Electronic Supplementary Material (ESI) for ChemComm.

Lead-Oriented Synthesis of Epigenetic Relevant Scaffolds

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1 General information

Solvents were removed under reduced pressure using an IKA rotary evaporator equipped with an IKA MVP10 basic vacuum pump. Dry solvents and reagents were purchased from commercial suppliers and used without further purification. Thin-layer chromatography was performed on Merck silica gel 60F254 plates. Flash column chromatography was conducted using a Teledyne ISCO CombiFlash NextGen 300+ purification system using 4g, 12g, or 24g RediSep Rf silica cartridges. Thermo Scientific silica gel (35-70 µm) was used for manual chromatography columns.

Analytical LC-MS was performed using a system comprised of an Agilent 1260 Infinity II HPLC instrument equipped with an Agilent InfinityLab LS/MSD XT MS detector with electrospray ionisation. The system ran with a positive and negative switching mode and UV diode array detector using an Agilent ZORBAX SB-C18 RRHT (50 mm × 4.6 mm × 1.8 µm) column and gradient elution with two binary solvent systems: MeCN/H_2O or MeCN/H_2O plus 0.1% formic acid. Preparative HPLC was performed using a system comprising an Agilent 1260 Infinity II HPLC system equipped with an Agilent 1290 Prep Bin Pump, an Agilent Prep-C18 column (250 mm × 21.2 mm × 10 µm), and an Agilent prep autosampler and fraction collector. The system ran using a UV diode array detector and purification was performed using a gradient elution using a MeCN/H_2O binary solvent system.

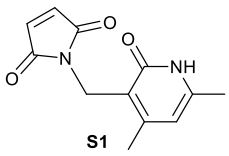
NMR analysis was conducted using a Bruker NEO400 spectrometer equipped with a 5mm BBFO IProbe (^{1}H = 400 MHz and ^{13}C = 100 MHz) or a Bruker NEO600 spectrometer equipped with a Prodigy BBO cryoprobe (^{1}H = 600 MHz and ^{13}C = 150 MHz). Chemical shifts are quoted in parts per million (ppm) and coupling constants are given in Hz. Splitting patterns have been abbreviated as follows: s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublets of doublets), td (triplet of doublets), t (triplet), q (quartet) and m (multiplet). NMR data is reported in the format: ppm (number of protons, splitting pattern, coupling constant).

Reactions were irradiated using a Kessil A160WE (Tuna Blue) LED lamp at 5cm.

2 Synthetic procedures

Compounds **1a** and **1c** have been previously reported in the literature.[1]

2.1 Synthesis of maleimide S1

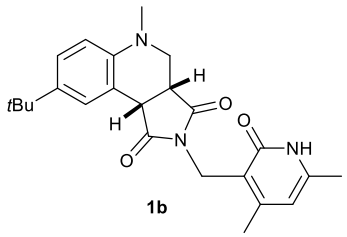


S1

Maleic anhydride (1 equiv., 500 mg, 5.1 mmol) and 3-(Aminomethyl)-4,6-dimethylpyridin-2(1H)-one (1.1 equiv., 854 mg, 5.61 mmol) were added to acetic acid (3 mL) and stirred at 120°C for 6 hours (checked by LC-MS). The mixture was cooled to room temperature and poured in cold water. The aqueous fraction was extracted three times with DCM, the organic phases were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude was purified by column chromatography (0% MeOH in DCM to 2.5% MeOH in DCM + 0.5% AcOH) to afford the desired product **S1** as a white powder (196 mg, 17%). Fractions need to be checked by LCMS as a side product co-elutes but cannot be detected by TLC.

^{1}H NMR (400 MHz, DMSO) δ 11.34 (s, 1H), 6.92 (s, 2H), 5.82 (s, 1H), 4.40 (s, 2H), 2.15 (s, 3H), 2.08 (s, 3H). **^{13}C NMR** (101 MHz, DMSO) δ 171.0, 162.6, 150.3, 143.6, 134.8, 119.3, 107.8, 34.3, 19.5, 18.6. **LCMS (ESI)**: Calculated for $C_{12}H_{13}N_2O_3$ $[M+H]^+$: 233.1; Found: 232.9.

2.2 Synthesis of tetrahydroquinoline 1b

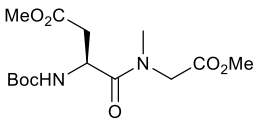


1b

S1 (1 equiv., 58 mg, 0.25 mmol) and 4-*tert*-butyl-*N*,*N*-dimethylaniline (5 equiv., 221 mg, 1.75 mmol) were added to a 10 mL vial and suspended in 1,4-dioxane (3 mL) with stirring. The reaction was then irradiated with a blue LED light (Kessil A160WE lamp), cooled with a fan and supplied with oxygen with an air balloon for 48 hours at room temperature. The solvent was then evaporated under reduced pressure and the crude product was dissolved in DMF/water (9:1) and purified by preparative reversed-phase HPLC chromatography (Agilent 1260 Infinity II HPLC) on a Agilent Prep-C18 column (250 mm × 21.2 mm × 10 µm) using a linear gradient (5% to 95% in 24 min, flow-rate of 20 mL.min^{-1}) of solvent B (0.1% TFA in ACN, v/v) in solvent A (0.1% TFA in H_2O, v/v). Detection was set at 254 and 280 nm. The volatiles were then evaporated in a SpeedVac Concentrator (Fisher Scientific). The compound was obtained as a yellow oil which showed a little degradation. It was then purified by silica gel column chromatography (0-3% MeOH in DCM) to afford the desired product as a light-yellow solid (29 mg, 28%).

^{1}H NMR (400 MHz, CDCl3) δ 13.10 (s, 1H), 7.49 (d, J = 2.5 Hz, 1H), 7.23 (dd, J = 8.6, 2.4 Hz, 1H), 6.65 (d, J = 8.6 Hz, 1H), 5.87 (s, 1H), 4.69 (d, J = 14.3 Hz, 1H), 4.53 (d, J = 14.1 Hz, 1H), 3.93 (d, J = 9.6 Hz, 1H), 3.43 (dd, J = 11.3, 3.2 Hz, 1H), 3.31 (ddd, J = 9.5, 4.7, 3.2 Hz, 1H), 3.03 (dd, J = 11.3, 4.7 Hz, 1H), 2.78 (s, 3H), 2.24 (d, J = 5.4 Hz, 6H), 1.33 (s, 9H); **^{13}C NMR** (101 MHz, CDCl3) δ 178.0, 176.2, 164.4, 151.7, 146.1, 143.7, 142.0, 127.5, 125.1, 118.7, 118.3, 111.9, 109.4, 50.9, 43.3, 42.0, 39.4, 35.7, 34.0, 31.5, 19.6, 18.5; **LCMS (ESI)**: Calculated $C_{24}H_{30}N_3O_3$ for $[M+H]^+$: 408.2; Found: 408.0; **R_f** = 0.37 (5% MeOH in DCM).

2.3 Synthesis of protected dipeptide S2

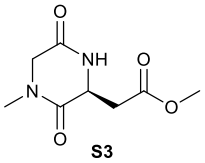


S2

To a solution of Boc-Asp(OMe)-OH (1 equiv., 1771 mg, 7.16 mmol) in DCM (35 mL) were added DMAP (1.2 equiv., 1051 mg, 8.6 mmol), EDCI (1 equiv., 1373 mg, 7.16 mmol) and sarcosine methyl ester hydrochloride (1 equiv., 1000 mg, 7.16 mmol). The reaction was stirred overnight at room temperature. The solution was then washed with 1N HCl, brine and dried over Na_2SO_4 and concentrated under reduced pressure. The crude was purified by silica gel chromatography (20%-50% EtOAc in hexanes) to afford the desired compound **S2** as a clear oil (770 mg, 32%) and as a mixture of rotamers.

^{1}H NMR (400 MHz, CDCl3) δ 5.40 (t, J = 9.3 Hz, 1H, M+m), 5.14 – 5.00 (m, 1H, M), 4.93 – 4.80 (m, 1H, m), 4.30 (d, J = 2.0 Hz, 2H, m), 4.21 (d, J = 17.3 Hz, 1H, M), 4.05 (d, J = 17.3 Hz, 1H, M), 3.78 (s, 3H, m), 3.75 (s, 3H, M), 3.71 (s, 3H, M), 3.70 (s, 3H, m), 3.22 (s, 3H, M), 3.00 (s, 3H, m), 2.86 – 2.74 (m, 2H, m+M), 2.64 (ddd, J = 15.6, 7.9, 5.9 Hz, 2H, M+m), 1.45 (s, 9H), 1.44 (s, 9H); **^{13}C NMR** (101 MHz, CDCl3) δ 171.6 (m), 171.5 (M), 171.2 (m), 171.1 (M), 169.4 (m), 169.2 (M), 154.97 (M), 154.89 (m), 80.26 (m), 80.16 (M), 52.4 (m), 52.2 (M), 52.0 (M), 51.9 (m), 51.3 (m), 49.8 (M), 47.38 (M), 47.33 (M), 37.5 (M), 37.3 (m), 36.5 (M), 35.4 (m), 28.27 (M), 28.25 (m); **LCMS (ESI):** Calculated for $C_{14}H_{24}N_2NaO_7$ $[M+H]^+$: 355.2 ;Found 354.8; **R_f** = 0.32 (50% EtOAc in Hexanes).

2.4 Synthesis of diketopiperazine methyl ester S3

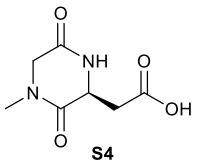


S3

S2 (1 eq., 770 mg, 2.32 mmol) was dissolved in a solution of 4 M HCl in dioxane (10 mL) and stirred at room temperature for one hour. The solvent was then evaporated under reduced pressure. The product was used without further purification. The crude was dissolved in MeOH (22 mL) and DIPEA was added until pH reached 8-9 (5 mL in this case). The reaction mixture was then stirred at 50 °C overnight. The solvent was evaporated under reduced pressure. The crude was purified by silica gel column chromatography (0-10% MeOH in DCM) to afford the pure product as a yellow oil (302 mg, 65%).

^{1}H NMR (400 MHz, CDCl3) δ 7.05 (bs, 1H), 4.35 (broad d, J = 8.3 Hz, 1H), 4.07 (dd, J = 17.7, 1.3 Hz, 1H), 3.96 (dd, J = 17.6, 1.3 Hz, 1H), 3.73 (s, 3H), 3.05 (dd, J = 17.5, 3.5 Hz, 1H), 3.00 (s, 3H), 2.85 (dd, J = 17.5, 8.1 Hz, 1H); **^{13}C NMR** (101 MHz, CDCl3) δ 171.1, 165.1, 164.8, 52.2, 51.7, 51.6, 38.0, 34.0; **LCMS (ESI):** Calculated for $C_8H_{13}N_2O_4$ $[M+H]^+$: 201.1; Found 201.1; **R_f** = 0.24 (5% MeOH in DCM).

2.5 Synthesis of diketopiperazine carboxylic acid S4

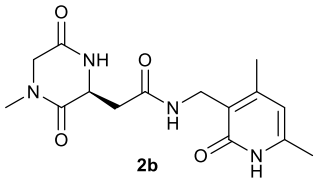


S4

S3 (1 eq., 186 mg, 0.93 mmol) was dissolved in THF (32 mL) and cooled to 0 °C. A solution of LiOH (2.5 eq., 56 mg, 2.32 mmol) in H_2O (16 mL) was added dropwise and the reaction mixture was stirred at 0 °C for 1 hour. Keeping the solution at room temperature, the reaction mixture was acidified with HCl to pH = 1 and extracted with DCM. Organic phases were combined, dried over Na_2SO_4 and concentrated *in vacuo*. The crude was purified by silica gel chromatography (0-10% MeOH in DCM + 1% AcOH) to afford the pure product as a white hygroscopic solid (180 mg, quant.).

^{1}H NMR (400 MHz, MeOD) δ 4.25 (t, J = 4.8 Hz, 1H), 4.11 (d, J = 17.6 Hz, 1H), 3.99 (d, J = 17.5 Hz, 1H), 2.97 (s, 3H), 2.80 (dd, J = 17.3, 4.2 Hz, 1H); **^{13}C NMR** (101 MHz, MeOD) δ 166.7, 166.4, 51.7, 51.1, 37.3, 32.7; **LCMS (ESI):** Calculated for $C_7H_{11}N_2O_4$ $[M+H]^+$: 187.1; Found 186.9; **R_f** = 0.17 (% MeOH in DCM + 1% AcOH).

2.6 Synthesis of diketopiperazine-pyridinone 2b

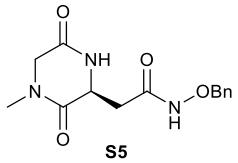


2b

To a solution of **S4** (1 eq., 50 mg, 0.269 mmol) in DMF (4 mL) were added triethylamine (2.5 eq., 94 µL, 0.67 mmol), HOBt (1.2 eq., 44 mg, 0.32 mmol), and EDCI (1.2 eq., 62 mg, 0.32 mmol) and stirred for 10 min. Then, 3-(Aminomethyl)-4,6-dimethylpyridin-2(1H)-one (1.1 eq., 41 mg, 0.27 mmol) was added. The reaction mixture was stirred overnight at room temperature. The solvent and volatiles were removed under reduced pressure. The residue was purified by silica gel chromatography (0%-20% MeOH in DCM + 1% AcOH) to afford the desired product as a yellow solid (71 mg, 89%).

^{1}H NMR (400 MHz, MeOD) δ 6.12 (s, 1H), 5.51 (s, 1H), 4.35 – 4.18 (m, 3H), 4.08 (d, J = 17.4 Hz, 1H), 3.96 (d, J = 17.4 Hz, 1H), 2.96 (s, 3H), 2.77 (qd, J = 15.6, 5.1 Hz, 2H), 2.27 (d, J = 10.1 Hz, 6H); **^{13}C NMR** (101 MHz, MeOD) δ 166.7, 152.5, 143.6, 109.8, 78.1, 52.0, 51.0, 38.4, 34.5, 32.7, 18.2, 17.2; **LCMS (ESI):** Calculated for $C_{15}H_{21}N_4O_4$ $[M+H]^+$: 321.2; Found 320.8; **R_f** = 0.2 (10% MeOH in DCM + 1% AcOH).

2.7 Synthesis of O-benzyl diketopiperazine hydroxamate S5

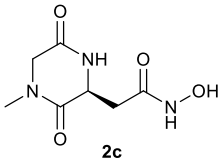


S5

To a solution of **S4** (1 eq., 50 mg, 0.27 mmol) in DMF (4 mL) were added HATU (1.1 eq., 112 mg, 0.3 mmol), DIPEA (1.3 eq., 108 µL, 0.62 mmol), and *O*-Benzylhydroxylamine hydrochloride (1 eq., 43 mg, 0.27 mmol). The reaction mixture was stirred at room temperature overnight. The volatiles were removed under reduced pressure. The residue was retaken in EtOAc and washed with an aqueous 1M HCl solution, saturated $NaHCO_3$ and brine. The organic phases were gathered, dried over Na_2SO_4 and concentrated under vacuo. The product could be seen in this residue so the aqueous phases was concentrated under vacuo. The solid thus obtained was extracted twice with DCM which allowed to obtain the desired product after evaporation of the volatiles. The residue was further purified by flash chromatography on silica gel (0-10% MeOH in DCM) to afford the desired product as a yellow oil (60 mg, 77%).

^{1}H NMR (400 MHz, MeOD) δ 7.48 – 7.19 (m, 5H), 4.25 (t, J = 4.9 Hz, 1H), 4.06 (dd, J = 17.6, 1.4 Hz, 1H), 3.93 (dd, J = 17.6, 1.2 Hz, 1H), 2.94 (s, 3H), 2.72 (dd, J = 15.7, 5.2 Hz, 1H), 2.55 (dd, J = 15.8, 4.8 Hz, 1H); **^{13}C NMR** (101 MHz, MeOD) δ 167.5, 166.4, 166.4, 135.5, 129.0, 128.3, 128.1, 77.8, 51.7, 51.0, 35.5, 32.8; **LCMS (ESI):** Calculated for $C_{14}H_{18}N_3O_4$ $[M+H]^+$: 292.1; Found 291.9.

2.8 Synthesis of hydroxamic acid 2c

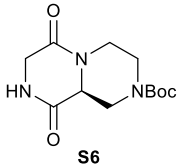


2c

To a solution of **S5** (1 eq., 60 mg, 0.206 mmol) in MeOH (5 mL) flushed with nitrogen was added 10% Pd/C (12 mg, 20% w/w). In the reaction mixture was bubbled hydrogen and it was stirred at room temperature for 6 hours. Completion was checked by TLC (10% MeOH in DCM). The reaction mixture was filtered over celite and washed with MeOH. The product was obtained as a white solid (39 mg, quant.) that could be used without further purification.

^{1}H NMR (400 MHz, DMSO) δ 10.46 (s, 1H), 8.79 (s, 1H), 8.12 – 7.94 (m, 1H), 4.10 – 4.03 (m, 1H), 3.91 – 3.85 (m, 1H), 3.82 (dd, J = 17.0, 1.0 Hz, 1H), 2.79 (d, J = 3.4 Hz, 3H), 2.55 – 2.36 (m, 3H); **^{13}C NMR** (101 MHz, DMSO) δ 166.5, 166.4, 165.5, 51.8, 51.7, 35.9, 33.5; **LCMS (ESI):** Calculated for $C_7H_{10}N_3O_4$ $[M-H]^-$: 200.1; Found 200.1.

2.9 Synthesis of Boc-protected diketopiperazine S6



S6

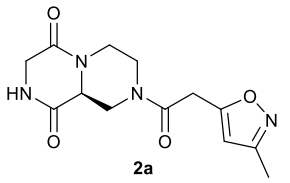
To a solution of Cbz-Gly-OH (1 eq., 236 mg, 1.13 mmol) in DCM (8 mL), under a nitrogen atmosphere and at 0 °C, was added HATU (473 mg, 1.24 mmol, 1.1 equiv) and DIPEA (394 µL, 2.26 mmol, 2 equiv). After 30 min, a solution of (S)-1-tert-Butyl 3-methyl piperazine-1,3-dicarboxylate (276 mg, 1.13 mmol, 1 equiv) in DCM (1.6 mL) was added and the reaction was stirred at 0 °C for 1 h and at rt for 24 h. The mixture was then diluted with EtOAc (100 mL) and the organic phase was washed in order with 1 M $KHSO_4$ (2 * 20 mL), aqueous $NaHCO_3$ (2 * 20 mL), and brine (2 * 20 mL) and dried over Na_2SO_4, then volatiles

were removed under reduced pressure. The residue was purified by flash chromatography on silica gel (n-hexanes/EtOAc) to afford the desired product as a clear/light-yellow oil (386 mg). The product was slightly contaminated with tetramethylurea but was telescoped into the next step.

The compound obtained above was dissolved in MeOH (8 mL) and ammonium formate (20 eq., 1.12 g, 17.7 mmol). The suspension was placed under an atmosphere of N_2 and 10% Pd/C (177 mg) was added portion wise. The suspension was then refluxed for 20 hours and conversion was checked by TLC & LC-MS. The reaction mixture was filtered over celite which was washed with MeOH (100 mL). The filtrate was then concentrated under vacuo. The residue was purified by flash chromatography on silica gel (0-6% MeOH in DCM) to afford the desired product **S6** as a white solid (150 mg, 49% over two steps).

^{1}H NMR (400 MHz, CDCl3) δ 6.85 (s, 1H), 4.66 – 4.50 (m, 2H), 4.22 – 4.10 (m, 1H), 4.08 (s, 2H), 4.00 – 3.91 (m, 1H), 2.87 – 2.66 (m, 3H), 1.48 (s, 9H); **^{13}C NMR** (101 MHz, CDCl3) δ 164.8, 162.0, 154.2, 81.0, 56.5, 44.7, 41.3, 28.3. **LCMS (ESI):** Calculated for $C_{12}H_{19}N_3NaO_4$ $[M+H]^+$: 292.1; Found 292.0; $\mathbf{R_f}$ = 0.32 (10% MeOH in DCM).

2.10 Synthesis of diketopiperazine isoxazole 2a



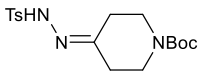
2a

S5 (1 equiv., 42 mg, 0.16 mmol) was placed in a round bottom flask in an ice-bath under argon and 4M HCl in dioxane (2 mL) was added. The ice-bath was removed and the reaction mixture was stirred at room temperature for 1 hour (Completion check by TLC). The solvent was removed under vacuo and the crude was used without purification.

Then to a solution of 3-Methyl-5-isoxazoleacetic acid (1.1 equiv., 24 mg, 0.17 mmol) in DMF (2.3 mL) were added triethylamine (3 equiv., 70 µL, 0.47 mmol), HOBt (1.25 equiv., 26 mg, 0.19 mmol), and EDCI (1.25 equiv., 37 mg, 0.19 mmmol) and stirred for 10 min. Then, the above crude product (1 equiv., 32 mg, 0.156 mmol) in DMF (2.3 mL) was added. The reaction mixture was stirred at room temperature overnight. The reaction mixture was evaporated under vacuo. The crude was purified by silica gel chromatography (0-8% MeOH in DCM) to afford the desired product in a mixture with HOBt as an oil. This oil was dissolved in DCM and washed with saturated $NaHCO_3$. The aqueous phase was extracted five times with DCM. The organic phases were combined, dried over Na_2SO_4 and concentrated in vacuo to afford the desired product as a white solid (20 mg, 45%). This compound was obtained as a mixture of rotamers visible by NMR.

^{1}H NMR (400 MHz, CDCl3) δ 8.31 (s, 1H, m), 8.25 (s, 1H, M), 6.22 (s, 1H, M), 6.21 (s, 1H, m), 4.69 (dd, J = 13.0, 3.8, 1H, M), 4.35 – 4.23 (m, 1H+2H, m+M), 4.21 – 3.77 (m, 6H, m+M), 3.26 (dd, J = 13.4, 10.8 Hz, 1H, m), 3.16 – 3.02 (m, 1H, M), 2.82 – 2.58 (m, 2H, m+M), 2.20 (s, 3H); **^{13}C NMR** (101 MHz, DMSO) δ 167.15 (M), 167.09 (m), 166.2 (M+m), 164.58 (m), 164.53 (M), 163.5 (m), 163.4 (M), 159.94 (M), 159.92 (m), 104.6 (M+m), 56.13 (M), 56.09 (m), 48.0 (m), 44.9 (M), 44.5 (M), 44.3 (m), 41.2 (M), 40.9 (m), 32.0 (m), 31.84 (M), 11.44 (M+m); **LCMS (ESI):** Calculated for $C_{13}H_{17}N_4O_4$ $[M+H]^+$: 293.1; Found 292.8.

2.11 Synthesis of hydrazone S7

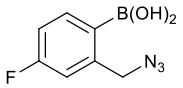


S7
CAS RN : 1046478-89-0

To a solution of sulfonyl hydrazide (1.0 eqv) in MeOH (0.5M) was added ketone (1.0 eqv). The rxn mix was stirred at RT until complete conversion was observed by TLC. Solvents were removed in vacuum to give the title compound as a white solid (9.7 g, 98%).

^{1}H NMR (400 MHz, CDCl3) δ 7.83 (dt, J = 8.6, 2.3 Hz, 2H), 7.36 – 7.29 (m, 2H), 3.57 – 3.40 (m, 4H), 2.49 – 2.32 (m, 7H), 1.44 (s, 9H); **LCMS (ESI):** Calculated for $C_{17}H_{26}N_3O_4S$ $[M+H]^+$: 368.2; Found 367.9.

2.12 Synthesis of boronic acid S8

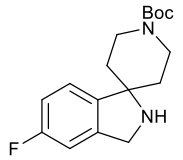


S8

(2-(bromomethyl)-4-fluorophenyl)boronic acid (1 eq., 1 g, 4.31 mmol) was dissolved in THF (7 mL) and distilled water (2 mL). After the addition of sodium azide (3 eq., 0.84 g, 12.9 mmol), the reaction mixture was vigorously stirred at room temperature for 12 h. The resulting mixture was extracted with diethyl ether (40 mL) and the organic layer was washed with water (3 x 10 mL) and dried over Na_2SO_4. Solvent was evaporated under reduced pressure to obtain **S8** as a white solid (620 mg, 75%).

^{1}H NMR (400 MHz, CDCl3) δ 8.31 – 8.21 (m, 1H), 7.26 – 7.16 (m, 2H), 4.87 (s, 2H); **^{13}C NMR** (101 MHz, CDCl3) δ 165.7 (d, J = 254.7 Hz), 145.7 (d, J = 8.0 Hz), 140.1 (d, J = 9.1 Hz), 116.9 (d, J = 21.4 Hz), 115.2 (d, J = 20.0 Hz), 53.7.

2.13 Synthesis of spiro-indoline S9



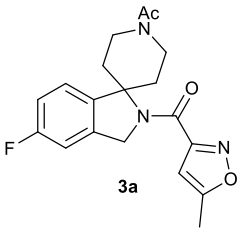
S9

A pressure tube provided with a stir bar was charged with boronic acid **S7** (3 eq., 478 mg, 2.452 mmol) and LiOMe (3 eq., 92 mg, 2.452 mmol) in dry dioxane (5 mL) under an argon atmosphere. The tube was heated at 120 °C and a solution of N-sulfonylhydrazone (1 eq., 300 mg, 0.82 mmol) dissolved in dry dioxane (5 mL) was added with a syringe pump (0.25 mL/h). Once the addition was finished, the tube was heated at 120 °C for 16 hours. The reaction mixture was then cooled down to room temperature and to the mixture was added 10 mL of EtOAc and 5 mL 1M HCl and vigorously stirred. The layers were separated, and the organic phase was extracted three more times with 5 mL of 1M HCl. The organic phase was discarded. The combined aqueous acidic phase was treated with EtOAc (10 mL). To the mixture was added a solution of aqueous 3M NaOH until the aqueous phase reached basic pH > 10. The layers were separated, and the basic aqueous phase was extracted three times with EtOAc (3 x 10 mL). The combined basic organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give the product. After column chromatography (EtOAc in hexanes) compound **S9** was obtained as a gummy solid (130 mg, 52 %).

^{1}H NMR (400 MHz, CDCl3) δ 7.10 – 7.03 (m, 1H), 6.97 – 6.88 (m, 2H), 4.17 (s, 2H), 4.15 – 3.99 (m, 2H), 3.11 (t, J = 11.5 Hz, 2H), 2.04 – 1.97 (m, 1H), 1.83 (td, J = 12.9, 4.0 Hz, 2H), 1.65 – 1.54 (m, 2H), 1.50 (s, 9H); **^{13}C NMR** (101 MHz, CDCl3) δ 162.5 (d, J = 244.1 Hz), 154.9, 144.2 (d, J = 2.2 Hz), 143.8 (d, J = 8.0 Hz), 122.3 (d, J = 9.1 Hz), 113.9 (d, J = 22.9 Hz), 109.6 (d, J = 22.5 Hz)., 79.6, 64.3, 50.2, 50.2, 40.7, 36.8, 28.5.; **LCMS (ESI):** Calculated for $C_{17}H_{24}FN_2O_2$ $[M+H]^+$: 307.2; Found 306.8.

LCMS (ESI): Calculated for $C_{22}H_{26}FN_3NaO_4$ $[M+Na]^+$: 438.2; Found 437.7.

2.14 Synthesis of Acetylated isoxazole spiro-indoline 3a



3a

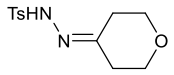
5-Methyl-isoxazole-3-carboxylic acid (1 eq., 6 mg, 0.049 mmol) was added to a solution of S8 (1 eq., 15 mg, 0.049 mmol), DIPEA (2.1 eq., 17.89 µL, 0.65 mmol), HATU (1.1 eq., 20.49 mg, 0.054 mmol) in DCM (0.5 mL). The resulting mixture was stirred at room temperature for 12 h. The mixture was concentrated and used without further purification.

The above crude was dissolved in DCM (0.3 mL), and 4 N HCl in 1,4-dioxane (0.1 mL) was added. After 12 h, the reaction mixture was concentrated to gave a white solid which was used in the next step without purification.

To a solution of the previous crude (1 eq., 16 mg, 0.051 mmol) and triethylamine (2 eq., 14.241 µl, 0.101 mmol) in DCM (0.3 mL) at 0 °C, acetyl chloride (1.2 eq., 4.333 µl, 0.0609 mmol) was added dropwise, and the resulting reaction mixture was allowed to warm to room temperature while stirring overnight (18 h). After this time, saturated $NaHCO_3$ was added, and the biphasic system was separated. The aqueous phase was extracted with DCM (three times) and the organic phases were combined, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting crude material was purified by flash column chromatography on silica gel (20% EtOAc on hexanes) to afford the desired compound **3a** (9 mg, 65%)

^{1}H NMR (400 MHz, CDCl3) δ 7.35 (dd, J = 8.6, 4.8 Hz, 1H), 7.04 (td, J = 8.8, 2.6 Hz, 1H), 7.00 – 6.92 (m, 1H), 6.41 (d, J = 1.0 Hz, 1H), 5.28 (d, J = 16.0 Hz, 1H), 5.21 (d, J = 16.0 Hz, 1H), 4.20 – 4.10 (m, 1H), 4.08-3.99 (m, 1H), 3.74 – 3.57 (m, 2H), 3.23-3.10 (m, 2H), 2.51 (d, J = 1.0 Hz, 4H), 2.19 (s, 3H), 1.93 (ddd, J = 14.0, 6.8, 4.4 Hz, 1H), 1.78 (dt, J = 13.6, 4.3 Hz, 1H); **^{13}C NMR** (101 MHz, CDCl3) δ 169.9, 169.8, 162.8 (d, J = 246.7 Hz), 160.6, 159.1, 140.4 (d, J = 2.9 Hz), 136.7 (d, J = 8.7 Hz), 124.1 (d, J = 8.7 Hz), 115.4 (d, J = 22.5 Hz), 109.8 (d, J = 23.6 Hz), 103.1, 68.8, 54.0 (d, J = 2.9 Hz), 42.4, 38.0, 33.9, 31.7, 21.8, 12.1**; LCMS (ESI):** Calculated for $C_{19}H_{21}FN_3O_3$ $[M+H]^+$: 358.15; Found 357.8.

