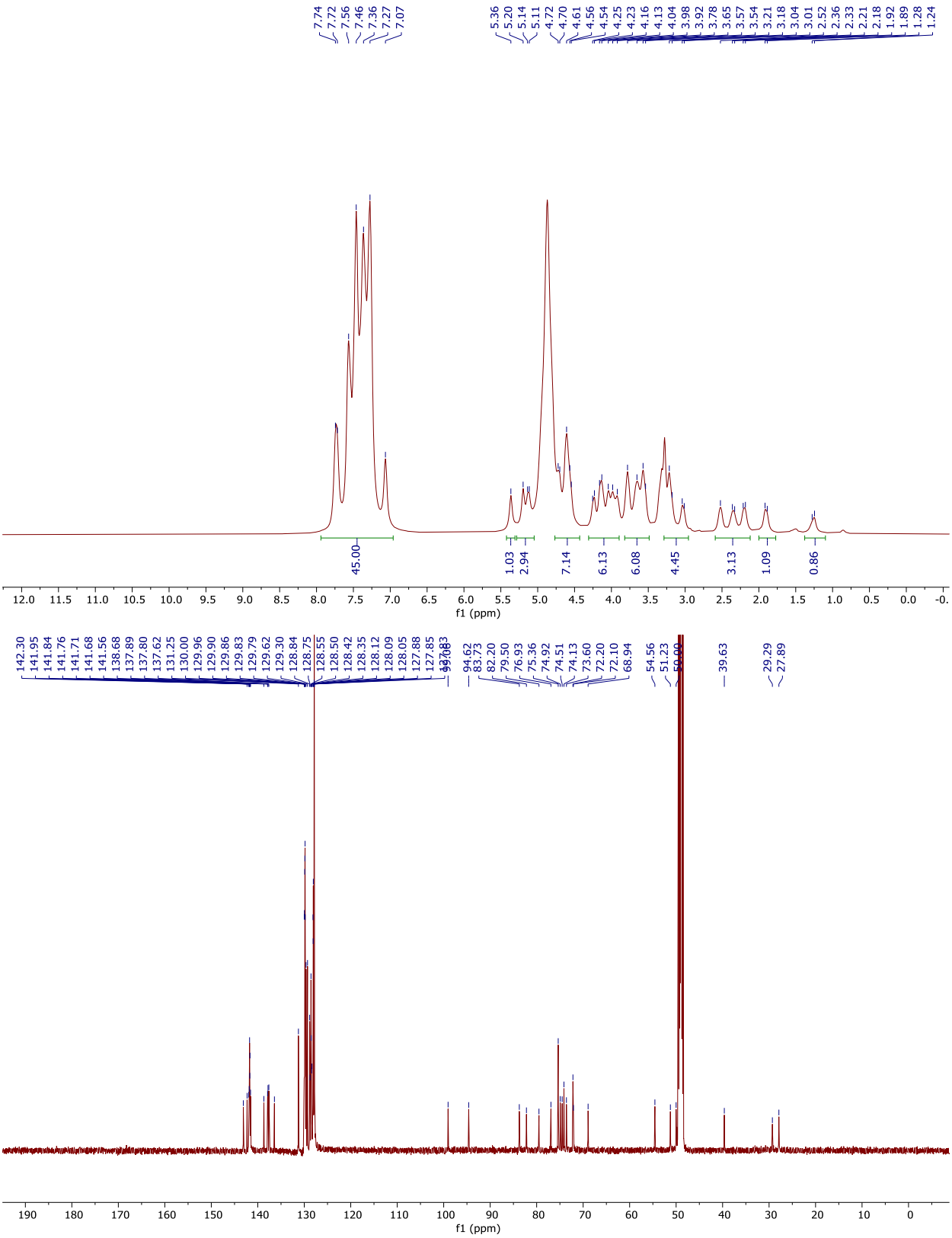




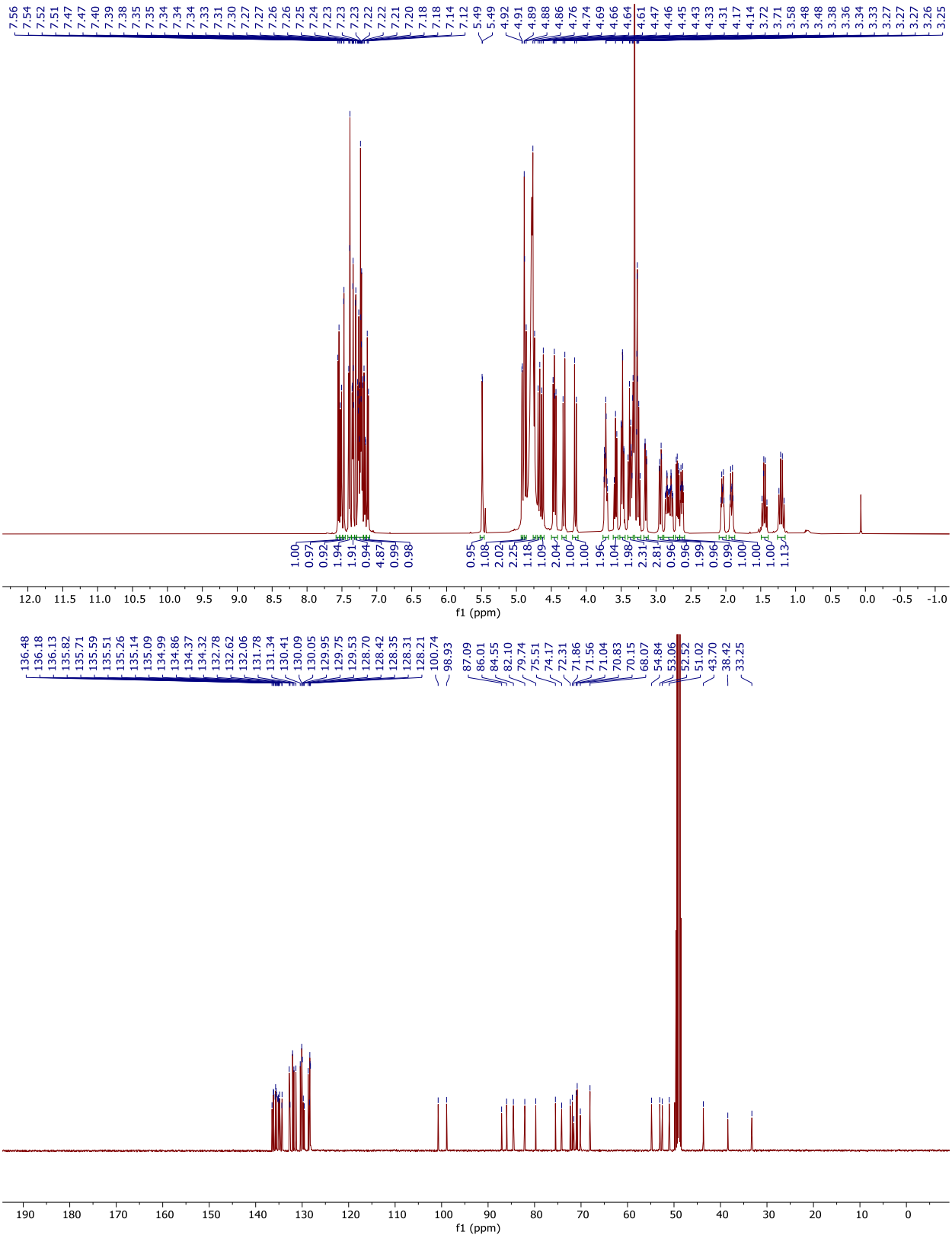
HSQC and HMBC of compound **7**



