Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Selectivity, ligand deconstruction, and cellular activity analysis of a BPTF bromodomain inhibitor.

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Materials:

All reagents used which were commercially available were used as received without further purification. Chloroacetyl chloride and N,N-diisopropylethylamine (Acros). R-3-(Boc-amino)pyrrolidine and S-3-(Boc-amino)pyrrolidine, iron powder (Alfa Aesar). Acetic acid, ammonium hydroxide (British Drug Houses). 2,4-Dichloropyrimidine (Chem-Impex). (R)-1-boc-3-aminopyrrolidine, (S)-1-boc-3-aminopyrrolidine, 4-Nitrophenylchloroformate (Combi-blocks). Acetic anhydride, diethyl ether, hexanes, isopropylamine, and sodium chloride (Fisher). Triethylamine (Mallinkrodt). Sodium bicarbonate (EMD Millipore). Ethanol (Pharmco-Aaper). 3-aminobenzoic acid, 4-fluoroaniline, N,N-dimethylaminopyridine, sodium hydroxide, dichloromethane, 1,2-dichloroethane, 1,4-dioxane, ethyl acetate, formaldehyde (37% solution in H_2O/MeOH), imidazole, magnesium sulfate, methanol, methyl 2-isocyanatobenzoate, methyl 3-isocyanatobenzoate, methyl 4-isocyanatobenzoate, methylamine (2.0 M in THF), N-methyl 4-fluoroaniline, sodium triacetoxyborohydride, tetrahydrofuran, thionyl chloride, trifluoroacetic acid, and tris base (Sigma-Aldrich). 3-Nitrobenzoyl chloride (TCI). Ethylamine (70% in H_2O) (VWR).

^{1}H and ^{13}C NMR:

All NMR spectra were acquired on a Bruker 500 MHz spectrometer equipped with a prodigy TCI cryoprobe. Small molecule NMR spectra were taken in $CDCl_3$ (^{1}H ref. 7.26 ppm, ^{13}C ref. 77.0 ppm), MeOD (^{1}H ref. 3.31 ppm), or DMSO-*d*6 (^{1}H ref. 2.50 ppm, ^{13}C ref. 39.51 ppm).

Protein-Observed Fluorine (PrOF) NMR:

Fluorinated BPTF was produced and purified as described elsewhere.[1] All ^{19}F NMR spectra were acquired at 25 °C and 471 MHz. Protein samples with or without ligand were prepared in a buffer comprised of 50 mM Tris, 100 mM NaCl, pH = 7.4, with 40-50 µM protein, 52 µM trifluoroacetic acid, 5% D_2O, and 1% DMSO. Protein spectra were acquired with 500-1000 scans, acquisition time of 0.05 s, relaxation delay of 0.7 s, and a transmitter offset of -125 ppm with a 20-40 ppm spectral window. A second spectrum was acquired for each sample to observe trifluoroacetate (^{19}F ref. -76.55 ppm) with 16 scans, acquisition time of 0.5 s, relaxation delay of 1 s, and a transmitter offset of -75 ppm with a 10 ppm spectral window. Each protein sample was referenced by applying the difference between trifluoroacetate and its reference chemical shift.

Binding affinity (K_d) was acquired by observing the change in chemical shift ($\Delta\delta$) compared with a no-ligand control spectrum. The values for $\Delta\delta$ were plotted and a curve was produced using GraphPad Prism software by fitting to the following equation:

$$Y = \Delta\delta_{max}\frac{(K_d+X+P)-\sqrt{(K_d+X+P)^2-(4PX)}}{2P} \quad (1)$$

X = ligand concentration (µM)

Y= experimental $\Delta\delta$ (ppm) as an absolute value

$\Delta\delta_{max}$ = maximum chemical shift (ppm)

K_d = dissociation constant (µM)

P = protein concentration (µM)

Mass Spectrometry:

HRMS Conditions: For high-resolution ESI-MS analysis of small molecules, either a Bruker BioTOF II ESI/TOS-MS direct infusion instrument was used in conjunction with polyethylene glycol standards for mass calibration, or a Thermo-Fisher Orbitrap-Velos equipped with an autosampler.

UPLC/MS Conditions: For UPLC/MS analysis of proteins, a Waters Acquity UPLC coupled to a Waters Synapt G2 HDMS quadrupole orthogonal acceleration time of flight mass spectrometer was used (Waters Corp., Milford, MA USA). A Waters Acquity UPLC Protein BEH C_4 2.1 mm x 100 mm column (1.7 μm diameter particles) at 35°C was used for the following 15 min linear gradient separation at a flow rate of 0.400 mL/min using A: water containing 0.1% formic acid and B: acetonitrile containing 0.1% formic acid: 3% B, 0 min to 3 min; 3% B to 97% B, 3 min to 9 min; 97% B, 9 min to 11 min; 97% B to 3% B, 11 min to 13 min; 3% B 13 min to 15 min. Mass spectra were collected in profile mode over the range *m/z* 300-2500 every 0.1s during the chromatographic separation. MS parameters in positive electrospray ionization mode were as follows: capillary, 0.3 kV; sampling cone, 35.0 V; extraction cone, 4.0 V; desolvation gas flow, 800 L/h; source temperature, 100°C; desolvation temperature, 350°C; cone gas flow, 20 L/h; trap CE, off. Lockspray (on-the-fly mass calibration) configuration consisted of infusion of a 5 μg/mL solution of leucine-enkephalin and acquisition of one mass spectrum (0.2s scan, *m/z* 50-1200) every 10s. Three lockspray *m/z* measurements of protonated (positive ionization mode) leucine-enkephalin were averaged and used to apply a mass correction to measured *m/z* values during the course of the analysis.

Table S1: MS of fully fluorinated proteins

Protein	Calculated m/z (Da)	Observed m/z (Da)	% Fully-fluorinated
5FW BPTF (BD)	14455	14455.9	100%
5FW BRD4 (BD1)	15137	15137.0	93%
5FW BRDT (BD1)	14184	14183.2	97%
5FW PCAF (BD)	17182	17181.5	86%
5FW *Pf* GCN5 (BD)	12640	12639.3	96%

Cell lines: Cell lines were purchased from ATCC and further certified at CSHL tissue culture shared resources. For this specific study we utilized MCF-7 cells (Hormone positive breast cancer cell lines) A549 cells (Lung adenocarcinoma cell line), HepG2 (Liver hepatocellular carcinoma cell line), K562 (Chronic myelogenous leukemia cell line), Suit2 (Metastatic liver tumor of pancreatic carcinoma), and 293ft-NEO (virus production). Cells were grown according to ATCC recommendations.

mRNA quantification. Cells were resuspended in Trizol (Thermo Fisher) for RNA purification. cDNA was synthesized with SuperScript III (Thermo Fisher) according to manufacturer's instructions. cDNA was utilized on qPCR analysis for the quantification of *BPTF* mRNA levels (3 primer sets: 1F: ACC CAG AGA ATT TGC ATT GG-3',1R: ATT TTA CCC ATG TCG CTT GC- 3'; 2F: AGC AGA AGC CGA CAG TGA TT-3', 2R: GGC CTT GCT TAA CCC ATG TA- 3'; 3F: TTG GCA TCT TGC AAA GTG AG- 3', 3R:TTA TGG GCC TGT AAG GAA CG-3'), *GAPDH* mRNA (F: 5'CCA CAT CGC TCA GAC ACC AT 3', R: 5' CCA GGC GCC CAA TAC G 3'), and *β-ACTIN* mRNA (normalization, 5' AGA GCT ACG AGC TGC CTG AC 3', 5' AGC ACT GTG TTG GCG TAC AG 3'). qPCR reactions were performed on a QuantStudio 6 (Thermo Fisher).

Cell Viability assay. Cell lines were treated for 72 hours with DMSO control, **(*S*)-1** (5 μM) or **(*R*)-1** (5 μM). Following treatment, cells were incubated for 3 hours with AlamarBlue (Thermo Fisher), a resazurin dye. The AlamarBlue is actively reduced to another compound, resorufin, which is fluorescent in 580 nm excitation and 590 nm emission. Fluorescence was measured using a plate reader. All data was normalized to the control which were the DMSO treated wells.

CRISPR/Cas-9 genomic editing. Short-guide RNA (sgRNA) sequences were predicted utilizing previously published algorithm.[5] sgRNAs targeting hRPA3 and hBPTF were cloned into the all-in-one CRISPR-CAS9-GFP plasmid. All in one plasmids and virus packaging/envelope coding plasmids (VSVG and Pax2) were transfected into 293 ft-Neo cells using PEI (Sigma). Lentivirus supernatant was collected every 24 hours, for a total of 72 hours, and further utilized to infect HepG2, MCF-7 and K562 cells. GFP measurements (a readout for virus infection) were taken using MACSQuant (Miltenyi Biotec) every 3 days, or when culture plates were ~70 confluent. FlowJo (Tree Star) was used to analyze flow cytometry data.

CRISPR/Cas-9 genomic editing quantification: Genomic DNA from sgRNA infected cells was utilized on qPCR for quantification of editing efficiency, utilizing previously published methods.[6] qPCR reactions were carried out on a QuantStudio 6 (Thermo Fisher) utilizing the following primer sequences for BPTF genomic locus (gBPTF primer #1 - 1F 5' - CGT GAA GAA GAC ACT TCC AAT AC - 3', 1R 5' -CCA CGT CAT CCC ATC TAT GAA- 3'; gBPTF primer #2 - 2F 5' -AGC GTT AAT TCC ACA CTG TAT TTC- 3', 2R 5' -AAG AAC GTG ATG GTA CTC CTT ATC- 3'; gBPTF primer #3 - 3F 5' -CTC GAG AGG AAT TGA TGT CTG AA- 3', 3R 5' -ATG TCT CAC AGC AAA GCA AAT C- 3'; gBPTF primer #4 - 4F 5' -TGT GAG ACA TGT TCA GCA GTA T- 3', 4R 5' -CCT TGT GTG CTA CAC AGA CT- 3')

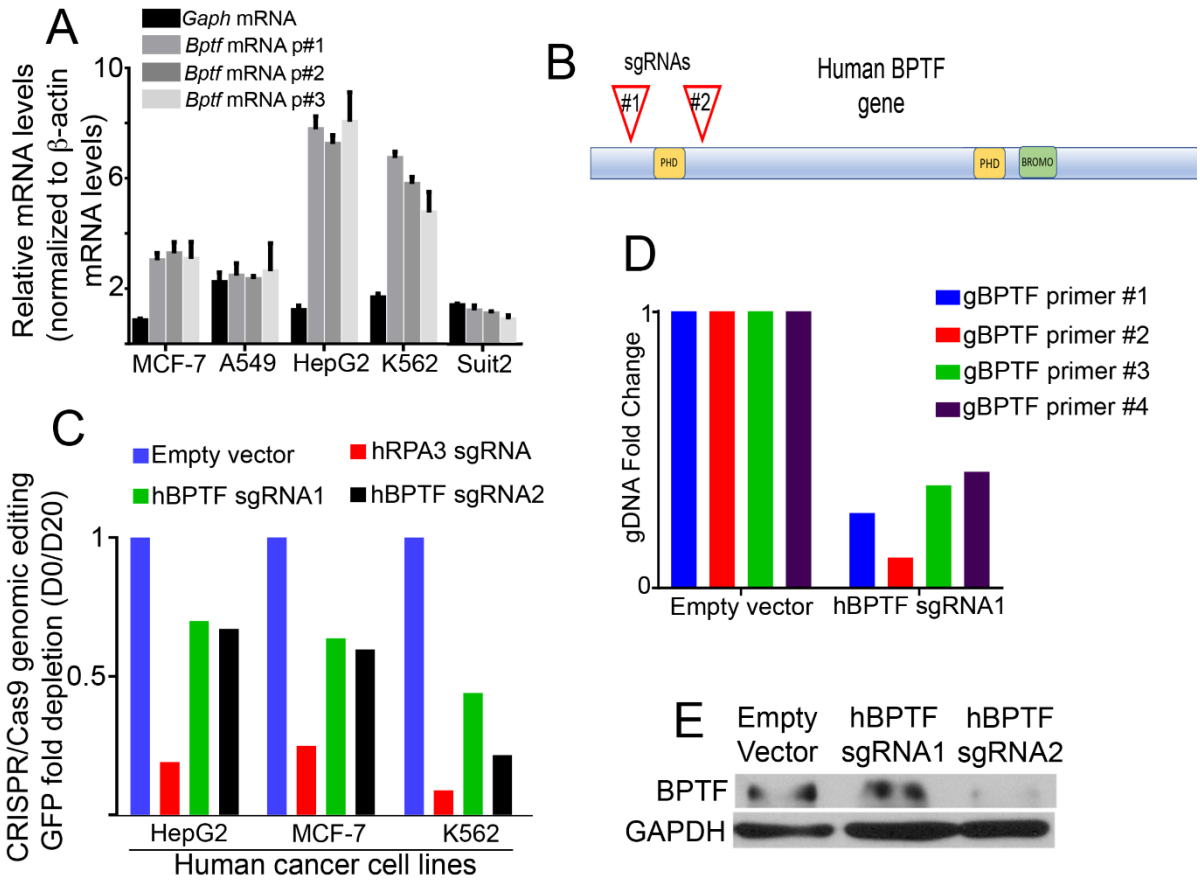


Figure S1: Characterizing the effects of BPTF inhibition and S-AU-1 treatment. (A) *BPTF* mRNA quantification across a series of human cancer cell lines. (**B**) BPTF genomic scheme showing location of sgRNAs. (**C**) Summary of CRISPR-Cas9 genomic editing in HepG2, MCF-7 and K562 cells. Cells were infected with all in one CRISPR-Cas9-GFP lentiviral particles, expressing sgRNAs targeting RPA3 gene (depletion control) and two differed domains of BPTF gene. Fold change was calculated by comparing final measure of GFP to initial infection GFP. n=2 technical replicate. (**D**) CRISPR-Cas9 genomic editing efficiency quantification. Genomic DNA from HepG2 cells infected with control lentivirus (Empty vector) or with lentiviral particles expressing BPTF sgRNA1 was purified and utilized for qPCR analysis with primer sets surrounding the BPTF genomic region targeted by sgRNAs. (**E**) Western blot showing BPTF protein levels and GAPDH protein levels (loading control) in HepG2 cells infected with control lentivirus (Empty vector) or lentiviral expressing sgRNAs against BPTF.

Pharmacokinetic (PK) studies. To assess the PK of AU1, female BALBc mice were injected intraperitoneal with 5 mg/kg of AU1. Blood samples were collected at 5, 15, 30 min and 1, 5, 12, 24 h after i.p. administration. Three mice were used per time point. For each time point, the mice were rendered unconscious by CO_2, followed by surgically thoracotomy. The pooled blood (~1 mL) from the chest cavity was collected into heparinized tubes. The plasma fraction was separated by centrifugation (12,000 rpm, 10 min, 4°C) and stored at -20°C until LC/MS/MS analysis was carried out by the company Ianalytical using standard approaches.

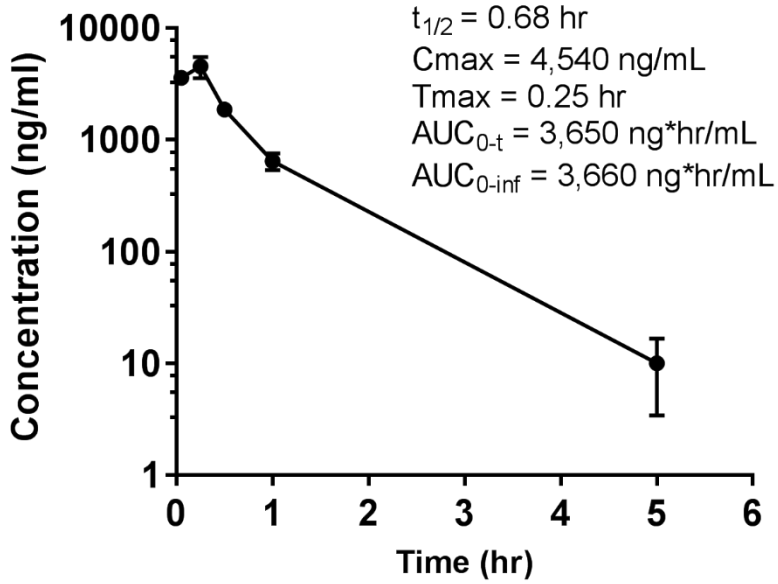


Figure S2. Plasma concentration-time curve for AU1 (ng/mL) and calculated PK parameters. Due to complications with injections, the 5 and 30 minute data points only represent data from one mouse

Computational modeling

The following additional supplementary information is available on Zenodo (DOI: 10.5281/zenodo.2542912): 1) A network file, in GEXF format, of the conformation space network; 2) A PDB file showing the structure of the protein, ligand and water box used in this simulation; 3) An archive file containing a representative structure of every state in the network; and, 4) A trajectory of 10 randomly chosen frames from the lowest free-energy cluster.

Initial docked structures

Autodock Vina[2] was used to generate starting poses of **(*S*)-1** bound to BPTF. The structure of BPTF from PDB ID 2RIV[3], where the binding pocket is occupied only by a molecule of isopropyl alcohol, which is removed prior to docking. The Y17E mutation was inconsequential as it is far from the binding pocket, and it is in a segment that was not included in the molecular dynamics simulation. For docking we use a

cubic grid with 80 points along each axis, with a 0.375 Å spacing, centered at the position of the C3 atom of the isopropyl alcohol. Crystallographic water molecules were not included during docking. This procedure was chosen to follow prior retrospective predictions of bromodomain-ligand structure as described previously.[4] As the ligand is roughly linear in shape, we chose as initial structures two poses of opposite orientation: one with the *p*-fluoroaniline bound to the recognition pocket (pose A) and one with the methyl ester bound to the recognition pocket (pose B). In both cases we chose the pose with the lowest predicted binding free energy.