2.15 Synthesis of hydrazone S10

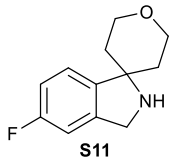


S10

To a solution of sulfonyl hydrazide (1.0 equiv., 5.02 g, 29.2 mmol) in MeOH (0.5 M) was added the tetrahydro-4H-pyran-4-one (1.0 equiv., 2.92 g, 29.2 mmol). The reaction mixture was stirred at room temperature until complete conversion was observed by TLC. Solvents were removed in vacuum to give the title compound as a white solid (7.6 g, 97 %).

Analyses were in accordance with literature.[2]

2.16 Synthesis of spiro-indoline S10



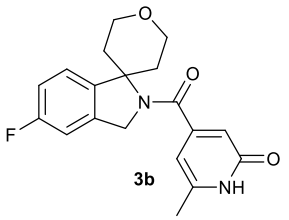
S11

A pressure tube provided with a stir bar was charged with boronic acid S7 (3 eq., 478 mg, 2.452 mmol) and LiOMe (3 eq., 92 mg, 2.452 mmol) in dry dioxane (5 mL) under an argon atmosphere. The tube was heated at 120 °C and a solution of N-sulfonylhydrazone (1 eq., 300 mg, 0.82 mmol) dissolved in dry dioxane (5 mL) was added with a syringe pump (0.25 mL/h). Once the addition was finished, the tube was heated at 120 °C for 16 hours. The reaction mixture was then cooled down to room temperature and to the mixture was added 10 mL of EtOAc and 5 mL 1M HCl and vigorously stirred. The layers were separated, and the organic phase was extracted three more times with 5 mL of 1M HCl. The organic phase was discarded. The combined aqueous acidic phase was treated with EtOAc (10 mL). To the mixture was added a solution of aqueous 3M NaOH until the aqueous phase reached basic pH > 10. The layers were separated, and the basic aqueous phase was extracted three times with EtOAc (3 x 10 mL). The combined basic organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give the product. After column chromatography (EtOAc in hexanes) compound **S10** was obtained as a brown oil (118 mg, 51%).

^{1}H NMR (400 MHz, CDCl3) δ 7.09 (dd, J = 6.9, 4.9 Hz, 1H), 6.98 – 6.81 (m, 1H), 4.16 (d, J = 1.1 Hz, 2H), 4.00 – 3.87 (m, 2H), 3.83 – 3.72 (m, 2H), 2.01 (dddd, J = 13.7, 12.3, 5.0, 1.5 Hz, 2H), 1.52 (ddd, J = 13.6, 2.4, 1.3 Hz, 2H); **^{13}C NMR** (101 MHz, CDCl3)

δ 162.5 (d, J = 244.5 Hz), 144.0, 143.4 (d, J = 8.0 Hz), 122.4 (d, J = 9.1 Hz), 114.0 (d, J = 22.9 Hz), 109.6 (d, J = 22.5 Hz), 64.8, 63.7, 50.1 (d, J = 2.5 Hz), 37.6; **LCMS (ESI):** Calculated for $C_{12}H_{15}FNO$ $[M+H]^+$: 208.1; Found: 207.9.

2.17 Synthesis of spiroindoline pyridinone 3b

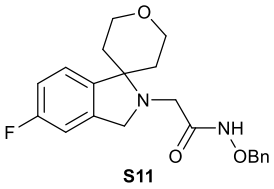


3b

To a solution of 2-Hydroxy-6-methylpyridine-4-carboxylic acid (1 eq., 25 mg, 0.163 mmol) in DMF (2 mL) were added HATU (1.1 eq., 68 mg, 0.18 mmol), DIPEA (1.3 eq., 37 µL, 0.21 mmol), and **S11** (1 eq., 34 mg, 0.16 mmol). The reaction mixture was stirred at room temperature overnight. Then a solution of HATU (0.55 eq., 34 mg, 0.09 mmol) in DMF (0.5 mL) was added to 2-Hydroxy-6-methylpyridine-4-carboxylic acid (0.5 eq., 12 mg, 0.08 mmol). DIPEA (0.65 eq., 19 µL, 0.1 mmol) was added to that solution which was added to the reaction mixture. Stirring was continued for another 24 hours. The volatiles were removed under reduced pressure. The residue was retaken in DCM and washed with an aqueous HCl solution (pH = 4-5). The organic phase was dried over Na_2SO_4 and concentrated under vacuo. The residue was purified by flash chromatography on silica gel (0-6% MeOH in DCM) to afford the desired product as an off-white solid (20 mg, 36%).

^{1}H NMR (400 MHz, DMSO) δ 11.77 (s, 1H), 7.93 (dd, J = 8.8, 5.0 Hz, 1H), 7.41 – 6.96 (m, 2H), 6.14 (dd, J = 1.5, 0.8 Hz, 1H), 6.04 – 5.98 (m, 1H), 4.68 (s, 2H), 3.92 (dd, J = 9.9, 2.3 Hz, 4H), 3.22 (dt, J = 13.8, 9.3 Hz, 2H), 2.21 (s, 3H), 1.67 – 1.49 (m, 2H); **^{13}C NMR** (101 MHz, DMSO) δ 167.4, 163.5, 162.3 (d, J = 243.0 Hz), 150.6, 147.8, 141.6 (d, J = 2.5 Hz), 137.7 (d, J = 9.1 Hz), 125.5 (d, J = 8.7 Hz), 115.0 (d, J = 22.2 Hz), 112.7, 110.4 (d, J = 23.6 Hz), 101.8, 67.3, 63.5, 54.0, 33.0, 19.1, 18.5, 17.2; **LCMS (ESI):** Calculated for $C_{19}H_{20}FN_2O_3$ $[M+H]^+$: 343.2; Found: 342.9.

2.18 Synthesis of O-benzyl spiro-indoline hydroxamate S11

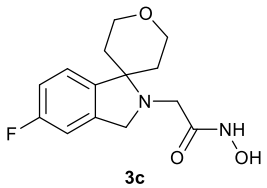


S11

A pressure tube provided with a stir bar was charged with boronic acid **S10** (3 eq., 478 mg, 2.452 mmol) and LiOMe (3 eq., 92 mg, 2.452 mmol) in dry dioxane (5 mL) under an argon atmosphere. The tube was heated at 120 °C and a solution of N-sulfonylhydrazone (1 eq., 300 mg, 0.82 mmol) dissolved in dry dioxane (5 mL) was added with a syringe pump (0.25 mL/h). Once the addition was finished, the tube was heated at 120 °C for 16 hours. The reaction mixture was then cooled down to room temperature and to the mixture was added 10 mL of EtOAc and 5 mL 1M HCl and vigorously stirred. The layers were separated, and the organic phase was extracted three more times with 5 mL of 1M HCl. The organic phase was discarded. The combined aqueous acidic phase was treated with EtOAc (10 mL). To the mixture was added a solution of aqueous 3M NaOH until the aqueous phase reached basic pH > 10. The layers were separated, and the basic aqueous phase was extracted three times with EtOAc (3 x 10 mL). The combined basic organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give the product. After column chromatography (EtOAc in hexanes) compound **S11** was obtained as a gummy solid (130 mg, 52 %).

^{1}H NMR (400 MHz, CDCl3) δ 9.24 (s, 1H), 7.53 (dd, J = 8.4, 4.8 Hz, 1H), 7.43 – 7.30 (m, 5H), 7.03 – 6.77 (m, 2H), 4.97 (s,2H), 4.01 – 3.72 (m, 6H), 3.42 (s, 2H), 1.90 – 1.73 (m, 2H), 1.46 (d, J = 14.8 Hz, 2H); **^{13}C NMR** (101 MHz, CDCl3) δ 167.3, 162.2 (d, J = 246.0 Hz), 142.5, 140.3, 135.1, 129.4, 128.9, 128.6, 123.8 (d, J = 8.7 Hz), 113.7 (d, J = 22.5 Hz), 110.2 (d, J = 23.3 Hz), 78.0, 64.4, 56.0 (d, J = 2.5 Hz), 50.8, 32.5; **LCMS (ESI):** Calculated for $C_{21}H_{24}FN_2O_3$ $[M+H]^+$: 371.2; Found: 370.9.

2.19 Synthesis of spiro-indoline hydroxamic acid 3c



3c

To a solution of **S11** (1 eq., 10 mg, 0.027 mmol) in MeOH (2 mL) flushed with nitrogen was added 10% Pd/C (6 mg, 60% w/w). In the reaction mixture was bubbled hydrogen and it was stirred at room temperature for 6 hours. Completion was checked by TLC (10% MeOH in DCM). The reaction mixture was filtered over celite and washed with MeOH. The product was purified by semi-prep HPLC (6 min gradient from 5% ACN in H_2O to 95% ACN in H_2O) to give **3c** as a white solid (5.5 mg, 73%).

^{1}H NMR (400 MHz, DMSO) δ 10.42 b(s, 1H), 8.74 (bs, 1H), 7.72 (dd, J = 8.4, 5.1 Hz, 1H), 7.17 (dd, J = 8.9, 2.7 Hz, 1H), 7.02 (ddd, J = 9.4, 8.5, 2.8 Hz, 1H), 3.98 (s, 2H), 3.92 – 3.71 (m, 4H), 3.21 (s, 2H), 1.86 (ddd, J = 13.6, 10.9, 5.8 Hz, 2H), 1.53 – 1.42 (m, 2H); **^{13}C NMR** (101 MHz, DMSO) δ 167.2, 161.8 (d, J = 241.6 Hz), 144.0 (d, J = 2.5 Hz), 142.1 (d, J = 8.7 Hz), 124.6 (d, J = 8.7 Hz), 113.3 (d, J = 21.8 Hz), 110.3 (d, J = 22.5 Hz), 64.5, 64.1, 55.9, 50.1, 32.7; **LCMS (ESI):** Calculated for $C_{14}H_{18}FN_2O_3$ $[M+H]^+$: 281.1; Found: 280.8.

3 Computational procedures

3.1 General information

PDB files for each protein (.cif, .mtz and .pdb) were downloaded from the PDB website (HDAC6: 5EDU[3], BRD4: 4BW1[4] and EZH2: 5IJ7[5]). Receptors were prepared using the MakeReceptor software from the OpenEye suite. Water molecules were included during the preparation of the receptor and no constraints were selected.

Molecular properties were calculated using the RDKit Descriptor Calculation node in Knime.

3.2 Selection and tuning of the evaluation sub-functions λ

Example of the influence of parameters **a** and **b** for evaluation sub-functions based on $\boldsymbol{\lambda = 1 + \tanh[a * (x - b)]}$

- Parameter **b** defines where the sub-evaluation function λ will reach 50% of its maximal value.
- Parameter **a** define the slope of the curve between both "plateau" (red dotted line).

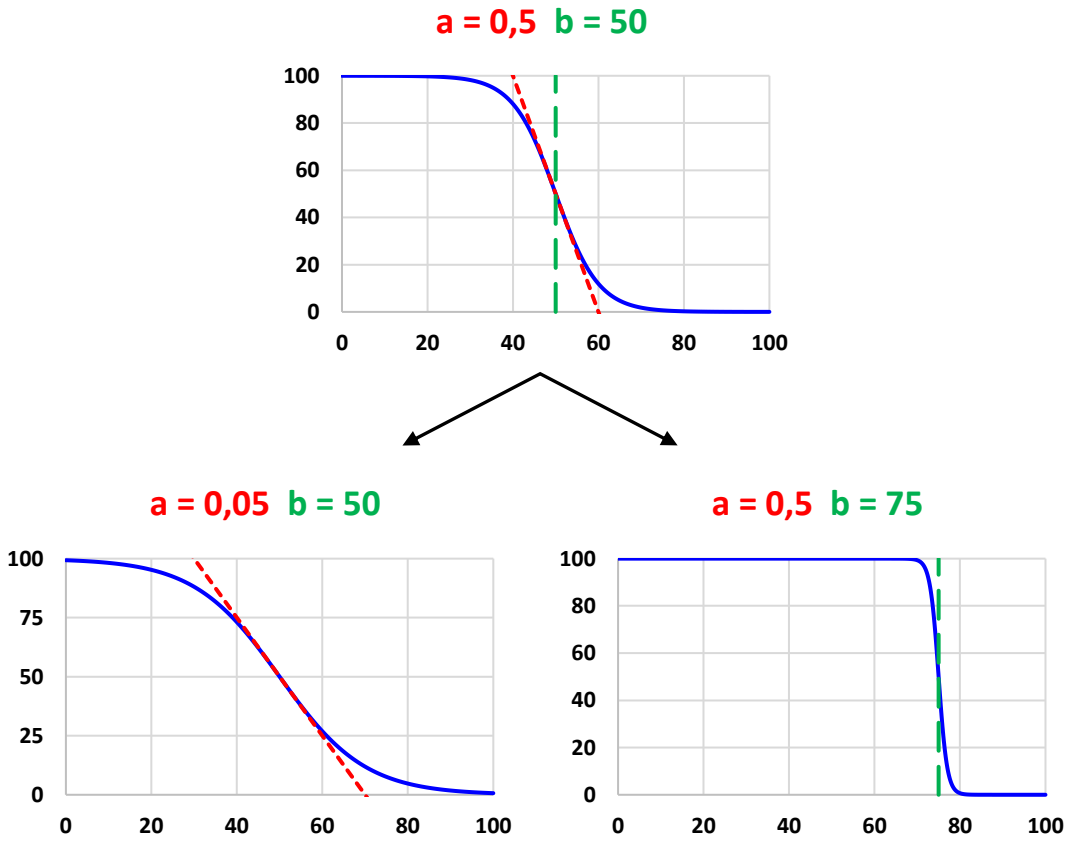


Figure S1. Impact of parameters **a** and **b** on our evaluation sub-functions.

Those parameters were tuned as follows:

- The parameter **b** was selected around the middle point between the limits for lead-likeness and drug-likeness. Taking molecular weight as an example, as the "limits" for lead-likeness and drug-likeness are 300 g.mol^{-1} and 500 g.mol^{-1} respectively, we selected **b** = 400 in the corresponding evaluation sub-function.
- Then the parameter **a** was tuned to obtain an evaluation score of $\boldsymbol{\lambda = 95}$ when the molecular property was equal to the lead-likeness limit. For the molecular weight, with **a** ≈ 0.015, $\boldsymbol{\lambda_{MW}}$ ≈ 95 for a molecular weight of 300 g.mol^{-1}.

In the end, the following evaluation sub-functions λ_i were used:

Molecular weight:	$\boldsymbol{\lambda_{MW} = 50 * (1 - \tanh(0.015 * [x - 400]))}$
H-bond donor:	$\boldsymbol{\lambda_{HBD} = 50 * (1 - \tanh(1.18 * [x - 4.25]))}$
H-bond acceptor:	$\boldsymbol{\lambda_{HBA} = 50 * (1 - \tanh(0.37 * [x - 7]))}$
Number of rotatable bonds:	$\boldsymbol{\lambda_{RotB} = 50 * (1 - \tanh(0.37 * [x - 7]))}$
Number of aromatic rings:	$\boldsymbol{\lambda_{ArRing} = 50 * (1 - \tanh(1.47 * [x - 3]))}$
Fraction sp^3:	$\boldsymbol{\lambda_{Fsp3} = 50 * (1 + \tanh(14.7 * [x - 0.4]))}$
TPSA:	$\boldsymbol{\lambda_{TPSA} = 50 * (1 - \tanh(0.037 * [x - 100]))}$
LogP:	$\boldsymbol{\lambda_{LogP} = 50 * (1 - \tanh(1.5 * [x - 4]))\ for\ LogP > 0}$
	$\boldsymbol{\lambda_{LogP} = 50 * (1 - \tanh(5.76 * [x + 1]))\ for\ LogP \geq 0}$

3.3 Design of the sub-evaluation function for global 3D-ness

As explained in the main text, we also aimed to reward sphere-like compounds against rod- or disk-like molecules. To do so, we decided to score each candidate on its position in a PMI plot. We thus designed an evaluation function based on the following equation:

$$\lambda_{PMI} = e^{-\frac{\left(\frac{I_1}{I_3}-1\right)^2}{0,6} - \frac{\left(\frac{I_2}{I_3}-1\right)^2}{0,2}}$$

Equation S1. PMI Scoring function 2D-representation.

This function can be represented in a 2D-environment using level sets where we can see that compounds in the up-right corner will obtain the highest scores and the further molecules get from this corner, the lower their score.

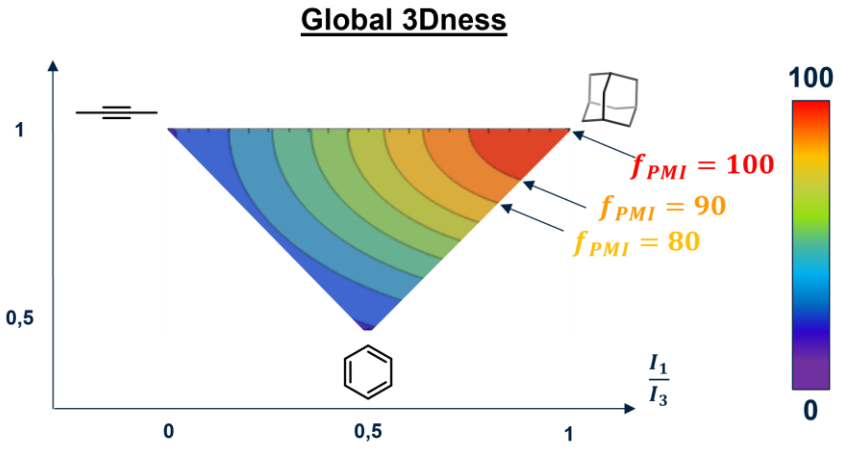


Figure S2. PMI Scoring function 2D-representation.

3.4 Optimisation and Expression of the evaluation function Λ

To select an efficient and rationale expression for our evaluation function $\mathbf{\Lambda}$, we first tested three different possibilities:

- An arithmetic average: $\frac{\mathbf{1}}{\boldsymbol{n}} * \sum_{\boldsymbol{i=1}}^{\boldsymbol{n}} \boldsymbol{\lambda_i}$
- A quadratic average: $\sqrt{\frac{\mathbf{1}}{\boldsymbol{n}} * \sum_{\boldsymbol{i=1}}^{\boldsymbol{n}} \boldsymbol{\lambda_i}^2}$
- A geometric average: $\sqrt[n]{\prod_{\boldsymbol{i=1}}^{\boldsymbol{n}} \boldsymbol{\lambda_i}}$

All three expressions were able to rank compounds as expected (Figure S4-6) but we decided on employing an arithmetic average as it gave the most balanced results amongst the three. The quadratic average was discarded as not strict enough since it awarded high $\mathbf{\Lambda}$ scores for even larger and complex molecules as seen in Figure S3-4. This can also be observed in the repartition of the compounds for different values of $\mathbf{\Lambda}$ where most of the molecules ended up in the first two intervals (Figure S5). On the other hand, the geometric average showed excellent ranking abilities but also proved to be too strict for high values of $\mathbf{\Lambda}$ with most compounds getting scores lower than 75. The geometric average also presented some issues by design as it consisted in the multiplication all evaluation sub-functions $\boldsymbol{\lambda}$ together, if any $\boldsymbol{\lambda}$ gave a score of 0 then $\mathbf{\Lambda}$ would directly be 0 as well.

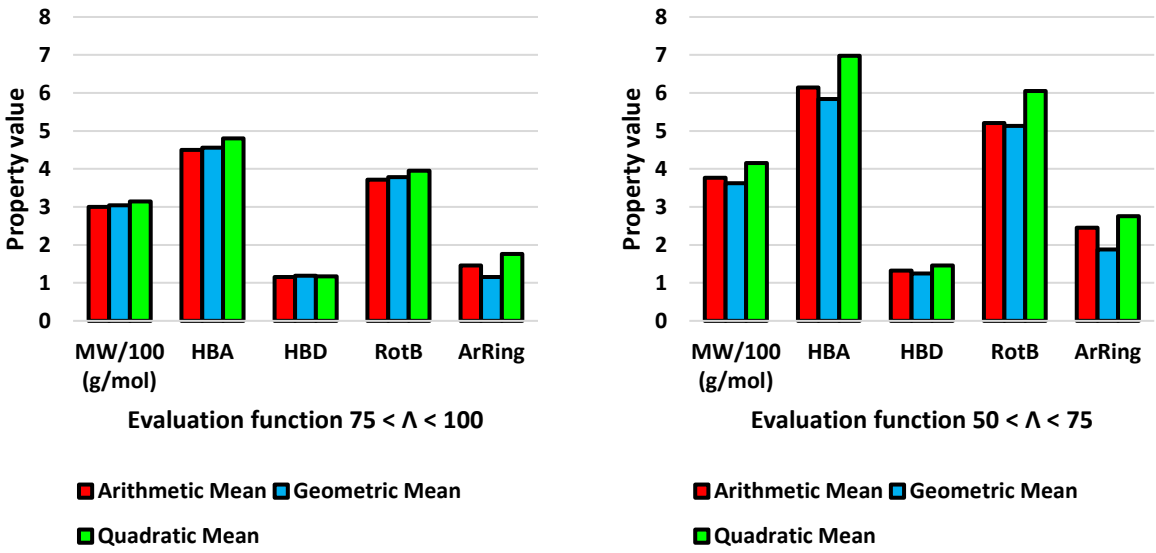


Figure S3. Mean properties obtained for 75 < $\mathbf{\Lambda}$ <100 (left) and 50 < $\mathbf{\Lambda}$ <75 (right) for the three averages considered.

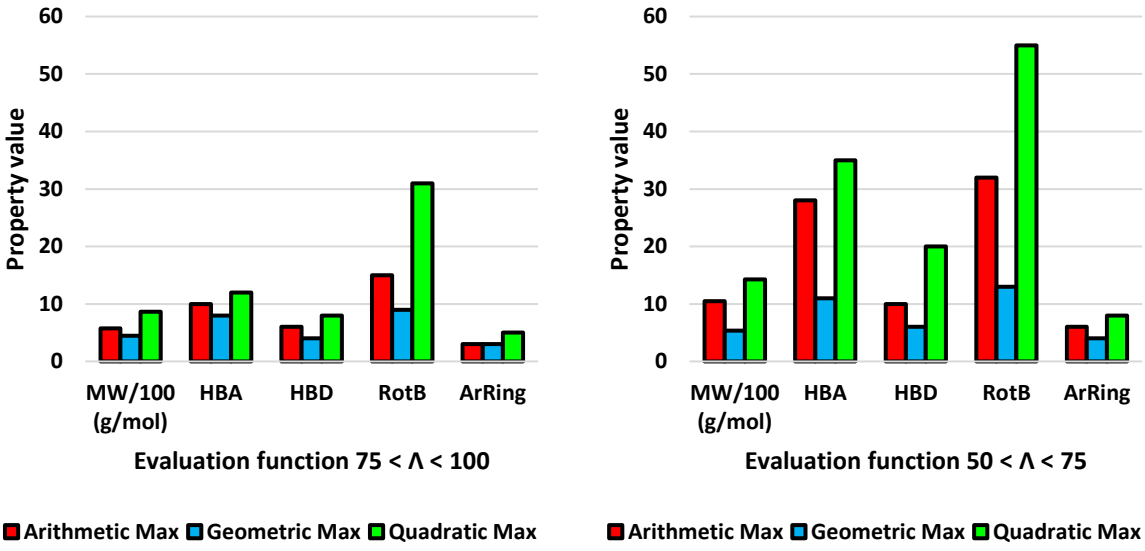


Figure S4. Max properties obtained for 75< Λ <100 and 50< Λ <75 (right) for the three averages considered.

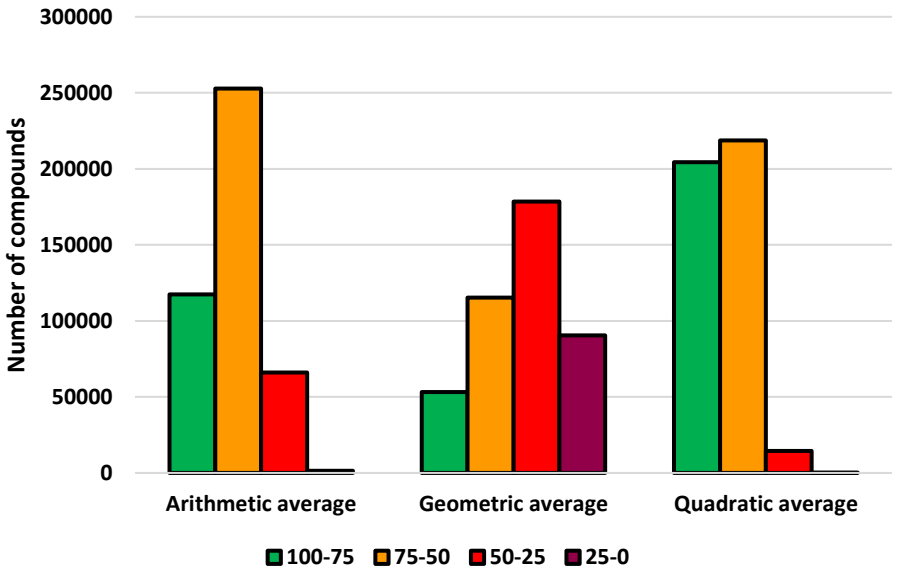


Figure S5. Repartition of the compounds of the MLSMR library amongst each interval of Λ for the three averages considered.

3.5 Application on the MLSMR

When comparing the properties of the starting MLSMR library with the properties of the molecules with a Λ > 75, we can also observe an improvement for all properties towards higher lead-likeness. The improvement is moderate with this example as the starting library was already mainly containing molecules with adequate properties.

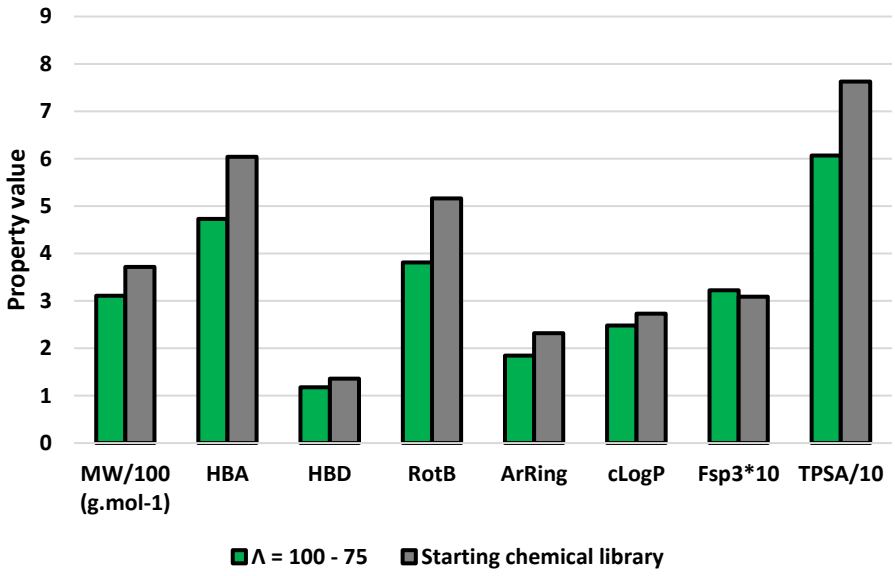


Figure S6. Improvement of the properties when selecting compounds with Λ > 75 in the MLSMR

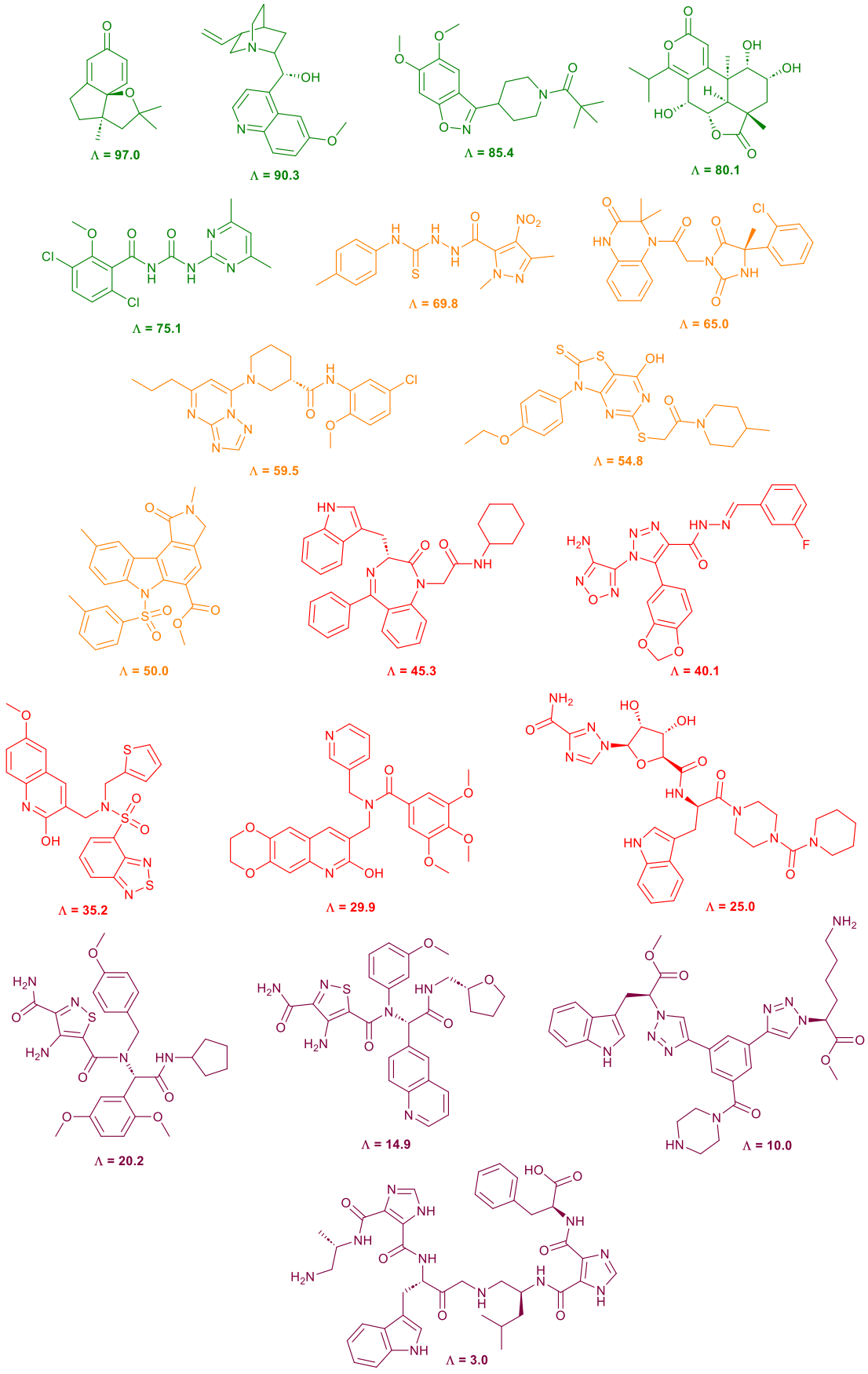


Figure S7. Examples of compounds from the MLSMR for different values of **Λ**

3.6 Relation between PMI scoring and other properties

We explored the properties of compounds with sphere-like 3D shape and observed a shift towards degraded molecular properties (less lead-like) which seems to validate the idea that PMI properties are driven by the main skeleton. However, we can also see that λ_{PMI} could be an interesting approach to target sphere-like compounds for chemical library design.

