A mechanistic model for colibactin-induced genotoxicity.

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Supporting Information

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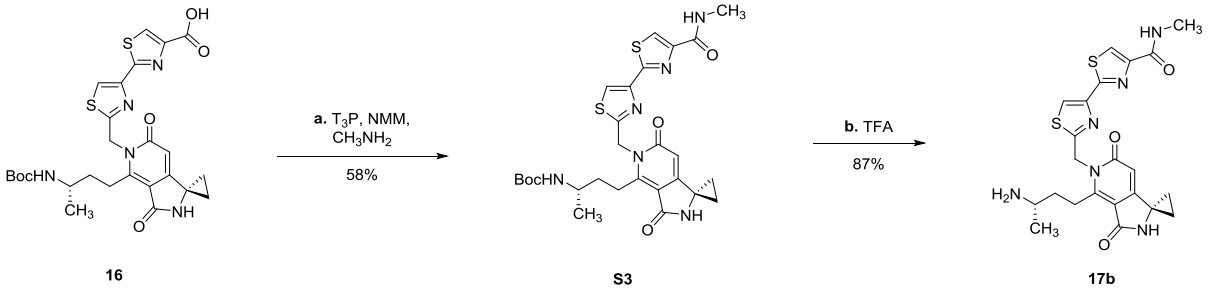
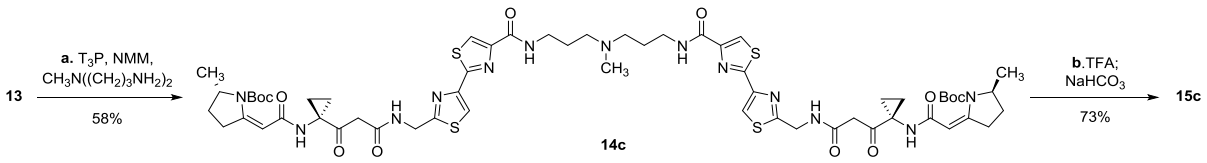
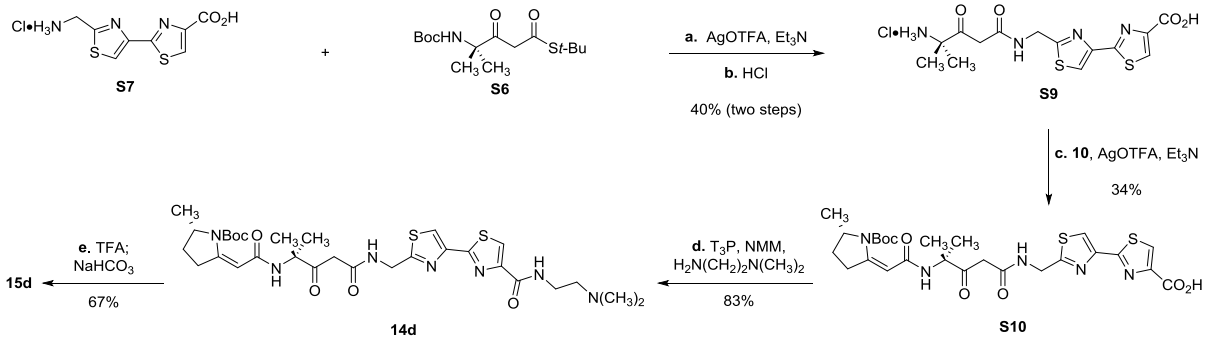


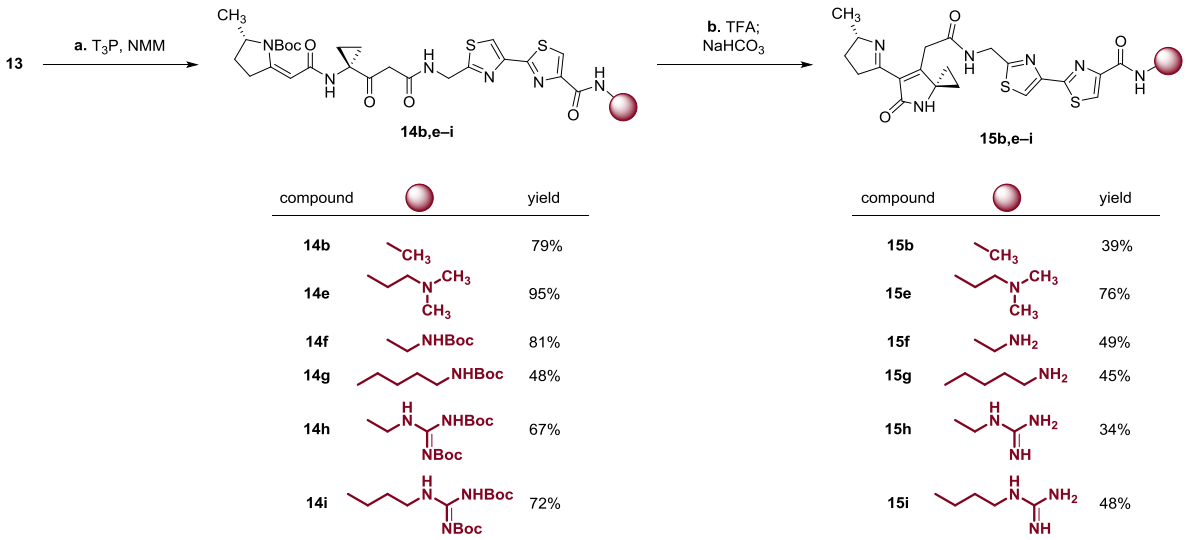
Scheme S1. Synthesis of the pyridone derivative **17b**. Reagents and conditions: (a) propylphosphonic anhydride solution (T3P), *N*-methylmorpholine, methylamine, THF, 23 °C, 58%; (b) trifluoroacetic acid (TFA), CH_2Cl_2, 0 °C, 87%.



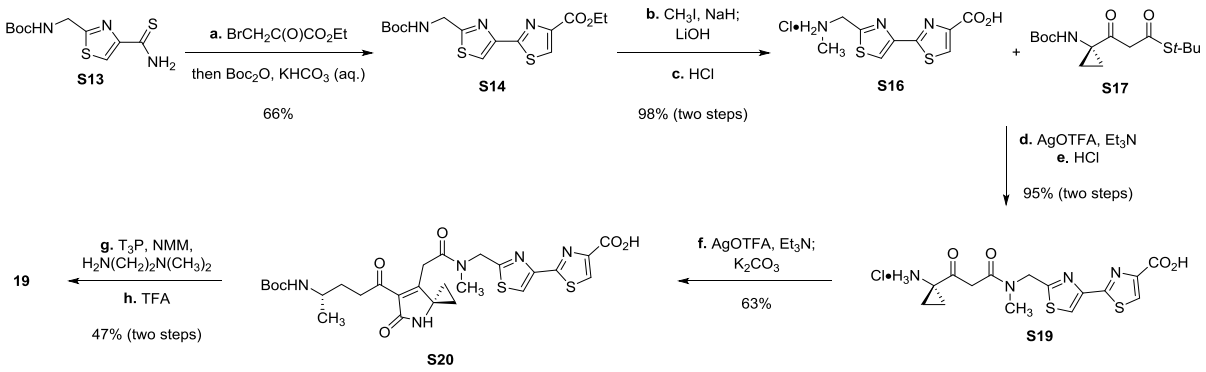
Scheme S2. Synthesis of the dimeric unsaturated imine **15c**. Reagents and conditions: (a) propylphosphonic anhydride solution (T3P), *N*-methylmorpholine, *N*,*N*-bis(3-aminopropyl)methylamine, THF, 23 °C, 58%; (b) trifluoroacetic acid (TFA), CH_2Cl_2, 0 °C, then aqueous $NaHCO_3$, 23 °C, 73%.



Scheme S3. Synthesis of the unsaturated imine **15d**. Reagents and conditions: (a) silver trifluoroacetate (AgOTFA), Et_3N, DMF, 0 °C, 40%; (b) HCl, CH_2Cl_2–1,4-dioxane (1:1), 0→23 °C, >99%; (c) **10**, AgOTFA, Et_3N, DMF, 0 °C, 34%; (d) propylphosphonic anhydride solution (T3P), *N*-methylmorpholine, *N*,*N*-dimethylethylenediamine, THF, 23 °C, 83%; (e) trifluoroacetic acid (TFA), CH_2Cl_2, 0 °C, then aqueous $NaHCO_3$, 23 °C, 67%.



Scheme S4. Synthesis of the unsaturated imines **15b**, **15e**–**i**. Reagents and conditions: (a) methylamine, *N*,*N*-dimethyl-1,3-diaminopropane, *N*-(*tert*-butoxycarbonyl)-1,2-diaminoethane, *N*-(*tert*-butoxycarbonyl)-1,5-diaminopentane, *N*,*N*′-bis-(*tert*-butoxycarbonyl)-*N*″-(2-aminoethyl)-guanidine (**S11**), or *N*,*N*′-bis-(*tert*-butoxycarbonyl)-*N*″-(4-aminobutyl)-guanidine (**S12**), propylphosphonic anhydride solution (T3P), *N*-methylmorpholine, THF, 23 °C; 79% (**14b**), 95% (**14e**), 81% (**14f**), 48% (**14g**), 67% (**14h**), 72% (**14i**); (b) trifluoroacetic acid (TFA), CH_2Cl_2, 0 °C, then aqueous $NaHCO_3$, 23 °C; 39% (**15b**), 76% (**15e**), 49% (**15f**), 45% (**15g**), 34% (**15h**), 48% (**15i**).



Scheme S5. Synthesis of the unsaturated imine **19**. Reagents and conditions: (a) ethyl bromopyruvate, *iso*-propanol, 83 °C, then di-*tert*-butyl dicarbonate, aqueous $KHCO_3$, 1,4-dioxane, 0 °C, 66%; (b) NaH, iodomethane, DMF, –5→15 °C, then LiOH, H_2O, 15 °C, 98%; (c) HCl, CH_2Cl_2–1,4-dioxane (3:1), 23 °C, >99%; (d) AgOTFA, Et_3N, DMF, 0 °C, 95%; (e) HCl, CH_2Cl_2–1,4-dioxane (3:1), 23 °C, >99%; (f) AgOTFA, Et_3N, DMF, 0 °C, then K_2CO_3, CH_3OH, 0→23 °C, 63% (g) propylphosphonic anhydride solution (T3P), *N*-methylmorpholine, *N*,*N*-dimethylethylenediamine, THF, 23 °C, 58%; (b) trifluoroacetic acid (TFA), CH_2Cl_2, 0 °C, 81%.

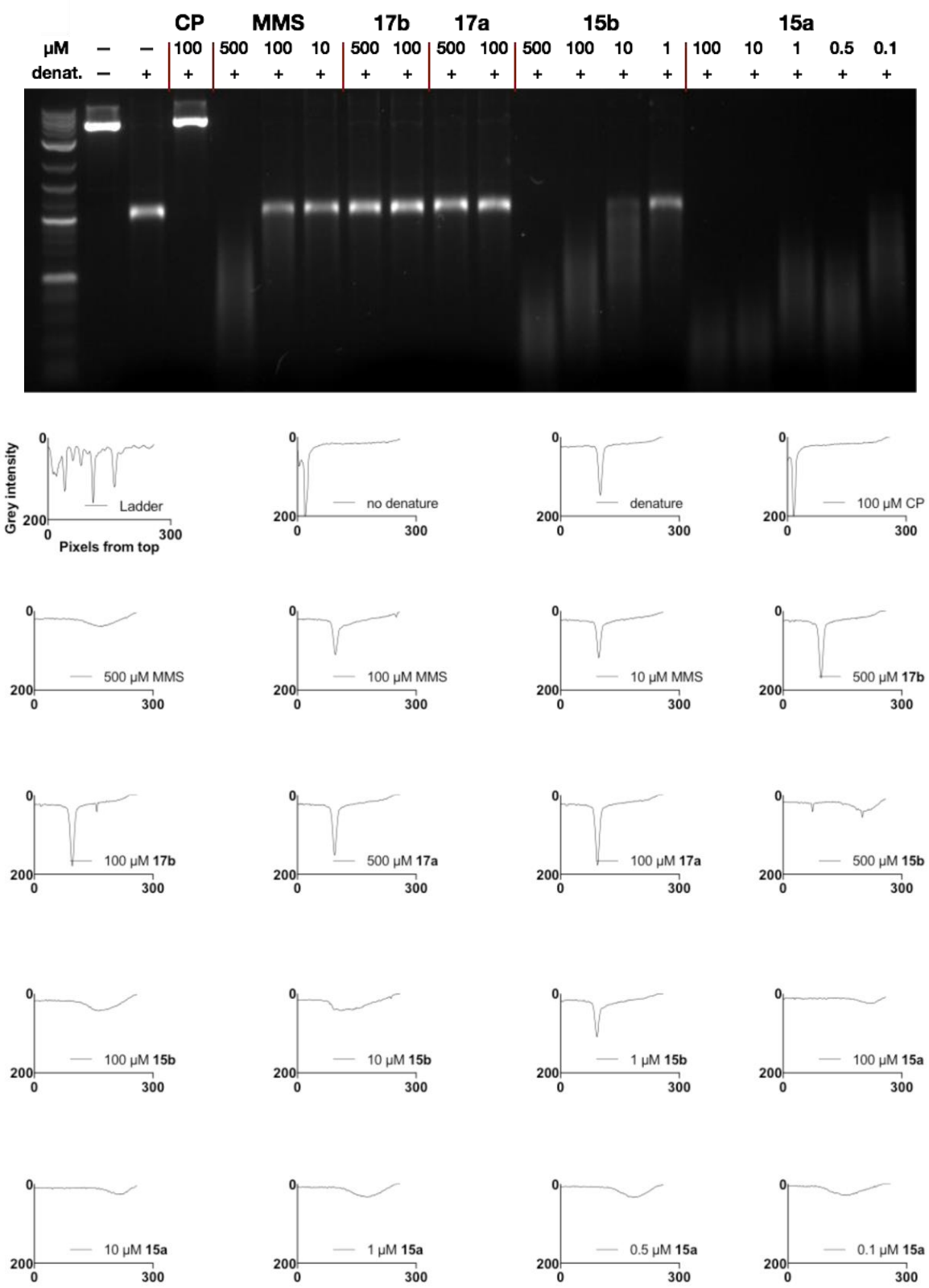


Figure S1. Unaltered image (top) and grayscale values (bottom) of the DNA agarose electrophoresis gel shown in Figure 2A of the manuscript, stained with SybrGold. 0 pixels on the x-axis of the graphs corresponds to the top edge of the gel. Generated using ImageJ.

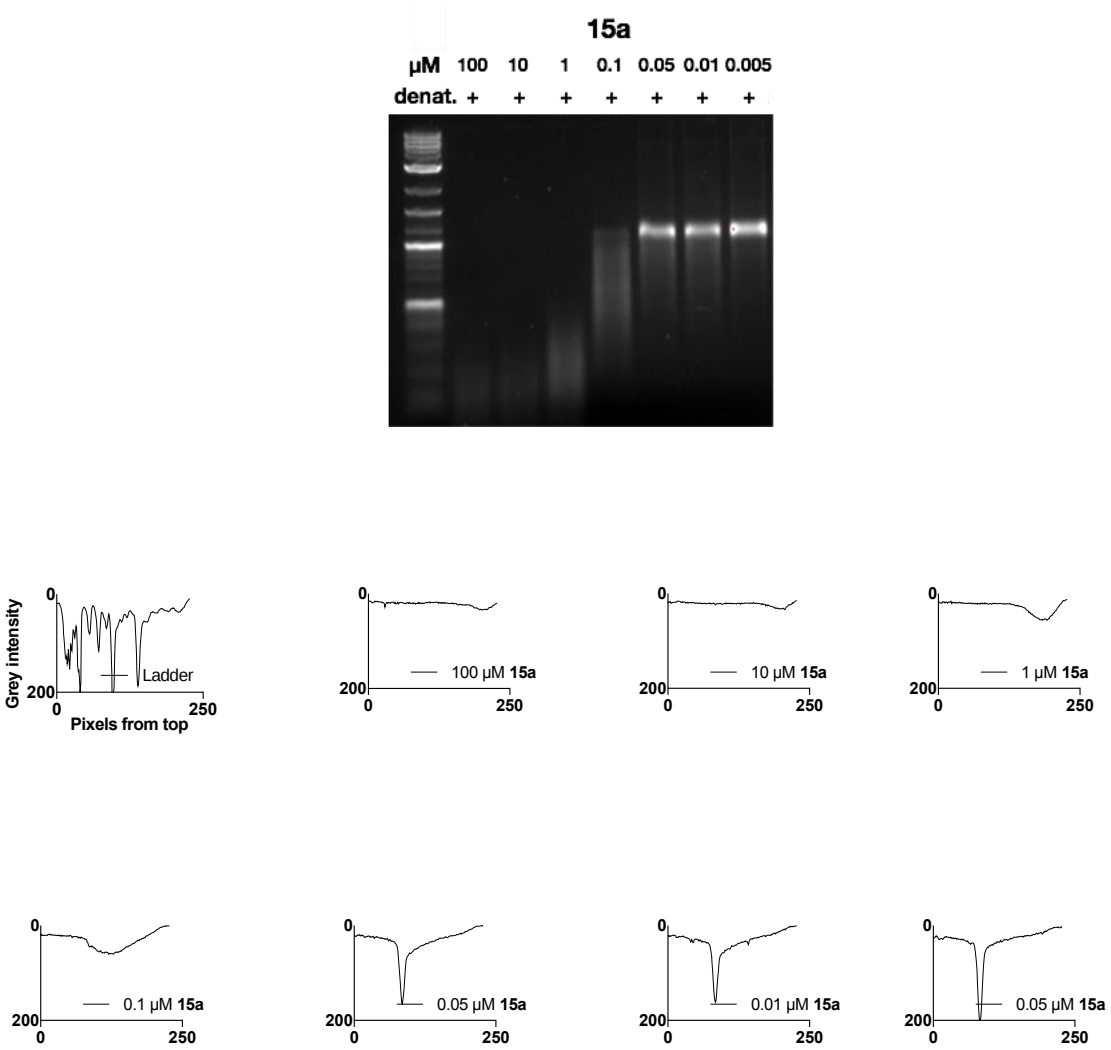


Figure S2. Unaltered image (top) and grayscale values (bottom) of the DNA agarose electrophoresis gel shown in Figure 2B of the manuscript, stained with SybrGold. 0 pixels on the x-axis of the graphs corresponds to the top edge of the gel. Generated using ImageJ.

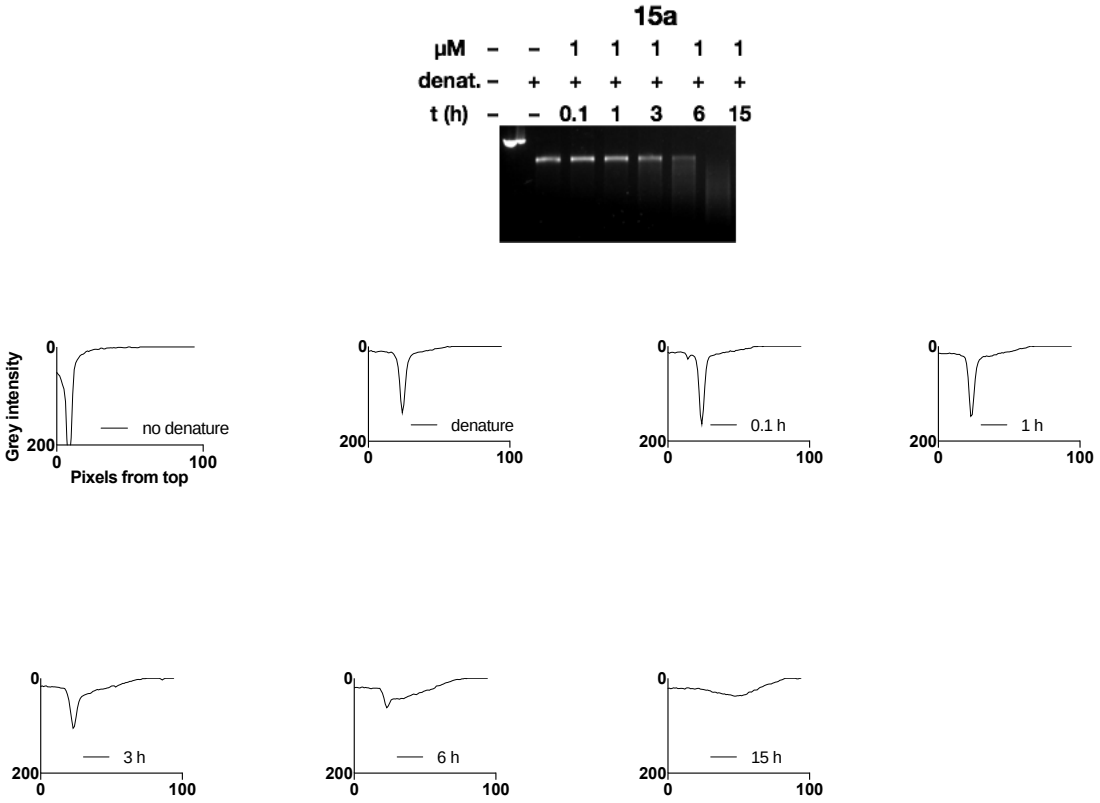


Figure S3. Unaltered image (top) and grayscale values (bottom) of the DNA agarose electrophoresis gel shown in Figure 3C of the manuscript, stained with SybrGold. 0 pixels on the x-axis of the graphs corresponds to the top edge of the gel. Generated using ImageJ.

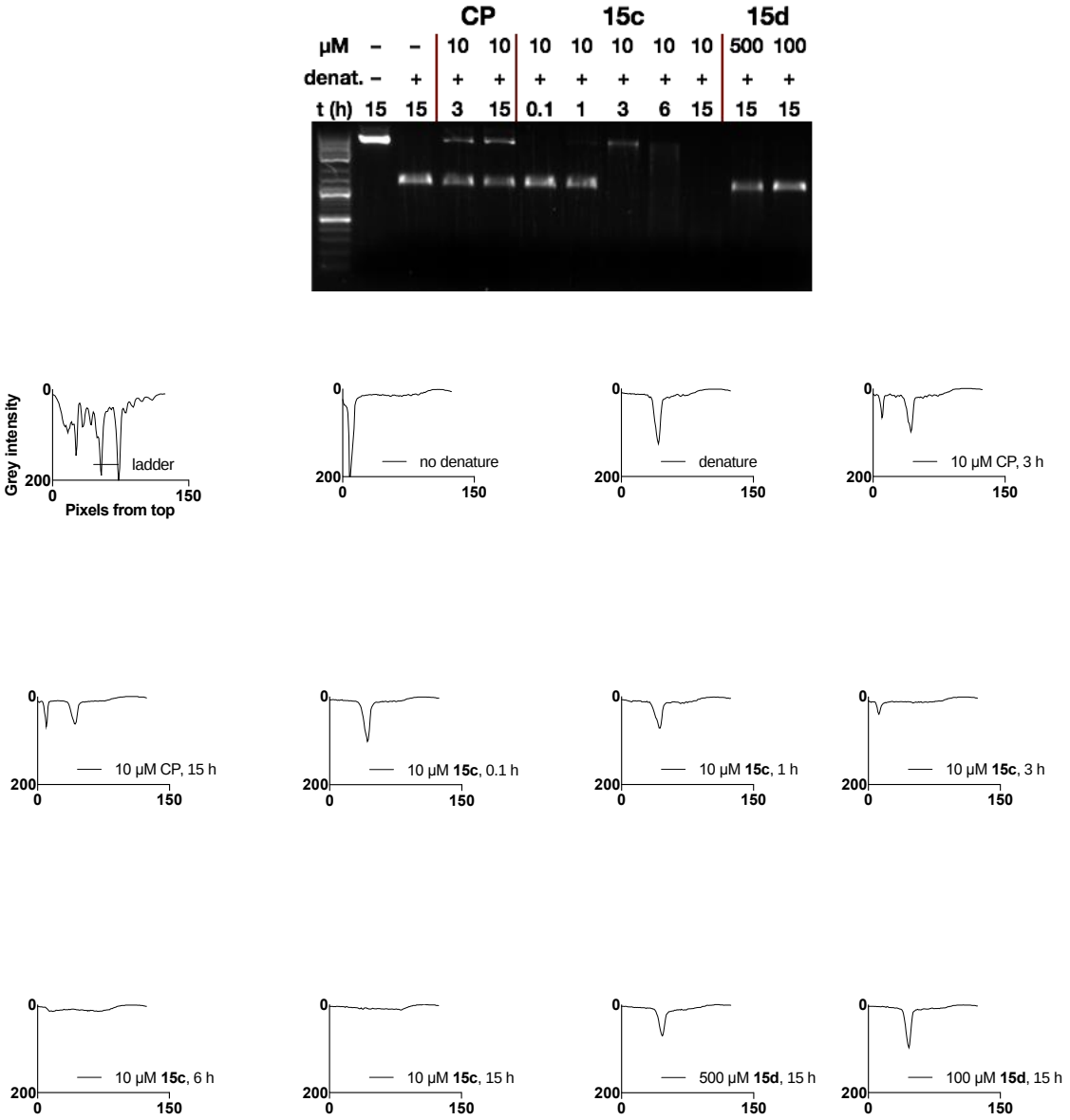


Figure S4. Unaltered image (top) and grayscale values (bottom) of the DNA agarose electrophoresis gel shown in Figure 4B of the manuscript, stained with SybrGold. 0 pixels on the x-axis of the graphs corresponds to the top edge of the gel. Generated using ImageJ.

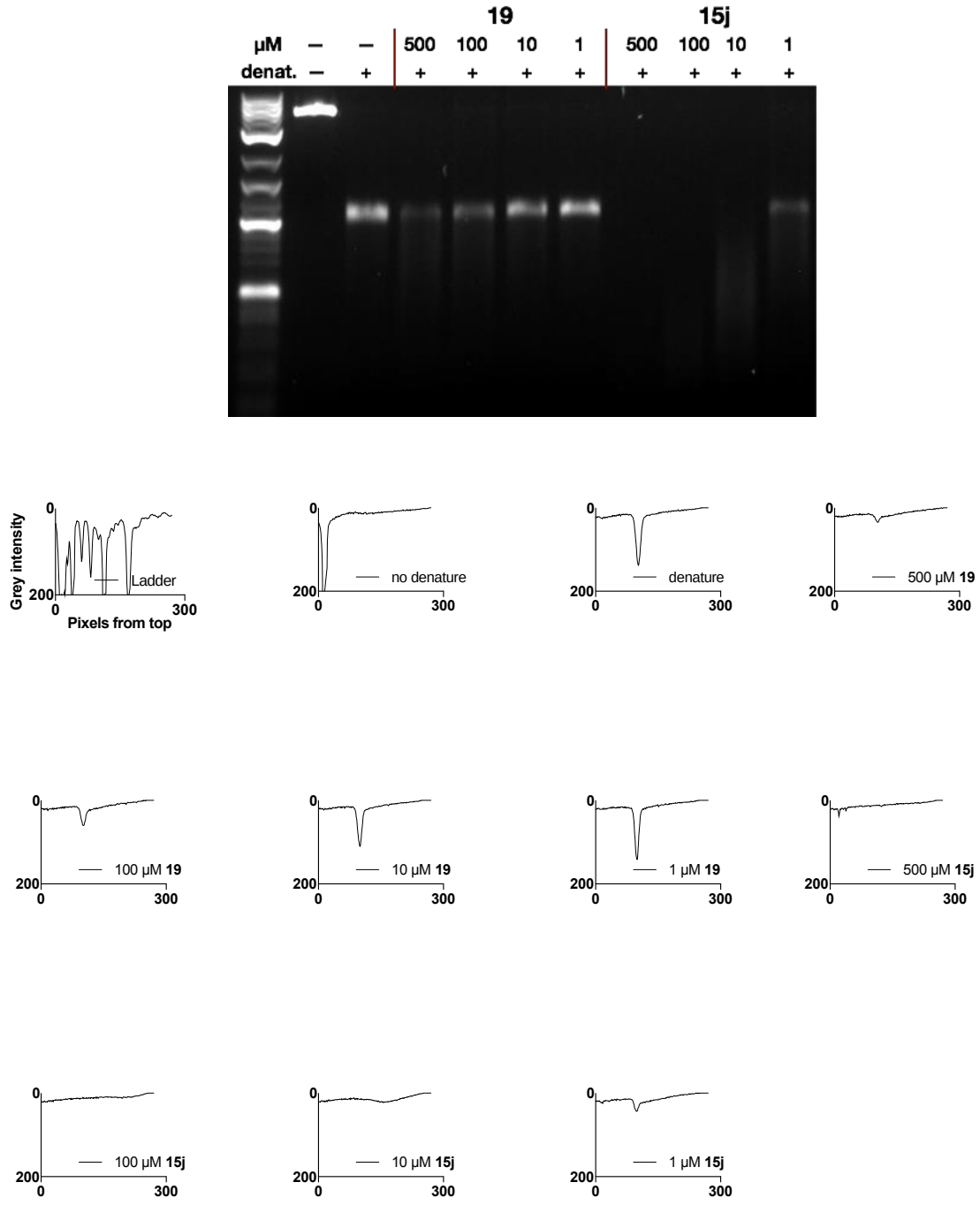


Figure S5. Unaltered image (top) and grayscale values (bottom) of the DNA agarose electrophoresis gel shown in Figure 5 of the manuscript, stained with SybrGold. 0 pixels on the x-axis of the graphs corresponds to the top edge of the gel. Generated using ImageJ.

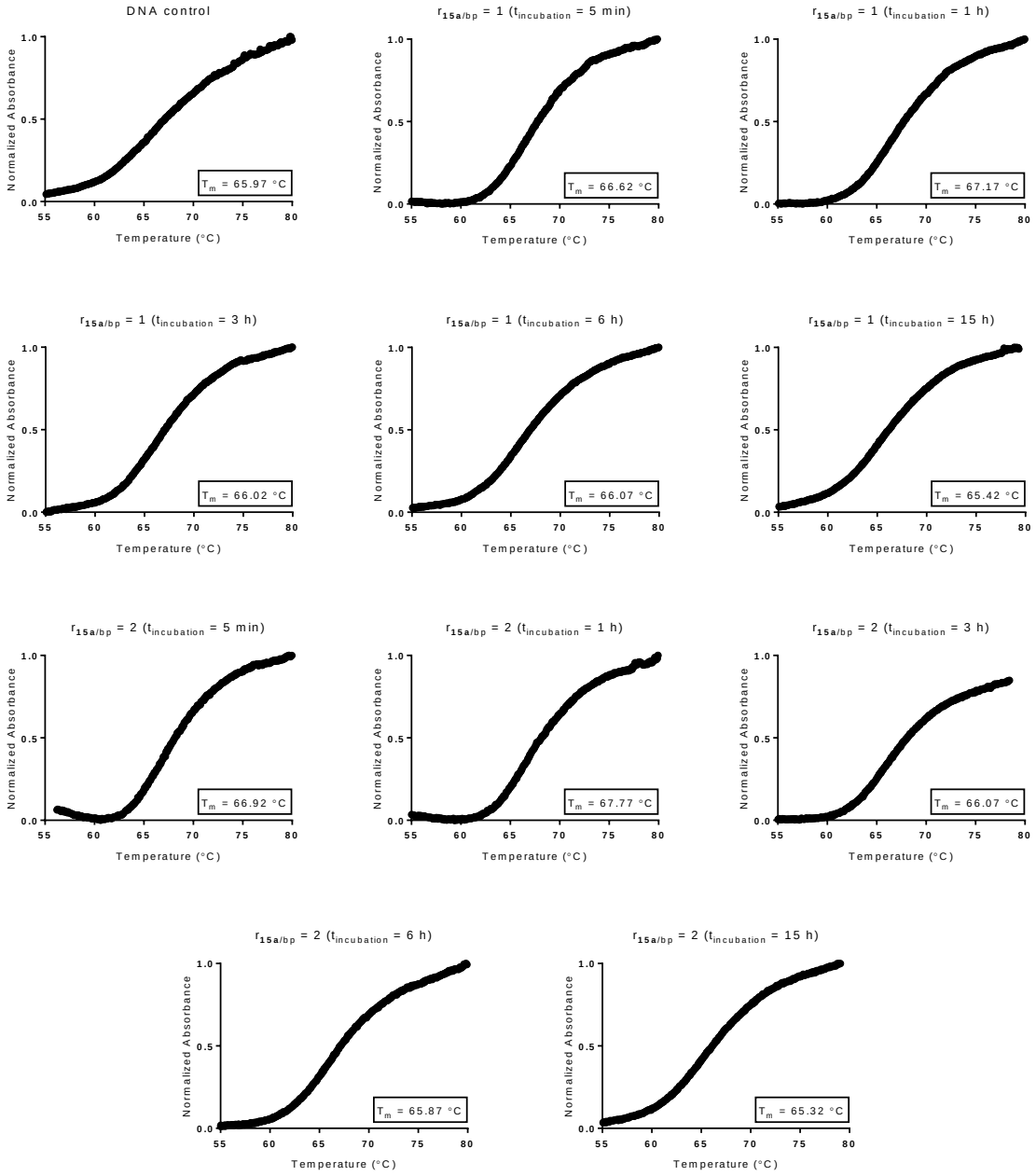


Figure S6. Time-dependent modulation of the melting temperature of calf thymus DNA treated with 1 or 2 bp equiv of the imine **15a**. Conditions: 2.09 mM NaH_2PO_4, 7.13 mM Na_2HPO_4, 928 μM Na_2EDTA, 1.01 mM DMSO, pH 7.18. The imine **15a** was incubated with ctDNA for 5 min, 1 h, 3 h, 6 h, or 15 h prior to UV thermal denaturation experiments (260 nm, heating rate: 0.5 °C/min). [DNA] = 32.0 mM bps. The T_m was defined as the temperature at which half of the duplex DNA was unwound, and was determined by the maximum of the first derivative of the thermal denaturation profile.

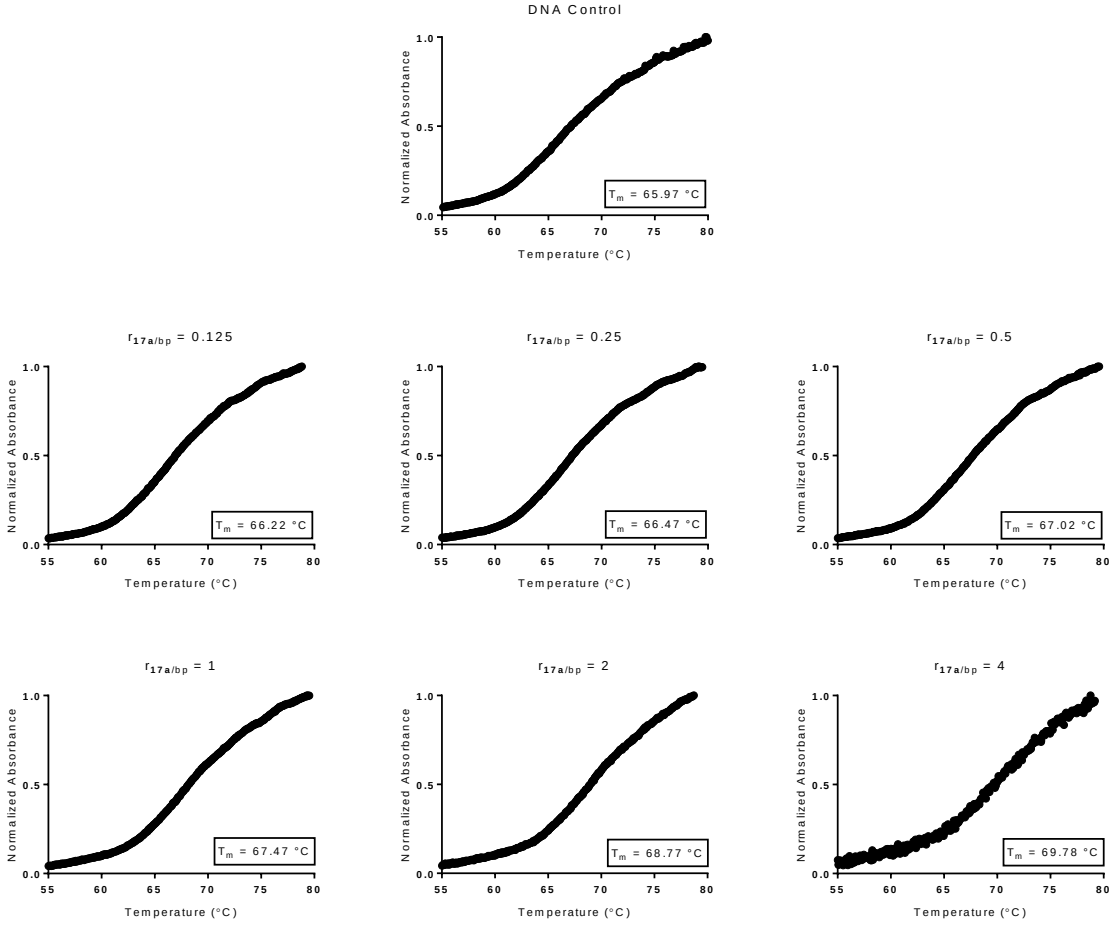


Figure S7. Increase in the melting temperature of calf thymus DNA after treatment with increasing amounts of the pyridone **17a**. Conditions: 2.09 mM NaH_2PO_4, 7.13 mM Na_2HPO_4, 928 μM Na_2EDTA, 1.01 mM DMSO, pH 7.18. The pyridone **17a** was incubated with ctDNA for 3 h prior to UV thermal denaturation experiments (260 nm, heating rate: 0.5 °C/min). [DNA] = 32.0 mM bps. The T_m was defined as the temperature at which half of the duplex DNA was unwound, and was determined by the maximum of the first derivative of the thermal denaturation profile.

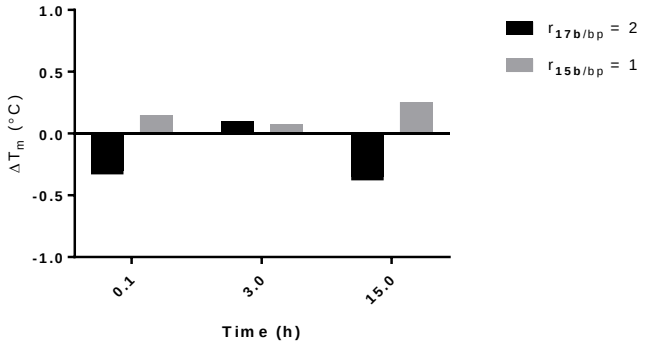


Figure S8. No significant modulation of the melting temperature of calf thymus DNA was observed on treatment with 2 bp equiv of the pyridone **17b** or 1 bp equiv of the imine **15b**. Conditions: 2.09 mM NaH_2PO_4, 7.13 mM Na_2HPO_4, 928 μM Na_2EDTA, 1.01 mM DMSO, pH 7.18. The pyridone **17b** and the imine **15b** were incubated with ctDNA for 5 min, 3 h, or 15 h prior to UV thermal denaturation experiments (260 nm, heating rate: 0.5 °C/min). [DNA] = 32.0 mM bps.

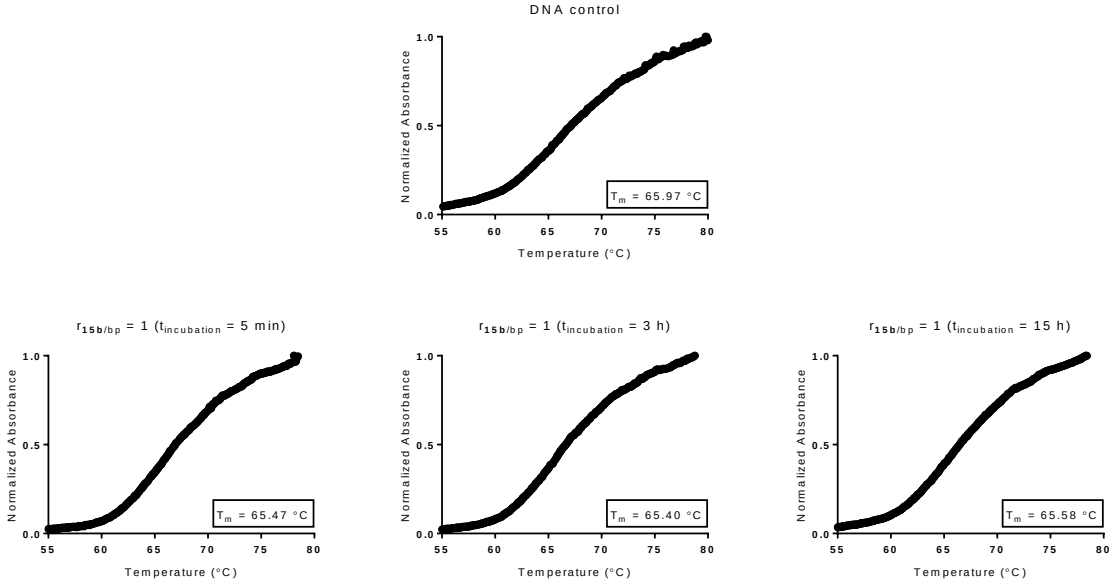


Figure S9. No significant modulation of the melting temperature of calf thymus DNA was observed on treatment with 2 bp equiv of the unsaturated imine **15b**. Conditions: 2.09 mM NaH_2PO_4, 7.13 mM Na_2HPO_4, 928 μM Na_2EDTA, 1.01 mM DMSO, pH 7.18. The imine **15b** was incubated with ctDNA for 5 min, 1 h, 3 h, 6 h, or 15 h prior to UV thermal denaturation experiments (260 nm, heating rate: 0.5 °C/min). [DNA] = 32.0 mM bps. The T_m was defined as the temperature at which half of the duplex DNA was unwound, and was determined by the maximum of the first derivative of the thermal denaturation profile.

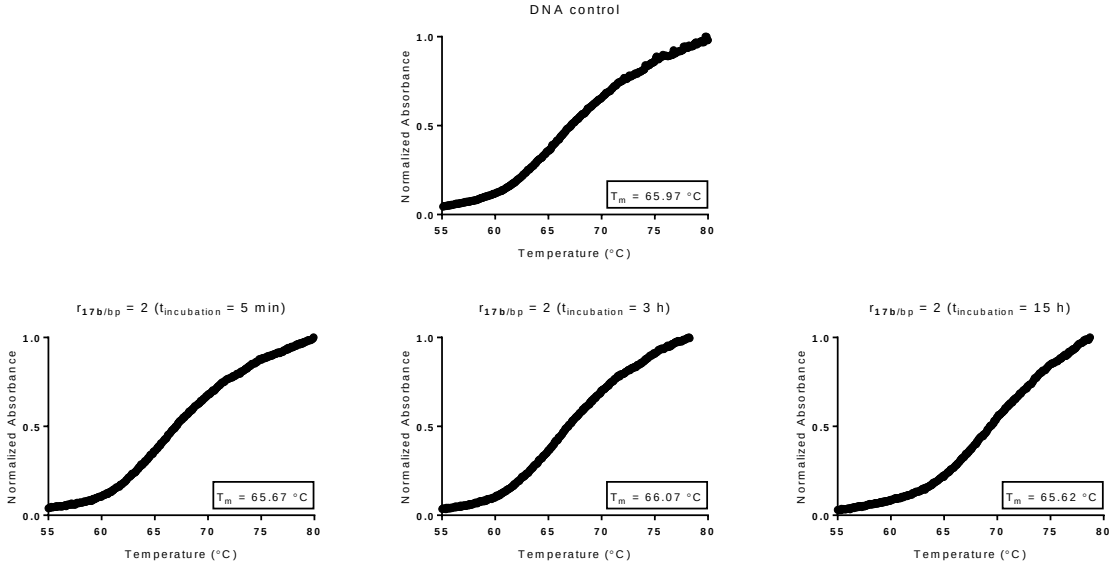


Figure S10. No significant modulation of the melting temperature of calf thymus DNA was observed on treatment with 2 bp equiv of the pyridone **17b**. Conditions: 2.09 mM NaH_2PO_4, 7.13 mM Na_2HPO_4, 928 μM Na_2EDTA, 1.01 mM DMSO, pH 7.18. The pyridone **17b** was incubated with ctDNA for 5 min, 1 h, 3 h, 6 h, or 15 h prior to UV thermal denaturation experiments (260 nm, heating rate: 0.5 °C/min). [DNA] = 32.0 mM bps. The T_m was defined as the temperature at which half of the duplex DNA was unwound, and was determined by the maximum of the first derivative of the thermal denaturation profile.

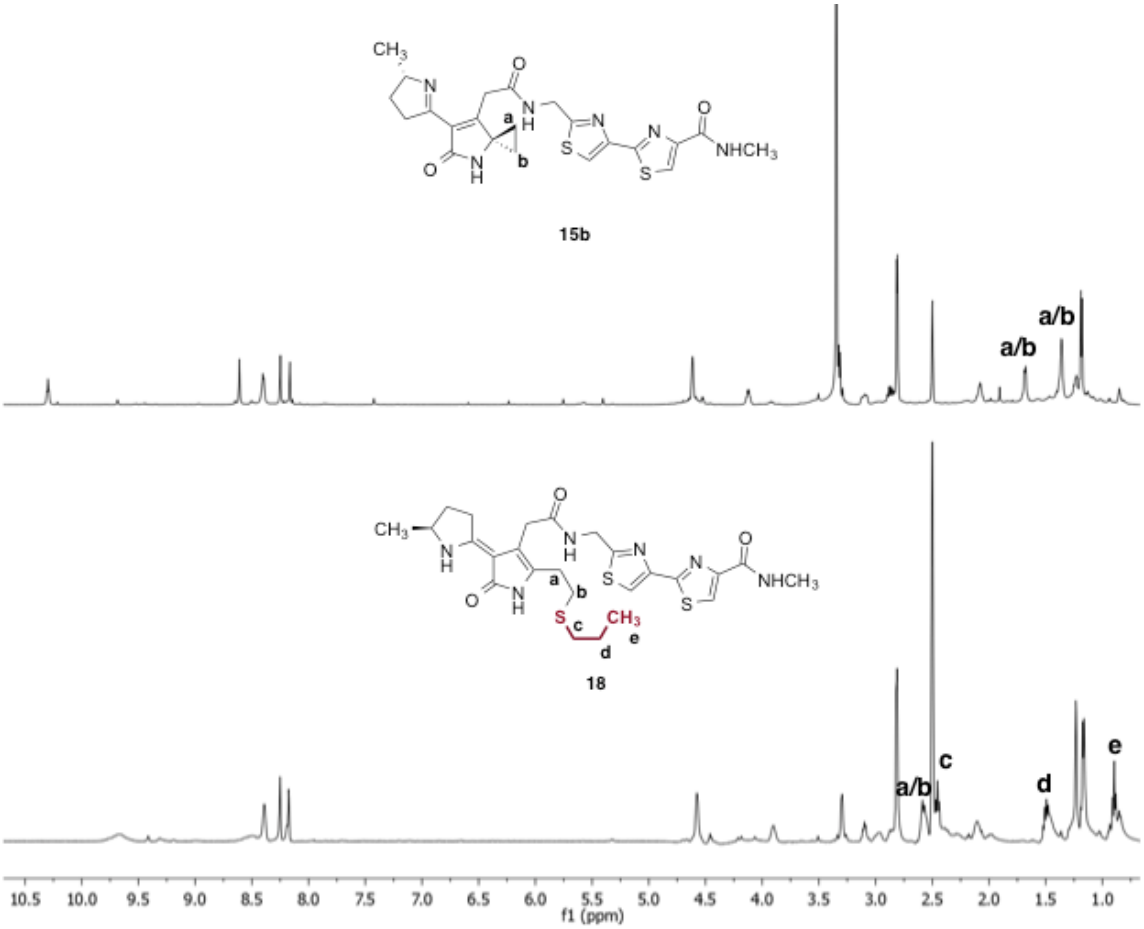


Figure S11. A comparison of the ^{1}H NMR of the unsaturated imine **15b** (top) and the propanethiol adduct product **18** (bottom). ^{1}H spectroscopic data were recorded in DMSO-d_6 (600 MHz (**15b**), 500 MHz (**18**), 23 °C).

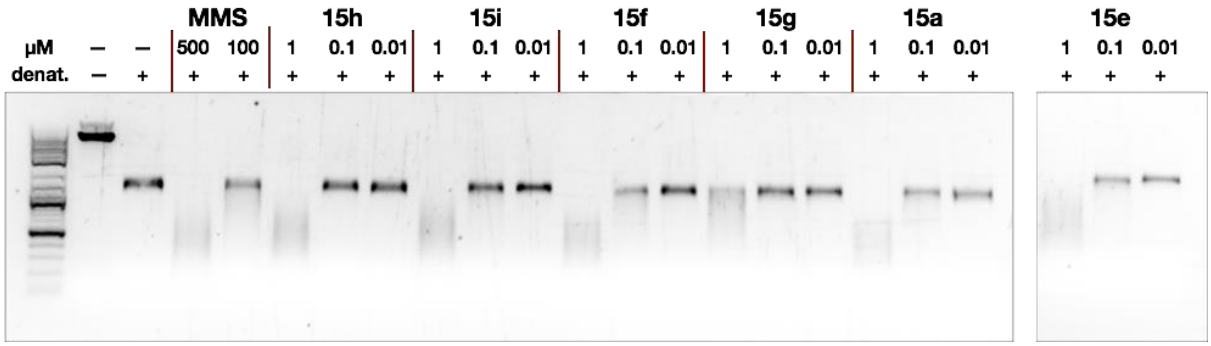
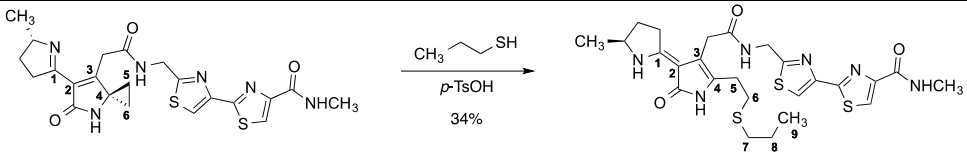


Figure S12. DNA alkylation assay employing linearized pBR322 DNA and the derivatives **15a** and **15e–i** to probe the influence of the cationic residue on DNA alkylation activity. Conditions: Linearized pBR322 DNA (20 μM in base pairs), **15h** (1, 0.1, or 0.01 μM), **15i** (1, 0.1, or 0.01 μM), **15f** (1, 0.1, or 0.01 μM), **15g** (1, 0.1, or 0.01 μM), **15a** (1, 0.1, or 0.01 μM), **15e** (1, 0.1, or 0.01 μM), 37 °C, 15 h. Methyl methanesulfonate (MMS; 500 or 100 μM) was used as a positive control for DNA alkylation. DNA was visualized using SybrGold.