Molecular dynamics

Only residues 2797-2907 were used for the dynamics. The two systems (pose A and pose B) were both solvated with a 12 Å cutoff, and neutralized with three sodium ions. Parameters from CGENFF were used for the AU1 ligand.[7,8], The systems were prepared for dynamics using energy minimization with harmonic restraints on the protein and ligand atoms (500 steps of steepest descent followed by 500 steps of the adopted basis Newton Raphson method) and this procedure was then repeated with the restraints removed. The CHARMM program with an OpenMM interface for GPU dynamics was used to run dynamics, with a 2 fs timestep. The SHAKE algorithm, with a tolerance of 10^{-8} is used to constrain covalent bonds to hydrogen. Nonbonded interactions are computed with the particle mesh Ewald method with a Gaussian width of 0.32 and 96 grid points along the x, y and z dimensions. An 8.5 Å cutoff is used for Lennard-Jones interactions.

The energy-minimized systems were then heated gradually from 50 to 300 K by increments of 25 K, using 5000 dynamics steps at each temperature. Temperature was controlled using a Langevin heatbath with a friction coefficient of 1.0 ps^{-1}. Following heating, equilibration runs were performed at 300 K using constant pressure dynamics, implemented with a Monte Carlo barostat with a reference pressure of 1.0 atm and volume moves attempted every 50 timesteps. The resulting systems were used as starting points for WExplore simulations. All subsequent dynamics were run at 300 K at constant pressure.

WExplore sampling

The WExplore[9] implementation again followed previous work on predicting bromodomain-ligand poses,[4] and more information on the WExplore algorithm and its application to protein-ligand interactions can be found elsewhere.[4,9,10,11,12] Briefly, the WExplore algorithm enhances the sampling of ligand release pathways by running an ensemble of weighted trajectories forward simultaneously and periodically (here, every 20 ps) altering, or "resampling", the trajectory ensemble using cloning and merging operations. The weights (initialized to all be equal) govern the weight with which the trajectory at that time contributes to averages of observables. During cloning, a single trajectory is split in two, and its weight is evenly divided among the clones. When two trajectories are merged into one, their weight is combined, and a single trajectory is chosen for continuation with a probability that is proportional to its weight. Cloning and merging steps are a common feature of all weighted ensemble applications.[9]

WExplore is a variant of weighted ensemble where the total number of walkers is fixed (each cloning event is coupled to a merging event), and cloning and merging decisions are made using the occupancy of a set of hierarchical regions that are defined using Voronoi polyhedra. To determine which region a given trajectory is in, we measure the distances between that trajectory and the set of "images" that define the Voronoi polyhedra. The trajectory is then assigned to the region that has the closest image. Here we use a four-level region hierarchy, with critical distances of 2.5, 3.5, 5.0 and 10 Å (again following previous work[4,11,12]). To improve sampling efficiency we employ a minimum weight (10^{-12}) and a maximum weight (0.1) that a walker can achieve, enforced by disallowing cloning or merging operations that would violate these rules.

For both pose A and pose B we run four WExplore simulations, with 48 trajectories each. Each simulation was run for 830 cycles of dynamics and resampling. In total, 3.19 μs of dynamics was run for each starting pose (6.37 μs combined).

Network analysis and pose determination

To analyze the structures from WExplore sampling and predict an ensemble of final poses, we performed clustering and Markov state model analysis. Clustering was performed using a three-step process. In the first step, each frame from the MD simulation was "featurized" using a large set of protein-ligand distances. This distance set was the set of all possible distances between two subsets of atoms: 1) the ligand atoms N2, C10, C14, C21, N4, H1, N1, C18, O2, C23, O3, N6, H10, and F1, which were chosen to evenly cover the ligand, and 2) all protein CA atoms that were within 8 A of the ligand center of mass. There were 938 distances in the feature set. In the second step, the feature space was projected onto five dimensions using time-lagged independent coordinate analysis (or, tICA).[13] This was performed using a tICA lag time of 0.2 ns. In the third step, a k-means clustering was performed using the tICA projections, dividing the structures into 1200 states.

Markov state models were then constructed for a range of lag times (1, 2, 5, 10, 20, 50 sampling periods), and various subsets of the data (p = 0, 10, 20, 30, 40, 50, 60) where p is the percent of initial data discarded. For each Markov model the weights of each node were computed using the top eigenvector of the transition matrix. The highest such node is shown in Figure S1A. There is very little variation as a function of lag time, and three distinct states are predicted in the entire map (P1, P2, and P3). Figure S1B shows that these states are in the same community in the network model, which demonstrates that these predictions are relatively robust.

Each node in the network visualizations in Figure 9 (main text) and Figure S1B shows one of the clusters. The weights of the edges between the nodes were computed using the conditional transition probabilities between the clusters as follows:

$$e_{ij} = 100\frac{1}{2}\left(t_{ij} + t_{ji}\right) \quad (2)$$

where t_{ij} is an element of the transition probability matrix that represents the conditional probability of transitioning between states *i* and *j*. These weights are used in the Force Atlas algorithm in the Gephi program[14] to construct a minimized network layout.

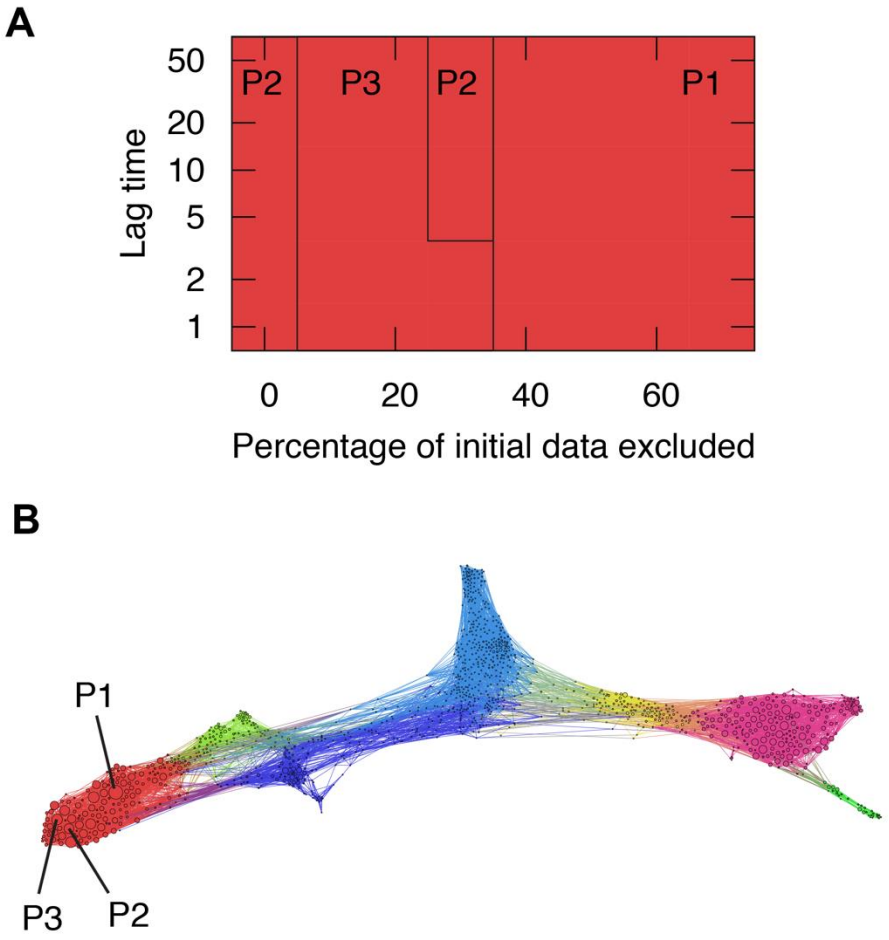


Figure S3: A. The highest weighted state is shown as a function of lag time and exclusion of initial data. Each square in the map is colored by the community that the highest weighted state belongs to for that set of parameters, which here is constant across the entire map. B. The network of poses is colored according to communities that were determined by modularity optimization in Gephi. The three highest weighted states are labeled and we observe that they are in the same community.

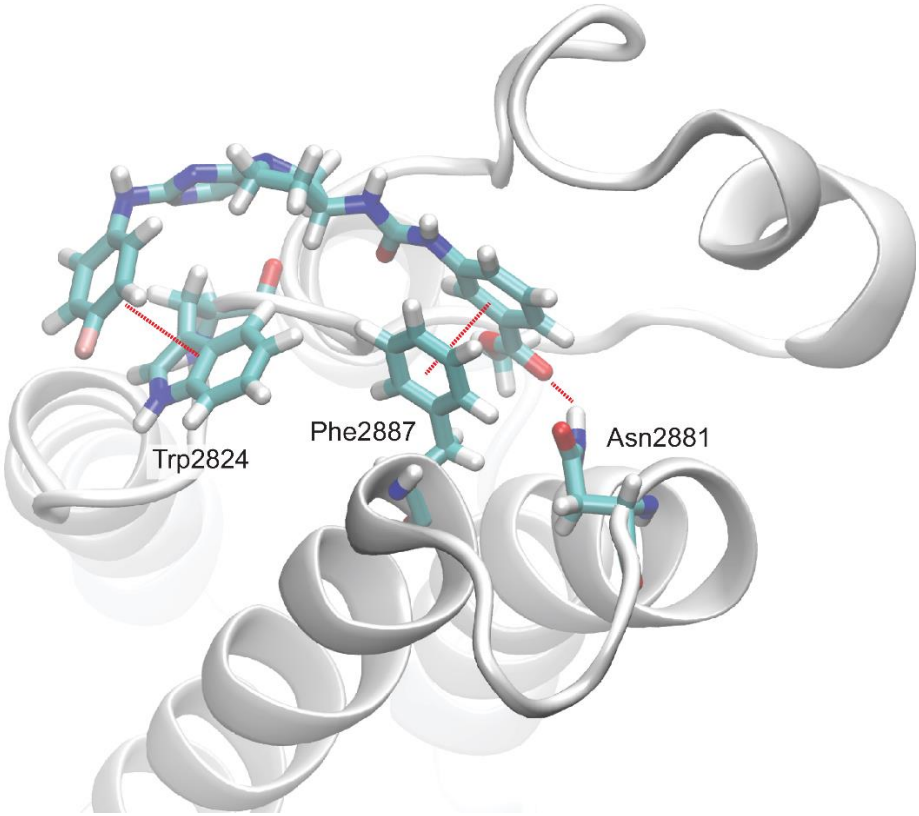


Figure S4: Exemplary Aryl ring - Phe2887 interaction taken from the ensemble of most probable poses. This ring-stacking interaction between the aryl ring and Phe2887 was consistently observed in the lowest-free energy cluster. Interactions with residues Trp2824 and Asn2881 are shown to help orient the viewer.

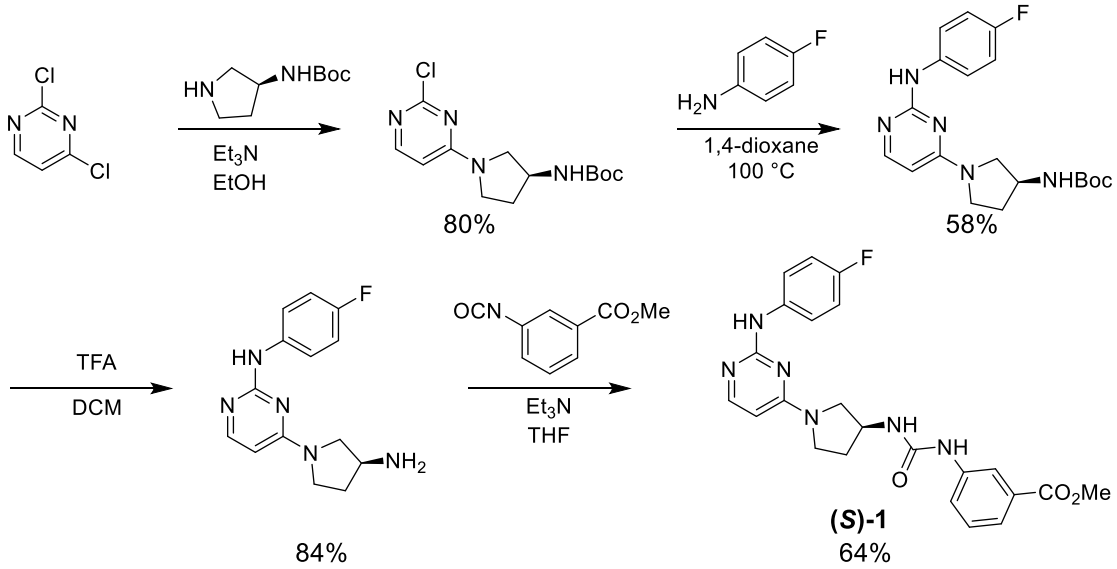
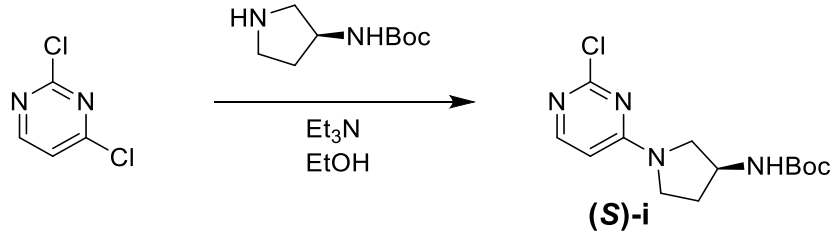


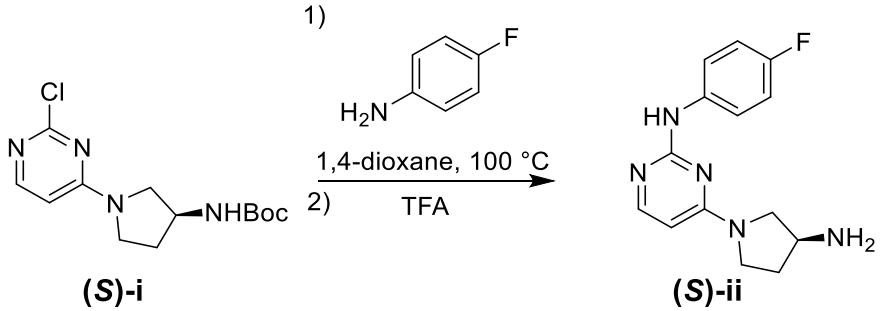
Figure S5: General synthetic scheme for (***S***)**-1**

Synthetic experimental



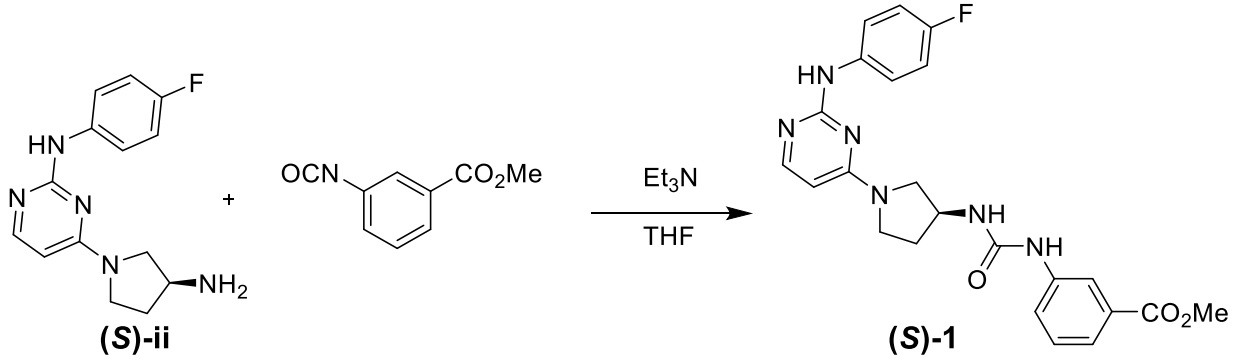
(*S*)-i

(*S*)-i was synthesized as reported previously.[15] ^{1}H NMR, as a mixture of rotational isomers (500 MHz, DMSO-d_6) δ 8.01 (dd, *J* = 11.0, 6.0 Hz, 1H), 7.26 – 7.20 (m, 1H), 6.49 (d, *J* = 6.0 Hz, 1H), 4.11 (dq, *J* = 43.4, 6.0 Hz, 1H), 3.59 (ddd, *J* = 25.8, 11.6, 6.4 Hz, 2H), 3.47 (dq, *J* = 13.8, 7.4, 7.0 Hz, 1H), 3.36 (ddd, *J* = 26.0, 10.1, 5.8 Hz, 1H), 3.17 (dd, *J* = 11.0, 4.5 Hz, 1H), 2.11 (ddq, *J* = 39.9, 13.7, 7.1 Hz, 1H), 1.88 (ddq, *J* = 25.6, 12.8, 6.2 Hz, 1H), 1.39 (s, 9H). ^{13}C NMR, as a mixture of rotational isomers (126 MHz, DMSO-d_6) δ 160.75, 160.63, 159.39, 156.27, 155.20, 103.16, 102.95, 77.96, 51.93, 51.83, 49.80, 49.20, 44.69, 30.43, 29.90, 28.19. HRMS (ESI-TOF) calculated for $C_{13}H_{19}ClN_4O_2Na^+$ [M+Na]$^+$: 321.1094, observed 321.1068.