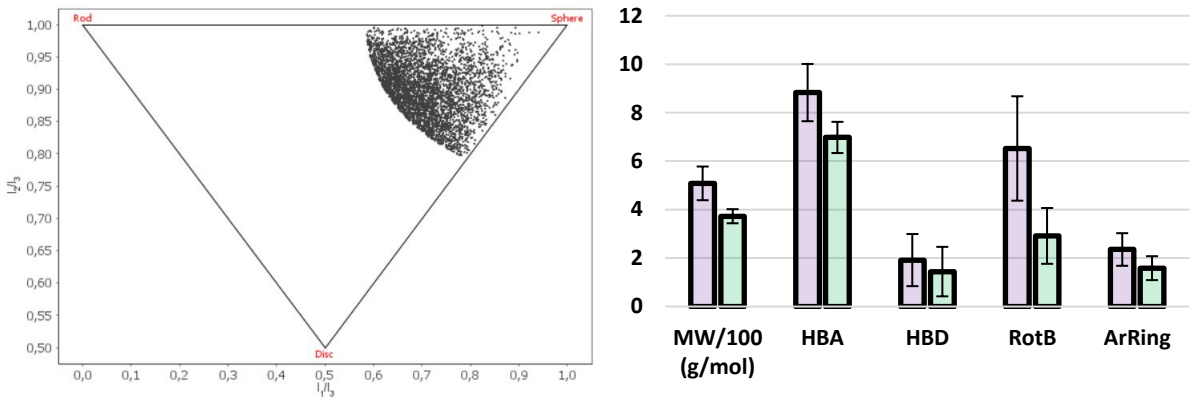


Figure S8. PMI plot and property distribution comparison for tetrahydroquinolines with λ_{PMI} > 75 (purple, left) and $\mathbf{\Lambda}$ > 75 (green, right).

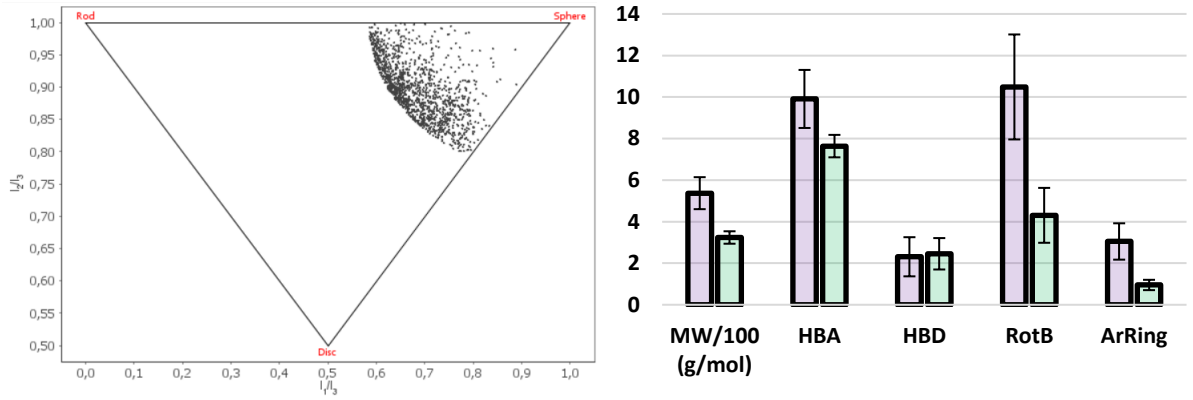


Figure S9. PMI plot and property distribution comparison for diketopiperazines with λ_{PMI} > 75 (purple, left) and $\mathbf{\Lambda}$ > 75 (green, right).

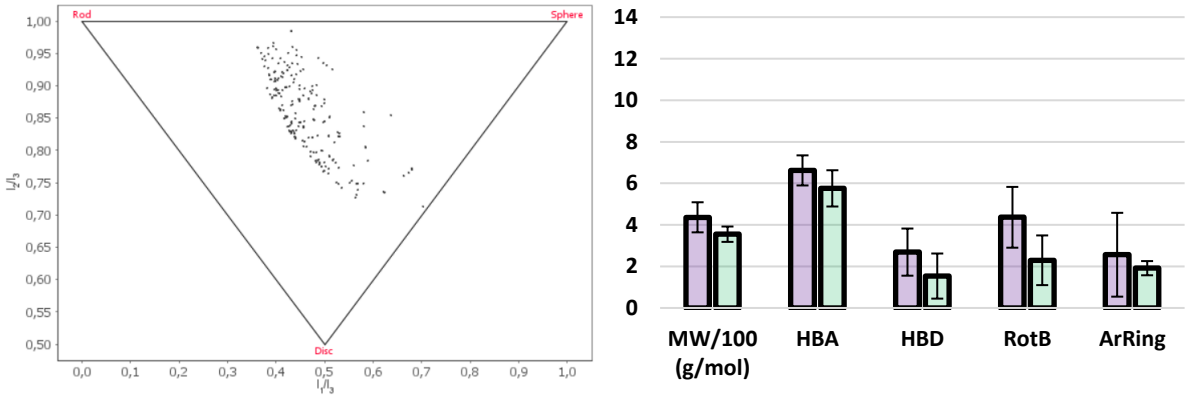


Figure S10. PMI plot and property distribution comparison for spiroindolines with λ_{PMI} > 50 (purple, left) and $\mathbf{\Lambda}$ > 75 (green, right).

3.7 Benchmarking against LLAMA

To further validate our method, we tried to benchmark it against one of the few existing methods published in the literature to assess for lead-likeness, the LLAMA method described by the group of Adam Nelson in 2016. We compared our method with LLAMA using the MLSMR and the following score limits: LLAMA penalty score lower than 2 and a **Λ** score above 75. A high overlap was found with 141,641 compounds succeeding with both methods of 223,305 molecules succeeding in at least one (Figure S11). We further analysed the remaining compounds (Figure S12) from each side and found that those selected by our method presented slightly better properties overall (except for cLogP) demonstrating the competitiveness of our method against one of the main strategies in the literature to drive synthetic efforts towards lead-likeness.

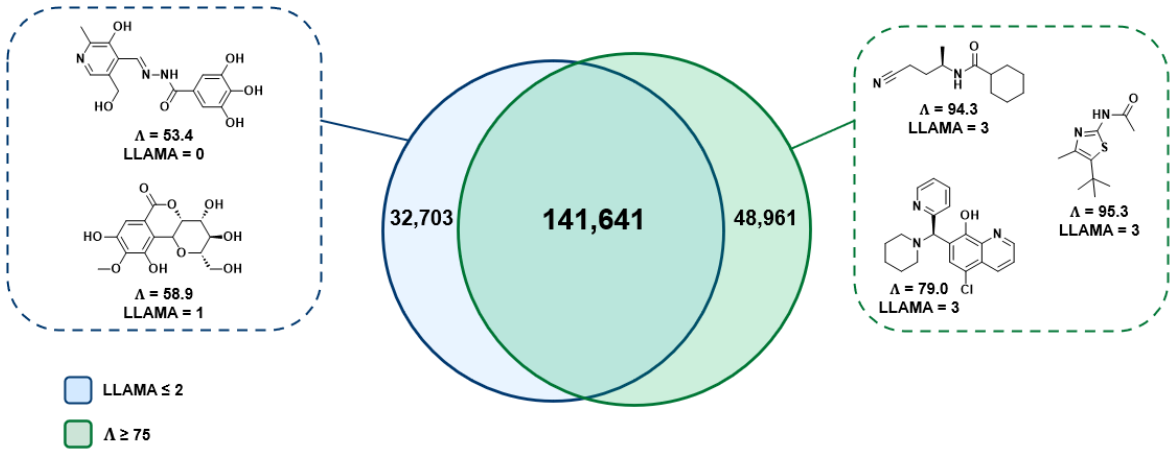


Figure S11. Examples of compounds from the MLSMR for different values of **Λ**

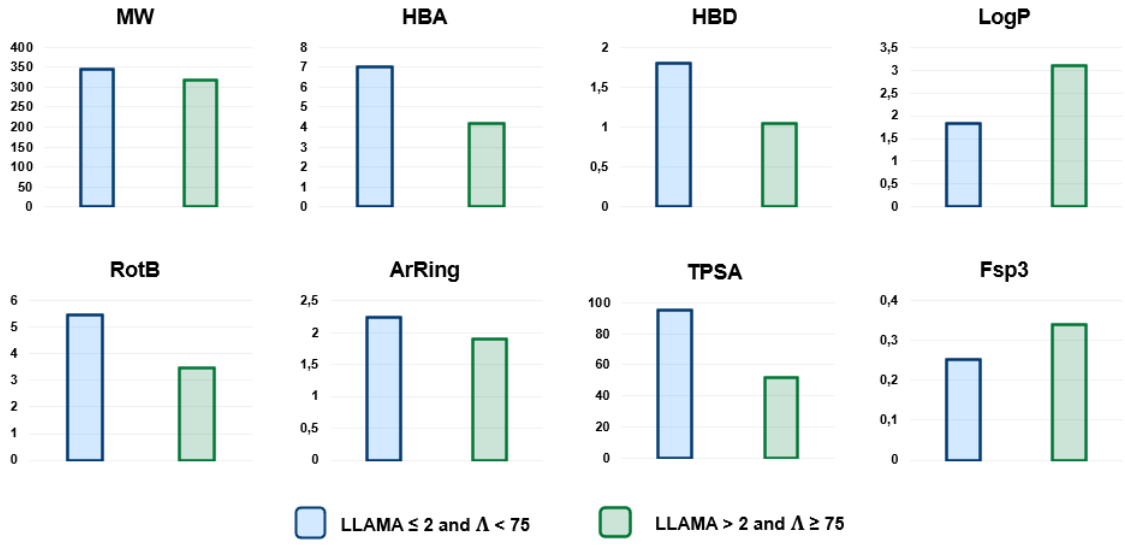


Figure S12. Examples of compounds from the MLSMR for different values of **Λ**

3.8 Correlation with Molecular complexity according to Böttcher[6]

Additionally, we checked for a potential correlation between our lead-likeness evaluation function **Λ** and the concept of molecular complexity as described by Thomas Böttcher in 2016.[6] This concept relies on information theory and doesn't depend on the parameters we used for our study (MW, HBD, HBA…). To do so, we randomly picked five hundred molecules from the MLSMR chemical library (five hundred numbers were generated using an RNG node in Knime, molecule indexes corresponding to those numbers were selected). Using the Böttcher Score Calculator available on the website of the ForliLab[7] from Scripps Research Institute we then plotted for each molecule their molecular complexity against their lead-likeness score **Λ**. Despite being two independent metrics, a correlation could be observed with an R^2 of 0.55. This comparison shows that a high lead-likeness score **Λ** correlates with a lower molecular complexity.

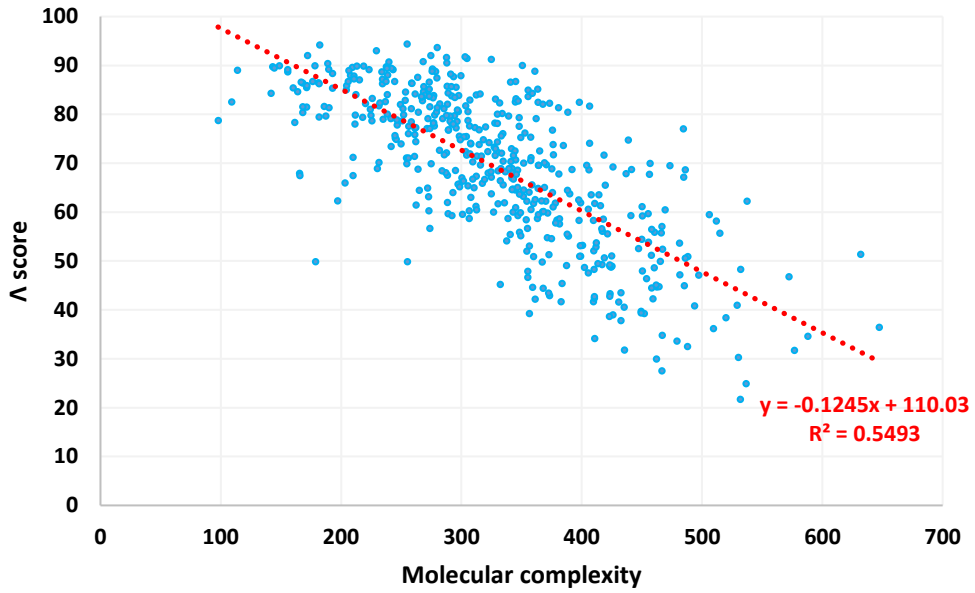


Figure S13. Correlation between molecular complexity and our lead-likeness score **Λ**

3.9 Lead-likeness distribution in all three chemical libraries

The distribution for our lead-likeness evaluation function was assessed in all three libraries. The tetrahydroquinolines and diketopiperazines ones showed a gaussian distribution centered around **Λ** = 40-50. The spiroindoline was slightly shifted towards more lead-like compounds but it was also composed of 10 times less molecules which may biased the statistics.

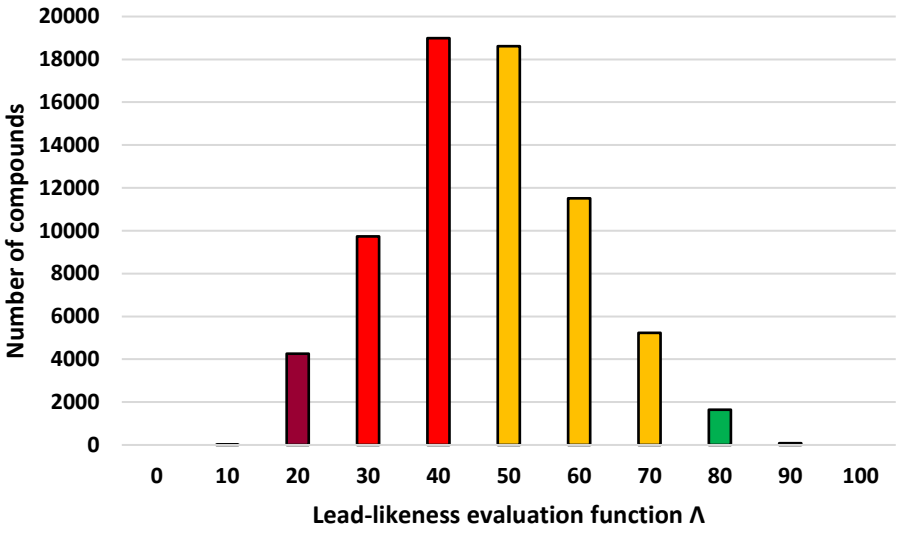


Figure S14. Lead-likeness distribution across the tetrahydroquinolines chemical library.

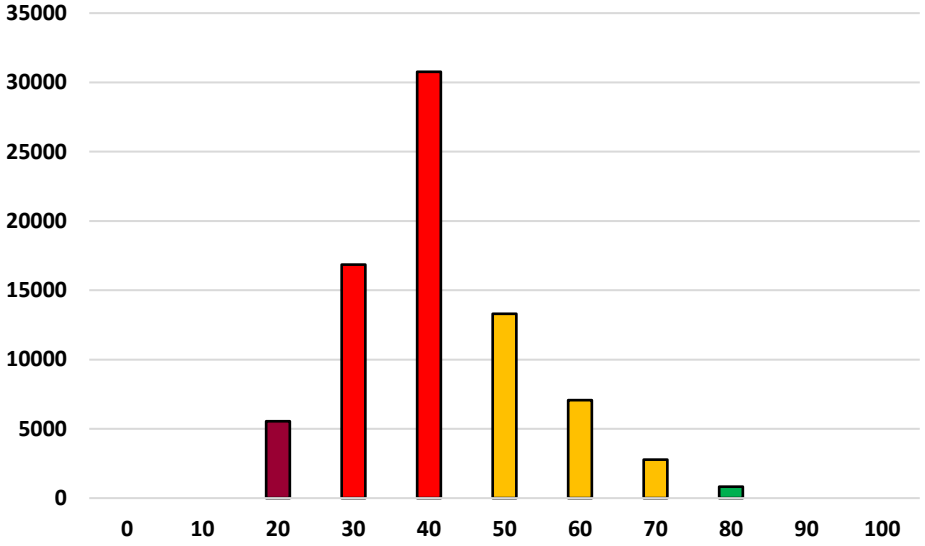


Figure S15. Lead-likeness distribution across the diketopiperazines chemical library.

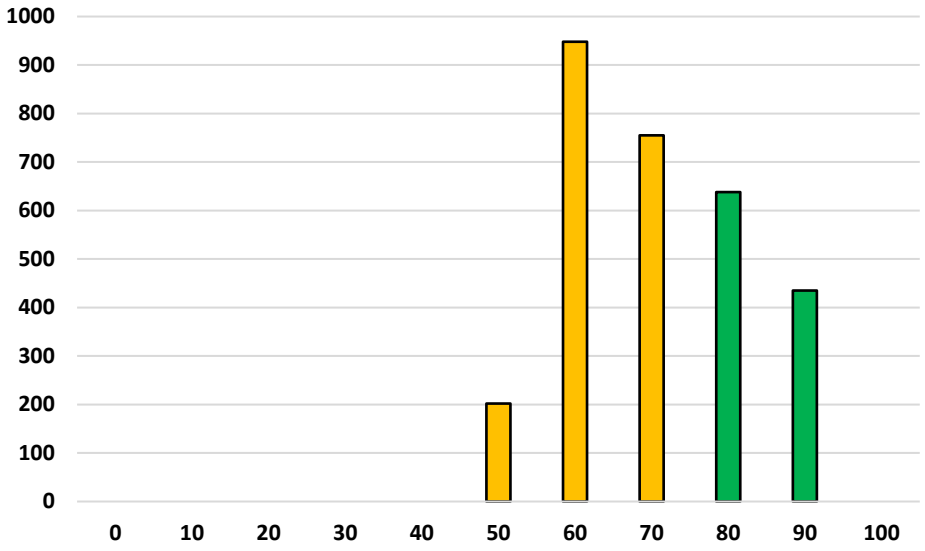


Figure S16. Lead-likeness distribution across the spiroindolines chemical library.

3.10 Generation of conformers using OEOMEGA

Conformers used in this study were generated using the OEOMEGA tool from the OpenEye suite. They were also modified to adopt the proper protonation state at a pH of 7.4 using the FILTER tool. To do so the following command line was used in OpenEye:

- filter -filter filter.txt -pkanorm -in XXX.sdf -out XXX_pka.oeb.gz
    - **# The normalisation was performed with a personalised "filter.txt" written to accept all molecules.**

One conformer was calculated for PMI plots while ten were calculated for docking studies using the following command line in OpenEye:

- oeomega classic -in XXX_pka.oeb.gz -out XXX_pka_conf.oeb.gz -flipper true -progress dots -maxconfs **Y**
    - **# The option "-flipper true" allowed to generate stereoisomers, particularly on protonated quaternary ammoniums.**
    - **# The number of generated conformers was selected with the "-maxconfs Y", Y being the number of conformers.**

3.11 Docking study

3.11.1 Command lines

We selected PDB files containing ligands exhibiting the isosteres of interests as shown in Figure S17 (green ligands).

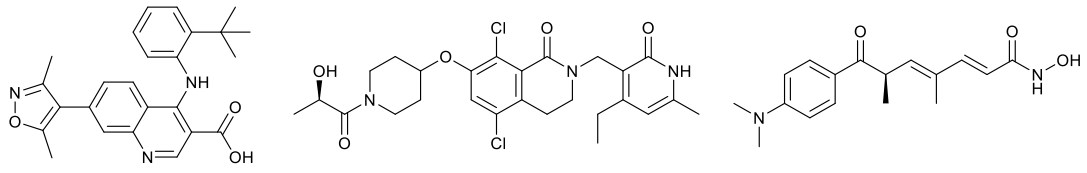


Figure S17. Structures of the ligands co-crystallised in each protein.

Docking experiments were performed with both FRED and HYBRID to obtain unbiased and biased results towards the isosteres we considered in this study. We used the following command lines in OpenEye for FRED and HYBRID respectively:

- fred -receptor rec_XXX.oedu -dbase XXX_pka_conf.oeb.gz -dock_resolution high -docked_molecule_file fred_docked_XXX.oeb.gz -score_file fred_score_XXX.txt -hitlist_size 0
- hybrid -receptor rec_XXX.oedu -dbase XXX_pka_conf.oeb.gz -dock_resolution high -docked_molecule_file hybrid_docked_XXX.oeb.gz -score_file hybrid_score_XXX.txt -hitlist_size 0
    - **# We used the option "-hitlist_size 0" to obtain the docking scores and poses of all compounds.**

Docking poses and scores were then analysed manually by VIDA.

3.11.2 Docking poses for selected compounds

- Docking poses into BRD4

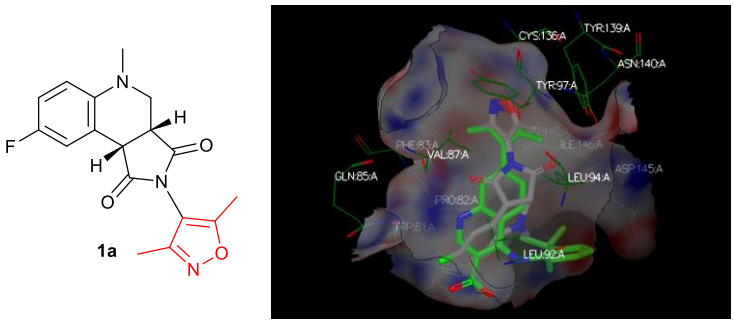


Figure S18. Best docking pose for **1a** in BRD4 (PDB = 4BW1) obtained with HYBRID.

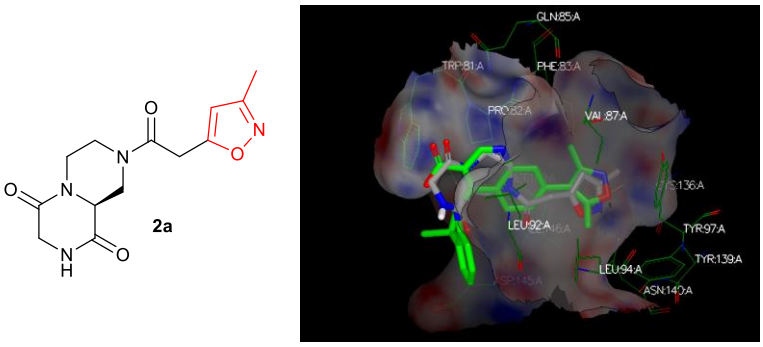


Figure S19. Best docking pose for **2a** in BRD4 (PDB = 4BW1) obtained with HYBRID.

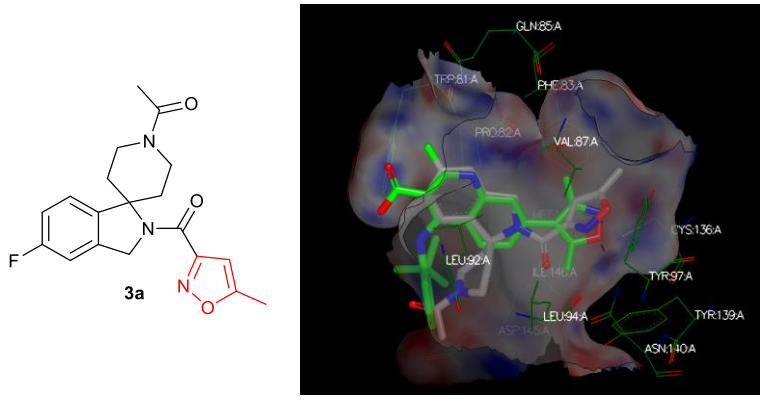


Figure S20. Best docking pose for **3a** in BRD4 (PDB = 4BW1) obtained with HYBRID.

- Docking poses into EZH2

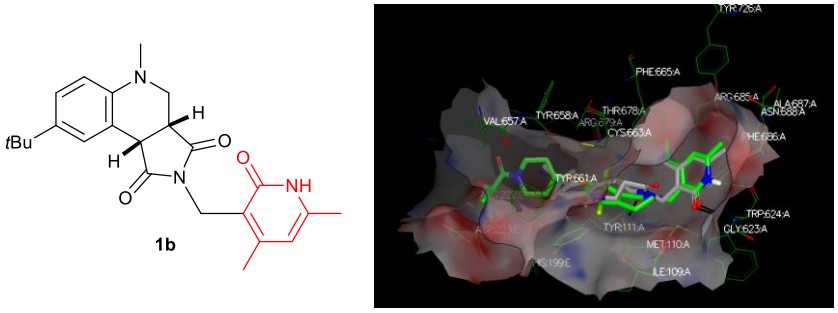


Figure S21. Best docking pose for **1b** in EZH2 (PDB = 5IJ7) obtained with HYBRID.

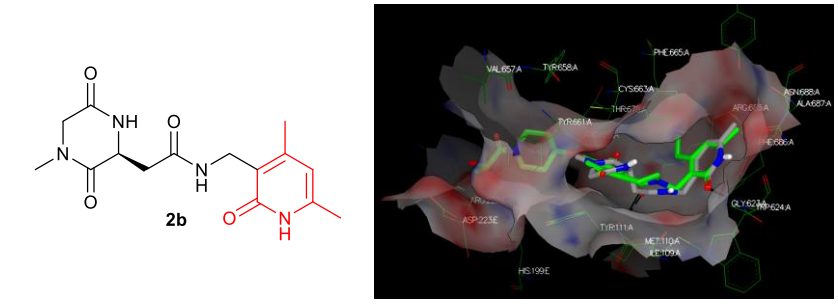


Figure S22. Best docking pose for **2b** in EZH2 (PDB = 5IJ7) obtained with HYBRID.

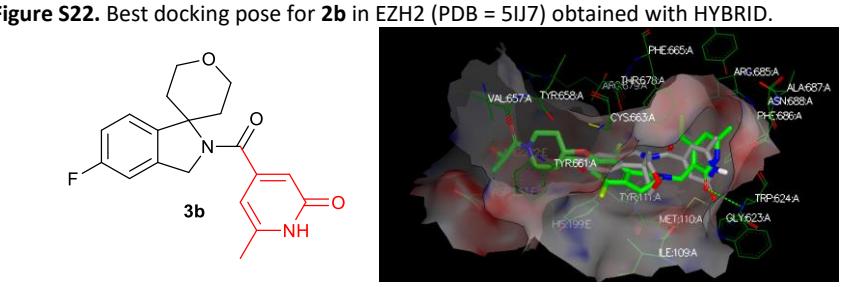


Figure S23. Best docking pose for **2c** in EZH2 (PDB = 5IJ7) obtained with HYBRID.

- <u>Docking poses into HDAC6</u>

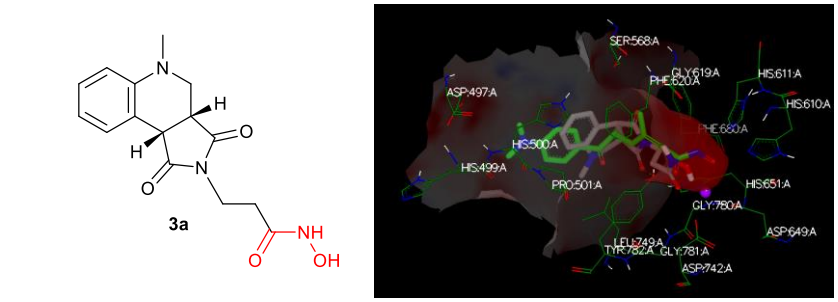


Figure S24. Best docking pose for **3a** in HDAC6 (PDB = 5EDU) obtained with HYBRID.

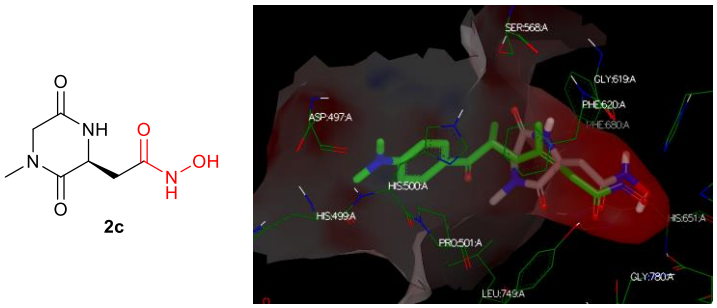


Figure S25. Best docking pose for **2c** in HDAC6 (PDB = 5EDU) obtained with HYBRID.