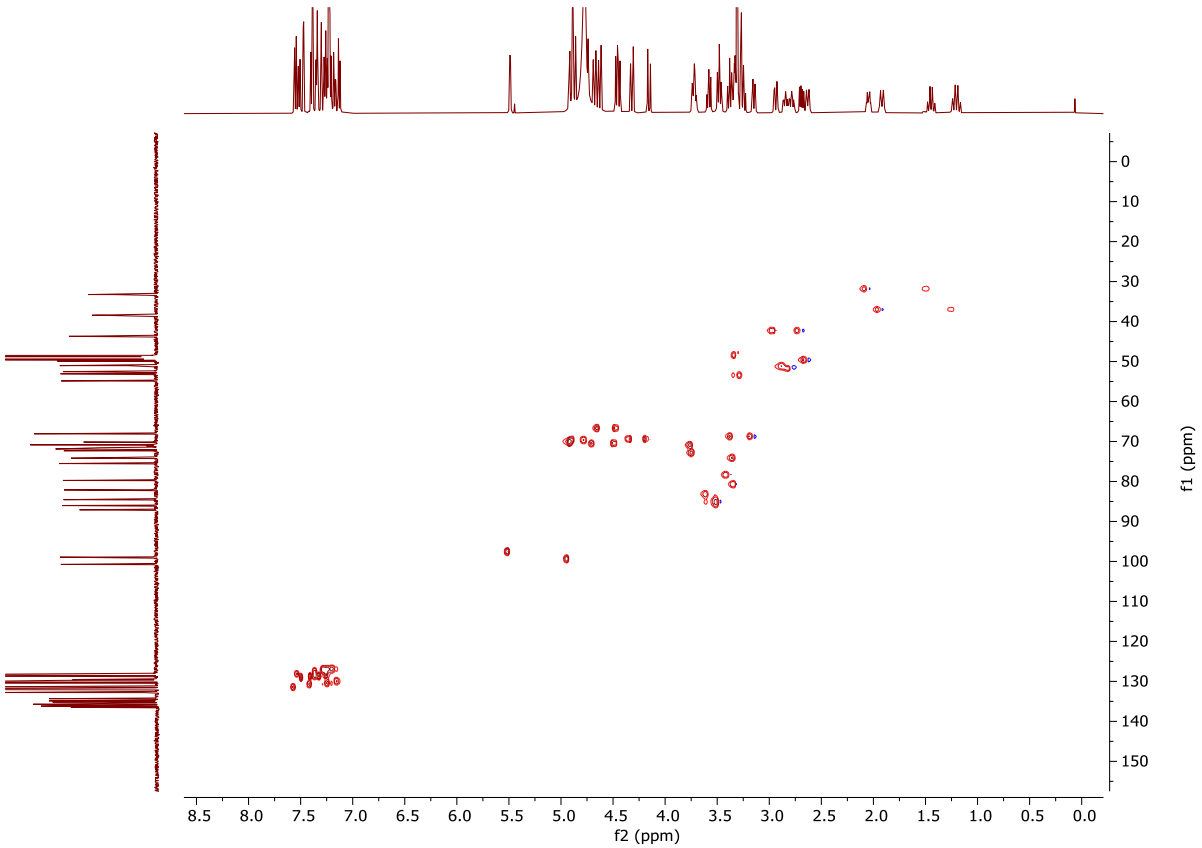
COSY of compound **7**

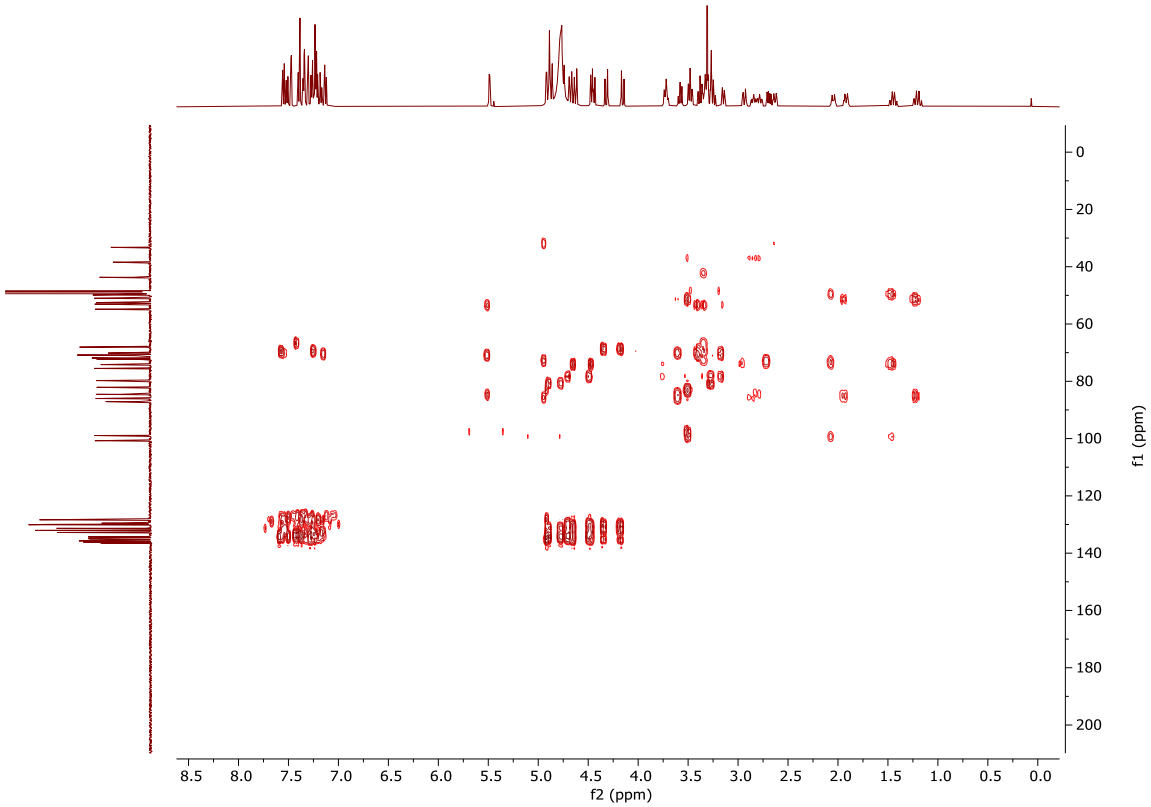


^{1}H and ^{13}C NMR of compound **7** (HCl salt)