(*S*)-i **(*S*)-ii**

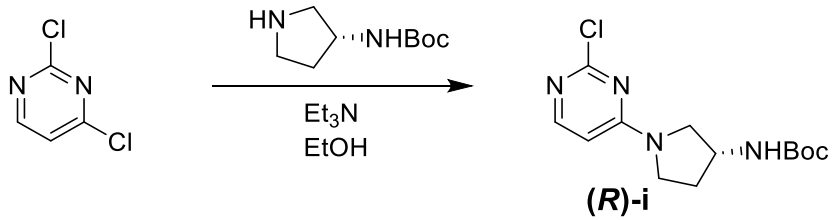
(*S*)-ii was synthesized as reported previously.[15] ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.01 (s, 1H), 7.88 (d, *J* = 5.9 Hz, 1H), 7.80 (dd, *J* = 8.9, 5.1 Hz, 2H), 7.05 (t, *J* = 8.8 Hz, 2H), 5.94 – 5.83 (m, 1H), 3.71 – 2.87 (m, 5H), 2.08 – 1.95 (m, 1H), 1.72 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.06, 158.36 (d, *J* = 244.9 Hz), 137.87 (d, *J* = 2.2 Hz), 119.65 (d, *J* = 7.3 Hz), 114.64 (d, *J* = 21.8 Hz), 95.48, 54.46, 50.91, 50.10, 44.59, 33.85. HRMS (ESI-TOF) calculated for $C_{14}H_{16}FN_5H^+$ [M+H]$^+$: 274.1423, observed 274.1504.



(*S*)-ii **(*S*)-1**

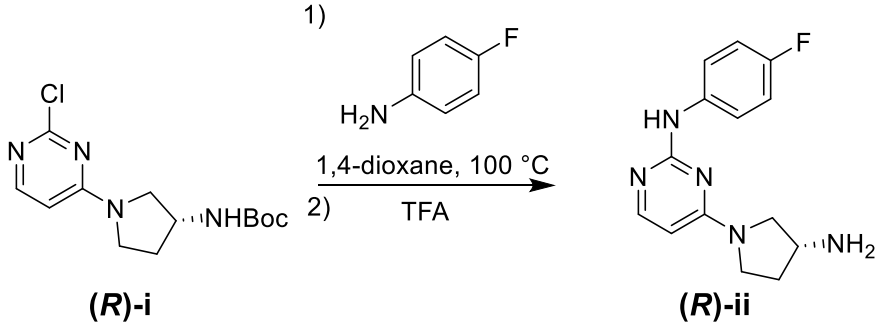
(*S*)-1 was synthesized as reported previously.[15] $[\alpha]_D^{22}$ = -22.500 (c = 0.938, 80% $CHCl_3$/ 20% MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.06 (s, 1H), 8.59 (s, 1H), 8.14 (s, 1H), 7.93 (d, *J* = 5.9 Hz, 1H), 7.79 (dd, *J* = 8.6, 5.0 Hz, 2H), 7.55 (dd, *J* = 8.1, 2.1 Hz, 1H), 7.52 – 7.47 (m, 1H), 7.37 (t, *J* = 7.9 Hz, 1H), 7.06 (t, *J* = 8.7 Hz, 2H), 6.60 (d, *J* = 6.7 Hz, 1H), 5.98 (d, *J* = 5.8 Hz, 1H), 4.33 (m, 1H), 3.84 (s, 3H), 3.75-3.18 (m, 4H), 2.21 (m, 1H), 1.96 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.26, 160.19, 158.40 (d, *J* = 237.5 Hz), 155.64,

155.58, 154.75, 140.64, 137.77, 130.05, 129.06, 122.20, 121.87, 119.72 (d, *J* = 7.3 Hz), 118.09, 114.69 (d, *J* = 21.9 Hz), 95.65, 52.09, 51.92, 44.27. HRMS (ESI-TOF) calculated for $C_{23}H_{23}FN_6O_3H^+$ [M+H]$^+$: 451.1849, observed 451.1860.



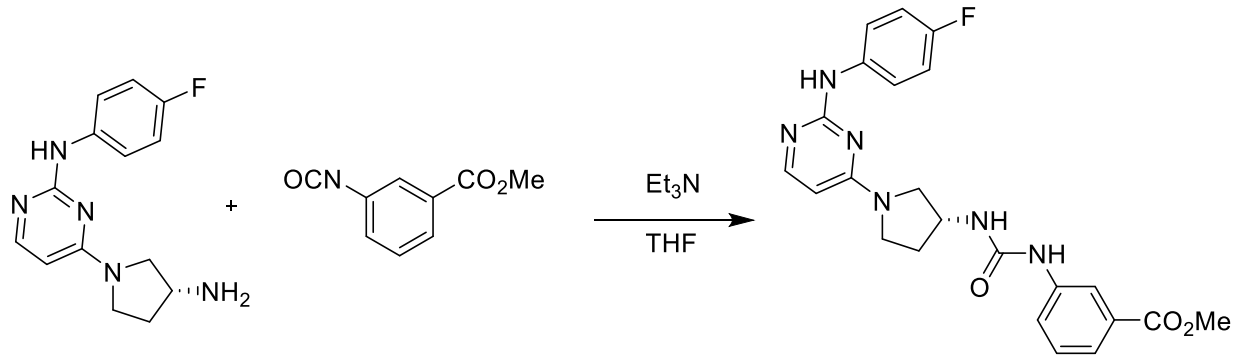
(*R*)-i

2,4-Dichloropyrimidine (292 mg, 1.96 mmol, 1 eq) was dissolved in 5 mL of EtOH. Triethylamine (300 µL, 1.1 eq) and (*R*)-3-(Boc-amino)pyrrolidine (400 mg, 2.15 mmol, 1.1 eq) were added and the reaction was stirred at ambient temperature for 4 h. The solvent was removed and the crude material was dissolved in EtOAc and washed 3 X with H_2O followed by brine and dried over $MgSO_4$. The crude material was filtered and concentrated followed by purification using a Combiflash Rf system (hexanes/EtOAc, 0-100% EtOAc) to afford **(*R*)-i** (105 mg, 18%). ^{1}H NMR as a mixture of rotational isomers (500 MHz, DMSO-d_6) δ 8.02 (d, *J* = 6.1 Hz, 0.5H), 8.00 (d, *J* = 6.2 Hz, 0.5H), 7.26 (d, *J* = 7.4 Hz, 0.5H), 7.23 (d, *J* = 7.3 Hz, 0.5H), 6.49 (d, *J* = 6.0 Hz, 1H), 4.15 (m, 0.5H), 4.05 (m, 0.5H), 3.61 (dd, *J* = 11.9, 6.1 Hz, 0.5H), 3.56 (dd, *J* = 11.1, 6.7 Hz, 0.5H), 3.47 (dq, *J* = 13.7, 7.0 Hz, 1H), 3.42 – 3.32 (m, 1H), 3.17 (d, *J* = 4.7 Hz, 0.5H), 3.15 (d, *J* = 4.7 Hz, 0.5H), 2.15 (dq, *J* = 13.8, 7.1 Hz, 0.5H), 2.06 (dt, *J* = 13.6, 6.7 Hz, 0.5H), 1.94 – 1.88 (m, 0.5H), 1.84 (dt, *J* = 12.8, 6.6 Hz, 0.5H), 1.39 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.65, 160.54, 159.29, 156.17, 155.05, 103.07, 102.85, 77.86, 51.83, 51.73, 49.70, 49.10, 44.59, 30.33, 29.80, 28.09. HRMS (ESI-TOF) calculated for $C_{13}H_{19}ClN_4O_2Na^+$ [M+Na]$^+$: 321.1094, observed 321.1141



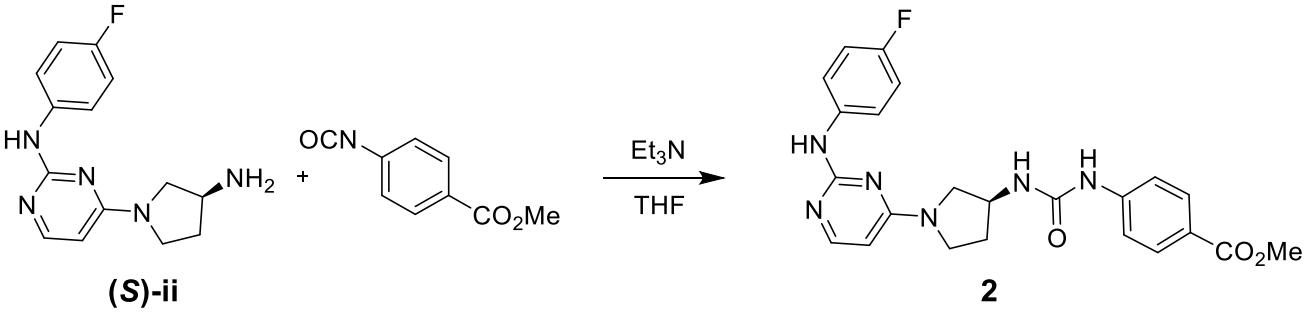
(*R*)-i **(*R*)-ii**

(*R*)-i (105 mg, 0.352 mmol) was dissolved in 3 mL of 1,4-dioxane followed by addition of 4-fluoroaniline (50 µL, 0.528 mmol) and heated to reflux for 18 h. The solvent was removed, and the crude material was redissolved in TFA and stirred at ambient temperature for 2 h. The TFA was removed under a stream of nitrogen, and the crude material was dissolved in H_2O and DCM. The aqueous layer was washed 3 X with DCM and separated, the pH was adjusted to ~12 using 1M NaOH and extracted into EtOAc. The organic layers were combined, washed with brine, and dried over $MgSO_4$. Following filtration, the solvent was removed under reduced pressure to afford **(*R*)-ii** as a white solid (60 mg, 62%, 2 steps). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.01 (s, 1H), 7.88 (d, *J* = 5.8 Hz, 1H), 7.79 (dd, *J* = 8.9, 5.0 Hz, 2H), 7.05 (t, *J* = 8.9 Hz, 2H), 5.89 (d, *J* = 5.8 Hz, 1H), 3.75 – 2.92 (m, 6H), 2.03 (s, 2H), 1.71 (s, 1H), 1.28 – 1.12 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.98, 158.26 (d, *J* = 244.5 Hz), 155.41, 155.29, 137.77, 137.75, 119.54 (d, *J* = 7.3 Hz), 114.54 (d, *J* = 21.8 Hz), 95.39, 54.25, 44.47. HRMS (ESI-TOF) calculated for $C_{14}H_{16}FN_5H^+$ [M+H]$^+$: 274.1423, observed 274.1429.



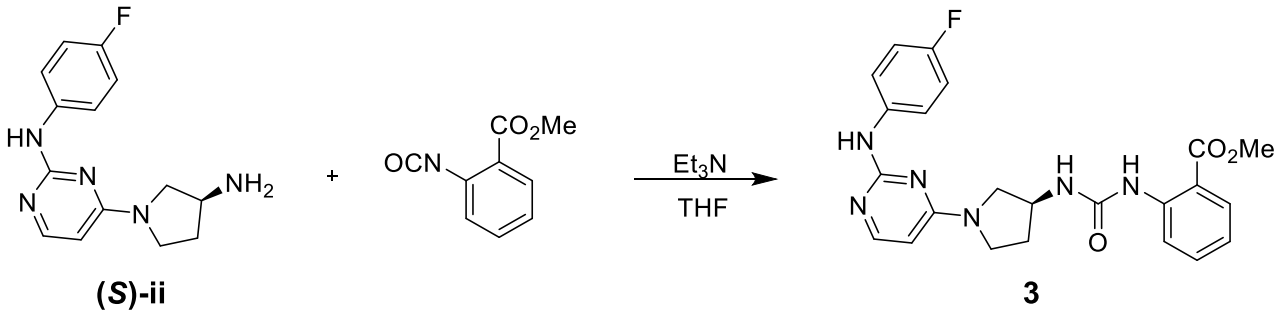
(*R*)-ii **(*R*)-1**

(*R*)-ii (60 mg, 0.22 mmol) was dissolved in 2 mL of THF, followed by addition of triethylamine (46 µL, 0.33 mmol) and methyl 3-isocyanatobenzoate (58 mg, 0.33 mmol). The reaction proceeded at ambient temperature for 2 h. The crude material was concentrated followed by purification using a Combiflash Rf system (Hexanes/EtOAc, 0-100% EtOAc) to isolate **(*R*)-1** (70 mg, 0.16 mmol, 71%). $[\alpha]_D^{22}$ = +16.500 (c = 0.938, 80% $CHCl_3$/ 20% MeOH). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.06 (s, 1H), 8.59 (s, 1H), 8.14 (t, *J* = 1.9 Hz, 1H), 7.93 (d, *J* = 6.0 Hz, 1H), 7.85 – 7.76 (m, 1H), 7.54 (dt, *J* = 8.1, 1.6 Hz, 1H), 7.52 – 7.48 (m, 1H), 7.37 (t, *J* = 7.9 Hz, 1H), 7.06 (t, *J* = 8.9 Hz, 2H), 6.60 (d, *J* = 6.8 Hz, 1H), 5.98 (d, *J* = 5.9 Hz, 1H), 4.33 (br. m, 1H), 3.84 (s, 3H), 3.71 – 3.39 (m, 4H), 2.21 (br. m, 1H), 1.93 (d, *J* = 25.9 Hz, 1H), 1.29 – 1.13 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.26, 160.19, 158.40 (d, *J* = 237.9 Hz), 155.65, 154.75, 140.64, 137.76, 130.05, 129.06, 122.20, 121.87, 119.72 (d, *J* = 7.3 Hz), 118.09, 114.69 (d, *J* = 21.9 Hz), 95.65, 52.09, 51.92, 44.27. HRMS (ESI-TOF) calculated for $C_{23}H_{23}FN_6O_3H^+$ $[M+H]^+$: 451.1849, observed 451.1883.



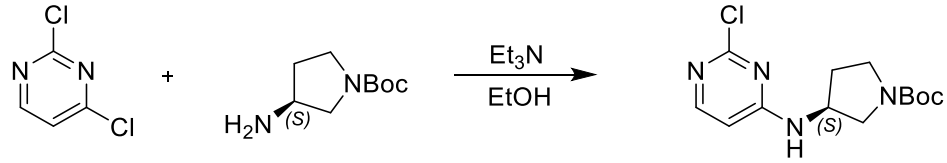
(*S*)-ii **2**

(*S*)-ii (40 mg, 0.15 mmol) was dissolved in 2 mL of THF and 30 µL of triethylamine (0.23 mmol). Methyl-4-isocyanatobenzoate (38.9 mg, 0.220 mmol) was added and the mixture was stirred at room temperature for 3 h. Upon reaction, the solution turned white and heterogeneous. Aliquots were taken and solubilized into methanol to monitor product formation via TLC (100% EtOAc, R_f = 0.2). Following completion of the reaction, the crude material was concentrated and purified via flash column chromatography (2:1 hexanes:EtOAc, to 100% EtOAc) was used to isolate **2** as a yellow solid (40.0 mg, 0.089 mmol, 57%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.06 (s, 1H), 8.76 (s, 1H), 7.93 (d, *J* = 5.9 Hz, 1H), 7.84 (d, *J* = 8.6 Hz, 2H), 7.79 (dd, *J* = 8.8, 5.0 Hz, 2H), 7.51 (d, *J* = 8.7 Hz, 2H), 7.06 (t, *J* = 8.9 Hz, 2H), 6.72 (d, *J* = 6.8 Hz, 1H), 5.98 (d, *J* = 5.9 Hz, 1H), 4.34 (s, 1H), 3.80 (s, 3H), 3.74 – 3.39 (m, 5H), 3.24 (s, 1H), 2.21 (s, 1H), 1.96 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.92, 160.18, 158.40 (d, *J* = 237.3 Hz), 155.66, 155.57, 154.39, 144.86, 137.76 (d, *J* = 2.2 Hz), 130.35, 121.81, 119.72 (d, *J* = 7.3 Hz), 116.75, 114.69 (d, *J* = 21.8 Hz), 95.64, 51.88, 51.69, 44.23. HRMS (ESI-TOF) calculated for $C_{23}H_{23}FN_6O_3H^+$ $[M+H]^+$: 451.1849, observed 451.1884.



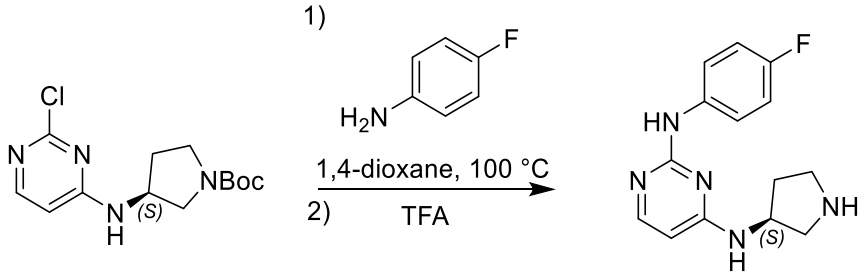
(*S*)-ii **3**

(*S*)-ii (30 mg, 0.11 mmol) was dissolved in 2 mL of THF and 25 µL of triethylamine (0.17 mmol). Methyl-2-isocyanatobenzoate (30 mg, 0.17 mmol) was added and the mixture was stirred at room temperature for 2 h. Following completion of the reaction, the crude material was concentrated and purified via flash column chromatography (9:1 EtOAc/10% NH_4OH in methanol) was used to isolate **3** as a white solid (20.0 mg, 0.044 mmol, 40%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.37 (s, 1H), 8.49 (dd, *J* = 8.6, 1.2 Hz, 1H), 7.98 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.84 (d, *J* = 6.1 Hz, 1H), 7.59 – 7.55 (m, 2H), 7.51 (ddd, *J* = 8.7, 7.2, 1.7 Hz, 1H), 6.98 (m, 3H), 5.78 (d, *J* = 6.1 Hz, 1H), 5.61 (s, 1H), 4.57 (q, *J* = 5.4 Hz, 1H), 3.88 (s, 3H), 3.82 – 3.28 (m, 5H), 2.53 (s, 3H), 2.32 (p, *J* = 6.9, 6.5 Hz, 1H), 2.10 (s, 1H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 169.14, 160.46, 158.35, 158.19 (d, *J* = 241.1 Hz), 154.48, 142.88, 135.86, 134.64, 130.78, 120.97, 120.72 (d, *J* = 7.7 Hz), 119.57, 115.26 (d, *J* = 22.3 Hz), 113.89, 95.76, 52.24, 44.60. HRMS (ESI-TOF) calculated for $C_{23}H_{23}FN_6O_3H^+$ $[M+H]^+$: 451.1849, observed 451.1884.