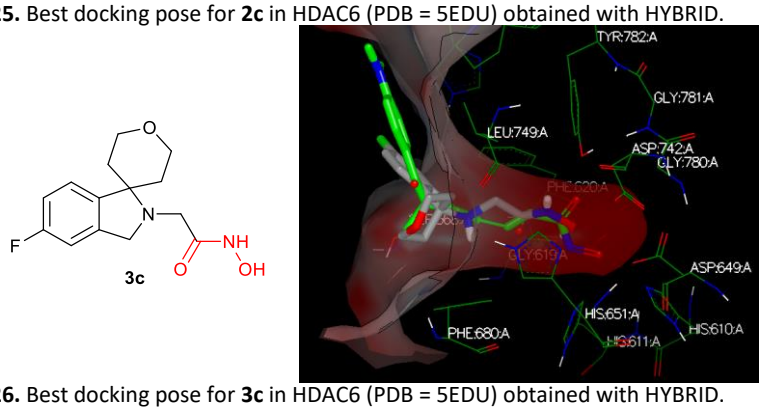


Figure S26. Best docking pose for **3c** in HDAC6 (PDB = 5EDU) obtained with HYBRID.

3.12 Biological evaluation

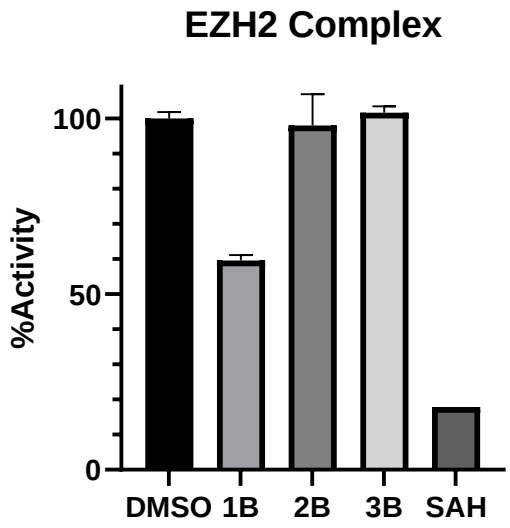


Figure S27. Activity Assay for EZH2 methyltranserase Activity on core histones. Compounds were tested in duplicate at 100 µM.

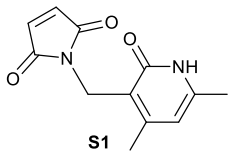
Compounds **1-3b** were evaluated in a EZH2 Methyltransferase Assay via radiometric HotSpot™ by Reaction Biology. Compounds were provided as a DMSO stock solution (10 mM) and tested in a single-dose mode, in duplicate, at 100 µM. Control compound, SAH (S-(5'-Adenosyl)-L-homocysteine) was tested in 10-dose IC_{50} mode with 3-fold serial dilution starting at 100 µM. Reactions were carried out at 1 µM SAM.

		Enzyme % Activity (relative to DMSO controls)	
	Methyltransferase:	EZH2 Complex	
	Methyltransferase Concentration (nM):	5	
	Substrate:	Core Histone	
	Substrate Concentration:	0.05 mg/mL	
Compound ID:	Testing Concentration (μM):	Data 1:	Data 2:
1b	100	60,60	58,46
2b	100	104,29	91,66
3b	100	100,34	102,91
SAH Control	100	17.87	

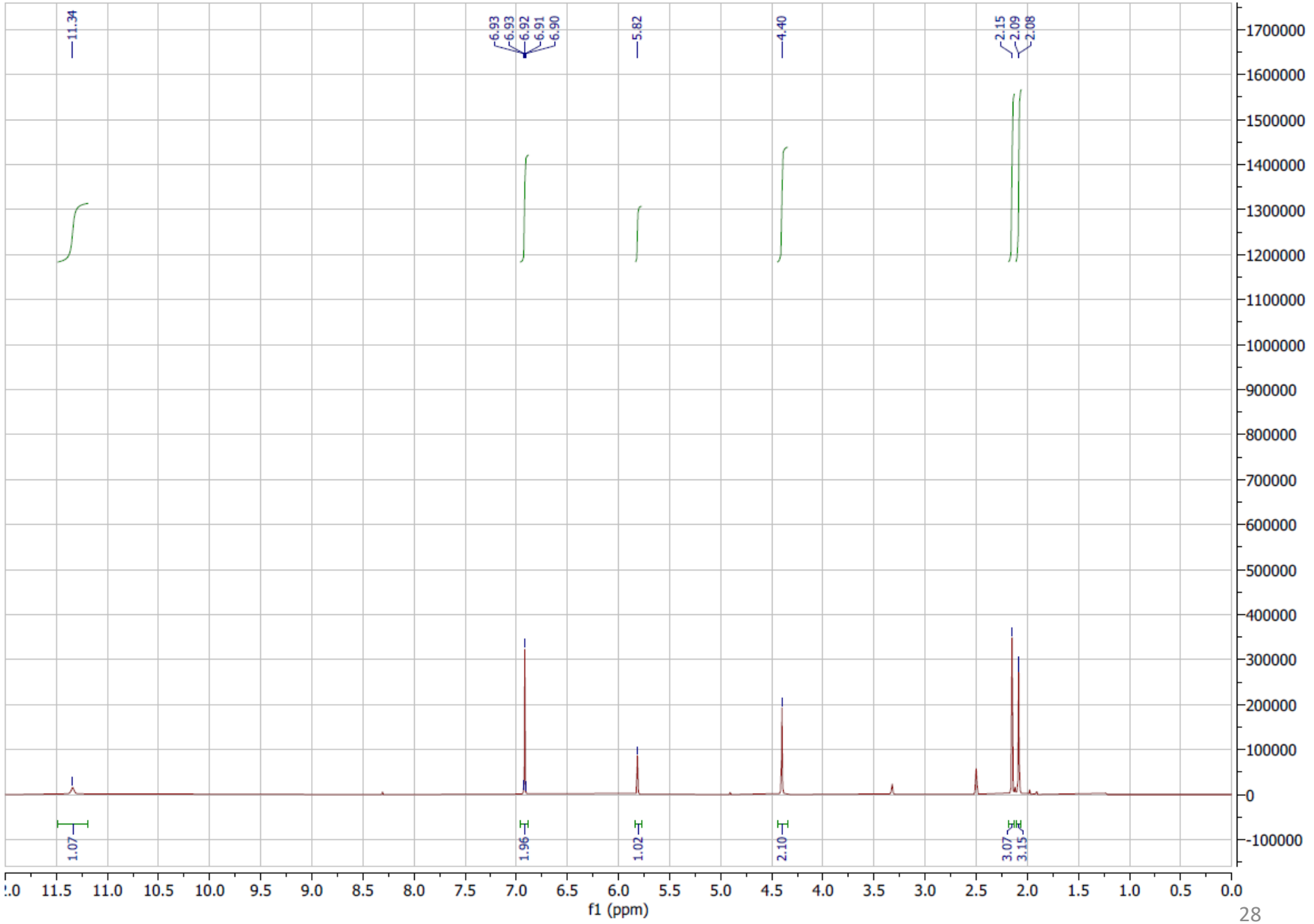
4 References

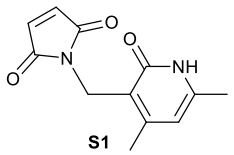
[1] A. I. Green and G. M. Burslem RSC Med. Chem., 2021, **12**, 1780-1786.
[2] (a) L. López, M.-P. Cabal and C. Valdés *Angew. Chem. Int. Ed.* 2022, **61**, e202113370. (b) R. Barroso, M. P. Cabal, R. Badía-Laiño and C. Valdés *Chem. Eur. J.* 2015, **21**, 16463–16473.
[3] Y. Hai and D. W. Christianson *Nat. Chem. Bio.* 2016, **12**, 741-747.
[4] O. Mirguet, Y. Lamotte, C.-W. Chung, P. Bamborough, D. Delannée, A. Bouillot, F. Gellibert, G. Krysa, A. Lewis, J. Witherington, P. Huet, Y. Dudit, L. Trotter and E. Nicodeme *ChemMedChem* 2013, **9**, 580-589.
[5] A. Brooun, K. S. Gajiwala, Y.-L. Deng, W. Liu, B. Bolaños, P. Bingham, Y.-A. He, W. Diehl, N. Grable, P.-P. Kung, S. Sutton, K. A. Maegley, X. Yu and A. E. Stewart *Nat. Comm.* 2016, **7**, e11384.
[6] T. Böttcher *J. Chem. Inf. Model.* 2016, **56**, 462–470.
[7] R. M. Demoret, M. A. Baker, M. Ohtawa, S. Chen, C. C. Lam, S. Khom, M. Roberto, S. Forli, K. N. Houk and R. A. Shenvi *J. Am. Chem. Soc.* 2020, **142**, 18599–18618.

5 NMR spectra

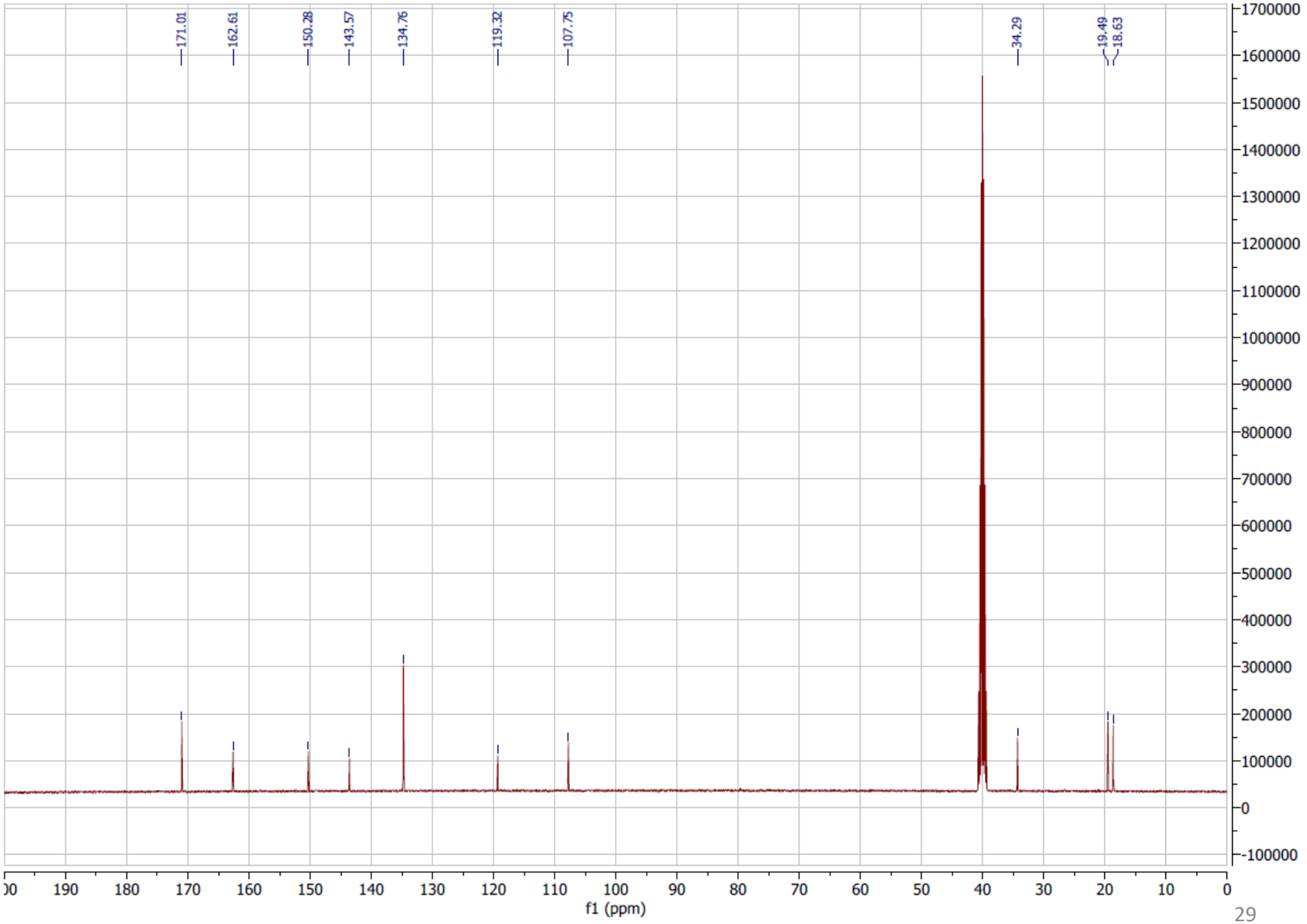

O
O
N
O
NH

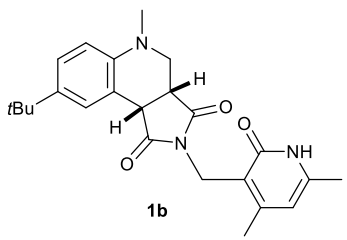
S1


11.34
6.93
6.93
6.92
6.91
6.90
5.82
4.40
2.15
2.09
2.08
1700000
1600000
1500000
1400000
1300000
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
-100000
1.07
1.96
1.02
2.10
3.07
3.15
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

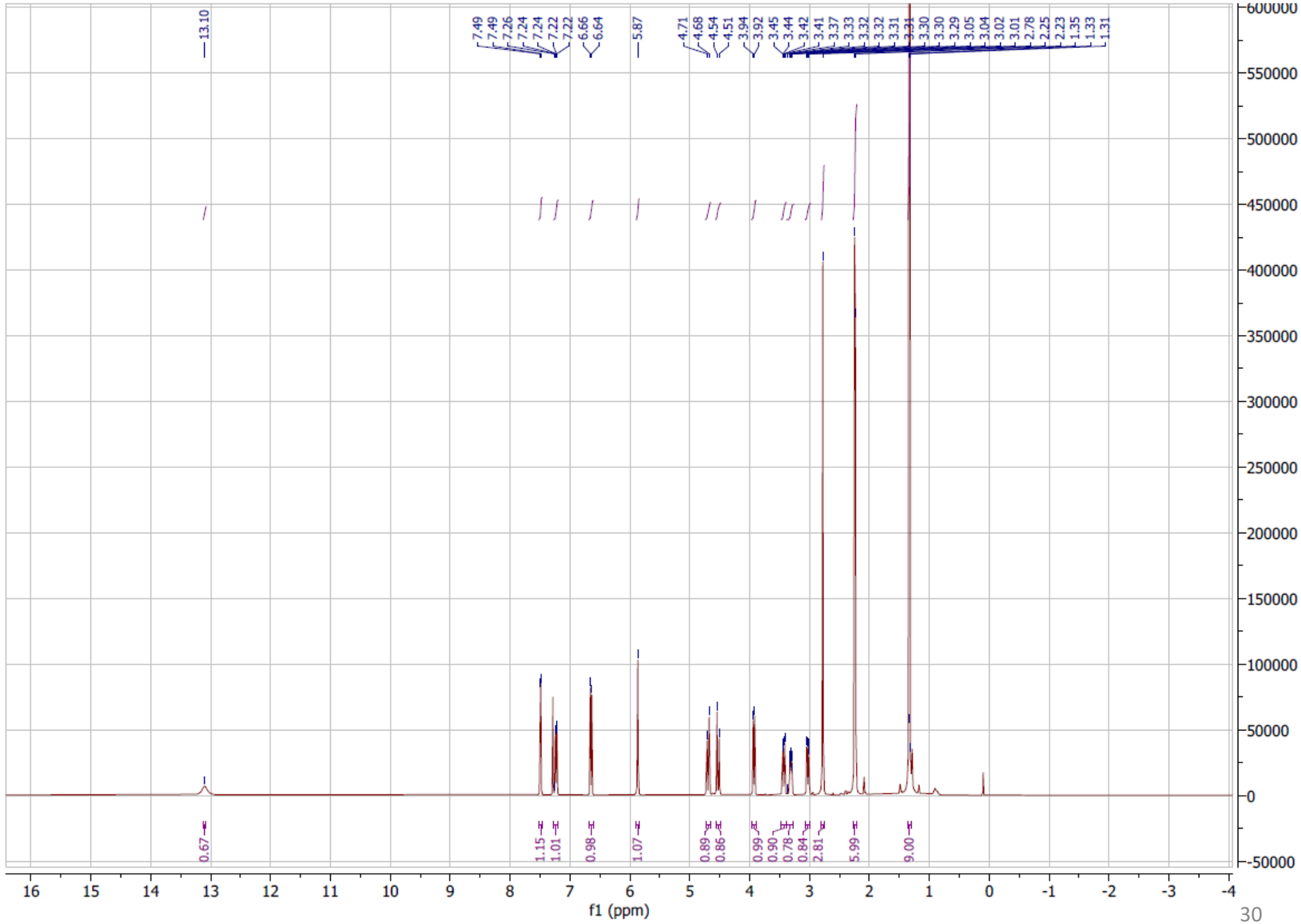

O
O
N
O
NH

S1


171.01
162.61
150.28
143.57
134.76
119.32
107.75
34.29
19.49
18.63
1700000
1600000
1500000
1400000
1300000
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
-100000
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


tBu
N
H
H
O
O
N
O
NH
O

1b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7.49
7.49
7.26
7.24
7.24
7.22
7.22
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6.64
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4.71
4.68
4.54
4.51
3.94
3.92
3.45
3.44
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3.41
3.37
3.33
3.32
3.32
3.31
3.31
3.30
3.30
3.29
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3.04
3.02
3.01
2.78
2.25
2.23
1.35
1.33
1.31
0.67
1.15
1.01
0.98
1.07
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0.86
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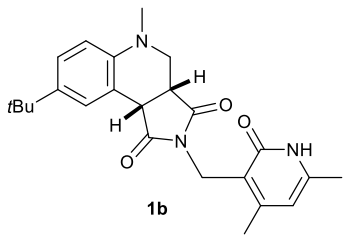
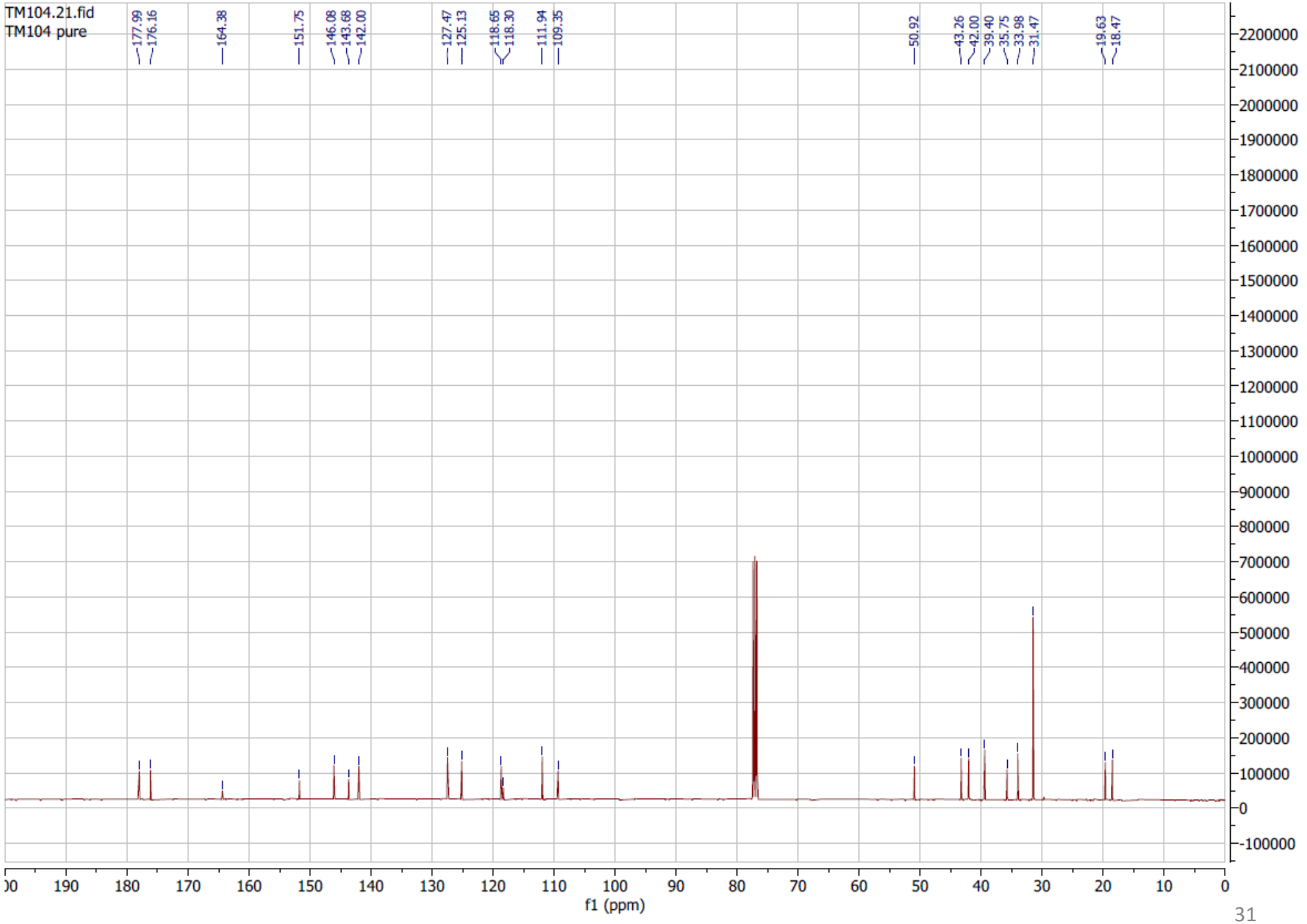
0.90
0.78
0.84
2.81
5.99
9.00
f1 (ppm)


tBu
N
H
H
O
O
N
O
NH

1b


TM104.21.fid
TM104 pure
177.99
176.16
164.38
151.75
146.08
143.68
142.00
127.47
125.13
118.65
118.30
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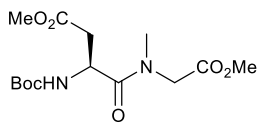
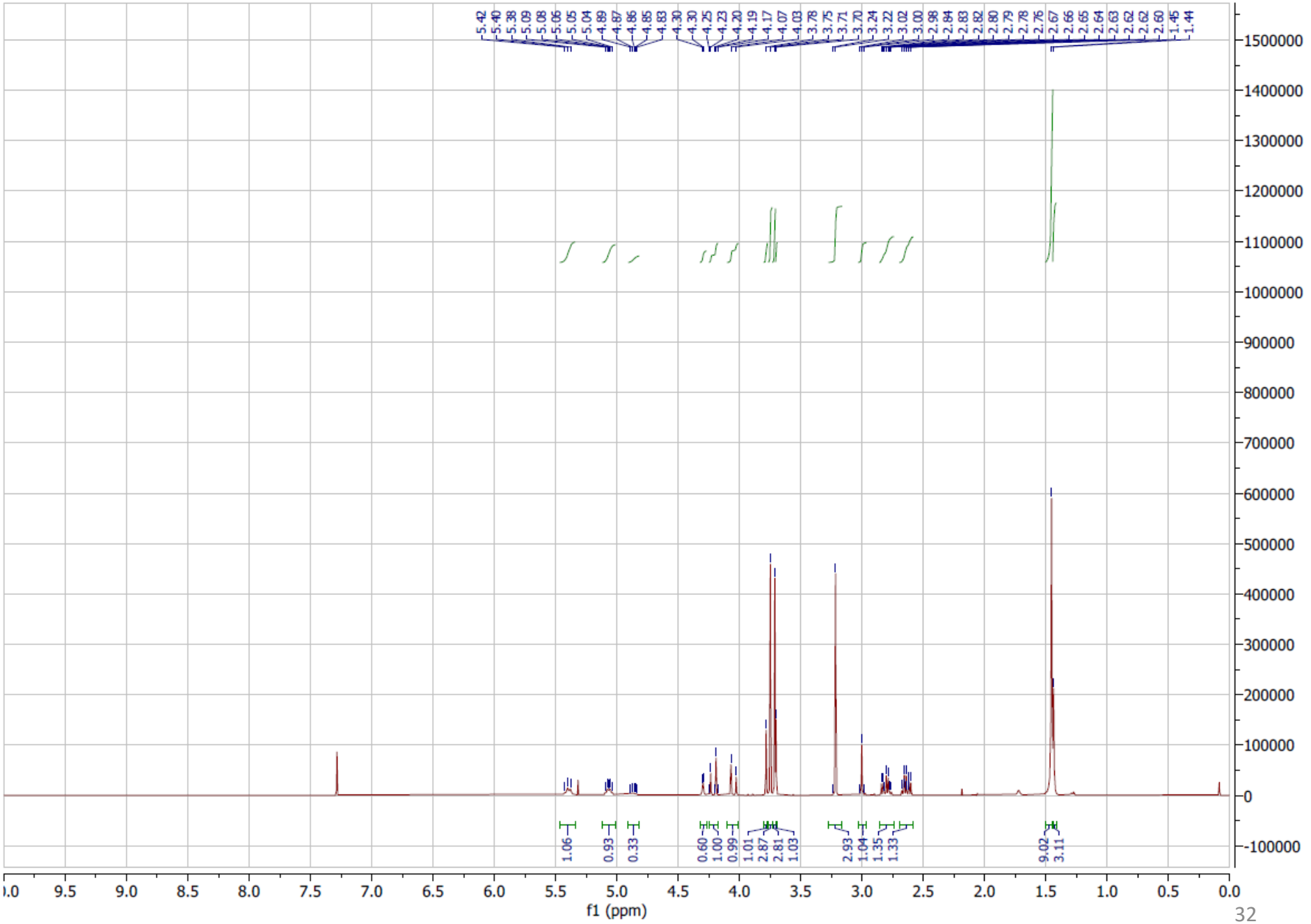
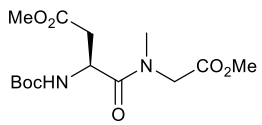
33.98
31.47
19.63
18.47
f1 (ppm)

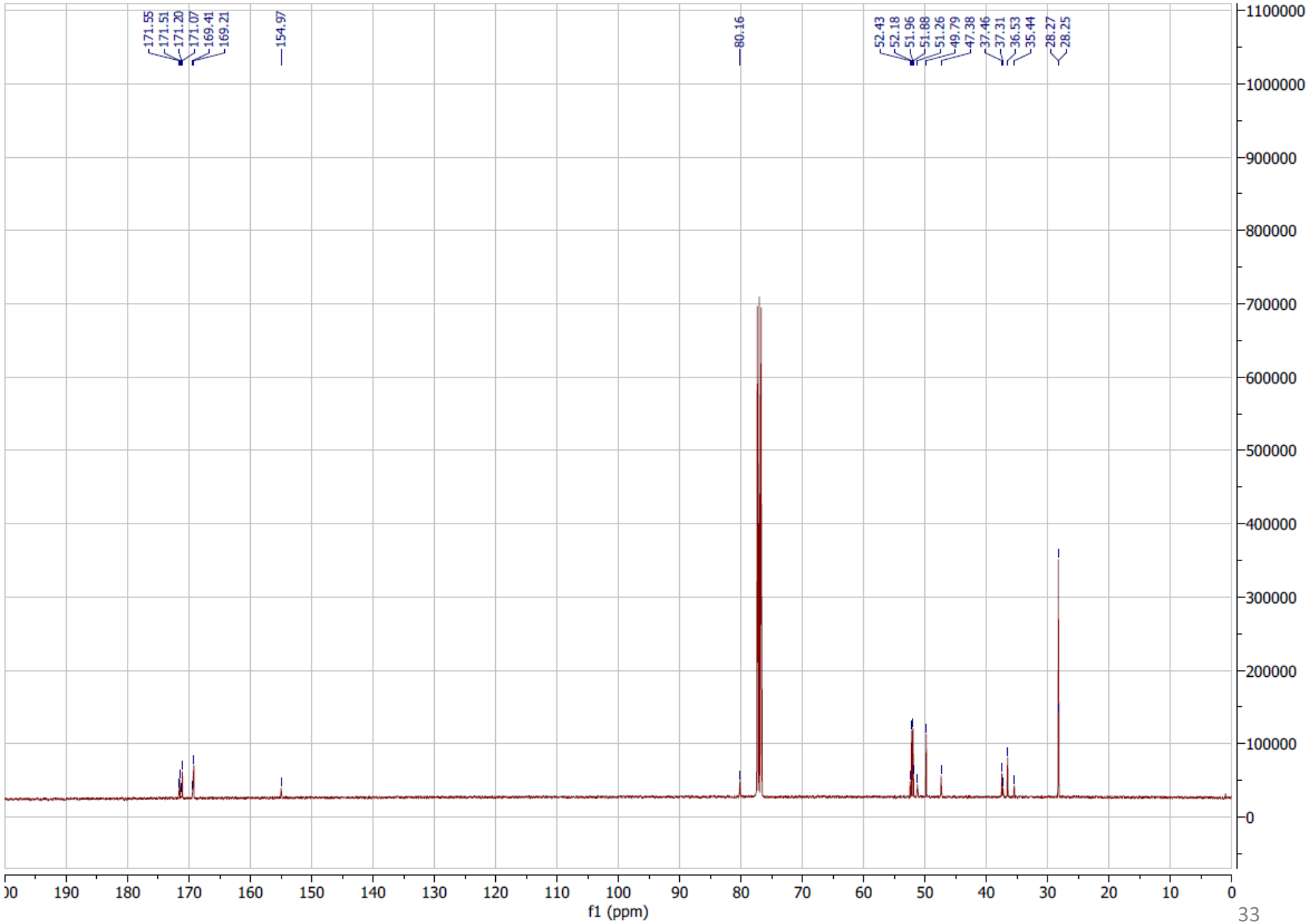

MeO2C
BocHN
N
CO2Me
O

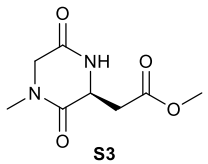
S2


5.42
5.40
5.38
5.09
5.08
5.06
5.05
5.04
4.89
4.87
4.86
4.85
4.83
4.30
4.30
4.25
4.23
4.20
4.19
4.17
4.07
4.03
3.78
3.75
3.71
3.70
3.24
3.22
3.02
3.00
2.98
2.84
2.83
2.82
2.80
2.79
2.78
2.76
2.67
2.66
2.65
2.64
2.63
2.62
2.62
2.60
1.45
1.44
1.06
0.93
0.33
0.60
1.00
0.99
1.01
2.87
2.81
1.03
2.93
1.04
1.35
1.33
9.02
3.11
f1 (ppm)