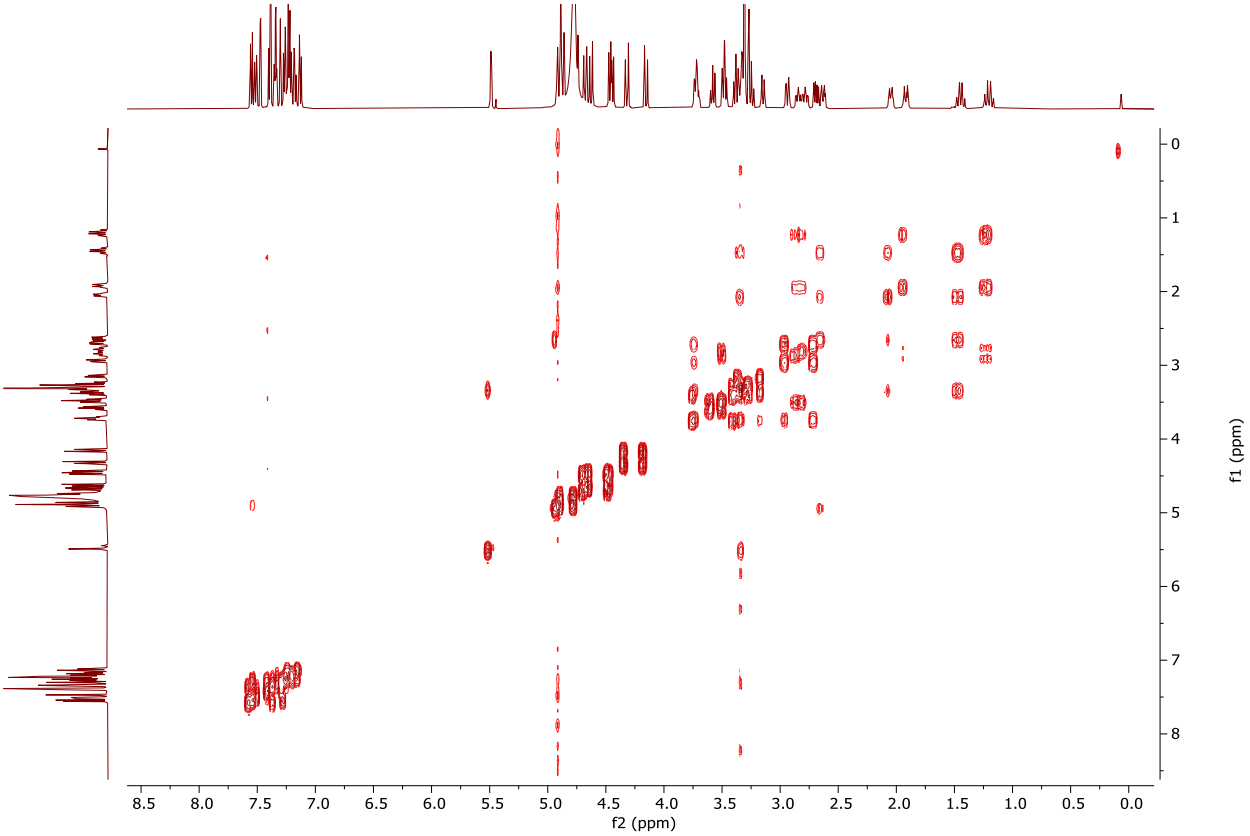


^{1}H and ^{13}C NMR of compound **8**

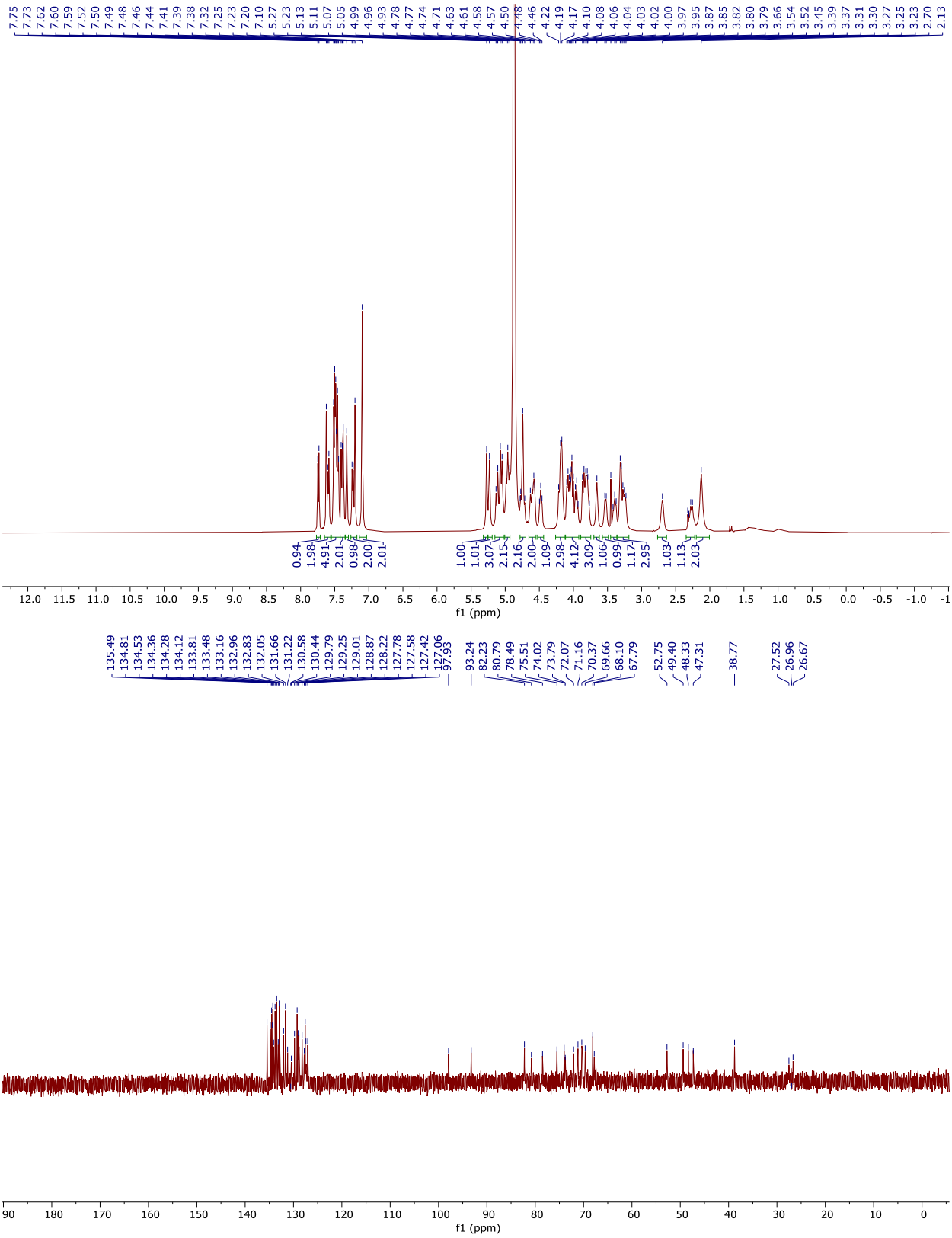