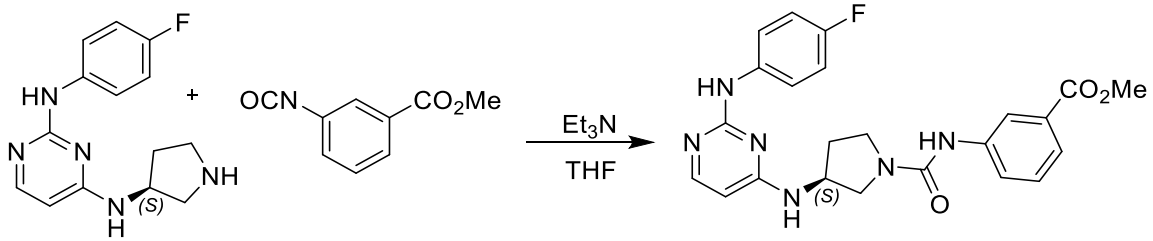
(*S*)4-i

2,4-Dichloropyrimidine (300 mg, 2.0 mmol) was dissolved in 5 mL of EtOH, the mixture was briefly warmed to 50 °C to solubilize. Triethylamine (308 µL, 2.2 mmol) was added, followed by (S)-1-boc-3-aminopyrrolidine (403 µL, 2.2 mmol). The reaction was stirred at ambient temperature for 18 h. The solvent was removed under reduced pressure, and the crude mixture was diluted in EtOAc followed by washing 3 X with H_2O, brine, and dried over $MgSO_4$. The mixture was concentrated and purified on a Combiflash Rf system (Hexanes/EtOAc, 0-100% EtOAc) to afford **(*S*)4-i** as a colorless oil (389 mg, 1.4 mmol, 65%). ^{1}H NMR, mixture of rotational isomers (500 MHz, DMSO-d_6) δ 8.15 (d, *J* = 6.3 Hz, 1H), 7.93 (d, *J* = 5.9 Hz, 1H), 6.46 (d, *J* = 5.9 Hz, 1H), 4.38 (d, *J* = 27.9 Hz, 1H), 3.54 (dt, *J* = 10.7, 5.0 Hz, 1H), 3.48 – 3.29 (m, 4H), 3.11 (dd, *J* = 10.9, 4.1 Hz, 1H), 2.11 (dq, *J* = 13.3, 6.5 Hz, 1H), 1.81 (tt, *J* = 12.2, 6.2 Hz, 1H), 1.40 (s, *J* = 4.8 Hz, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.00, 159.71, 155.65, 153.49, 105.23, 78.41, 50.98, 50.68, 49.97, 49.26, 43.89, 43.72, 30.68, 29.92, 28.15. HRMS (ESI-TOF) calculated for $C_{13}H_{19}ClN_4O_2H^+$ $[M+H]^+$: 299.1167, observed 299.1267.



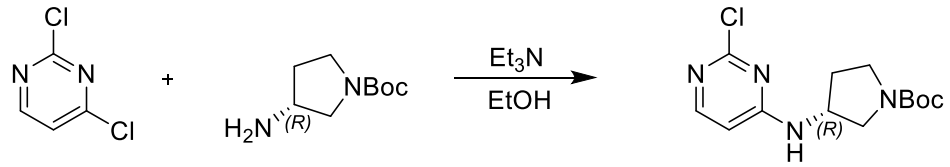
(*S*)4-i **(*S*)4-ii**

(*S*)4-i (268 mg, 0.899 mmol) was dissolved in 5 mL of 1,4-dioxane, followed by addition of 4-fluoroaniline (127 µL, 1.35 mmol). The mixture was heated to reflux for 5 h, followed by removal of solvent. Upon cooling to ambient temperature, diethyl ether was used to precipitate out the intermediate, and was collected via vacuum filtration. The filtered solid was dissolved in 3 mL of trifluoroacetic acid, and stirred for 1 h. The solvent was removed, and the mixture was dissolved in H_2O. The aqueous layer was washed 3 X with DCM and separated. The aqueous layer was adjusted to pH 12 using 1M NaOH, and extracted into EtOAc. The organic layer was washed with brine, and dried over $MgSO_4$ followed by filtration and concentration to isolate **(*S*)4-ii** (215 mg, 0.788 mmol, 87% over 2 steps). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.04 – 8.93 (m, 1H), 8.32 (s, 1H), 7.77 (dd, *J* = 9.0, 5.0 Hz, 3H), 7.48 – 7.19 (m, 1H), 7.05 (t, *J* = 8.9 Hz, 2H), 5.99 – 5.89 (m, 1H), 4.31 (s, 1H), 3.63 – 3.16 (m, 1H), 3.09 (dd, *J* = 11.3, 6.4 Hz, 1H), 2.95 (dt, *J* = 10.9, 7.3 Hz, 1H), 2.85 (ddd, *J* = 10.9, 8.1, 5.8 Hz, 1H), 2.70 (dd, *J* = 11.3, 4.4 Hz, 1H), 2.04 (dq, *J* = 14.6, 7.5 Hz, 1H), 1.64 (ddt, *J* = 12.9, 7.8, 5.2 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.95, 159.48, 156.40 (d, *J* = 236.8 Hz), 137.67, 119.73 (d, *J* = 7.3 Hz), 114.52 (d, *J* = 21.8 Hz), 79.06, 52.55, 45.01, 32.34. HRMS (ESI-TOF) calculated for $C_{14}H_{16}FN_5H^+$ $[M+H]^+$: 274.1423, observed 274.1461.



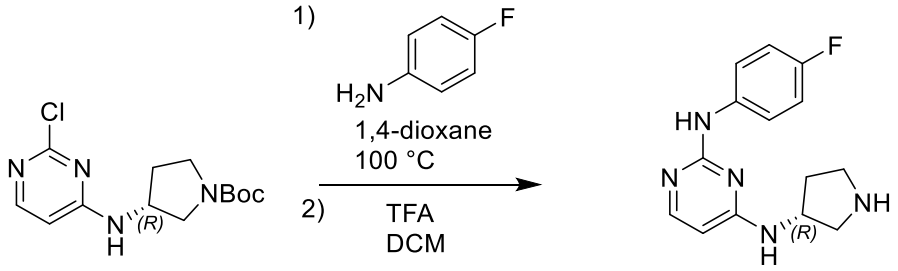
(*S*)4-ii **(*S*)-4**

(*S*)4-ii (84 mg, 0.31 mmol) was dissolved in 3 mL of THF, followed by addition of triethylamine (0.47 mmol) and methyl 3-isocyanatobenzoate (84 mg, 0.47 mmol). The reaction was stirred at ambient temperature for 2 h, followed by concentration and purification on a Combiflash Rf system (hexanes/EtOAc, 0-100% EtOAc) to afford **(*S*)-4** as a white solid (113 mg, 0.25 mmol, 82%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.03 (s, 1H), 8.46 (s, 1H), 8.19 (t, *J* = 1.9 Hz, 1H), 7.86 – 7.81 (m, 2H), 7.81 – 7.75 (m, 2H), 7.52 (dt, *J* = 7.7, 1.3 Hz, 1H), 7.47 (s, 1H), 7.37 (t, *J* = 7.9 Hz, 1H), 7.05 (t, *J* = 8.9 Hz, 2H), 5.98 (d, *J* = 5.8 Hz, 1H), 4.51 (s, 1H), 3.83 (s, 3H), 3.76 (dd, *J* = 10.7, 6.1 Hz, 1H), 3.58 (dt, *J* = 10.2, 7.3 Hz, 1H), 3.51 (td, *J* = 9.8, 8.9, 5.5 Hz, 1H), 3.36 (dd, *J* = 10.7, 4.3 Hz, 1H), 2.21 (dq, *J* = 13.5, 7.0 Hz, 1H), 1.95 (dq, *J* = 12.7, 6.1 Hz, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 166.23, 161.97, 159.49, 156.45 (d, *J* = 236.9 Hz), 154.73, 153.69, 140.91, 137.61 (d, *J* = 2.2 Hz), 129.59, 128.57, 123.62, 122.04, 119.81 (d, *J* = 7.4 Hz), 119.73, 114.56 (d, *J* = 21.8 Hz), 97.74, 51.93, 51.33, 49.66, 43.98, 30.42. HRMS (ESI-TOF) calculated for $C_{23}H_{23}FN_6O_3H^+$ $[M+H]^+$: 451.1849, observed 451.1883.



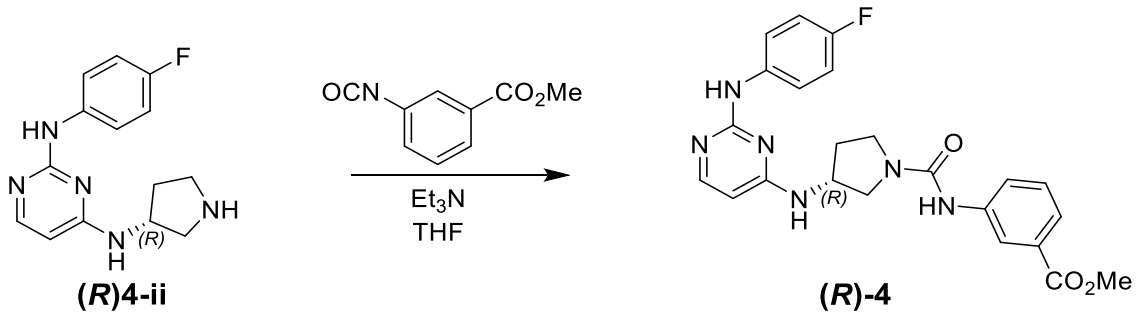
(*R*)4-i

2,4-Dichloropyrimidine (300 mg, 2.0 mmol) was dissolved in 5 mL of EtOH, the mixture was briefly warmed to 50 °C to solubilize. Triethylamine (2.2 mmol) was added, followed by (*R*)-1-boc-3-aminopyrrolidine (403 µL, 2.2 mmol). The reaction was stirred at ambient temperature for 18 h. The solvent was removed under reduced pressure, and the crude mixture was diluted in EtOAc followed by washing 3 X with H_2O, brine, and dried over $MgSO_4$. The mixture was concentrated and purified on a Combiflash Rf system (Hexanes/EtOAc, 0-100% EtOAc) to afford **(*R*)4-i** as a colorless oil (357 mg, 1.2 mmol, 60%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.05 – 7.96 (m, 1H), 6.30 (d, *J* = 5.9 Hz, 1H), 5.83 – 5.58 (m, 1H), 3.67 (dd, *J* = 11.5, 5.9 Hz, 1H), 3.53 – 3.40 (m, 2H), 3.36 – 3.20 (m, 1H), 2.21 (p, *J* = 6.8 Hz, 1H), 2.04 (s, 1H), 1.99 – 1.85 (m, 1H), 1.45 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 162.95, 160.72, 154.50, 79.86, 51.61, 51.05, 50.24, 43.89, 43.64, 31.61, 30.86, 28.45. HRMS (ESI-TOF) calculated for $C_{13}H_{19}ClN_4O_2H^+$ [M+H]$^+$: 299.1276, observed 299.1249.



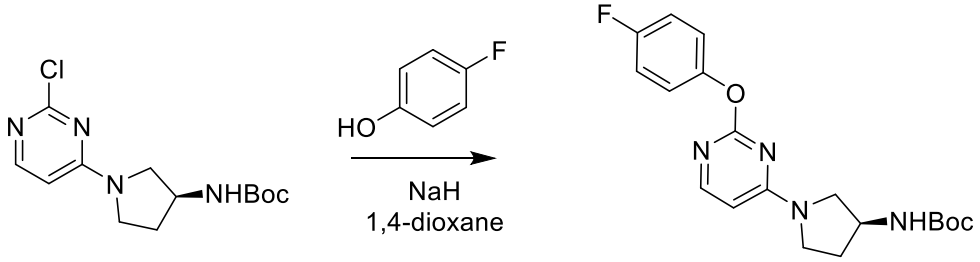
(*R*)4-i **(*R*)4-ii**

(*R*)4-i (330.7 mg, 1.107 mmol) was dissolved in 4 mL of 1,4-dioxane, to which 4-fluoroaniline (158 µL, 1.66 mmol) was added. The solution was heated to reflux for 18 h, followed by removal of solvent. When cooled to rt, diethyl ether was added, and the resulting precipitate was collected by vacuum filtration and washed with additional diethyl ether. The dried precipitate was dissolved in 3 mL of TFA and 1 mL of DCM and stirred at ambient temperature for 1 h. The solvent was removed, and the crude mixture was dissolved in H_2O and DCM. The aqueous layer was washed 3 X with DCM and separated. The pH was adjusted to > 10 using 1M NaOH and extracted 3 X with EtOAc. The combined organic layers were washed with brine and dried over $MgSO_4$. Following filtration, the solvent was removed yielding **(*R*)4-ii** as a brown oil (148.9 mg, 0.5448 mmol, 49%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.91 (d, *J* = 5.8 Hz, 1H), 7.52 (dd, *J* = 8.8, 4.8 Hz, 2H), 7.08 (d, *J* = 26.9 Hz, 1H), 6.98 (t, *J* = 8.6 Hz, 2H), 5.85 (d, *J* = 5.8 Hz, 1H), 5.22 (d, *J* = 6.8 Hz, 1H), 4.34 (s, 1H), 3.18 (ddd, *J* = 19.6, 13.0, 6.7 Hz, 2H), 3.04 – 2.99 (m, 1H), 2.97 (dd, *J* = 12.0, 3.6 Hz, 1H), 2.63 (d, *J* = 14.4 Hz, 3H), 2.21 (ddd, *J* = 15.5, 13.8, 7.4 Hz, 1H), 1.78 (td, *J* = 10.7, 8.0, 4.6 Hz, 1H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 162.28, 159.80, 158.22 (d, *J* = 240.8 Hz), 156.07, 135.98, 121.11 (d, *J* = 7.7 Hz), 115.23 (d, *J* = 22.3 Hz), 53.30, 51.63, 45.36, 33.06. HRMS (ESI-TOF) calculated for $C_{14}H_{16}FN_5H^+$ [M+H]$^+$: 274.1423, observed 274.1438.



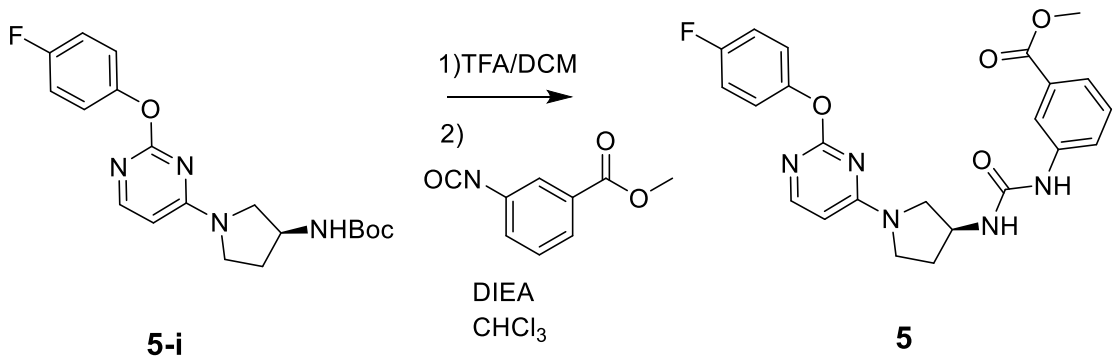
(*R*)4-ii **(*R*)-4**

(*R*)4-ii (120 mg, 0.44 mmol) was dissolved in 5 mL of THF, followed by addition of triethylamine (92 µL, 0.66 mmol) and methyl 3-isocyanatobenzoate (120 mg, 0.66 mmol). The reaction was stirred at ambient temperature for 2 h, followed by removal of solvent and purification on a Combiflash Rf System (hexanes/EtOAc, 0-100% EtOAc) to afford **(*R*)-4** as a white solid (110 mg, 55%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.03 (s, 1H), 8.46 (s, 1H), 8.19 (t, *J* = 1.9 Hz, 1H), 7.87 – 7.81 (m, 2H), 7.78 (dd, *J* = 9.0, 5.0 Hz, 2H), 7.52 (dt, *J* = 7.8, 1.2 Hz, 1H), 7.50 – 7.45 (m, 1H), 7.37 (t, *J* = 7.9 Hz, 1H), 7.05 (t, *J* = 8.9 Hz, 2H), 5.98 (d, *J* = 5.8 Hz, 1H), 4.50 (s, 1H), 3.84 (s, 3H), 3.76 (dd, *J* = 10.7, 6.1 Hz, 1H), 3.58 (dt, *J* = 10.2, 7.3 Hz, 1H), 3.51 (td, *J* = 10.2, 9.1, 5.6 Hz, 1H), 3.36 (dd, *J* = 10.7, 4.4 Hz, 1H), 2.21 (dq, *J* = 13.5, 7.0 Hz, 1H), 1.95 (qd, *J* = 11.2, 9.9, 3.9 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.23, 161.97, 159.47, 156.46 (d, *J* = 236.7 Hz), 153.68, 140.90, 137.59 (d, *J* = 2.3 Hz), 129.59, 128.57, 123.62, 122.04, 119.85, 119.76 (d, *J* = 8.6 Hz), 114.56 (d, *J* = 21.9 Hz), 51.93, 51.32, 49.66, 43.97, 30.41, 19.01. HRMS (ESI-TOF) calculated for $C_{23}H_{23}FN_6O_3H^+$ $[M+H]^+$: 451.1849, observed 451.1882.