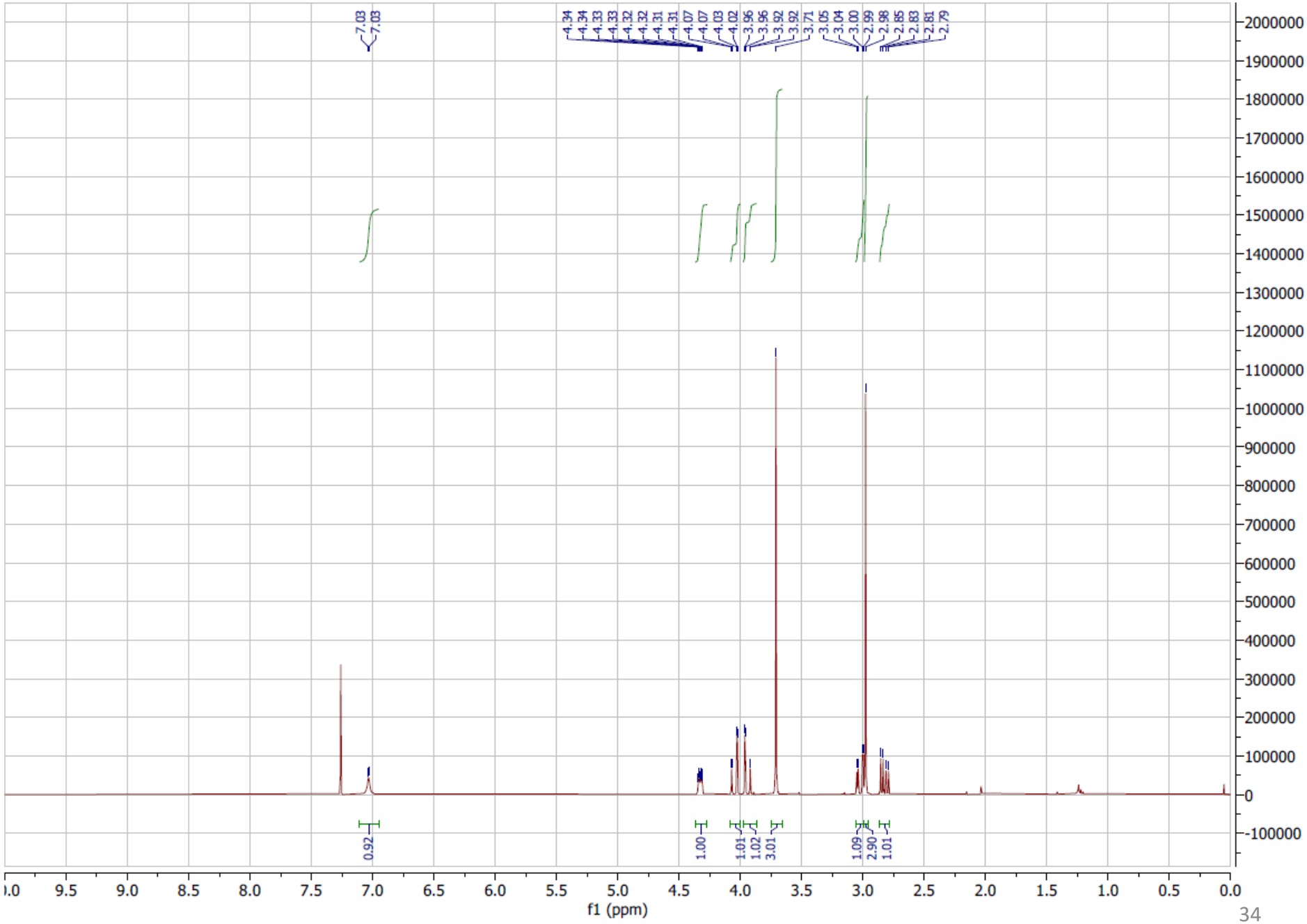

MeO2C
BocHN
N
CO2Me
O

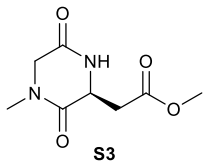
S2


171.55
171.51
171.20
171.07
169.41
169.21
154.97
80.16
52.43
52.18
51.96
51.88
51.26
49.79
47.38
37.46
37.31
36.53
35.44
28.27
28.25
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

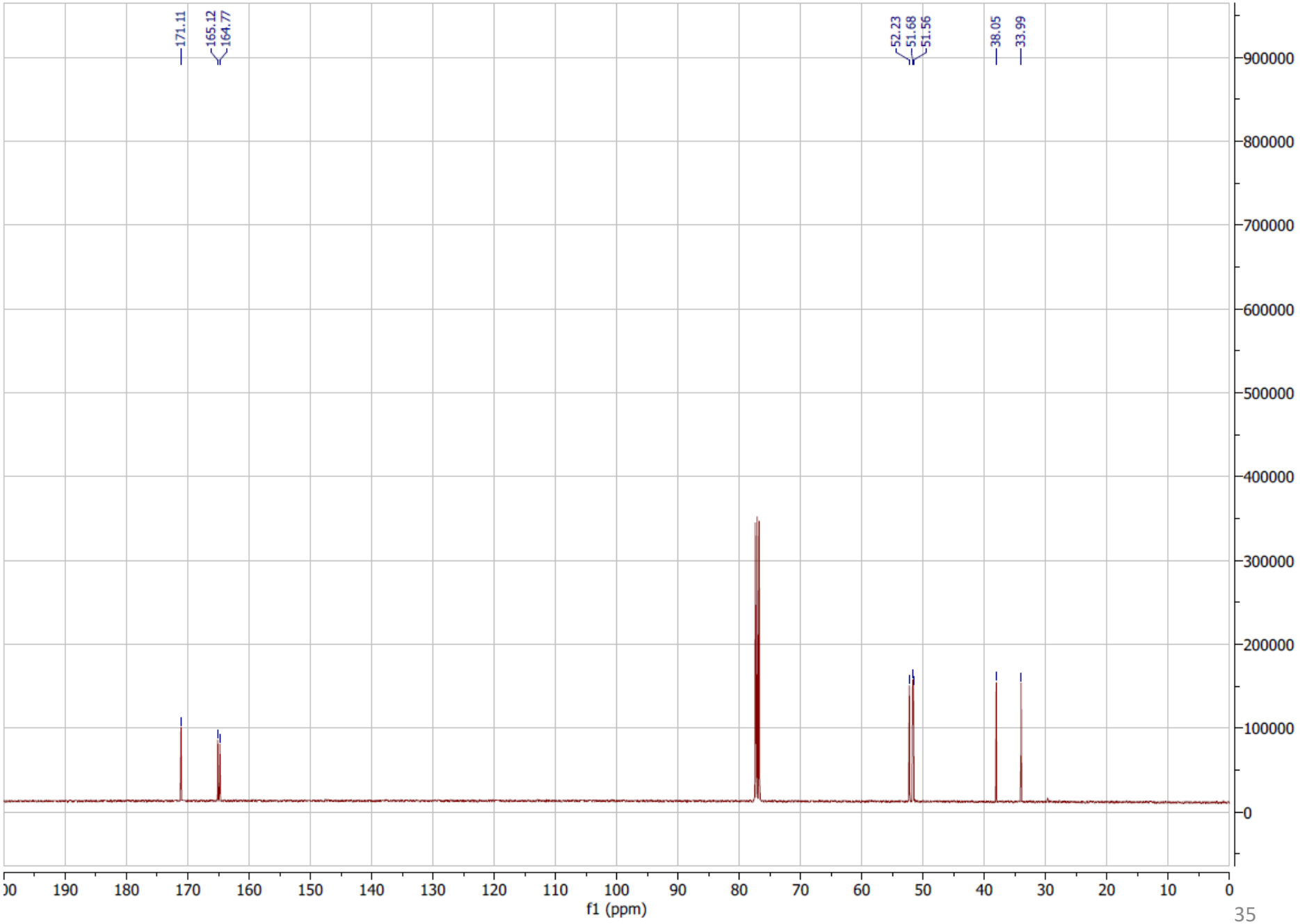

O
NH
O
N
O
O

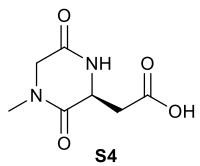
S3


7.03
7.03
4.34
4.34
4.33
4.33
4.32
4.32
4.31
4.31
4.07
4.07
4.03
4.02
3.96
3.96
3.92
3.92
3.71
3.05
3.04
3.00
2.99
2.98
2.85
2.83
2.81
2.79
0.92
1.00
1.01
1.02
3.01
1.09
2.90
1.01
f1 (ppm)

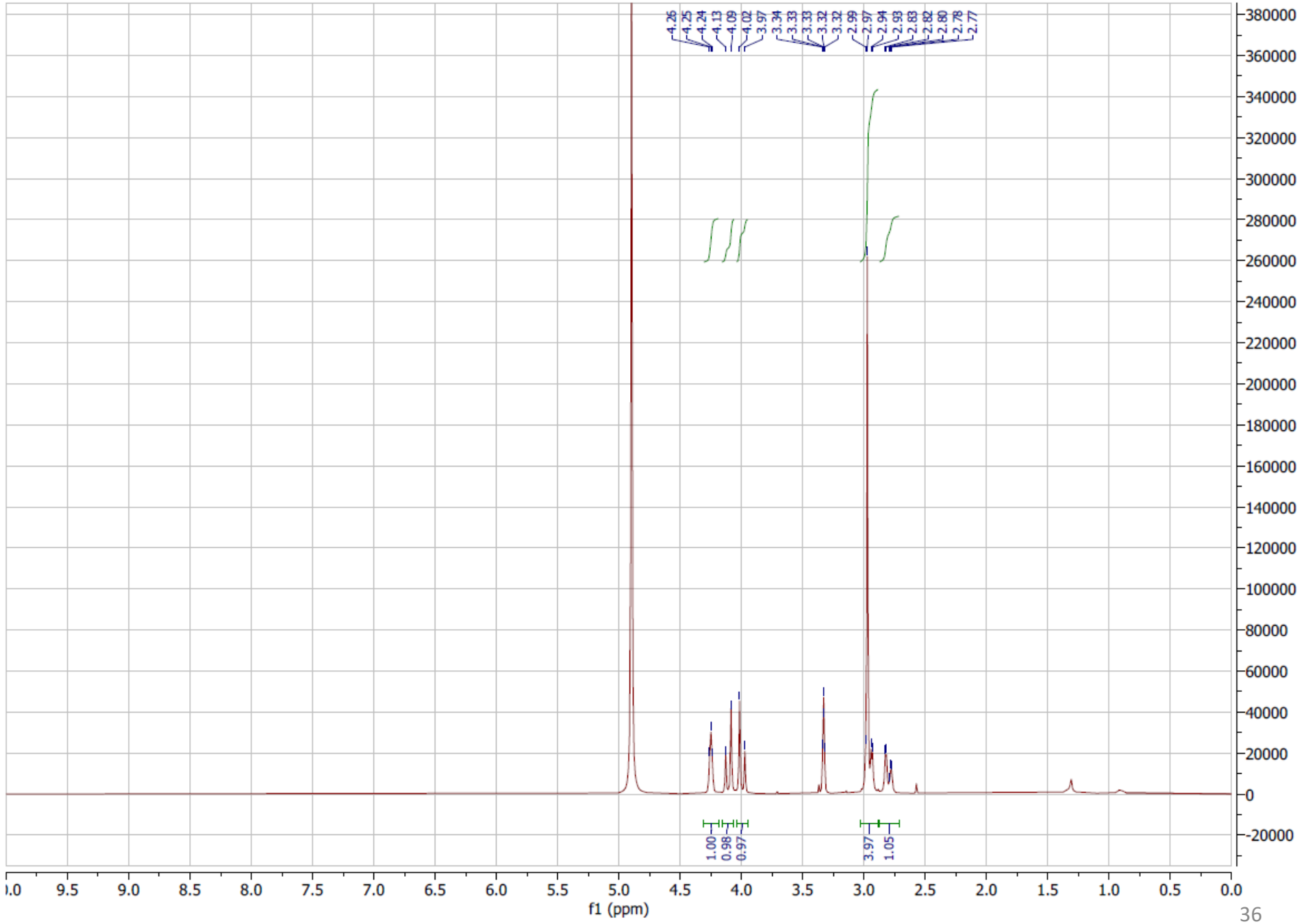

O
NH
O
N
O
O

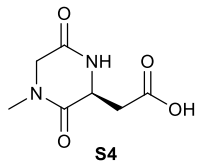
S3


171.11
165.12
164.77
52.23
51.68
51.56
38.05
33.99
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

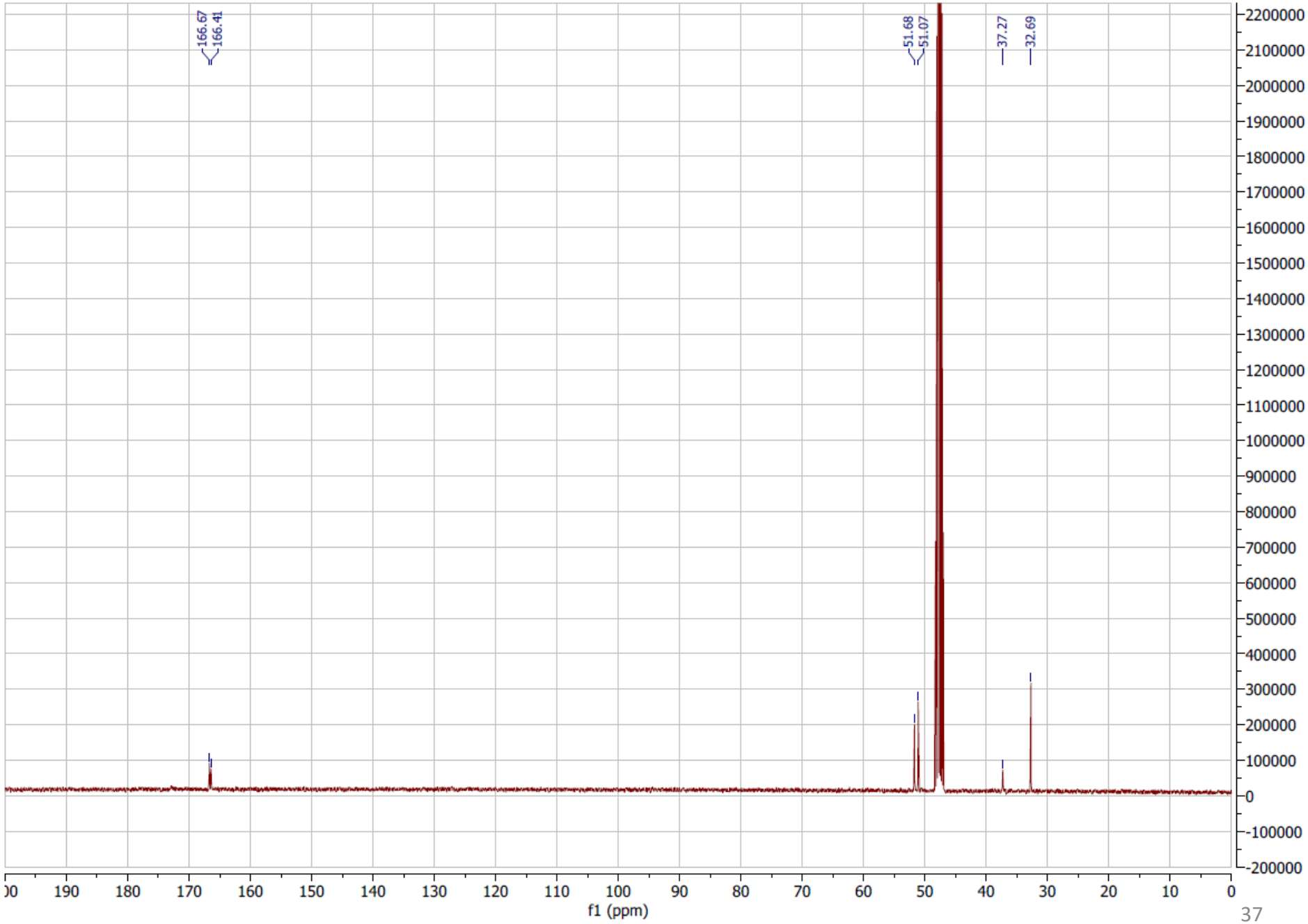

O
NH
O
N
OH
O

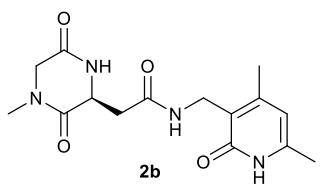
S4


4.26
4.25
4.24
4.13
4.09
4.02
3.97
3.34
3.33
3.33
3.32
3.32
2.99
2.97
2.94
2.93
2.83
2.82
2.80
2.78
2.77
380000
360000
340000
320000
300000
280000
260000
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
-20000
1.00
0.98
0.97
3.97
1.05
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

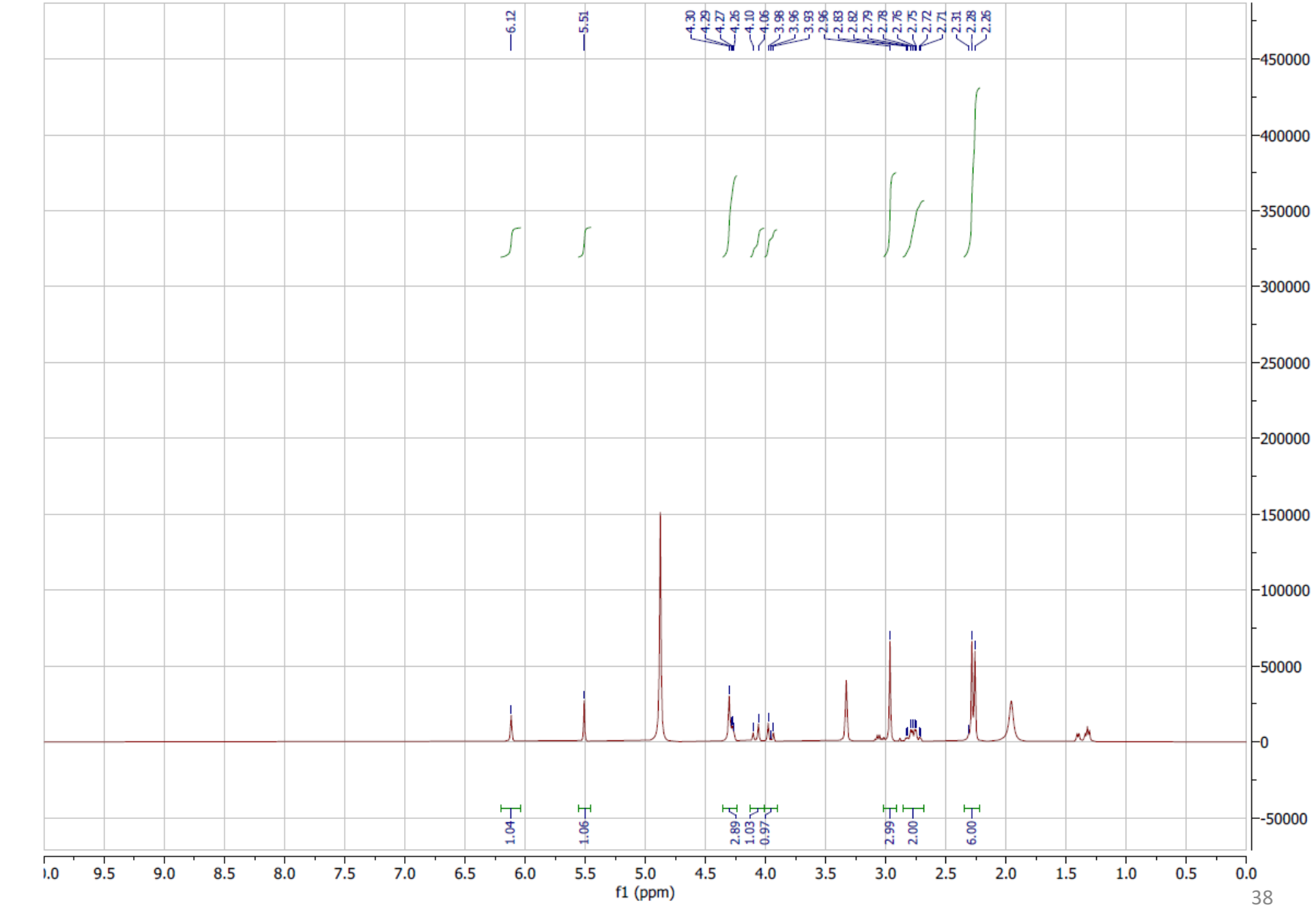

O
NH
O
N
OH
O

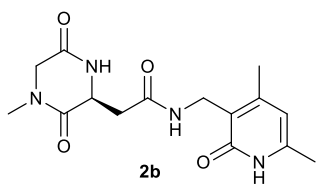
S4


166.67
166.41
51.68
51.07
37.27
32.69
2200000
2100000
2000000
1900000
1800000
1700000
1600000
1500000
1400000
1300000
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
-100000
-200000
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

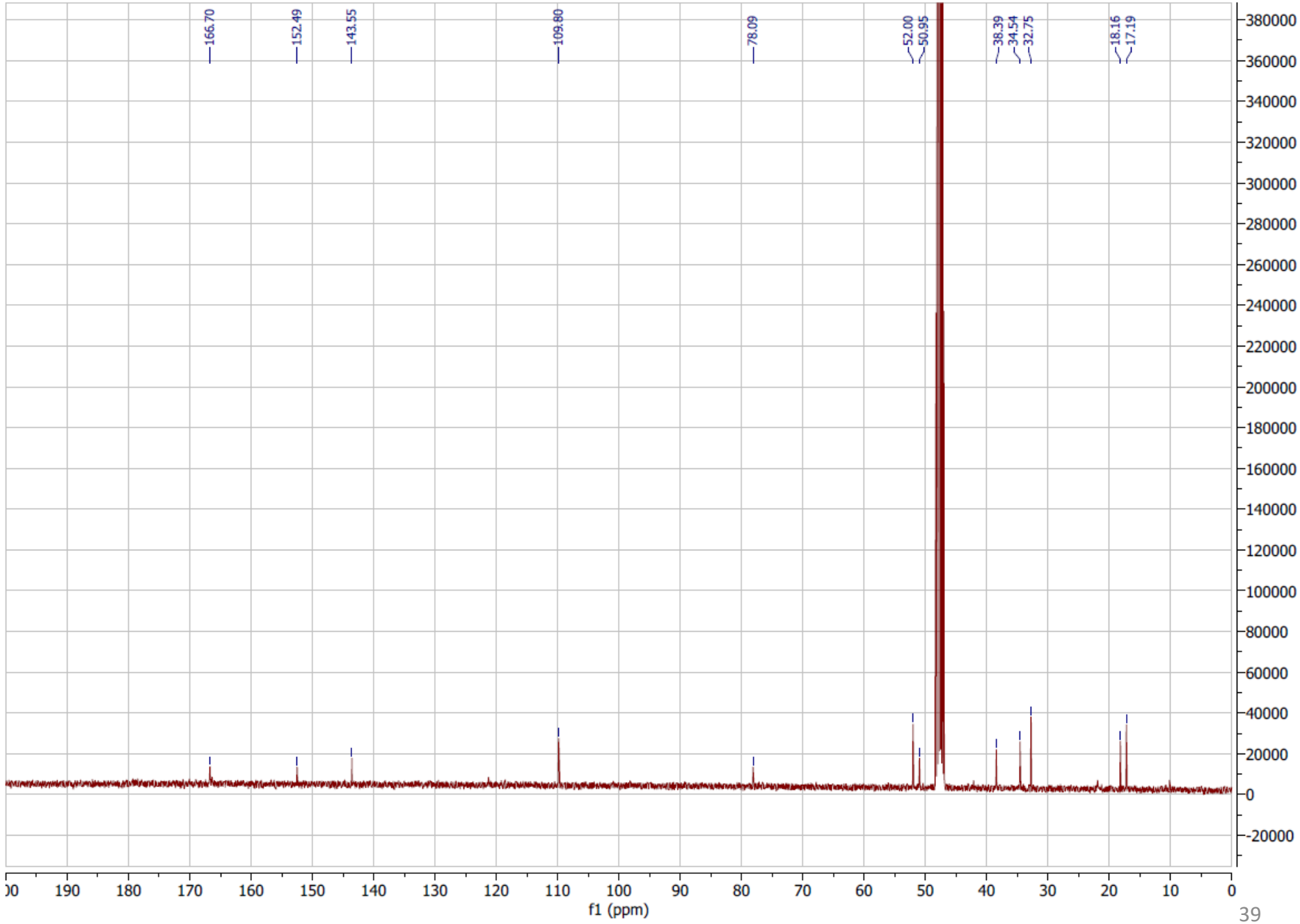

O
NH
O
N
N
H
O
O
N
H

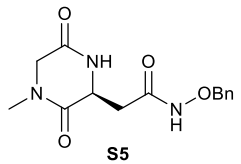
2b


6.12
5.51
4.30
4.29
4.27
4.26
4.10
4.06
3.98
3.96
3.93
2.96
2.83
2.82
2.79
2.78
2.76
2.75
2.72
2.71
2.31
2.28
2.26
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
-50000
1.04
1.06
2.89
1.03
0.97
2.99
2.00
6.00
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

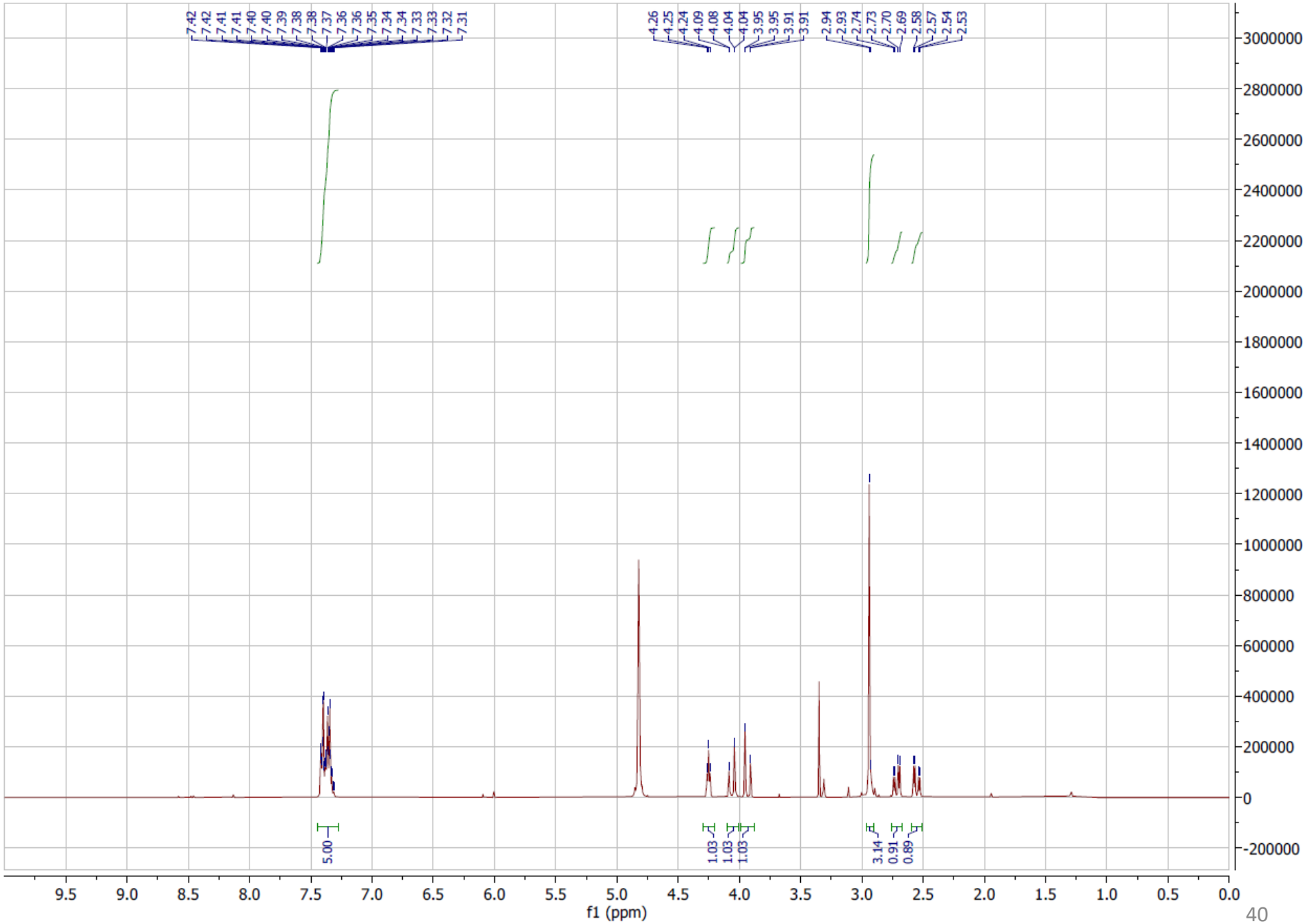

O
NH
O
N
N
H
O
O
N
H

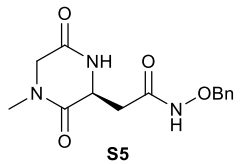
2b


166.70
152.49
143.55
109.80
78.09
52.00
50.95
38.39
34.54
32.75
18.16
17.19
380000
360000
340000
320000
300000
280000
260000
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
-20000
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