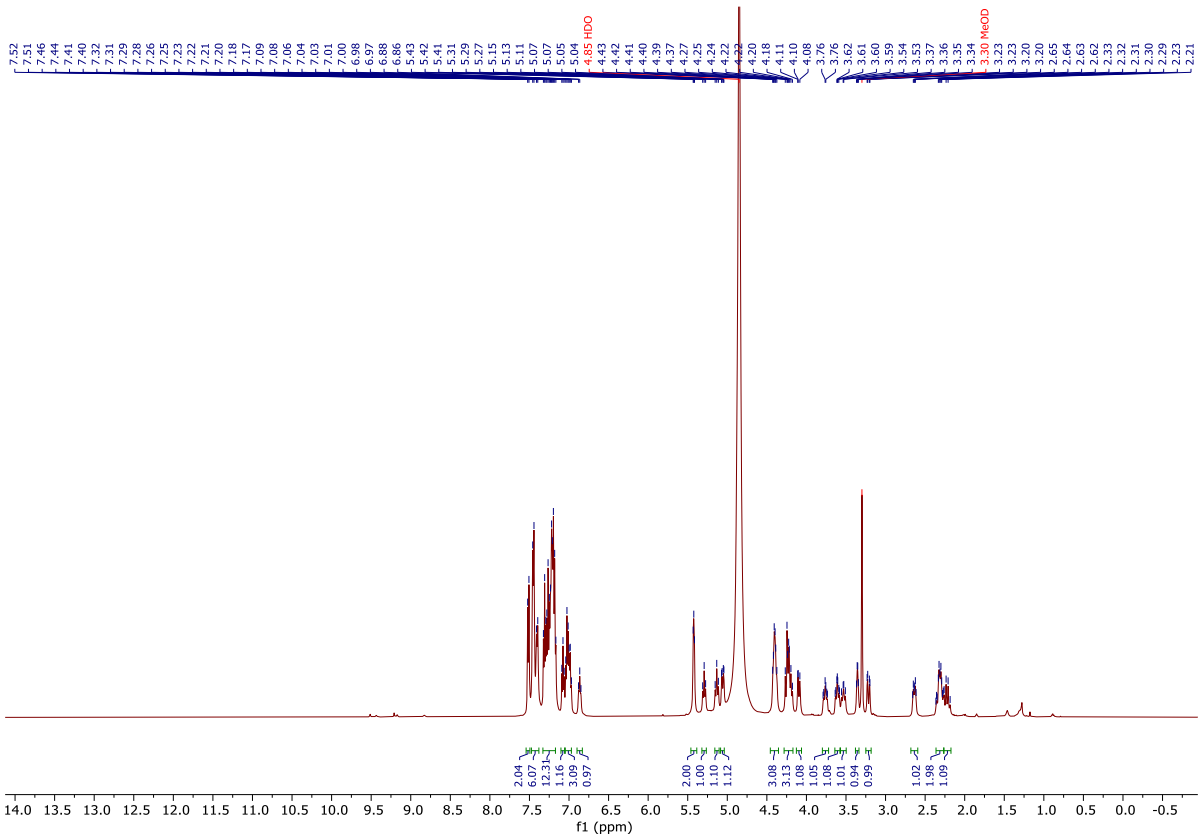
HSQC and HMBC of compound **8**

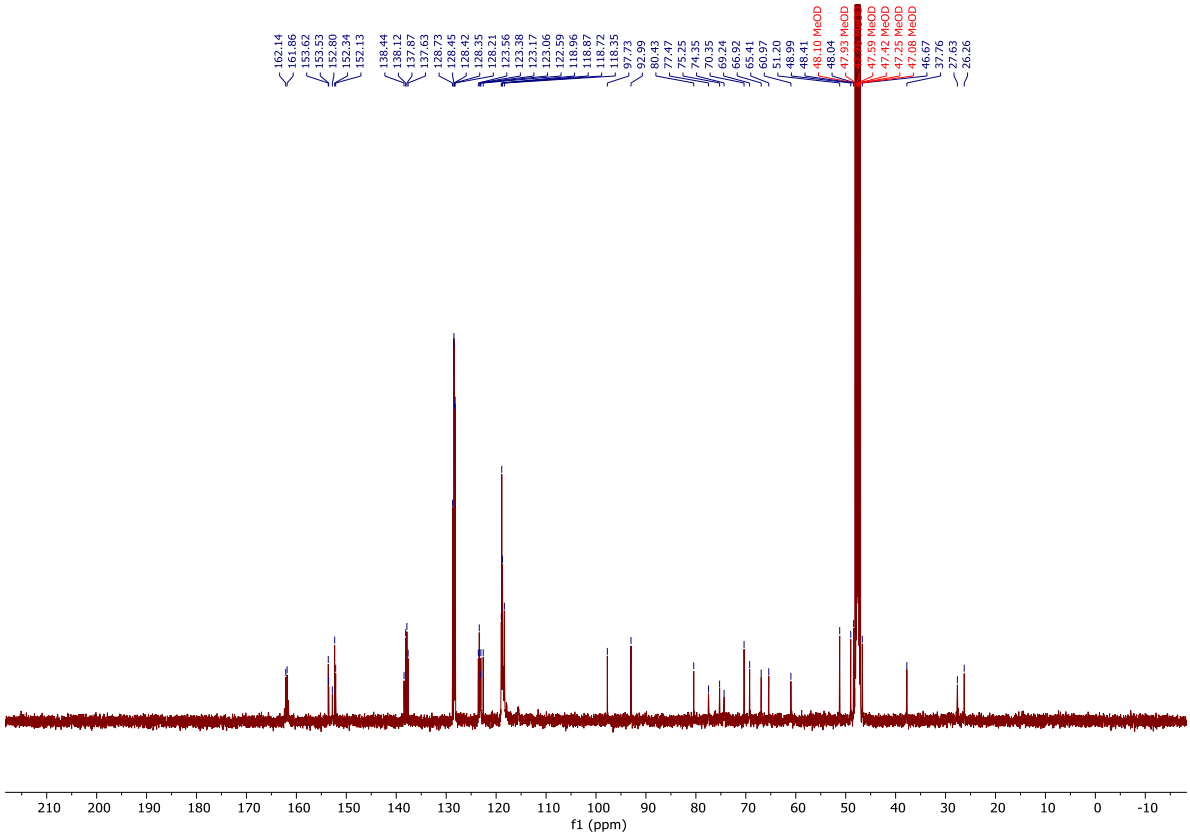