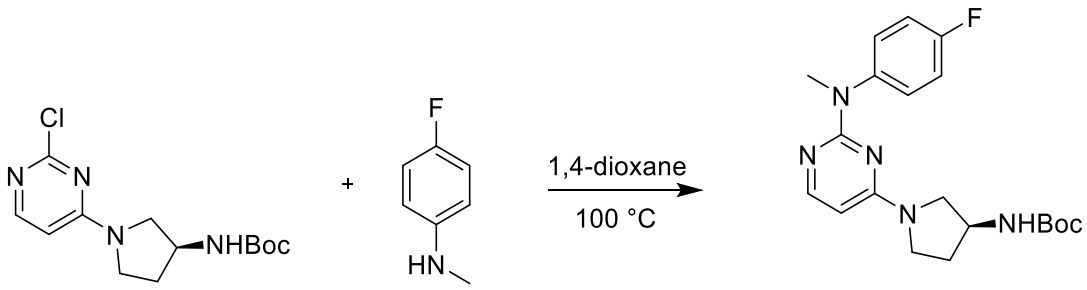
(*S*)-i **5-i**

In a sealed reaction vessel 16.4 mg (0.68 mmol) of NaH was dissolved in 0.5 mL of 1,4-dioxane followed by addition of 76.7 mg (0.68 mmol) of 4-fluorophenol and stirred for 0.25 h. 51.1 mg (0.17 mmol) of **(*S*)-i** was then added and the mixture was heated to 100° for 48 h. The solution was diluted with 10 mL H_2O and extracted with 10 mL of EtOAc (3 X). The crude material was concentrated and purified via column chromatography (hexanes:EtOAc) to afford **5-i**, used without further purification. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.98 (d, *J* = 6.0 Hz, 1H), 7.18 – 7.11 (m, 2H), 7.09 – 7.03 (m, 2H), 6.03 (d, *J* = 6.0 Hz, 1H), 4.84 (s, 1H), 4.32 (s, 1H), 3.81 – 3.17 (m, 4H), 2.25 (s, 1H), 2.04 (s, 1H), 1.45 (s, 9H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 171.18, 164.90, 161.91, 159.64 (d, *J* = 242.8 Hz), 156.58, 155.30, 148.99 (d, *J* = 2.8 Hz), 123.21 (d, *J* = 8.4 Hz), 115.82 (d, *J* = 23.3 Hz), 99.12, 60.40, 52.51, 50.39, 44.52, 40.90, 31.72, 30.80, 28.35, 21.04, 14.19. HRMS (ESI-TOF) calculated for $C_{19}H_{23}FN_4O_3H^+$ $[M+H]^+$: 375.1827, observed 375.1814.



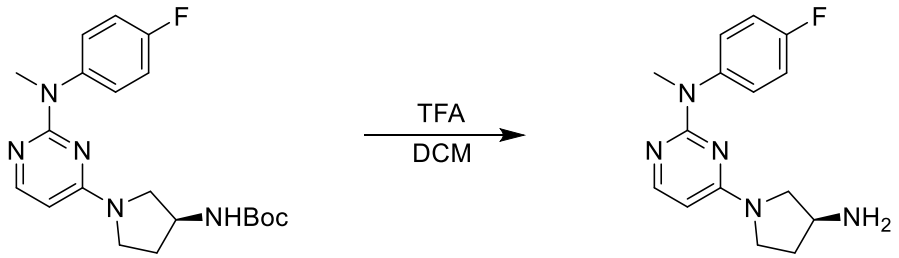
5-i **5**

41.6 mg (0.11 mmol) of **5-i** was titrated with 1 mL of trifluoroacetic acid for 12 hr. The solution was neutralized and the resulting amine was extracted with 5 mL of ethyl acetate. The ethyl acetate was removed under vacuum and the resulting oil was resuspended in 1 mL of chloroform. 19.7 mg (0.11 mmol) of methyl 3-isocyanobenzoate was added and the solution was stirred for 12 h. 200 µL of diisopropylethylamine (0.11 mmol) was added and the reaction was stirred for an additional 12 h. The mixture was diluted with 2 mL of DI water and extracted with 3 X 10 mL of DCM. The solvent was removed and **5** was isolated by column chromatography (hexanes:ethyl acetate), 44% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.61 (d, *J* = 11.6 Hz, 1H), 8.14 (s, 1H), 8.01 – 7.90 (m, 1H), 7.59 – 7.48 (m, 2H), 7.37 (t, *J* = 7.9 Hz, 1H), 7.25 – 7.12 (m, 4H), 6.67 – 6.52 (m, 1H), 6.31 (d, *J* = 5.9 Hz, 1H), 4.30 (d, *J* = 44.1 Hz, 1H), 3.84 (s, 3H), 3.61 (s, 1H), 3.46 (s, 2H), 3.38-3.26 (m, 1H), 2.19 (d, *J* = 38.8 Hz, 1H), 1.94 (d, *J* = 42.0 Hz, 1H) ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.73, 164.80, 159.33 (d, *J* = 240.1 Hz), 156.78, 155.23, 149.65, 141.10, 130.53, 129.53, 123.95 (d, *J* = 8.5 Hz), 122.71, 122.36, 118.60, 116.21 (d, *J* = 23.3 Hz), 99.99, 52.57, 52.12, 49.71, 49.05, 44.98, 44.80, 31.47, 30.70. HRMS (ESI-TOF) calculated for $C_{23}H_{22}FN_5O_4H^+$ $[M+H]^+$: 452.1729, observed 452.1710.



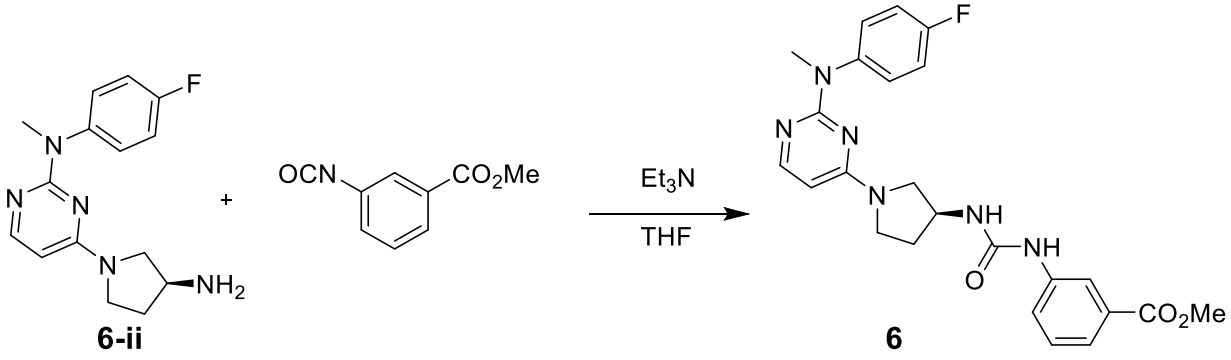
(*S*)-i **6-i**

(*S*)-i (200 mg, 0.67 mmol) was dissolved in 5 mL of 1,4-dioxane, followed by addition of N-methyl-4-fluoroaniline (180 µL, 2.0 mmol) and was heated to reflux for 18 h. The solvent was removed under reduced pressure and the mixture was redissolved in DCM. The organic layer was washed with 10% $NaHCO_3$, followed by H_2O and brine and dried over $MgSO_4$. Following filtration, the crude mixture was concentrated and purified by flash chromatography (1:1 Hexanes/EtOAc) to afford **6-i** as a beige solid (160 mg, 0.41 mmol, 62%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.81 (d, *J* = 5.8 Hz, 1H), 7.34 (dd, *J* = 8.8, 5.1 Hz, 2H), 7.14 (t, *J* = 8.8 Hz, 2H), 5.84 (d, *J* = 5.8 Hz, 1H), 4.06 (s, 1H), 3.78 – 3.02 (m, 3H), 3.39 (s, 3H), 2.06 (br. m, 1H), 1.82 (br. m, 1H), 1.38 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.76, 158.86 (d, *J* = 240.7 Hz), 155.35, 142.12, 128.02 (d, *J* = 8.2 Hz), 114.78 (d, *J* = 22.2 Hz), 94.70, 77.86, 51.18, 44.02, 37.66, 28.20. HRMS (ESI-TOF) calculated for $C_{20}H_{26}FN_5O_2H^+$ $[M+H]^+$: 388.2104, observed 388.2141.



6-i **6-ii**

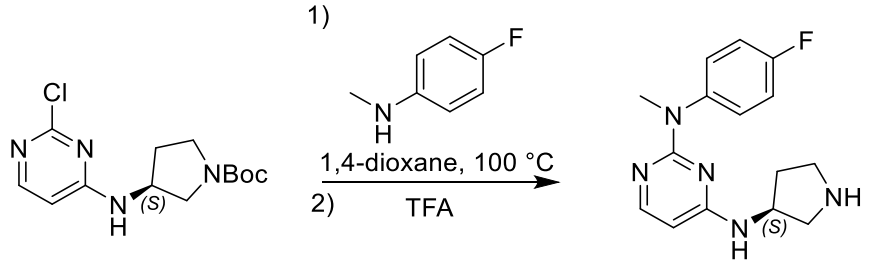
6-i (41.8 mg, 0.108 mmol) was dissolved in 3 mL of TFA and 1 mL of DCM. The reaction was stirred at ambient temperature for 1 h. The solvent was removed, and the crude mixture was dissolved in H_2O and DCM. The aqueous layer was washed 3 X with DCM and separated. The aqueous layer was basified to pH > 10 using 1M NaOH, and then extracted 3 X with EtOAc. The combined organic layers were washed with $MgSO_4$ and brine, followed by filtration and concentration to isolate **6-ii** as a brown oil (20.8 mg, 67%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.89 (d, *J* = 5.9 Hz, 1H), 7.30 – 7.26 (m, 2H), 7.02 (t, *J* = 8.7 Hz, 2H), 5.71 (d, *J* = 5.9 Hz, 1H), 3.69 – 3.49 (m, 3H), 3.46 (s, 3H), 3.41 – 3.00 (m, 1H), 2.18 – 2.09 (m, 1H), 1.88 – 1.70 (m, 3H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 161.36, 160.35, 159.88 (d, *J* = 243.6 Hz), 155.42, 142.08 (d, *J* = 3.0 Hz), 128.13 (d, *J* = 8.2 Hz), 115.28 (d, *J* = 22.3 Hz), 94.32, 54.31, 44.38, 38.27, 34.32. HRMS (ESI-TOF) calculated for $C_{15}H_{18}FN_5H^+$ $[M+H]^+$: 288.1580, observed 288.1624.



6-ii **6**

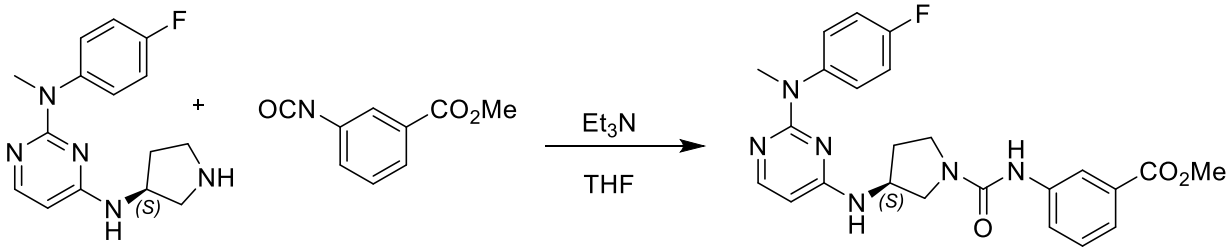
6-ii (68 mg, 0.24 mmol) was dissolved in 3 mL of THF, followed by addition of triethylamine (49 µL, 0.36 mmol) and methyl 3-isocyanatobenzoate (63 mg, 0.36 mmol). The reaction was stirred at ambient temperature for 2 h. The solvent was removed under reduced pressure and the crude mixture was redissolved in EtOAc and washed 3 X with H_2O followed by brine and $MgSO_4$. Following filtration, the organic layer was concentrated and purified on a Combiflash Rf system (Hexanes/EtOAc, 0-100% EtOAc) to afford **6** as a yellow solid (83 mg, 0.18 mmol, 75%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.67 (s, 1H), 8.13 (d, *J* = 1.9 Hz, 1H), 7.83 (d, *J* = 5.8 Hz, 1H), 7.55 (dd, *J* = 8.1, 2.2 Hz, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.37 (d, *J* = 8.2 Hz, 1H), 7.34 (d, *J* = 7.2 Hz, 2H), 7.15 (t, *J* = 8.7 Hz, 2H), 6.63 (d, *J* = 6.8 Hz, 1H), 5.89 (d, *J* = 5.8 Hz, 1H), 4.27 (s, 1H), 3.83 (s, 3H), 3.57 (dd, *J* = 11.2, 6.0 Hz, 1H), 3.40 (s, 3H), 2.14 (d, *J* = 12.6 Hz, 1H), 1.90 (s, 1H), 1.23 (s, 1H ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.27, 160.80, 159.96, 158.90 (d, *J* = 241.0 Hz), 155.47,

154.76, 142.13 (d, *J* = 2.9 Hz), 140.68, 130.04, 129.04, 128.07 (d, *J* = 8.4 Hz), 122.17, 121.83, 118.06, 114.83 (d, *J* = 22.2 Hz), 94.72, 52.08, 43.97, 37.69. HRMS (ESI-TOF) calculated for $C_{24}H_{25}FN_6O_3H^+$ $[M+H]^+$: 465.2006, observed 465.2041.



(*S*)4-i **(*S*)6-iii**

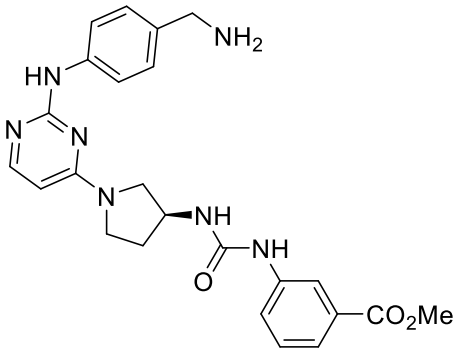
(*S*)4-i (304 mg, 1.02 mmol, 1 eq) was dissolved in 8 mL of 1,4-dioxane, followed by addition of N-methyl-4-fluoroaniline (200 µL, 1.53 mmol). The mixture was heated to reflux for 18 h, followed by removal of solvent. Upon cooling to ambient temperature, diethyl ether was used to precipitate out the intermediate, and was collected via vacuum filtration. The filtered solid was dissolved in 3 mL of trifluoroacetic acid and stirred for 1 h. The solvent was removed, and the mixture was dissolved in H_2O. The aqueous layer was washed 3 X with DCM and separated. The aqueous layer was adjusted to pH 12 using 1M NaOH and extracted into EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. The material was filtered and concentrated to isolate **(*S*)6-iii** as a brown oil (267 mg, used without further purification). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.79 – 7.66 (m, 1H), 7.41 – 7.26 (m, 2H), 7.18 – 7.10 (m, 2H), 5.84 (d, *J* = 5.8 Hz, 1H), 4.26 – 3.96 (m, 1H), 3.67 – 3.48 (m, 2H), 3.38 (s, 3H), 2.92 (dd, *J* = 11.3, 6.5 Hz, 1H), 2.86 (dt, *J* = 11.0, 7.3 Hz, 1H), 2.78 – 2.69 (m, 1H), 2.64 – 2.54 (m, 1H), 1.96 – 1.85 (m, 1H), 1.78 – 1.71 (m, 1H), 1.66 – 1.49 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.96, 158.80 (d, *J* = 240.8 Hz), 142.11 (d, *J* = 2.9 Hz), 128.07 (d, *J* = 8.2 Hz), 114.70 (d, *J* = 22.3 Hz), 66.90, 52.69, 45.11, 37.62, 32.35, 30.09, 25.01. HRMS (ESI-TOF) calculated for $C_{15}H_{18}FN_5H^+$ $[M+H]^+$: 288.1617, observed 288.1580.



(*S*)6-iii **(*S*)6-iv**

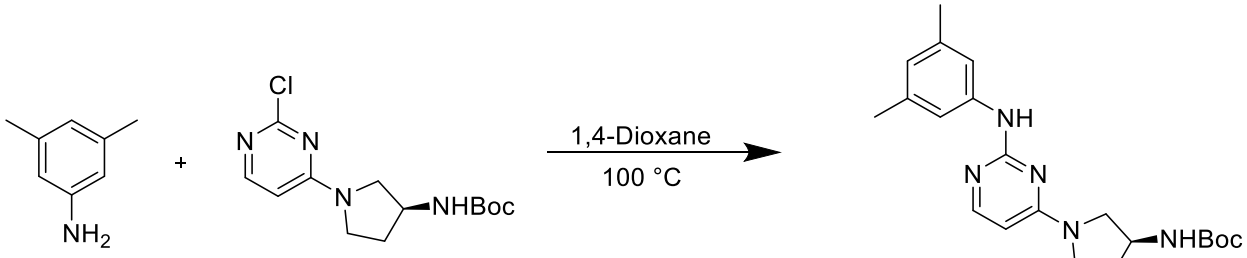
(*S*)6-iii (93 mg, 0.32 mmol, 1 eq) was dissolved in 2 mL of THF, followed by addition of triethylamine (68 µL, 0.49 mmol) and methyl 3-isocyanatobenzoate (86 mg, 0.49 mmol) and was stirred at ambient temperature for 2 h. The crude mixture was concentrated and purified on a Combiflash Rf system (hexanes/EtOAc, 0-100% EtOAc) to afford **(*S*)6-iv** as a yellow solid (103 mg, 0.22 mmol, 69%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.41 (s, 1H), 8.19 (d, *J* = 2.0 Hz, 1H), 7.84 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.74 (d, *J* = 5.5 Hz, 1H), 7.52 (dt, *J* = 7.8, 1.2 Hz, 1H), 7.40 – 7.31 (m, 4H), 7.14 (t, *J* = 8.8 Hz, 2H), 5.89 (d, *J* = 5.7 Hz, 1H),

4.26 (s, 1H), 3.84 (s, 3H), 3.63 (dd, *J* = 10.7, 6.2 Hz, 1H), 3.52 (dt, *J* = 10.2, 7.1 Hz, 1H), 3.44 (d, *J* = 18.4 Hz, 1H), 3.28 (dd, *J* = 10.7, 4.6 Hz, 1H), 2.09 (dq, *J* = 13.4, 7.0 Hz, 1H), 1.88 (dt, *J* = 12.5, 7.2 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.23, 161.66, 160.94, 158.84 (d, *J* = 241.0 Hz), 154.62, 153.64, 142.01 (d, *J* = 2.9 Hz), 140.92, 129.58, 128.55, 128.09 (d, *J* = 8.2 Hz), 123.61, 122.01, 119.70, 114.72 (d, *J* = 22.2 Hz), 96.74, 51.93, 51.21, 49.62, 43.91, 37.64, 30.22. HRMS (ESI-TOF) calculated for $C_{24}H_{25}FN_6O_3H^+$ [M+H]$^+$: 465.2006, observed 465.2070.