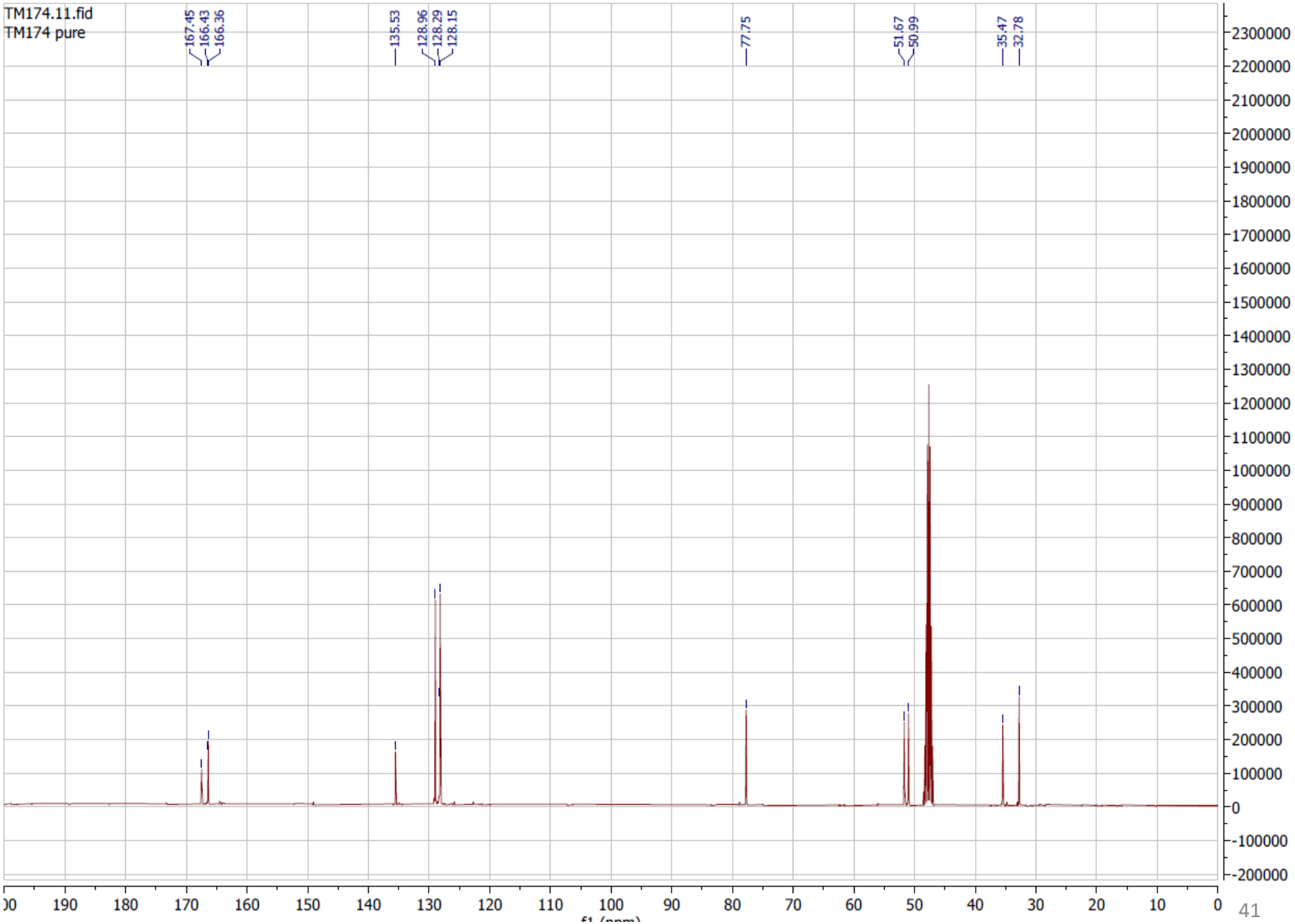

O
NH
O
N
OBn
N
H
O

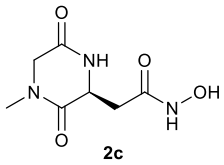
S5

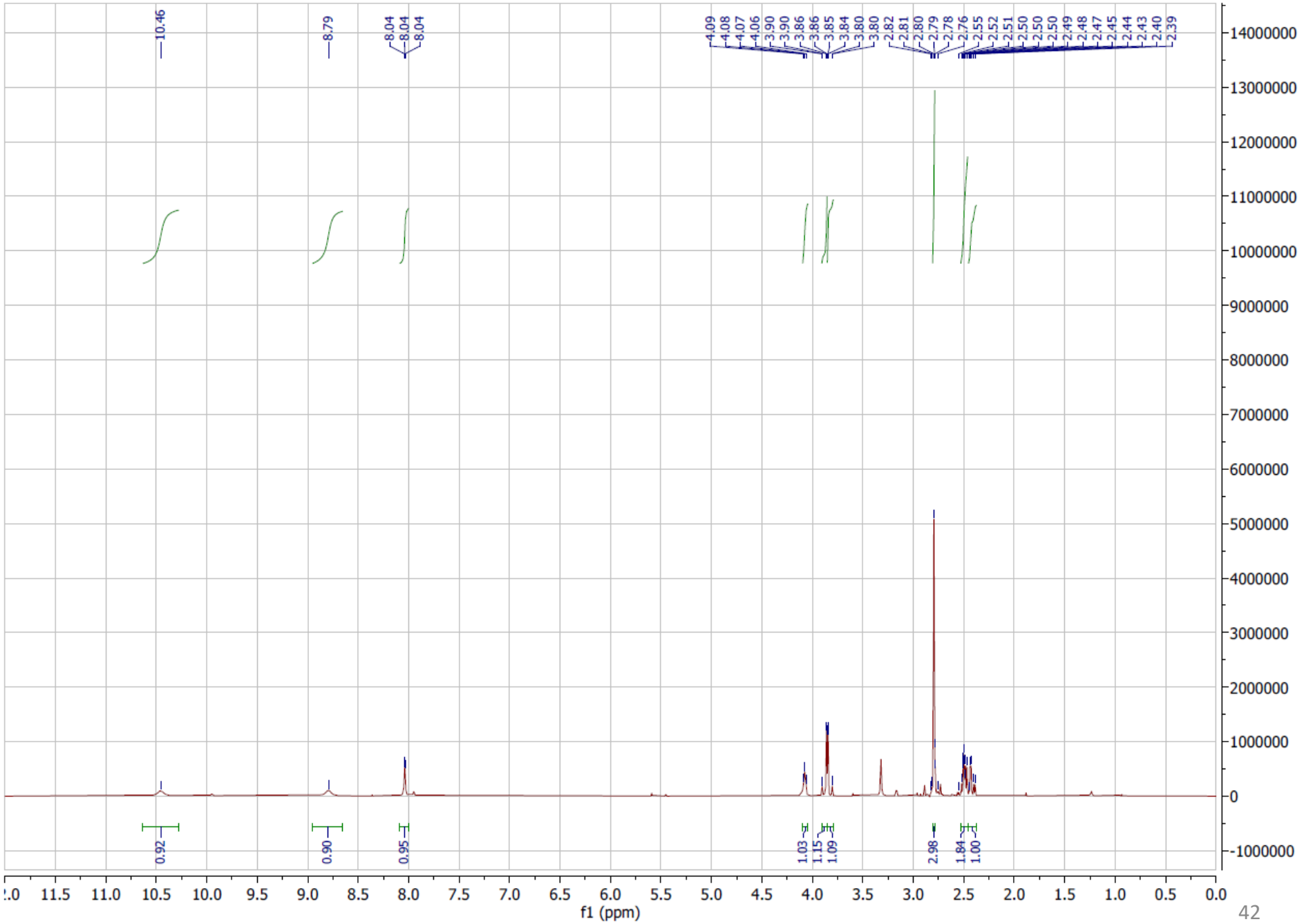

7.42
7.42
7.41
7.41
7.40
7.40
7.39
7.38
7.38
7.37
7.36
7.36
7.35
7.34
7.34
7.33
7.33
7.32
7.31
4.26
4.25
4.24
4.09
4.08
4.04
4.04
3.95
3.95
3.91
3.91
2.94
2.93
2.74
2.73
2.70
2.69
2.58
2.57
2.54
2.53
5.00
1.03
1.03
1.03
3.14
0.91
0.89
3000000
2800000
2600000
2400000
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
-200000
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

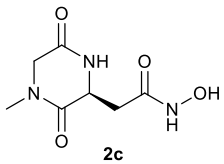

O
NH
O
N
N
OBn
H
O

S5

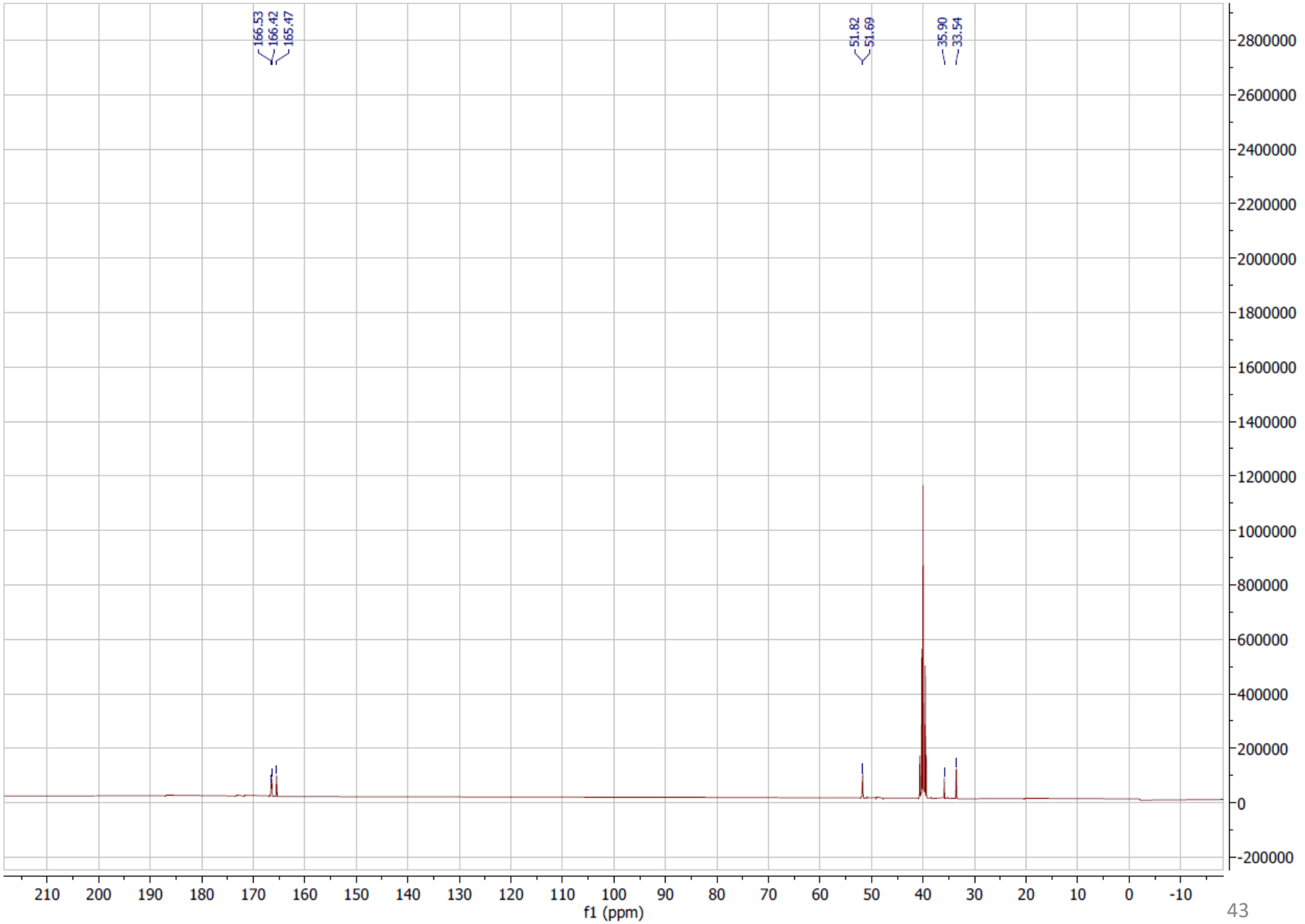

TM174.11.fid
TM174 pure
167.45
166.43
166.36
135.53
128.96
128.29
128.15
77.75
51.67
50.99
35.47
32.78
2300000
2200000
2100000
2000000
1900000
1800000
1700000
1600000
1500000
1400000
1300000
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
-100000
-200000
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

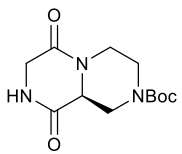

O
NH
O
N
N
OH
H
O
2c


10.46
8.79
8.04
8.04
8.04
4.09
4.08
4.07
4.06
3.90
3.90
3.86
3.86
3.85
3.84
3.80
3.80
2.82
2.81
2.80
2.79
2.78
2.76
2.55
2.52
2.51
2.50
2.50
2.50
2.49
2.48
2.47
2.45
2.44
2.43
2.40
2.39
0.92
0.90
0.95
1.03
1.15
1.09
2.98
1.84
1.00
f1 (ppm)

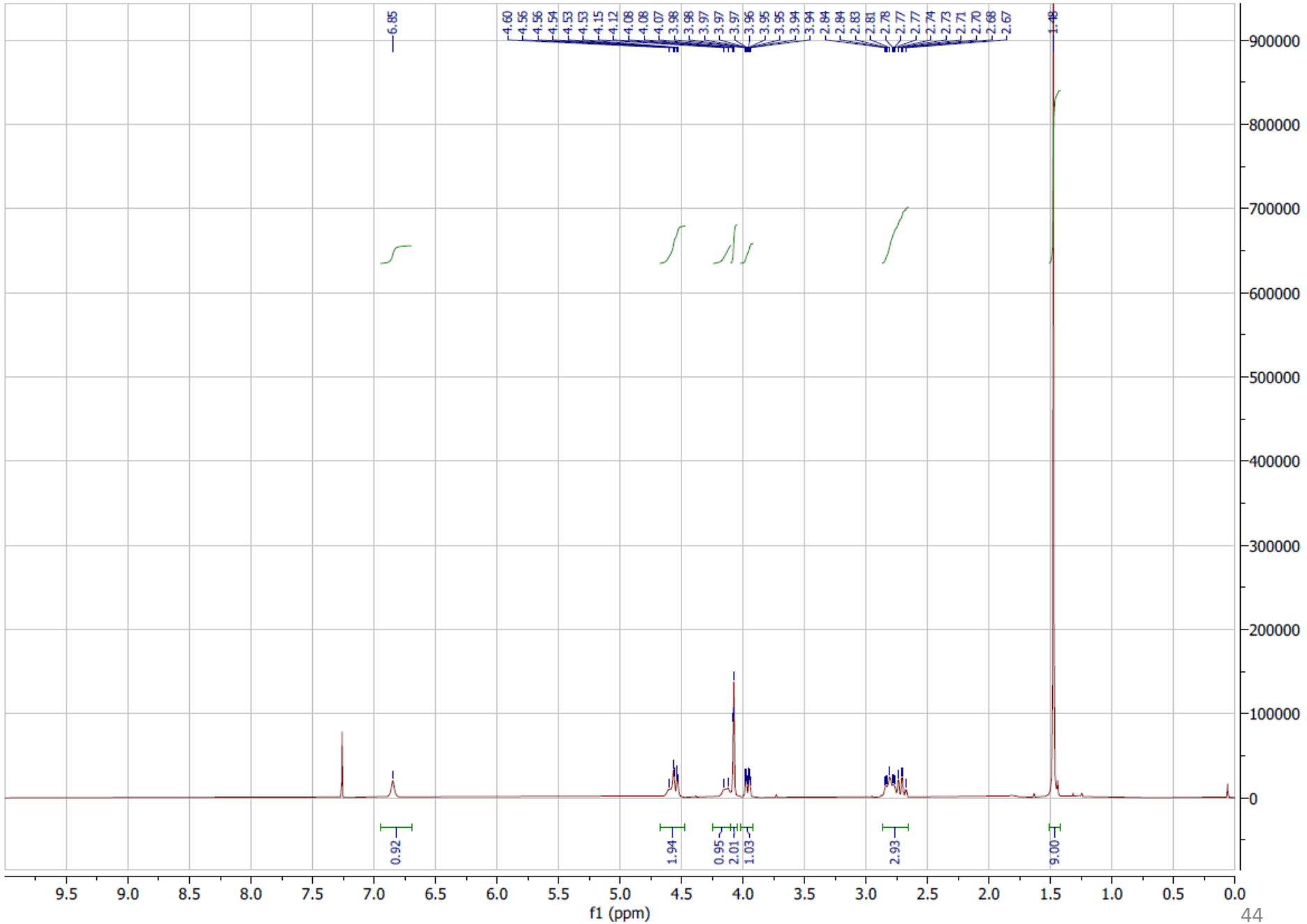

O
NH
O
N
OH
N
H
O

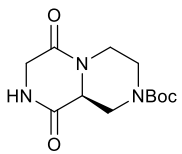
2c


166.53
166.42
165.47
51.82
51.69
35.90
33.54
2800000
2600000
2400000
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
-200000
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

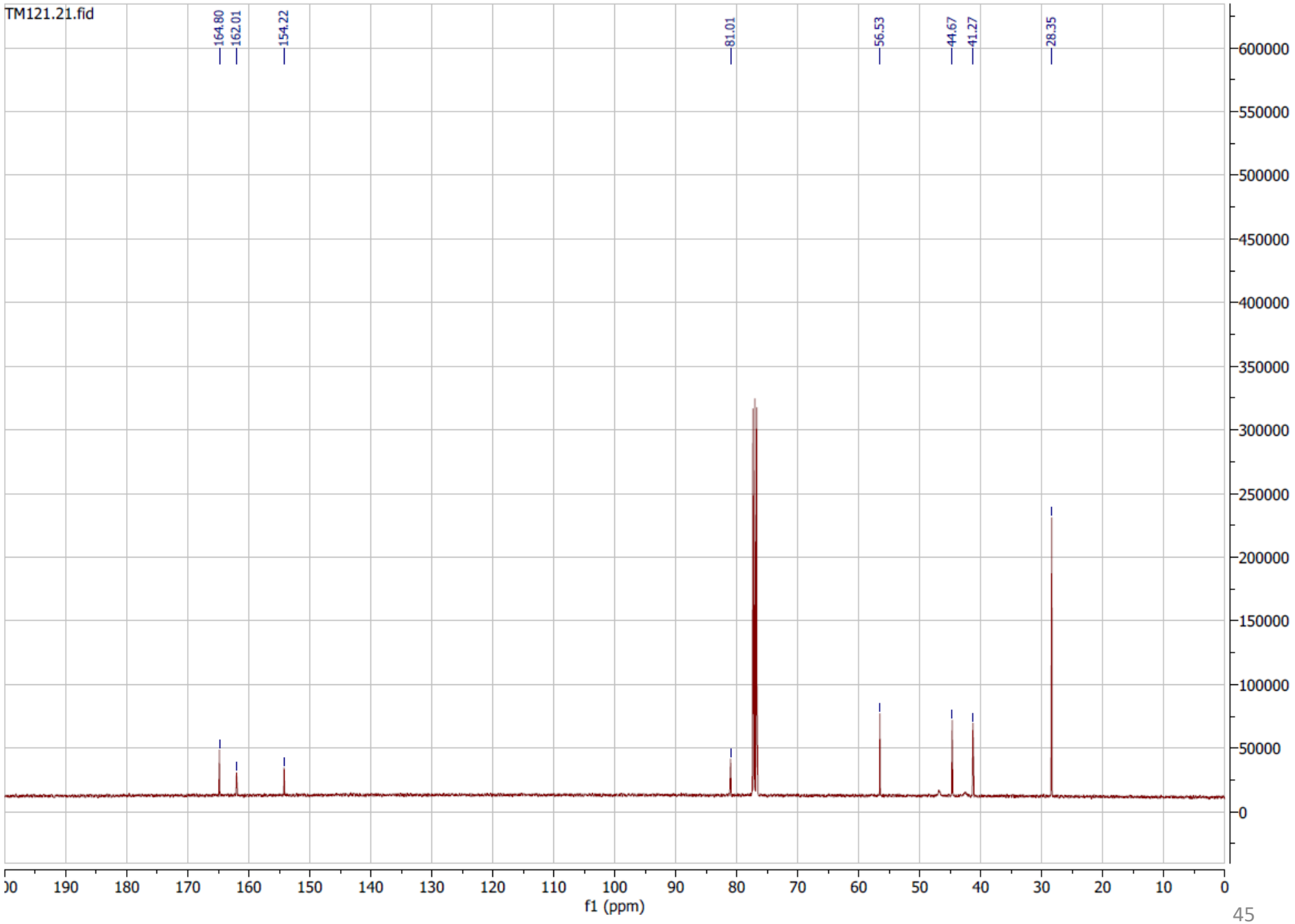

O
N
HN
NBoc
O

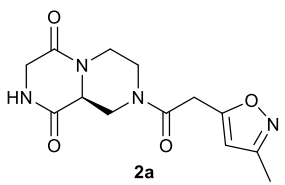
S5


6.85
4.60
4.56
4.56
4.54
4.54
4.53
4.53
4.15
4.12
4.08
4.08
4.07
3.98
3.98
3.97
3.97
3.97
3.96
3.95
3.95
3.94
3.94
2.84
2.84
2.83
2.81
2.78
2.77
2.77
2.74
2.73
2.71
2.70
2.68
2.67
1.48
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
0.92
1.94
0.95
2.01
1.03
2.93
9.00
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

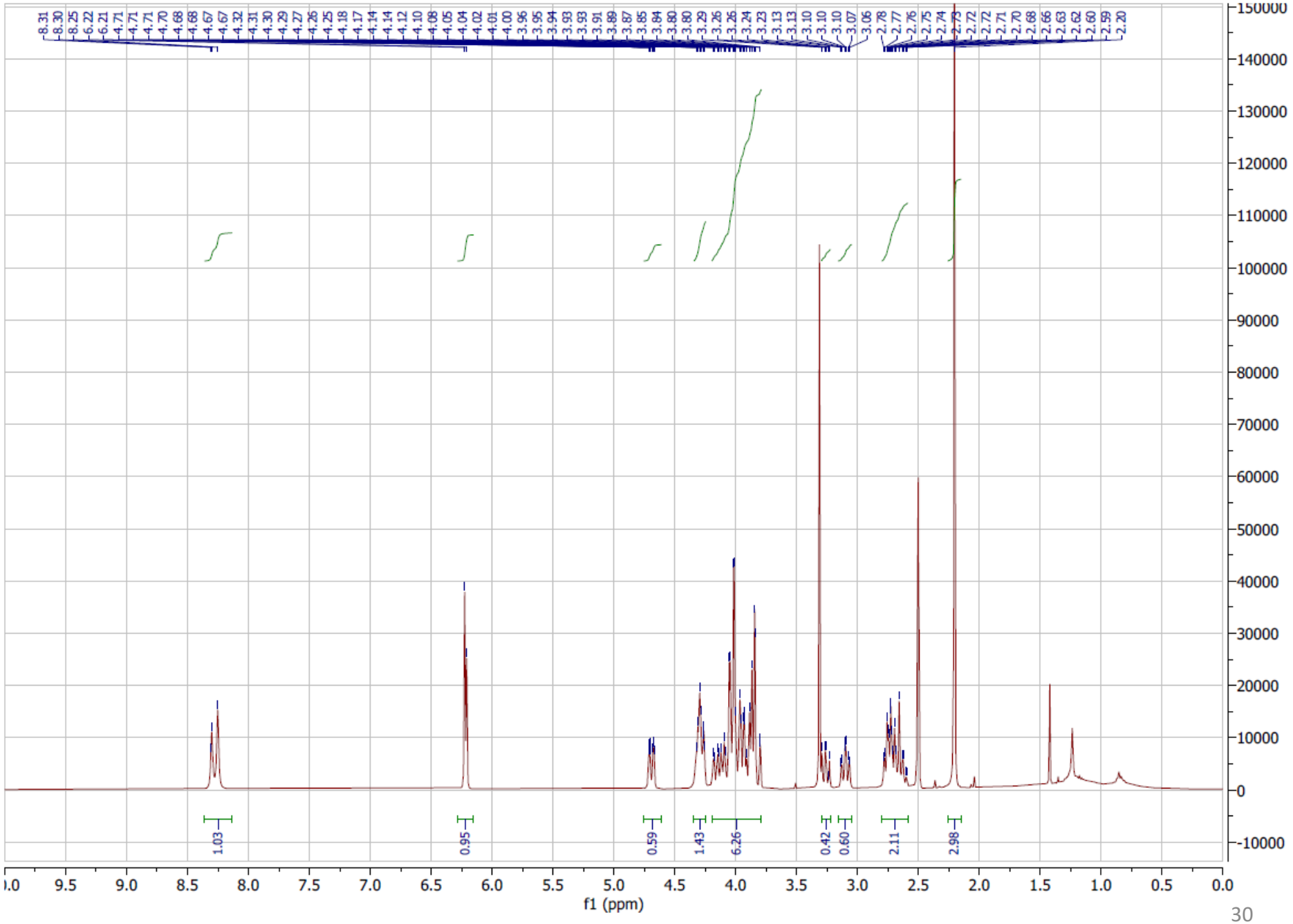

O
N
HN
NBoc
O

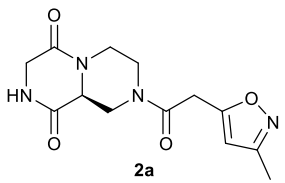
S5

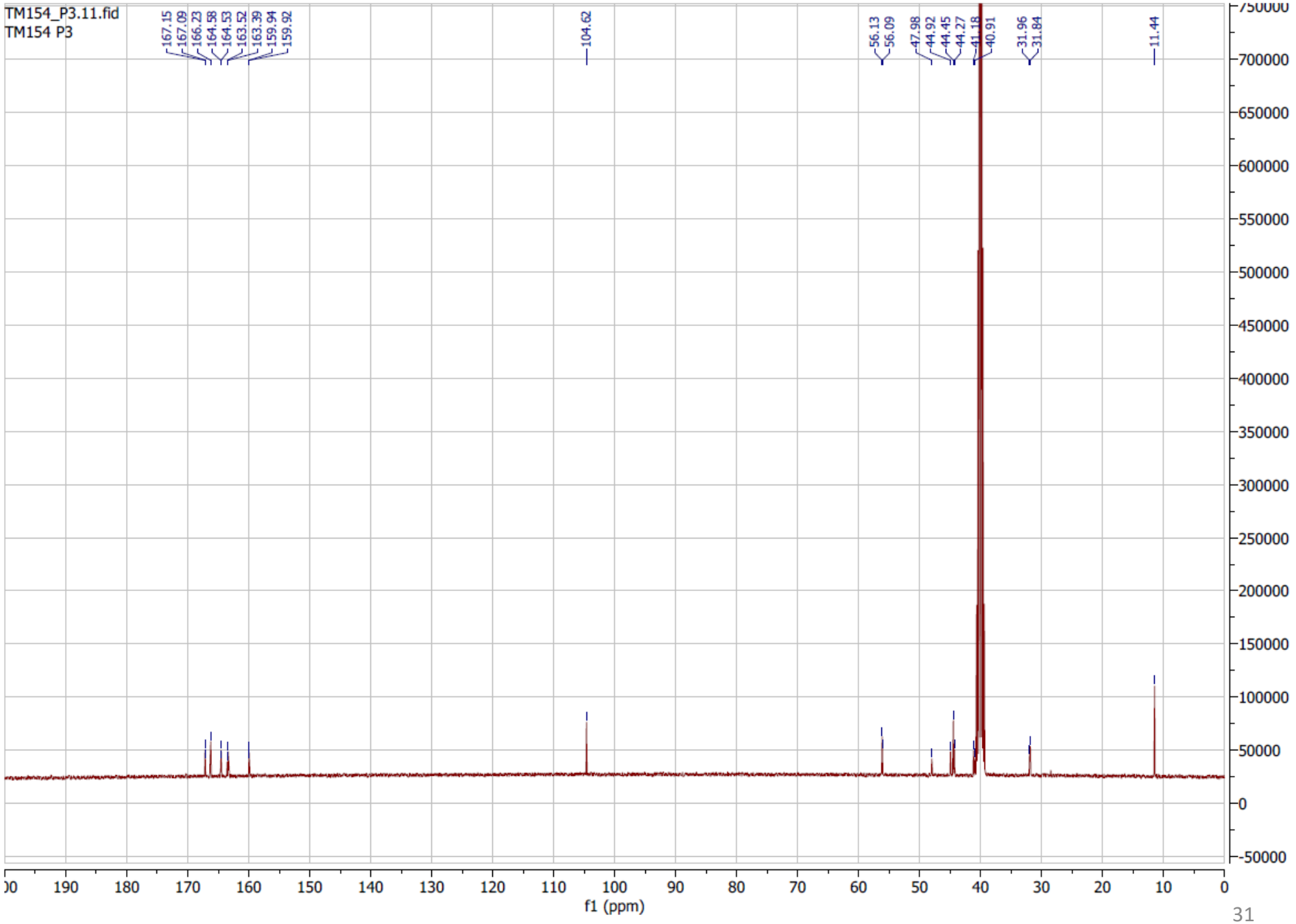

TM121.21.fid
164.80
162.01
154.22
81.01
56.53
44.67
41.27
28.35
600000
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