COSY of compound **8**

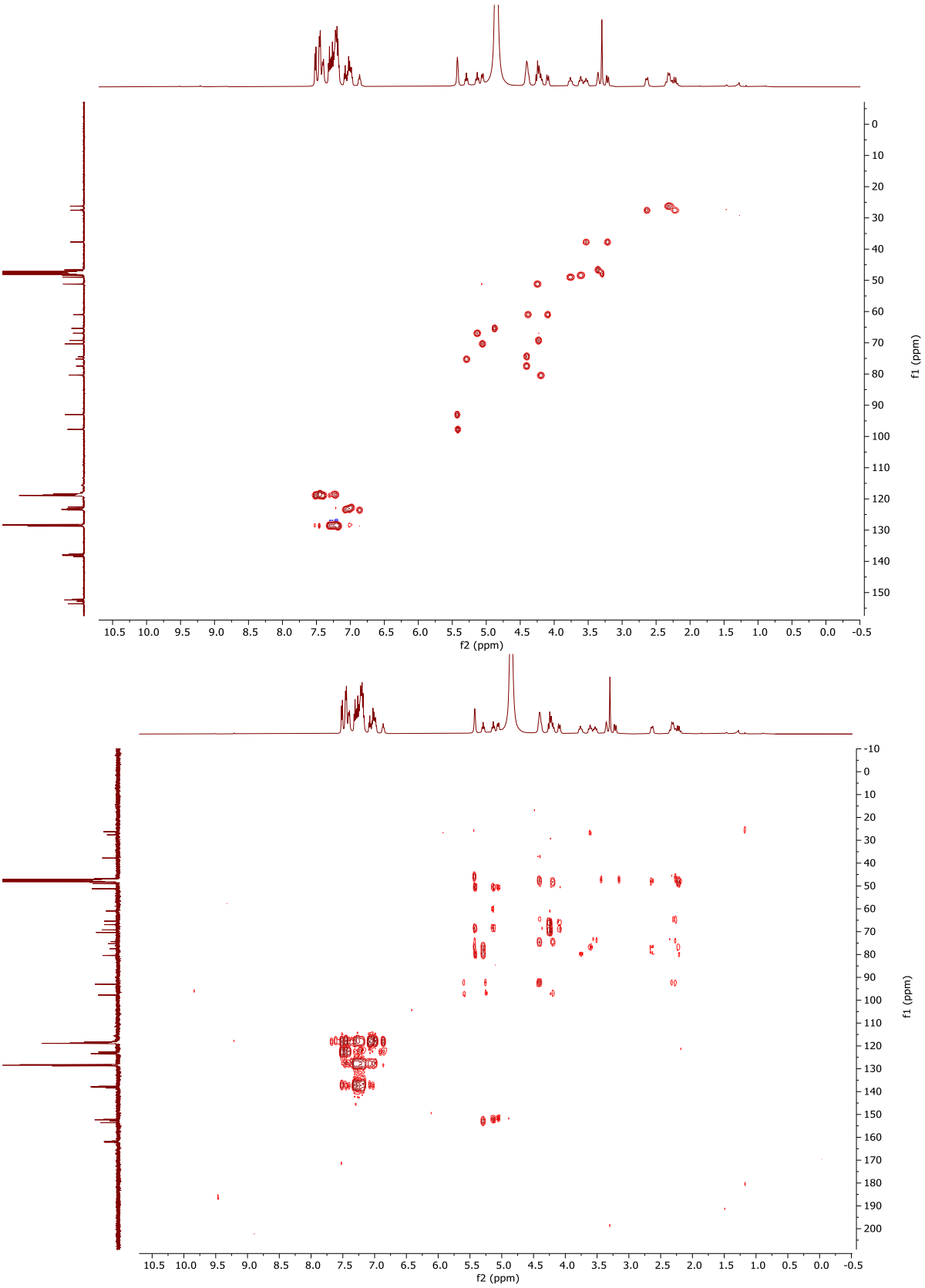


^{1}H and ^{13}C NMR of compound **8** (HCl salt)