Table S1. Comparison of selected ^{1}H and ^{13}C NMR Data of **15b** and **18**.



15b **18**

Position	δ_H (**15b**)[a]	δ_H (**18**)[b]	δ_C (**15b**)[a]	δ_C (**18**)[b]
1	—	—	168.4	160.8
2	—	—	127.4	96.1
3	—	—	157.7	104.3
4	—	—	45.3	125.8
5	1.31−1.42 (m) 1.62−1.73 (m)	2.61−2.57 (m)	11.7	30.4
6	1.31−1.42 (m) 1.62−1.73 (m)	2.61−2.57 (m)	11.7	25.7
7	—	2.45 (t, 7.3)	—	33.1
8	—	1.44−1.56 (m)	—	22.5
9	—	0.90 (t, 7.4)	—	13.3

[a]NMR spectra were obtained in DMSO-d_6 at 600 MHz for ^{1}H and 150 MHz for ^{13}C. [b]NMR spectra were obtained in DMSO-d_6 at 500 MHz for ^{1}H and 125 MHz for ^{13}C.

General Experimental Methods.

UV Spectroscopy. UV thermal denaturation samples were prepared by mixing calf thymus DNA [32.0 mM base pairs (bps)] in 2.09 mM NaH_2PO_4, 7.13 mM Na_2HPO_4, 928 μM Na_2EDTA, 1.01 mM DMSO, pH 7.18 to a final volume of 1.0 mL. Samples were subjected to sonication (6 h) at 25 ºC to effect complete dissolution. After incubation with **15a**, **15b**, **17a**, and **17b** for 5 min, 1 h, 3 h, 6 h, or 15 h, the UV thermal denaturation spectra of the samples were recorded at 260 nm as a function of temperature (55→80 °C, heating rate: 0.5 °C/min). First derivative plots were used to determine the denaturation temperature.

Electrophoretic gel assay. The 4,163 bp plasmid pBR322 was propogated in DH5a, isolated by MaxiPrep (Qiagen), and linearized with 5U/μg EcoRI (NEB). The cut plasmid was column purified and eluted into 10 mM Tris pH 8.0. For each reaction, 130 ng of DNA (20 μM base pairs) was incubated with compound in a 10 μL total volume. Reactions proceeded for 15 h at 37 ºC, unless otherwise noted. Compounds were diluted in DMSO such that each reaction consisted of a fixed 5% DMSO concentration. Pure MMS (Alfa Aesar) and cisplatin (Biovision) stock solutions were diluted into DMSO immediately prior to use. After incubation, 35 μL of denaturation buffer (6% sucrose, 1% sodium hydroxide, 0.04% bromophenol blue) was added to each reaction. Non-denatured control samples were diluted with 6% sucrose, 0.04% bromophenol blue. Samples were vortexed for 1 min, left at room temperature for 15 min, and then immediately frozen at –80 ºC. Thawed samples were then loaded onto a 1% agarose Tris-Borate-EDTA (TBE) gel stained with SybrGold (Molecular Probes) and run in TBE buffer for 1 hour at 120V.

General Experimental Procedures. All reactions were performed in single-neck, flame-dried, round-bottomed flasks fitted with rubber septa under a positive pressure of nitrogen unless otherwise noted. Air- and moisture-sensitive liquids were transferred via syringe or stainless steel cannula, or were handled in a nitrogen-filled drybox (working oxygen level <10 ppm). Organic solutions were concentrated by rotary evaporation at 28–32 °C. Flash-column chromatography was performed as described by Still et al.,[1] employing silica gel (60 Å, 40–63 μm particle size) purchased from Sorbent Technologies (Atlanta, GA). Anion-exchange chromatography was performed as described by Béland et al.,[2] employing trimethylamine acetate-functionalized silica gel (SiliaBond® TMA Acetate). Analytical thin-layered chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25 mm, 60 Å pore size) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV).

Materials. Commercial solvents and reagents were used as received with the following exceptions. Dichloromethane, ether and *N*,*N*-dimethylformamide were purified according to the method of Pangborn et al.[3] Triethylamine was distilled from calcium hydride under an atmosphere of argon immediately before use. Di-*iso*-propylamine was distilled from calcium hydride and was stored under nitrogen. Methanol was distilled from magnesium turnings under an atmosphere of nitrogen immediately before use. Tetrahydrofuran was distilled from sodium–benzophenone under an atmosphere of nitrogen immediately before use. Deoxyribonucleic acid sodium salt from calf thymus (Type I, fibers) was purchased from

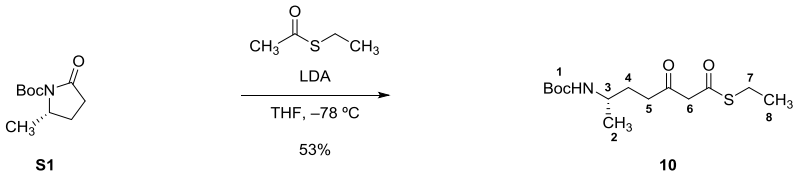
Sigma Aldrich. Trimethylamine acetate-functionalized silica gel (SiliaBond® TMA Acetate) was purchased from SiliCycle (Quebec City, CA). *tert*-butyl-(*S*)-2-methyl-5-oxopyrrolidine-1-carboxylate (**S1**),[4] 2'-((3-(1-aminocyclopropyl)-3-oxopropanamido)methyl)-[2,4'-bithiazole]-4-carboxylic acid hydrochloride (**11**),[5] 3-(*tert*-butylthio)-3-oxopropanoic acid (**S5**),[6] 2'-(aminomethyl)-[2,4'-bithiazole]-4-carboxylic acid hydrochloride (**S7**),[5] *N*,*N'*-bis-(*tert*-butoxycarbonyl)-*N''*-(2-aminoethyl)-guanidine (**S11**),[7] *N*,*N'*-bis-(*tert*-butoxycarbonyl)-*N''*-(4-aminobutyl)-guanidine (**S12**),[7] *tert*-butyl-((4-carbamothioylthiazol-2-yl)methyl)carbamate (**S13**),[5] and S-(*tert*-butyl)-3-(1-((*tert*-butoxycarbonyl)amino)cyclopropyl)-3-oxopropanethioate (**S17**)[5] were prepared according to published procedures.

Instrumentation. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 500 or 600 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent (CD_2Cl_2, δ 5.32; CD_3OD, δ 3.31; C_2D_6OS, δ 2.50). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances, br = broad, app = apparent), coupling constant in Hertz, integration, and assignment. Proton-decoupled carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded at 125 MHz at 24 °C, unless otherwise noted. Chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to the carbon resonances of the solvent (CD_2Cl_2, δ 54.0; CD_3OD, δ 49.0; C_2D_6OS, δ 39.5). Signals of protons and carbons were assigned, as far as possible, by using the following two dimensional NMR spectroscopy techniques: [^{1}H, ^{1}H] COSY (Correlation Spectroscopy), [^{1}H, ^{13}C] HSQC (Heteronuclear Single Quantum Coherence) and long range [^{1}H, ^{13}C] HMBC (Heteronuclear Multiple Bond Connectivity). Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra were obtained using a Thermo Electron Corporation Nicolet 6700 FTIR spectrometer referenced to a polystyrene standard. Data are represented as follows: frequency of absorption (cm^{-1}), intensity of absorption (s = strong, m = medium, w = weak, br = broad). Analytical ultra high-performance liquid chromatography/mass spectrometry (UPLC/MS) was performed on a Waters UPLC/MS instrument equipped with a reverse-phase C_{18} column (1.7 μm particle size, 2.1 × 50 mm), dual atmospheric pressure chemical ionization (API)/electrospray (ESI) mass spectrometry detector, and photodiode array detector. Samples were eluted with a linear gradient of 5% acetonitrile–water containing 0.1% formic acid→100% acetonitrile containing 0.1% formic acid over 0.75 min, followed by 100% acetonitrile containing 0.1% formic acid for 0.75 min, at a flow rate of 800 μL/min. High-resolution mass spectrometry (HRMS) were obtained on a Waters UPLC/HRMS instrument equipped with a dual API/ESI high-resolution mass spectrometry detector and photodiode array detector. Unless otherwise noted, samples were eluted over a reverse-phase C_{18} column (1.7 μm particle size, 2.1 × 50 mm) with a linear gradient of 5% acetonitrile–water containing 0.1% formic acid→95% acetonitrile–water containing 0.1% formic acid for 1 min, at a flow rate of 600 μL/min. Optical rotations were measured on a Perkin Elmer polarimeter equipped with a sodium (589 nm, D) lamp. Optical rotation data are represented as follows: specific rotation ($[\alpha]_\lambda^T$),

concentration (g/100 mL), and solvent. UV spectra were recorded on a Cary 3E UV/Vis spectrophotometer equipped with a thermoelectrically controlled 12-cell holder. High precision quartz SUPRASIL cells with a 1 cm path length were used for all absorbance studies.

Synthetic Procedures.

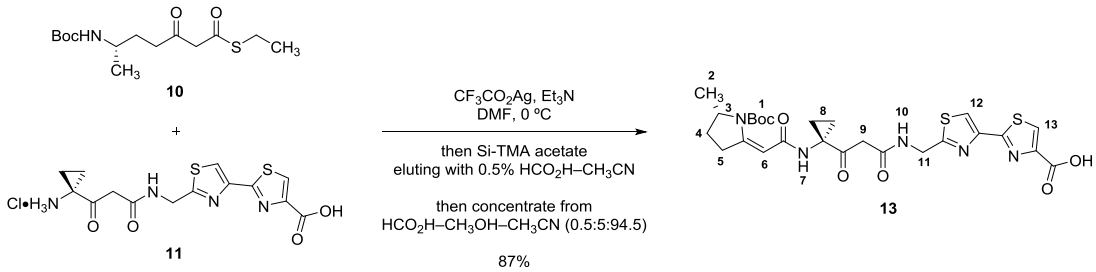
*Synthesis of the β-ketothioester **10**:*



Ethyl thioacetate (2.08 mL, 19.5 mmol, 1.30 equiv) was added dropwise via syringe to a solution of lithium di-*iso*-propylamide (19.5 mmol, 1.30 equiv) in tetrahydrofuran (75 mL) at –78 °C. The reaction mixture was stirred for 30 min at –78 °C. A solution of the imide **S1** (3.00 g, 15.1 mmol, 1 equiv) in tetrahydrofuran (28 mL) was added dropwise via cannula to the reaction mixture. The resulting mixture was stirred for 3 h at –78 °C. The product mixture was diluted sequentially with saturated aqueous ammonium chloride solution (30 mL) and ethyl acetate (50 mL). The diluted product mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (2 × 50 mL). The organic layers were combined and the combined organic layers were washed with saturated aqueous sodium chloride solution (30 mL). The washed organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue obtained was purified by flash-column chromatography (eluting with 5% ethyl acetate–hexanes initially, grading to 20% ethyl acetate–hexanes, linear gradient) to provide the β-ketothioester **10** as a light pink solid (2.40 g, 53%).

^{1}H NMR (500 MHz, CD_2Cl_2) δ 4.38 (bs, 1H), 3.66 (s, 2H, H_6), 3.58 (m, 1H, H_3), 2.90 (q, J = 6.9 Hz, 2H, H_7), 2.65 – 2.50 (m, 2H, H_5), 1.77 – 1.67 (m, 1H, H_4), 1.62 – 1.52 (m, 1H, H_4), 1.41 (s, 9H, H_1), 1.25 (t, J = 7.8 Hz, 3H, H_8), 1.10 (d, J = 6.6 Hz, 3H, H_2). ^{13}C NMR (126 MHz, CD_2Cl_2) δ 202.4 (C), 192.6 (C), 155.9 (C), 79.3 (C), 58.2 (CH_2), 46.4 (CH), 40.4 (CH_2), 31.2 (CH_2), 28.7 (CH_3), 24.5 (CH_2), 21.8 (CH_3), 14.9 (CH_3). IR (ATR-FTIR), cm^{-1}: 3387 (m), 2797 (w), 2929 (w), 1717 (w), 1683 (s), 1512 (s), 1310 (m), 1170 (m), 1051 (s), 541 (m). HRMS-CI (m/z): $[M + Na]^+$ calcd for $C_{14}H_{25}NNaO_4S$, 326.1397; found, 326.1399. $[\alpha]_D^{20}$ +8.0 (*c* 1.0, CH_2Cl_2).

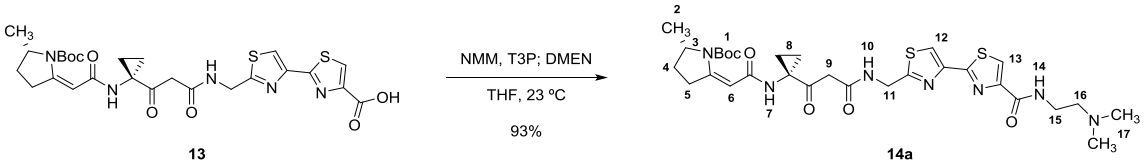
*Synthesis of the acid **13**:*



Silver trifluoroacetate (164 mg, 742 μmol, 2.00 equiv) was added to a solution of triethylamine (207 μL, 1.48 mmol, 4.00 equiv) and the amine **11** (149 mg, 371 μmol, 1 equiv) in *N*,*N*-dimethylformamide (2.7 mL) at 0 °C. A solution of the β-ketothioester **10** (146 mg, 482 μmol, 1.30 equiv) in *N*,*N*-dimethylformamide (1.2 mL) was added dropwise via syringe to the reaction mixture. The reaction vessel was covered with foil to exclude light and the reaction mixture was stirred for 1 h at 0 °C. The heterogeneous product mixture was filtered through a fritted funnel the filtrate was concentrated. The residue obtained was applied to a column containing trimethylamine acetate-functionalized silica gel (Si-TMA acetate; eluting with 0.5% formic acid–acetonitrile). The fractions containing product were collected, combined, and concentrated. The concentrated product was diluted with a solution containing 0.5% formic acid–5% methanol–acetonitrile (600 mL). The diluted product solution was concentrated. This process was repeated until LC/MS analysis indicated full conversion to the acid **13** (white solid, 185 mg, 87%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 13.12 (bs, 1H), 8.94 (t, J = 6.0 Hz, 1H, H_{10}), 8.47 (bs, 1H, H_7), 8.46 (s, 1H, H_{13}), 8.23 (s, 1H, H_{12}), 6.55 (s, 1H, H_6), 4.59 (d, J = 5.9 Hz, 2H, H_{11}), 4.17 (app p, J = 6.6 Hz, 1H, H_3), 3.55 (s, 2H, H_9), 3.41 (dd, J = 18.2, 8.7 Hz, 1H, H_5), 2.82 (dt, J = 18.6, 9.9 Hz, 1H, H_5), 1.89 (ddd, J = 20.6, 12.2, 8.6 Hz, 1H, H_4), 1.55 (app t, J = 10.4 Hz, 1H, H_4), 1.47 (s, 9H, H_1), 1.39 – 1.34 (m, 2H, H_8), 1.15 (d, J = 6.3 Hz, 3H, H_2), 1.05 – 0.99 (m, 2H, H_8). ^{13}C NMR (126 MHz, DMSO-d_6) δ 204.7 (C), 171.4 (C), 169.0 (C), 167.0 (C), 163.1 (C), 162.1 (C), 153.3 (C), 151.2 (C), 148.3 (C), 147.1 (C), 128.8 (CH), 118.2 (CH), 98.4 (CH), 80.9 (C), 56.0 (CH), 46.3 (CH_2), 40.6 (C), 40.5 (CH_2), 28.9 (CH_2), 27.8 (CH_2), 27.8 (CH_3), 19.5 (CH_2), 19.5 (CH_2), 19.3 (CH_3). IR (ATR-FTIR), cm^{-1}: 3329 (m), 2978 (w), 1722 (w), 1711 (w), 1673 (s), 1641 (w), 1586 (m), 1543 (m), 1518 (m), 1286 (s), 1230 (s), 1180 (s), 1157 (s), 1142 (s), 780 (m). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{26}H_{32}N_5O_7S_2$, 590.1738; found, 590.1731. $[\alpha]_D^{20}$ +13.0 (*c* 1.0, DMSO).

*Synthesis of the amide **14a**:*

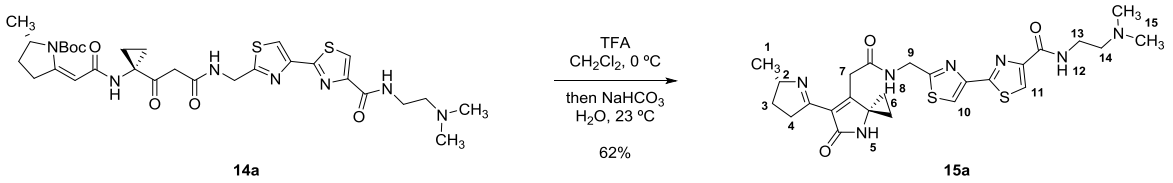


13 14a

A solution of T3P in ethyl acetate (50 wt%, 10.6 μL, 17.8 μmol, 1.50 equiv) and 4-methylmorpholine (6.5 μL, 59.4 μmol, 5.00 equiv) were added in sequence to a solution of the acid **13** (7.0 mg, 11.9 μmol, 1 equiv) in tetrahydrofuran (240 μL) at 23 °C. The reaction mixture was stirred for 20 min at 23 °C. A solution of *N*,*N*-dimethylethylenediamine (3.2 μL, 29.7 μmol, 2.50 equiv) in tetrahydrofuran (50 μL) was added to the reaction mixture. The resulting mixture was stirred for 7 h at 23 °C. The product mixture was concentrated. The concentrated product mixture was diluted with ethyl acetate (10 mL). The diluted product mixture was poured into a separatory funnel that had been charged with saturated aqueous sodium bicarbonate solution (5.0 mL) and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (2 × 10 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the amide **14a** as a white solid (7.3 mg, 93%). The product so obtained was used without further purification.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.96 (t, J = 5.7 Hz, 1H, H_{10}), 8.48 (bs, 1H, H_7), 8.26 (s, 1H, H_{13}), 8.24 (bs, 1H, H_{14}), 8.19 (s, 1H, H_{12}), 6.55 (s, 1H, H_6), 4.59 (d, J = 5.4 Hz, 2H, H_{11}), 4.17 (app p, J = 5.7 Hz, 1H, H_3), 3.55 (s, 2H, H_9), 3.47 – 3.35 (m, 3H, H_5, H_{15}), 2.82 (dt, J = 19.0, 9.9 Hz, 1H, H_5), 2.41 (t, J = 6.2 Hz, 2H, H_{16}), 2.18 (s, 6H, H_{17}), 1.97 – 1.81 (m, 1H, H_4), 1.55 (app t, J = 10.4 Hz, 1H, H_4), 1.47 (s, 9H, H_1), 1.39 – 1.33 (m, 2H, H_8), 1.15 (d, J = 5.8 Hz, 3H, H_2), 1.06 – 0.96 (m, 2H, H_8). ^{13}C NMR (101 MHz, DMSO-d_6) δ 204.7 (C), 171.6 (C), 169.0 (C), 167.1 (C), 161.9 (C), 160.2 (C), 153.3 (C), 151.2 (C), 150.8 (C), 147.2 (C), 124.1 (CH), 118.2 (CH), 98.4 (CH), 80.9 (C), 58.1 (CH_2), 56.0 (CH), 46.4 (CH_2), 45.2 (CH_3), 40.7 (C), 40.1 (CH_2), 36.7 (CH_2), 28.9 (CH_2), 27.8 (CH_2), 27.8 (CH_3), 19.5 (CH_2), 19.5 (CH_2), 19.3 (CH_3). IR (ATR-FTIR), cm^{-1}: 3278 (br w), 2975 (w), 2931 (w), 1703 (m), 1648 (s), 1603 (m), 1549 (m), 1291 (m), 1158 (s). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{30}H_{42}N_7O_6S_2$, 660.2633; found, 660.2631. $[\alpha]_D^{20}$ +25.0 (*c* 1.0, CH_3OH).

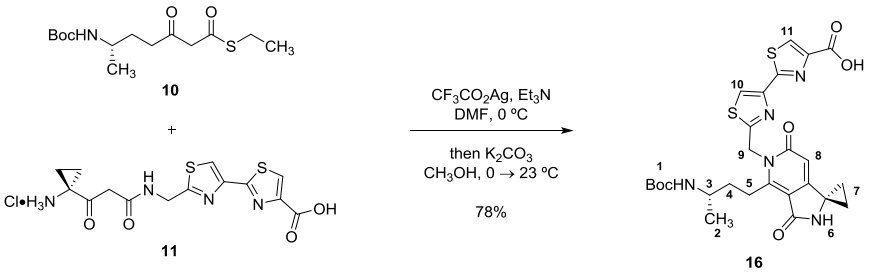
*Synthesis of the lactam **15a**:*



Trifluoroacetic acid (751 μL, 9.82 mmol, 120 equiv) was added dropwise via syringe to a solution of the amide **14a** (54.0 mg, 81.8 μmol, 1 equiv) in dichloromethane (1.6 mL) at 0 °C. The reaction mixture was stirred for 14 h at 0 °C. The reaction mixture was concentrated and the concentrated reaction mixture was diluted with saturated aqueous sodium bicarbonate solution (4.0 mL). The diluted reaction mixture was stirred for 1 h at 23 °C. The product mixture was diluted sequentially with water (10 mL) and ethyl acetate (30 mL). The diluted product mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (5 × 30 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the lactam **15a** as a light yellow solid (27.5 mg, 62%). The product so obtained was used without further purification.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.28 (t, J = 6.0 Hz, 1H, H_8), 8.60 (bs, 1H, H_5), 8.28 – 8.23 (m, 2H, H_{11}, H_{12}), 8.19 (s, 1H, H_{10}), 4.66 – 4.57 (m, 2H, H_9), 4.18 – 4.07 (m, 1H, H_2), 3.39 (app q, J = 6.5 Hz, 2H, H_{13}), 3.35 – 3.30 (m, 2H, H_7), 3.15 – 3.06 (m, 1H, H_4), 2.87 (dt, J = 17.8, 8.8 Hz, 1H, H_4), 2.45 (t, J = 6.7 Hz, 2H, H_{14}), 2.21 (s, 6H, H_{15}), 2.12 – 2.06 (m, 1H, H_3), 1.76 – 1.61 (m, 2H, H_6), 1.42 – 1.33 (m, 3H, H_3, H_6), 1.19 (d, J = 6.6 Hz, 3H, H_1). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.1 (C), 169.6 (C), 168.3 (C), 168.2 (C), 161.8 (C), 160.2 (C), 157.6 (C), 150.8 (C), 147.4 (C), 127.4 (C), 124.2 (CH), 118.0 (CH), 66.5 (CH), 58.0 (CH_2), 45.3 (C), 45.1 (CH_3), 40.4 (CH_2), 36.6 (CH_2), 36.5 (CH_2), 33.5 (CH_2), 29.7 (CH_2), 21.9 (CH_3), 11.8 (CH_2), 11.7 (CH_2). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{25}H_{32}N_7O_3S_2$, 542.2003; found, 542.2016. $[\alpha]_D^{20}$ –6.0 (*c* 1.5, DMSO- d_6).

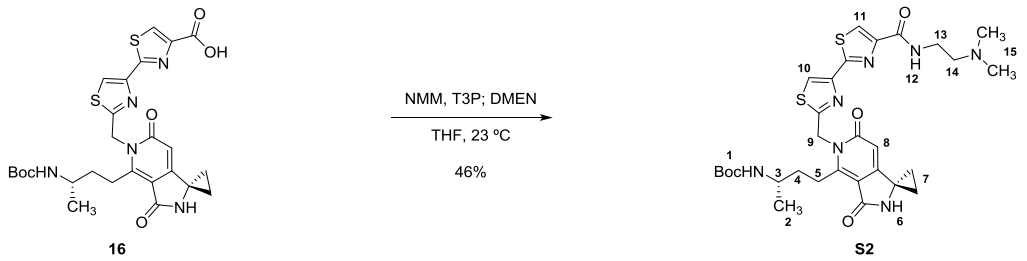
*Synthesis of the pyridone **16**:*



Silver trifluoroacetate (410 mg, 1.86 mmol, 2.00 equiv) was added to a solution of triethylamine (518 µL, 3.71 mmol, 4.00 equiv) and the amine **11** (374 mg, 930 µmol, 1 equiv) in *N*,*N*-dimethylformamide (6.0 mL) at 0 °C. A solution of the β-ketothioester **10** (366 mg, 1.21 mmol, 1.30 equiv) in *N*,*N*-dimethylformamide (2.0 mL) was added dropwise via syringe to the reaction mixture. The reaction vessel was covered with foil to exclude light and the reaction mixture was stirred for 1 h at 0 °C. Potassium carbonate (385 mg, 2.79 mmol, 3.00 equiv) and methanol (8.0 mL) were then added in sequence to the reaction mixture at 0 °C. The reaction mixture was stirred for 30 min at 0 °C. The heterogeneous product mixture was filtered through a fritted funnel. The filter cake was washed with methanol (10 mL). The filtrates were combined and the combined filtrates were concentrated. The residue obtained was applied to a trimethylamine acetate-functionalized silica column (Si-TMA acetate; eluting with 0.5% formic acid–acetonitrile). The fractions containing the product **16** were collected, combined, and concentrated to provide the pyridone **16** as a white solid (414 mg, 78%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.48 (bs, 1H, H_6), 8.39 (s, 1H, H_{11}), 8.25 (s, 1H, H_{10}), 6.80 (d, J = 8.0 Hz, 1H), 6.17 (s, 1H, H_8), 5.60 (d, J = 16.0 Hz, 1H, H_9), 5.49 (d, J = 15.9 Hz, 1H, H_9), 3.63 – 3.52 (m, 1H, H_3), 3.53 – 3.45 (m, 1H, H_5), 3.32 – 3.14 (m, 1H, H_5), 1.75 – 1.60 (m, 2H, H_4), 1.37 (app t, J = 2.7 Hz, 2H, H_7), 1.35 (app t, J = 2.8 Hz, 1H, H_7), 1.30 (s, 9H, H_1), 1.06 (d, J = 6.6 Hz, 3H, H_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.9 (C), 166.7 (C), 162.3 (C), 161.8 (C), 161.6 (C), 159.9 (C), 155.1 (C), 153.0 (C), 149.3 (C), 147.2 (C), 128.3 (CH), 118.7 (CH), 109.7 (C), 103.3 (CH), 77.4 (C), 46.1 (CH), 44.2 (CH_2), 39.7 (C), 35.5 (CH_2), 28.2 (CH_3), 24.1 (CH_2), 20.7 (CH_3), 15.2 (CH_2). IR (ATR-FTIR), cm^{-1}: 3327 (w), 3121 (w), 2971 (w), 2355 (br w), 1720 (w), 1702 (w), 1674 (m), 1649 (s), 1571 (m), 1518 (m), 1171 (m), 578 (s). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{26}H_{30}N_5O_6S_2$, 572.1632; found, 572.1630. $[\alpha]_D^{20}$ –64.0 (*c* 0.5, DMSO).

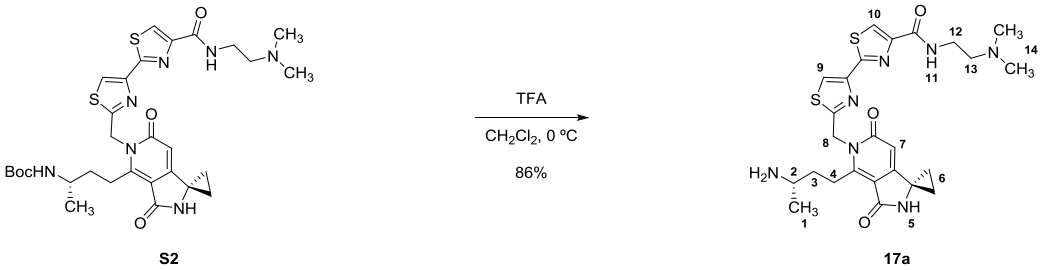
*Synthesis of the amide **S2**:*



A solution of T3P in ethyl acetate (50 wt%, 54.7 μL, 91.8 μmol, 1.50 equiv) and 4-methylmorpholine (33.7 μL, 306 μmol, 5.00 equiv) were added in sequence to a solution of the acid **16** (35.0 mg, 61.2 μmol, 1 equiv) in tetrahydrofuran (790 μL) at 23 °C. *N*,*N*-Dimethylethylenediamine (16.7 μL, 153 μmol, 2.50 equiv) was then added to the reaction mixture. The resulting mixture was stirred for 7 h at 23 °C. The product mixture was concentrated. The concentrated product mixture was diluted with ethyl acetate (10 mL). The diluted product mixture was poured into a separatory funnel that had been charged with saturated aqueous sodium bicarbonate solution (5.0 mL) and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (2 × 10 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the amide **S2** as a white solid (18.1 mg, 46%). The product so obtained was used without further purification.

^{1}H NMR (500 MHz, CD_2Cl_2) δ 8.06 (s, 1H, H_{11}), 8.01 (s, 1H, H_{10}), 7.64 (bs, 1H, H_{12}), 6.33 (bs, 1H, H_6), 6.01 (s, 1H, H_8), 5.62 (d, *J* = 15.0 Hz, 1H, H_9), 5.55 (d, *J* = 14.2 Hz, 1H, H_9), 5.38 (d, *J* = 7.4 Hz, 1H), 3.82 – 3.74 (m, 1H, H_3), 3.66 – 3.58 (m, 1H, H_5), 3.51 (app q, *J* = 5.9 Hz, 2H, H_{13}), 3.48 – 3.41 (m, 1H, H_5), 2.51 (t, *J* = 6.1 Hz, 2H, H_{14}), 2.27 (s, 6H, H_{15}), 1.89 – 1.81 (m, 1H, H_4), 1.80 – 1.69 (m, 1H, H_4), 1.52 – 1.45 (m, 2H, H_7), 1.41 (s, 9H, H_1), 1.37 – 1.32 (m, 2H, H_7), 1.19 (d, *J* = 6.5 Hz, 3H, H_2). ^{13}C NMR (126 MHz, CD_2Cl2) δ 168.4 (C), 166.0 (C), 163.1 (C), 162.7 (C), 161.2 (C), 160.5 (C), 156.1 (C), 154.4 (C), 151.8 (C), 148.5 (C), 123.8 (CH), 119.2 (CH), 110.3 (C), 103.9 (CH), 79.2 (C), 58.7 (CH_2), 47.1 (CH), 45.7 (CH_3), 45.1 (CH_2), 40.6 (C), 37.5 (CH_2), 36.1 (CH_2), 28.7 (CH_3), 25.0 (CH_2), 21.3 (CH_3), 16.2 (CH_2). IR (ATR-FTIR), cm^{-1}: 3327 (br w), 2972 (w), 1694 (w), 1651 (s), 1541 (m), 1250 (m), 1165 (m), 568 (m). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{30}H_{40}N_7O_5S_2$, 642.2527; found, 642.2532. $[\alpha]_D^{20}$ –125.8 (*c* 0.93, CH_3OH).

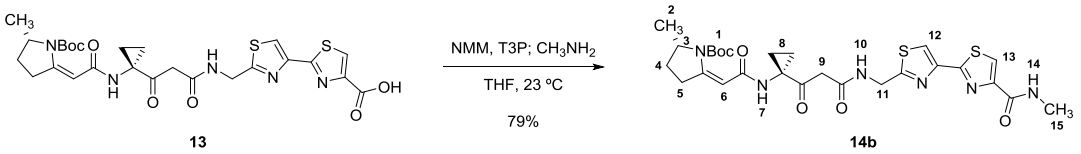
*Synthesis of the amide **17a**:*



Trifluoroacetic acid (206 μL, 2.69 mmol, 120 equiv) was added dropwise via syringe to a solution of the amide **S2** (14.4 mg, 22.4 μmol, 1 equiv) in dichloromethane (560 μL) at 0 °C. The reaction mixture was stirred for 14 h at 0 °C. The reaction mixture was concentrated. The concentrated product mixture was diluted with chloroform (10 mL). The diluted product mixture was poured into a separatory funnel that had been charged with saturated aqueous sodium bicarbonate solution (5.0 mL) and the layers that formed were separated. The aqueous layer was extracted with chloroform (2 × 10 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the amide **17a** as a white solid (10.5 mg, 86%). The product so obtained was used without further purification.

^{1}H NMR (400 MHz, CD_3OD) δ 8.24 (s, 1H, H_{10}), 8.18 (s, 1H, H_9), 6.19 (s, 1H, H_7), 5.73 (d, *J* = 15.4 Hz, 1H, H_8), 5.68 (d, *J* = 15.5 Hz, 1H, H_8), 3.75 – 3.62 (m, 1H, H_4), 3.60 – 3.50 (m, 3H, H_4, H_{12}), 3.10 – 3.02 (m, 1H, H_2), 2.59 (t, *J* = 6.7 Hz, 2H, H_{13}), 2.32 (s, 6H, H_{14}), 1.87 – 1.72 (m, 2H, H_3), 1.58 – 1.50 (m, 2H, H_6), 1.47 – 1.40 (m, 2H, H_6), 1.18 (d, *J* = 6.3 Hz, 3H, H_1). ^{13}C NMR (151 MHz, CD_3OD) δ 169.5 (C), 167.5 (C), 165.0 (C), 163.7 (C), 163.4 (C), 162.5 (C), 154.9 (C), 151.7 (C), 149.1 (C), 125.2 (CH), 120.2 (CH), 112.3 (C), 104.4 (CH), 59.3 (CH_2), 47.7 (CH), 46.1 (CH_2), 45.5 (CH_3), 41.5 (C), 39.0 (CH_2), 38.0 (CH_2), 25.5 (CH_2), 22.5 (CH_3), 16.3 (CH_2). IR (ATR-FTIR), cm^{-1}: 3355 (br w), 2956 (w), 1691 (w), 1648 (s), 1572 (w), 1545 (w), 1288 (m), 568 (m). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{25}H_{32}N_7O_3S_2$, 542.2003; found, 542.2004. $[\alpha]_D^{20}$ –13.0 (*c* 1.0, CH_3OH).

*Synthesis of the amide **14b**:*

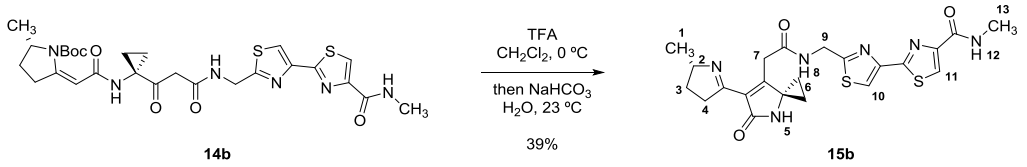


13 14b

A solution of T3P in ethyl acetate (50 wt%, 22.7 μL, 38.2 μmol, 1.50 equiv) and 4-methylmorpholine (14.0 μL, 127 μmol, 5.00 equiv) were added in sequence to a solution of the acid **13** (15.0 mg, 25.4 μmol, 1 equiv) in tetrahydrofuran (330 μL) at 23 °C. The reaction mixture was stirred for 20 min at 23 °C. A solution of methylamine in tetrahydrofuran (2.00 M, 23 μL, 63.6 μmol, 2.50 equiv) was then added to the reaction mixture. The resulting mixture was stirred for 7 h at 23 °C. The product mixture was concentrated. The residue obtained was purified by flash-column chromatography (eluting with dichloromethane initially, grading to 10% methanol–dichloromethane, linear gradient) to provide the amide **14b** as an off-white solid (12.1 mg, 79%).

^{1}H NMR (500 MHz, CD_2Cl_2) δ 8.05 (s, 1H, H_{13}), 8.04 (bs, 1H, H_{10}), 7.93 (s, 1H, H_{12}), 7.38 (bs, 1H, H_{14}), 6.55 (s, 1H, H_6), 6.23 (bs, 1H, H_7), 4.76 (d, *J* = 6.0 Hz, 2H, H_{11}), 4.23 (app p, *J* = 6.8 Hz, 1H, H_3), 3.66 (s, 2H, H_9), 3.47 (dd, *J* = 18.3, 8.7 Hz, 1H, H_5), 2.98 (d, *J* = 5.1 Hz, 3H, H_{15}), 2.89 (dddd, *J* = 18.3, 11.1, 8.3, 2.2 Hz, 1H, H_5), 1.92 (tt, *J* = 12.1, 8.5 Hz, 1H, H_4), 1.62 (app q, *J* = 4.4 Hz, 2H, H_8), 1.56 (dd, *J* = 12.2, 8.6 Hz, 1H, H_4), 1.18 (d, *J* = 6.5 Hz, 3H, H_2), 1.17 – 1.13 (m, 2H, H_8). ^{13}C NMR (126 MHz, CD_2Cl_2) δ 206.1 (C), 170.6 (C), 170.4 (C), 167.2 (C), 163.0 (C), 161.9 (C), 156.9 (C), 152.4 (C), 151.6 (C), 148.9 (C), 123.5 (CH), 117.6 (CH), 97.2 (CH), 82.2 (C), 57.5 (CH), 45.6 (CH_2), 42.0 (C), 41.7 (CH_2), 30.2 (CH_2), 28.9 (CH_2), 28.5 (CH_3), 26.3 (CH_3), 21.6 (CH_2), 21.6 (CH_2), 19.9 (CH_3). IR (ATR-FTIR), cm^{-1}: 3295 (br w), 2975 (w), 2931 (w), 1706 (m), 1651 (s), 1606 (m), 1547 (m), 1289 (m), 1156 (s), 771 (m). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{27}H_{35}N_6O_6S_2$, 603.2054; found, 603.2053. $[\alpha]_D^{20}$ +36.1 (*c* 0.83, CH_2Cl_2).

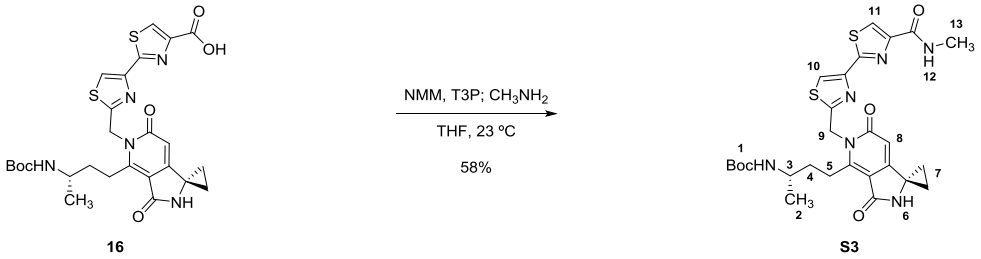
*Synthesis of the lactam **15b**:*



Trifluoroacetic acid (216 μL, 2.83 mmol, 120 equiv) was added dropwise via syringe to a solution of the amide **14b** (14.2 mg, 23.6 μmol, 1 equiv) in dichloromethane (590 μL) at 0 °C. The reaction mixture was stirred for 14 h at 0 °C. The reaction mixture was concentrated. The concentrated reaction mixture was diluted with saturated aqueous sodium bicarbonate solution (400 μL). The diluted reaction mixture was stirred for 1 h at 23 °C. The product mixture was diluted sequentially with water (1.0 mL) and ethyl acetate (3.0 mL). The diluted product mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (5 × 3.0 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the lactam **15b** as a light yellow solid (4.5 mg, 39%). The product so obtained was used without further purification.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.28 (t, J = 6.0 Hz, 1H, H_8), 8.60 (bs, 1H, H_5), 8.41 – 8.34 (m, 1H, H_{12}), 8.25 (s, 1H, H_{11}), 8.17 (s, 1H, H_{10}), 4.65 – 4.57 (m, 2H, H_9), 4.17 – 4.07 (m, 1H, H_2), 3.38 – 3.26 (m, 2H, H_7), 3.15 – 3.05 (m, 1H, H_4), 2.91 – 2.85 (m, 1H, H_4), 2.81 (d, J = 4.8 Hz, 3H, H_{13}), 2.14 – 2.03 (m, 1H, H_3), 1.73 – 1.61 (m, 2H, H_6), 1.42 – 1.29 (m, 3H, H_3, H_6), 1.19 (d, J = 6.7 Hz, 3H, H_1). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.1 (C), 169.6 (C), 168.4 (C), 168.2 (C), 161.7 (C), 160.9 (C), 157.7 (C), 150.9 (C), 147.5 (C), 127.4 (C), 123.8 (CH), 117.8 (CH), 66.5 (CH), 45.3 (C), 40.4 (CH_2), 36.5 (CH_2), 33.5 (CH_2), 29.7 (CH_2), 25.8 (CH_3), 21.9 (CH_3), 11.8 (CH_2), 11.8 (CH_2). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{22}H_{25}N_6O_3S_2$, 485.1424; found, 485.1418. $[\alpha]_D^{20}$ –2.3 (*c* 1.3, DMSO-d_6).

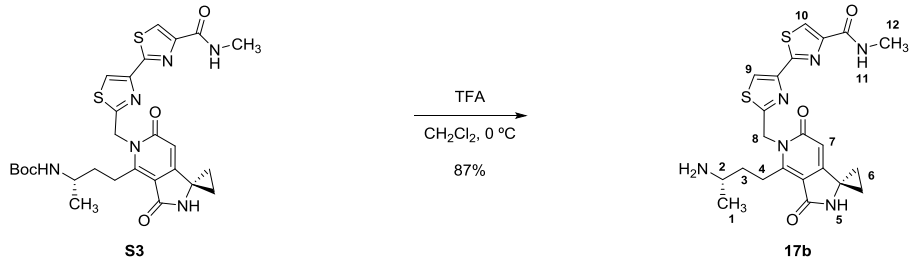
*Synthesis of the amide **S3**:*



A solution of T3P in ethyl acetate (50 wt%, 54.7 μL, 91.8 μmol, 1.50 equiv) and 4-methylmorpholine (33.7 μL, 306 μmol, 5.00 equiv) were added in sequence to a solution of the acid **16** (35.0 mg, 61.2 μmol, 1 equiv) in tetrahydrofuran (790 μL) at 23 °C. A solution of methylamine in tetrahydrofuran (2.00 M, 77 μL, 153 μmol, 2.50 equiv) was then added to the reaction mixture. The resulting mixture was stirred for 7 h at 23 °C. The product mixture was concentrated. The concentrated product mixture was diluted with ethyl acetate (10 mL). The diluted product mixture was poured into a separatory funnel that had been charged with saturated aqueous sodium bicarbonate solution (5.0 mL) and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (2 × 10 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the amide **S3** as a white solid (20.8 mg, 58%). The product so obtained was used without further purification.

^{1}H NMR (600 MHz, CD_3OD) δ 8.22 (s, 1H, H_{11}), 8.16 (s, 1H, H_{10}), 6.16 (s, 1H, H_8), 5.71 (d, J = 15.8 Hz, 1H, H_9), 5.65 (d, J = 15.8 Hz, 1H, H_9), 3.74 (app h, J = 6.4 Hz, 1H, H_3), 3.60 – 3.50 (m, 2H, H_5), 2.96 (s, 3H, H_{13}), 1.89 – 1.75 (m, 2H, H_4), 1.54 – 1.51 (m, 2H, H_7), 1.43 – 1.40 (m, 2H, H_7), 1.37 (s, 9H, H_1), 1.17 (d, J = 6.7 Hz, 3H, H_2). ^{13}C NMR (151 MHz, CD_3OD) δ 169.4 (C), 167.4 (C), 164.9 (C), 164.0 (C), 163.7 (C), 162.5 (C), 157.9 (C), 155.1 (C), 151.8 (C), 149.3 (C), 124.9 (CH), 119.8 (CH), 112.2 (C), 104.3 (CH), 79.9 (C), 47.8 (CH), 45.9 (CH_2), 41.5 (C), 36.8 (CH_2), 28.9 (CH_3), 26.3 (CH_3), 25.9 (CH_2), 21.2 (CH_3), 16.3 (CH_2). IR (ATR-FTIR), cm^{-1}: 3284 (br w), 2971 (w), 1694 (m), 1652 (s), 1573 (m), 1550 (m), 1167 (m), 570 (s). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{27}H_{33}N_6O_5S_2$, 585.1948; found, 585.1948. $[\alpha]_D^{20}$ –101.2 (*c* 0.85, CH_3OH).

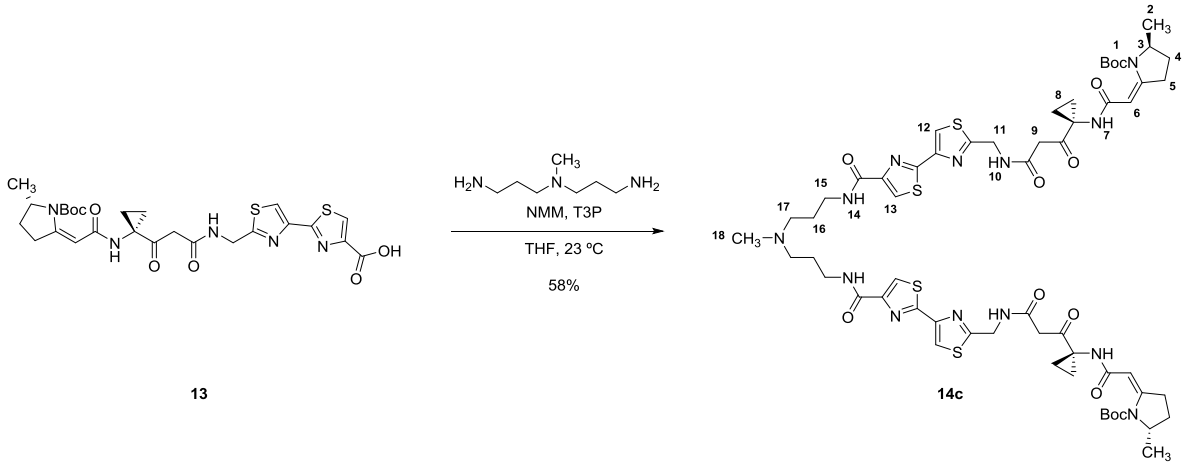
*Synthesis of the amide **17b**:*



Trifluoroacetic acid (273 μL, 3.57 mmol, 120 equiv) was added dropwise via syringe to a solution of the amide **S3** (17.4 mg, 29.8 μmol, 1 equiv) in dichloromethane (740 μL) at 0 °C. The reaction mixture was stirred for 14 h at 0 °C. The reaction mixture was concentrated. The concentrated product mixture was diluted with chloroform (10 mL). The diluted product mixture was poured into a separatory funnel that had been charged with saturated aqueous sodium bicarbonate solution (5.0 mL) and the layers that formed were separated. The aqueous layer was extracted with chloroform (2 × 10 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the amide **17b** as a white solid (12.6 mg, 87%). The product so obtained was used without further purification.

^{1}H NMR (600 MHz, CD_3OD) δ 8.24 (s, 1H, H_9), 8.16 (s, 1H, H_{10}), 6.21 (s, 1H, H_7), 5.72 (d, J = 15.7 Hz, 1H, H_8), 5.67 (d, J = 15.7 Hz, 1H, H_8), 3.74 – 3.65 (m, 1H, H_4), 3.62 – 3.51 (m, 1H, H_4), 3.31 – 3.26 (m, 1H, H_2), 2.96 (s, 3H, H_{12}), 2.04 – 1.86 (m, 2H, H_3), 1.58 – 1.50 (m, 2H, H_6), 1.48 – 1.41 (m, 2H, H_6), 1.30 (d, J = 6.6 Hz, 3H, H_1). ^{13}C NMR (151 MHz, CD_3OD) δ 169.6 (C), 167.3 (C), 164.8 (C), 164.0 (C), 163.7 (C), 162.3 (C), 153.7 (C), 151.8 (C), 149.1 (C), 124.8 (CH), 120.3 (CH), 112.5 (C), 104.8 (CH), 48.1 (CH), 46.0 (CH_2), 41.6 (C), 36.4 (CH_2), 26.4 (CH_3), 25.0 (CH_2), 20.3 (CH_3), 16.4 (CH_2). IR (ATR-FTIR), cm^{-1}: 3419 (br w), 2926 (w), 2857 (w), 1688 (w), 1647 (s), 1556 (m), 1289 (w), 568 (m). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{22}H_{25}N_6O_3S_2$, 485.1424; found, 485.1425. $[\alpha]_D^{20}$ –29.0 (*c* 1.0, CH_3OH).

*Synthesis of the dimeric amide **14c**:*

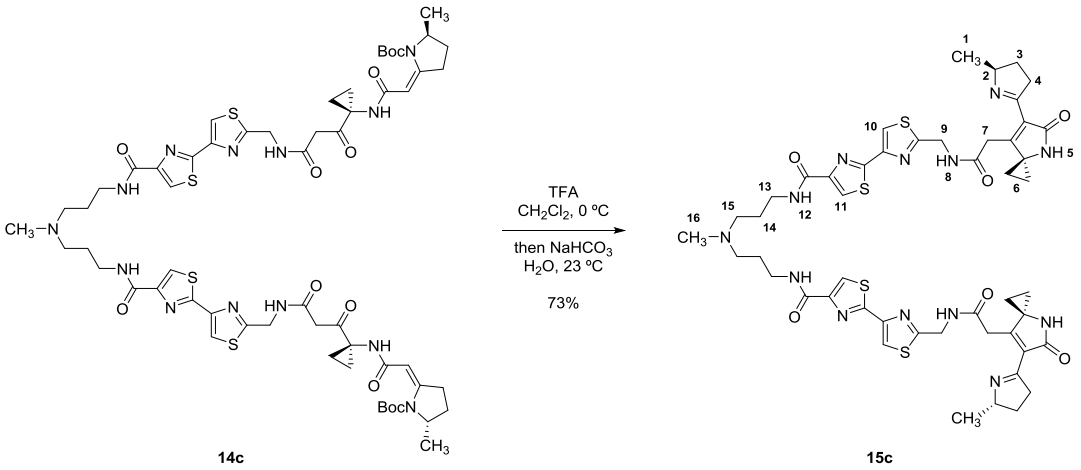


13 14c

A solution of T3P in ethyl acetate (50 wt%, 50.5 μL, 84.8 μmol, 2.50 equiv) and 4-methylmorpholine (37.3 μL, 339 μmol, 10.00 equiv) were added in sequence to a solution of the acid **13** (20.0 mg, 33.9 μmol, 1 equiv) in tetrahydrofuran (680 μL) at 23 °C. The reaction mixture was stirred for 20 min at 23 °C. A solution of *N*,*N*-bis(3-aminopropyl)methylamine (2.7 μL, 17.0 μmol, 0.50 equiv) in tetrahydrofuran (50 μL) was then added to the reaction mixture. The resulting mixture was stirred for 7 h at 23 °C. The product mixture was concentrated. The concentrated product mixture was diluted with ethyl acetate (10 mL). The diluted product mixture was poured into a separatory funnel that had been charged with saturated aqueous sodium bicarbonate solution (5.0 mL) and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (2 × 10 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the dimeric amide **14c** as a white solid (12.5 mg, 58%). The product so obtained was used without further purification.