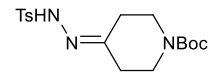

O
N
HN
N
O
O
O
N

2a

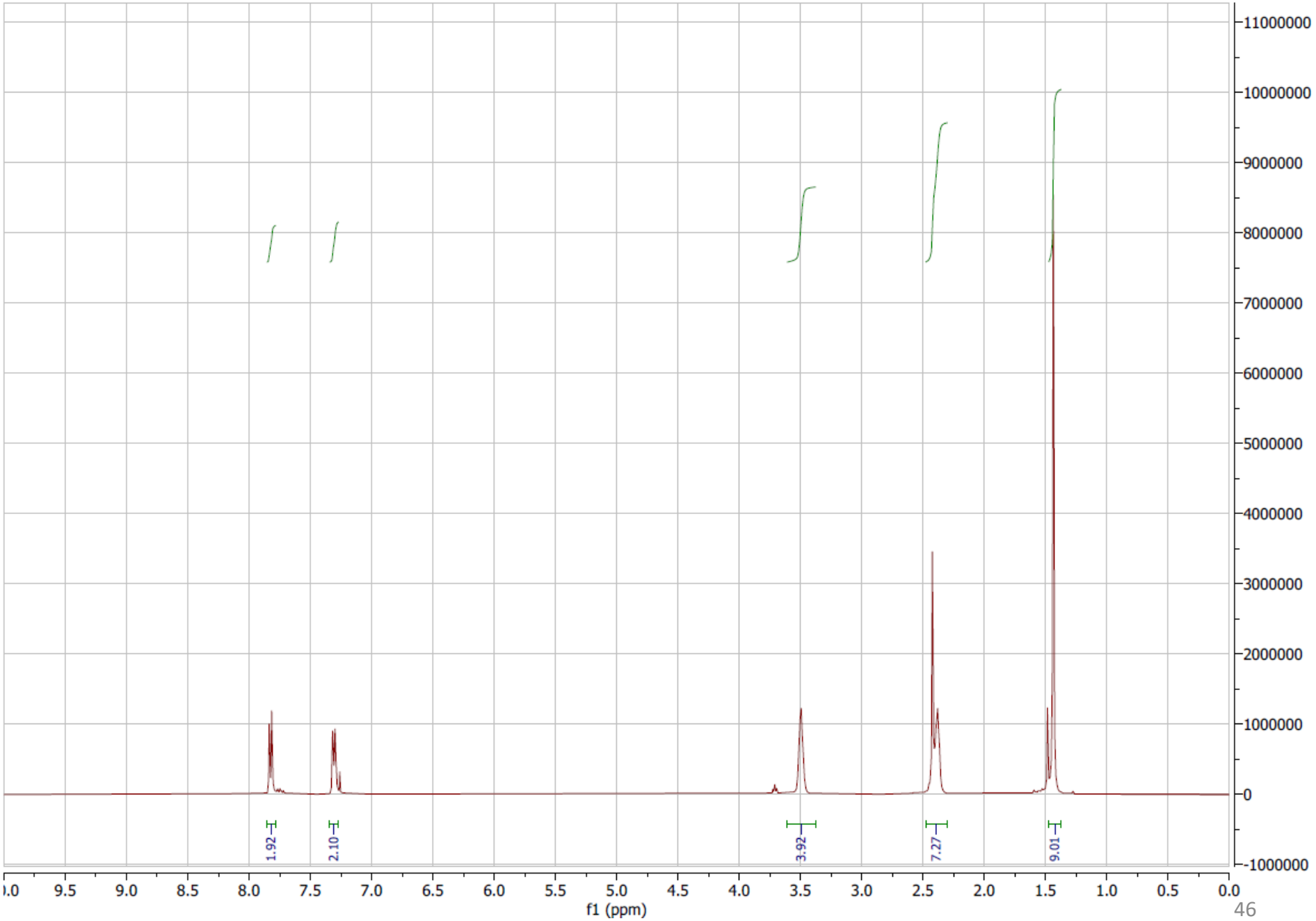

8.31 8.30 8.25 6.22 6.21 4.71 4.71 4.71 4.70 4.68 4.68 4.67 4.67 4.32 4.31 4.30 4.29 4.27 4.26 4.25 4.18 4.17 4.14 4.14 4.12 4.10 4.08 4.05 4.04 4.02 4.01 4.00 3.96 3.95 3.94 3.93 3.93 3.91 3.89 3.87 3.85 3.84 3.80 3.80 3.29 3.26 3.26 3.24 3.23 3.13 3.13 3.10 3.10 3.07 3.06 2.78 2.77 2.76 2.75 2.74 2.73 2.72 2.72 2.71 2.70 2.68 2.66 2.63 2.62 2.60 2.59 2.20
1.03
0.95
0.59
1.43
6.26
0.42
0.60
2.11
2.98
150000
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

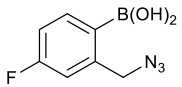

O
N
HN
N
O
O
O
N
2a

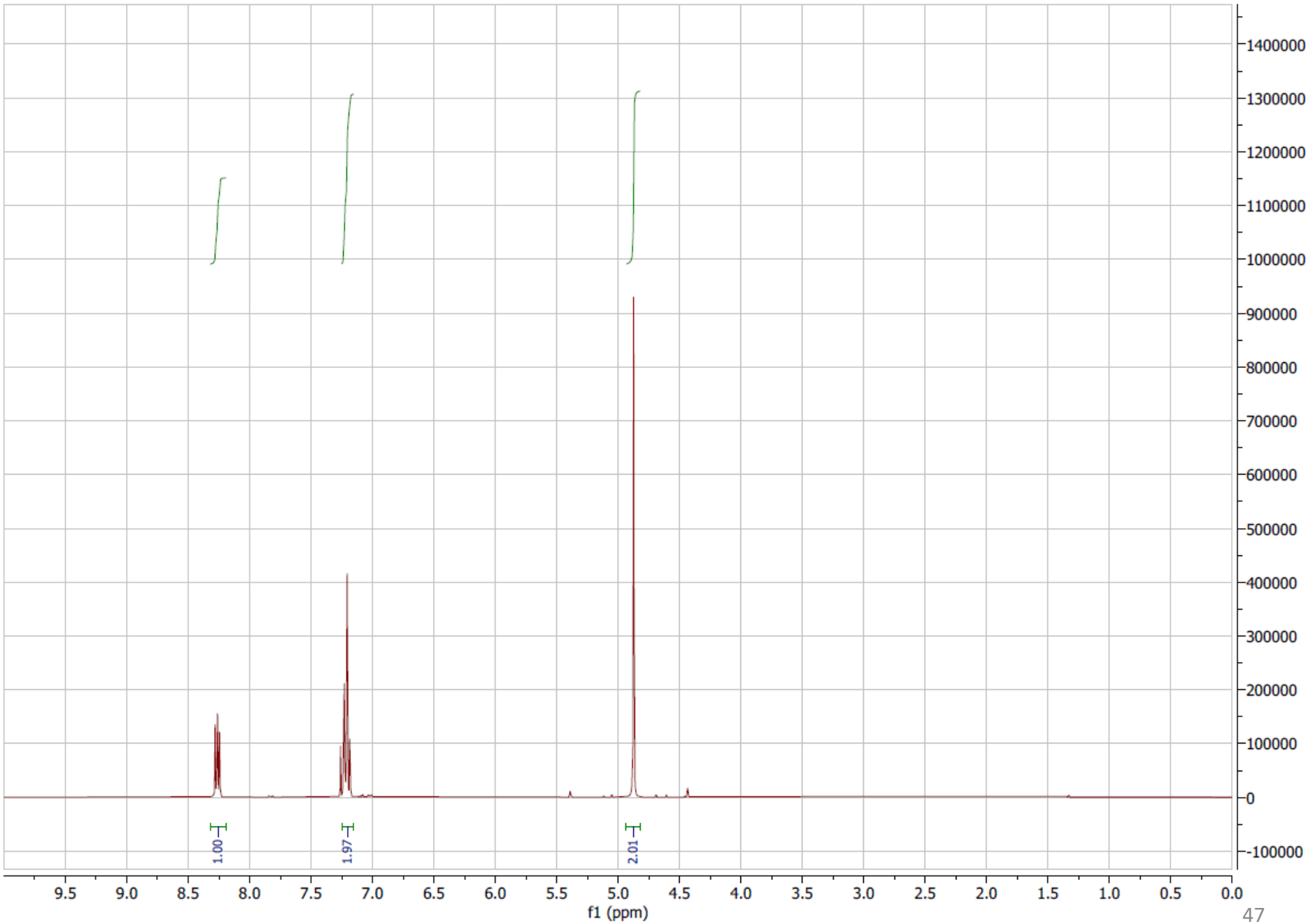

TM154_P3.11.fid
TM154 P3
167.15
167.09
166.23
164.58
164.53
163.52
163.39
159.94
159.92
104.62
56.13
56.09
47.98
44.92
44.45
44.27
41.18
40.91
31.96
31.84
11.44
f1 (ppm)



S6
CAS RN : 1046478-89-0




B(OH)2
N3
F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1100000
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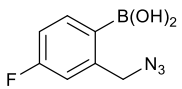
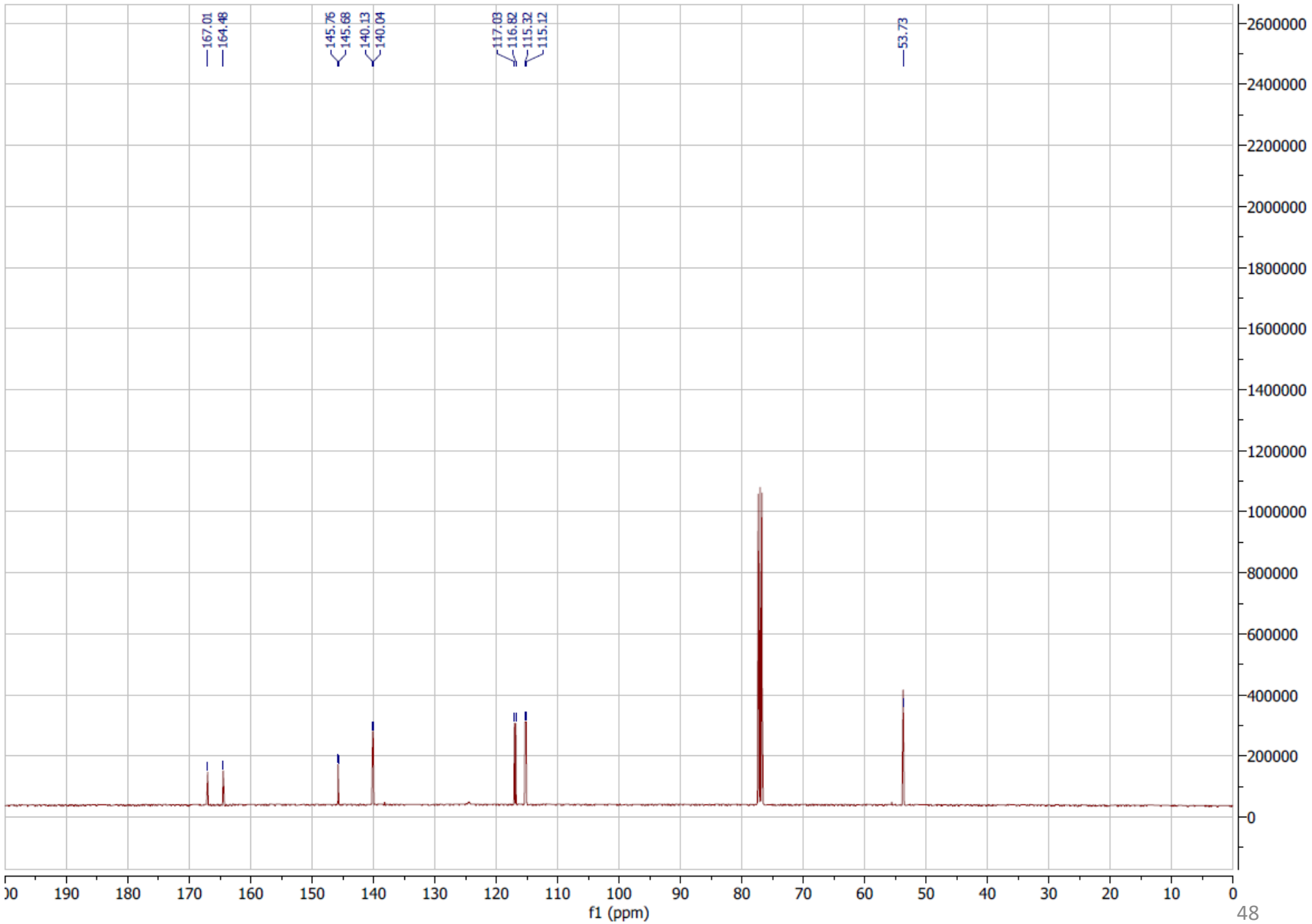
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
-100000
1.00
1.97
2.01
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


B(OH)2
N3
F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164.48
145.76
145.68
140.13
140.04
117.03
116.82
115.32
115.12
53.73
2600000
2400000
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
190
180
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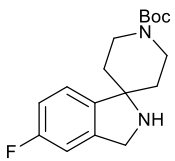
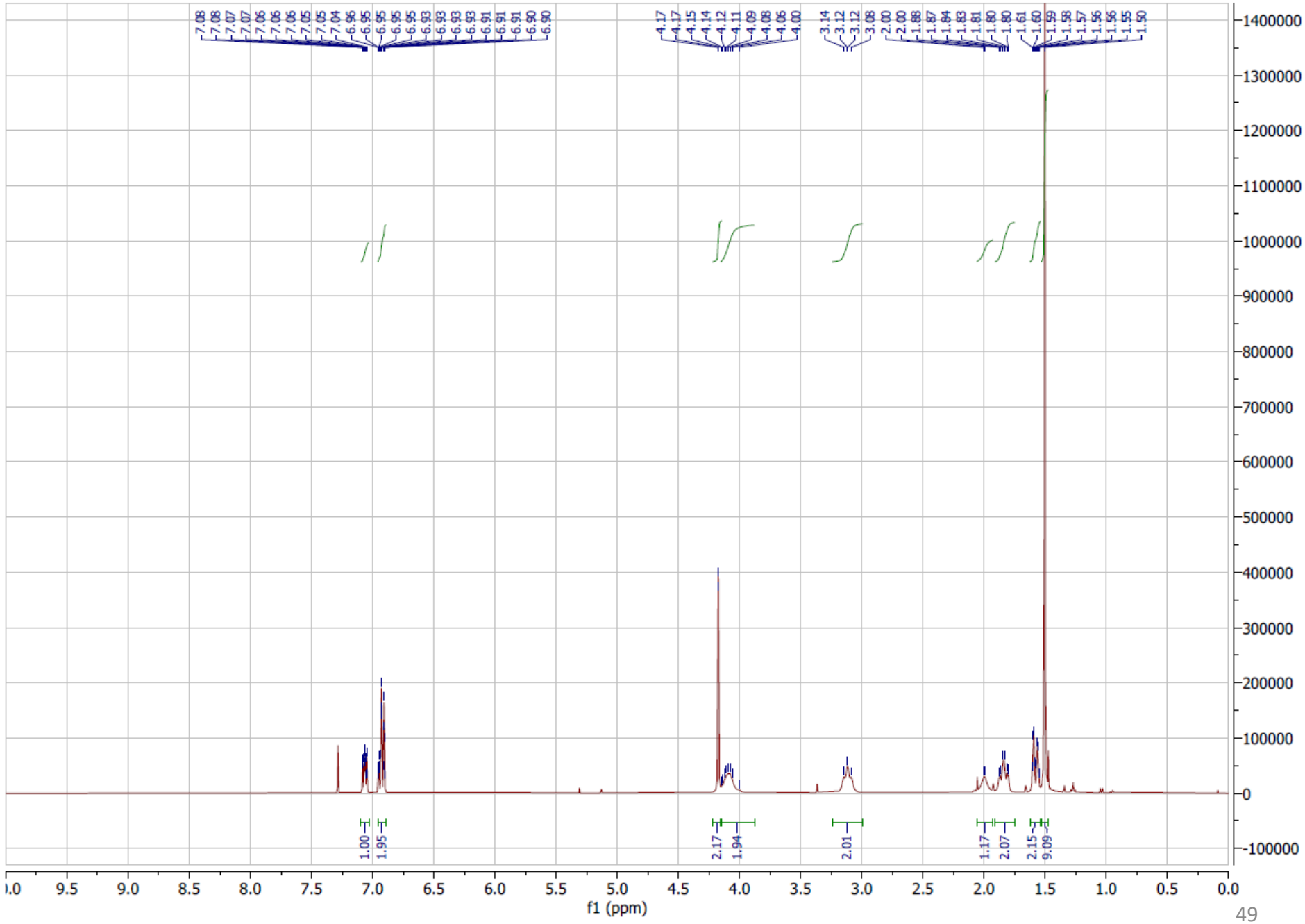
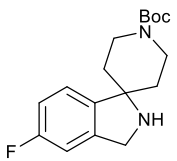
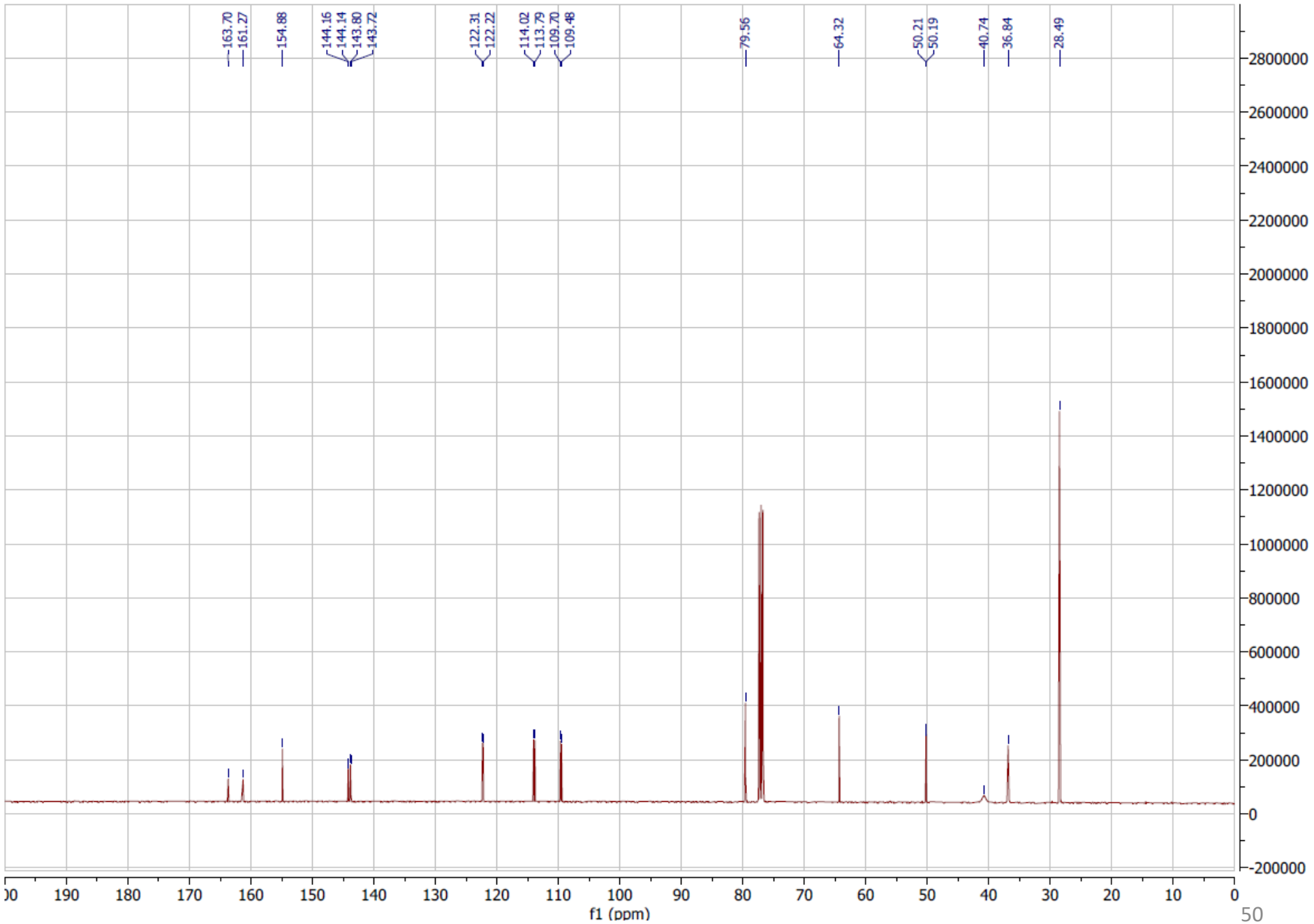
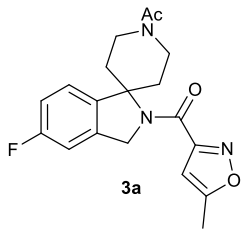
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


Boc
N
NH
F


7.08 7.08 7.07 7.07 7.06 7.06 7.06 7.05 7.05 7.04 6.96 6.95 6.95 6.95 6.93 6.93 6.93 6.91 6.91 6.90 6.90
4.17 4.17 4.15 4.14 4.12 4.11 4.09 4.08 4.06 4.00
3.14 3.12 3.12 3.08
2.00 2.00 1.88 1.87 1.84 1.83 1.81 1.80 1.80 1.61 1.60 1.59 1.58 1.57 1.56 1.56 1.55 1.50
1.00 1.95 2.17 1.94 2.01 1.17 2.07 2.15 9.09
f1 (ppm)

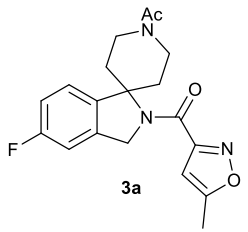

Boc
N
NH
F

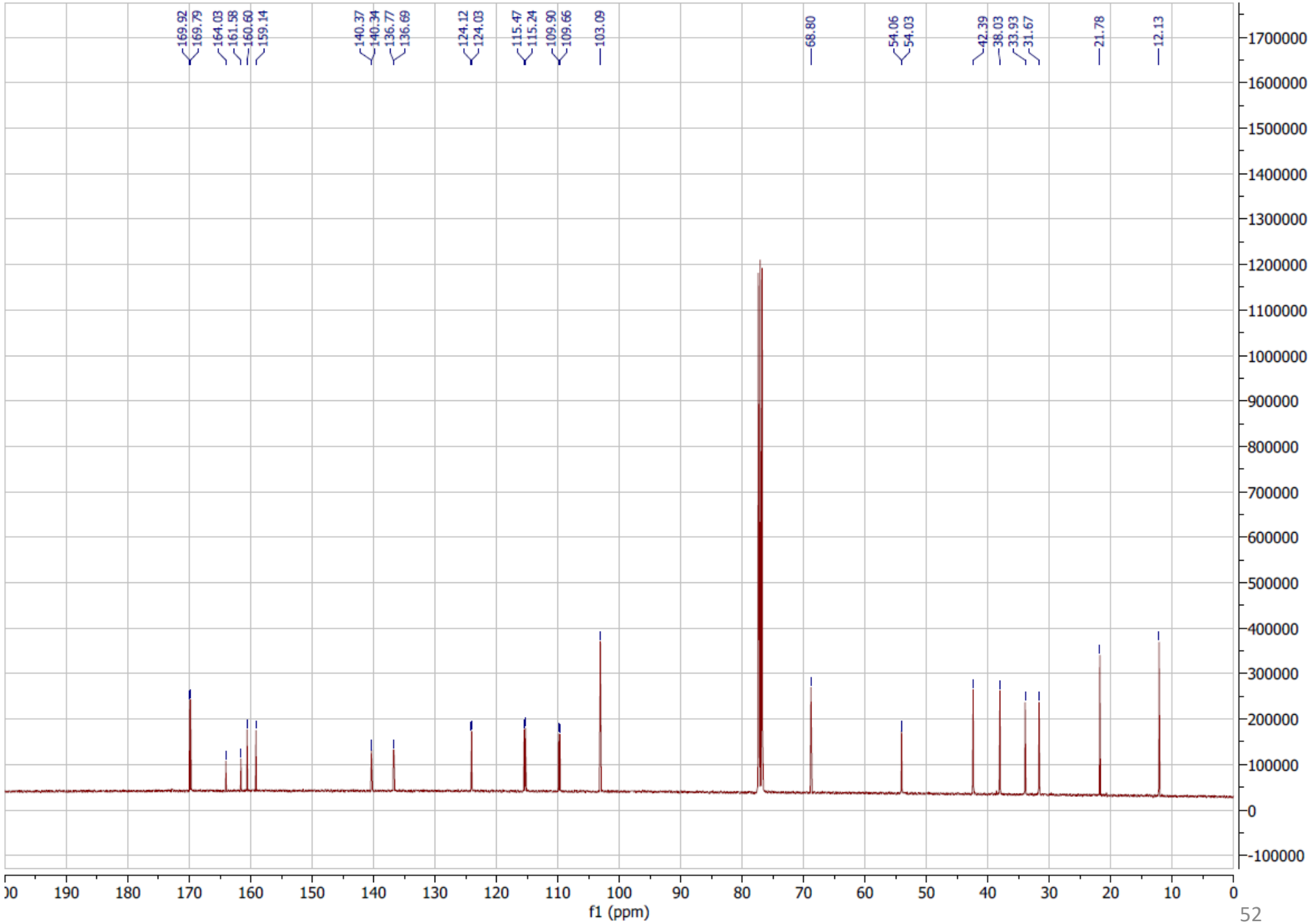

163.70
161.27
154.88
144.16
144.14
143.80
143.72
122.31
122.22
114.02
113.79
109.70
109.48
79.56
64.32
50.21
50.19
40.74
36.84
28.49
2800000
2600000
2400000
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
-200000
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

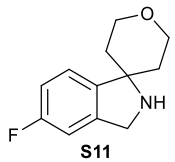

Ac
N
O
N
F
N
O

3a

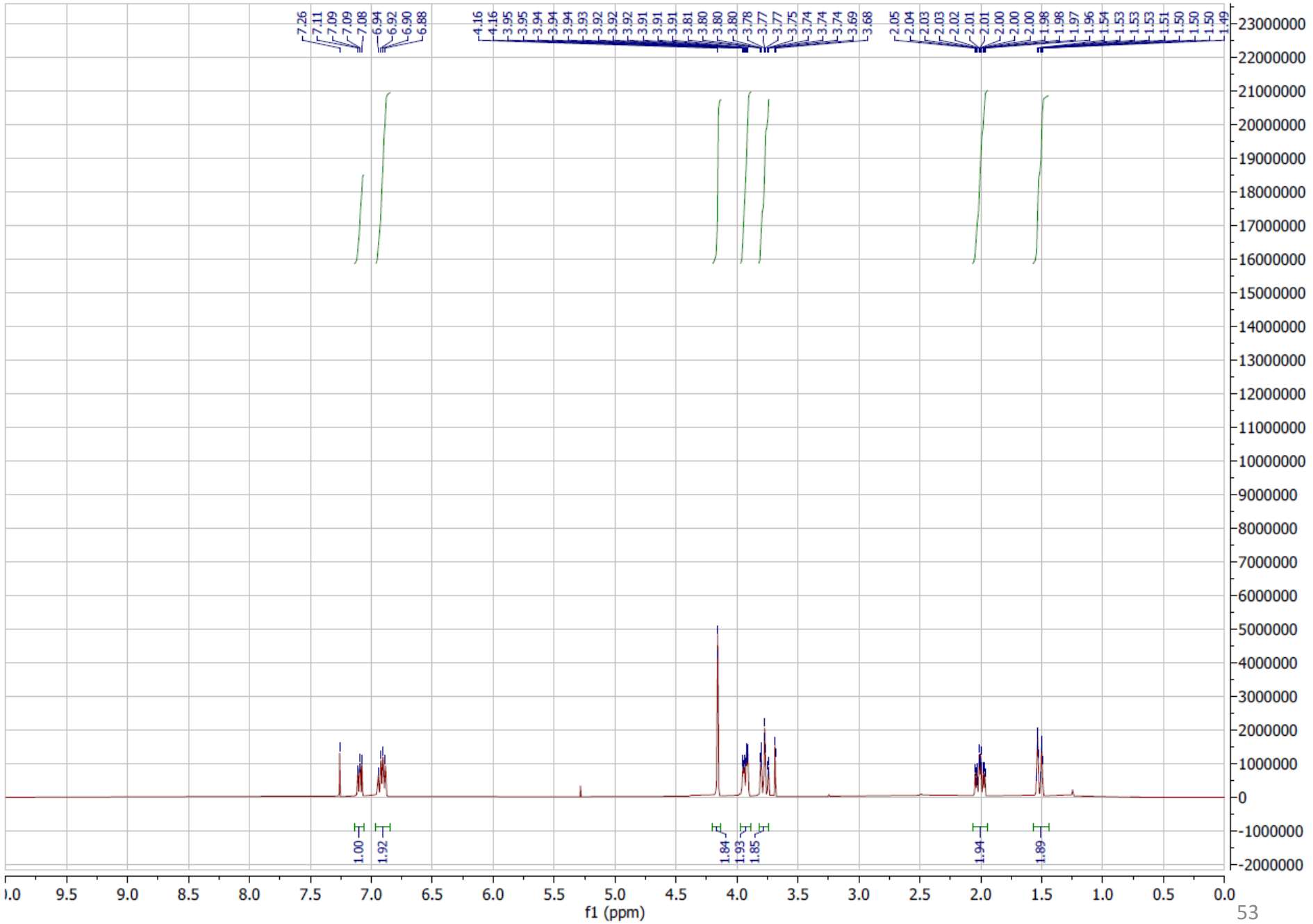
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
0.98
0.99
0.96
0.93
1.02
0.96
1.04
1.00
2.00
1.98
2.99
2.99
1.15
1.10
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

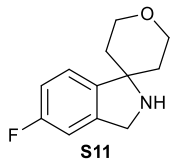

Ac
N
O
N
F
N
O
3a


169.92
169.79
164.03
161.58
160.60
159.14
140.37
140.34
136.77
136.69
124.12
124.03
115.47
115.24
109.90
109.66
103.09
68.80
54.06
54.03
42.39
38.03
33.93
31.67
21.78
12.13
1700000
1600000
1500000
1400000
1300000
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
-100000
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