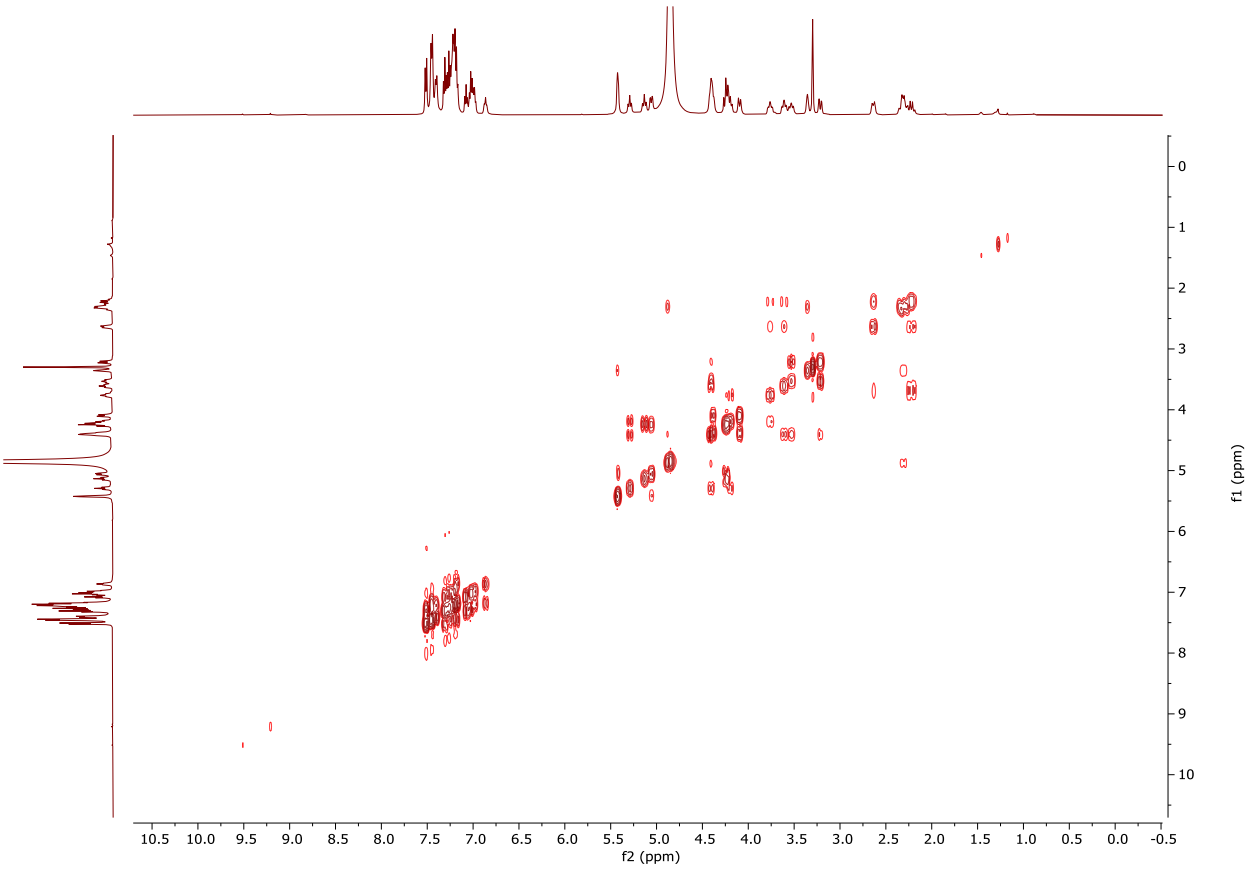

4.85 HDO
3.30 MeOD
f1 (ppm)


48.10 MeOD
48.04
47.93 MeOD
47.59 MeOD
47.42 MeOD
47.25 MeOD
47.08 MeOD
f1 (ppm)