^{1}H NMR (500 MHz, CD_2Cl_2) δ 8.21 – 8.14 (m, 2H, H_{10}), 8.07 – 8.01 (m, 2H, H_{14}), 8.01 (s, 2H, H_{13}), 7.80 (s, 2H, H_{12}), 6.76 (bs, 2H, H_7), 6.57 (s, 2H, H_6), 4.68 (d, J = 5.9 Hz, 4H, H_{11}), 4.28 – 4.16 (m, 2H, H_3), 3.68 (s, 4H, H_9), 3.55 – 3.49 (m, 4H, H_{15}), 3.50 – 3.42 (m, 2H, H_5), 2.95 – 2.77 (m, 2H, H_5), 2.51 (t, J = 6.6 Hz, 4H, H_{17}), 2.28 (s, 3H, H_{18}), 1.98 – 1.86 (m, 2H, H_4), 1.87 – 1.80 (m, 4H, H_{16}), 1.64 – 1.58 (m, 4H, H_8), 1.60 – 1.52 (m, 2H, H_4), 1.49 (s, 18H, H_1), 1.17 (d, J = 6.5 Hz, 6H, H_2), 1.14 – 1.01 (m, 4H, H_8). ^{13}C NMR (126 MHz, CD_2Cl_2) δ 206.2 (C), 170.6 (C), 170.2 (C), 167.4 (C), 162.8 (C), 161.3 (C), 156.6 (C), 152.4 (C), 151.8 (C), 148.7 (C), 123.6 (CH), 117.6 (CH), 97.5 (CH), 82.1 (C), 57.4 (CH), 56.7 (CH_2), 46.0 (CH_2), 42.5 (CH_3), 41.9 (C), 41.6 (CH_2), 38.9 (CH_2), 30.1 (CH_2), 28.9 (CH_2), 28.5 (CH_3), 27.5 (CH_2), 21.6 (CH_2), 21.6 (CH_2), 19.8 (CH_3). IR (ATR-FTIR), cm^{-1}: 3282 (br w), 2974 (w), 2933 (w), 1708 (m), 1651 (s), 1608 (m), 1541 (s), 1315 (m), 1290 (s), 1156 (s), 1026 (m), 770 (m), 755 (m), 620 (w). HRMS-CI (m/z): [M + H]$^+$ calcd for $C_{59}H_{78}N_{13}O_{12}S_4$, 1288.4770; found, 1288.4768. $[\alpha]_D^{20}$ +19.0 (*c* 1.0, CH_2Cl_2).

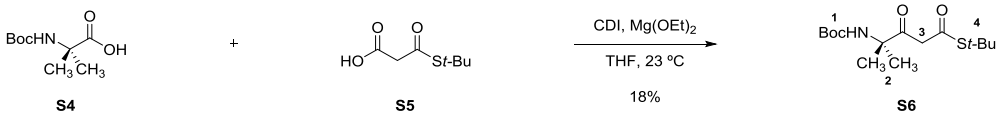
*Synthesis of the dimeric lactam **15c**:*



Trifluoroacetic acid (57.0 μL, 745 μmol, 120 equiv) was added dropwise via syringe to a solution of the amide **14c** (8.0 mg, 6.21 μmol, 1 equiv) in dichloromethane (200 μL) at 0 °C. The reaction mixture was stirred for 14 h at 0 °C. The reaction mixture was concentrated. The concentrated reaction mixture was diluted with saturated aqueous sodium bicarbonate solution (650 μL). The diluted reaction mixture was stirred for 1 h at 23 °C. The product mixture was diluted sequentially with water (1.0 mL) and ethyl acetate (3.0 mL). The diluted product mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (5 × 3.0 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the dimeric lactam **15c** as a light yellow solid (4.8 mg, 73%). The product so obtained was used without further purification.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.28 (t, J = 5.9 Hz, 2H, H_8), 8.61 (bs, 2H, H_5), 8.55 (t, J = 5.9 Hz, 2H, H_{12}), 8.22 (s, 2H, H_{11}), 8.15 (s, 2H, H_{10}), 4.60 (d, J = 6.0 Hz, 4H, H_9), 4.16 – 4.07 (m, 2H, H_2), 3.38 – 3.29 (m, 8H, H_7, H_{13}), 3.16 – 3.05 (m, 2H, H_4), 2.86 (dt, J = 18.1, 9.1 Hz, 2H, H_4), 2.39 (t, J = 6.8 Hz, 4H, H_{15}), 2.19 (s, 3H, H_{16}), 2.10 – 2.04 (m, 2H, H_3), 1.77 – 1.65 (m, 8H, H_6, H_{14}), 1.38 – 1.34 (m, 6H, H_3, H_6), 1.17 (d, J = 7.0 Hz, 6H, H_1). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.0 (C), 169.6 (C), 168.4 (C), 168.2 (C), 161.7 (C), 160.2 (C), 157.7 (C), 151.0 (C), 147.4 (C), 127.4 (C), 123.9 (CH), 117.8 (CH), 66.5 (CH), 55.4 (CH_2), 45.3 (C), 41.8 (CH_3), 40.4 (CH_2), 37.7 (CH_2), 36.5 (CH_2), 33.5 (CH_2), 29.7 (CH_2), 26.7 (CH_2), 21.9 (CH_3), 11.8 (CH_2), 11.8 (CH_2). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{49}H_{58}N_{13}O_6S_4$, 1052.3510; found, 1052.3514. $[\alpha]_D^{20}$ +1.3 (*c* 1.5, DMSO-d_6).

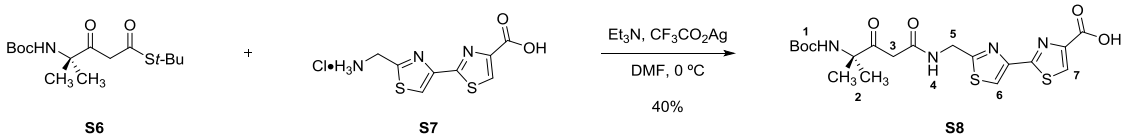
*Synthesis of the β-ketothioester **S6**:*



1,1′-Carbonyldiimidazole (1.20 g, 7.38 mmol, 1.50 equiv) was added to a solution of 2-((*tert*-butoxycarbonyl)amino)-2-methylpropanoic acid (**S4**, 1.00 g, 4.92 mmol, 1 equiv) in tetrahydrofuran (25 mL) at 23 °C. The resulting mixture was stirred for 6 h at 23 °C. In a second round-bottomed flask, magnesium ethoxide (845 mg, 7.38 mmol, 1.50 equiv) was added to a solution of 3-(*tert*-butylthio)-3-oxopropanoic acid (**S5**, 2.60 g, 14.8 mmol, 3.00 equiv) in tetrahydrofuran (13 mL) at 23 °C. The resulting mixture was stirred for 6 h at 23 °C, and then was concentrated to dryness. The activated carboxylic acid prepared in the first flask was transferred via cannula to the dried magnesium salt prepared in the second flask. The resulting mixture was stirred for 14 h at 23 °C. The product mixture was diluted sequentially with saturated aqueous ammonium chloride solution (20 mL) and ethyl acetate (30 mL). The diluted product mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (2 × 30 mL). The organic layers were combined and the combined organic layers were washed with saturated aqueous sodium chloride solution (30 mL). The washed organic layer was dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue obtained was purified by flash-column chromatography (eluting with 75% dichloromethane–hexanes initially, grading to dichloromethane, linear gradient) to provide the β-ketothioester **S6** as a white solid (275 mg, 18%).

^{1}H NMR (500 MHz, CD_2Cl_2) δ 5.04 (bs, 1H), 3.72 (s, 2H, H_3), 1.46 (s, 9H, H_4), 1.42 (s, 9H, H_1), 1.34 (s, 6H, H_2). ^{13}C NMR (126 MHz, CD_2Cl_2) δ 203.7 (C), 193.7 (C), 155.2 (C), 80.7 (C), 61.6 (C), 51.7 (CH_2), 49.1 (C), 29.9 (CH_3), 28.6 (CH_3), 24.2 (CH_3). IR (ATR-FTIR), cm^{-1}: 3348 (m), 2974 (m), 2942 (w), 1723 (s), 1697 (s), 1668 (s), 1522 (s), 1452 (m), 1366 (m), 1273 (s), 1166 (s), 1080 (s), 1022 (m), 995 (s), 686 (s). HRMS-CI (m/z): $[M + Na]^+$ calcd for $C_{15}H_{27}NNaO_4S$, 340.1553; found, 340.1553.

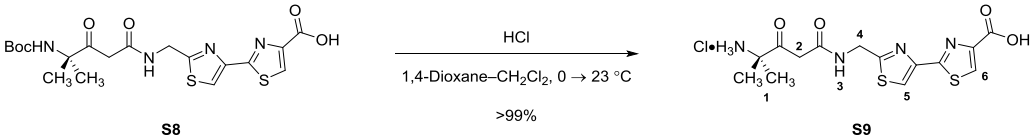
*Synthesis of the β-ketoamide **S8**:*



A solution of the thioester **S6** (821 mg, 2.59 mmol, 1.30 equiv) in *N*,*N*-dimethylformamide (5.4 mL) was added dropwise via syringe over 20 min to a solution of silver trifluoroacetate (879 mg, 3.98 mmol, 2.00 equiv), triethylamine (1.11 mL, 7.96 mmol, 4.00 equiv), and the amine **S7** (480 mg, 1.99 mmol, 1 equiv) in *N*,*N*-dimethylformamide (21 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C. The product mixture was filtered through a fritted funnel and the filtrate was concentrated. The concentrated product mixture was applied to a column containing trimethylamine acetate-functionalized silica gel (Si-TMA acetate; eluting with 2% acetic acid–methanol). The fractions containing product were collected, combined, and concentrated. The residue obtained was triturated with dichloromethane (50 mL) to provide the β-ketoamide **S8** as a white solid (368 mg, 40%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 13.01 (bs, 1H), 8.94 – 8.86 (m, 1H, H_4), 8.47 (s, 1H, H_7), 8.25 (s, 1H, H_6), 7.49 (bs, 1H), 4.60 (d, J = 5.9 Hz, 2H, H_5), 3.49 (s, 2H, H_3), 1.39 (s, 9H, H_1), 1.23 (s, 6H, H_2). ^{13}C NMR (126 MHz, DMSO-d_6) δ 205.4 (C), 171.3 (C), 167.2 (C), 162.1 (C), 162.0 (C), 155.0 (C), 148.2 (C), 147.1 (C), 128.9 (CH), 118.3 (CH), 78.6 (C), 60.2 (C), 43.0 (CH_2), 40.5 (CH_2), 28.2 (CH_3), 23.2 (CH_3). IR (ATR-FTIR), cm^{-1}: 3308 (br m), 2962 (w), 2933 (w), 1713 (m), 1670 (s), 1650 (s), 1516 (s), 1293 (s), 1163 (s), 1053 (m), 754 (m). HRMS-CI (m/z): [M + H]$^+$ calcd for $C_{19}H_{25}N_4O_6S_2$, 469.1210; found, 469.1212.

*Synthesis of the hydrochloride salt **S9**:*

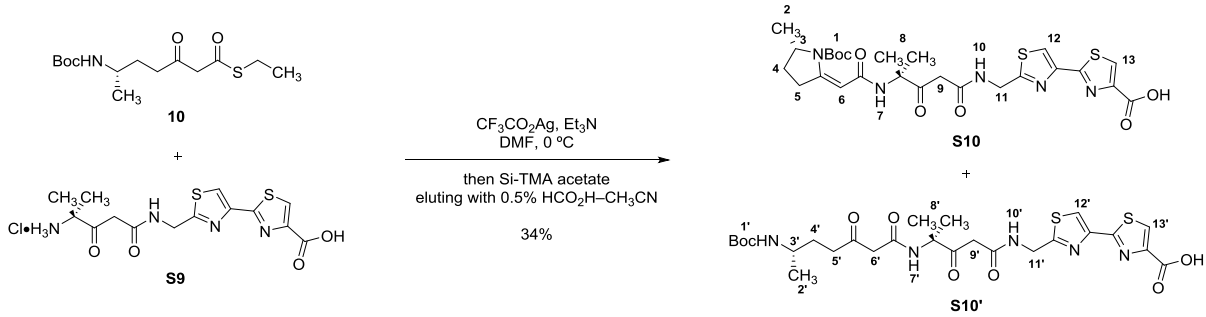


S8 **S9**

A solution of hydrogen chloride in 1,4-dioxane (4.0 N, 3.74 mL, 14.5 mmol, 70.2 equiv) was added dropwise via syringe to a solution of the β-ketoamide **S8** (100 mg, 213 μmol, 1 equiv) in dichloromethane (4.0 mL) at 0 °C. The reaction mixture was allowed to warm to 23 °C and stirred at this temperature for 1 h. The product mixture was concentrated to provide the hydrochloride salt **S9** as a white solid (86.4 mg, >99%). The product **S9** obtained in this way was used directly in the following step.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.22 (t, J = 6.1 Hz, 1H, H_3), 8.49 (s, 1H, H_6), 8.41 (bs, 3H), 8.29 (s, 1H, H_5), 4.65 (d, J = 5.8 Hz, 2H, H_4), 3.77 (s, 2H, H_2), 1.49 (s, 6H, H_1). ^{13}C NMR (126 MHz, DMSO-d_6) δ 202.7 (C), 170.7 (C), 165.9 (C), 162.0 (C), 162.0 (C), 148.1 (C), 147.1 (C), 128.9 (CH), 118.4 (CH), 61.5 (C), 43.7 (CH_2), 40.5 (CH_2), 21.9 (CH_3).

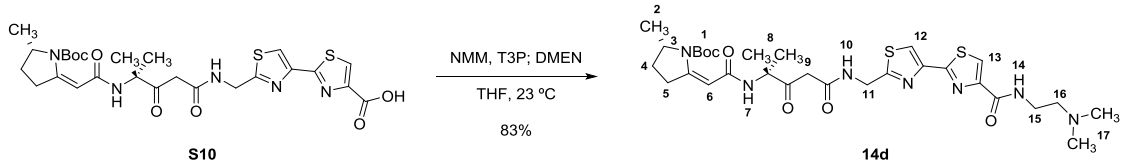
*Synthesis of the acid **S10**:*



Silver trifluoroacetate (164 mg, 742 μmol, 2.00 equiv) was added to a solution of triethylamine (207 μL, 1.48 mmol, 4.00 equiv) and the amine **S9** (149 mg, 371 μmol, 1 equiv) in *N*,*N*-dimethylformamide (2.7 mL) at 0 °C. A solution of the β-ketothioester **10** (146 mg, 482 μmol, 1.30 equiv) in *N*,*N*-dimethylformamide (1.2 mL) was added dropwise via syringe to the reaction mixture. The reaction vessel was covered with foil to exclude light and the reaction mixture was stirred for 1 h at 0 °C. The heterogeneous product mixture was filtered through a fritted funnel and the filtrate was concentrated. The residue obtained was applied to a column containing trimethylamine acetate-functionalized silica gel (Si-TMA acetate; eluting with 0.5% formic acid–acetonitrile). The fractions containing product were collected, combined, and concentrated to provide a 2.3:1 mixture of the acids **S10** and **S10'** as a white solid (146 mg, 34%). The product mixture so obtained was used without further purification.

^{1}H NMR (**S10**, 400 MHz, DMSO-d_6) δ 8.81 (t, J = 6.0 Hz, 1H, H_{10}), 8.47 (s, 1H, H_{13}), 8.29 (bs, 1H, H_7), 8.25 (s, 1H, H_{12}), 6.59 (s, 1H, H_6), 4.60 (d, J = 5.9 Hz, 2H, H_{11}), 4.15 (app p, J = 6.5 Hz, 1H, H_3), 3.43 (s, 2H, H_9), 3.40 – 3.26 (m, 1H, H_5), 2.75 (dt, J = 18.9, 10.0 Hz, 1H, H_5), 1.95 – 1.77 (m, 1H, H_4), 1.56 – 1.47 (m, 1H, H_4), 1.47 (s, 9H, H_1), 1.23 (s, 6H, H_8), 1.13 (d, J = 6.4 Hz, 3H, H_2). ^{1}H NMR (**S10'**, 400 MHz, DMSO-d_6) δ 8.87 (t, J = 6.1 Hz, 1H, $H_{10'}$), 8.61 (bs, 1H, $H_{7'}$), 8.47 (s, 1H, $H_{13'}$), 8.25 (s, 1H, $H_{12'}$), 6.71 – 6.64 (m, 1H), 4.60 (d, J = 5.9 Hz, 2H, $H_{11'}$), 3.51 (s, 2H, $H_{9'}$), 3.39 – 3.27 (m, 3H, $H_{3'}$, $H_{6'}$), 2.54 – 2.44 (m, 2H, $H_{5'}$), 1.56 – 1.45 (m, 2H, $H_{4'}$), 1.37 (s, 9H, $H_{1'}$), 1.27 (s, 6H, $H_{8'}$), 0.98 (d, J = 6.5 Hz, 3H, $H_{2'}$). ^{13}C NMR (**S10**, 101 MHz, DMSO-d_6) δ 204.9 (C), 171.4 (C), 167.6 (C), 167.5 (C), 163.1 (C), 162.0 (C), 153.3 (C), 151.2 (C), 148.1 (C), 147.1 (C), 128.9 (CH), 118.3 (CH), 98.2 (CH), 80.8 (C), 59.9 (C), 56.0 (CH), 43.0 (CH_2), 40.6 (CH_2), 28.8 (CH_2), 27.8 (CH_2), 27.8 (CH_3), 23.4 (CH_3), 23.3 (CH_3), 19.3 (CH_3). ^{13}C NMR (**S10'**, 101 MHz, DMSO-d_6) δ 204.7 (C), 204.6 (C), 171.4 (C), 167.3 (C), 166.5 (C), 163.1 (C), 162.0 (C), 155.1 (C), 148.1 (C), 147.1 (C), 128.9 (CH), 118.3 (CH), 77.4 (C), 60.4 (C), 50.0 (CH_2), 45.2 (CH), 43.4 (CH_2), 40.6 (CH_2), 38.8 (CH_2), 29.9 (CH_2), 28.3 (CH_3), 23.4 (CH_3), 23.3 (CH_3), 20.9 (CH_3). IR (ATR-FTIR), cm^{-1}: 3297 (br w), 2976 (w), 2933 (w), 1711 (s), 1648 (s), 1244 (m), 1158 (s), 754 (m), 635 (w). HRMS-CI (m/z): [M + H]$^+$ calcd for $C_{26}H_{34}N_5O_7S_2$, 592.1894; found, 592.1891. $[\alpha]_D^{20}$ +15.0 (*c* 1.0, CH_3OH).

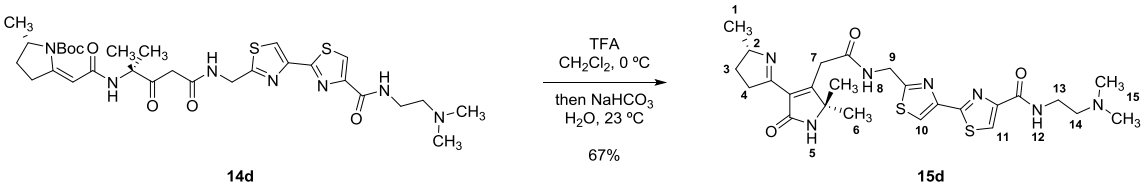
*Synthesis of the amide **14d**:*



A solution of T3P in ethyl acetate (50 wt%, 154 μL, 259 μmol, 1.50 equiv) and 4-methylmorpholine (94.9 μL, 863 μmol, 5.00 equiv) were added in sequence to a solution of the acid **S10** (102.1 mg, 173 μmol, 1 equiv) in tetrahydrofuran (1.2 mL) at 23 °C. The reaction mixture was stirred for 20 min at 23 °C. *N*,*N*-Dimethylethylenediamine (47.1 μL, 431 μmol, 2.50 equiv) was added to the reaction mixture. The resulting mixture was stirred for 7 h at 23 °C. The product mixture was concentrated. The concentrated product mixture was diluted with ethyl acetate (10 mL). The diluted product mixture was poured into a separatory funnel that had been charged with saturated aqueous sodium bicarbonate solution (5.0 mL) and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (2 × 10 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The residue obtained was purified by flash-column chromatography (eluting with 15% methanol–dichloromethane initially, grading to 40% methanol–dichloromethane, linear gradient) to provide the amide **14d** as a white solid (94.7 mg, 83%).

^{1}H NMR (500 MHz, CD_2Cl_2) δ 8.05 (bs, 2H, H_{12}, H_{13}), 7.92 – 7.85 (m, 2H, H_{10}, H_{14}), 6.55 (s, 1H, H_6), 5.97 (bs, 1H, H_7), 4.75 (d, *J* = 6.0 Hz, 2H, H_{11}), 4.20 (app p, *J* = 6.7 Hz, 1H, H_3), 3.67 – 3.60 (m, 2H, H_{15}), 3.54 (s, 2H, H_9), 3.37 (dd, *J* = 18.3, 9.0 Hz, 1H, H_5), 2.86 – 2.71 (m, 3H, H_5, H_{16}), 2.50 (s, 6H, H_{17}), 1.86 (ddd, *J* = 20.7, 12.1, 8.6 Hz, 1H, H_4), 1.50 (s, 10H, H_1, H_4), 1.34 (s, 6H, H_8), 1.14 (d, *J* = 6.4 Hz, 3H, H_2). ^{13}C NMR (126 MHz, CD_2Cl_2) δ 206.1 (C), 170.7 (C), 169.3 (C), 168.0 (C), 163.2 (C), 161.7 (C), 156.9 (C), 152.4 (C), 151.4 (C), 148.8 (C), 123.9 (CH), 118.1 (CH), 96.9 (CH), 82.2 (C), 61.2 (C), 58.7 (CH_2), 57.4 (CH), 45.2 (CH_3), 43.3 (CH_2), 41.7 (CH_2), 36.7 (CH_2), 30.1 (CH_2), 28.8 (CH_2), 28.5 (CH_3), 24.3 (CH_3), 24.3 (CH_3), 19.8 (CH_3). IR (ATR-FTIR), cm^{-1}: 3306 (br w), 2974 (w), 2934 (w), 1712 (m), 1654 (s), 1601 (w), 1540 (m), 1245 (m), 1156 (s), 620 (w). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{30}H_{44}N_7O_6S_2$, 662.2789; found, 662.2787. $[\alpha]_D^{20}$ –3.0 (*c* 1.0, CH_2Cl_2).

*Synthesis of the lactam **15d**:*

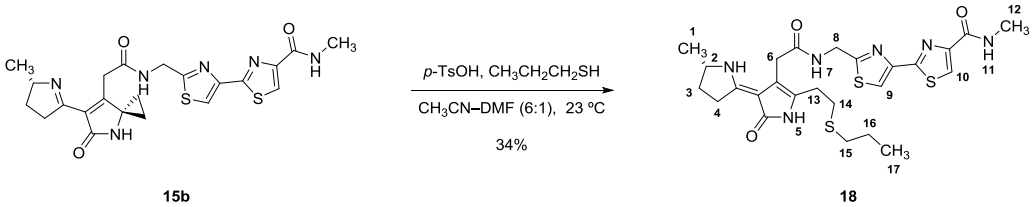


14d **15d**

Trifluoroacetic acid (31.9 μL, 417 μmol, 120 equiv) was added dropwise via syringe to a solution of the amide **14d** (2.3 mg, 3.48 μmol, 1 equiv) in dichloromethane (200 μL) at 0 °C. The reaction mixture was stirred for 14 h at 0 °C. The reaction mixture was concentrated. The concentrated reaction mixture was diluted with saturated aqueous sodium bicarbonate solution (200 μL). The diluted reaction mixture was stirred for 1 h at 23 °C. The product mixture was diluted sequentially with water (1.0 mL) and ethyl acetate (3.0 mL). The diluted product mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (5 × 3.0 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. To induce complete cyclization, the residue obtained was diluted with dichloromethane (1.0 mL) and left to stand for 3 h at 23 °C. The diluted product mixture was concentrated to provide the lactam **15d** as an off-white solid (1.3 mg, 67%).

^{1}H NMR (500 MHz, CD_2Cl_2) δ 10.84 (t, *J* = 6.0 Hz, 1H, H_8), 8.05 (s, 1H, H_{11}), 7.92 (s, 1H, H_{10}), 7.66 (bs, 1H, H_{12}), 6.23 (bs, 1H, H_5), 4.71 – 4.65 (m, 2H, H_9), 4.23 – 4.15 (m, 1H, H_2), 3.59 – 3.45 (m, 4H, H_7, H_{13}), 3.21 – 3.11 (m, 1H, H_4), 3.03 – 2.90 (m, 1H, H_4), 2.54 – 2.47 (m, 2H, H_{14}), 2.27 (s, 6H, H_{15}), 2.21 – 2.13 (m, 1H, H_3), 1.44 – 1.40 (m, 7H, H_3, H_6), 1.25 (d, *J* = 6.8 Hz, 3H, H_1). ^{13}C NMR (126 MHz, CD_2Cl_2) δ 171.2 (C), 169.8 (C), 169.8 (C), 169.2 (C), 163.6 (C), 163.0 (C), 161.3 (C), 151.7 (C), 149.0 (C), 127.9 (C), 123.7 (CH), 117.4 (CH), 67.9 (CH), 62.1 (C), 58.8 (CH_2), 45.7 (CH_3), 41.4 (CH_2), 37.5 (CH_2), 37.5 (CH_2), 36.5 (CH_2), 30.8 (CH_2), 25.5 (CH_3), 25.5 (CH_3), 22.4 (CH_3). IR (ATR-FTIR), cm^{-1}: 3277 (br w), 2961 (w), 1655 (s), 1604 (w), 1543 (s), 1187 (m), 619 (m). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{25}H_{34}N_7O_3S_2$, 544.2159; found, 544.2164. $[\alpha]_D^{20}$ –15.0 (*c* 1.0, CH_2Cl_2).

*Synthesis of the thioether **18**:*

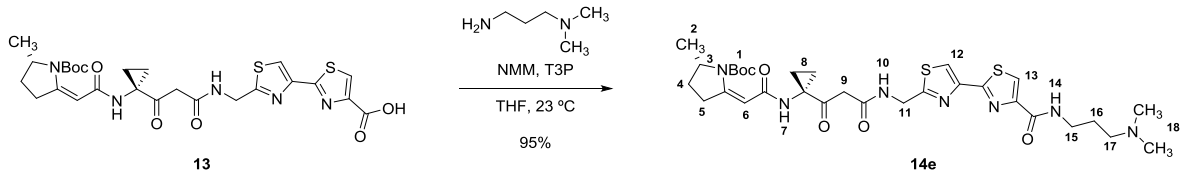


15b **18**

Propanethiol (100 μL) and *p*-toluenesulfonic acid monohydrate (2.0 mg, 10.3 μmol, 1.00 equiv) were added in sequence to a solution of the amide **15b** (5.0 mg, 10.3 μmol, 1 equiv) in acetonitrile (300 μL) at 23 °C. The reaction mixture was stirred for 30 min at 23 °C. *N*,*N*-Dimethylformamide (50 μL) was added to the reaction mixture at 23 °C. The reaction mixture was stirred for 3 h at 23 °C. The product mixture was diluted sequentially with toluene (5.0 mL) and hexanes (3.0 mL). The diluted product mixture was filtered and the filtrate was concentrated. The residue obtained was dried by azeotropic distillation with toluene (10 mL) to provide the thioether **18** as a light yellow solid (1.7 mg, 34%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.34 – 9.26 (m, 1H, H_7), 8.41 – 8.36 (m, 2H, H_5, H_{11}), 8.25 (s, 1H, H_{10}), 8.18 (s, 1H, H_9), 4.60 – 4.55 (m, 2H, H_8), 3.93 – 3.85 (m, 1H, H_2), 3.32 (d, *J* = 17.0 Hz, 1H, H_6), 3.28 (d, *J* = 16.8 Hz, 1H, H_6), 3.02 – 2.92 (m, 1H, H_4), 2.91 – 2.84 (m, 1H, H_4), 2.82 (bs, 3H, H_{12}), 2.62 – 2.54 (m, 4H, H_{13}, H_{14}), 2.49 – 2.42 (m, 2H, H_{15}), 2.14 – 2.07 (m, 1H, H_3), 1.55 – 1.41 (m, 3H, H_3, H_{16}), 1.17 (d, *J* = 6.8 Hz, 3H, H_1), 0.90 (t, *J* = 7.2 Hz, 3H, H_{17}). ^{1}H NMR (400 MHz, CD_3OD) δ 8.19 – 8.14 (m, 2H, H_9, H_{10}), 4.71 (d, *J* = 5.2 Hz, 2H, H_8), 4.12 – 3.99 (m, 1H, H_2), 3.49 (s, 2H, H_6), 3.04 – 2.89 (m, 5H, H_4, H_{12}), 2.77 – 2.65 (m, 4H, H_{13}, H_{14}), 2.44 (t, *J* = 7.2 Hz, 2H, H_{15}), 2.32 – 2.19 (m, 1H, H_3), 1.67 – 1.56 (m, 1H, H_3), 1.54 (app q, *J* = 7.3 Hz, 2H, H_{16}), 1.34 – 1.24 (m, 3H, H_1), 0.93 (t, *J* = 7.3 Hz, 3H, H_{17}). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.8 (C), 171.3 (C), 161.7 (C), 160.9 (C), 160.8 (C), 150.9 (C), 147.2 (C), 125.8 (C), 123.7 (CH), 117.9 (CH), 104.3 (C), 96.1 (C), 54.6 (CH), 40.6 (CH_2), 33.1 (CH_2), 32.0 (CH_2), 30.4 (CH_2), 30.2 (CH_2), 29.2 (CH_2), 25.8 (CH_3), 25.7 (CH_2), 22.5 (CH_2), 21.3 (CH_3), 13.3 (CH_3). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{25}H_{33}N_6O_3S_3$, 561.1771; found, 561.1776. $[\alpha]_D^{20}$ +60.5 (*c* 0.22, DMSO-d_6).

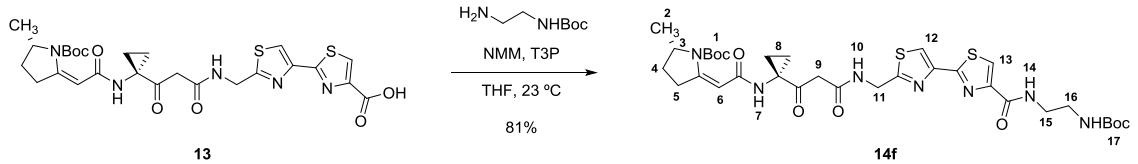
*Synthesis of the amide **14e**:*



A solution of T3P in ethyl acetate (50 wt%, 30.3 μL, 50.9 μmol, 1.50 equiv) and 4-methylmorpholine (18.6 μL, 170 μmol, 5.00 equiv) were added in sequence to a solution of the acid **13** (20.0 mg, 33.9 μmol, 1 equiv) in tetrahydrofuran (680 μL) at 23 °C. The reaction mixture was stirred for 20 min at 23 °C. *N*,*N*-Dimethyl-1,3-diaminopropane (10.7 μL, 84.8 μmol, 2.50 equiv) was then added to the reaction mixture. The resulting mixture was stirred for 7 h at 23 °C. The product mixture was concentrated. The concentrated product mixture was diluted with ethyl acetate (10 mL). The diluted product mixture was poured into a separatory funnel that had been charged with saturated aqueous sodium bicarbonate solution (5.0 mL) and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (2 × 10 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the amide **14e** as a white solid (21.8 mg, 95%). The product so obtained was used without further purification.

^{1}H NMR (500 MHz, CD_2Cl_2) δ 8.33 – 8.22 (m, 1H, H_{14}), 8.11 – 8.05 (m, 1H, H_{10}), 8.03 (s, 1H, H_{13}), 7.90 (s, 1H, H_{12}), 6.55 (s, 1H, H_6), 6.29 (bs, 1H, H_7), 4.76 (d, *J* = 5.9 Hz, 2H, H_{11}), 4.30 – 4.16 (m, 1H, H_3), 3.66 (s, 2H, H_9), 3.53 – 3.45 (m, 2H, H_{15}), 3.48 – 3.42 (m, 1H, H_5), 2.99 – 2.80 (m, 1H, H_5), 2.41 (t, *J* = 6.5 Hz, 2H, H_{17}), 2.25 (s, 6H, H_{18}), 1.92 (tt, *J* = 11.9, 8.5 Hz, 1H, H_4), 1.83 – 1.70 (m, 2H, H_{16}), 1.62 (app q, *J* = 4.4 Hz, 2H, H_8), 1.56 (dd, *J* = 12.4, 8.4 Hz, 1H, H_4), 1.49 (s, 9H, H_1), 1.17 (d, *J* = 6.4 Hz, 3H, H_2), 1.18 – 1.12 (m, 2H, H_8). ^{13}C NMR (126 MHz, CD_2Cl_2) δ 206.1 (C), 170.6 (C), 170.4 (C), 167.2 (C), 162.8 (C), 161.3 (C), 156.8 (C), 152.4 (C), 152.1 (C), 149.0 (C), 123.4 (CH), 117.3 (CH), 97.3 (CH), 82.2 (C), 58.7 (CH_2), 57.4 (CH), 45.8 (CH_3), 45.6 (CH_2), 42.0 (C), 41.7 (CH_2), 39.2 (CH_2), 30.2 (CH_2), 28.5 (CH_3), 28.5 (CH_2), 27.3 (CH_2), 21.6 (CH_2), 21.6 (CH_2), 19.8 (CH_3). IR (ATR-FTIR), cm^{-1}: 3282 (w), 2976 (w), 1709 (m), 1650 (s), 1606 (w), 1542 (m), 1290 (m), 1155 (s), 770 (w). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{31}H_{44}N_7O_6S_2$, 674.2789; found, 674.2795. $[\alpha]_D^{20}$ +33.0 (*c* 1.0, CH_2Cl_2).

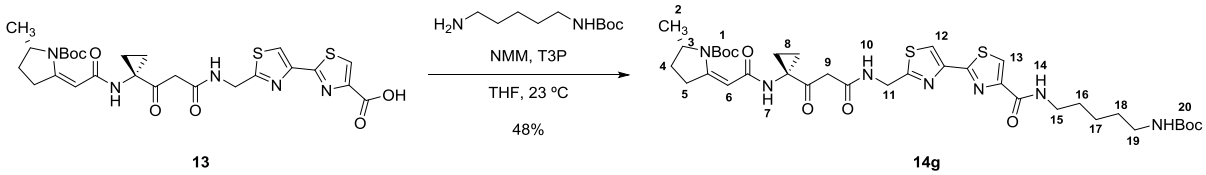
*Synthesis of the amide **14f**:*



A solution of T3P in ethyl acetate (50 wt%, 22.7 μL, 38.2 μmol, 1.50 equiv) and 4-methylmorpholine (14.0 μL, 127 μmol, 5.00 equiv) were added in sequence to a solution of the acid **13** (15.0 mg, 25.4 μmol, 1 equiv) in tetrahydrofuran (510 μL) at 23 °C. The reaction mixture was stirred for 20 min at 23 °C. *tert*-Butyl-*N*-(2-aminoethyl)carbamate (9.1 μL, 57.2 μmol, 2.25 equiv) was added to the reaction mixture. The resulting mixture was stirred for 7 h at 23 °C. The product mixture was concentrated. The residue obtained was purified by flash-column chromatography (eluting with dichloromethane initially, grading to 10% methanol–dichloromethane, linear gradient) to provide the amide **14f** as an off-white solid (15.1 mg, 81%).

^{1}H NMR (600 MHz, CD_2Cl_2) δ 8.10 – 8.04 (m, 2H, H_{10}, H_{13}), 7.96 (s, 1H, H_{12}), 7.76 (bs, 1H, H_{14}), 6.55 (s, 1H, H_6), 6.24 (bs, 1H, H_7), 5.08 (bs, 1H), 4.76 (d, *J* = 4.1 Hz, 2H, H_{11}), 4.32 – 4.19 (m, 1H, H_3), 3.67 (s, 2H, H_9), 3.57 – 3.50 (m, 2H, H_{15}), 3.48 (dd, *J* = 18.3, 8.8 Hz, 1H, H_5), 3.38 – 3.33 (m, 2H, H_{16}), 2.96 – 2.81 (m, 1H, H_5), 2.03 – 1.85 (m, 1H, H_4), 1.65 – 1.59 (m, 2H, H_8), 1.61 – 1.52 (m, 1H, H_4), 1.50 (s, 9H, H_1), 1.40 (s, 9H, H_{17}), 1.20 – 1.13 (m, 5H, H_2, H_8). ^{13}C NMR (126 MHz, CD_2Cl_2) δ 206.2 (C), 170.6 (C), 170.4 (C), 167.3 (C), 163.0 (C), 162.1 (C), 156.8 (C), 156.8 (C), 152.4 (C), 151.3 (C), 148.8 (C), 123.9 (CH), 117.8 (CH), 97.3 (CH), 82.2 (C), 79.7 (C), 57.4 (CH), 45.7 (CH_2), 42.0 (C), 41.6 (CH_2), 41.2 (CH_2), 40.5 (CH_2), 30.2 (CH_2), 28.8 (CH_2), 28.6 (CH_3), 28.5 (CH_3), 21.6 (CH_2), 21.6 (CH_2), 19.8 (CH_3). IR (ATR-FTIR), cm^{-1}: 3313 (br w), 2970 (w), 2930 (w), 1704 (m), 1650 (m), 1524 (w), 1154 (s), 1025 (w), 802 (m). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{33}H_{46}N_7O_8S_2$, 732.2844; found, 732.2852. $[\alpha]_D^{20}$ +4.0 (*c* 1.0, CH_2Cl_2).

*Synthesis of the amide **14g**:*

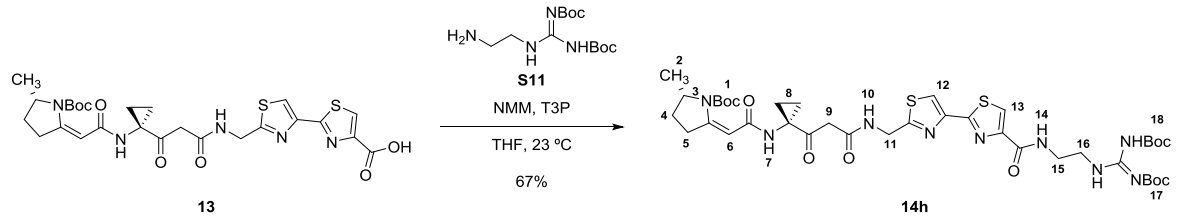


13 14g

A solution of T3P in ethyl acetate (50 wt%, 30.3 μL, 50.9 μmol, 1.50 equiv) and 4-methylmorpholine (18.6 μL, 170 μmol, 5.00 equiv) were added in sequence to a solution of the acid **13** (20.0 mg, 33.9 μmol, 1 equiv) in tetrahydrofuran (670 μL) at 23 °C. The reaction mixture was stirred for 20 min at 23 °C. *tert*-Butyl-*N*-(2-aminopentyl)carbamate (17.7 μL, 84.8 μmol, 2.50 equiv) was added to the reaction mixture. The resulting mixture was stirred for 7 h at 23 °C. The product mixture was concentrated. The residue obtained was purified by flash-column chromatography (eluting with dichloromethane initially, grading to 10% methanol–dichloromethane, linear gradient) to provide the amide **14g** as an off-white solid (12.6 mg, 48%).

^{1}H NMR (500 MHz, CD_2Cl_2) δ 8.09 – 8.04 (m, 1H, H_{10}), 8.05 (s, 1H, H_{13}), 7.96 (s, 1H, H_{12}), 7.42 (t, *J* = 6.2 Hz, 1H, H_{14}), 6.55 (s, 1H, H_6), 6.27 (bs, 1H, H_7), 4.76 (d, *J* = 6.0 Hz, 2H, H_{11}), 4.64 (bs, 1H), 4.23 (app p, *J* = 6.7 Hz, 1H, H_3), 3.66 (s, 2H, H_9), 3.51 – 3.44 (m, 1H, H_5), 3.43 (app q, *J* = 6.7 Hz, 2H, H_{15}), 3.13 – 3.05 (m, 2H, H_{19}), 2.95 – 2.84 (m, 1H, H_5), 1.92 (tt, *J* = 12.6, 8.6 Hz, 1H, H_4), 1.68 – 1.60 (m, 4H, H_8, H_{16}), 1.60 – 1.53 (m, 1H, H_4), 1.54 – 1.50 (m, 2H, H_{18}), 1.50 (s, 9H, H_1), 1.43 – 1.37 (m, 11H, H_{17}, H_{20}), 1.18 (d, *J* = 6.5 Hz, 3H, H_2), 1.18 – 1.12 (m, 2H, H_8). ^{13}C NMR (126 MHz, CD_2Cl_2) δ 206.1 (C), 170.6 (C), 170.4 (C), 167.2 (C), 163.0 (C), 161.3 (C), 156.8 (C), 156.4 (C), 152.4 (C), 151.7 (C), 148.8 (C), 123.6 (CH), 117.7 (CH), 97.3 (CH), 82.2 (C), 79.2 (C), 57.4 (CH), 45.6 (CH_2), 42.0 (C), 41.7 (CH_2), 41.0 (CH_2), 39.7 (CH_2), 30.3 (CH_2), 30.2 (CH_2), 30.1 (CH_2), 28.9 (CH_2), 28.7 (CH_3), 28.5 (CH_3), 24.7 (CH_2), 21.6 (CH_2), 21.6 (CH_2), 19.9 (CH_3). IR (ATR-FTIR), cm^{-1}: 3305 (br m), 2970 (w), 2932 (w), 1699 (s), 1653 (s), 1541 (m), 1242 (m), 1156 (s), 1026 (m), 621 (w). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{36}H_{52}N_7O_8S_2$, 774.3313; found, 774.3309. $[\alpha]_D^{20}$ +30.0 (*c* 0.9, CH_2Cl_2).

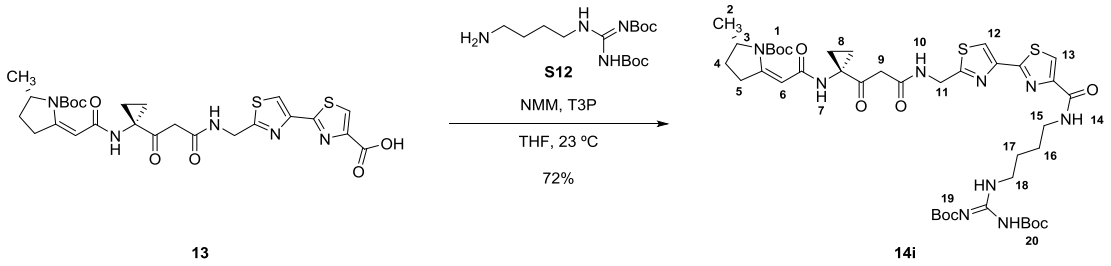
*Synthesis of the amide **14h**:*



A solution of T3P in ethyl acetate (50 wt%, 22.7 μL, 38.2 μmol, 1.50 equiv) and 4-methylmorpholine (14.0 μL, 127 μmol, 5.00 equiv) were added in sequence to a solution of the acid **13** (15.0 mg, 25.4 μmol, 1 equiv) in tetrahydrofuran (510 μL) at 23 °C. The reaction mixture was stirred for 20 min at 23 °C. The amine **S11** (17.3 mg, 57.2 μmol, 2.25 equiv) was added to the reaction mixture. The resulting mixture was stirred for 7 h at 23 °C. The product mixture was concentrated. The residue obtained was purified by flash-column chromatography (eluting with dichloromethane initially, grading to 10% methanol–dichloromethane, linear gradient) to provide the amide **14h** as an off-white solid (14.9 mg, 67%).

^{1}H NMR (500 MHz, CD_2Cl_2) δ 11.50 (bs, 1H), 8.49 (bs, 1H), 8.11 – 8.03 (m, 2H, H_{10}, H_{13}), 7.96 (s, 1H, H_{12}), 7.70 – 7.64 (m, 1H, H_{14}), 6.55 (s, 1H, H_6), 6.25 (bs, 1H, H_7), 4.75 (d, *J* = 5.9 Hz, 2H, H_{11}), 4.29 – 4.14 (m, 1H, H_3), 3.66 (s, 2H, H_9), 3.65 – 3.58 (m, 4H, H_{15}, H_{16}), 3.47 (dd, *J* = 18.3, 8.7 Hz, 1H, H_5), 2.94 – 2.82 (m, 1H, H_5), 1.98 – 1.86 (m, 1H, H_4), 1.65 – 1.59 (m, 2H, H_8), 1.60 – 1.52 (m, 1H, H_4), 1.49 (s, 9H, H_1), 1.48 (s, 9H, H_{17}), 1.44 (s, 9H, H_{18}), 1.17 (d, *J* = 6.2 Hz, 3H, H_2), 1.18 – 1.12 (m, 2H, H_8). ^{13}C NMR (126 MHz, CD_2Cl_2) δ 206.1 (C), 170.6 (C), 170.3 (C), 167.2 (C), 164.0 (C), 163.0 (C), 161.8 (C), 157.2 (C), 156.9 (C), 153.6 (C), 152.4 (C), 151.3 (C), 148.8 (C), 123.9 (CH), 117.8 (CH), 97.2 (CH), 83.7 (C), 82.2 (C), 79.5 (C), 57.4 (CH), 45.6 (CH_2), 42.0 (C), 41.6 (CH_2), 40.9 (CH_2), 39.2 (CH_2), 30.2 (CH_2), 28.8 (CH_3), 28.8 (CH_2), 28.6 (CH_3), 28.5 (CH_3), 21.6 (CH_2), 21.6 (CH_2), 19.8 (CH_3). IR (ATR-FTIR), cm^{-1}: 3323 (br w), 2976 (w), 2934 (w), 1716 (m), 1639 (s), 1611 (s), 1544 (w), 1316 (m), 1289 (m), 1133 (s), 1024 (m), 771 (w). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{39}H_{56}N_9O_{10}S_2$, 874.3586; found, 874.3583. $[\alpha]_D^{20}$ +27.0 (*c* 1.0, CH_2Cl_2).

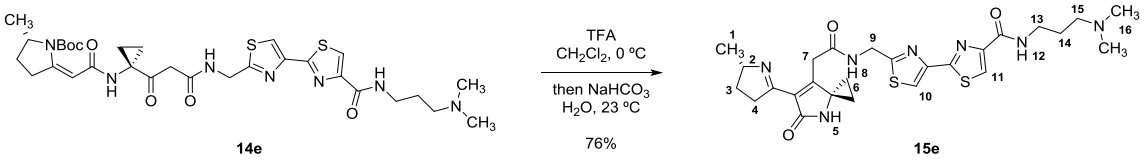
*Synthesis of the amide **14i**:*



A solution of T3P in ethyl acetate (50 wt%, 30.3 μL, 50.9 μmol, 1.50 equiv) and 4-methylmorpholine (18.6 μL, 170 μmol, 5.00 equiv) were added in sequence to a solution of the acid **13** (20.0 mg, 33.9 μmol, 1 equiv) in tetrahydrofuran (680 μL) at 23 °C. The reaction mixture was stirred for 20 min at 23 °C. The amine **S12** (39.2 mg, 119 μmol, 3.50 equiv) was added to the reaction mixture. The resulting mixture was stirred for 7 h at 23 °C. The product mixture was concentrated. The residue obtained was purified by flash-column chromatography (eluting with dichloromethane initially, grading to 10% methanol–dichloromethane, linear gradient) to provide the amide **14i** as an off-white solid (22.1 mg, 72%).

^{1}H NMR (600 MHz, CD_2Cl_2) δ 11.50 (bs, 1H), 8.29 (t, *J* = 5.5 Hz, 1H), 8.11 (t, *J* = 6.0 Hz, 1H, H_{10}), 8.06 (s, 1H, H_{13}), 7.95 (s, 1H, H_{12}), 7.49 (t, *J* = 6.2 Hz, 1H, H_{14}), 6.56 (s, 1H, H_6), 6.39 (bs, 1H, H_7), 4.76 (d, *J* = 6.2 Hz, 2H, H_{11}), 4.34 – 4.16 (m, 1H, H_3), 3.66 (s, 2H, H_9), 3.56 – 3.43 (m, 3H, H_5, H_{15}), 3.41 (app q, *J* = 6.1 Hz, 2H, H_{18}), 2.89 (dt, *J* = 18.6, 9.8 Hz, 1H, H_5), 1.92 (ddd, *J* = 20.9, 12.0, 8.4 Hz, 1H, H_4), 1.71 – 1.63 (m, 4H, H_{16}, H_{17}), 1.64 – 1.59 (m, 2H, H_8), 1.60 – 1.52 (m, 1H, H_4), 1.49 (s, 9H, H_1), 1.49 (s, 9H, H_{19}), 1.44 (s, 9H, H_{20}), 1.17 (d, *J* = 6.6 Hz, 3H, H_2), 1.16 – 1.13 (m, 2H, H_8). ^{13}C NMR (126 MHz, CD_2Cl_2) δ 206.1 (C), 170.6 (C), 170.3 (C), 167.2 (C), 164.1 (C), 163.0 (C), 161.4 (C), 156.9 (C), 156.7 (C), 153.8 (C), 152.4 (C), 151.7 (C), 148.8 (C), 123.7 (CH), 117.7 (CH), 97.2 (CH), 83.6 (C), 82.2 (C), 79.3 (C), 57.5 (CH), 45.6 (CH_2), 42.0 (C), 41.7 (CH_2), 41.0 (CH_2), 39.5 (CH_2), 30.2 (CH_2), 28.9 (CH_2), 28.6 (CH_3), 28.5 (CH_3), 28.4 (CH_3), 27.8 (CH_2), 27.2 (CH_2), 21.6 (CH_2), 21.6 (CH_2), 19.9 (CH_3). IR (ATR-FTIR), cm^{-1}: 3324 (br w), 2974 (w), 2935 (w), 1716 (m), 1639 (s), 1612 (s), 1366 (m), 1316 (m), 1290 (m), 1155 (s), 1132 (s), 1051 (m), 1026 (m), 771 (w). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{41}H_{60}N_9O_{10}S_2$, 902.3899; found, 902.3901. $[\alpha]_D^{20}$ +15.0 (*c* 1.0, CH_2Cl_2).