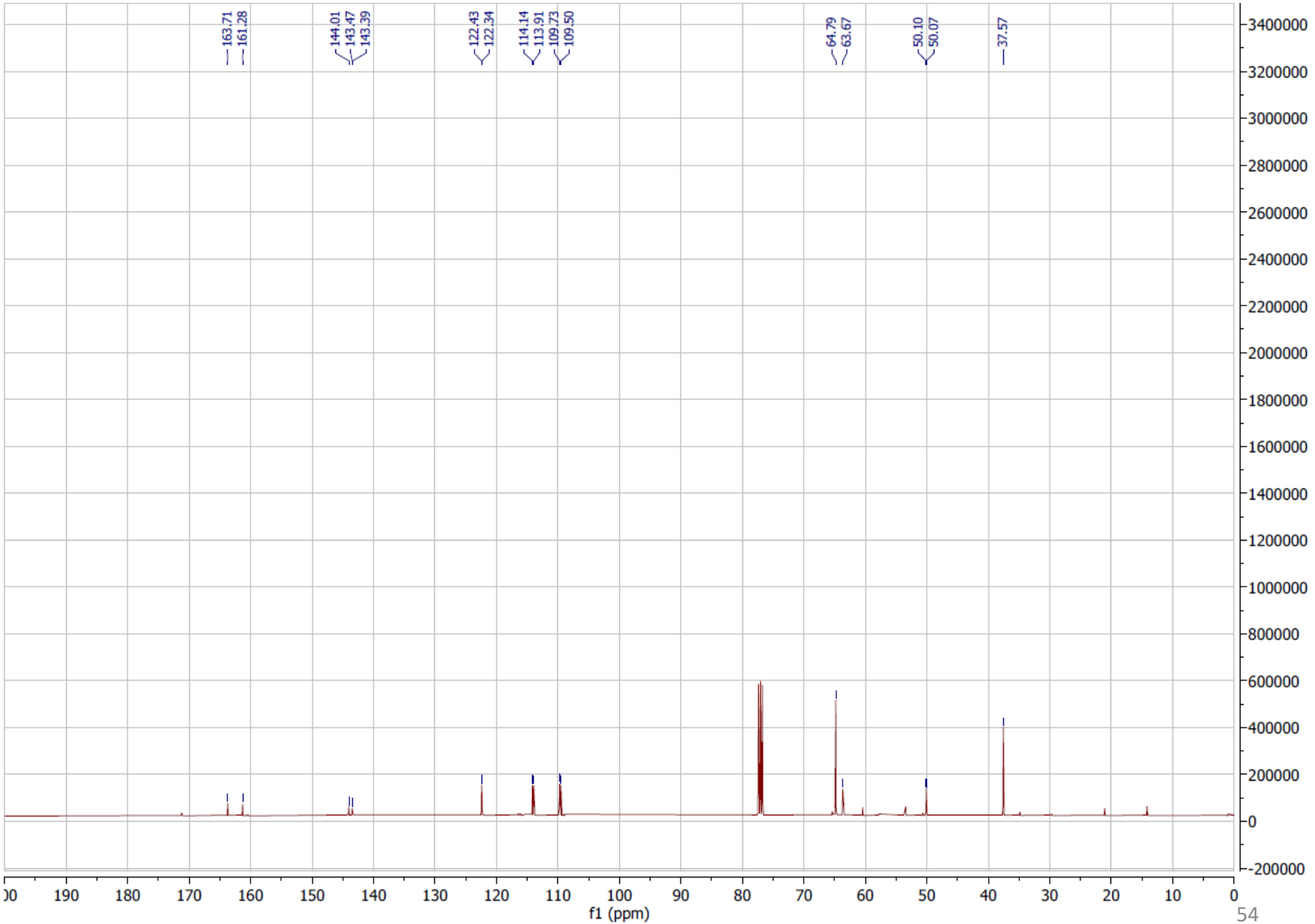


S11




O
NH
F

S11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144.01
143.47
143.39
122.43
122.34
114.14
113.91
109.73
109.50
64.79
63.67
50.10
50.07
37.57
3400000
3200000
3000000
2800000
2600000
2400000
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
-200000
190
180
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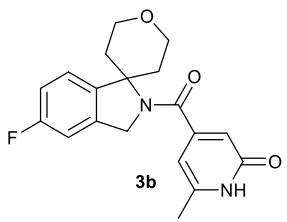
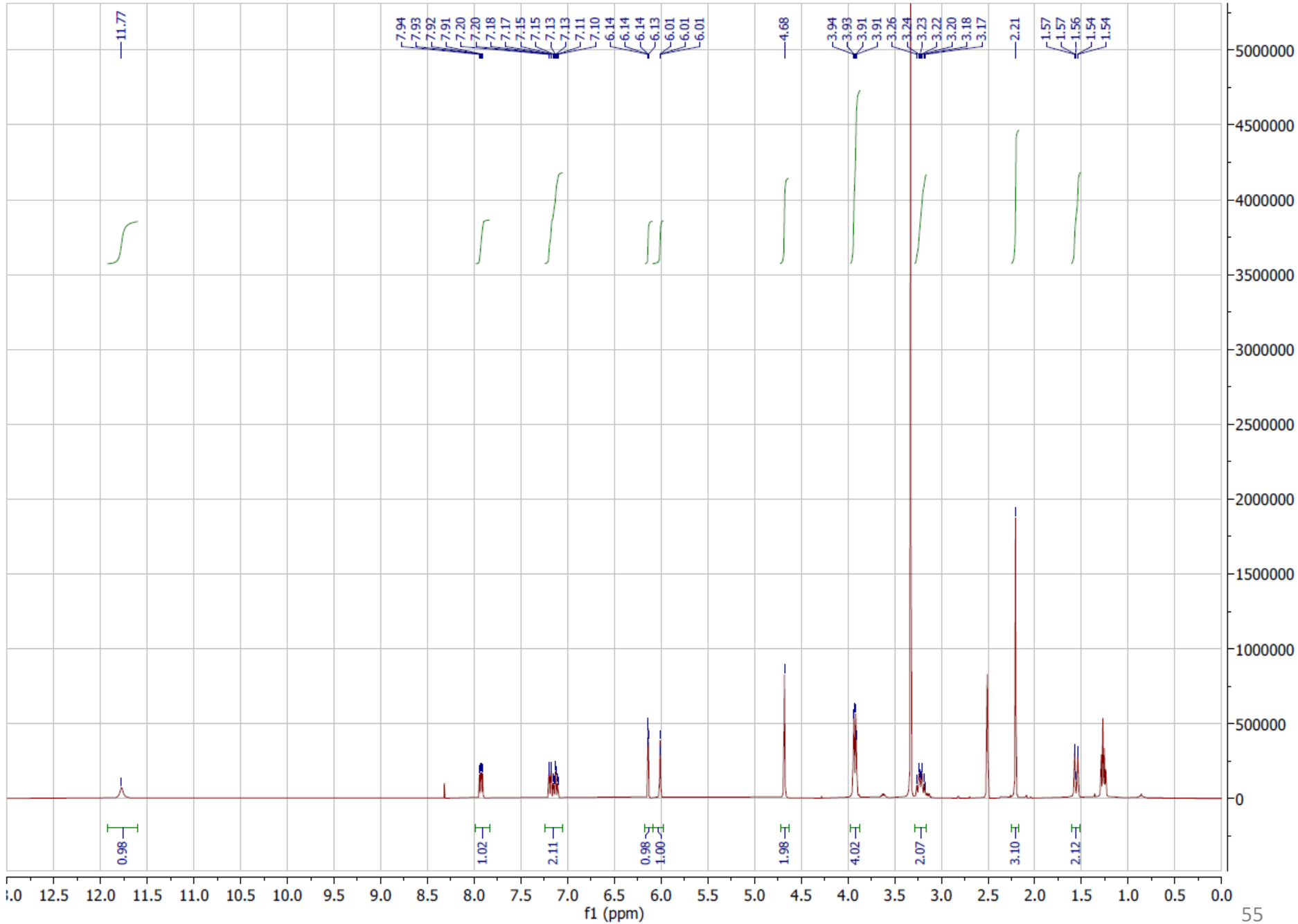
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


O
O
N
F
O
NH

3b


11.77
7.94
7.93
7.92
7.91
7.20
7.20
7.18
7.17
7.15
7.15
7.13
7.13
7.11
7.10
6.14
6.14
6.13
6.01
6.01
6.01
4.68
3.94
3.93
3.91
3.91
3.26
3.24
3.23
3.22
3.20
3.18
3.17
2.21
1.57
1.57
1.56
1.54
1.54
0.98
1.02
2.11
0.98
1.00
1.98
4.02
2.07
3.10
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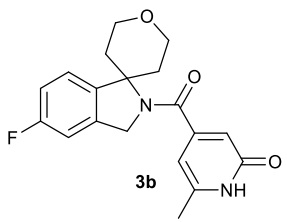
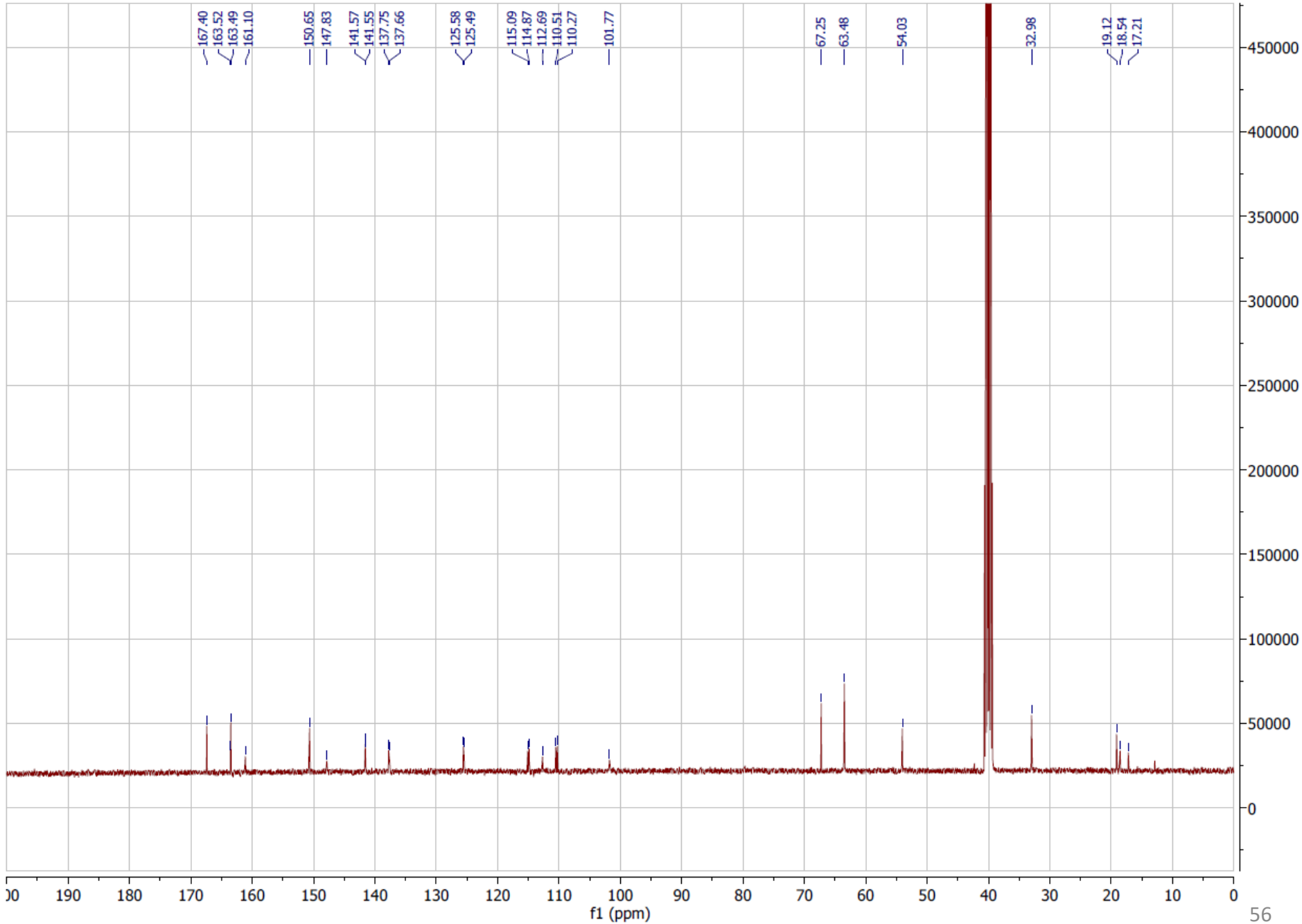
2000000
1500000
1000000
500000
0
f1 (ppm)


O
O
N
F
O
NH

3b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147.83
141.57
141.55
137.75
137.66
125.58
125.49
115.09
114.87
112.69
110.51
110.27
101.77
67.25
63.48
54.03
32.98
19.12
18.54
17.21
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
190
180
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120
110
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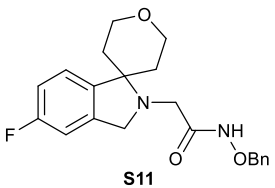
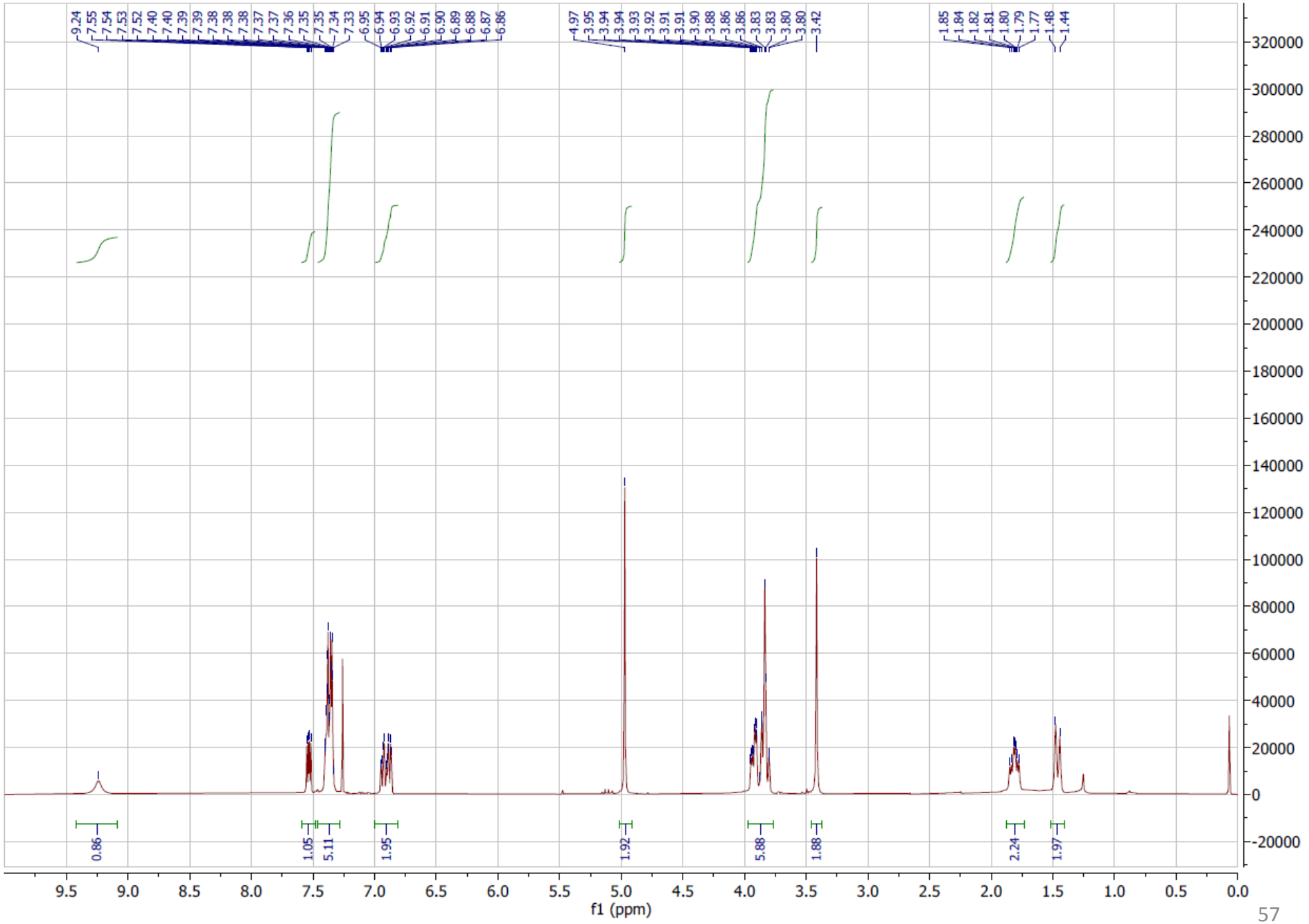
50
40
30
20
10
0
f1 (ppm)


O
N
F
NH
O
OBn

S11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7.53
7.52
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7.39
7.39
7.38
7.38
7.38
7.37
7.37
7.36
7.35
7.35
7.34
7.33
6.95
6.94
6.93
6.92
6.91
6.90
6.89
6.88
6.87
6.86
4.97
3.95
3.94
3.94
3.93
3.92
3.91
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3.90
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3.86
3.86
3.83
3.83
3.80
3.80
3.42
1.85
1.84
1.82
1.81
1.80
1.79
1.77
1.48
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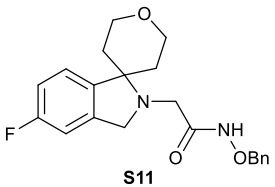
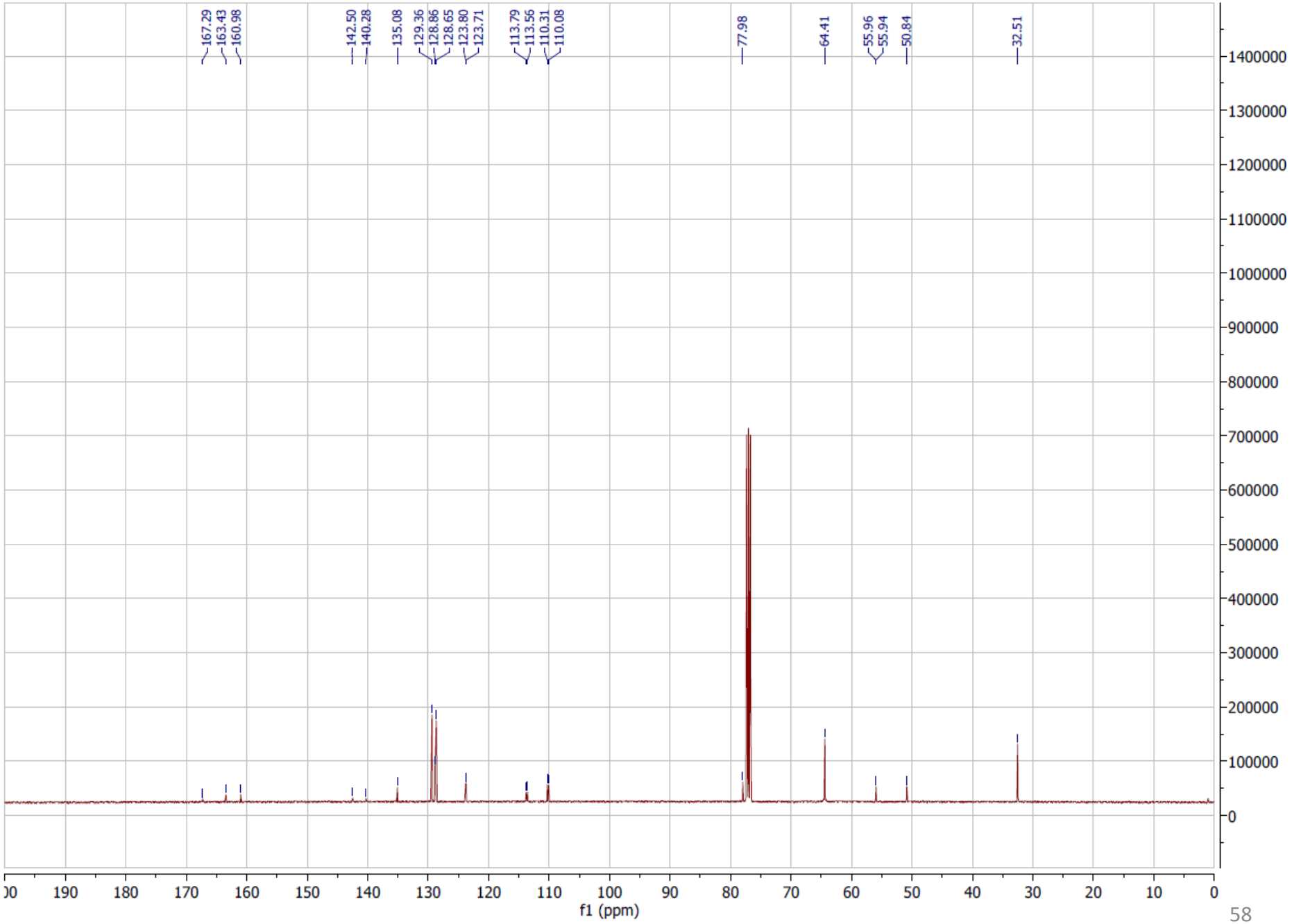
1.05
5.11
1.95
1.92
5.88
1.88
2.24
1.97
f1 (ppm)


O
N
F
NH
O
OBn

S11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128.65
123.80
123.71
113.79
113.56
110.31
110.08
77.98
64.41
55.96
55.94
50.84
32.51
1400000
1300000
1200000
1100000
1000000
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
190
180
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160
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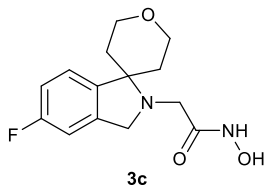
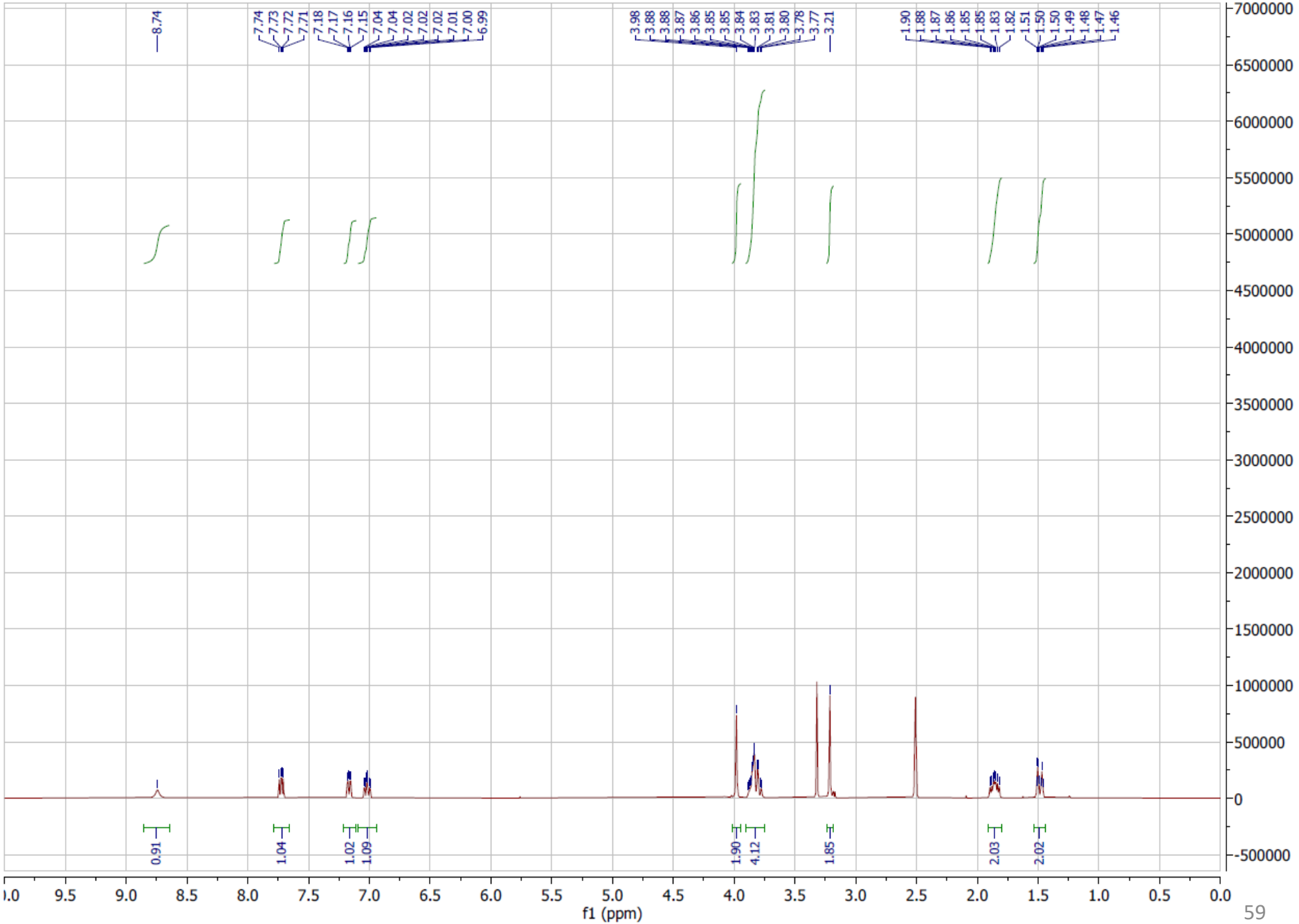
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


O
N
F
NH
O
OH

3c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7.72
7.71
7.18
7.17
7.16
7.15
7.04
7.04
7.02
7.02
7.02
7.01
7.00
6.99
3.98
3.88
3.88
3.87
3.86
3.85
3.85
3.84
3.83
3.81
3.80
3.78
3.77
3.21
1.90
1.88
1.87
1.86
1.85
1.85
1.83
1.82
1.51
1.50
1.50
1.49
1.48
1.47
1.46
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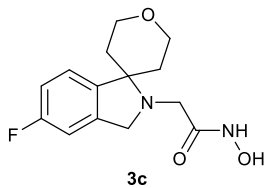
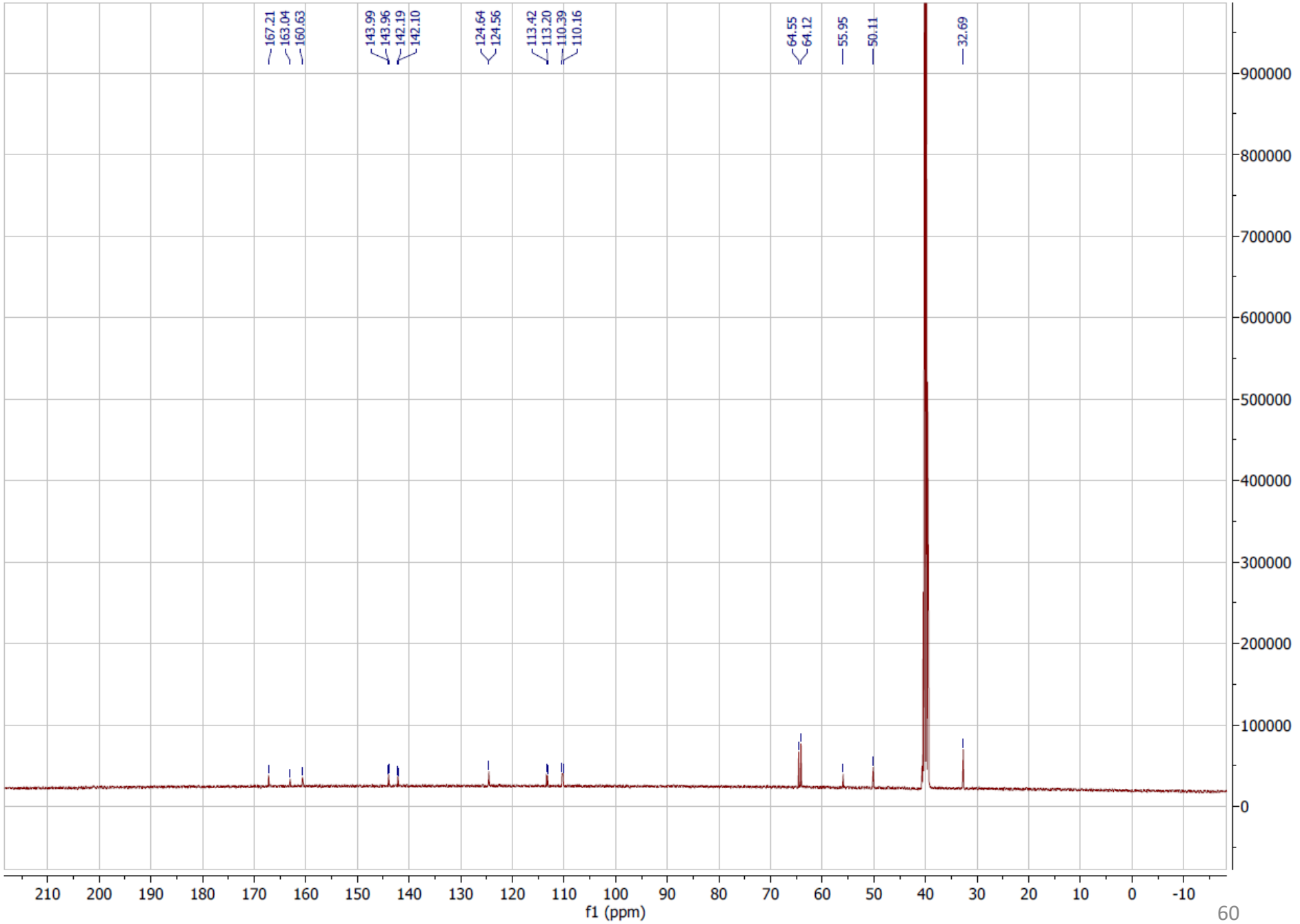
1.04
1.02
1.09
1.90
4.12
1.85
2.03
2.02
f1 (ppm)


O
N
F
NH
O
OH

3c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142.10
124.64
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113.42
113.20
110.39
110.16
64.55
64.12
55.95
50.11
32.69
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)