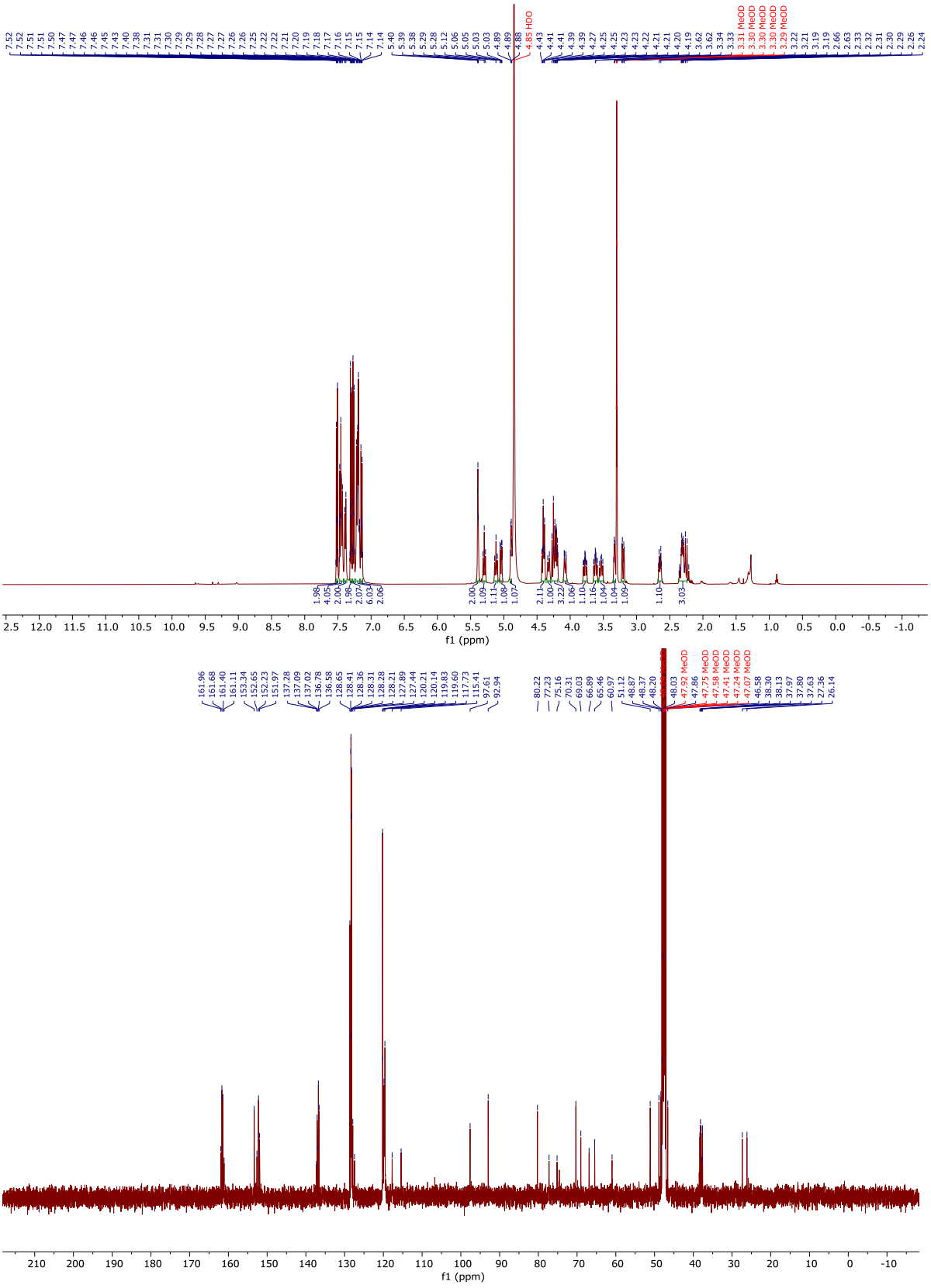
^{1}H and ^{13}C NMR of compound **9** (TFA salt)



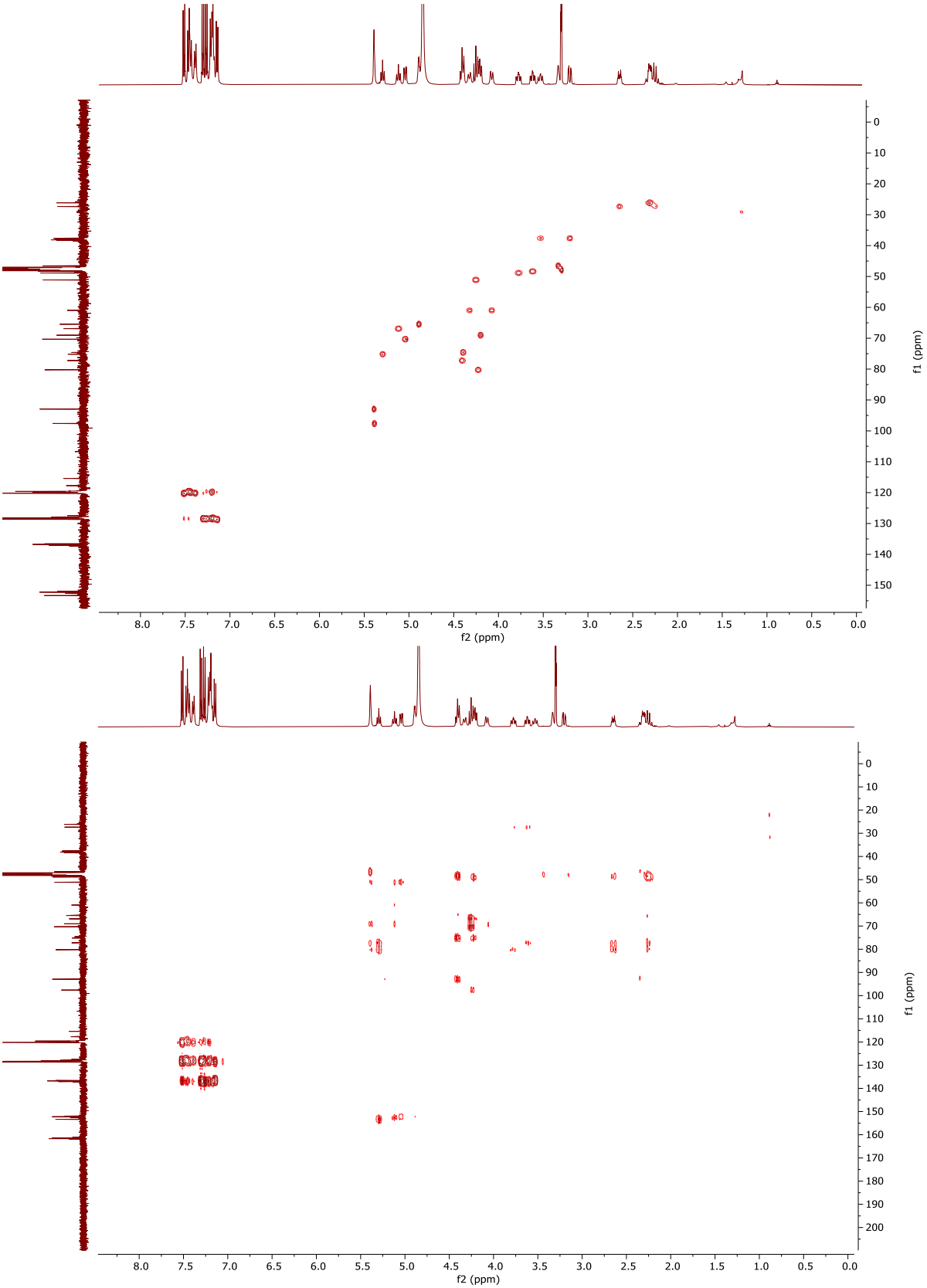
HSQC and HMBC of compound **9** (TFA salt)