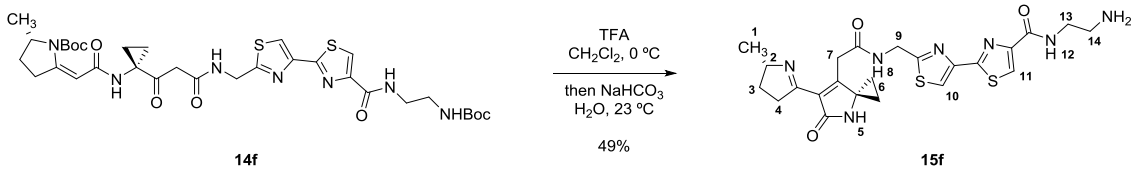
*Synthesis of the lactam **15e**:*



Trifluoroacetic acid (219 μL, 2.87 mmol, 120 equiv) was added dropwise via syringe to a solution of the amide **14e** (16.1 mg, 23.9 μmol, 1 equiv) in dichloromethane (600 μL) at 0 °C. The reaction mixture was stirred for 14 h at 0 °C. The reaction mixture was concentrated. The concentrated reaction mixture was diluted with saturated aqueous sodium bicarbonate solution (1.3 mL). The diluted reaction mixture was stirred for 1 h at 23 °C. The product mixture was diluted sequentially with water (1.0 mL) and ethyl acetate (3.0 mL). The diluted product mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (5 × 3.0 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the lactam **15e** as a light yellow solid (10.1 mg, 76%). The product so obtained was used without further purification.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.29 (t, J = 5.9 Hz, 1H, H_8), 8.70 – 8.58 (m, 2H, H_5, H_{12}), 8.24 (s, 1H, H_{11}), 8.17 (s, 1H, H_{10}), 4.65 – 4.57 (m, 2H, H_9), 4.16 – 4.08 (m, 1H, H_2), 3.35 – 3.29 (m, 4H, H_7, H_{13}), 3.15 – 3.02 (m, 1H, H_4), 2.86 (dt, J = 17.7, 8.8 Hz, 1H, H_4), 2.28 (t, J = 6.9 Hz, 2H, H_{15}), 2.15 (s, 6H, H_{16}), 2.12 – 2.04 (m, 1H, H_3), 1.71 – 1.63 (m, 4H, H_6, H_{14}), 1.41 – 1.31 (m, 3H, H_3, H_6), 1.18 (d, J = 6.6 Hz, 3H, H_1). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.2 (C), 169.7 (C), 168.4 (C), 168.3 (C), 161.7 (C), 160.3 (C), 157.7 (C), 151.0 (C), 147.5 (C), 127.4 (C), 123.9 (CH), 117.9 (CH), 66.6 (CH), 57.3 (CH_2), 45.3 (C), 45.2 (CH_3), 40.4 (CH_2), 37.7 (CH_2), 36.6 (CH_2), 33.5 (CH_2), 29.7 (CH_2), 26.9 (CH_2), 21.9 (CH_3), 11.9 (CH_2), 11.8 (CH_2). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{26}H_{34}N_7O_3S_2$, 556.2159; found, 556.2151. $[\alpha]_D^{20}$ –5.5 (*c* 3.1, DMSO-d_6).

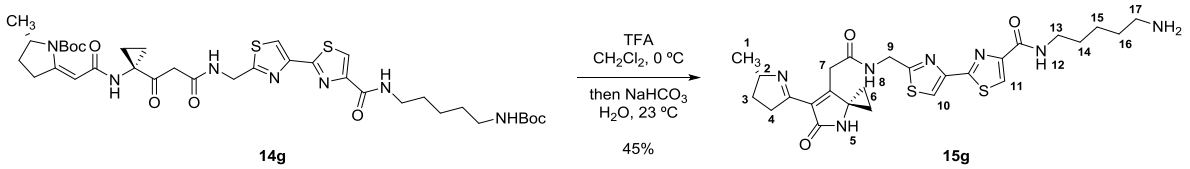
*Synthesis of the lactam **15f**:*



Trifluoroacetic acid (125 μL, 1.64 mmol, 120 equiv) was added dropwise via syringe to a solution of the amide **14f** (10.0 mg, 13.7 μmol, 1 equiv) in dichloromethane (340 μL) at 0 °C. The reaction mixture was stirred for 14 h at 0 °C. The reaction mixture was concentrated. The concentrated reaction mixture was diluted with saturated aqueous sodium bicarbonate solution (700 μL). The diluted reaction mixture was stirred for 1 h at 23 °C. The product mixture was diluted sequentially with water (1.0 mL) and ethyl acetate (3.0 mL). The diluted product mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (5 × 3.0 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the lactam **15f** as a light yellow solid (3.4 mg, 49%). The product so obtained was used without further purification.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.30 (t, J = 6.0 Hz, 1H, H_8), 8.62 (bs, 1H, H_5), 8.44 (t, J = 5.9 Hz, 1H, H_{12}), 8.28 (s, 1H, H_{11}), 8.19 (s, 1H, H_{10}), 4.64 – 4.58 (m, 2H, H_9), 4.17 – 4.08 (m, 1H, H_2), 3.38 – 3.29 (m, 4H, H_7, H_{13}), 3.14 – 3.06 (m, 1H, H_4), 2.87 (dt, J = 17.8, 8.7 Hz, 1H, H_4), 2.77 (t, J = 6.5 Hz, 2H, H_{14}), 2.10 – 2.04 (m, 1H, H_3), 1.71 – 1.66 (m, 2H, H_6), 1.41 – 1.30 (m, 3H, H_3, H_6), 1.19 (d, J = 6.7 Hz, 3H, H_1). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.2 (C), 169.7 (C), 168.4 (C), 168.3 (C), 161.7 (C), 160.6 (C), 157.7 (C), 150.9 (C), 147.4 (C), 127.4 (C), 124.2 (CH), 118.0 (CH), 66.6 (CH), 45.3 (C), 40.9 (CH_2), 40.7 (CH_2), 40.4 (CH_2), 36.6 (CH_2), 33.6 (CH_2), 29.7 (CH_2), 21.9 (CH_3), 11.9 (CH_2), 11.8 (CH_2). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{23}H_{28}N_7O_3S_2$, 514.1690; found, 514.1690. $[\alpha]_D^{20}$ –1.7 (*c* 1.2, DMSO-d_6).

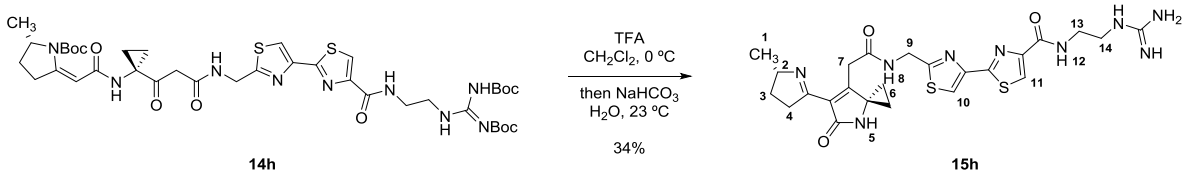
*Synthesis of the lactam **15g**:*



Trifluoroacetic acid (108 μL, 1.41 mmol, 120 equiv) was added dropwise via syringe to a solution of the amide **14g** (9.1 mg, 11.8 μmol, 1 equiv) in dichloromethane (290 μL) at 0 °C. The reaction mixture was stirred for 14 h at 0 °C. The reaction mixture was concentrated. The concentrated reaction mixture was diluted with saturated aqueous sodium bicarbonate solution (650 μL). The diluted reaction mixture was stirred for 1 h at 23 °C. The product mixture was diluted sequentially with water (1.0 mL) and ethyl acetate (3.0 mL). The diluted product mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (5 × 3.0 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the lactam **15g** as a light yellow solid (3.4 mg, 45%). The product so obtained was used without further purification.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.34 – 10.26 (m, 1H, H_8), 8.61 (bs, 1H, H_5), 8.44 – 8.38 (m, 1H, H_{12}), 8.25 (s, 1H, H_{11}), 8.19 (s, 1H, H_{10}), 4.64 – 4.59 (m, 2H, H_9), 4.17 – 4.07 (m, 1H, H_2), 3.35 – 3.26 (m, 4H, H_7, H_{13}), 3.15 – 3.05 (m, 1H, H_4), 2.87 (dt, J = 18.0, 8.9 Hz, 1H, H_4), 2.73 (t, J = 7.5 Hz, 2H, H_{17}), 2.15 – 2.05 (m, 1H, H_3), 1.72 – 1.66 (m, 2H, H_6), 1.58 – 1.50 (m, 4H, H_{14}, H_{16}), 1.39 – 1.32 (m, 5H, H_3, H_6, H_{15}), 1.19 (d, J = 6.7 Hz, 3H, H_1). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.1 (C), 169.6 (C), 168.4 (C), 168.3 (C), 161.7 (C), 160.3 (C), 157.7 (C), 151.0 (C), 147.5 (C), 127.4 (C), 124.0 (CH), 117.9 (CH), 66.5 (CH), 45.3 (C), 40.4 (CH_2), 39.3 (CH_2), 38.5 (CH_2), 36.5 (CH_2), 33.5 (CH_2), 29.7 (CH_2), 28.8 (CH_2), 27.9 (CH_2), 23.4 (CH_2), 21.9 (CH_3), 11.8 (CH_2), 11.8 (CH_2). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{26}H_{34}N_7O_3S_2$, 556.2159; found, 556.2167. $[\alpha]_D^{20}$ +2.5 (*c* 1.2, DMSO-d_6).

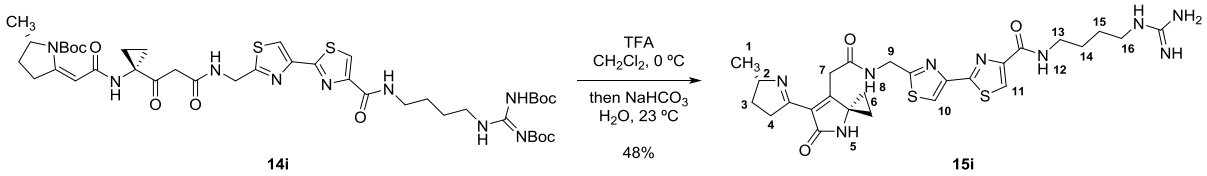
*Synthesis of the lactam **15h**:*



Trifluoroacetic acid (105 μL, 1.37 mmol, 120 equiv) was added dropwise via syringe to a solution of the amide **14h** (10.0 mg, 11.4 μmol, 1 equiv) in dichloromethane (290 μL) at 0 °C. The reaction mixture was stirred for 14 h at 0 °C. The reaction mixture was concentrated. The concentrated reaction mixture was diluted with saturated aqueous sodium bicarbonate solution (650 μL). The diluted reaction mixture was stirred for 1 h at 23 °C. The product mixture was diluted sequentially with water (1.0 mL) and ethyl acetate (3.0 mL). The diluted product mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (5 × 3.0 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the lactam **15h** as a light yellow solid (2.2 mg, 34%). The product so obtained was used without further purification.

^{1}H NMR (600 MHz, DMSO-d_6) δ 10.31 (t, J = 6.1 Hz, 1H, H_8), 8.58 (bs, 2H, H_5, H_{12}), 8.31 (s, 1H, H_{11}), 8.16 (s, 1H, H_{10}), 7.42 (bs, 1H), 4.65 – 4.60 (m, 2H, H_9), 4.17 – 4.08 (m, 1H, H_2), 3.47 – 3.41 (m, 2H, H_{13}), 3.36 – 3.30 (m, 4H, H_7, H_{14}), 3.14 – 3.07 (m, 1H, H_4), 2.87 (dt, J = 18.2, 8.9 Hz, 1H, H_4), 2.12 – 2.04 (m, 1H, H_3), 1.71 – 1.65 (m, 2H, H_6), 1.53 – 1.43 (m, 1H, H_3), 1.38 – 1.33 (m, 2H, H_6), 1.19 (d, J = 6.6 Hz, 3H, H_1). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.2 (C), 169.6 (C), 168.4 (C), 168.3 (C), 161.9 (C), 160.9 (C), 157.6 (C), 157.0 (C), 150.5 (C), 147.4 (C), 127.4 (C), 124.7 (CH), 117.9 (CH), 66.5 (CH), 45.3 (C), 40.4 (CH_2), 40.4 (CH_2), 38.0 (CH_2), 36.5 (CH_2), 33.5 (CH_2), 29.7 (CH_2), 21.9 (CH_3), 11.8 (CH_2), 11.7 (CH_2). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{24}H_{30}N_9O_3S_2$, 556.1908; found, 556.1913. $[\alpha]_D^{20}$ –5.5 (*c* 1.3, DMSO-d_6).

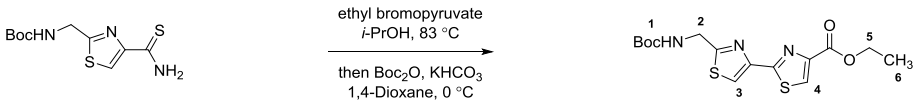
*Synthesis of the lactam **15i**:*



Trifluoroacetic acid (164 μL, 2.14 mmol, 120 equiv) was added dropwise via syringe to a solution of the amide **14i** (16.1 mg, 17.8 μmol, 1 equiv) in dichloromethane (450 μL) at 0 °C. The reaction mixture was stirred for 14 h at 0 °C. The reaction mixture was concentrated. The concentrated reaction mixture was diluted with saturated aqueous sodium bicarbonate solution (1.0 mL). The diluted reaction mixture was stirred for 1 h at 23 °C. The product mixture was diluted sequentially with water (1.0 mL) and ethyl acetate (3.0 mL). The diluted product mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (5 × 3.0 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the lactam **15i** as a light yellow solid (5.0 mg, 48%). The product so obtained was used without further purification.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.34 – 10.28 (m, 1H, H_8), 8.63 (bs, 1H, H_5), 8.50 (t, J = 6.2 Hz, 1H, H_{12}), 8.26 (s, 1H, H_{11}), 8.19 (s, 1H, H_{10}), 7.88 (bs, 1H), 4.64 – 4.58 (m, 2H, H_9), 4.16 – 4.08 (m, 1H, H_2), 3.34 – 3.27 (m, 4H, H_7, H_{13}), 3.15 – 3.05 (m, 3H, H_4, H_{16}), 2.87 (dt, J = 17.8, 8.8 Hz, 1H, H_4), 2.11 – 2.04 (m, 1H, H_3), 1.70 – 1.63 (m, 2H, H_6), 1.60 – 1.52 (m, 2H, H_{14}), 1.53 – 1.46 (m, 2H, H_{15}), 1.38 – 1.33 (m, 3H, H_3, H_6), 1.18 (d, J = 6.6 Hz, 3H, H_1). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.2 (C), 169.7 (C), 168.4 (C), 168.3 (C), 161.7 (C), 160.5 (C), 157.7 (C), 156.9 (C), 150.9 (C), 147.5 (C), 127.4 (C), 124.1 (CH), 117.9 (CH), 66.6 (CH), 45.3 (C), 40.4 (CH_2), 40.4 (CH_2), 38.2 (CH_2), 36.6 (CH_2), 33.6 (CH_2), 29.7 (CH_2), 26.5 (CH_2), 26.1 (CH_2), 21.9 (CH_3), 11.9 (CH_2), 11.8 (CH_2). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{26}H_{34}N_9O_3S_2$, 584.2221; found, 584.2221. $[\alpha]_D^{20}$ +3.1 (*c* 2.6, DMSO-d_6).

*Synthesis of the ethyl ester **S14**:*

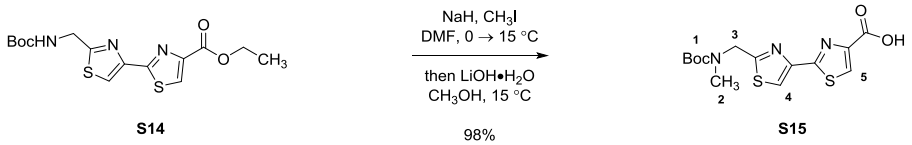


S13 **S14**

Ethyl bromopyruvate (3.01 g, 15.4 mmol, 1.50 equiv) was added to a solution of the thioamide **S13** (2.82 g, 10.3 mmol, 1 equiv) in *iso*-propanol (100 mL) at 23 °C. The reaction mixture was stirred for 16 h at 83 °C. The reaction mixture was concentrated. The residue obtained was dissolved in 1,4-dioxane (45 mL) and the resulting solution was cooled to 0 °C. Di-*tert*-butyl dicarbonate (3.60 g, 16.5 mmol, 1.60 equiv) and a solution of aqueous potassium bicarbonate (1N, 15 mL) were then added sequentially to the cooled solution. The reaction mixture was stirred for 16 h at 0 °C. The product mixture was concentrated and the residue obtained was applied to a trimethylamine acetate-functionalized silica column (Si-TMA acetate; eluting with 2% acetic acid–methanol) to provide the bithiazole **S14** as a white solid (2.51 g, 66%).

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.55 (s, 1H, H_4), 8.27 (s, 1H, H_3), 7.87 (t, J = 6.2 Hz, 1H), 4.45 (d, J = 6.1 Hz, 2H, H_2), 4.34 (q, J = 7.1 Hz, 2H, H_5), 1.42 (s, 9H, H_1), 1.33 (t, J = 7.1 Hz, 3H, H_6). ^{13}C NMR (151 MHz, DMSO-d_6) δ 172.9 (C), 162.5 (C), 160.7 (C), 155.8 (C), 147.1 (C), 147.0 (C), 129.4 (CH), 118.2 (CH), 78.7 (C), 60.9 (CH_2), 41.9 (CH_2), 28.2 (CH_3), 14.2 (CH_3). IR (ATR-FTIR), cm^{-1}: 3343 (m), 3130 (w), 3109 (w), 2983 (w), 1721 (s), 1686 (s), 1526 (s), 1298 (m), 1284 (m), 1204 (s), 1164 (s), 1100 (s), 819 (m), 770 (m), 621 (m). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{15}H_{20}N_3O_4S_2$, 370.0890; found, 370.0882.

*Synthesis of the acid **S15**:*

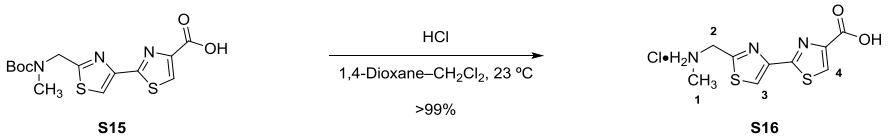


A dispersion of sodium hydride in mineral oil (60%, 109 mg, 2.83 mmol, 2.00 equiv) was added slowly to a solution of the bithiazole **S14** (523 mg, 1.42 mmol, 1 equiv) and iodomethane (793 μL, 12.7 mmol, 9.00 equiv) in *N*,*N*-dimethylformamide (6.0 mL) at –5 °C. The reaction mixture was stirred for 30 min at –5 °C and then was warmed to 15 °C. The warmed mixture was stirred for 14 h at 15 °C. Lithium hydroxide (891 mg, 21.2 mmol, 15.0 equiv) and water (6.0 mL) were then added in sequence to the reaction mixture. The reaction mixture was stirred for 3 h at 15 °C. The heterogeneous product mixture was filtered through a fritted funnel. The filter cake was washed with methanol (10 mL). The filtrates were combined and the combined filtrates were concentrated. The product mixture was diluted sequentially with saturated aqueous ammonium chloride solution (10 mL) and ethyl acetate (10 mL). The diluted product mixture was transferred to a separatory funnel and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (3 × 5.0 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated. The concentrated product mixture was applied to a column containing trimethylamine acetate-functionalized silica gel (Si-TMA acetate; eluting with 2% acetic acid–methanol). The fractions containing product were collected, combined, and concentrated to provide the acid **S15** as a white solid (495 mg, 98%).

In DMSO-d_6 at room temperature the title compound exists as an approximate 1:1 mixture of amide-bond rotamers.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.36 (s, 1H, H_5), 8.28 (s, 1H, H_4), 4.71 (s, 2H, H_3), 2.92 (d, 3H, H_2), 1.42 (d, 9H, H_1). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.9 (C), 162.4 (C), 161.6 (C), 154.7 (d, C), 150.1 (C), 147.5 (C), 127.6 (CH), 118.1 (CH), 79.6 (C), 49.8 (d, CH_2), 34.7 (d, CH_3), 27.9 (d, CH_3). IR (ATR-FTIR), cm^{-1}: 3113 (w), 2975 (w), 1681 (s), 1484 (m), 1392 (m), 1295 (m), 1240 (m), 1167 (s), 751 (m), 564 (w). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{14}H_{17}N_3NaO_4S_2$, 378.0553; found, 378.0554.

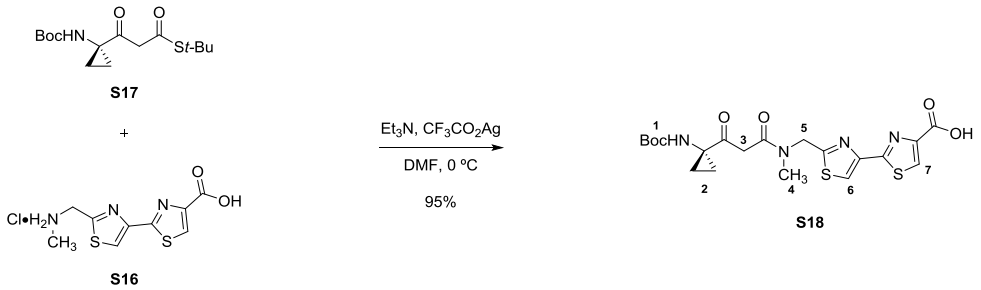
*Synthesis of the hydrochloride salt **S16**:*



A solution of hydrogen chloride in 1,4-dioxane (4.0 N, 8.0 mL, 32.0 mmol, 24.1 equiv) was added dropwise via syringe to a solution of the bithiazole **S15** (472 mg, 1.33 mmol, 1 equiv) in dichloromethane (24 mL) at 23 °C. The resulting mixture was stirred for 1 h at 23 °C. The product mixture was concentrated to provide the hydrochloride salt **S16** as a white solid (387 mg, >99%). The product **S16** obtained in this way was used directly in the following step.

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.72 (bs, 2H), 8.52 (s, 1H, H_4), 8.45 (s, 1H, H_3), 4.61 (t, J = 5.8 Hz, 2H, H_2), 2.66 (t, J = 5.3 Hz, 3H, H_1). ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.0 (C), 161.9 (C), 161.6 (C), 148.2 (C), 147.5 (C), 129.3 (CH), 120.7 (CH), 47.3 (CH_2), 32.5 (CH_3).

*Synthesis of the acid **S18**:*

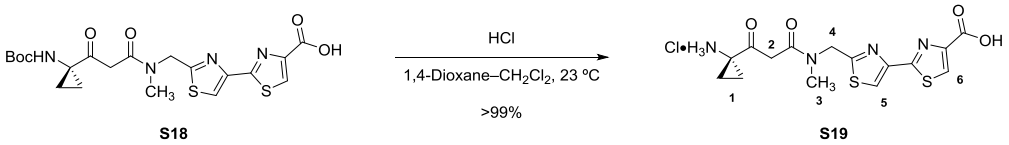


A solution of the β-ketothioester **S17** (506 mg, 1.60 mmol, 1.30 equiv) in *N*,*N*-dimethylformamide (3.0 mL) was added dropwise via syringe over 20 min to a mixture of silver trifluoroacetate (545 mg, 2.47 mmol, 2.00 equiv), triethylamine (688 µL, 4.94 mmol, 4.00 equiv), and the acid **S16** (360 mg, 1.23 mmol, 1 equiv) in *N*,*N*-dimethylformamide (12 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C. The heterogeneous product mixture was filtered through a fritted funnel. The filter cake was washed with methanol (12 mL). The filtrates were combined and the combined filtrates were concentrated. The concentrated product mixture was applied to a column containing trimethylamine acetate-functionalized silica gel (Si-TMA acetate; eluting with 2% acetic acid–methanol). The fractions containing product were collected, combined, and concentrated. The residue obtained was recrystallized from dichloromethane–hexanes (1:4; 50 mL) to provide the acid **S18** as a white solid. (565 mg, 95%).

In DMSO-d_6 at room temperature the title compound exists as a mixture (3.5:1) of amide-bond rotamers. The NMR signals are reported for the major isomer.

^{1}H NMR (600 MHz, DMSO-d_6) δ 13.14 (bs, 1H), 8.48 (s, 1H, H_7), 8.30 (s, 1H, H_6), 7.75 (bs, 1H), 4.83 (s, 2H, H_5), 3.82 (s, 2H, H_3), 2.98 (s, 3H, H_4), 1.41 (s, 9H, H_1), 1.41 – 1.36 (m, 2H, H_2), 1.09 (q, J = 4.4 Hz, 2H, H_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 204.8 (C), 168.9 (C), 167.7 (C), 162.1 (C), 162.0 (C), 156.2 (C), 148.1 (C), 147.1 (C), 129.0 (CH), 118.9 (CH), 78.8 (C), 48.4 (CH_2), 45.3 (CH_2), 41.2 (C), 36.0 (CH_3), 28.1 (CH_3), 19.8 (CH_2). IR (ATR-FTIR), cm^{-1}: 3328 (m), 2935 (w), 1704 (s), 1680 (s), 1643 (s), 1506 (s), 1287 (m), 1236 (m), 1160 (s), 746.2 (m), 457 (m). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{20}H_{25}N_4O_6S_2$, 481.1210; found, 481.1215.

*Synthesis of the hydrochloride salt **S19**:*

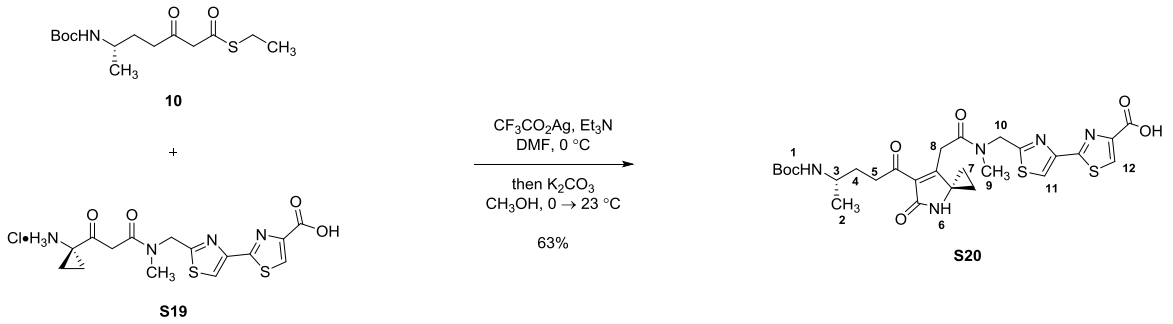


A solution of hydrogen chloride in 1,4-dioxane (4.0 N, 15.0 mL, 60.0 mmol, 54.5 equiv) was added dropwise via syringe to a solution of the bithiazole **S18** (527 mg, 1.10 mmol, 1 equiv) in dichloromethane (45.0 mL) at 23 °C. The resulting mixture was stirred for 3 h at 23 °C. The product mixture was concentrated to provide the hydrochloride salt **S19** as a white solid (457 mg, >99%). The product **S19** obtained in this way was used directly in the following step.

In DMSO-d_6 at room temperature the title compound exists as a mixture (3:1) of amide-bond rotamers. The NMR signals are reported for the major isomer.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.85 (bs, 3H), 8.49 (s, 1H, H_6), 8.32 (s, 1H, H_5), 4.84 (s, 2H, H_4), 3.78 (s, 2H, H_2), 3.05 (s, 3H, H_3) 1.83 – 1.74 (m, 2H, H_1), 1.56 – 1.44 (m, 2H, H_1).
^{13}C NMR (151 MHz, DMSO-d_6) δ 199.8 (C), 168.5 (C), 167.2 (C), 162.0 (C), 162.0 (C), 148.1 (C), 147.1 (C), 129.0 (CH), 118.9 (CH), 48.5 (CH_2), 42.0 (C), 41.1 (CH_2), 36.1 (CH_3), 13.6 (CH_2).

*Synthesis of the lactam **S20**:*

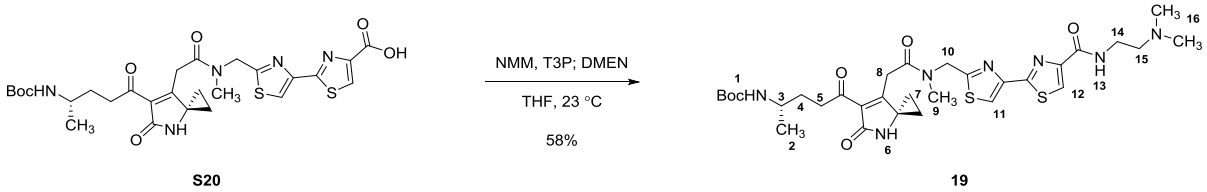


A solution of the thioester **10** (397 mg, 1.31 mmol, 1.30 equiv) in *N*,*N*-dimethylformamide (2.0 mL) was added dropwise via syringe over 20 min to a mixture of silver trifluoroacetate (445 mg, 2.01 mmol, 2.00 equiv), triethylamine (562 µL, 4.03 mmol, 4.00 equiv), and the amine **S19** (420 mg, 1.01 mmol, 1 equiv) in *N*,*N*-dimethylformamide (10 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C. Potassium carbonate (418 mg, 3.02 mmol, 3.00 equiv) and methanol (10 mL) were then added in sequence to the reaction mixture at 0 °C. The reaction mixture was allowed to warm to 23 °C and stirred at this temperature for 6 h. The heterogeneous product mixture was filtered through a fritted funnel. The filter cake was washed with methanol (10 mL). The filtrates were combined and the combined filtrates were concentrated. The residue obtained was applied to a trimethylamine acetate-functionalized silica column (Si-TMA acetate; eluting with 0.5% formic acid–acetonitrile). The fractions containing the product **S20** were collected, combined, and concentrated to provide the lactam **S20** as a white solid (380 mg, 63%).

In DMSO-d_6 at room temperature the title compound exists as a mixture (3:1) of amide-bond rotamers. The NMR signals are reported for the major isomer.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.62 (bs, 1H, H_6), 8.44 (s, 1H, H_{12}), 8.28 (s, 1H, H_{11}), 6.63 (bs, 1H), 4.82 (d, J = 15.7 Hz, 1H, H_{10}), 4.78 (d, J = 15.8 Hz, 1H, H_{10}), 3.62 (d, J = 16.2 Hz, 1H, H_8), 3.55 (d, J = 16.2 Hz, 1H, H_8), 3.46 – 3.38 (m, 1H, H_3), 3.18 (s, 3H, H_9), 2.91 – 2.83 (m, 2H, H_5), 1.62 – 1.49 (m, 4H, H_4, H_7), 1.50 – 1.40 (m, 2H, H_7), 1.36 (s, 9H, H_1), 1.00 (d, J = 6.3 Hz, 3H, H_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 197.2 (C), 169.0 (C), 168.9 (C), 168.4 (C), 167.7 (C), 166.8 (C), 162.2 (C), 155.0 (C), 147.2 (C), 130.5 (C), 128.5 (CH), 118.7 (CH), 77.3 (C), 48.8 (CH_2), 45.5 (C), 45.4 (CH), 38.4 (CH_2), 35.9 (CH_3), 29.9 (CH_2), 29.7 (CH_2), 28.3 (CH_3), 20.9 (CH_3), 13.3 (CH_2). IR (ATR-FTIR), cm^{-1}: 3328 (br w), 2975 (w), 1792 (w), 676 (s), 1631 (s), 1501 (m), 1391 (m), 1169 (s), 1152 (s), 674 (w), 556 (m). HRMS-CI (m/z): [M + Na]$^+$ calcd for $C_{27}H_{33}N_5NaO_7S_2$, 626.1714; found, 626.1716. $[\alpha]_D^{20}$ +8.0 (*c* 1.0, DMSO).

*Synthesis of the amide **19**:*



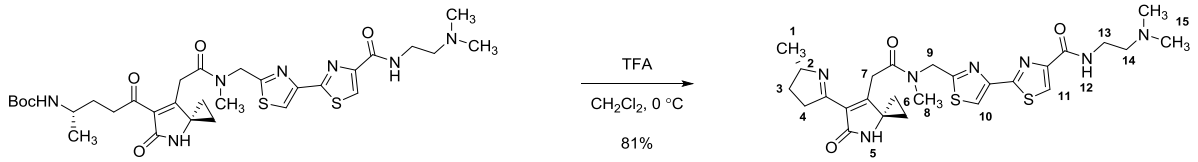
S20 **19**

A solution of T3P in ethyl acetate (50 wt%, 118 μL, 199 μmol, 1.50 equiv) and 4-methylmorpholine (72.8 μL, 663 μmol, 5.00 equiv) were added in sequence to a solution of the acid **S20** (80.0 mg, 133 μmol, 1 equiv) in tetrahydrofuran (2.7 mL) at 23 °C. The reaction mixture was stirred for 20 min at 23 °C. *N*,*N*-Dimethylethylenediamine (36.2 μL, 331 μmol, 2.50 equiv) was added to the reaction mixture. The resulting mixture was stirred for 14 h at 23 °C. The product mixture was concentrated. The concentrated product mixture was diluted with ethyl acetate (10 mL). The diluted product mixture was poured into a separatory funnel that had been charged with saturated aqueous sodium bicarbonate solution (5.0 mL) and the layers that formed were separated. The aqueous layer was extracted with ethyl acetate (2 × 10 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the amide **19** as a white solid (52.0 mg, 58%).

The product **19** obtained in this way was estimated to be of >95% purity by ^{1}H and ^{13}C NMR analysis (see accompanying spectra) and was used without further purification. In DMSO-d_6 at room temperature the title compound exists as a mixture (3:1) of amide-bond rotamers. The NMR signals are reported for the major isomer.

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.62 (s, 1H, H_6), 8.27 (bs, 1H, H_{12}), 8.24 (s, 1H, H_{11}), 8.23 (bs, 1H, H_{13}), 6.63 (bs, 1H), 4.82 (d, J = 15.9 Hz, 1H, H_{10}), 4.79 (d, J = 15.9 Hz, 1H, H_{10}), 3.62 (d, J = 16.2 Hz, 1H, H_8), 3.55 (d, J = 16.1 Hz, 1H, H_8), 3.50 – 3.40 (m, 1H, H_3), 3.39 (app q, J = 6.4 Hz, 2H, H_{14}), 3.18 (s, 3H, H_9), 2.91 – 2.80 (m, 2H, H_5), 2.42 (td, J = 6.6, 1.8 Hz, 2H, H_{15}), 2.18 (s, 6H, H_{16}), 1.64 – 1.48 (m, 4H, H_4, H_7), 1.47 – 1.38 (m, 2H, H_7), 1.36 (s, 9H, H_1), 1.00 (d, J = 6.5 Hz, 3H, H_2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 197.2 (C), 169.1 (C), 169.0 (C), 168.4 (C), 166.8 (C), 161.8 (C), 160.2 (C), 155.0 (C), 150.8 (C), 147.1 (C), 130.5 (C), 124.1 (CH), 118.7 (CH), 77.3 (C), 58.1 (CH_2), 48.8 (CH_2), 45.5 (C), 45.4 (CH), 45.2 (CH_3), 38.4 (CH_2), 36.7 (CH_2), 36.0 (CH_3), 29.9 (CH_2), 29.7 (CH_2), 28.3 (CH_3), 20.9 (CH_3), 13.3 (CH_2). IR (ATR-FTIR), cm^{-1}: 3325 (br w), 2975 (w), 2931 (w), 1681 (s), 1654 (s), 1543 (m), 1453 (w), 1165 (m), 766 (w), 621 (w). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{31}H_{44}N_7O_6S_2$, 674.2789; found, 674.2795. $[\alpha]_D^{20}$ –33.0 (*c* 1.0, CH_2Cl_2).

*Synthesis of the amide **15j**:*



19 **15j**

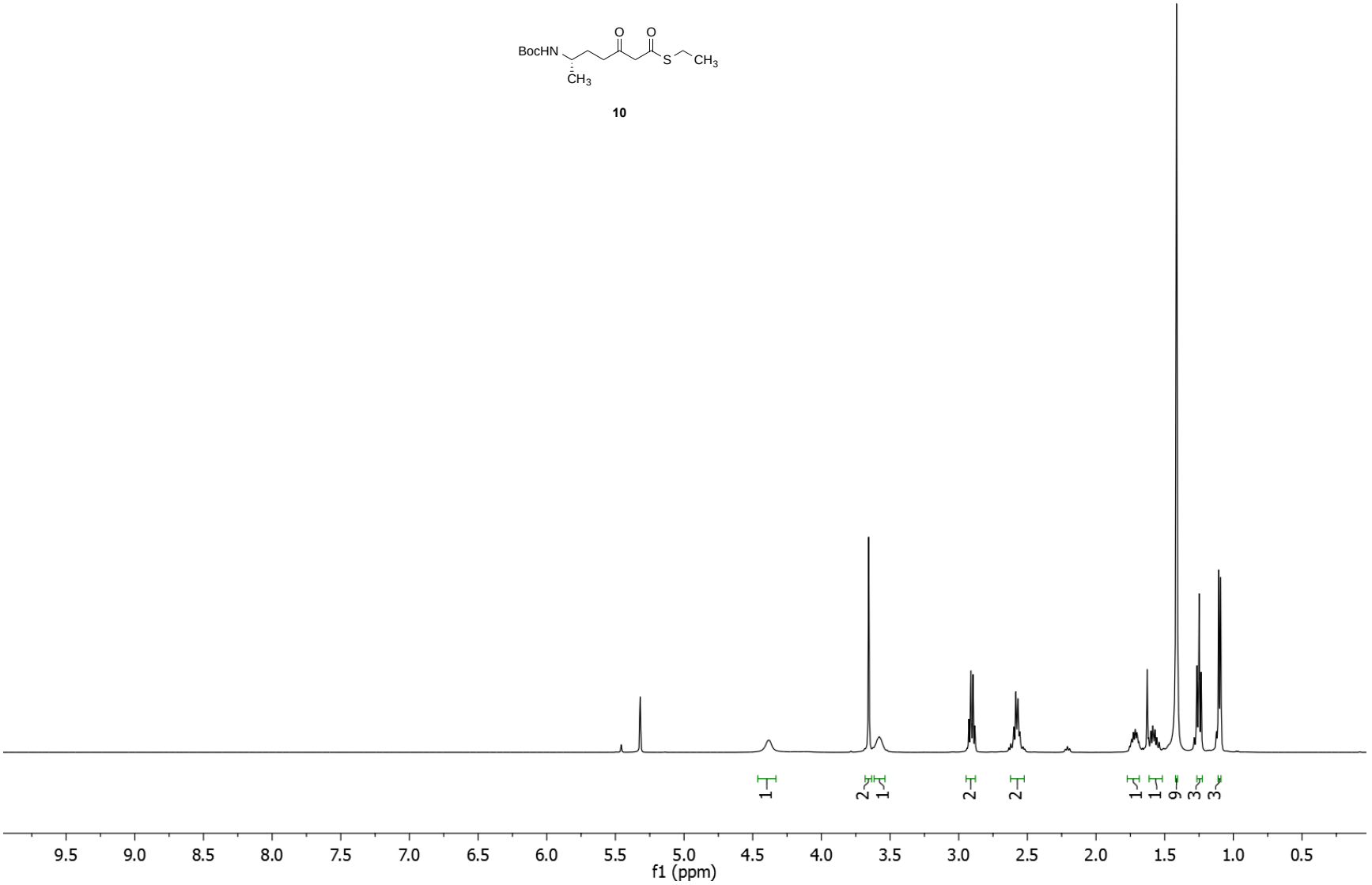
Trifluoroacetic acid (200 μL, 2.61 mmol, 176 equiv) was added dropwise via syringe to a solution of the amide **19** (10.0 mg, 14.8 μmol, 1 equiv) in dichloromethane (400 μL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C. The product mixture was concentrated. The concentrated product mixture was diluted with dichloromethane (10 mL). The diluted product mixture was poured into a separatory funnel that had been charged with saturated aqueous sodium bicarbonate solution (5.0 mL) and the layers that formed were separated. The aqueous layer was extracted with dichloromethane (2 × 10 mL). The organic layers were combined and the combined organic layers were dried over sodium sulfate. The dried solution was filtered and the filtrate was concentrated to provide the amide **15j** as a white solid (6.9 mg, 81%).

In DMSO-d_6 at room temperature the title compound exists as a mixture (3:1) of amide-bond rotamers. The NMR signals are reported for the major isomer.

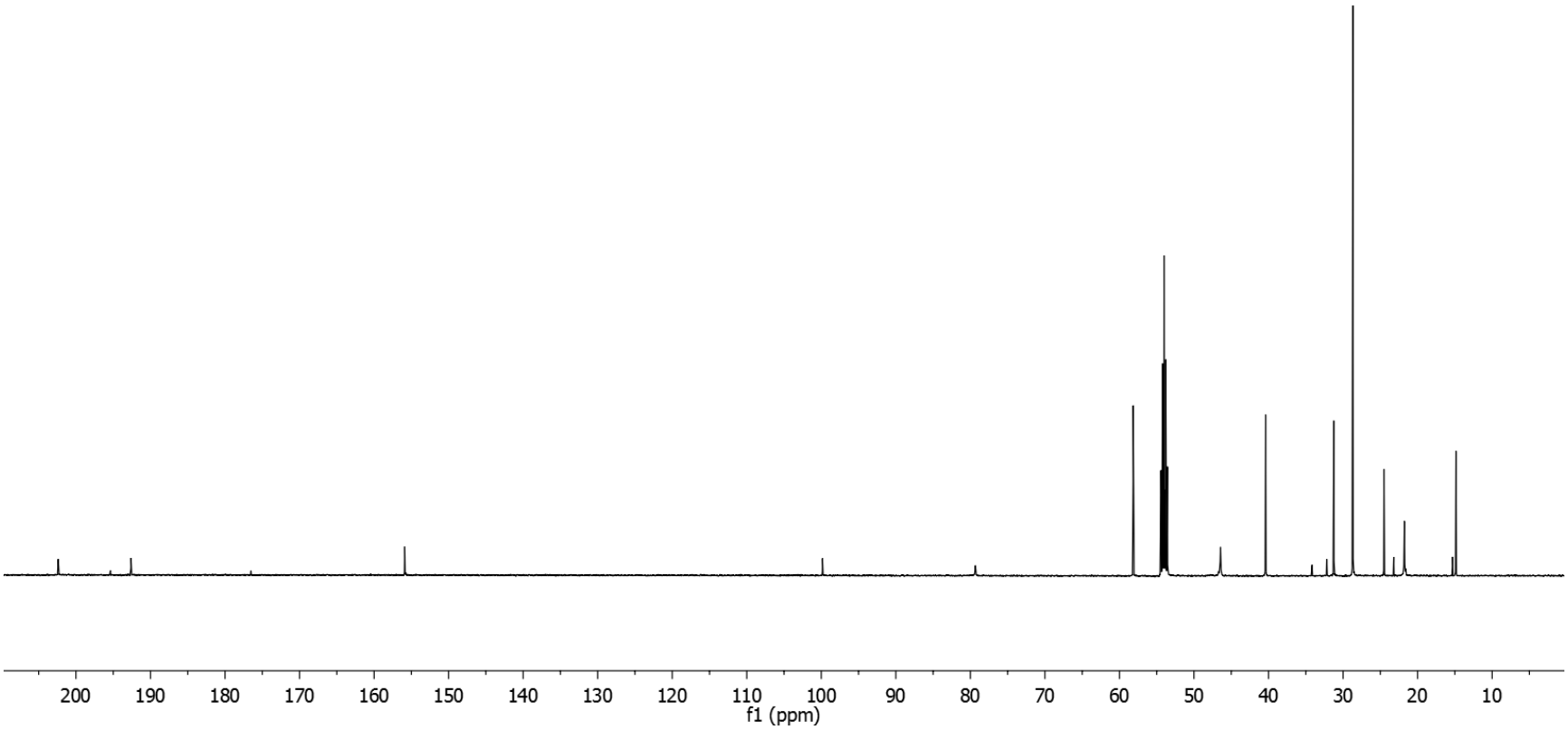
^{1}H NMR (600 MHz, DMSO-d_6) δ 8.46 (bs, 1H, H_5), 8.27 (s, 1H, H_{11}), 8.25 (bs, 1H, H_{12}), 8.24 (s, 1H, H_{10}), 4.84 (d, J = 15.6 Hz, 1H, H_9), 4.70 (d, J = 15.6 Hz, 1H, H_9), 3.95 – 3.88 (m, 1H, H_2), 3.71 (d, J = 15.8 Hz, 1H, H_7), 3.60 (d, J = 15.8 Hz, 1H, H_7), 3.39 (app q, J = 6.4 Hz, 2H, H_{13}), 3.17 (s, 3H, H_8), 3.01 – 2.92 (m, 1H, H_4), 2.81 – 2.69 (m, 1H, H_4), 2.41 (t, J = 6.6 Hz, 2H, H_{14}), 2.18 (s, 6H, H_{15}), 2.02 – 1.92 (m, 1H, H_3), 1.51 – 1.43 (m, 2H, H_6), 1.39 – 1.30 (m, 2H, H_6), 1.28 – 1.21 (m, 1H, H_3), 1.07 (d, J = 6.7 Hz, 3H, H_1). ^{13}C NMR (151 MHz, DMSO-d_6) δ 170.3 (C), 169.0 (C), 168.9 (C), 166.8 (C), 161.8 (C), 160.2 (C), 159.3 (C), 150.8 (C), 147.1 (C), 127.2 (C), 124.1 (CH), 118.7 (CH), 66.9 (CH), 58.1 (CH_2), 48.8 (CH_2), 45.4 (C), 45.2 (CH_3), 36.7 (CH_2), 36.1 (CH_3), 29.7 (CH_2), 29.6 (CH_2), 22.0 (CH_3), 12.2 (CH_2), 12.0 (CH_2). HRMS-CI (m/z): $[M + H]^+$ calcd for $C_{26}H_{34}N_7O_3S_2$, 556.2159; found, 556.2167. $[\alpha]_D^{20}$ –38.0 (*c* 1.0, CH_2Cl_2).