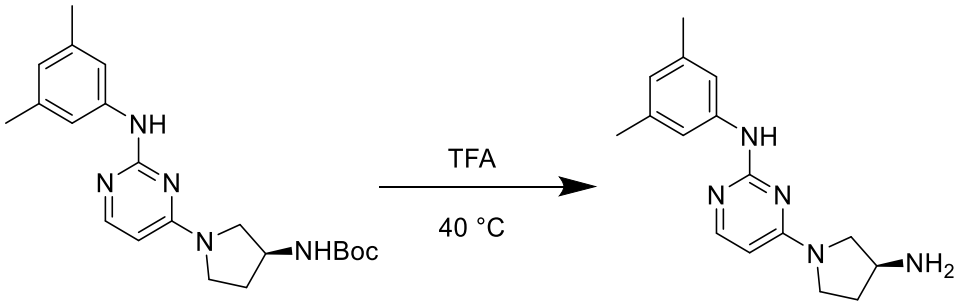
7

Compound **7** was synthesized as reported previously.[16]



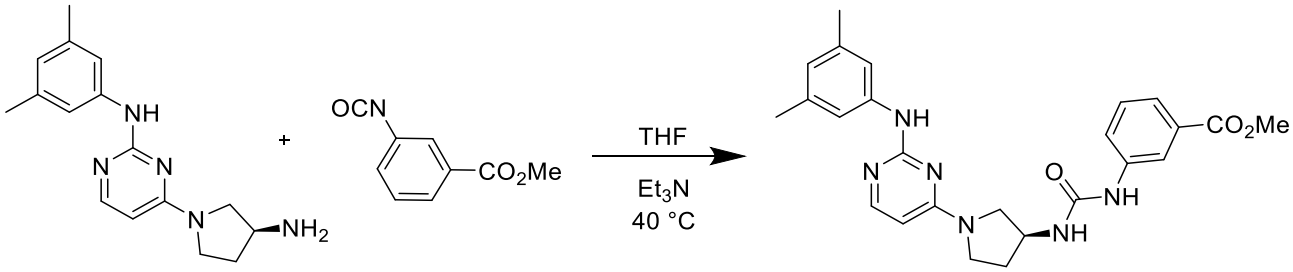
(*S*)-i **8-i**

(*S*)-i (302 mg, 1.01 mmol) was dissolved in 8 mL of 1,4-dioxane. 3,5-Dimethylaniline (302.4 µL, 1.18 mmol) was added and the reaction mixture was stirred at 100 ˚C for 6 h. The temperature was reduced to rt and the reaction mixture was stirred for 72 h. Upon completion of the reaction the solvent was removed and the orange/white solid was dissolved in EtOAc. The organic layer was washed 3 X with water, 1 X with brine, and dried over $MgSO_4$. The crude material was filtered and concentrated followed by purification using a Combiflash Rf system (hexanes/EtOAc, 0-100% EtOAc) to give the white solid **8-i** (172.7 mg, 46.0%). ^{1}H NMR as a mixture of rotational isomers (500 MHz, Chloroform-*d*) δ 7.59 (s, 1H), 7.33 (s, 1H), 6.77 (s, 2H), 5.90 (d, *J* = 7.1 Hz, 0.5H (rot.)), 5.86 (d, *J* = 7.1 Hz, 0.5H (rot.) 5.14 (s, 0.5H (rot.)), 5.06 (s, 0.5H (rot.)), 4.93 (s, 0.5H (rot.)), 4.38 (m, 1H), 3.95 (m, 0.5H (rot.)), 3.88 – 3.71 (m, 1H), 3.66 – 3.51 (m, 1H), 3.40 (d, *J* = 11.0 Hz, 0.5H (rot.)), 3.17 (s, 0.5H (rot.)), 2.31 (s, 6H), 2.15 (s, 1H), 1.86 (s, 1H), 1.47 (s, 9H). ^{13}C NMR as a mixture of rotational isomers (126 MHz, Chloroform-*d*) δ 159.98, 138.26, 125.96, 125.00, 118.59, 95.28, 95.04, 53.05, 50.84, 45.81, 45.21, 44.82, 32.18, 31.44, 30.47, 29.69, 28.28, 26.38, 23.42, 21.45 HRMS (ESI-TOF) calculated for $C_{21}H_{29}N_5O_2H^+$ [M+H]$^+$: 384.2355, observed 384.2412.



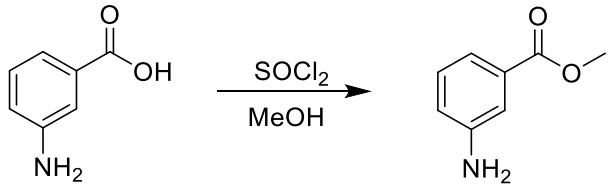
8-i **8-ii**

8-i (173 mg, 0.450 mmol) was dissolved in 8 mL neat TFA. The reaction was stirred at 40 °C for 2 h. The solvent was removed. The white solid was dissolved in EtOAc and the organic layer was washed 3 X with 1M NaOH, 1 X brine, and dried over $MgSO_4$. The material was filtered and concentrated to the white solid **8-ii** (109.6 mg, 85.6%) that was taken forward without further purification. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.95 (d, *J* = 5.8 Hz, 1H), 7.32 (s, 2H), 7.01 (s, 1H), 6.65 (s, 1H), 5.82 (d, *J* = 5.9 Hz, 1H), 3.89-3.02 (m, 4H), 3.75 (t, *J* = 5.6 Hz, 1H), 2.32 (s, 6H), 2.22 (dd, *J* = 12.9, 6.6 Hz, 1H), 1.92 – 1.79 (m, 1H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 160.70, 159.60, 155.59, 140.27, 138.20, 123.33, 116.70, 95.50, 54.78, 44.71, 34.50, 21.58. HRMS (ESI-TOF) calculated for $C_{16}H_{21}N_5H^+$[M+H]$^+$: 284.1821, observed 284.1877.



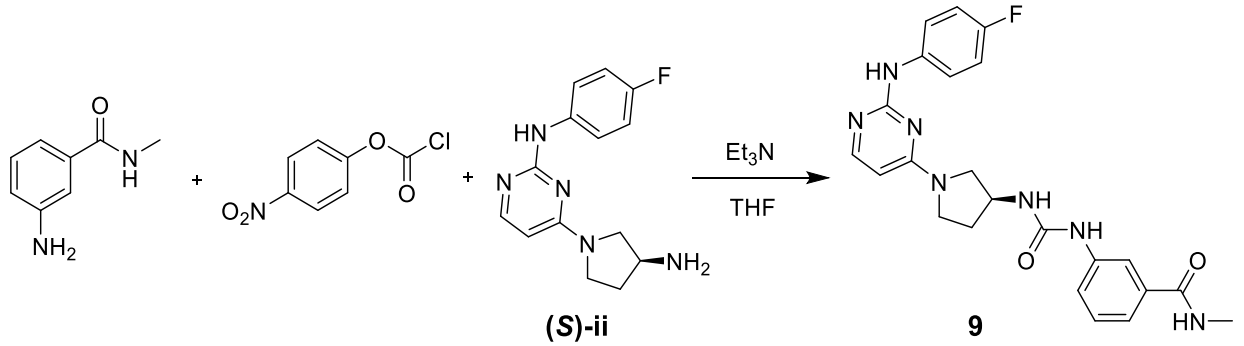
8-ii **8**

8-ii (100 mg, 0.353 mmol) and methyl-3-isocyanatobenzoate (83.3 mg, 0.470 mmol) were dissolved in 7 mL of THF. Triethylamine (60 µL, 0.462 mmol) was added and the reaction mixture was stirred at 40 °C for 90 min. Upon reaction completion the solvent was removed and the white solid that remained was dissolved in EtOAc. The organic layer was washed 3 X with water, 1 X brine, dried over $MgSO_4$. The material was filtered and concentrated followed by purification using a Combiflash Rf system (hexanes/EtOAc, 0-100% EtOAc) to give the white solid **8** (70.2 mg, 44.5%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.87 (s, 1H), 8.65 (s, 1H), 8.15 (s, 1H), 7.93 (d, *J* = 5.9 Hz, 1H), 7.55 (dd, 8.0 Hz, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.45 (s, 2H), 7.37 (t, *J* = 7.9 Hz, 1H), 6.66 (d, *J* = 6.6 Hz, 1H), 6.52 (s, 1H), 5.96 (d, *J* = 5.9 Hz, 1H), 4.32 (s, 1H), 3.84 (s, 3H), 3.77-3.38 (m, 4H), 2.22 (s, 6H), 2.00 (s, 1H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 167.04, 159.85, 139.53, 138.59, 137.89, 130.81, 129.03, 123.61, 123.14, 119.49, 117.26, 52.14, 21.40. HRMS(ESI-TOF) calculated for $C_{25}H_{28}N_6O_3H^+$ [M+H]$^+$: 461.2256, observed 461.2327.



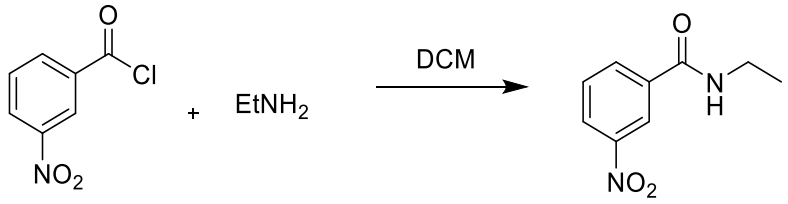
9-i

3-Aminobenzoic acid (500 mg, 3.64 mmol) was dissolved in 12 mL of MeOH. Briefly, the solution was warmed to improve solubility. Thionyl chloride (660 µL, 10.9 mmol) was added dropwise, and the solution was stirred at ambient temperature for 3 h. The solvent was removed, and the material was dissolved in EtOAc, washed with 10% $NaHCO_3$, followed by brine and dried over $MgSO_4$. Following filtration, the solvent was removed to afford **9-i** as a brown oil (220 mg, 40%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.21 – 7.18 (m, 1H), 7.13 (t, *J* = 7.6 Hz, 1H), 7.09 (dt, *J* = 7.7, 1.5 Hz, 1H), 6.79 (ddd, *J* = 7.8, 2.4, 1.3 Hz, 1H), 5.37 (s, 2H), 3.79 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.64, 148.88, 130.06, 128.95, 118.20, 116.19, 113.96, 51.70. HRMS (ESI-TOF) calculated for $C_8H_9NO_2H^+$ [M+H]$^+$: 152.0667, observed 152.0705.



(*S*)-ii **9**

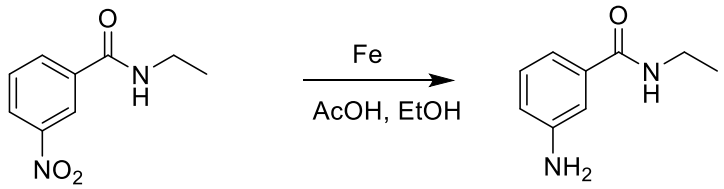
4-Nitrophenylchloroformate (161 mg, 0.798 mmol) was dissolved in 2 mL of anhydrous THF and cooled to 0 °C. Separately, 3-aminomethylbenzamide (70 mg, 0.798 mmol) and triethylamine (112 µL, 0.798 mmol) were dissolved in 1 mL of anhydrous THF. The second solution was added dropwise to the first, and the reaction was warmed to ambient temperature for 15 minutes. The mixture was diluted with EtOAc and washed 3 X with H_2O. The organic fraction was washed with brine and dried over $MgSO_4$ followed by filtration. **(*S*)-ii** (110 mg, 0.40 mmol) and triethylamine (112 µL) were added to the EtOAc fraction, and the reaction was stirred at ambient temperature for 1 h. The organic layer was washed 3 X with 1M NaOH, followed by brine and $MgSO_4$. The material was filtered and concentrated followed by purification via column chromatography (9:1 EtOAc/MeOH) to isolate **9** as a white solid (40 mg, 40%, 2 steps) ^{1}H NMR, mixture of rotational isomers (500 MHz, DMSO-d_6) δ 9.09 (s, 1H), 8.48 (s, 1H), 8.32 (dd, *J* = 4.5 Hz, 1H), 7.93 (d, *J* = 5.9 Hz, 1H), 7.82 – 7.76 (m, 3H), 7.56 – 7.51 (m, 1H), 7.33 (d, *J* = 7.7 Hz, 1H), 7.29 (t, *J* = 7.8 Hz, 1H), 7.07 (t, *J* = 8.7 Hz, 2H), 6.60 (d, *J* = 6.8 Hz, 1H), 5.99 (d, *J* = 5.9 Hz, 1H), 4.33 (s, 1H), 3.80 – 3.19 (m, 4H), 2.76 (s, 1H), 2.75 (s, 2H), 2.21 (s, 1H), 2.01 – 1.89 (m, 1H).^{13}C NMR (126 MHz, DMSO-d_6) δ 166.75, 166.67, 160.17, 159.15, 156.58 (d, *J* = 236.7 Hz), 155.36, 154.80, 140.28, 140.18, 137.67, 135.28, 128.52, 120.16, 119.83 (d, *J* = 7.4 Hz), 119.57, 116.79, 116.69, 114.72 (d, *J* = 21.9 Hz), 95.69, 51.99, 44.31, 26.25. HRMS (ESI-TOF) calculated for $C_{23}H_{24}FN_7O_2H^+$ [M+H]$^+$: 450.2009, observed 450.2044.



10-i

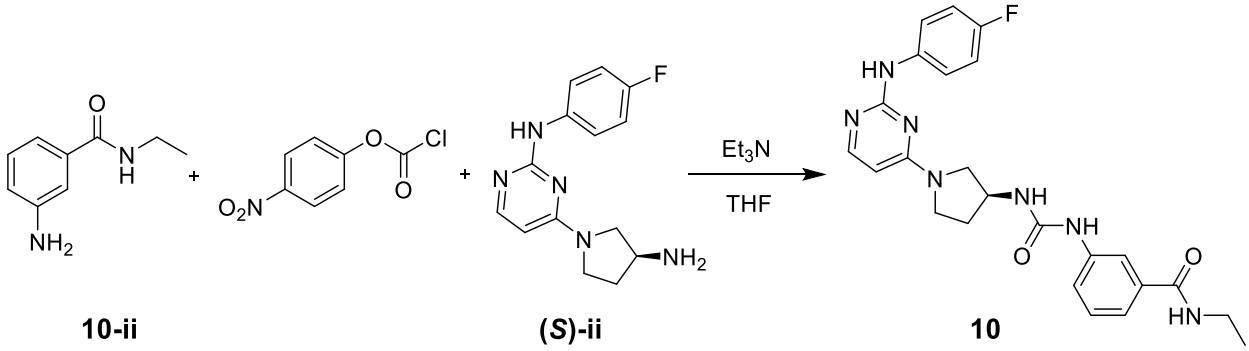
3-Nitrobenzoyl chloride (543.3 mg, 2.93 mmol) was dissolved in 5 mL of DCM. Next, Ethylamine (500 µL, 70% in H_2O, 5.86 mmol) was added, and an exotherm was observed. The reaction was stirred at

ambient temperature for 1 h, followed by washing 3 X with H_2O and brine, then dried over $MgSO_4$. The material was filtered and concentrated followed by purification on a Combiflash Rf system (Hexanes/EtOAc, 0-100% EtOAc) to afford **10-i** as a white solid (278.8 mg, 49%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.85 (t, *J* = 5.5 Hz, 1H), 8.67 (t, *J* = 2.0 Hz, 1H), 8.42 – 8.34 (m, 1H), 8.28 (dt, *J* = 7.7, 1.3 Hz, 1H), 7.77 (t, *J* = 8.0 Hz, 1H), 3.32 (qd, *J* = 7.2, 5.5 Hz, 2H), 1.15 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.60, 147.64, 135.89, 133.46, 129.95, 125.57, 121.72, 34.21, 14.47. HRMS (ESI-TOF) calculated for $C_9H_{10}N_2O_3H^+$ $[M+H]^+$: 195.0725, observed 195.0763.