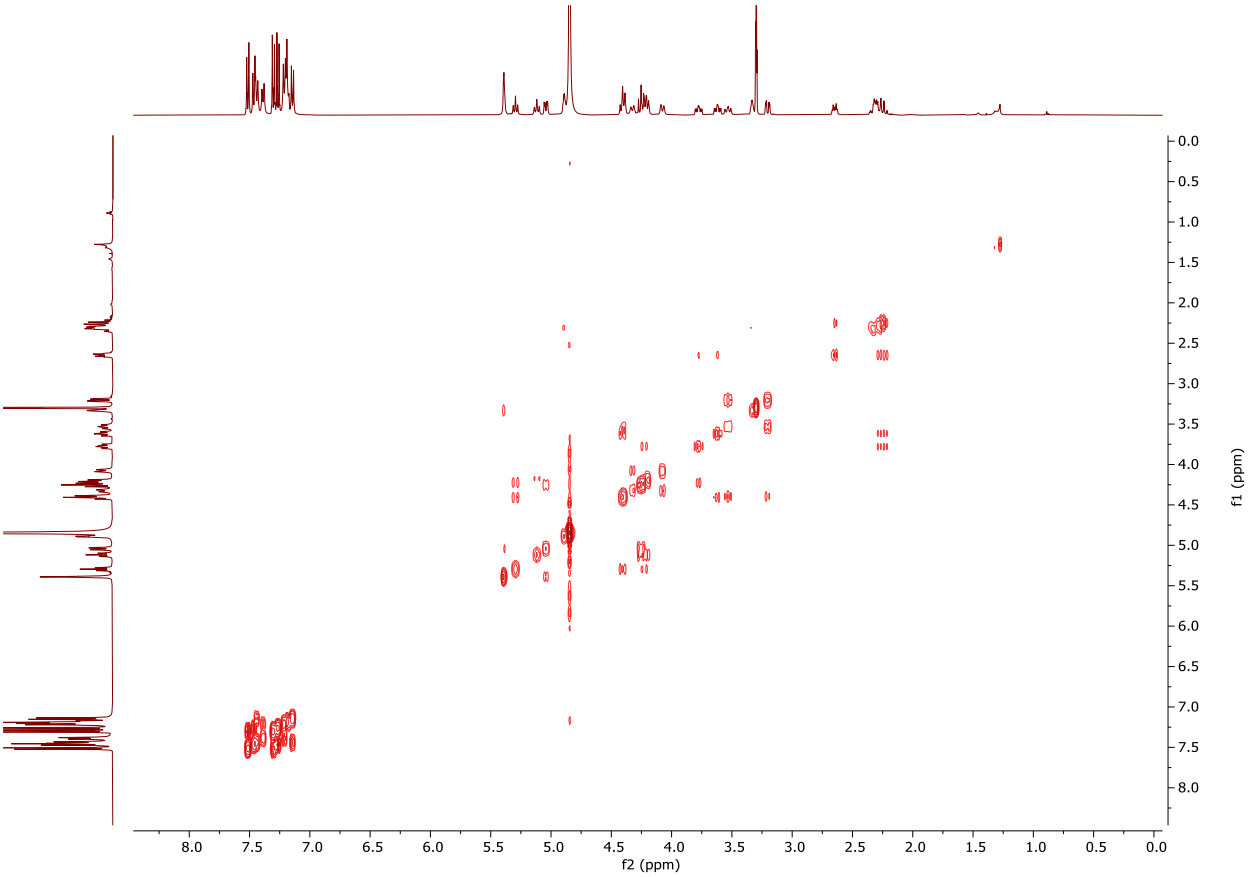
COSY of compound **9** (TFA salt)



^{1}H and ^{13}C NMR of compound **10** (TFA salt)



HSQC and HMBC of compound **10** (TFA salt)



COSY of compound **10** (TFA salt)

References

1 Y. Berkov-Zrihen, I. M. Herzog, R. I. Benhamou, M. Feldman, K. B. Steinbuch, P. Shaul, S. Lerer, A. Eldar and M. Fridman, *Chem. - A Eur. J.*, 2015, **21**, 4340–4349.

2 S. Bera, G. G. Zhanel and F. Schweizer, *J. Med. Chem.*, 2010, **53**, 3626–3631.