Catalog of Nuclear Magnetic Resonance Spectra

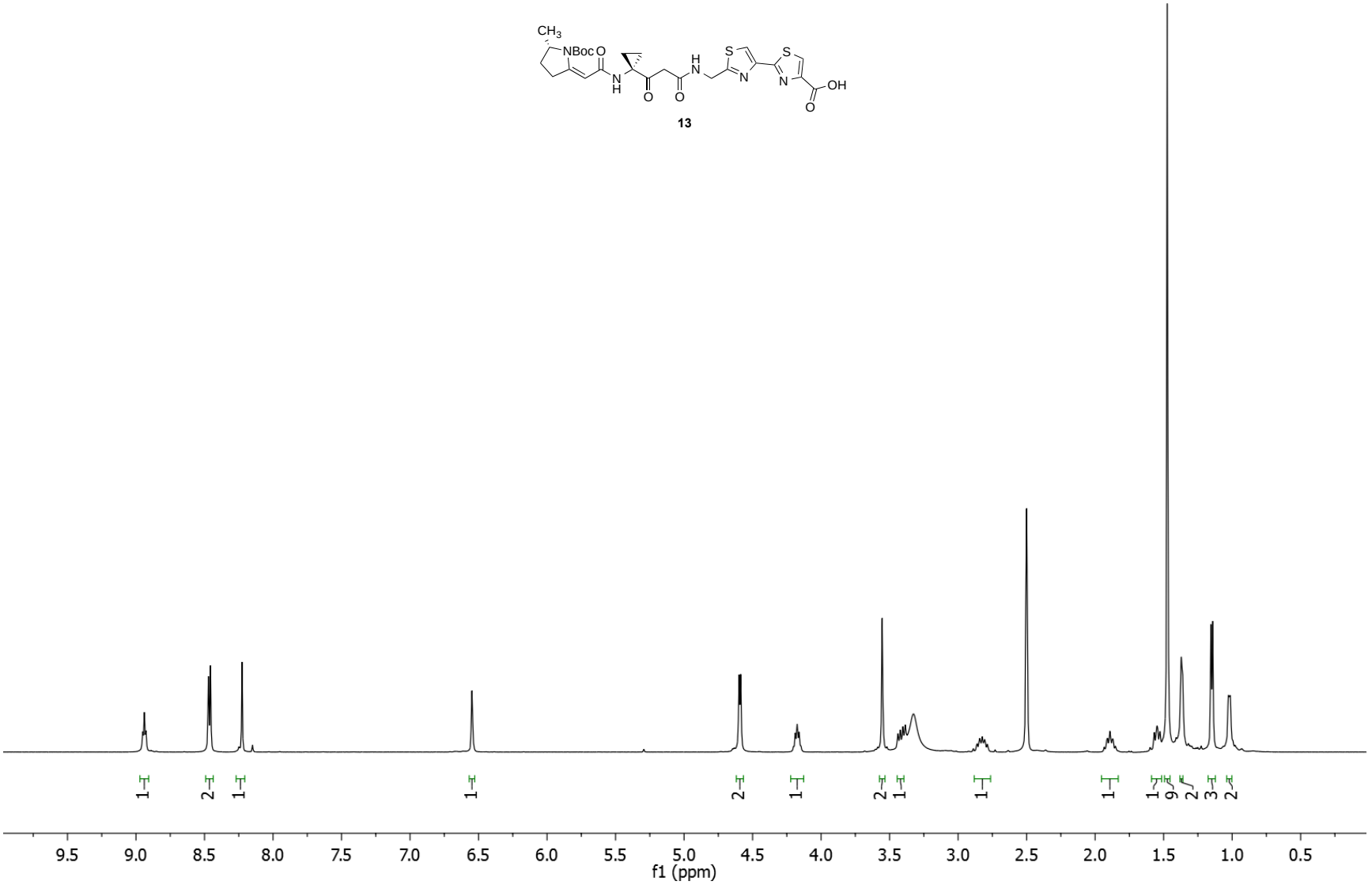
^{1}H NMR, 500 MHz, CD_2Cl_2

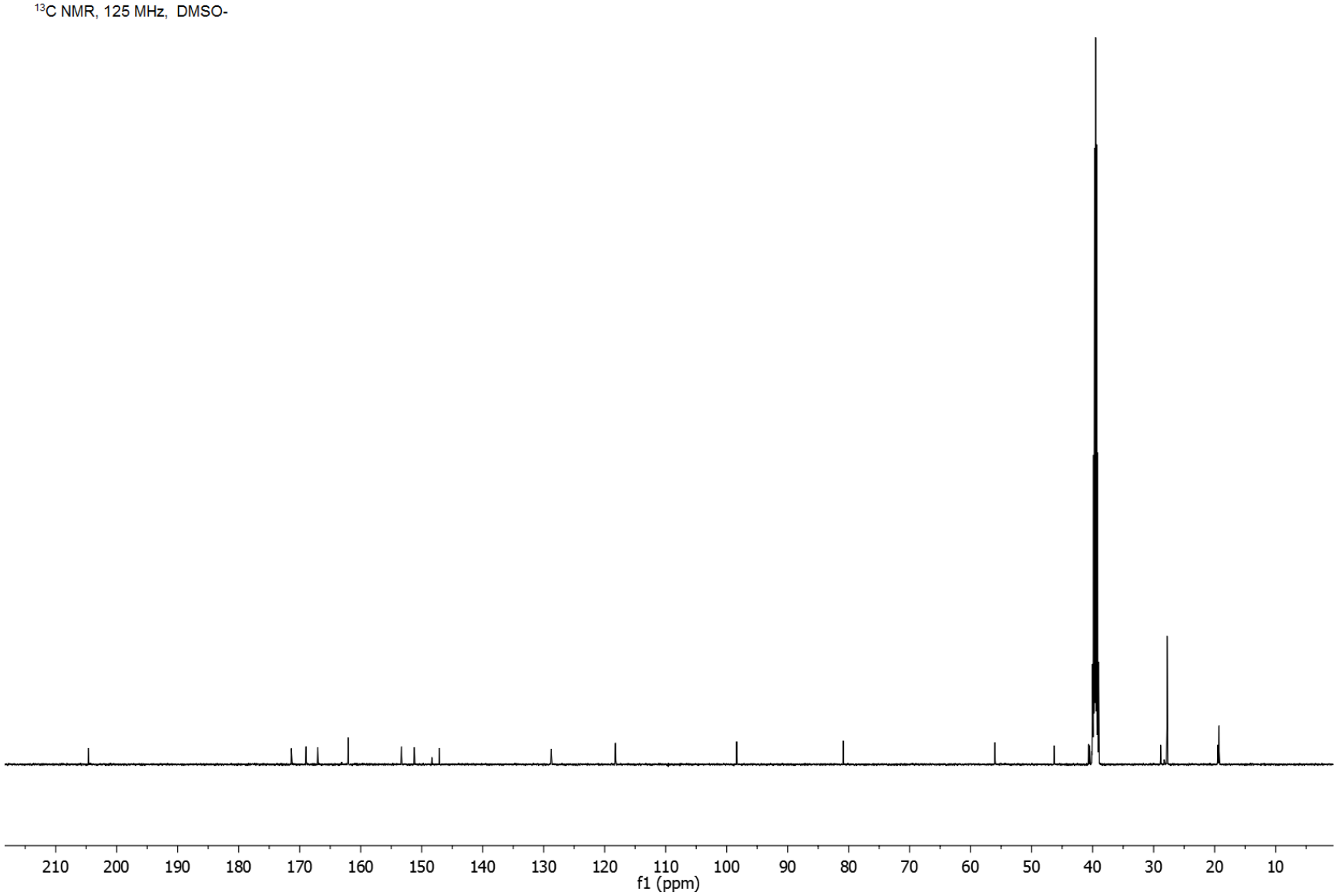


^{13}C NMR, 125 MHz, CD_2Cl_2

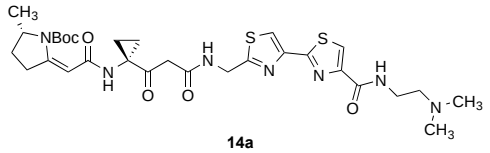


^{1}H NMR, 500 MHz, DMSO-d_6

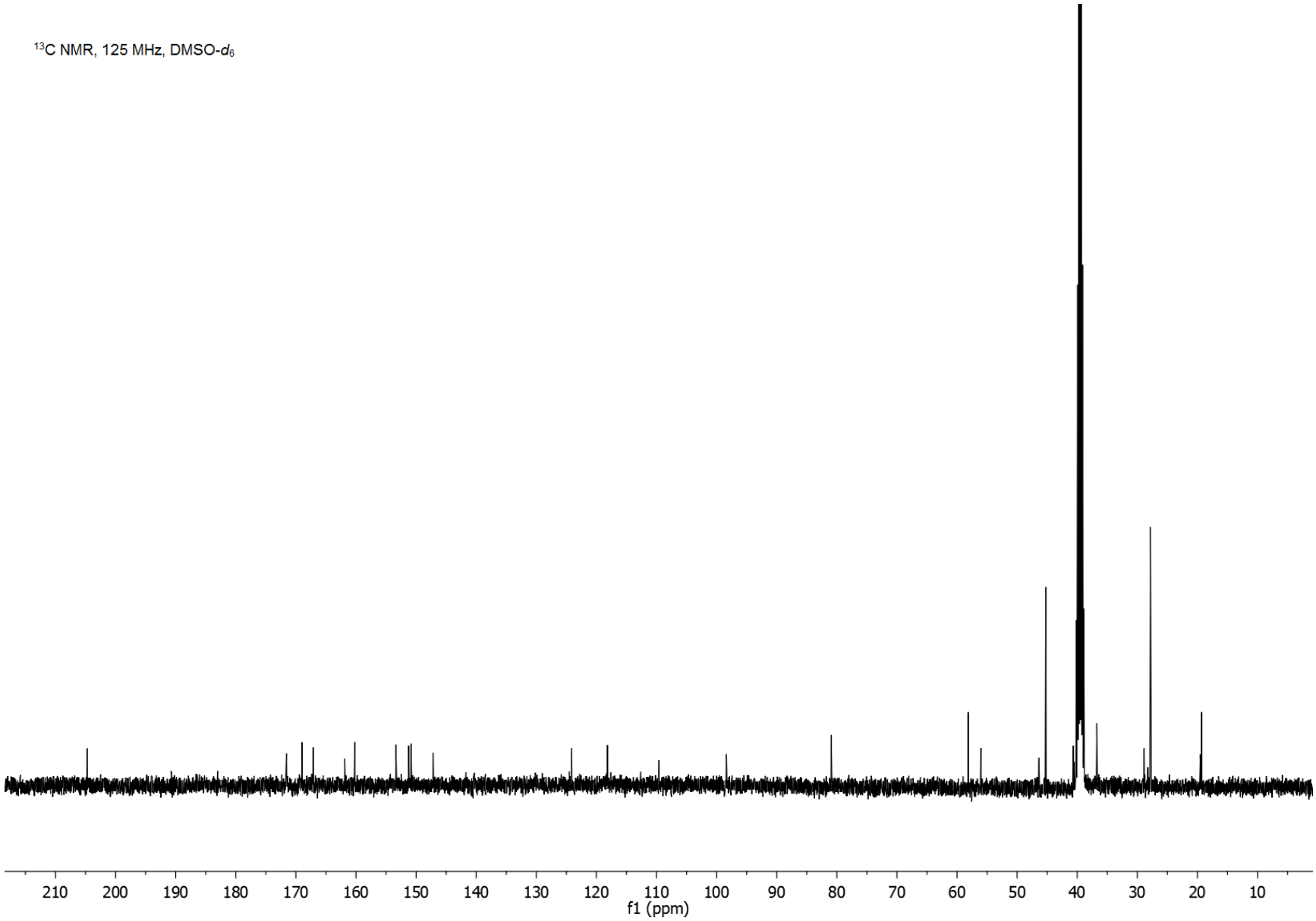



^{13}C NMR, 125 MHz, DMSO-
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

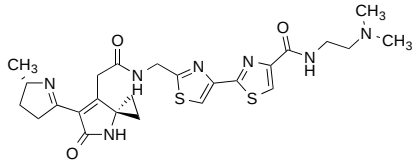
^{1}H NMR, 500 MHz, DMSO-d_6



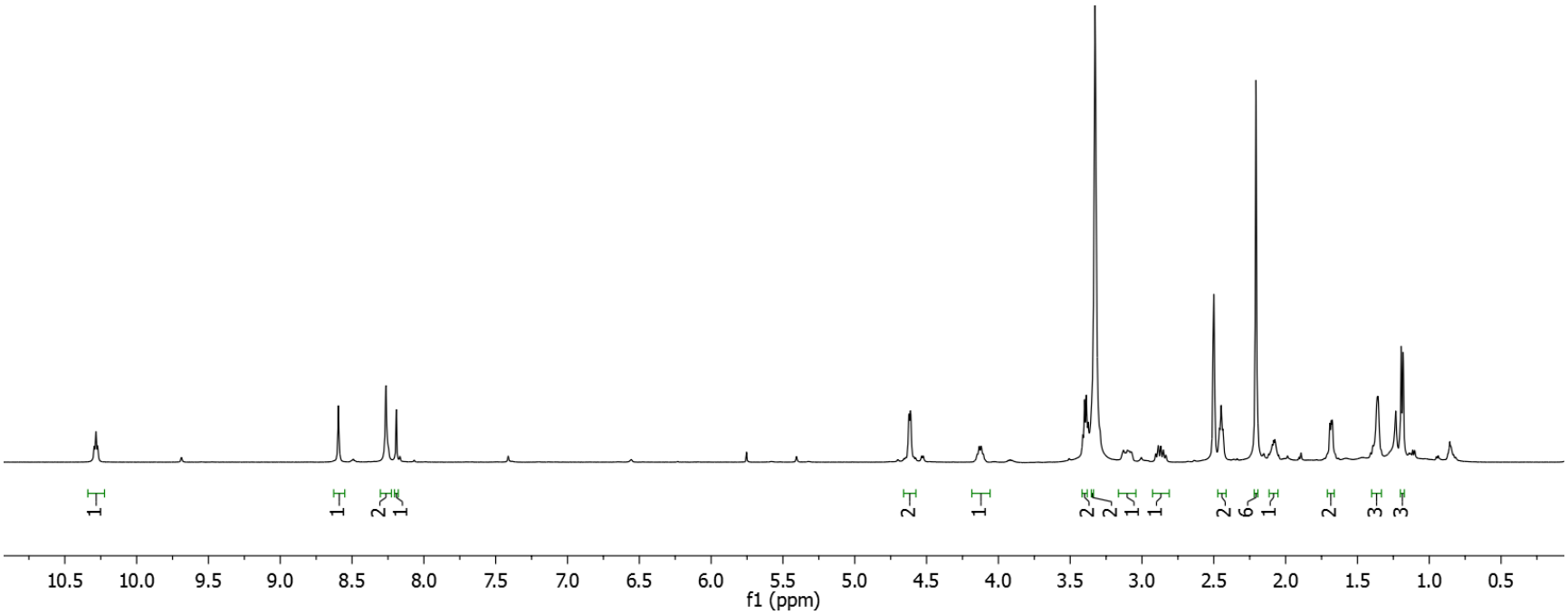
14a


^{13}C NMR, 125 MHz, DMSO-d_6
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

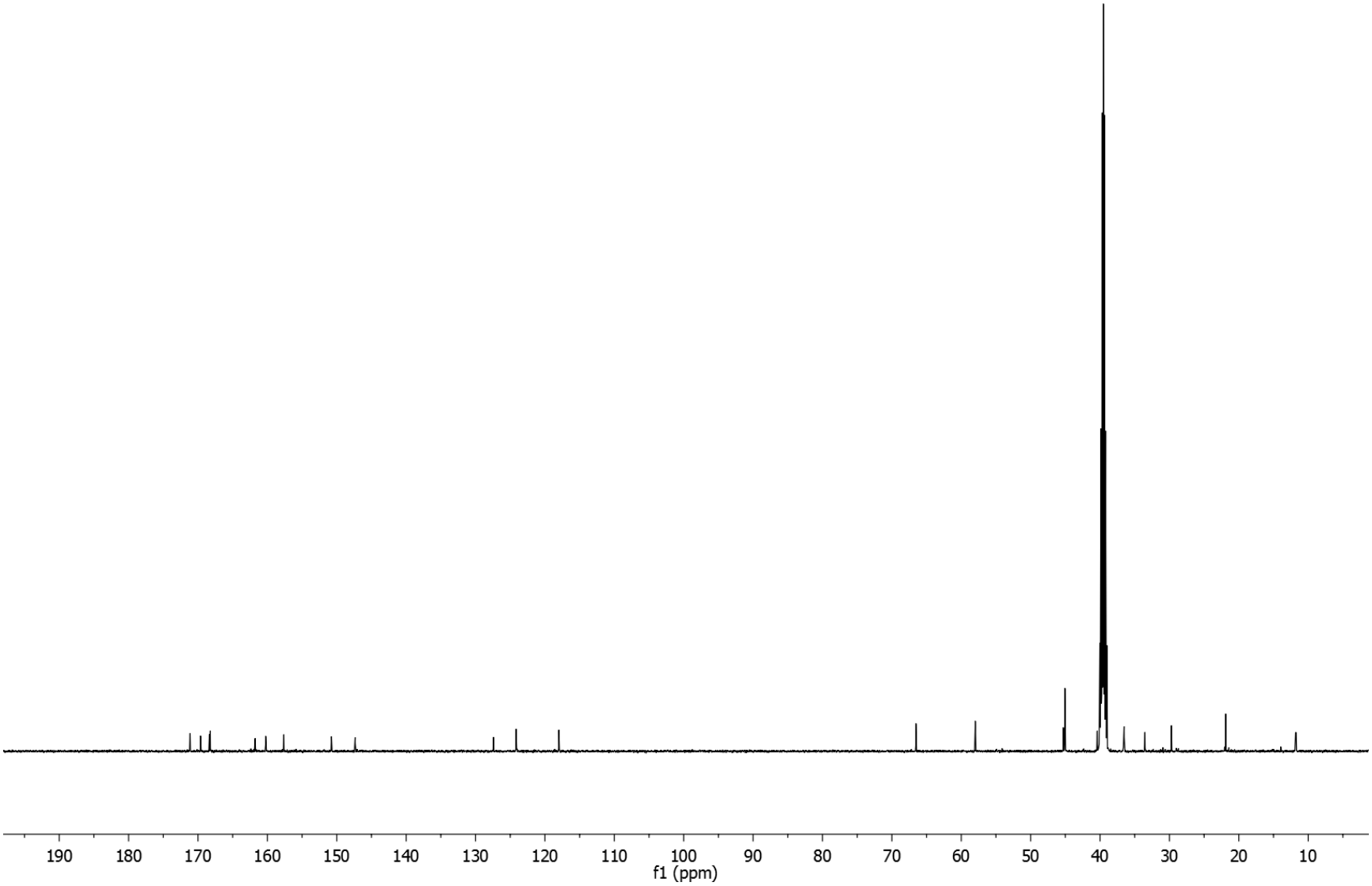
^{1}H NMR, 500 MHz, DMSO-d_6



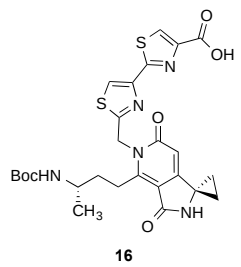
15a



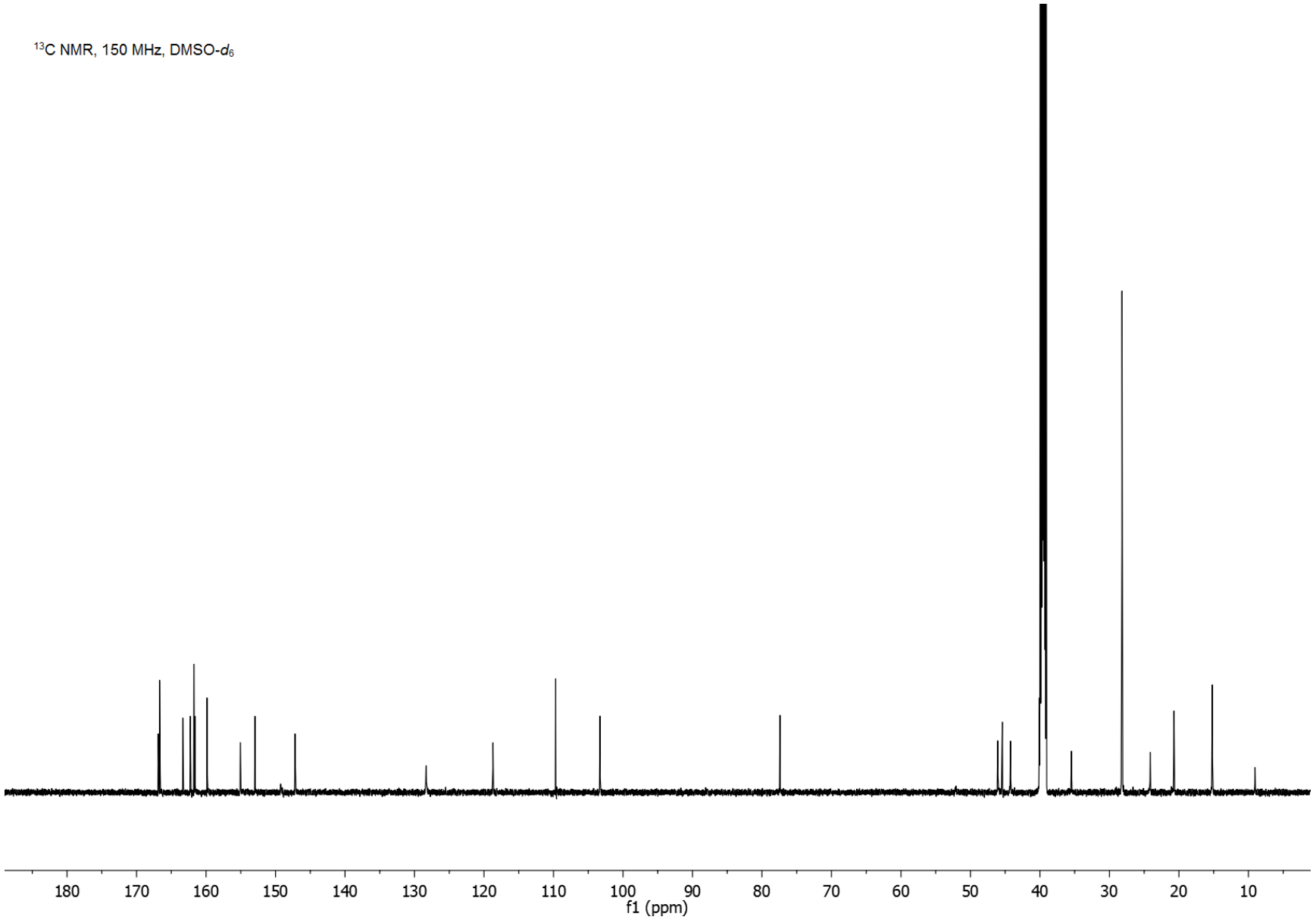
^{13}C NMR, 125 MHz, DMSO-d_6



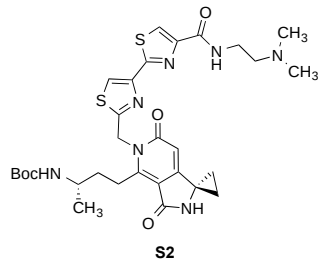
^{1}H NMR, 600 MHz, DMSO-d_6



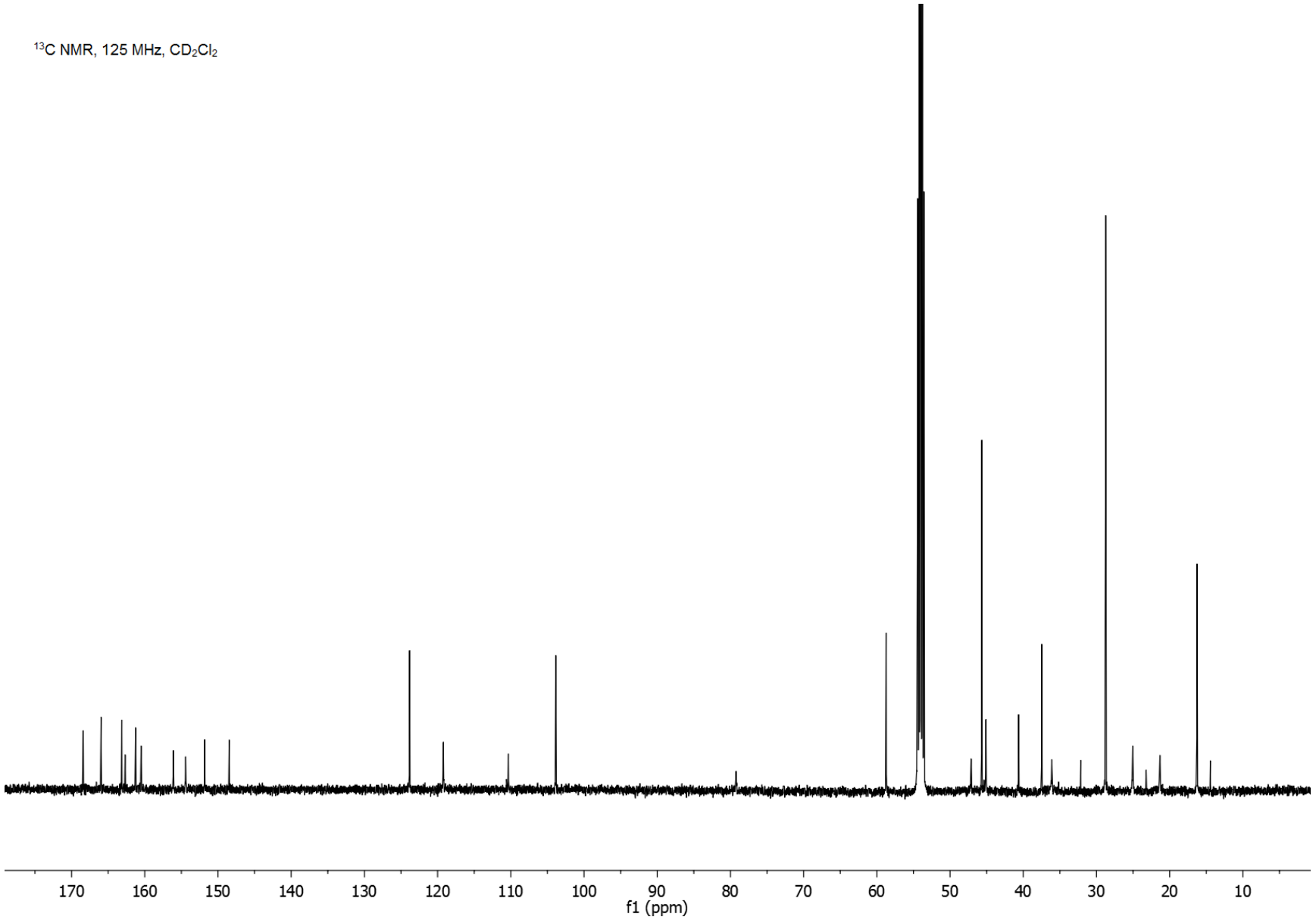
16


^{13}C NMR, 150 MHz, DMSO-d_6
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

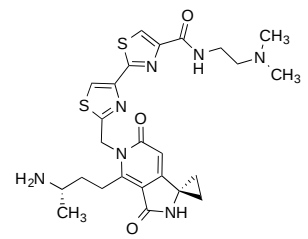
^{1}H NMR, 500 MHz, CD_2Cl_2



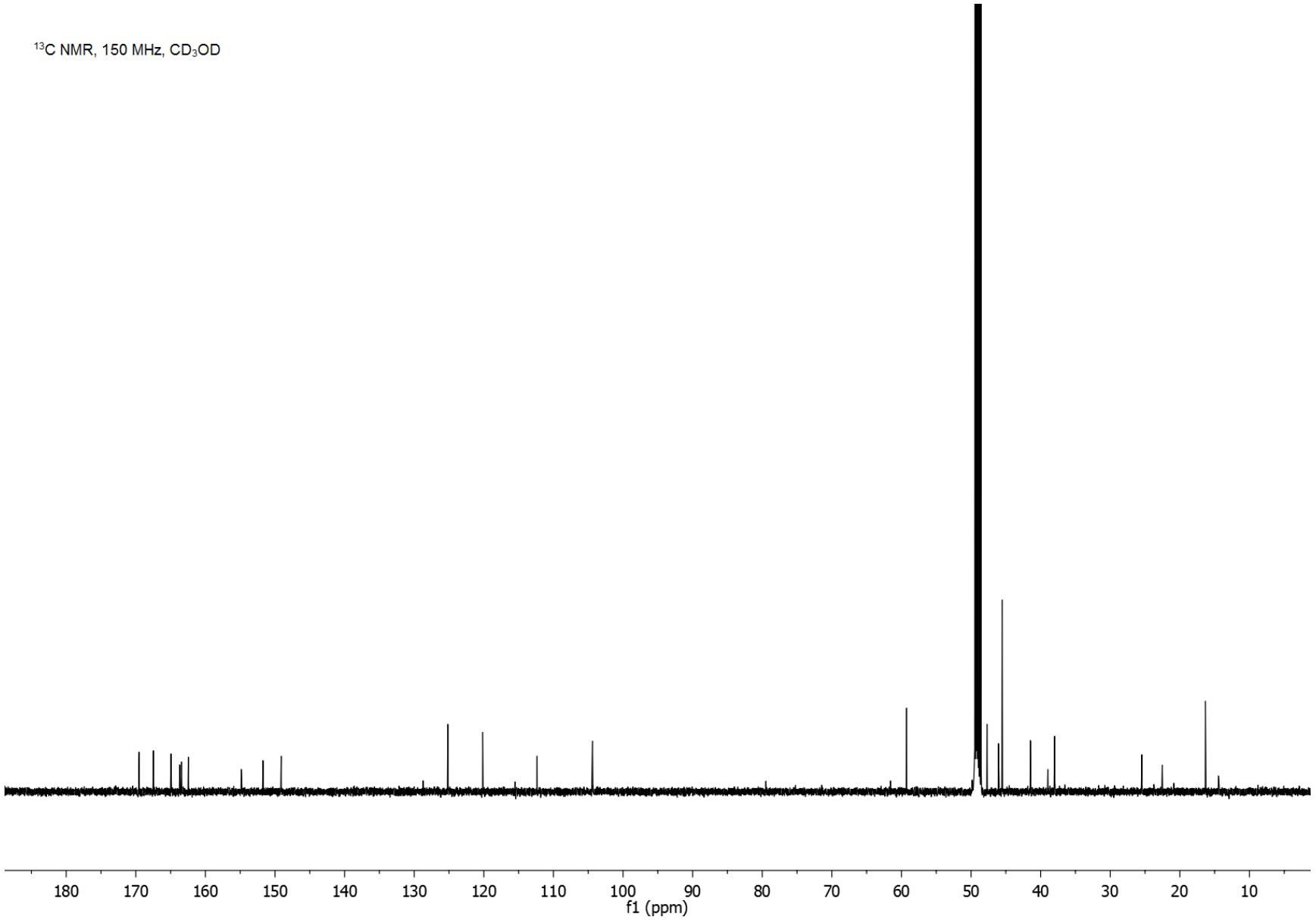
S2


^{13}C NMR, 125 MHz, CD_2Cl_2
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

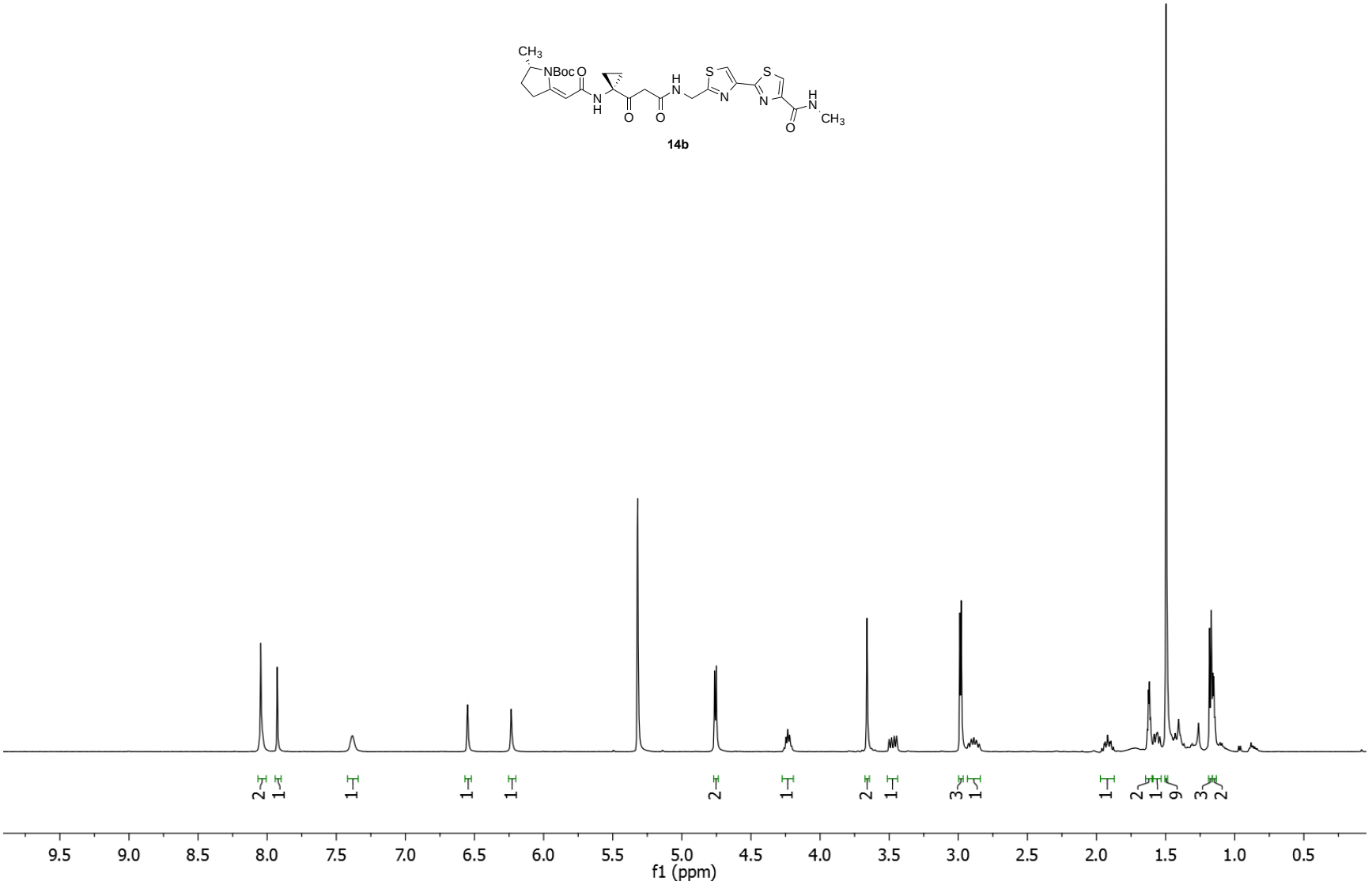
^{1}H NMR, 500 MHz, CD_3OD

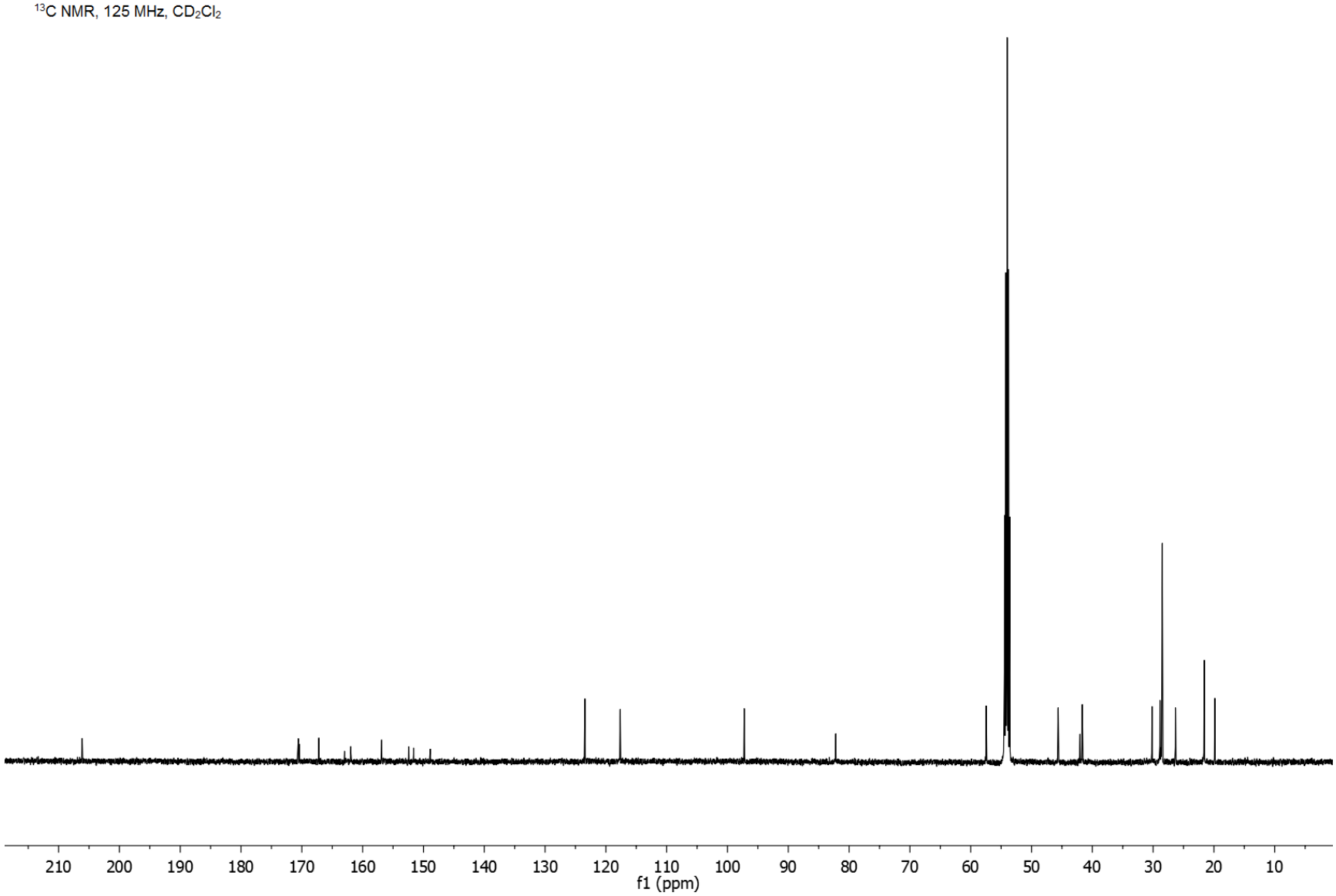


17a

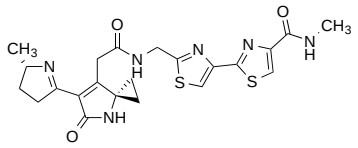

^{13}C NMR, 150 MHz, CD_3OD
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 500 MHz, CD_2Cl_2



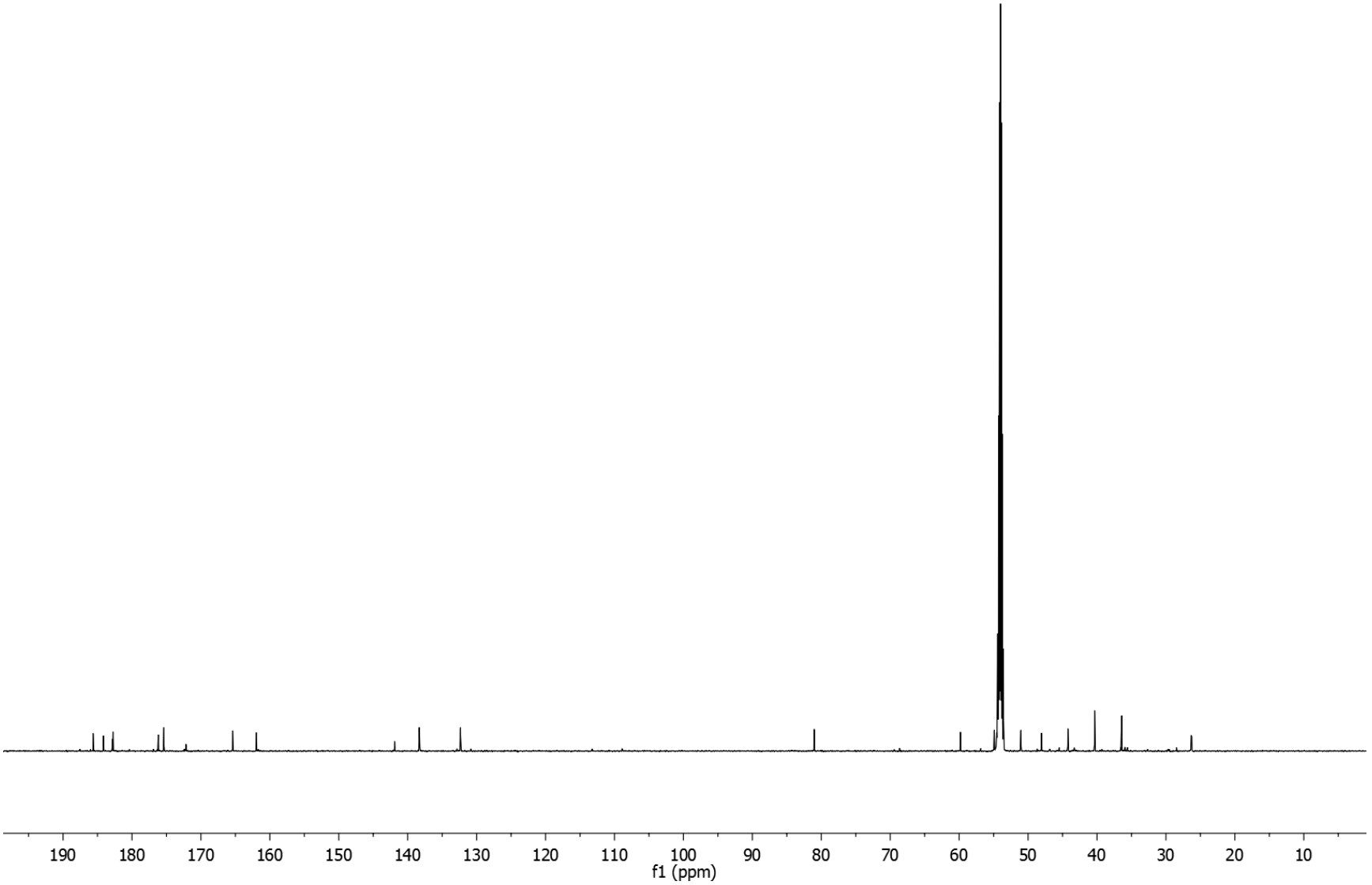

^{13}C NMR, 125 MHz, CD_2Cl_2
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