10-i **10-ii**

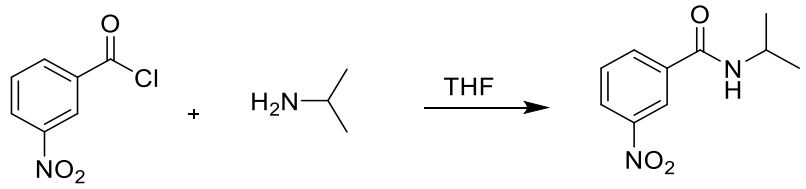
3-Nitroethylbenzamide(**10-i**) (230 mg, 1.19 mmol) was dissolved in 3 mL of EtOH. Iron powder (331 mg, 5.95 mmol) and acetic acid (677 µL, 5.95 mmol) were added, and the solution was stirred at ambient temperature for 3 h. The mixture was filtered, and the filtrate was diluted with EtOAc, followed by washing with 10% $NaHCO_3$. The organic layer was washed with brine and dried over $MgSO_4$. Following filtration and concentration **10-ii** was obtained as a brown oil (150 mg, 77%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.05 (t, *J* = 7.8 Hz, 1H), 7.00 (t, *J* = 2.0 Hz, 1H), 6.94 – 6.90 (m, 1H), 6.66 (dd, *J* = 7.9, 2.3 Hz, 1H), 5.21 (s, 1H), 3.23 (qd, *J* = 7.2, 5.5 Hz, 2H), 1.08 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.56, 148.48, 135.58, 128.42, 116.07, 114.05, 112.68, 33.75, 14.74. HRMS (ESI-TOF) calculated for $C_9H_{12}N_2OH^+$ $[M+H]^+$: 165.0983, observed 165.1017.



10-ii **(*S*)-ii** **10**

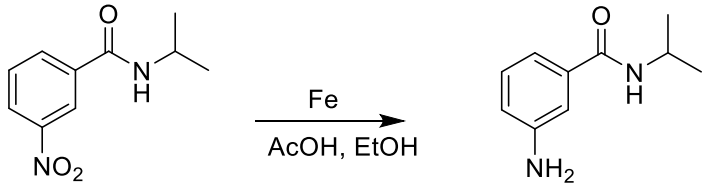
3-Aminoethylbenzamide(**10-ii**) (26.0 mg, 0.159 mmol) and triethylamine (33 µL, 0.236 mmol) were dissolved in 1 mL of THF. Separately, 4-nitrophenylchloroformate (47.6 mg, 0.236 mmol,) was dissolved in 2 mL of THF. The solutions were cooled to 0 °C, and the aniline solution was added dropwise to the chloroformate solution. The mixture was stirred at ambient temperature for 2h. **(*S*)-ii** was then added, and the reaction was stirred for 18 h. The solvent was removed, and it was diluted with EtOAc, followed by washing 3 X with H_2O. The organic layer was washed with brine and dried over $MgSO_4$. The material was filtered and concentrated followed by purification on a Combiflash Rf system (DCM/MeOH, 0-10% MeOH) to afford **10** (16.3 mg, 22%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.06 (s, 1H), 8.46 (s, 1H), 8.36 (t, *J* = 5.6 Hz, 1H), 7.93 (d, *J* = 5.8 Hz, 1H), 7.83 – 7.75 (m, 3H), 7.53 (dt, *J* = 7.9, 1.5 Hz, 1H), 7.36 – 7.32 (m, 1H),

7.28 (t, *J* = 7.8 Hz, 1H), 7.06 (t, *J* = 8.9 Hz, 2H), 6.58 (d, *J* = 6.8 Hz, 1H), 5.98 (d, *J* = 5.9 Hz, 1H), 4.33 (s, 1H), 3.53 (d, *J* = 95.3 Hz, 3H), 3.29 – 3.22 (m, 2H), 2.21 (s, 1H), 1.95 (s, 1H), 1.10 (t, *J* = 7.2 Hz, 3H).^{13}C NMR (126 MHz, DMSO-d_6) δ 165.96, 160.09, 158.30 (d, *J* = 238.2 Hz), 155.55, 155.47, 154.69, 140.13, 137.66, 135.37, 128.36, 120.04, 119.62 (d, *J* = 7.3 Hz), 119.55, 116.76, 114.59 (d, *J* = 21.8 Hz), 95.55, 51.87, 44.17, 33.88, 14.69. HRMS (ESI-TOF) calculated for $C_{24}H_{26}FN_7O_2H^+$ [M+H]$^+$: 464.2166, observed 464.2202.



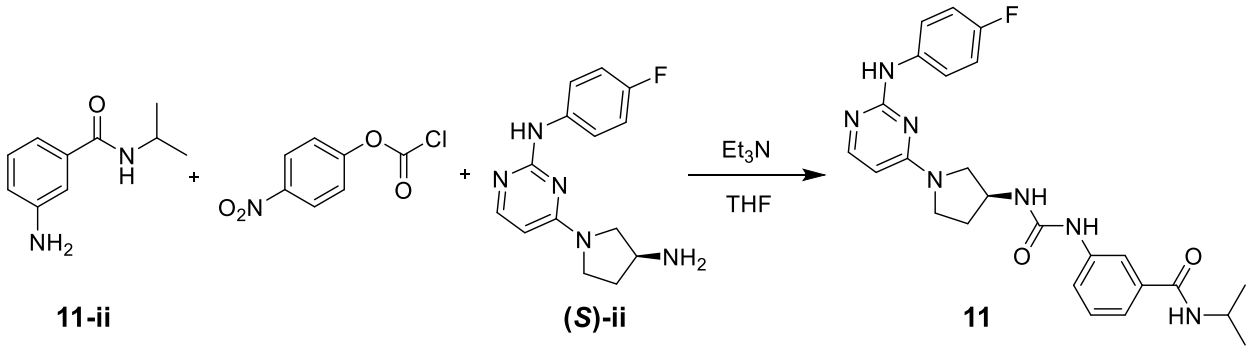
11-i

3-Nitrobenzoyl chloride (289.6 mg, 1.561 mmol) was dissolved in 2 mL of THF followed by dropwise addition of isopropylamine (667 µL, 7.797 mmol). The reaction was stirred at ambient temperature for 1.5 h. The mixture was diluted with EtOAc and washed 3 X with H_2O followed by brine and dried over $MgSO_4$. The material was filtered and the solvent was removed under reduced pressure to afford **11-i** as a white solid (65.6 mg, 0.315 mmol, 20%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.55 (t, *J* = 1.9 Hz, 1H), 8.36 – 8.31 (m, 1H), 8.15 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.63 (t, *J* = 8.0 Hz, 1H), 6.14 (s, 1H), 4.31 (dq, *J* = 13.5, 6.7 Hz, 1H), 1.30 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 164.20, 148.09, 136.52, 133.25, 129.79, 125.86, 121.54, 42.46, 22.71. HRMS (ESI-TOF) calculated for $C_{10}H_{12}N_2O_3Na^+$ [M+Na]$^+$: 231.0746, observed 231.0745.



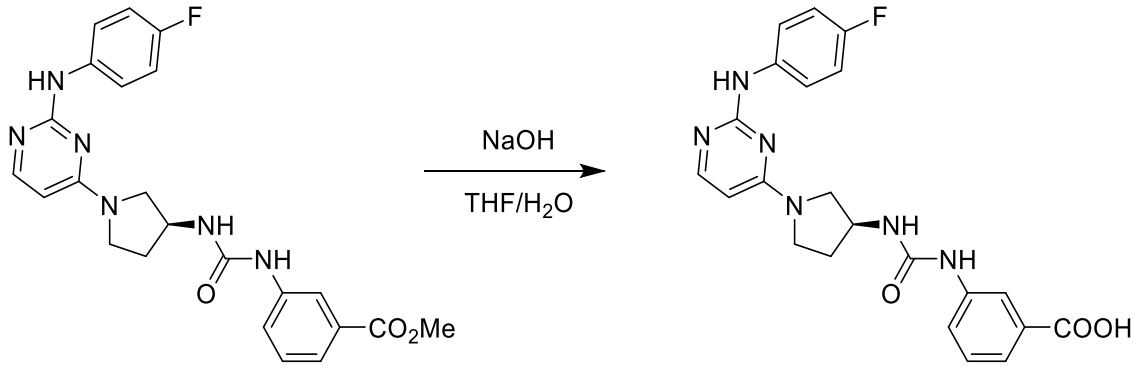
11-i **11-ii**

11-i (322 mg, 1.55 mmol) was dissolved in 3 mL of EtOH. Iron powder (743 mg, 7.75 mmol), and acetic acid (1.54 mL, 15.5 mmol) were added and the reaction was stirred at ambient temperature for 2 h. After completion, the solution was filtered, and the filtrate was diluted with EtOAc. The organic layer was washed 3 X with 10% $NaHCO_3$, followed by brine and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure to afford **11-ii** as a white solid (80 mg, 29%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.95 (d, *J* = 7.9 Hz, 1H), 7.05 (t, *J* = 7.8 Hz, 1H), 7.00 (t, *J* = 2.0 Hz, 1H), 6.97 – 6.90 (m, 1H), 6.66 (dd, *J* = 7.9, 2.3 Hz, 1H), 4.14 – 3.99 (m, 1H), 1.13 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.15, 148.52, 135.89, 128.44, 116.11, 114.36, 112.85, 40.70, 22.33. HRMS (ESI-TOF) calculated for $C_{10}H_{14}N_2OH^+$ [M+H]$^+$: 179.1140, observed 179.1177.



11-ii **(*S*)-ii** **11**

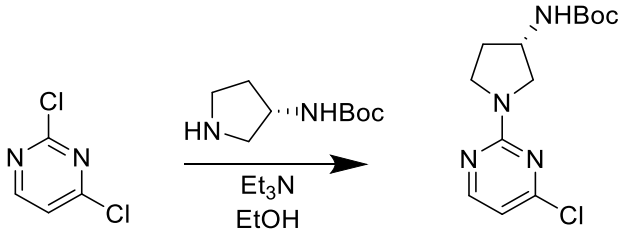
4-Nitrophenylchloroformate (96 mg, 0.48 mmol) was dissolved in 2 mL of anhydrous THF and cooled to 0 °C. Separately, **11-ii** (50 mg, 0.48 mmol) and triethylamine (66 µL, 0.48 mmol) were dissolved in 1 mL of anhydrous THF. The second solution was added dropwise to the first, and the reaction was warmed to ambient temperature for 15 minutes. The mixture was diluted with EtOAc and washed 3 X with H_2O. The organic fraction was washed with brine and dried over $MgSO_4$ followed by filtration. **(*S*)-ii** (76 mg, 0.28 mmol) and triethylamine (100 µL, 0.718 mmol) were added to the EtOAc fraction, and the reaction was stirred at 50 °C temperature for 1 h. The organic layer was washed 3 X with 1M NaOH, followed by brine and $MgSO_4$. Following filtration and concentration, the material was purified via column chromatography (9:1 EtOAc/MeOH) to isolate **11** as a white solid (30 mg, .063 mmol, 23%, 2 steps). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.08 (s, 1H), 8.47 (s, 1H), 8.12 (d, *J* = 7.8 Hz, 1H), 7.93 (d, *J* = 7.5 Hz, 1H), 7.79 (t, *J* = 6.9 Hz, 2H), 7.75 (t, *J* = 1.9 Hz, 1H), 7.54 (dd, *J* = 8.1, 2.1 Hz, 1H), 7.35 (dt, *J* = 7.7, 1.3 Hz, 1H), 7.28 (t, *J* = 7.8 Hz, 1H), 7.06 (t, *J* = 8.9 Hz, 2H), 6.58 (d, *J* = 6.8 Hz, 1H), 5.99 (d, *J* = 5.9 Hz, 1H), 4.33 (s, 1H), 4.13 – 4.01 (m, *J* = 6.7 Hz, 1H), 3.80 – 3.14 (m, 4H), 2.21 (s, 1H), 1.95 (s, 1H), 1.14 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.44, 160.07, 159.08, 156.46 (d, *J* = 237.1 Hz), 155.29, 154.70, 140.05, 137.57, 135.57, 128.27, 119.99, 119.70 (d, *J* = 6.9 Hz), 116.88, 114.61 (d, *J* = 21.9 Hz), 95.57, 51.90, 44.19, 40.78, 22.20. HRMS (ESI-TOF) calculated for $C_{25}H_{28}FN_7O_2H^+$ $[M+H]^+$: 478.2322, observed 478.2357.



(*S*)-1 **12**

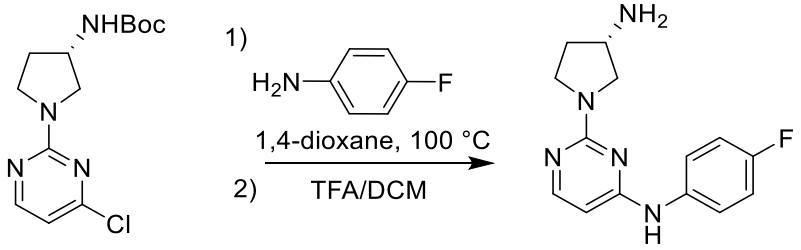
(*S*)-1 (101.6 mg, 0.372 mmol) was suspended in 5 mL of THF. Next, 1 mL of 1M NaOH was added, and the solution became clear. The reaction proceeded for 18 h, the solvent was removed under a stream of nitrogen and the crude material was dissolved in EtOAc and MeOH. The organic layers were washed 3 X

with 1M HCl followed by brine and dried over $MgSO_4$. The material was filtered and concentrated followed by purification on a Combiflash Rf system (EtOAc/MeOH, 0-30% MeOH) to afford **12** as a white solid (12.9 mg, 13%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.73 (s, 1H), 10.57 (m, 1H), 9.69 (m, 1H), 8.00 (s, 2H), 7.75 – 7.64 (m, 3H), 7.61 (d, *J* = 8.2 Hz, 1H), 7.44 (d, *J* = 7.5 Hz, 1H), 7.30 (t, *J* = 7.9 Hz, 1H), 7.17 (t, *J* = 8.7 Hz, 2H), 6.25 (d, *J* = 6.8 Hz, 1H), 4.40 – 4.25 (m, 1H), 3.81 – 3.30 (m, 5H), 2.29 – 2.14 (m, 1H), 2.00 – 1.84 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.36, 159.61, 157.99 (d, *J* = 239.8 Hz), 155.26, 140.83, 134.89, 131.34, 128.84, 121.80, 121.62 (d, *J* = 7.8 Hz), 121.39, 118.00, 115.45 (d, *J* = 22.3 Hz), 96.57, 53.07, 49.07, 48.35, 45.66, 45.46, 31.12, 30.18. HRMS (ESI-TOF) calculated for $C_{22}H_{21}FN_6O_3H^+$ $[M+H]^+$: 437.1693, observed 437.1727



13-i

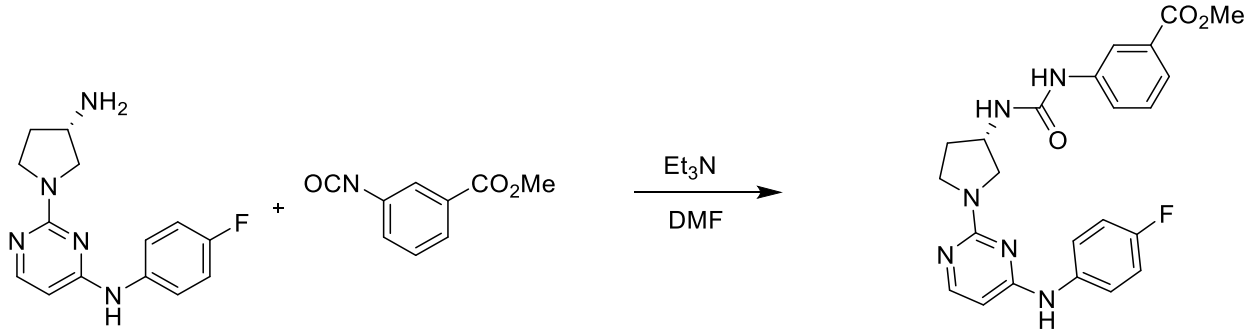
Following the synthetic protocol to form **(*S*)-i**, **13-i** can be isolated as an approximately 20% minor product, observed to be less polar than **(*S*)-i** (R_f of **13-i** = 0.4, 2:1 hexanes/EtOAc). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.28 (d, *J* = 5.1 Hz, 1H), 7.22 (d, *J* = 6.5 Hz, 1H), 6.69 (d, *J* = 5.1 Hz, 1H), 4.07 (p, *J* = 6.0 Hz, 1H), 3.67 – 3.60 (m, 1H), 3.60 – 3.50 (m, 1H), 3.46 (q, *J* = 9.0 Hz, 1H), 3.35 – 3.26 (m, 2H), 2.11 (ddd, *J* = 14.3, 12.8, 6.9 Hz, 1H), 1.86 (dq, *J* = 12.8, 6.3 Hz, 1H), 1.39 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.62, 108.18, 77.77, 51.89, 49.59, 44.72, 44.60, 30.34, 28.10. HRMS (ESI-TOF) calculated for $C_{13}H_{19}ClN_4O_2Na^+$ $[M+Na]^+$: 321.1094, observed 321.1086.