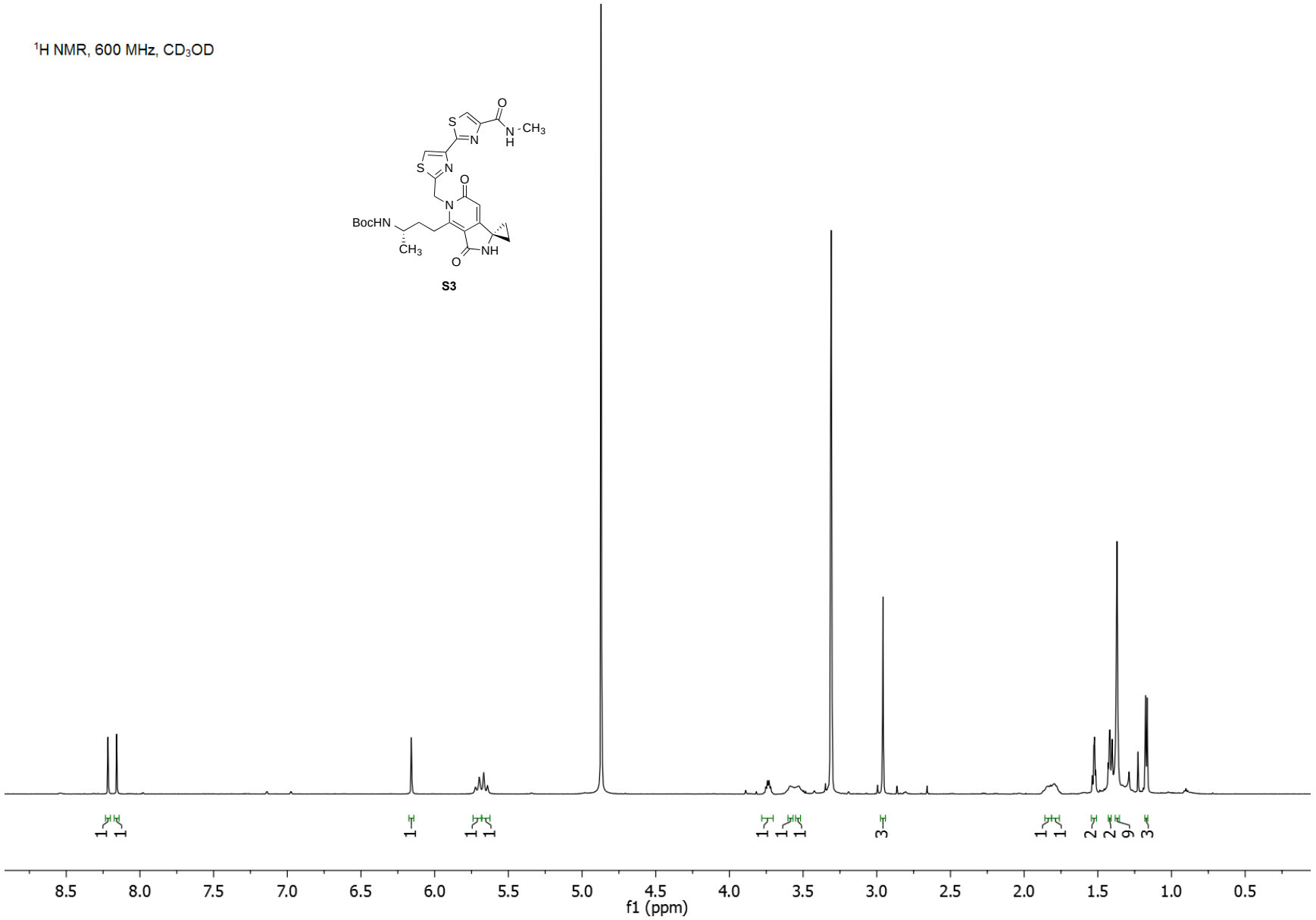
^{1}H NMR, 600 MHz, DMSO-d_6

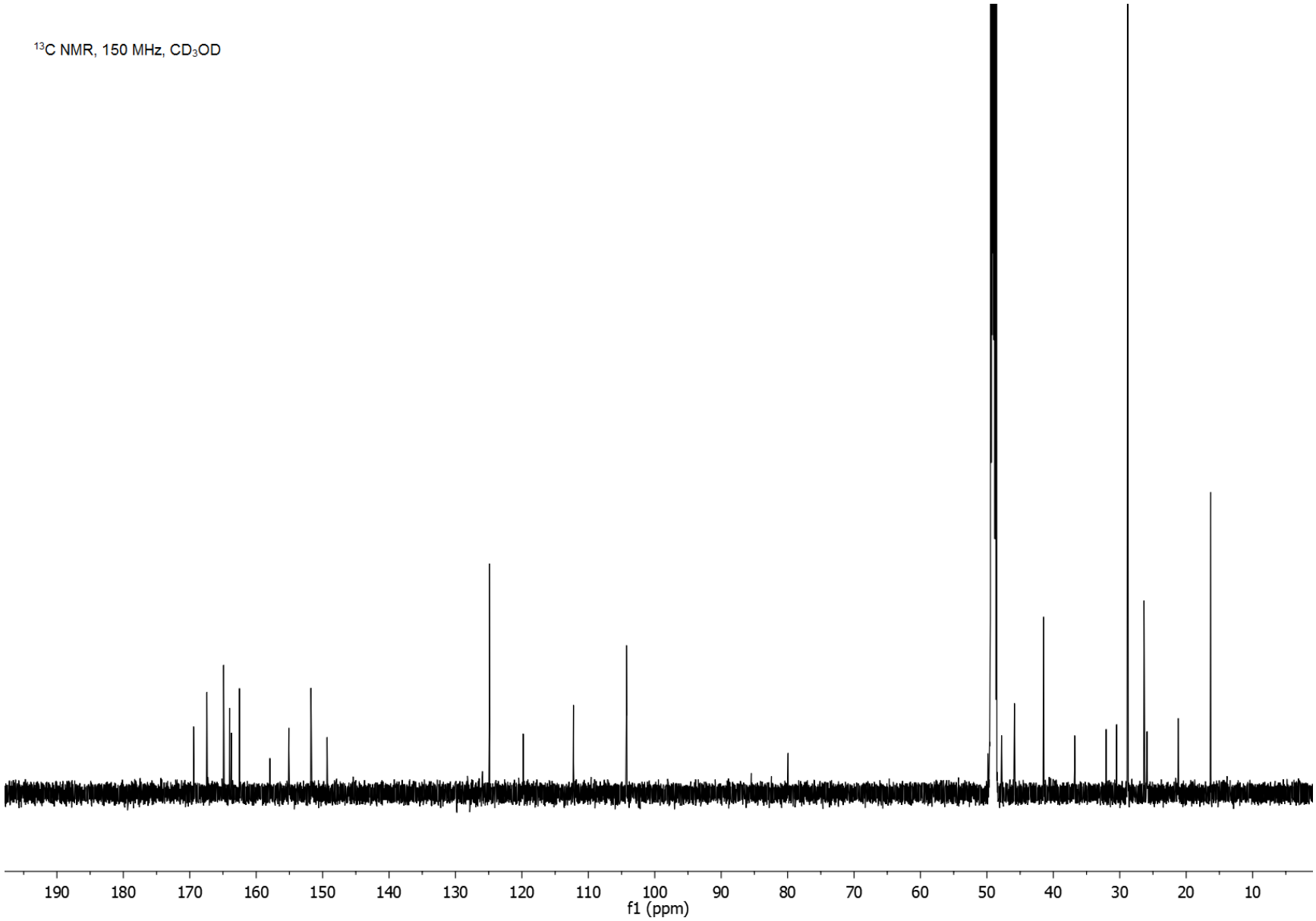


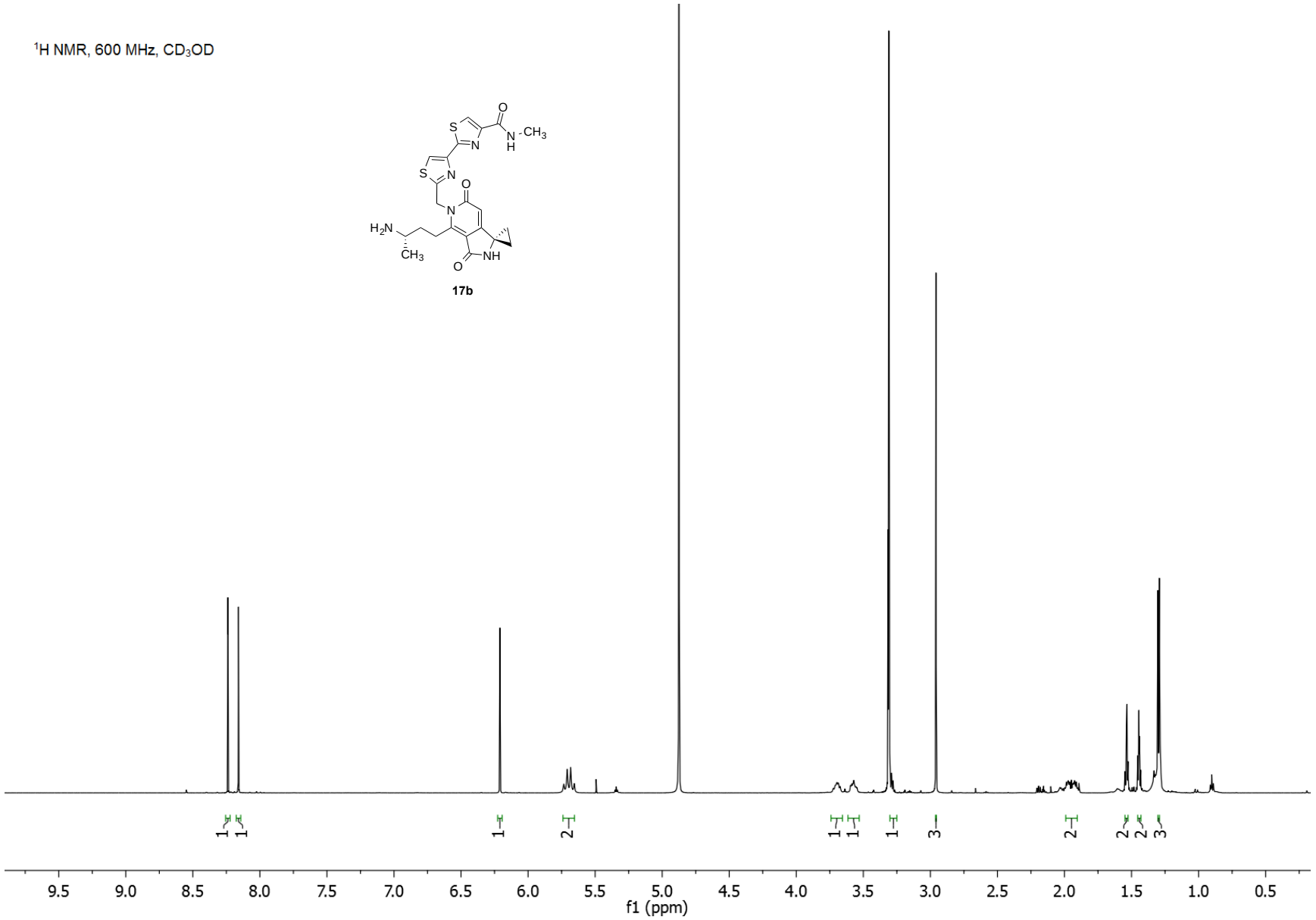
15b

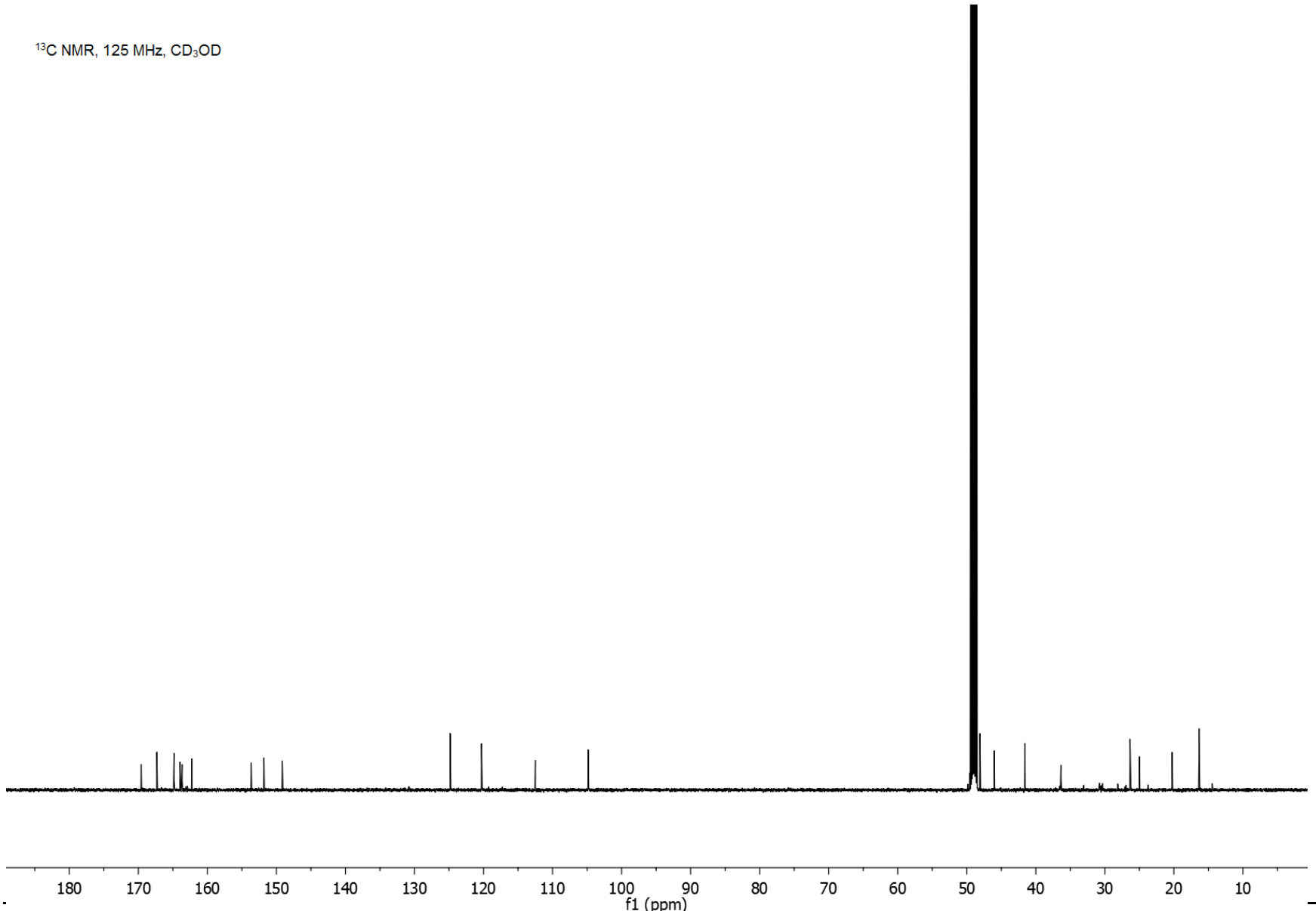
^{13}C NMR, 125 MHz, DMSO-d_6



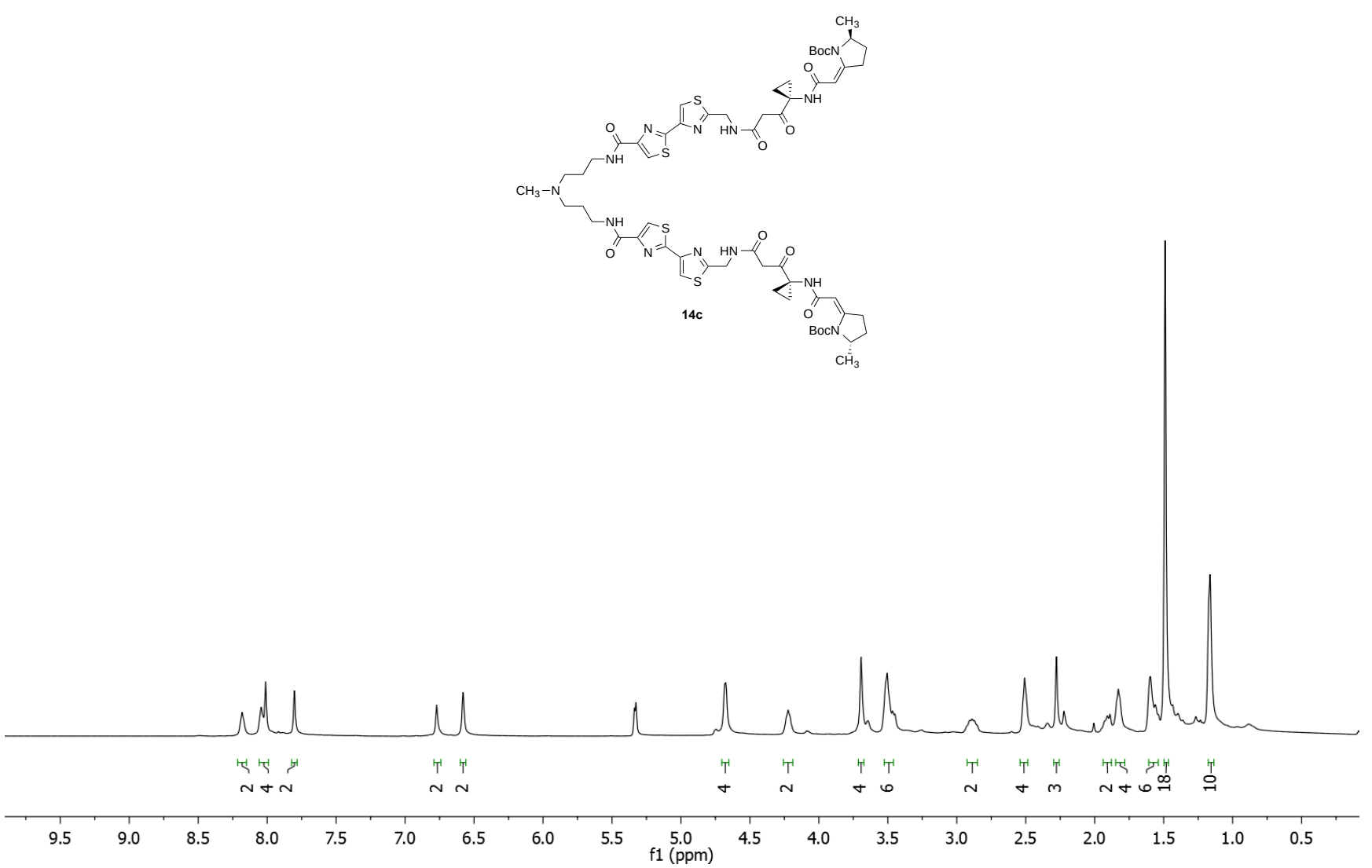

^{1}H NMR, 600 MHz, CD_3OD
S3
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


^{13}C NMR, 150 MHz, CD_3OD
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

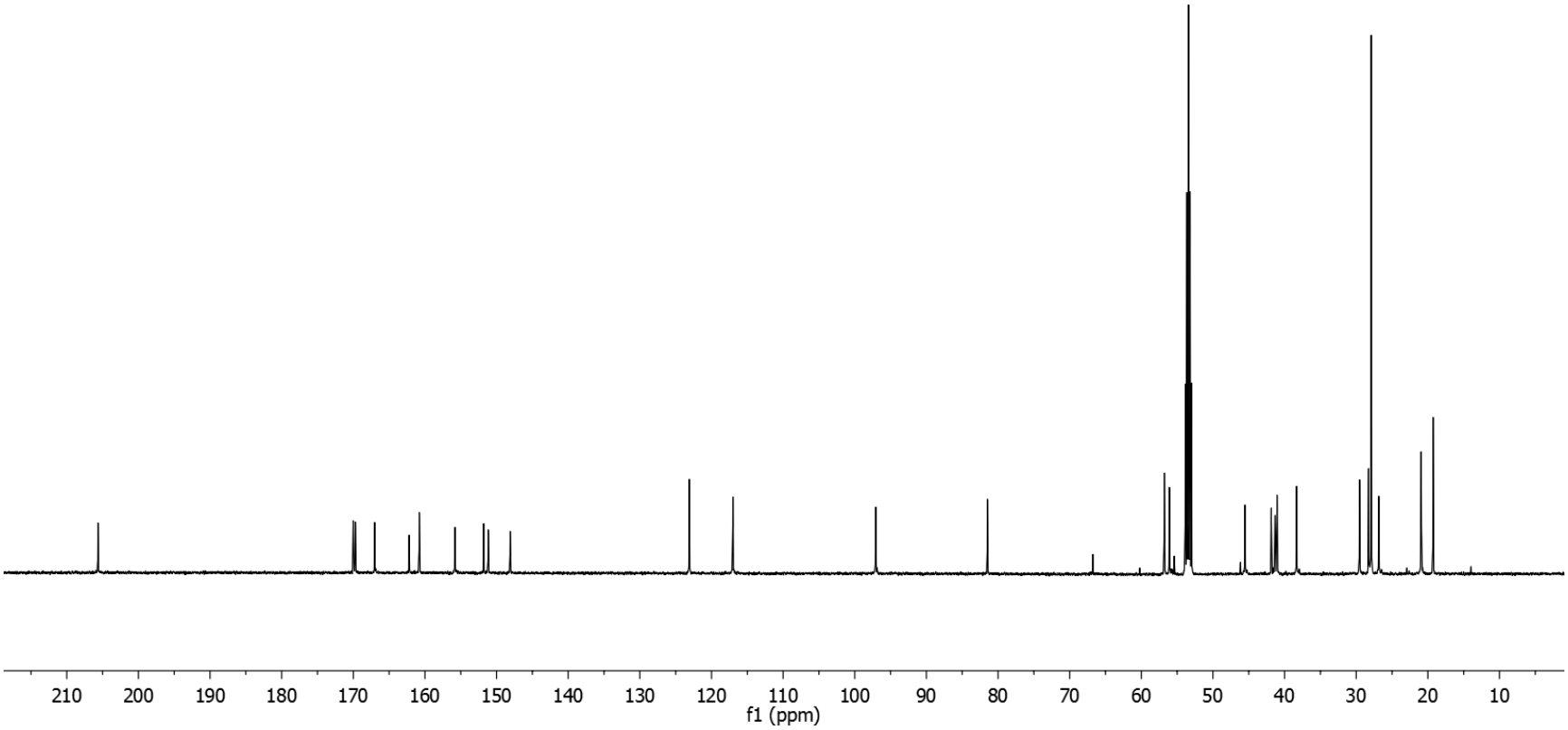

^{1}H NMR, 600 MHz, CD_3OD
17b
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


^{13}C NMR, 125 MHz, CD_3OD
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

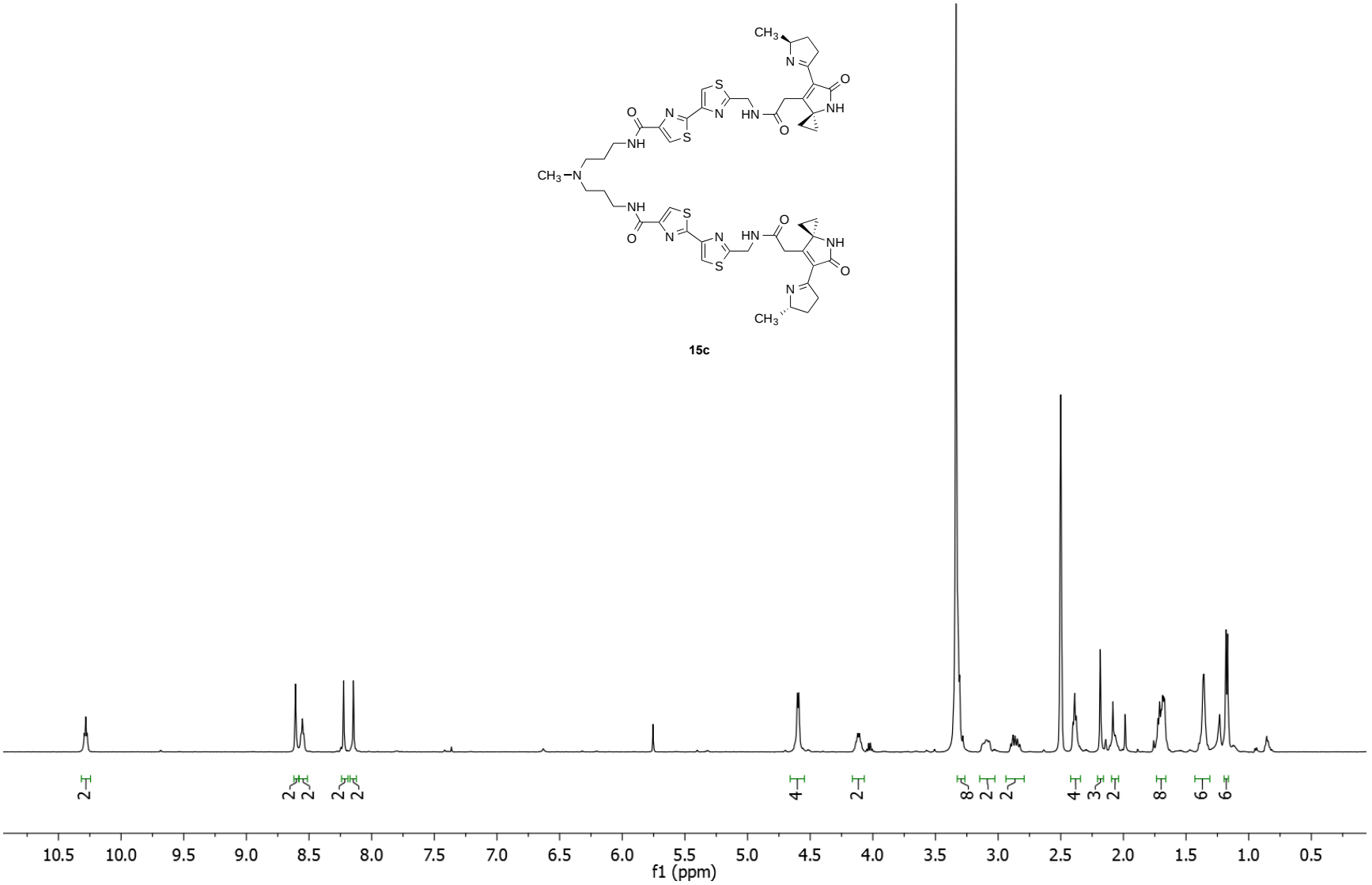
^{1}H NMR, 500 MHz, CD_2Cl_2

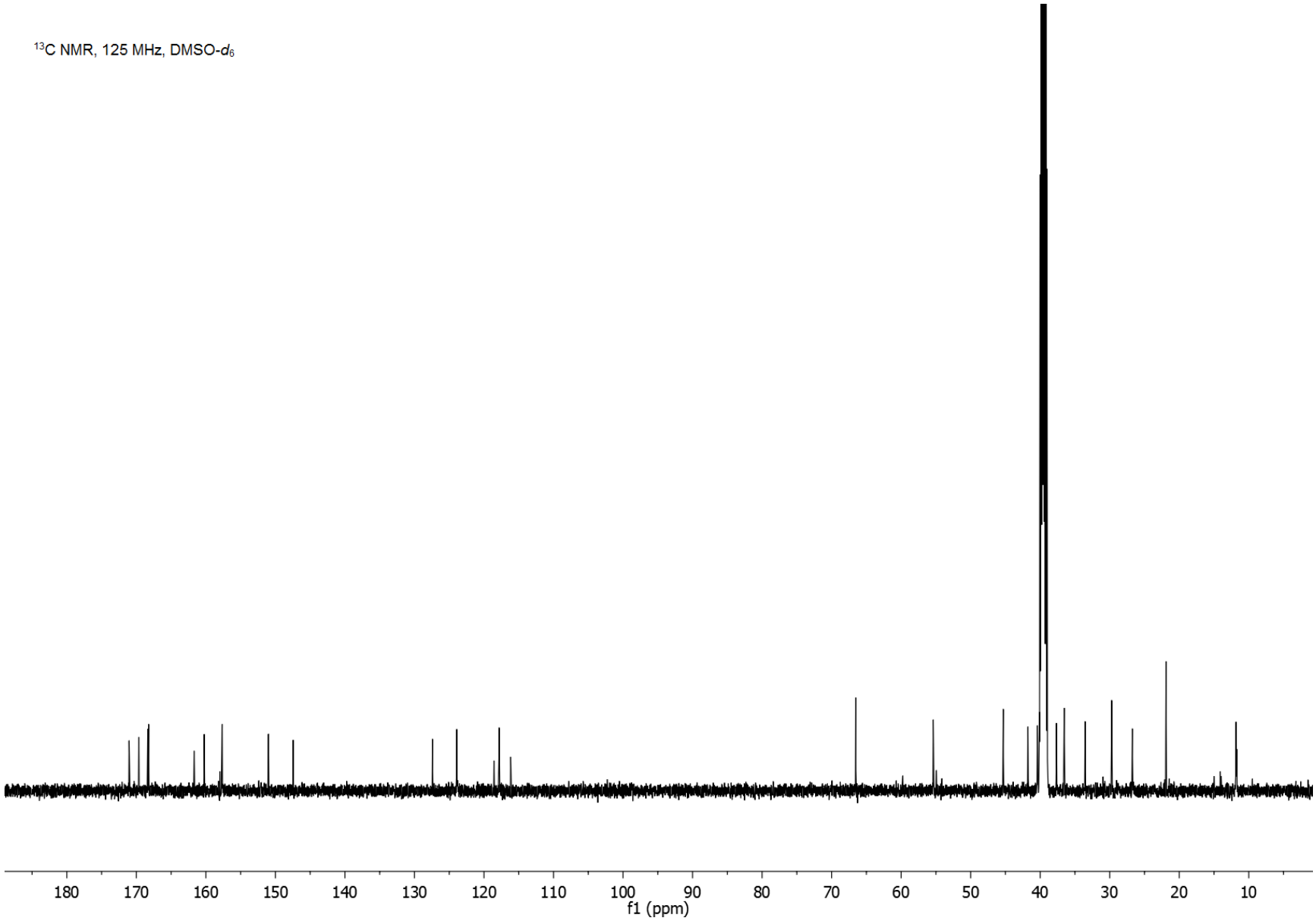


^{13}C NMR, 125 MHz, CD_2Cl_2

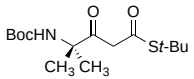


^{1}H NMR, 500 MHz, DMSO-d_6

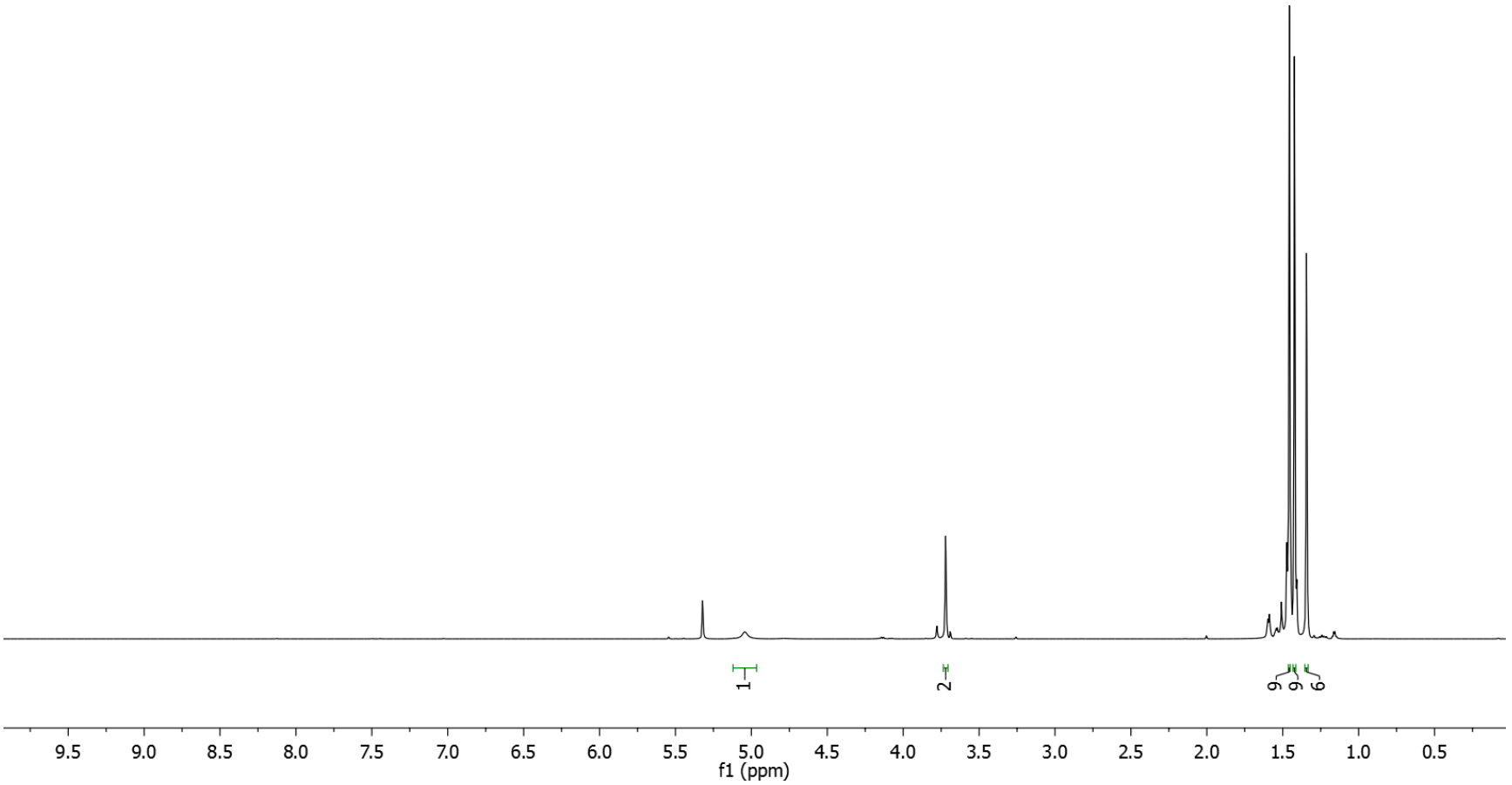


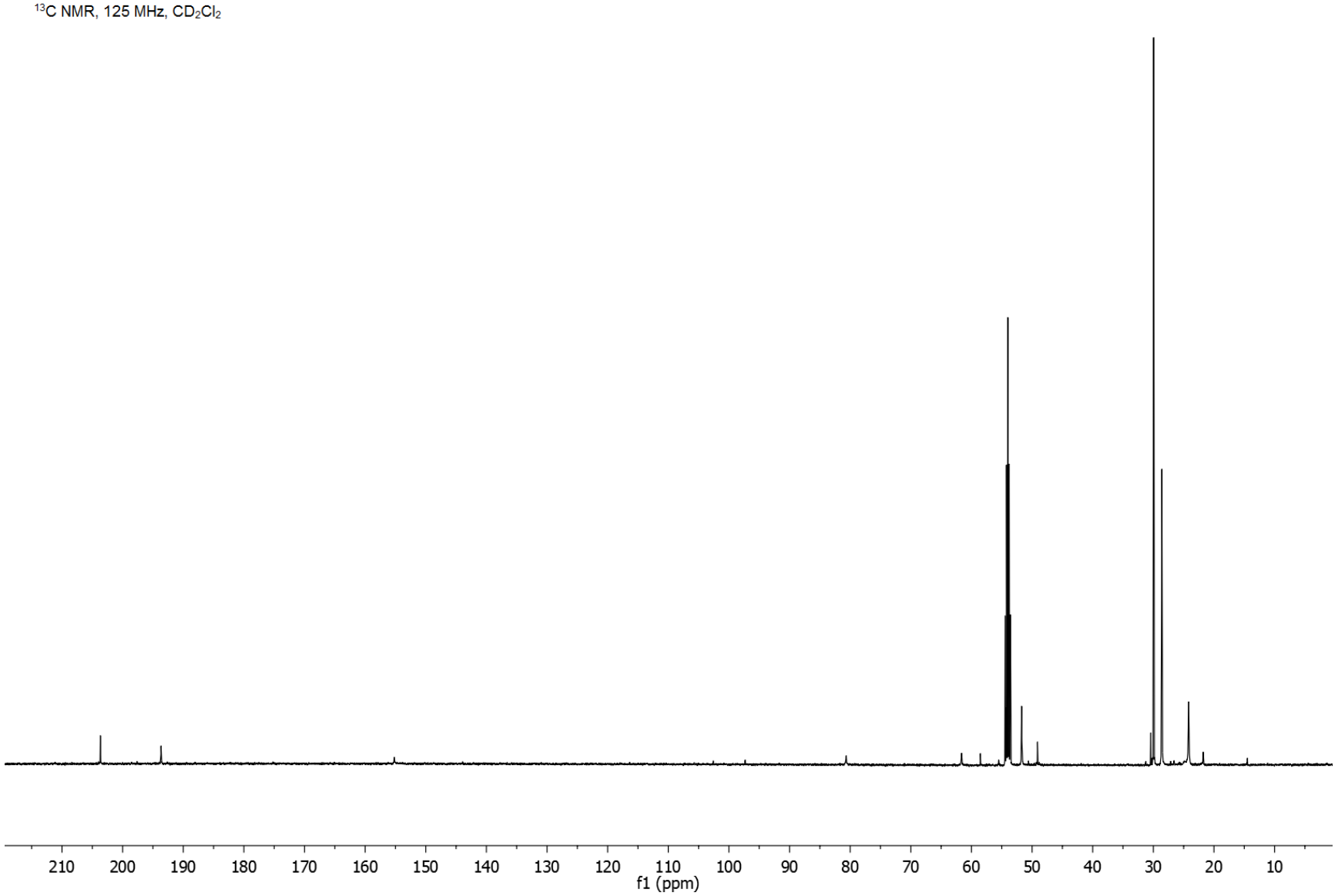

^{13}C NMR, 125 MHz, DMSO-d_6
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 500 MHz, CD_2Cl_2

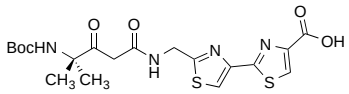


S6

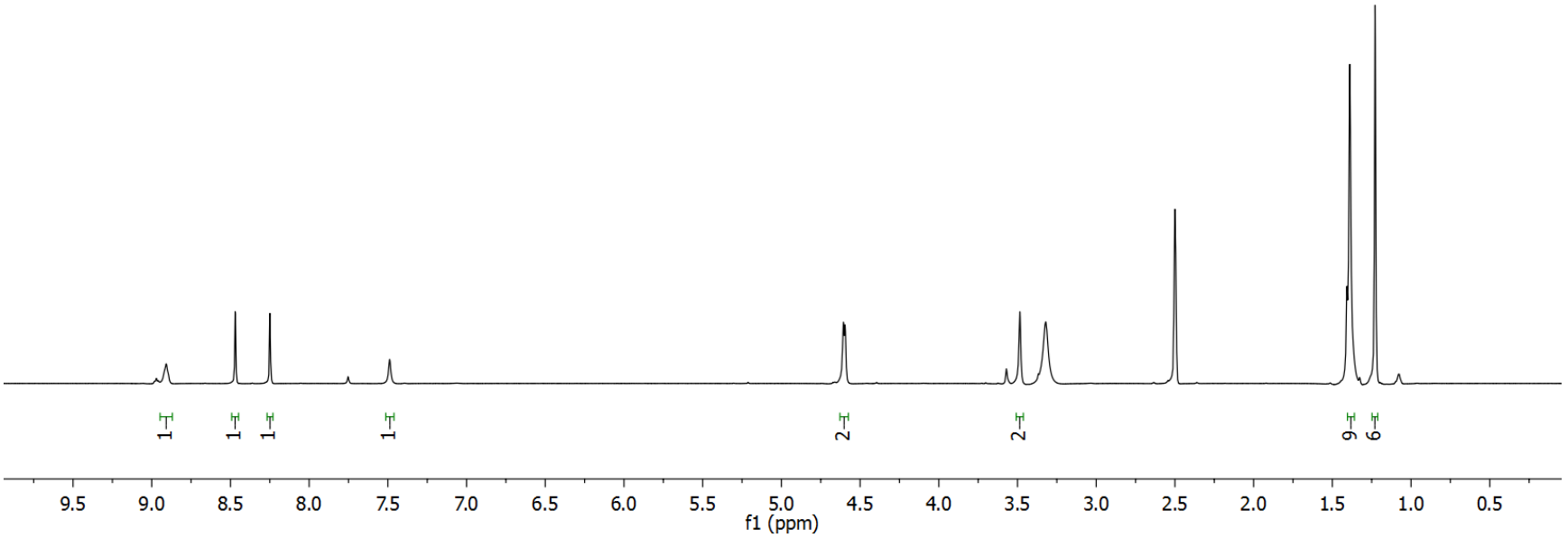


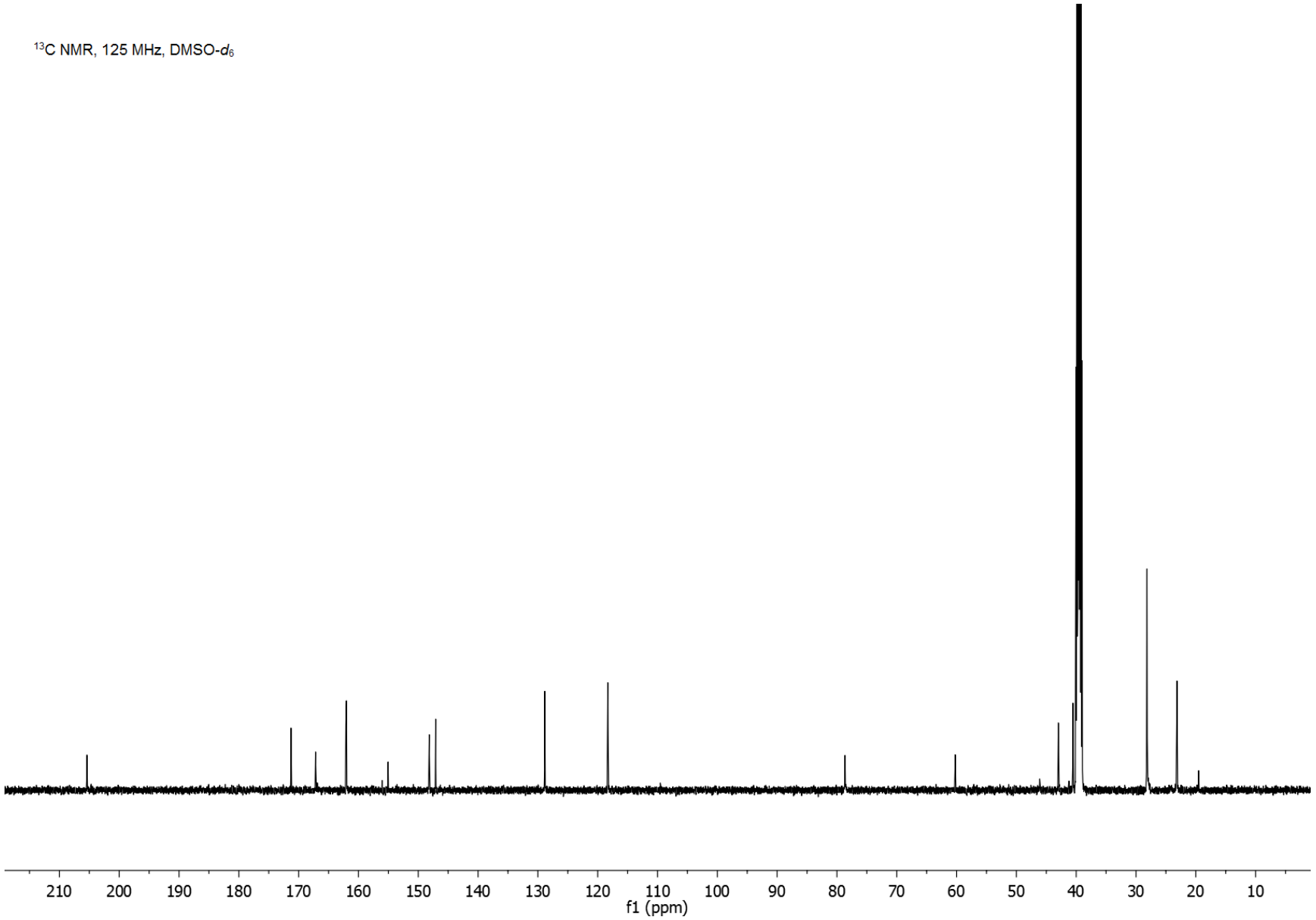

^{13}C NMR, 125 MHz, CD_2Cl_2
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 500 MHz, DMSO-d_6

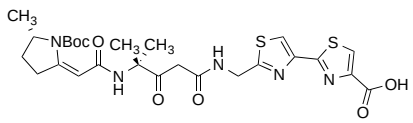


S8



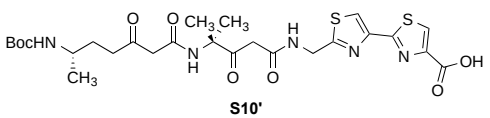

^{13}C NMR, 125 MHz, DMSO-d_6
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 400 MHz, DMSO-d_6

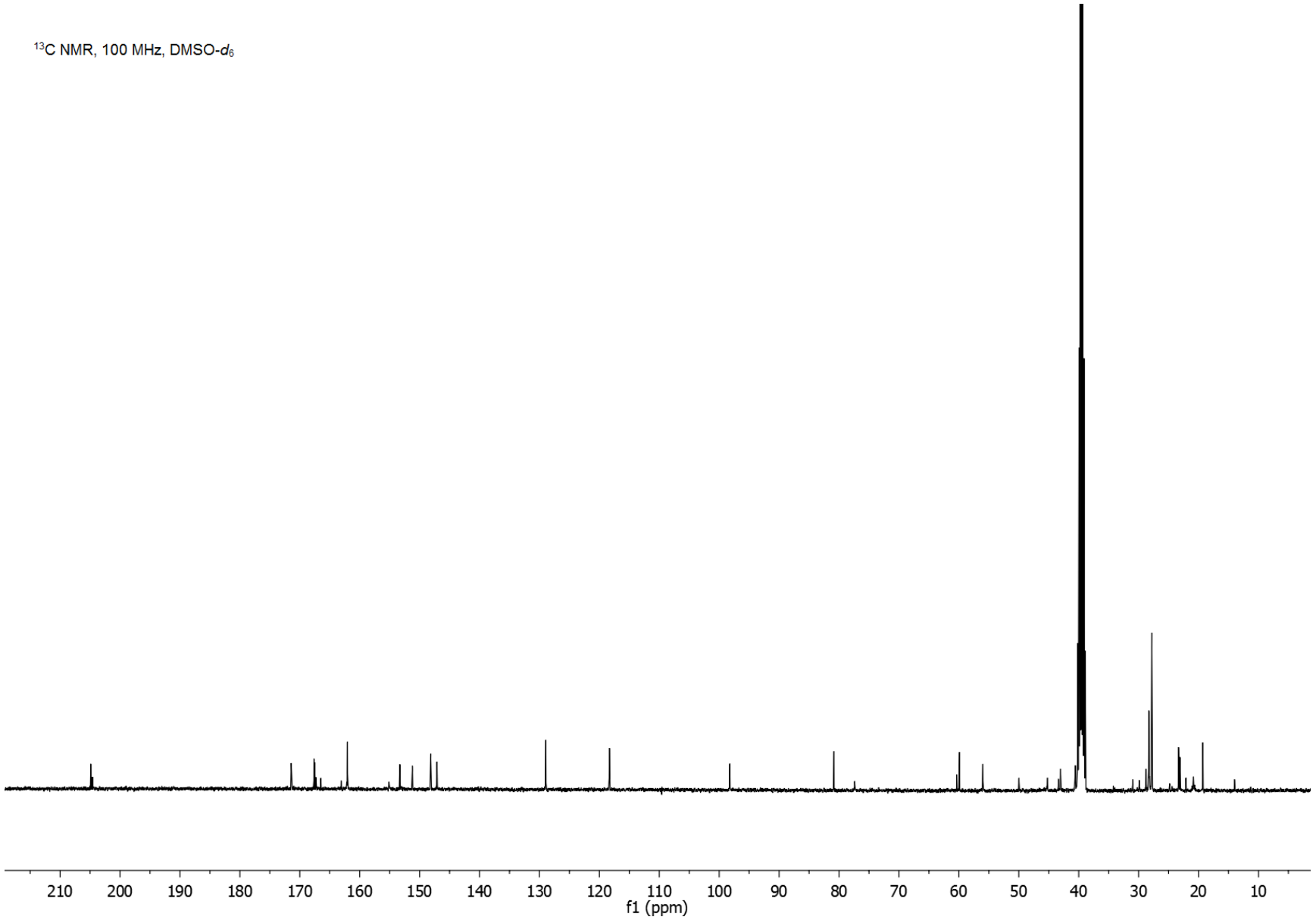


S10

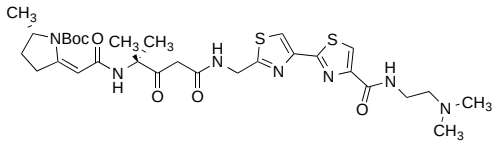
^{1}H NMR, 400 MHz, DMSO-d_6



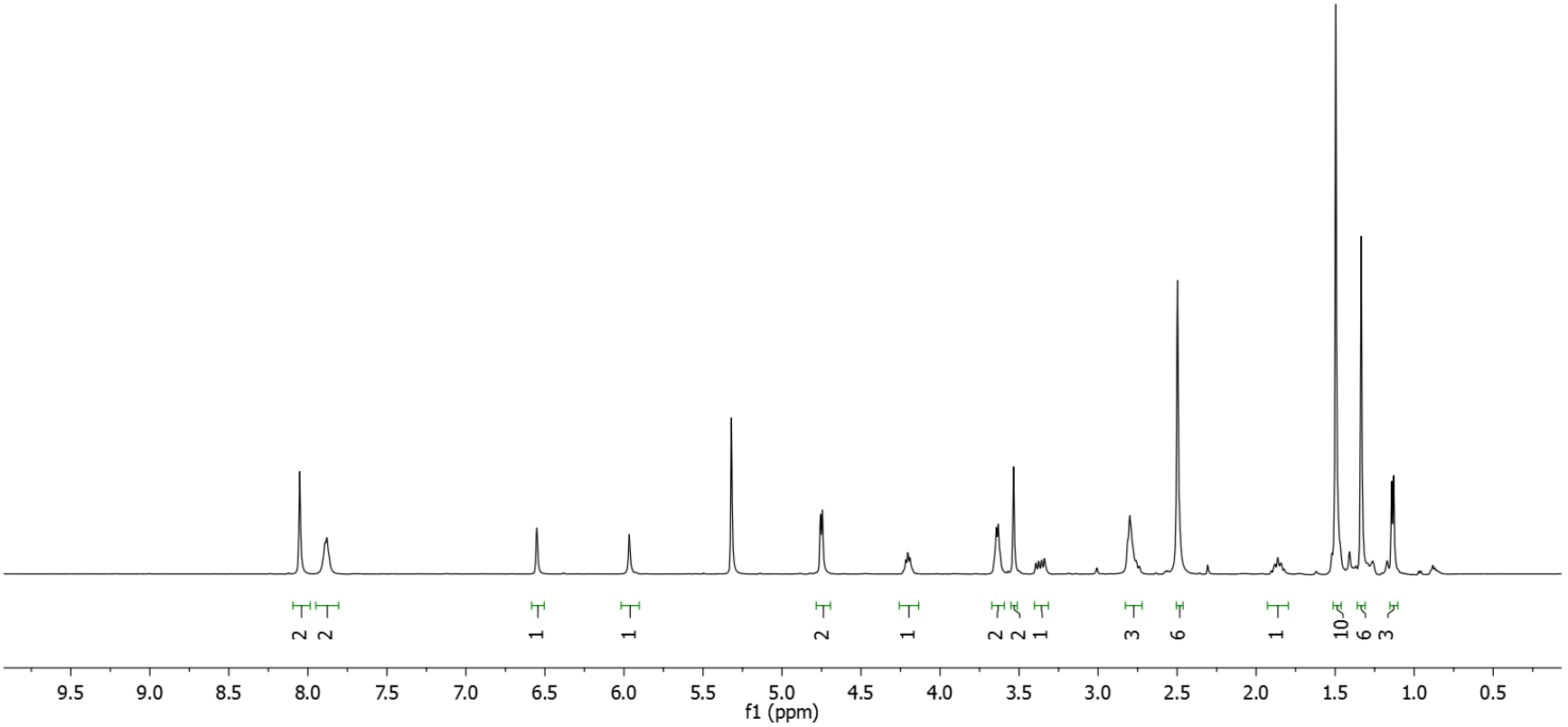
S10'

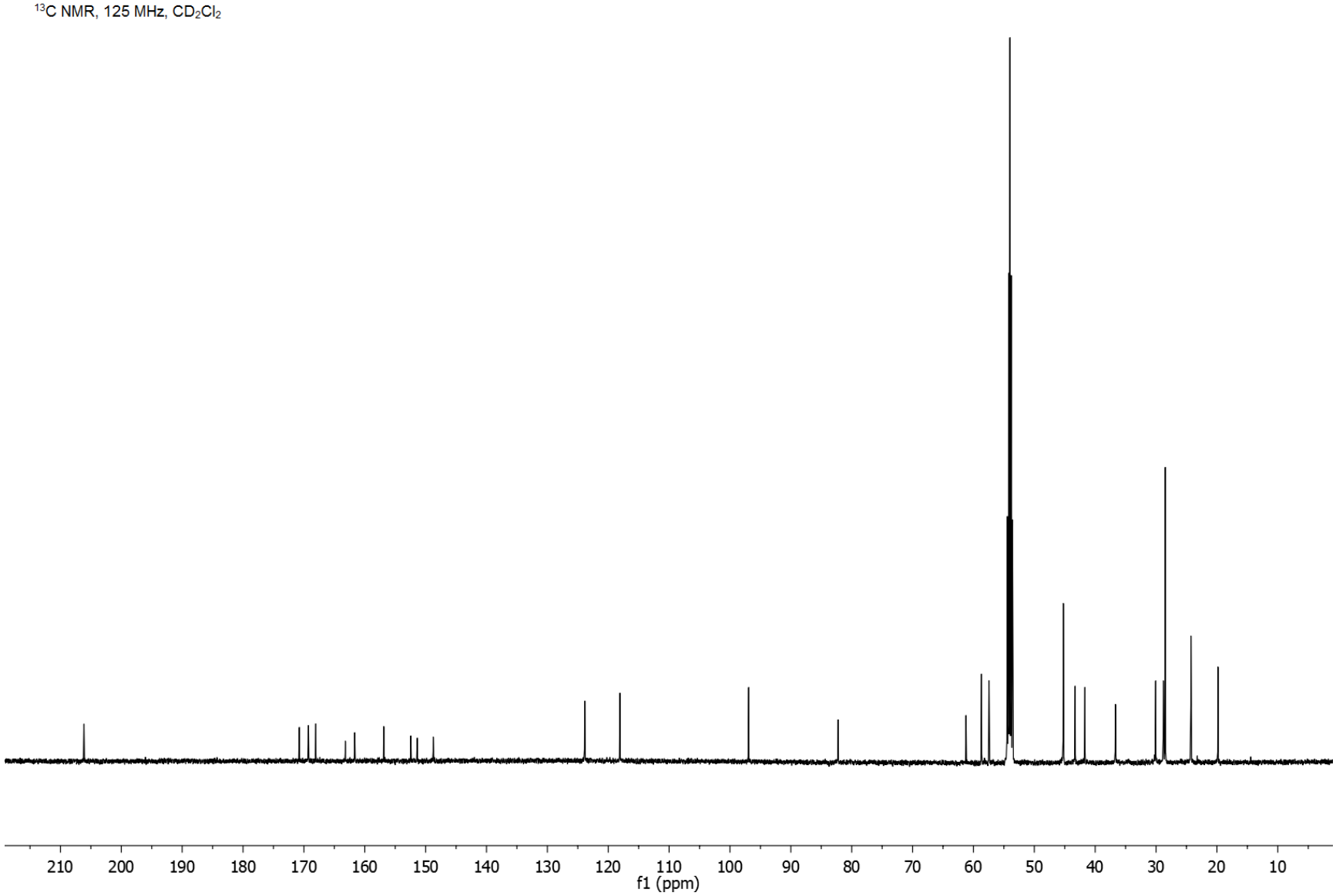

^{13}C NMR, 100 MHz, DMSO-d_6
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

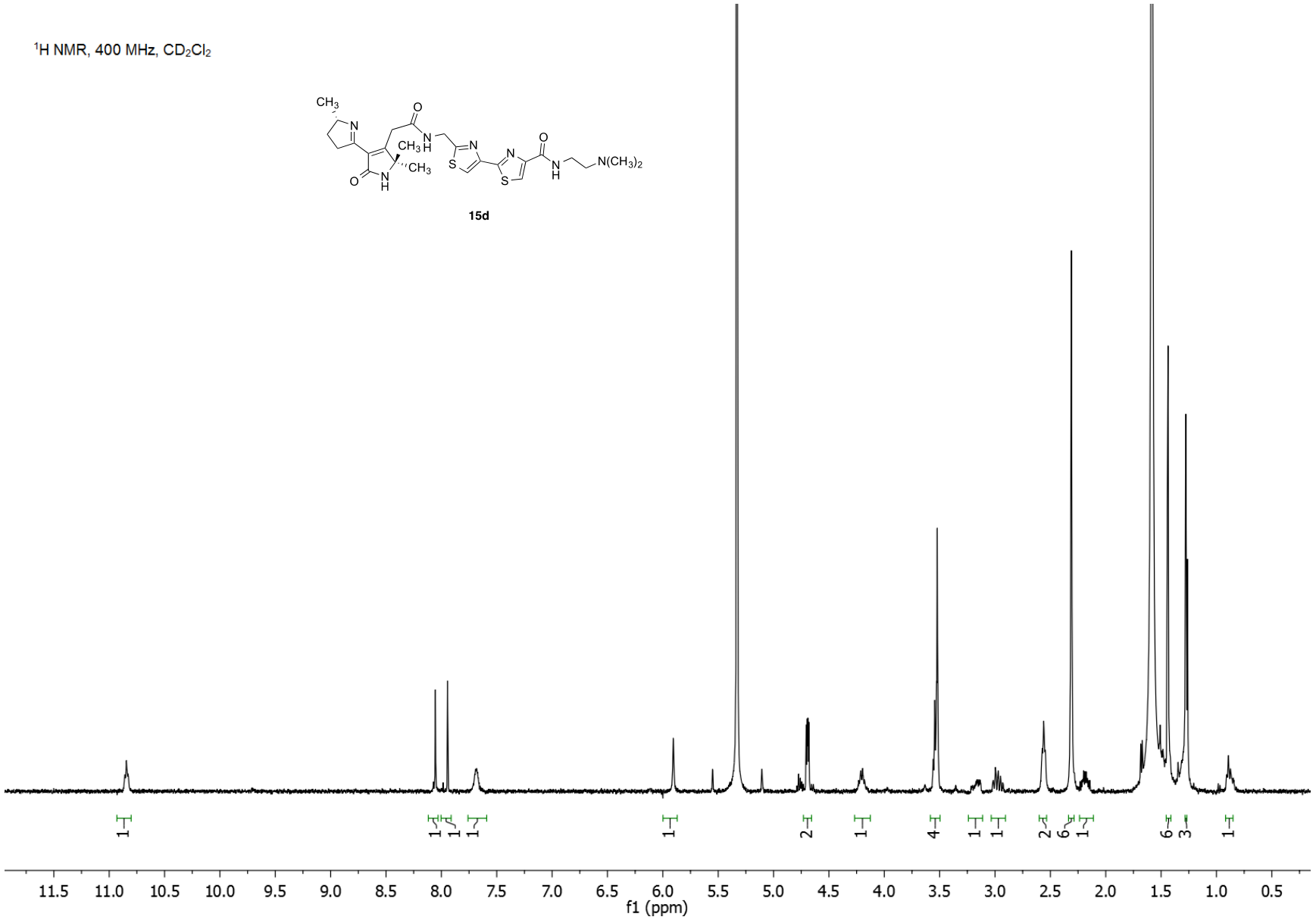
^{1}H NMR, 500 MHz, CD_2Cl_2



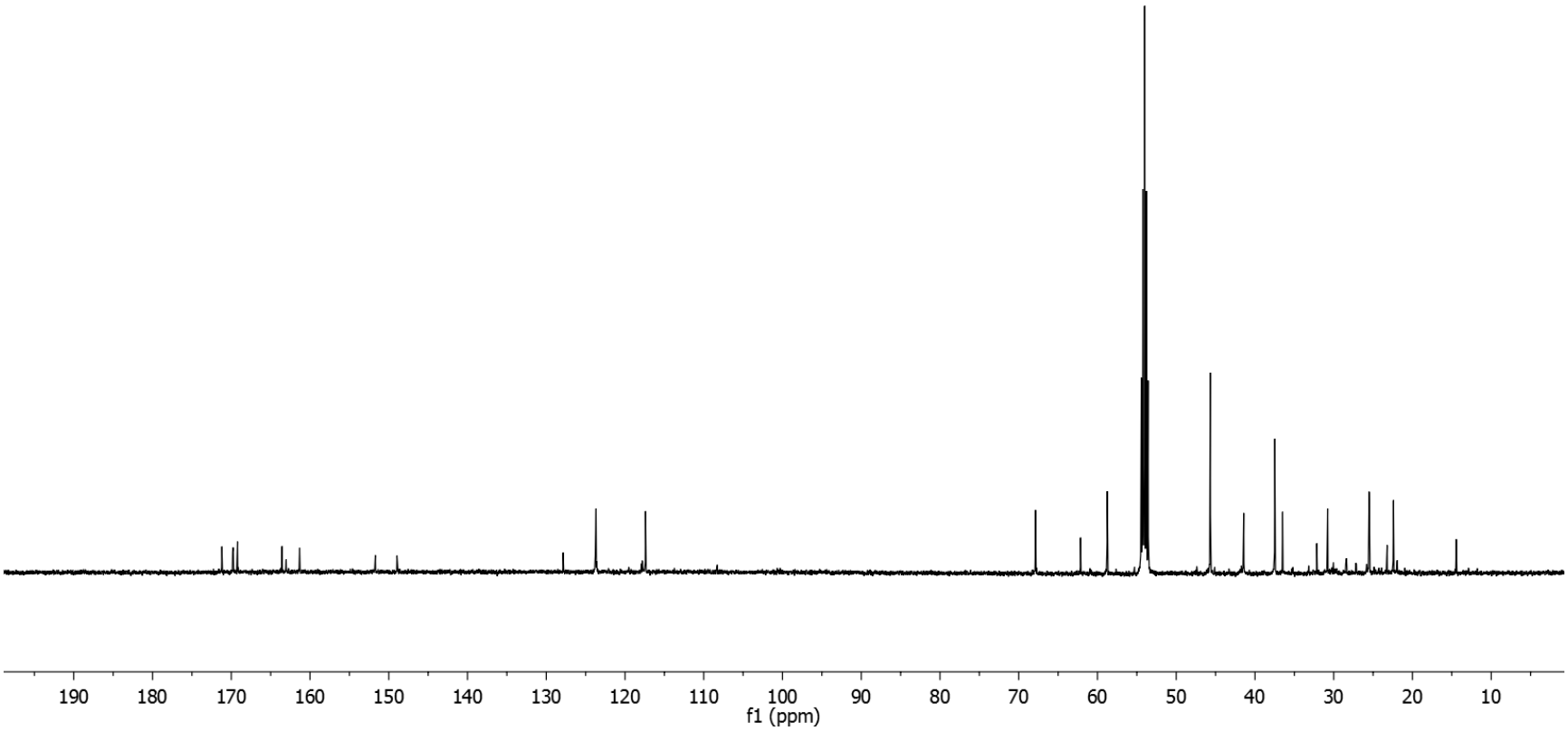
14d



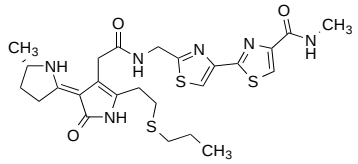

^{13}C NMR, 125 MHz, CD_2Cl_2
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


^{1}H NMR, 400 MHz, CD_2Cl_2
15d
f1 (ppm)

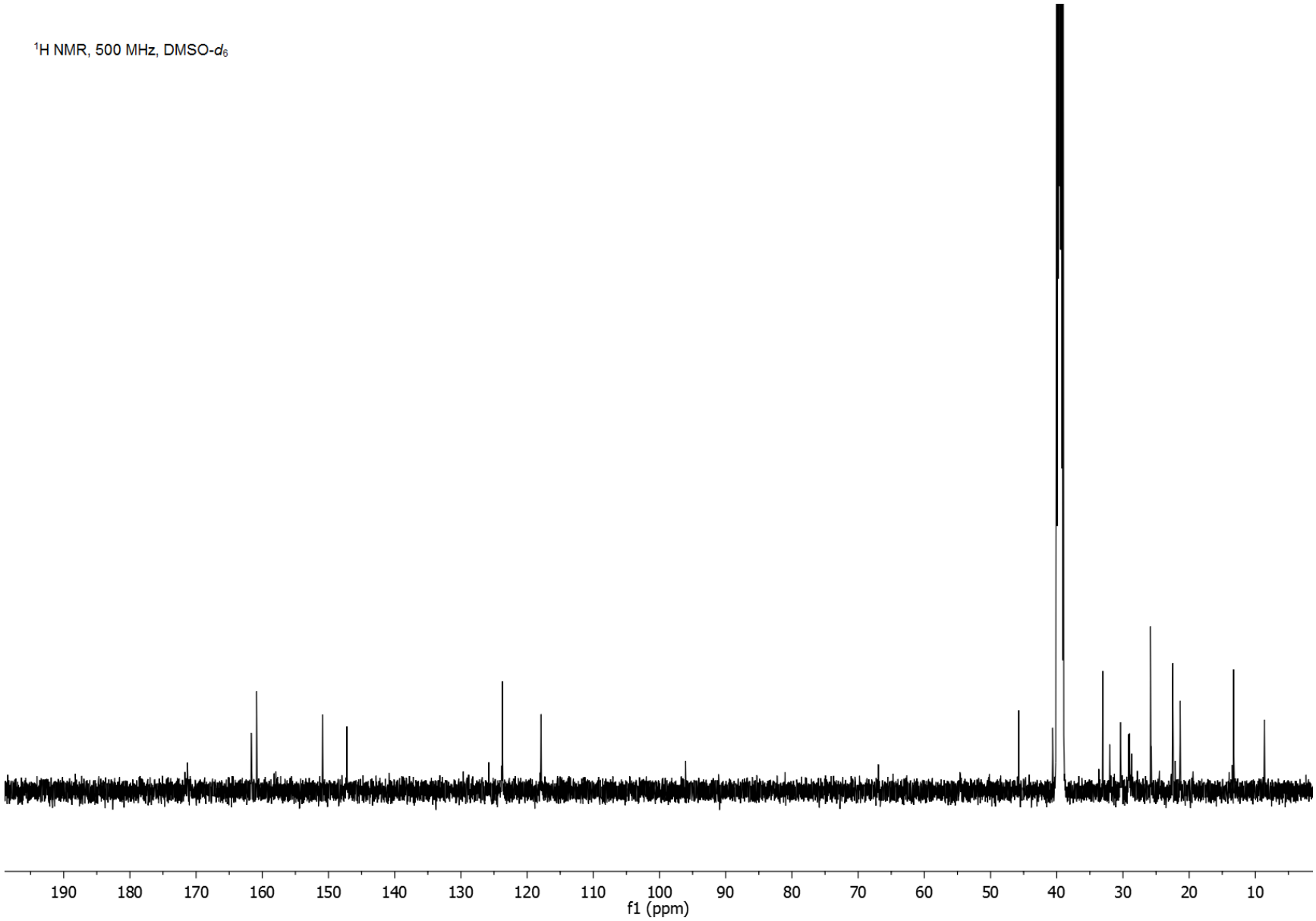
^{13}C NMR, 125 MHz, CD_2Cl_2



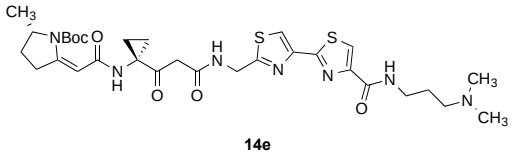
^{1}H NMR, 500 MHz, DMSO-d_6



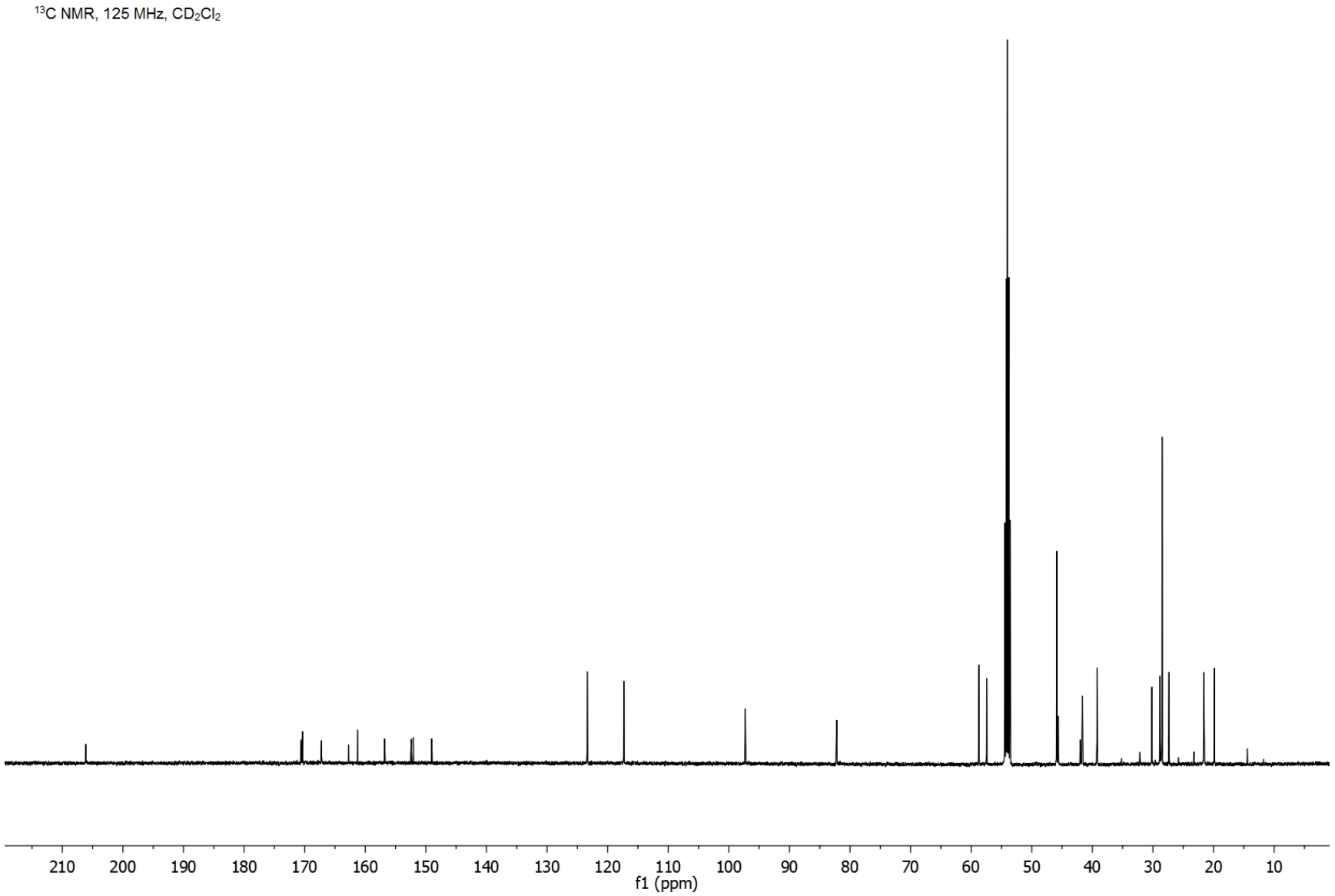
18


^{1}H NMR, 500 MHz, DMSO-d_6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

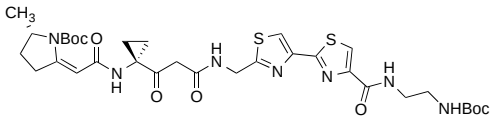
^{1}H NMR, 500 MHz, CD_2Cl_2



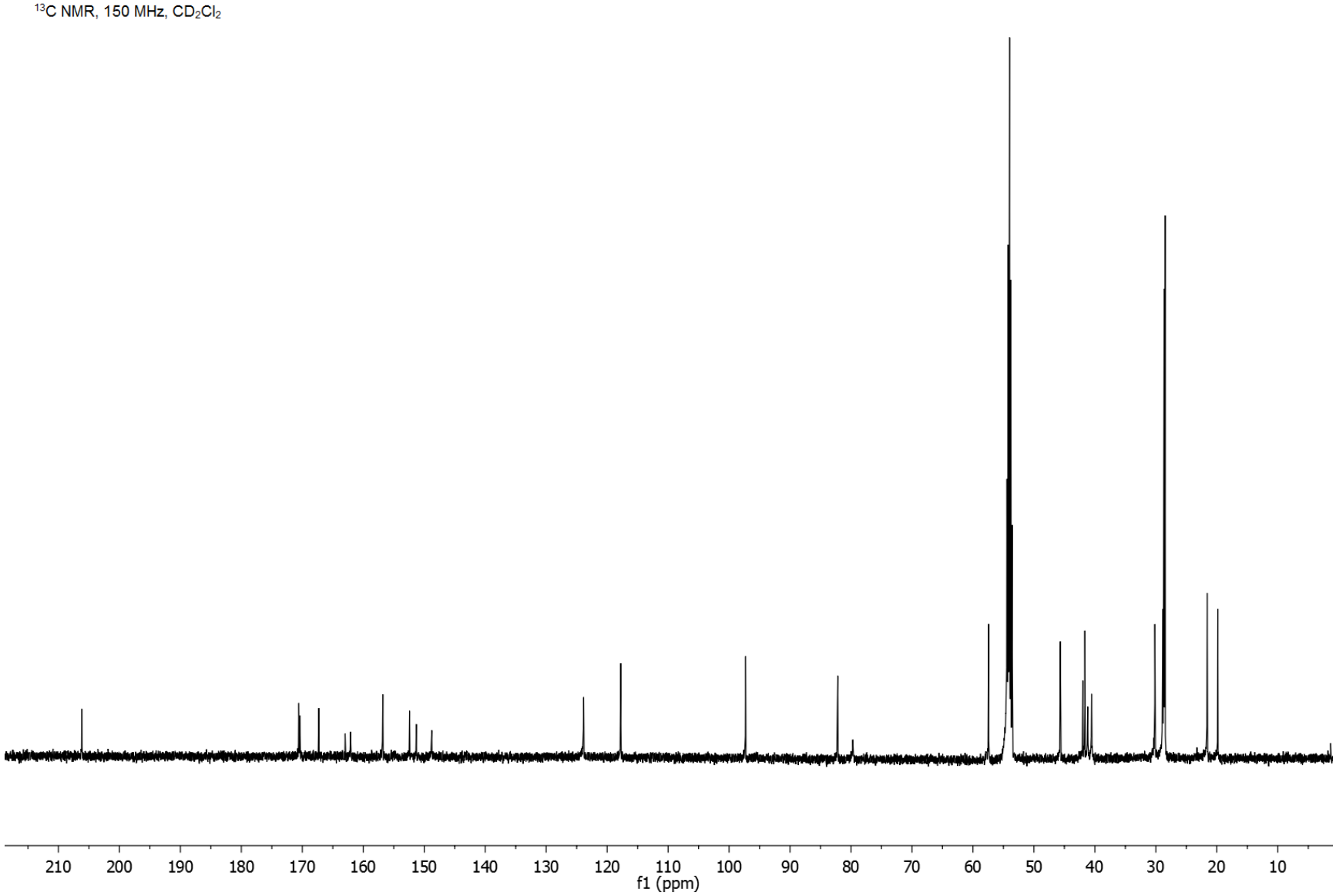
14e


^{13}C NMR, 125 MHz, CD_2Cl_2
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

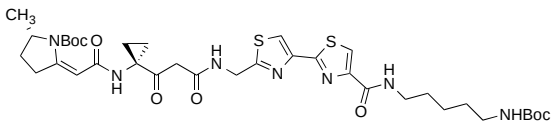
^{1}H NMR, 600 MHz, CD_2Cl_2



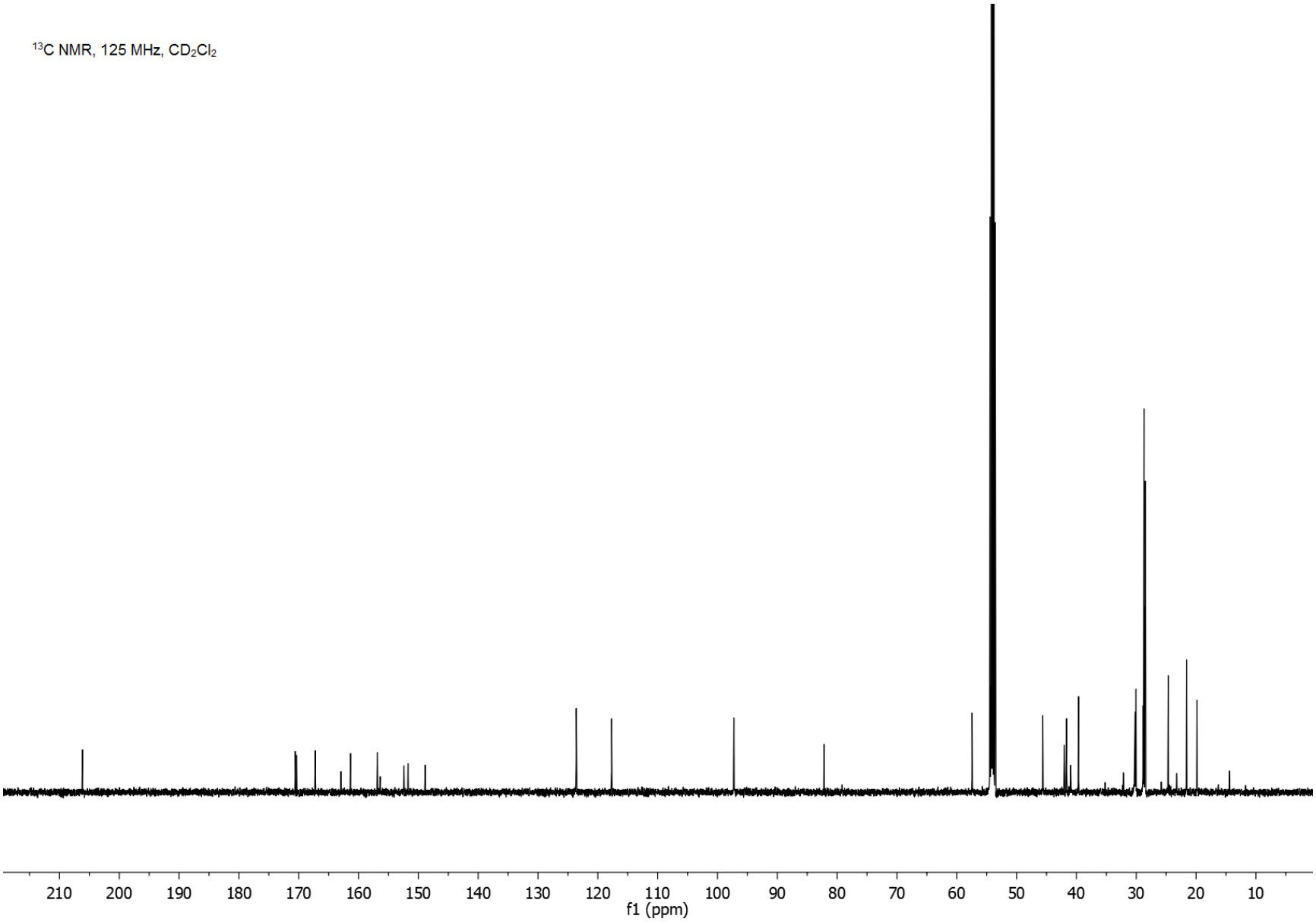
14f


^{13}C NMR, 150 MHz, CD_2Cl_2
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

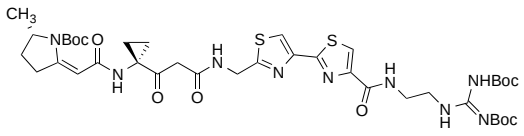
^{1}H NMR, 500 MHz, CD_2Cl_2



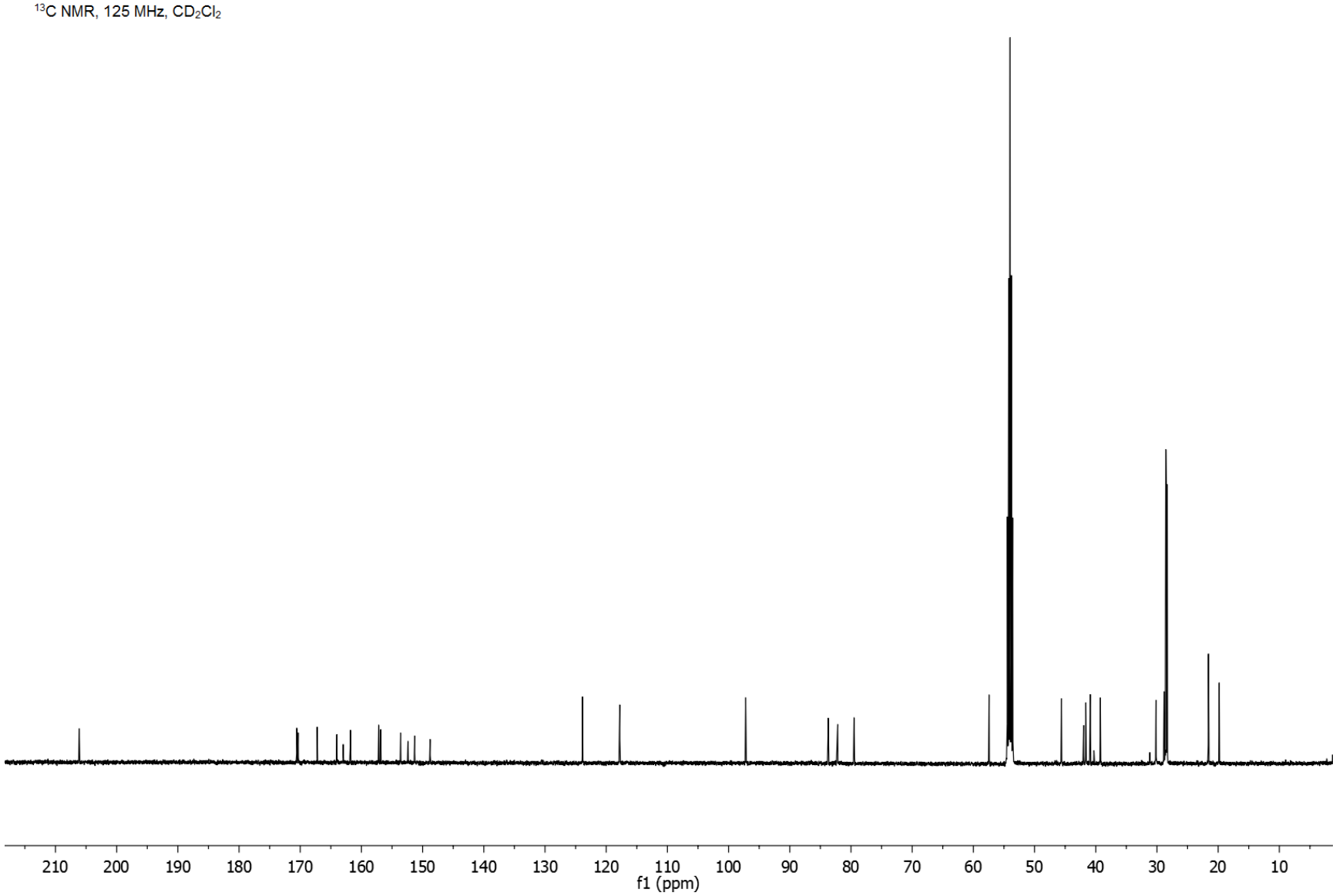
14g


^{13}C NMR, 125 MHz, CD_2Cl_2
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

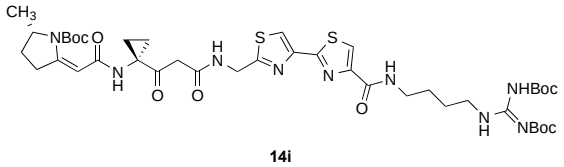
^{1}H NMR, 500 MHz, CD_2Cl_2



14h

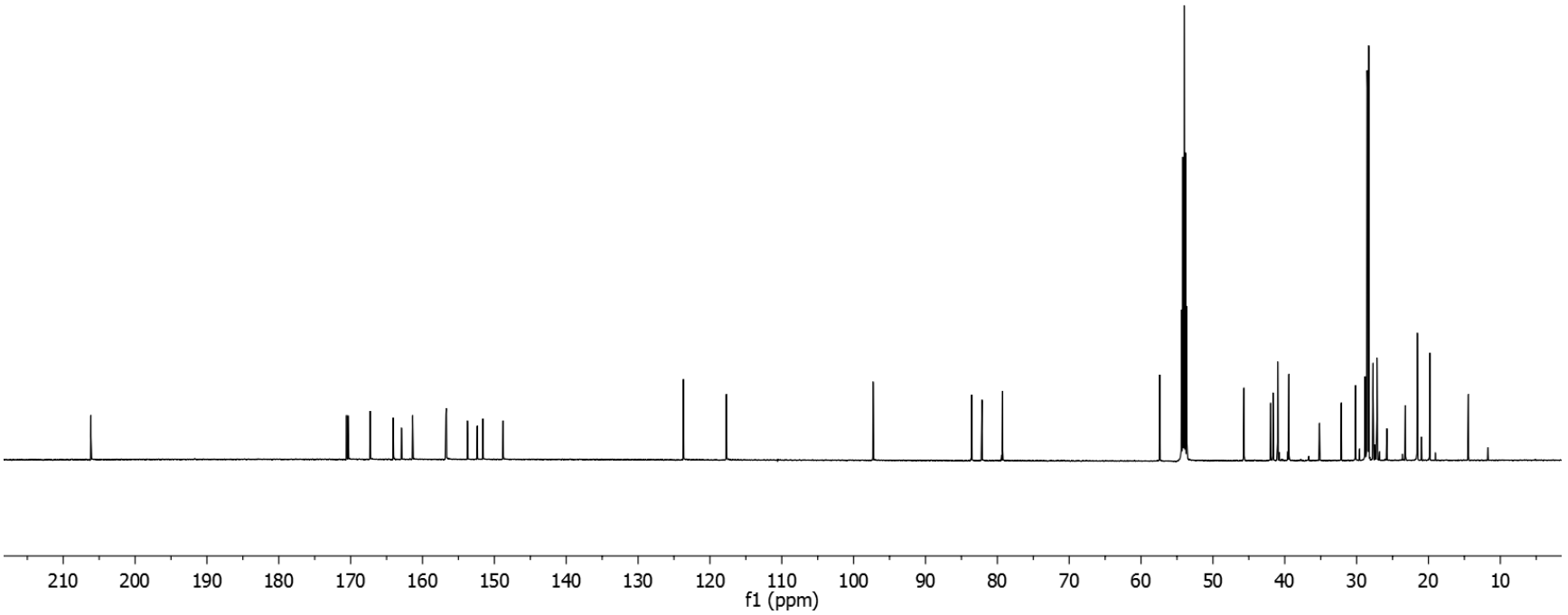

^{13}C NMR, 125 MHz, CD_2Cl_2
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 600 MHz, CD_2Cl_2

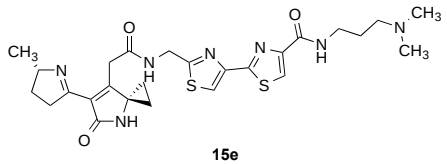


14i

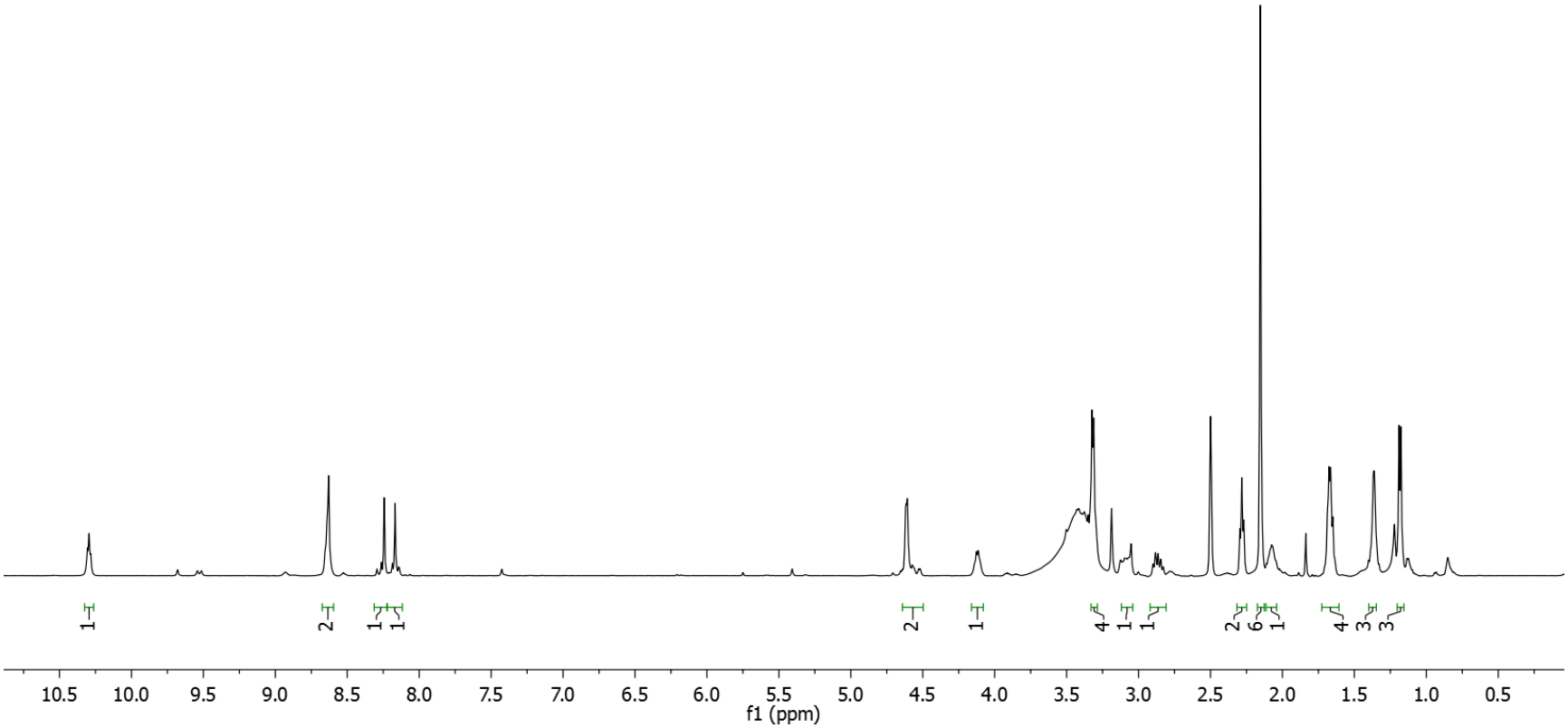
^{13}C NMR, 150 MHz, CD_2Cl_2

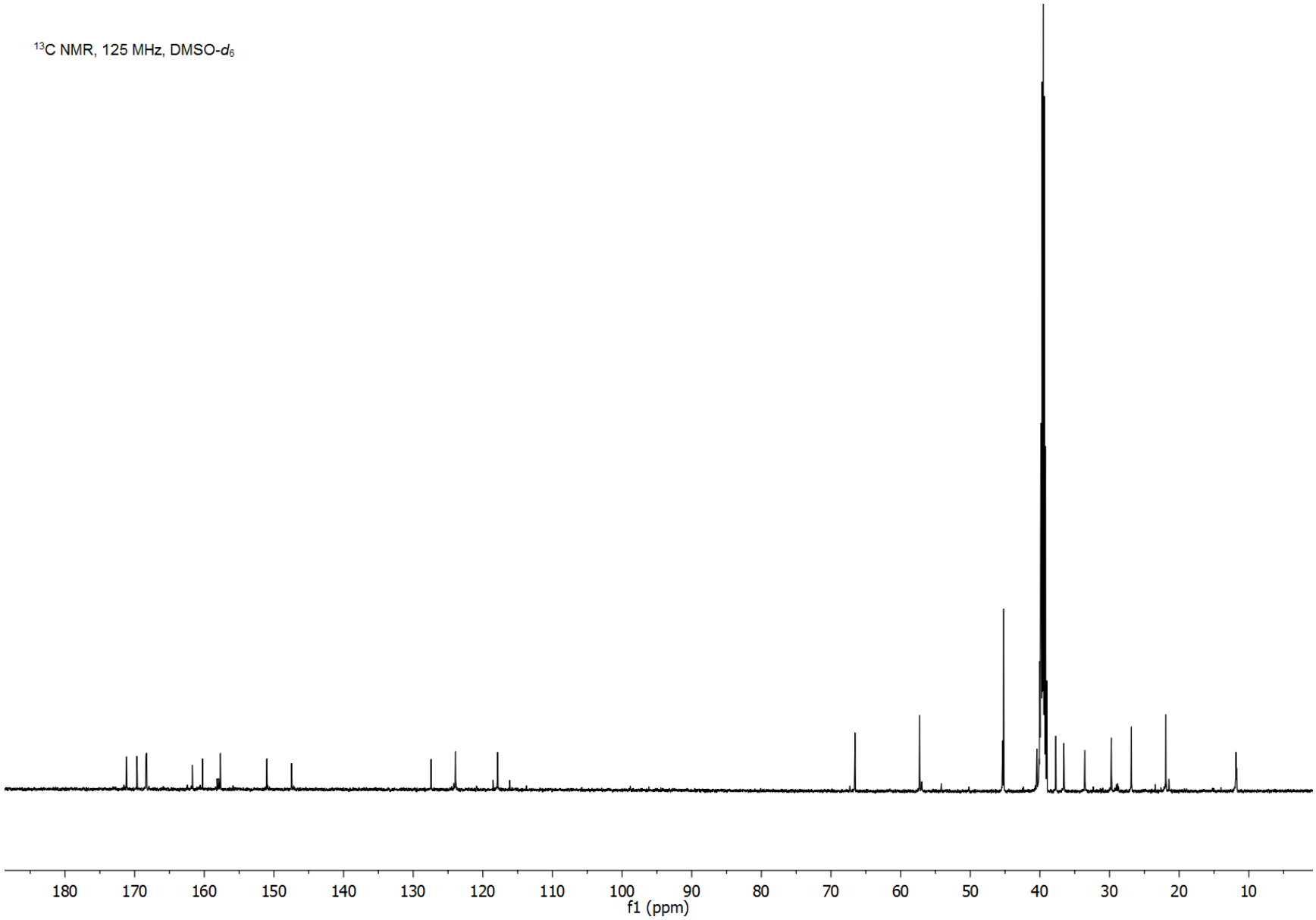


^{1}H NMR, 500 MHz, DMSO-d_6



15e




^{13}C NMR, 125 MHz, DMSO-d_6
180
170
160
150
140
130
120
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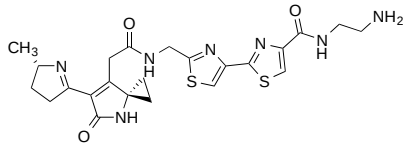
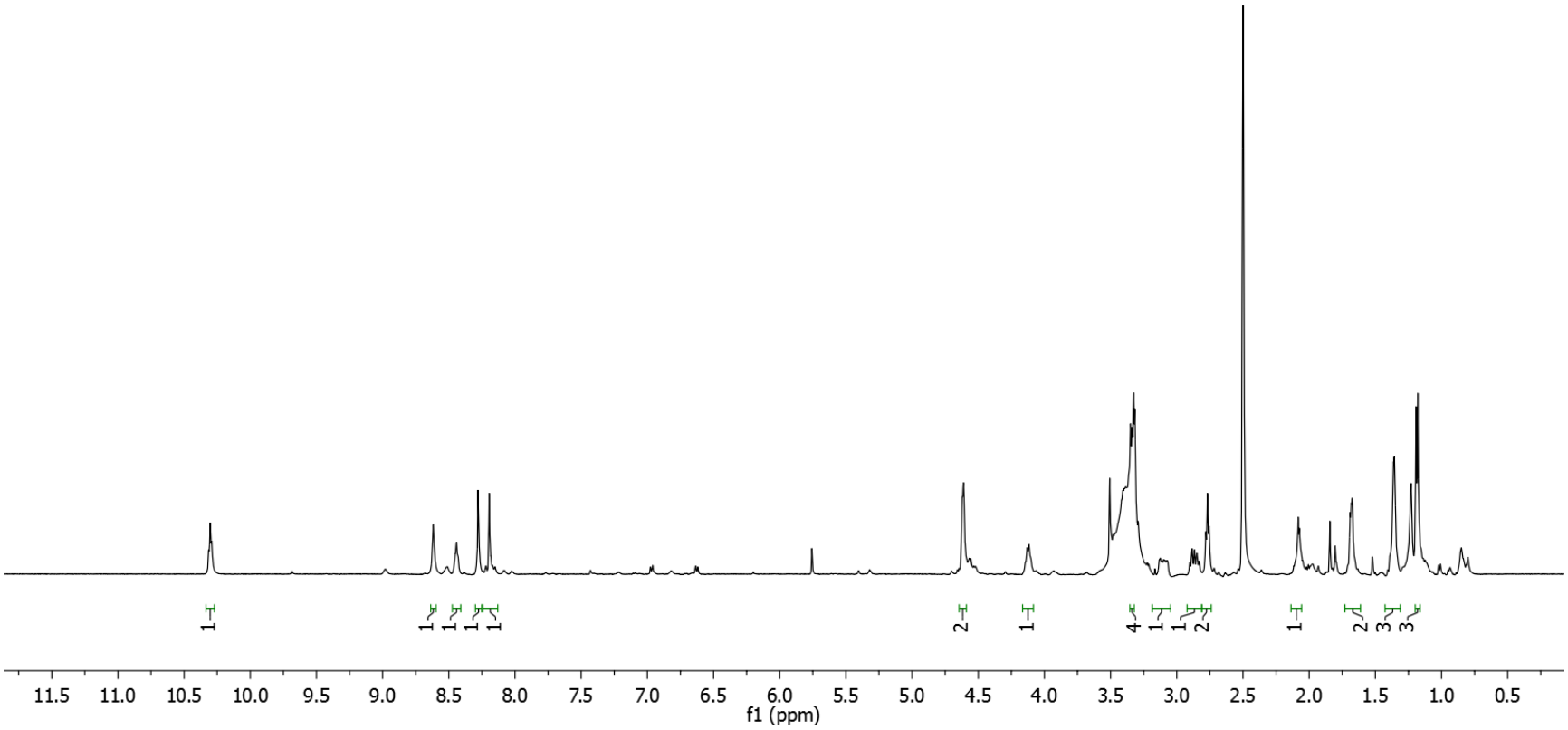
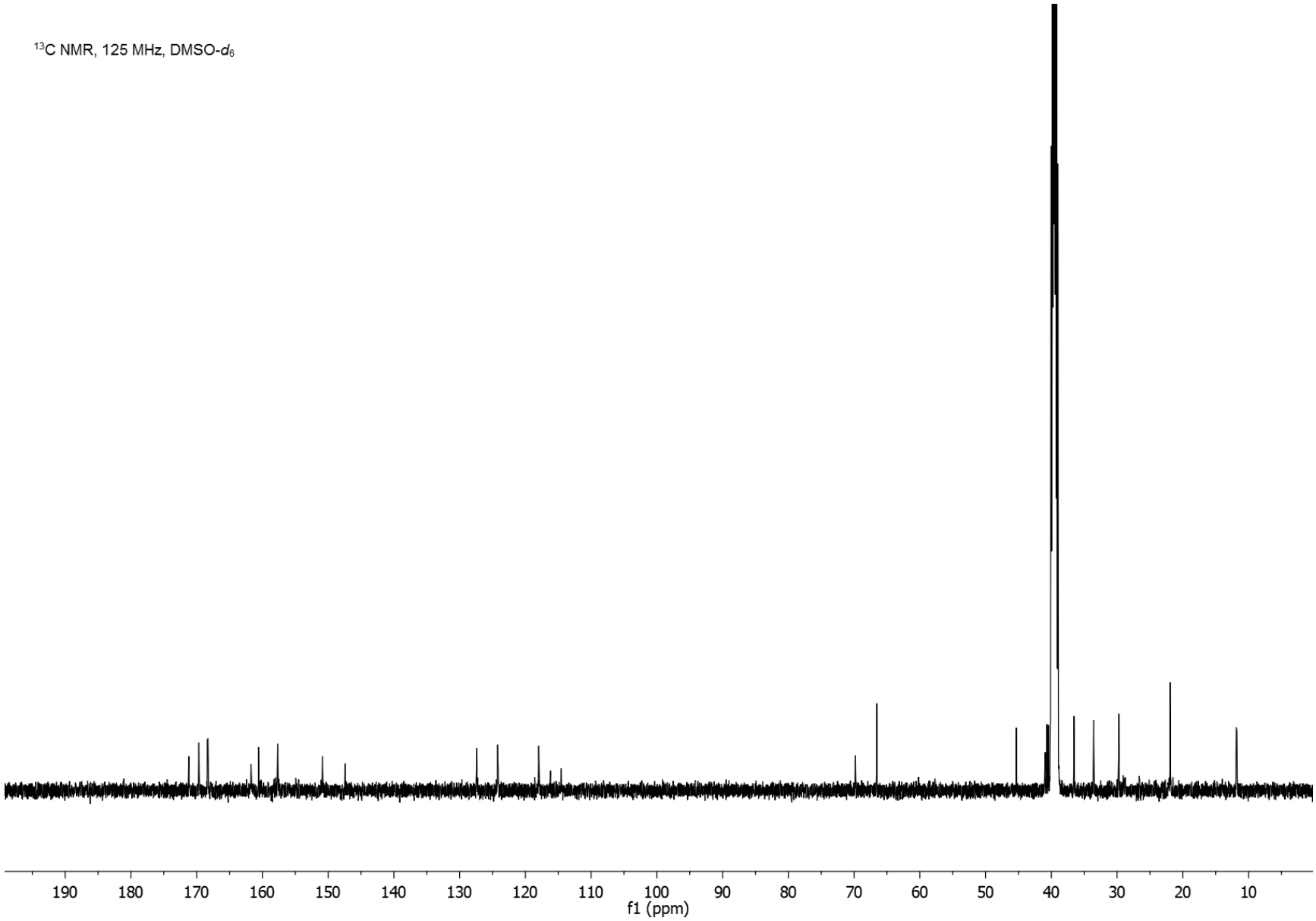
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 500 MHz, DMSO-d_6

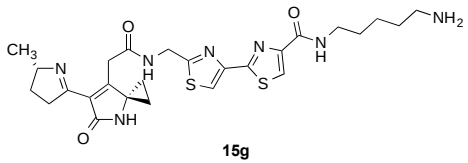


15f

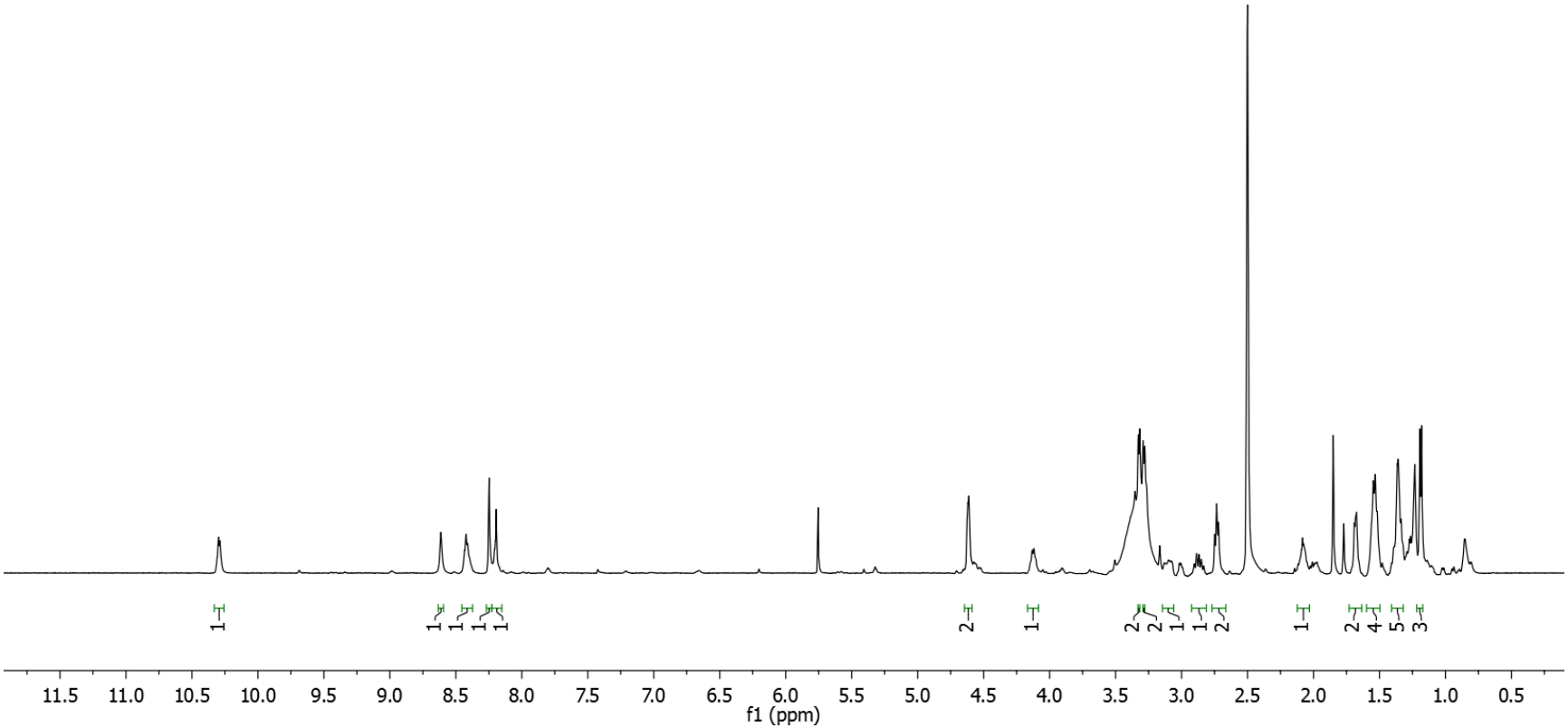


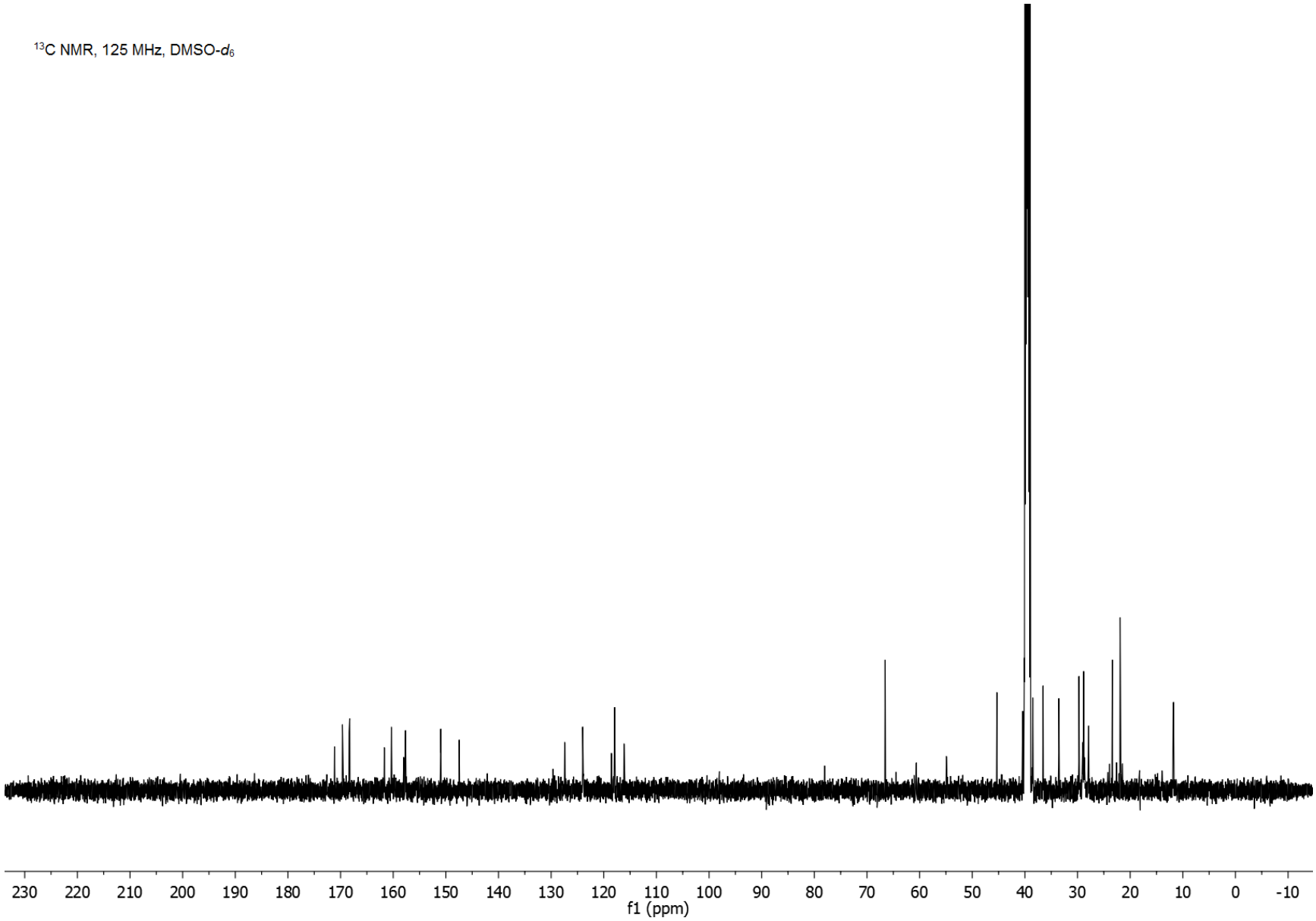

^{13}C NMR, 125 MHz, DMSO-d_6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 500 MHz, DMSO-d_6

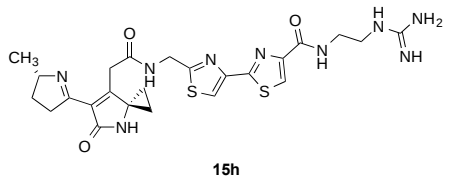


15g

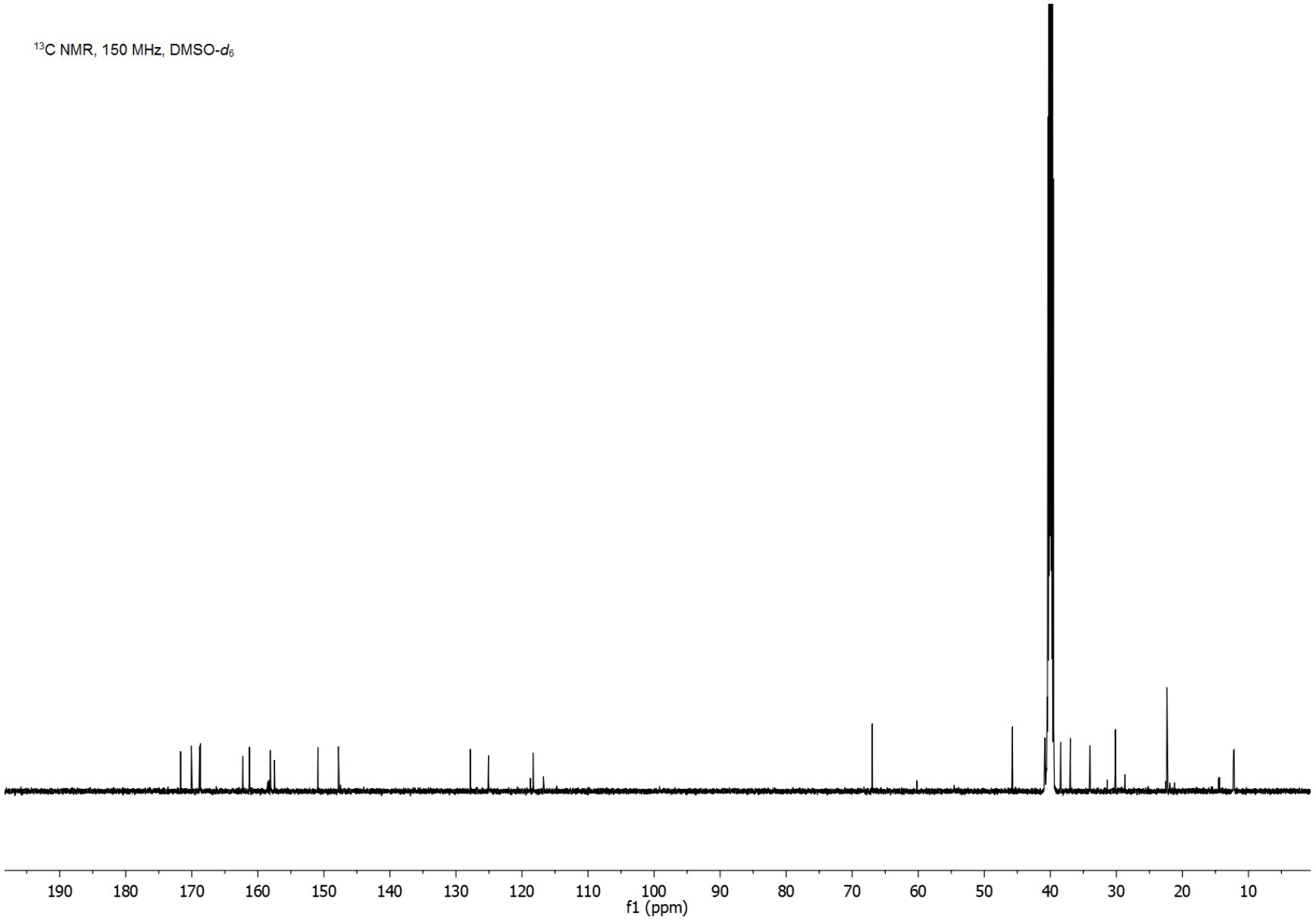



^{13}C NMR, 125 MHz, DMSO-d_6
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

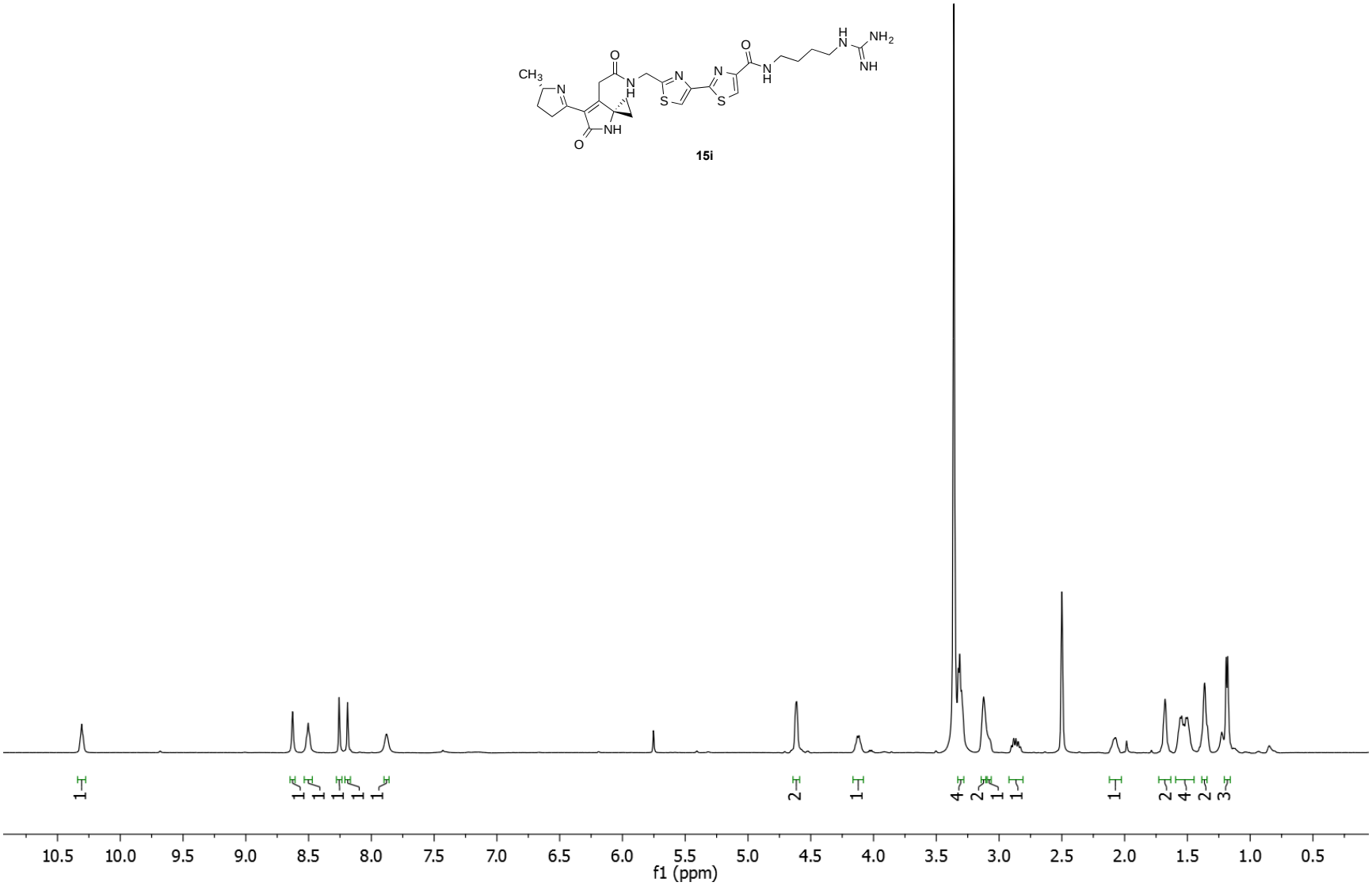
^{1}H NMR, 600 MHz, DMSO-d_6



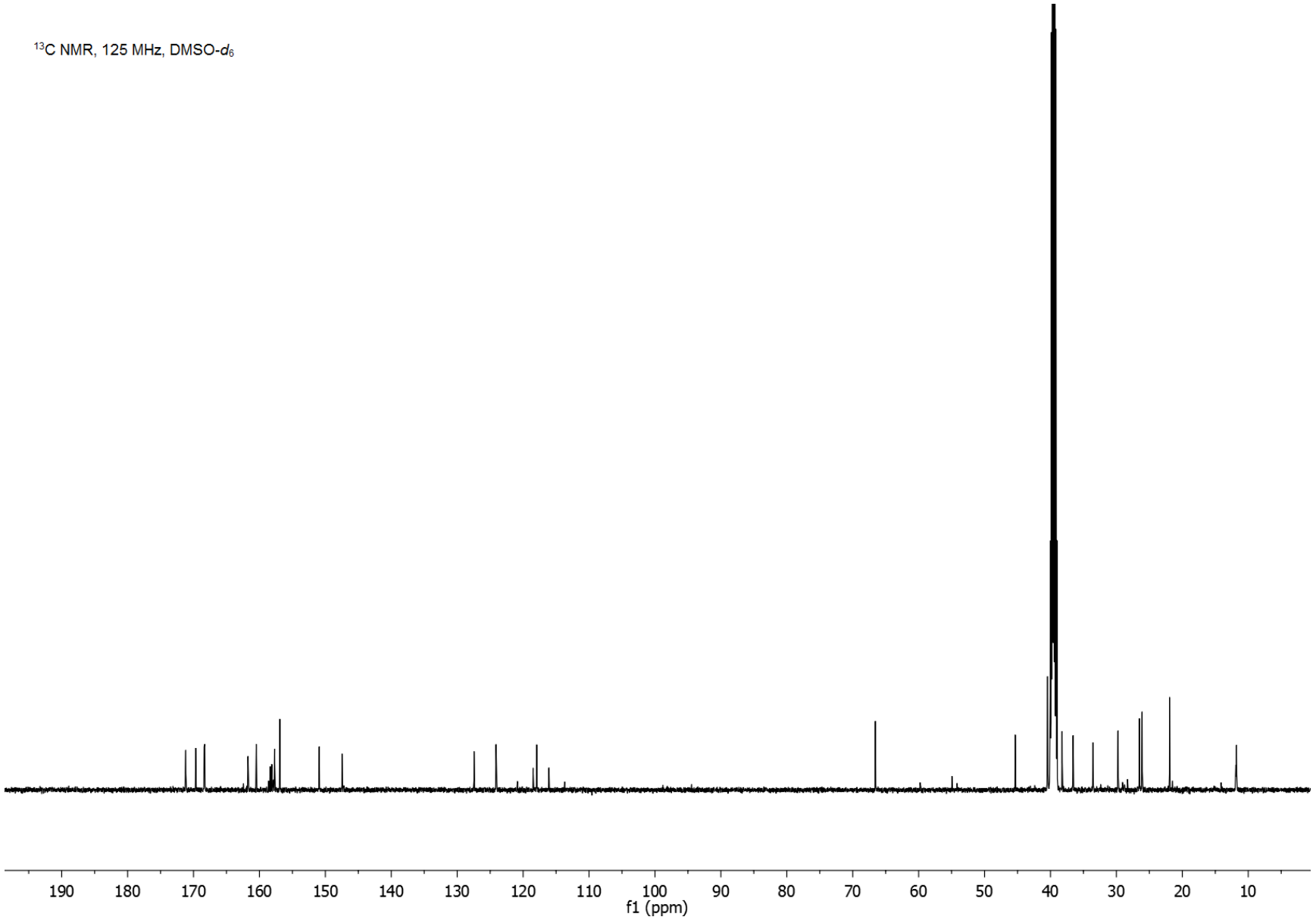
15h


^{13}C NMR, 150 MHz, DMSO-d_6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

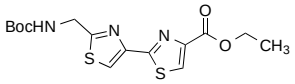
^{1}H NMR, 500 MHz, DMSO-d_6



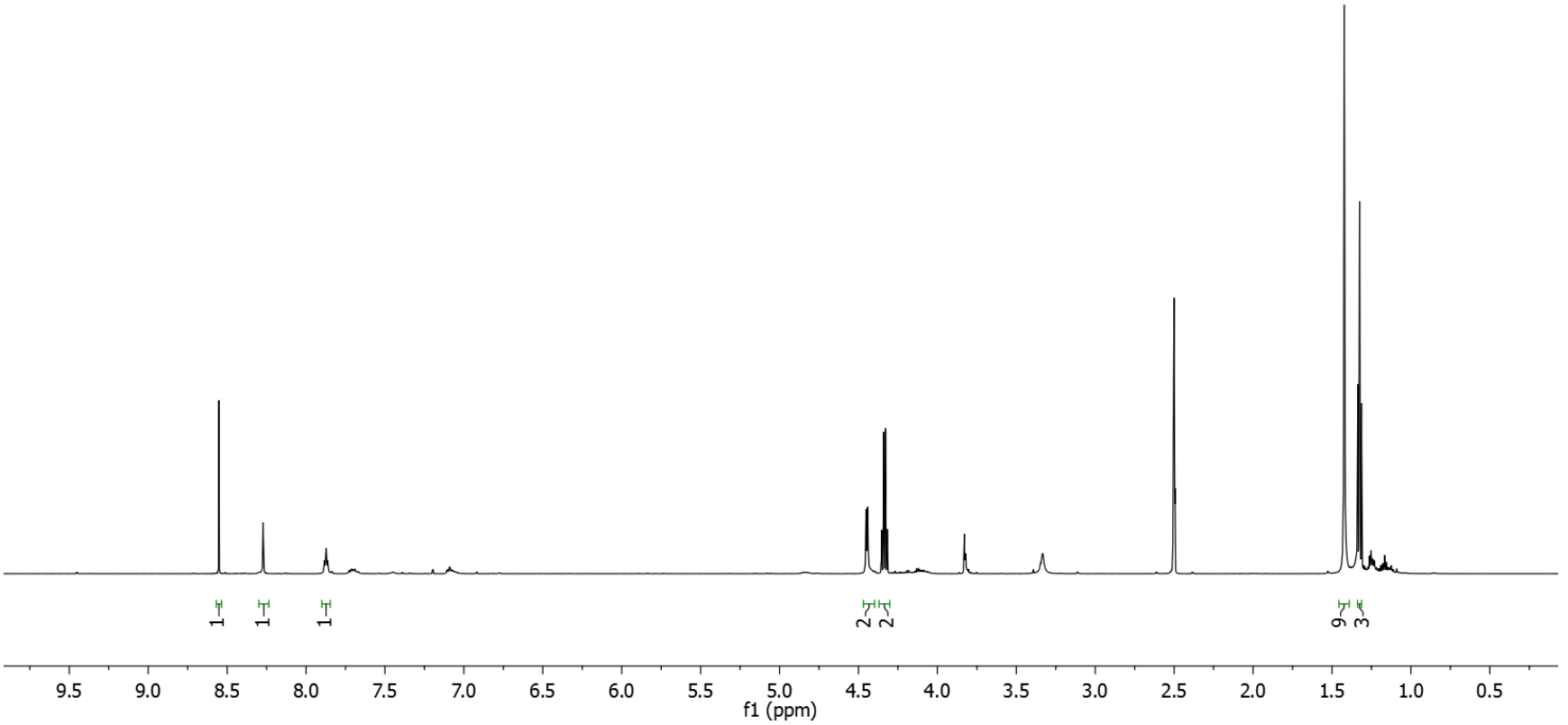
15i

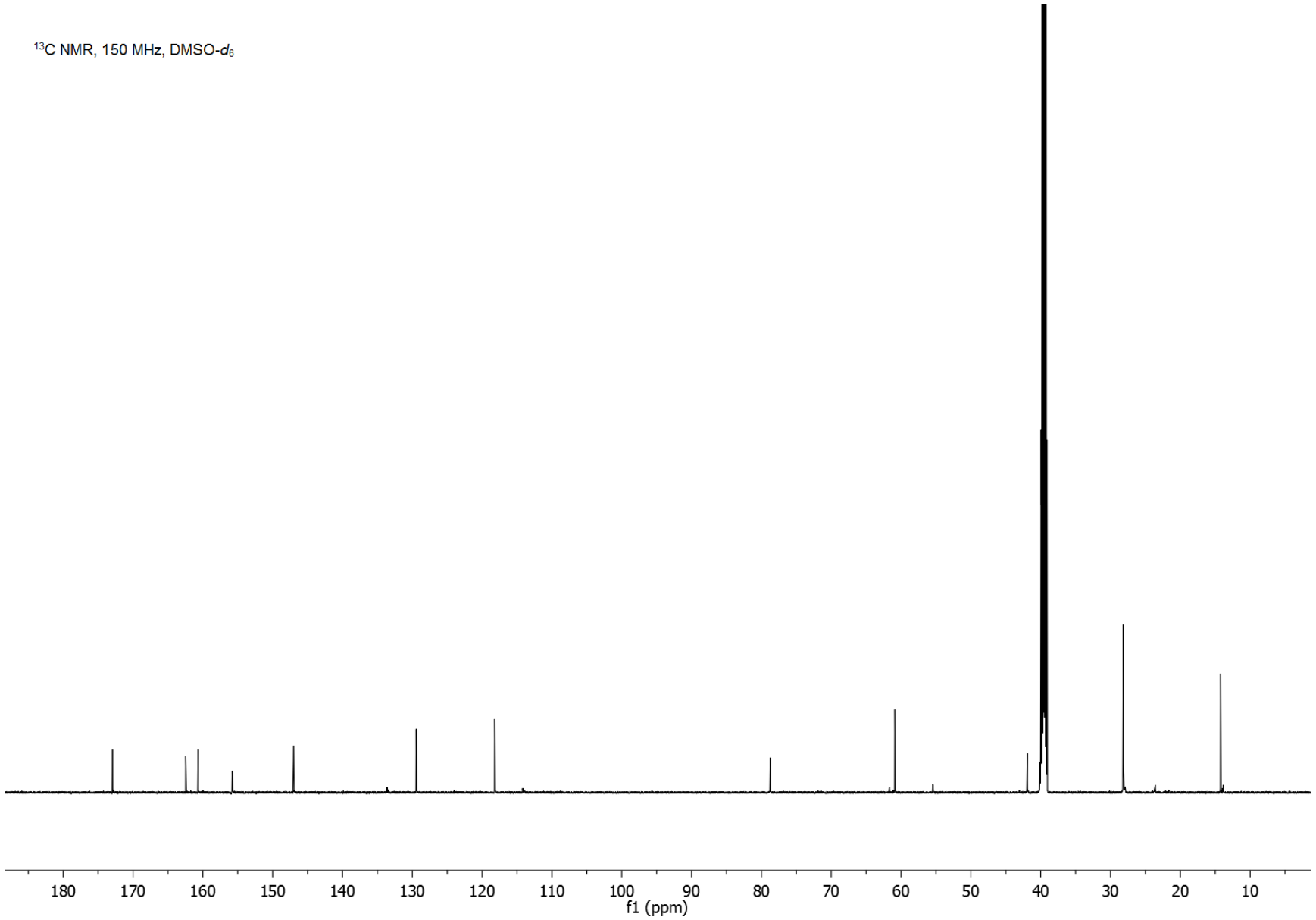

^{13}C NMR, 125 MHz, DMSO-d_6
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 600 MHz, DMSO-d_6

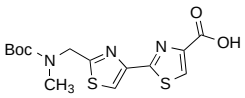


S14

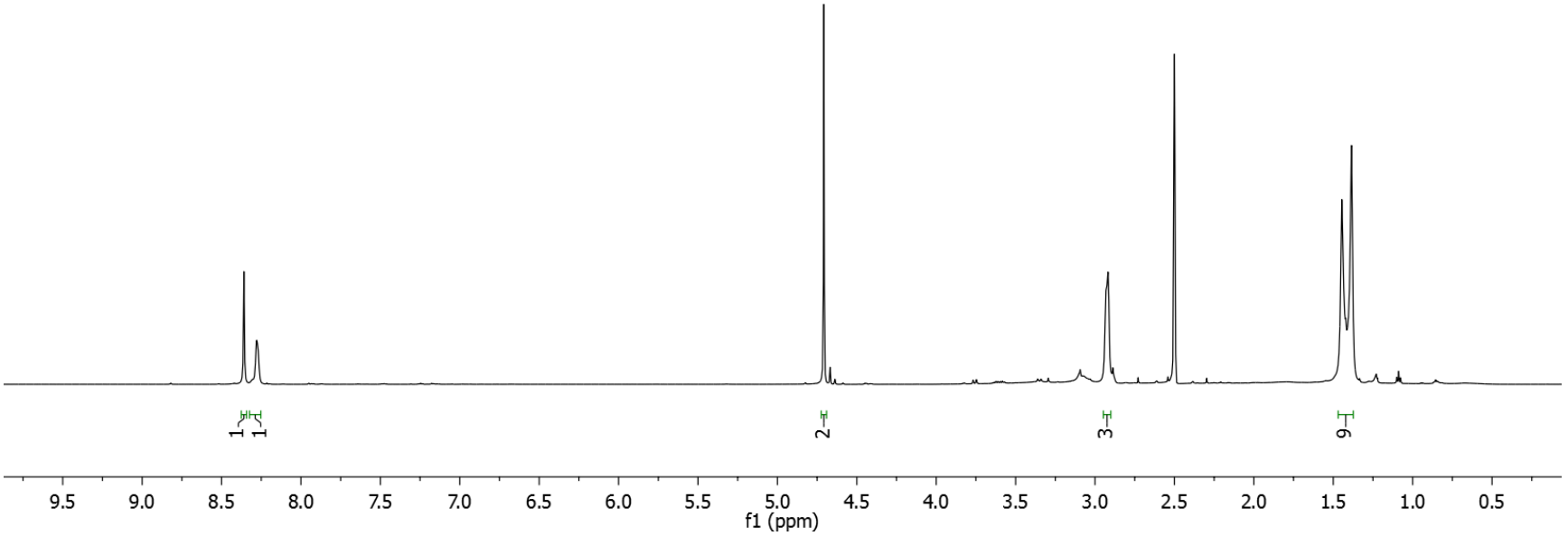


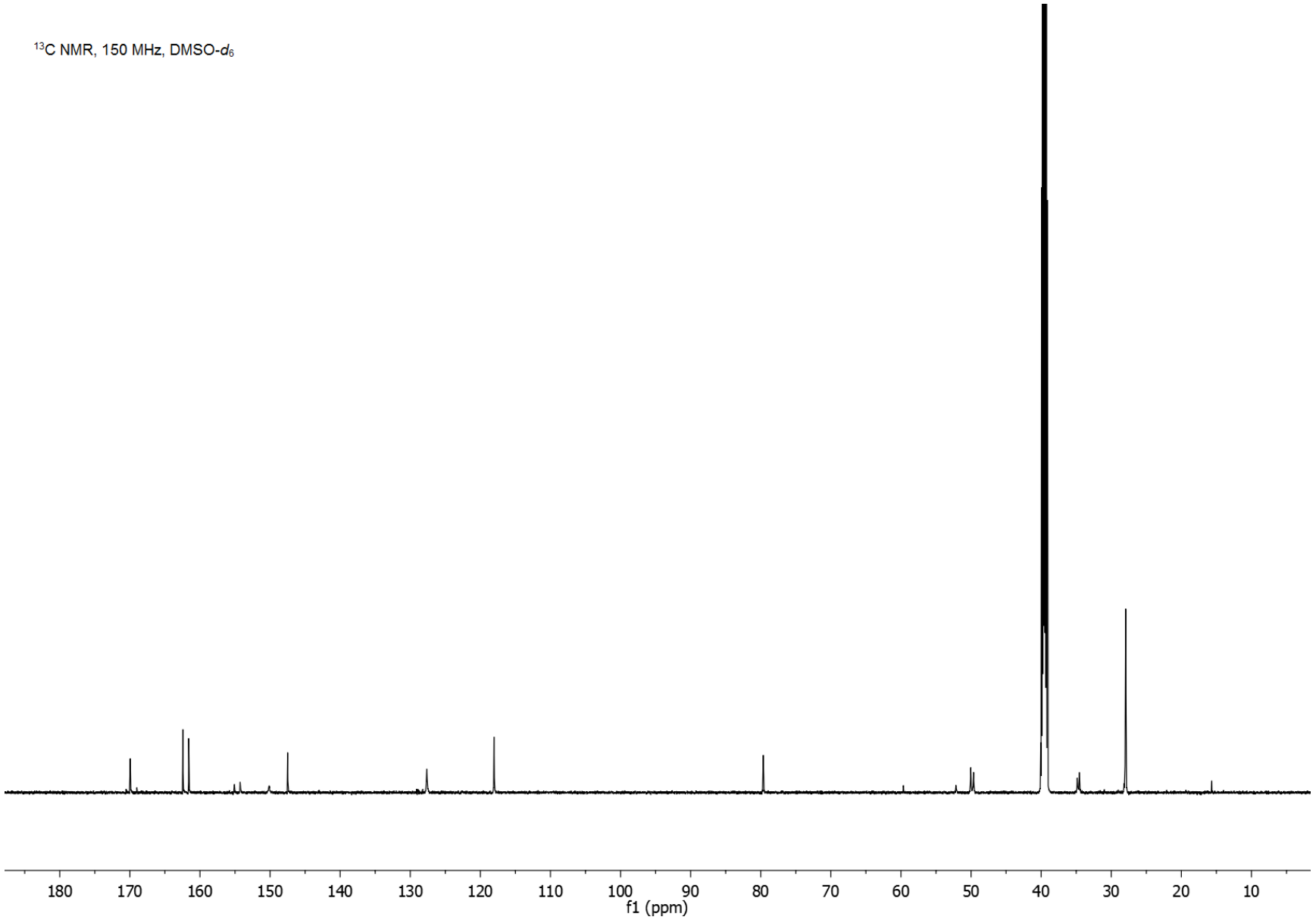

^{13}C NMR, 150 MHz, DMSO-d_6
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 600 MHz, DMSO-d_6

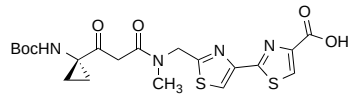


S15

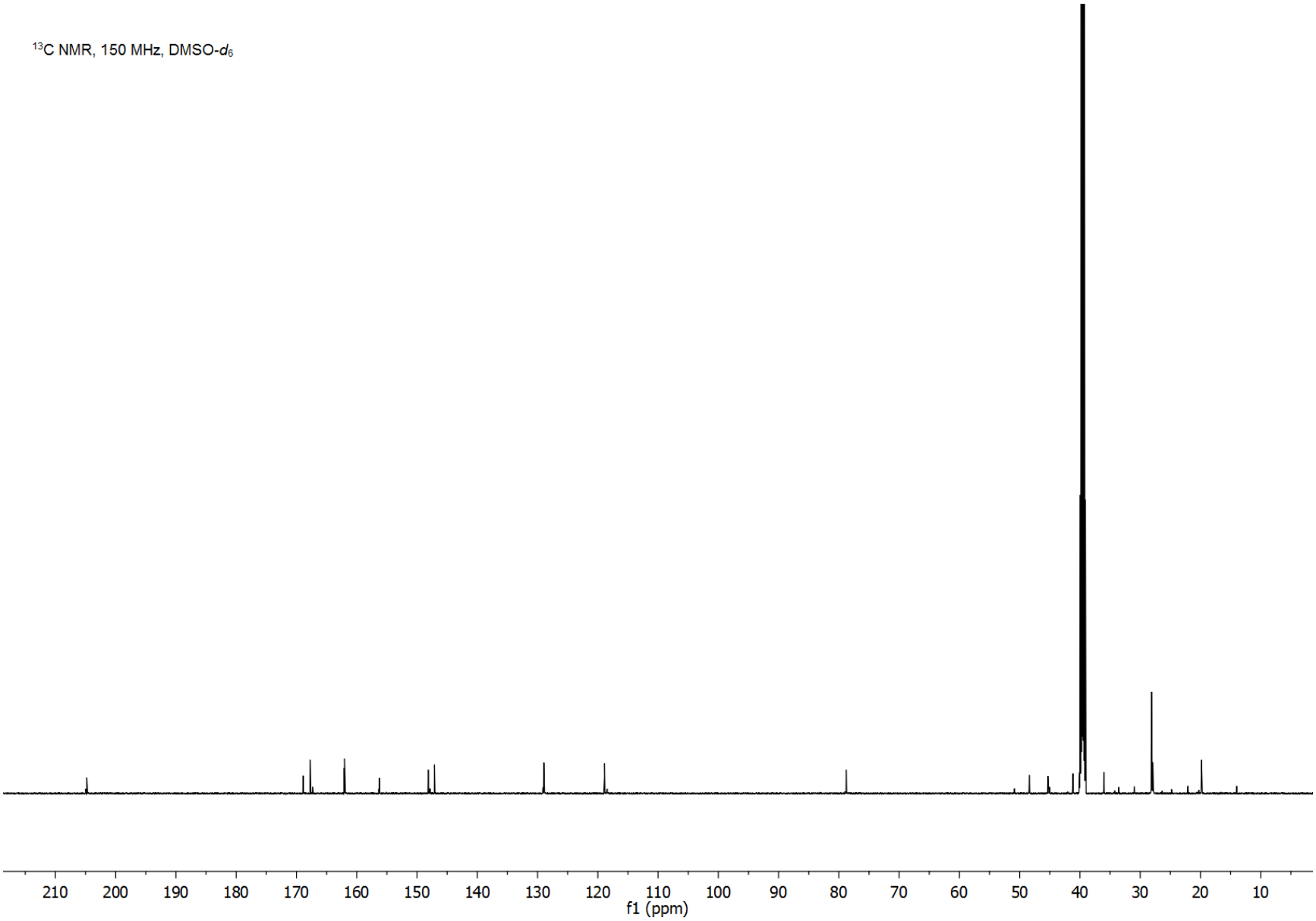



^{13}C NMR, 150 MHz, DMSO-d_6
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 600 MHz, DMSO-d_6



S18


^{13}C NMR, 150 MHz, DMSO-d_6
210
200
190
180
170
160
150
140
130
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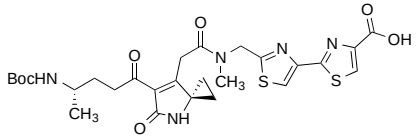
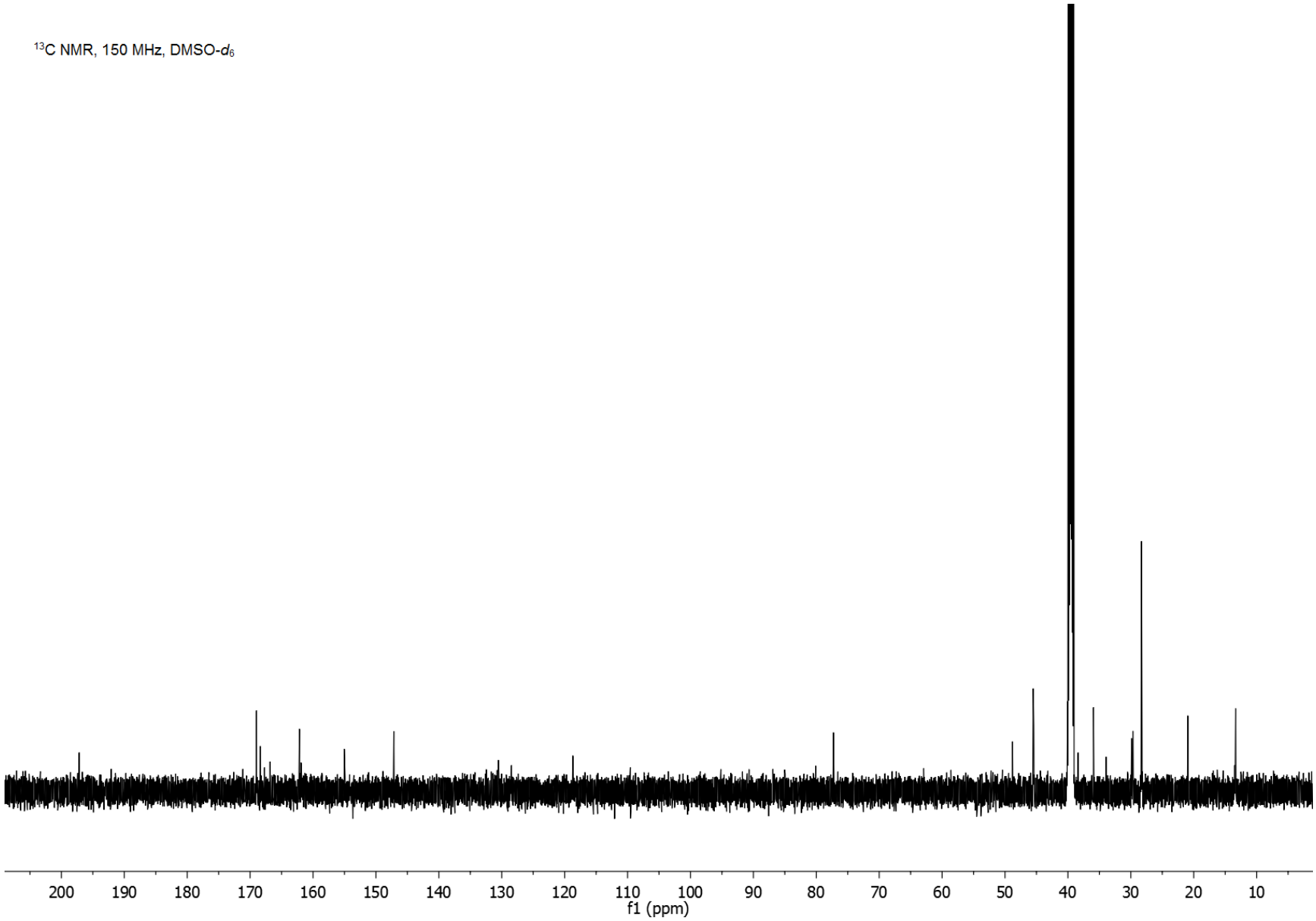
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 600 MHz, DMSO-d_6



S20


^{13}C NMR, 150 MHz, DMSO-d_6
200
190
180
170
160
150
140
130
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110
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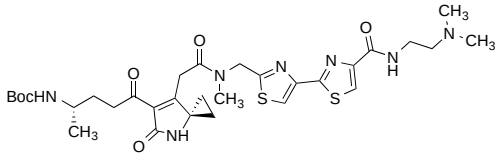
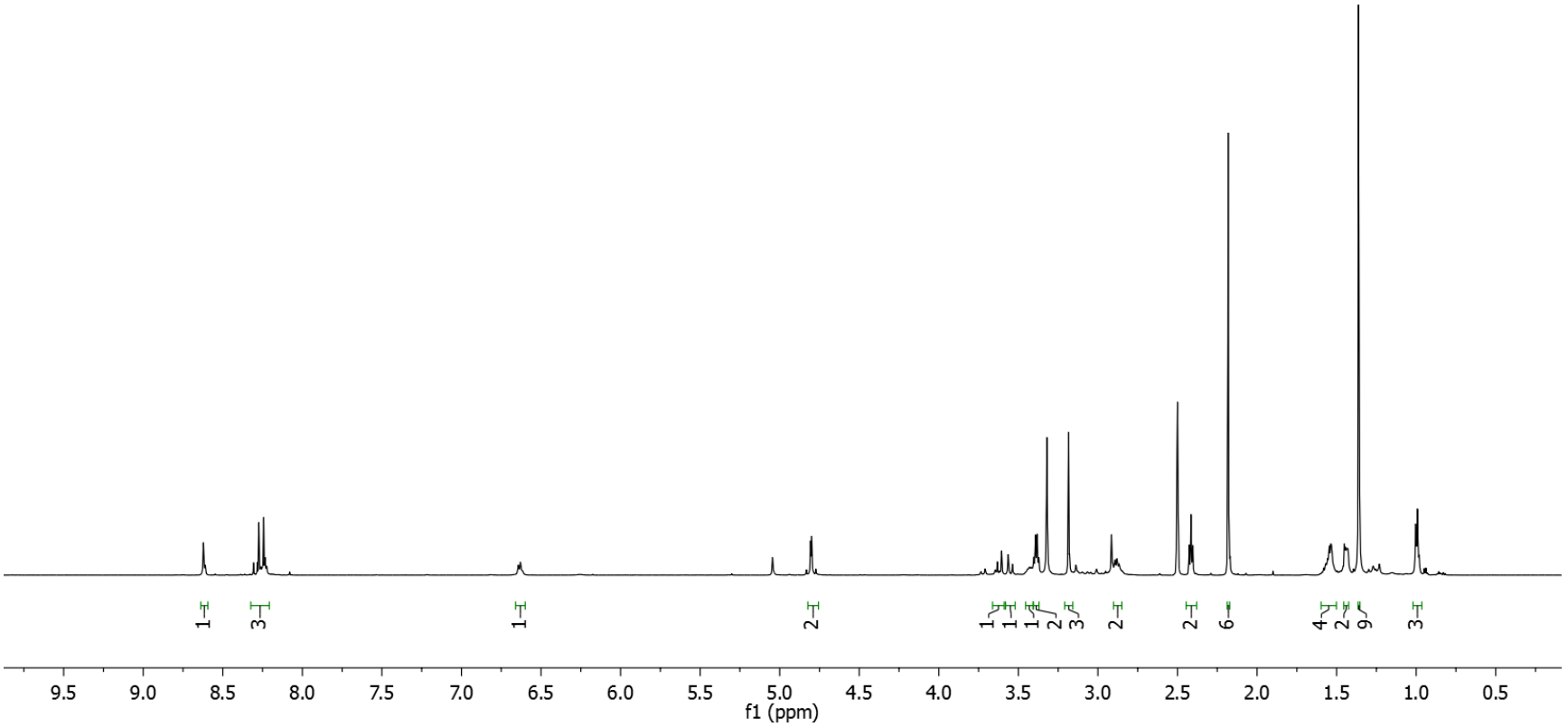
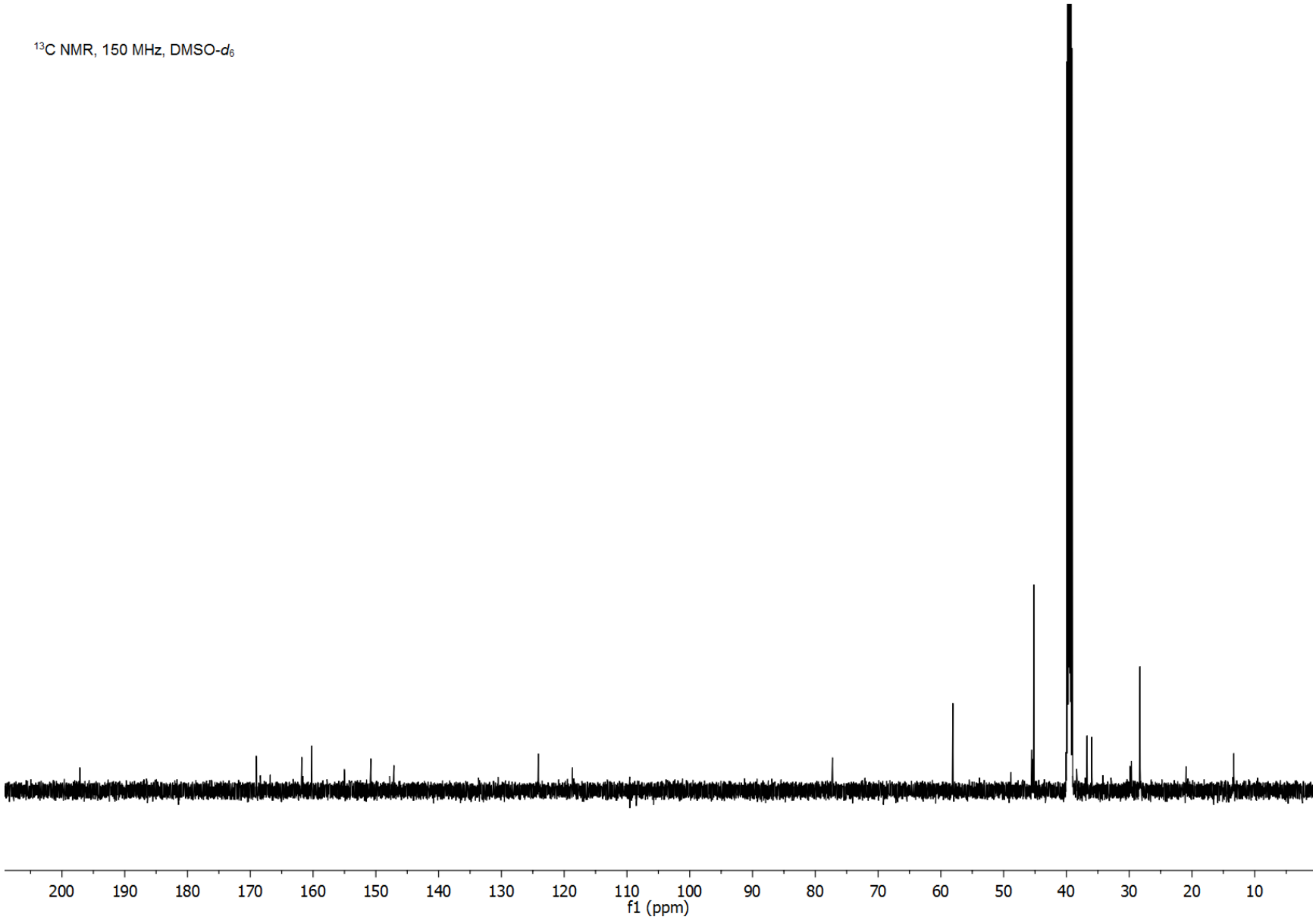
90
80
70
60
50
40
30
20
10
f1 (ppm)

^{1}H NMR, 600 MHz, DMSO-d_6



19




^{13}C NMR, 150 MHz, DMSO-d_6
200
190
180
170
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150
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100
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80
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60
50
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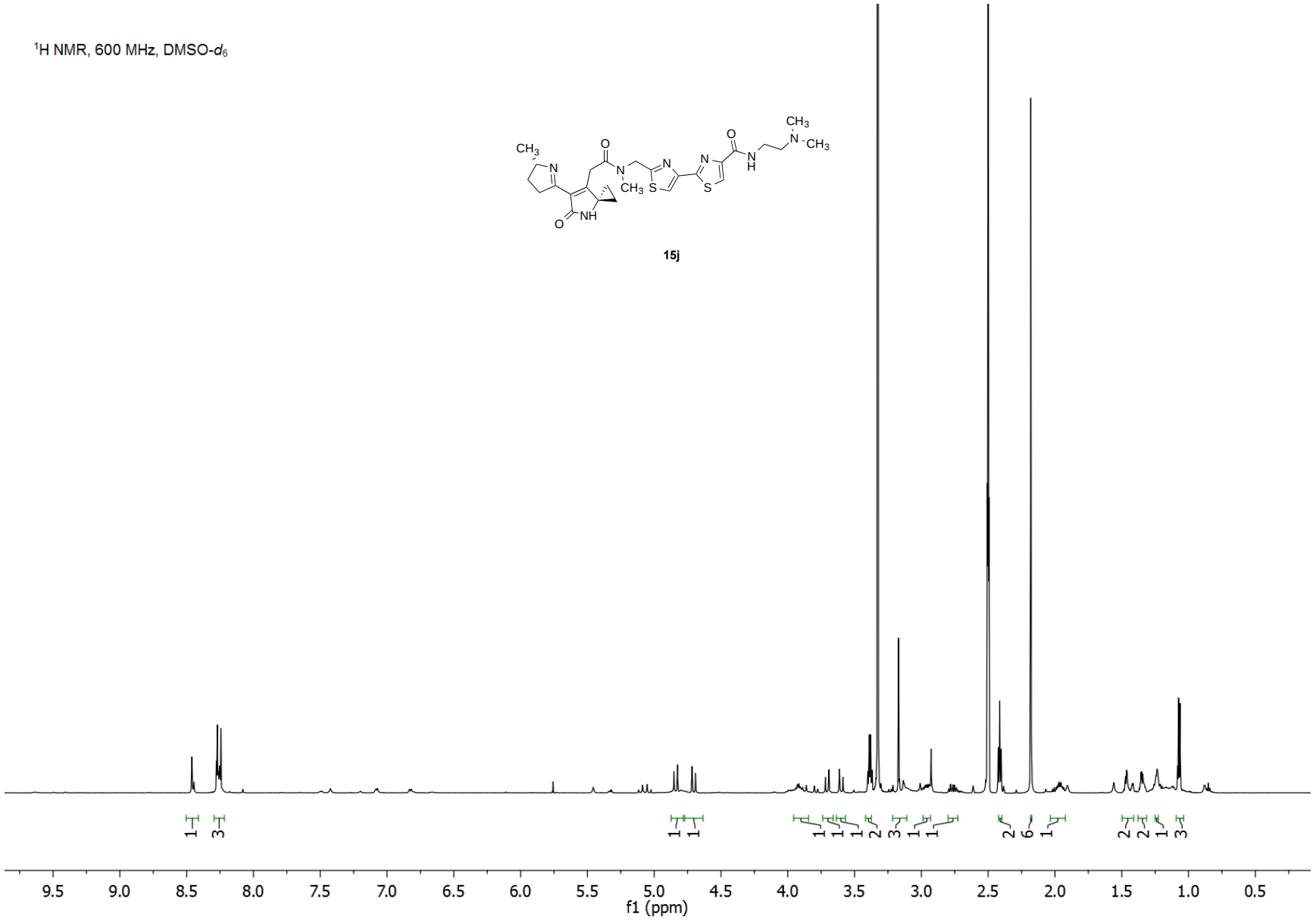
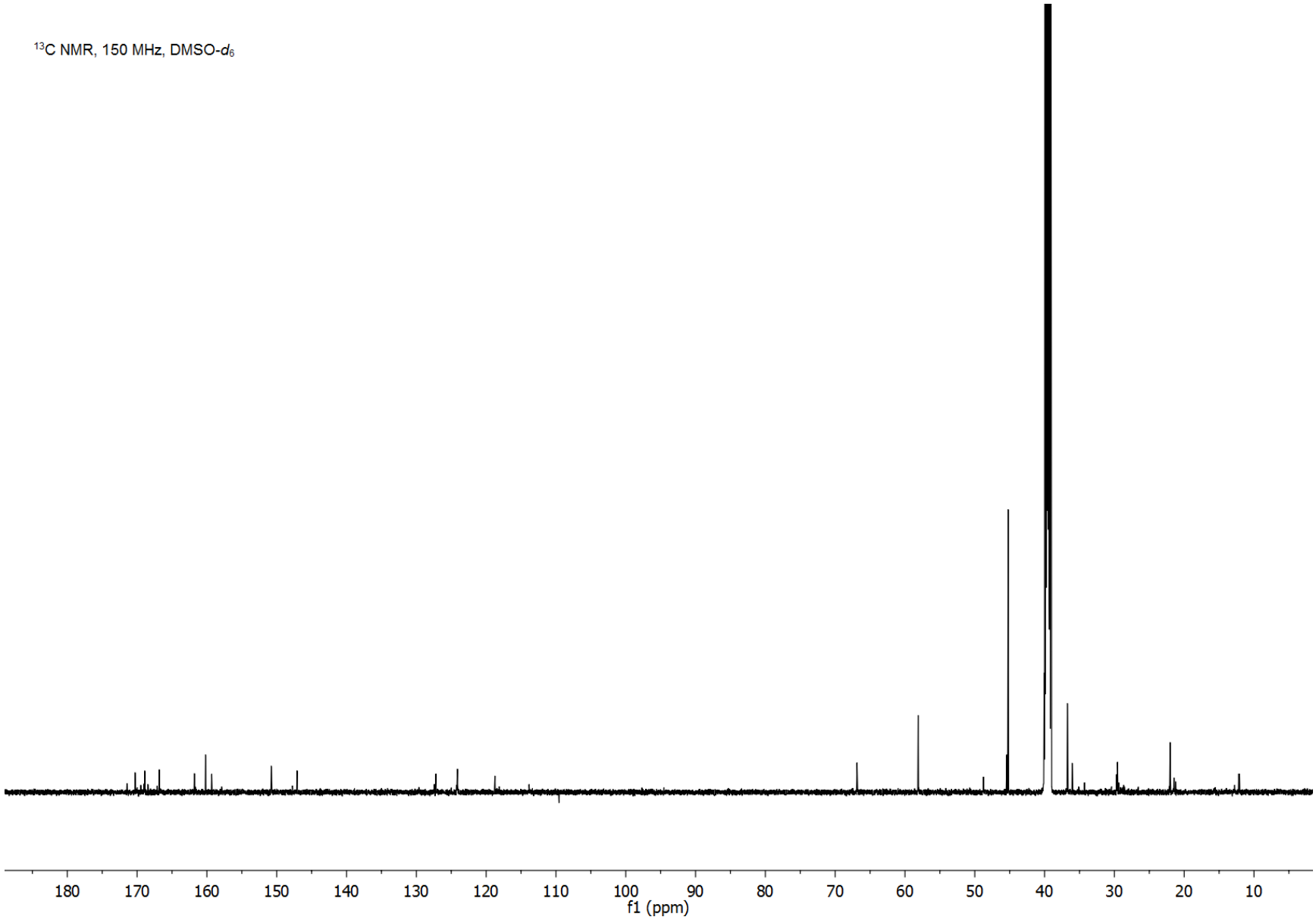
20
10
f1 (ppm)


^{1}H NMR, 600 MHz, DMSO-d_6
15j
1 3 1 1 1 1 1 2 3 1 1 2 6 1 2 2 1 3
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
f1 (ppm)


^{13}C NMR, 150 MHz, DMSO-d_6
180
170
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150
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130
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90
80
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60
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20
10
f1 (ppm)

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