13-i **13-ii**

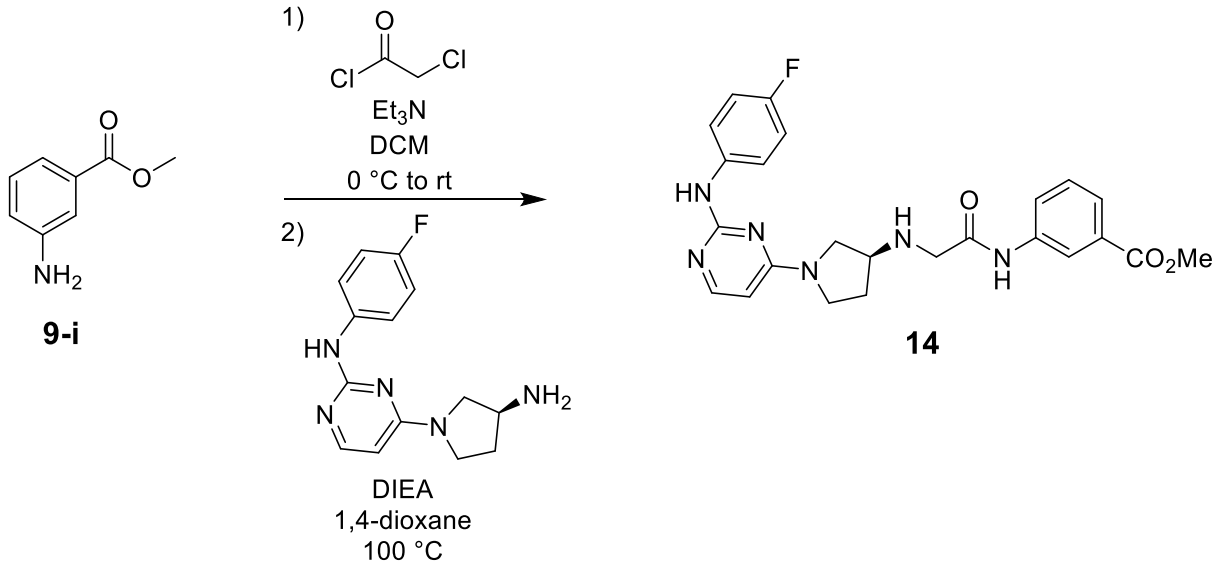
13-i (100.8 mg, 0.337 mmol) was dissolved in 3 mL of 1,4-dioxane, to which 4-fluoroaniline (48 µL, 0.506 mmol) was added. The solution was heated to reflux for 18 h, followed by removal of solvent. When cooled, the material was subjected to Et_2O and the precipitate was collected via vacuum filtration and washed with subsequent portions of Et_2O. The dried solid was then dissolved in 3 mL of TFA and 1 mL of DCM and stirred for 1h. The solvent was removed, and the crude mixture was dissolved in H_2O and DCM. The aqueous layer was washed 3 X with DCM and separated. The aqueous layer was basified to pH > 10 using 1M NaOH, and then extracted 3 X with EtOAc. The combined organic layers were washed with brine and dried over $MgSO_4$. The material was filtered followed by concentration to isolate **13-ii** as a white solid (17.7 mg, 2 steps, 19%) ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.20 (s, 1H), 7.88 (d, *J* = 5.6 Hz, 1H), 7.76 (dd, *J* = 9.0, 5.0 Hz, 2H), 7.11 (t, *J* = 8.7 Hz, 2H), 5.96 (d, *J* = 5.7 Hz, 1H), 3.67 – 3.55 (m, 2H), 3.55 (s,

1H), 3.50 – 3.41 (m, 1H), 3.19 – 3.12 (m, 1H), 2.02 (dq, *J* = 13.7, 6.9 Hz, 1H), 1.68 (dq, *J* = 12.7, 6.3 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.97, 158.74 (d, *J* = 241.0 Hz), 155.86, 137.02 (d, *J* = 2.3 Hz), 120.41 (d, *J* = 7.3 Hz), 114.93 (d, *J* = 21.9 Hz), 95.50, 54.40, 50.54, 44.61, 33.73, 30.31. HRMS (ESI-TOF) calculated for $C_{14}H_{16}FN_5H^+$: $[M+H]^+$: 274.1423, observed 274.1444.



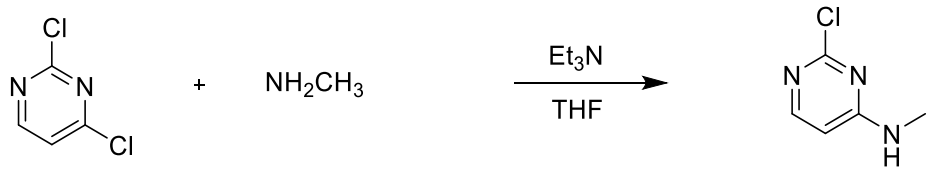
13-ii **13**

13-ii (135 mg, 0.494 mmol) was dissolved in 3 mL of DMF, followed by triethylamine (0.740 mmol). Methyl 3-isocyanatobenzoate (131 mg, 0.740 mmol) was added and the reaction was stirred for 3 h. The solvent was removed and the reaction was purified via flash chromatography (9:1 EtOAc/MeOH) to afford **13** as a yellow solid (98.9 mg, 44%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.41 (s, 1H), 8.78 (s, 1H), 8.12 (t, *J* = 1.9 Hz, 1H), 7.90 (d, *J* = 5.7 Hz, 1H), 7.80 – 7.71 (m, 2H), 7.56 (dd, *J* = 8.1, 2.1 Hz, 1H), 7.49 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.36 (t, *J* = 7.9 Hz, 1H), 7.13 (t, *J* = 8.7 Hz, 2H), 6.75 (d, *J* = 6.8 Hz, 1H), 6.04 (d, *J* = 5.8 Hz, 1H), 4.30 (q, *J* = 6.0, 5.5 Hz, 1H), 3.83 (s, 3H), 3.70 (dd, *J* = 11.8, 6.0 Hz, 1H), 3.64 – 3.53 (m, 2H), 3.43 (dd, *J* = 11.3, 3.9 Hz, 1H), 2.19 (dq, *J* = 13.6, 7.1 Hz, 1H), 1.92 (dq, *J* = 12.0, 5.8 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.75, 160.60, 159.64, 157.62 (d, *J* = 238.3 Hz), 155.28, 141.22, 137.27, 130.52, 129.51, 122.56, 122.23, 121.26 (d, *J* = 7.5 Hz), 118.44, 115.60 (d, *J* = 22.1 Hz), 96.65, 52.68, 52.56, 49.58, 44.99, 31.63. HRMS (ESI-TOF) calculated for $C_{23}H_{23}FN_6O_3H^+$ $[M+H]^+$: 451.1849, observed 451.1900.



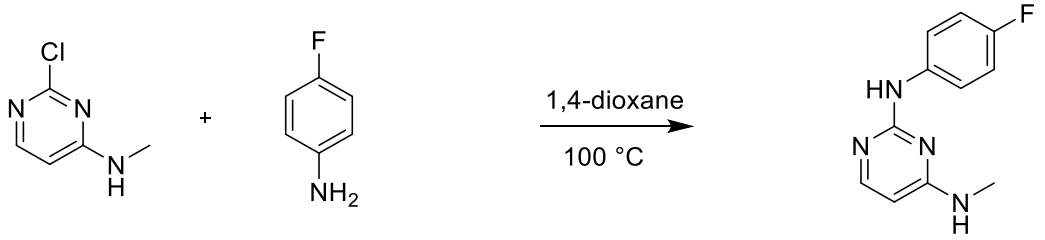
9i (100 mg, 0.66 mmol) was dissolved in 2.5 mL of DCM and cooled to 0 °C followed by addition of triethylamine (101 µL, 0.73 mmol). Separately, chloroacetyl chloride (58 µL, 0.73 mmol) was dissolved in

2 mL of DCM and was added slowly to the first solution. The mixture was warmed to ambient temperature and stirred for 1 h. Upon completion, the reaction was washed 3 X with H_2O, followed by brine and dried over $MgSO_4$. Upon filtration and concentration, and orange solid was isolated and used without further purification. The crude product was dissolved in 3 mL of 1,4-dioxane, followed by addition of N,N-diisopropylethylamine (0.99 mmol) and **(*S*)-ii** (216 mg, 0.79 mmol) and heated to reflux for 18 h. The solvent was removed under reduced pressure, and the product was purified on a Combiflash RF system (9:1 EtOAc/MeOH) to isolate **14** as a brown solid (80 mg, 0.172 mmol, 26%, 2 steps). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.05 (s, 1H), 9.00 (s, 1H), 8.29 (t, *J* = 2.0 Hz, 1H), 7.88 (d, *J* = 5.8 Hz, 1H), 7.86 – 7.81 (m, 1H), 7.81 – 7.74 (m, 3H), 7.63 (d, *J* = 7.9 Hz, 1H), 7.43 (t, *J* = 7.9 Hz, 1H), 7.04 (t, *J* = 8.9 Hz, 2H), 6.20 – 5.85 (m, 1H), 3.84 (s, 3H), 3.46-3.19 (m, 4H), 2.09 (s, 1H), 1.98 – 1.84 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.49, 165.93, 159.99, 158.26 (d, *J* = 239.2 Hz), 155.42, 155.35, 138.88, 137.72, 129.97, 129.05, 123.79, 123.54, 119.61, 119.55, 114.54 (d, *J* = 21.8 Hz), 95.40, 52.06, 51.46, 50.83, 44.43. HRMS (ESI-TOF) calculated for $C_{24}H_{25}FN_6O_3H^+$ $[M+H]^+$: 465.2006, observed 465.2041.



F-i

2,4-Dichloropyrimidine (500.2 mg, 3.36 mmol) was dissolved in 10 mL of THF, followed by addition of triethylamine (702 µL, 5.04 mmol). Methylamine (2 mL of a solution 2M in THF, 4.03 mmol) was added dropwise yielding a turbid mixture. The reaction was stirred at ambient temperature for 18 h, followed by removal of solvent. The crude material was redissolved in EtOAc and washed 3 X with H_2O and brine, and dried over $MgSO_4$. The material was filtered and the solvent was removed followed by purification on a Combiflash Rf system (Hexanes/EtOAc, 0-100% EtOAc) to afford **F-i** as a white solid (172.1 mg, 36%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.86 (d, *J* = 5.9 Hz, 1H), 6.42 (d, *J* = 5.9 Hz, 1H), 2.78 (d, *J* = 4.7 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.78, 157.68, 154.97, 104.93, 26.82. HRMS (ESI-TOF) calculated for $C_5H_6ClN_3H^+$ $[M+H]^+$: 144.0329, observed 144.0322.



F-i **F1**

F-i (67.1 mg, 0.467 mmol) was dissolved in 3 mL of 1,4-dioxane followed by addition of 4-fluoroaniline (66.4 µL, 0.701 mmol). The solution was heated to reflux for 12 h, followed by removal of solvent. The

crude material was dissolved in EtOAc and washed with 1 M NaOH followed by brine and dried over $MgSO_4$. Following filtration, the solvent was removed and the material was purified on a Combiflash Rf system (Hexanes/EtOAc, 0-100% EtOAc) to afford **F1** as a tan solid (77.6 mg, 76%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.97 (s, 1H), 7.83 – 7.74 (m, 2H), 7.10 (s, 1H), 7.04 (t, *J* = 8.9 Hz, 2H), 5.91 (d, *J* = 5.7 Hz, 1H), 2.88 – 2.74 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.97, 159.59, 156.37 (d, *J* = 236.6 Hz), 137.77, 137.76, 119.66 (d, *J* = 7.3 Hz), 114.52 (d, *J* = 21.8 Hz), 26.97. HRMS (ESI-TOF) calculated for $C_{11}H_{11}FN_4H^+$ $[M+H]^+$: 219.1001, observed 219.1038.

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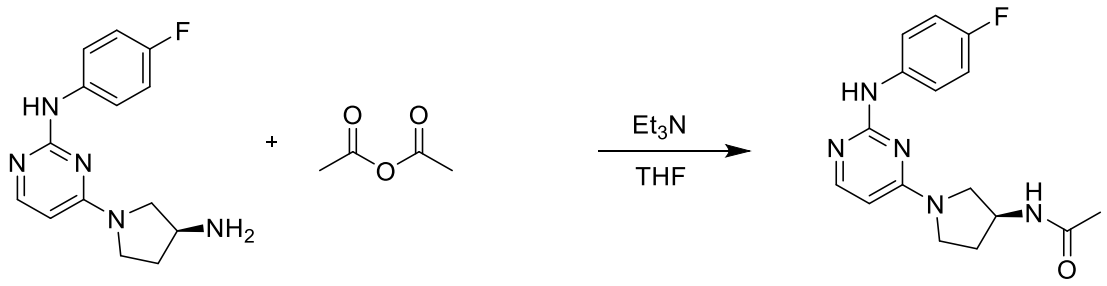
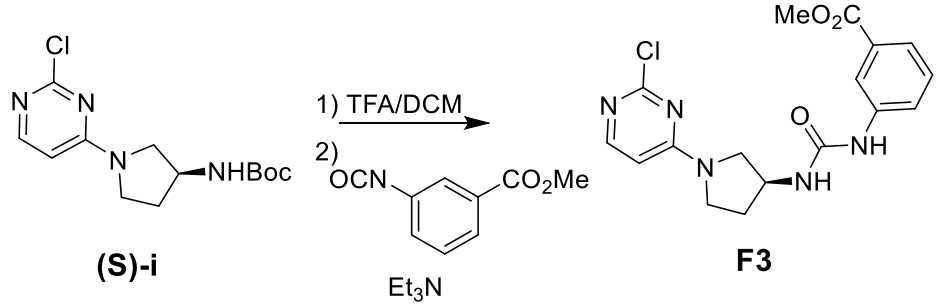


(*S*)-ii **F2**

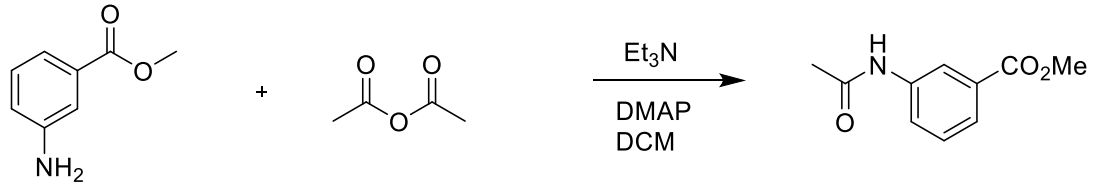
(*S*)-ii (30 mg, 0.11 mmol) was dissolved in 2 mL of THF, followed by addition of triethylamine (17 µL, 0.12 mmol) and acetic anhydride (12 µL, 0.12 mmol) and the solution was stirred at ambient temperature for 18 h. The solvent was removed, and the crude material was redissolved in DCM and washed with 0.1 M HCl, followed by brine and dried over $MgSO_4$. Following filtration, the solvent was removed under reduced pressure to afford **F2** as a white solid (31 mg, 90%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.40 (s, 1H), 8.17 (d, *J* = 6.7 Hz, 1H), 7.91 (d, *J* = 6.2 Hz, 1H), 7.73 (dd, *J* = 9.0, 4.9 Hz, 2H), 7.11 (t, *J* = 8.9 Hz, 2H), 6.05 (d, *J* = 6.4 Hz, 1H), 4.34 (s, 1H), 3.63 (s, 2H), 3.46-3.15 (m, 2H), 2.16 (d, *J* = 17.7 Hz, 1H), 1.94 – 1.86 (m, 1H), 1.81 (s, 3H). ^{13}C NMR, broadening prevented detection of some ^{13}C resonances (126 MHz, DMSO-d_6) δ 169.05, 159.78, 157.05 (d, *J* = 237.5 Hz), 136.48, 120.65 (d, *J* = 7.6 Hz), 114.86 (d, *J* = 21.9 Hz), 95.94, 51.71, 44.60, 22.47. HRMS (ESI-TOF) calculated for $C_{16}H_{18}FN_5OH^+$ $[M+H]^+$: 316.1529, observed 316.1565.



(S)-i **F3**

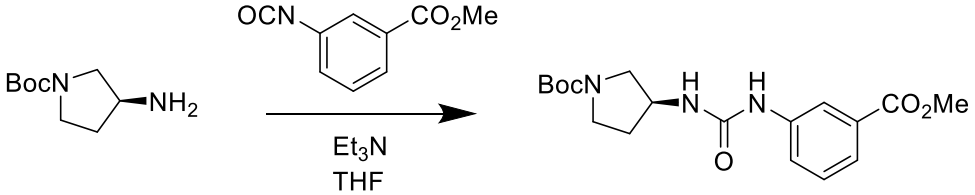
448 mg (1.49 mmol) of **(*S*)-i** was stirred in 3 mL of TFA in 1 mL of DCM for 1h. The TFA was removed and the solution was neutralized with 2 mL of triethylamine in 15 mL of DCM. 532 mg (3.00 mmol) of methyl 3-isocyanobenzoate was added and stirred for 12 h. The reaction mixture was diluted with 10 mL of water and extracted 3 X with 15 mL of EtOAc. The solvent was removed and **F3** was isolated with silica chromatography (hexanes:ethyl acetate), 77% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.62 (d, *J* = 9.9 Hz, 1H), 8.25 (s, 1H), 8.14 (s, 1H), 8.12 – 8.01 (m, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.46 (t, *J* = 7.9 Hz, 1H), 6.56 (s, 1H), 4.87 (d, *J* = 62.8 Hz, 1H), 3.85 (s, 3H), 3.81 – 3.72 (m, 1H), 3.66 – 3.46 (m, 3H),

2.39 – 2.22 (m, 1H), 2.19 – 2.02 (m, 1H). ^{13}C NMR (126 MHz, DMSO) δ 181.13, 166.45, 161.28, 159.90, 156.94, 140.49, 130.23, 129.35, 124.95, 103.71, 103.52, 52.69, 52.09, 45.26, 30.70, 30.21. HRMS (ESI-TOF) calculated for $C_{17}H_{18}ClN_5O_3H^+$ [M+H]$^+$: 376.1171, observed 376.1168.



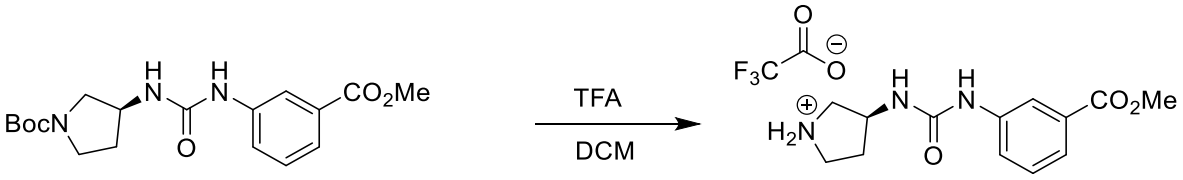
9-i **F4**

9-i (94.8 mg, 0.627 mmol) was dissolved in 3 mL of DCM. Acetic anhydride (88.7 µL, 0.941 mmol, 1), triethylamine (131.2 µL, 0.941 mmol), and N,N-dimethylaminopyridine (5.5 mg, 0.045 mmol) were added. The reaction was stirred at ambient temperature for 18 h. Upon completion, the solution was added to a separatory funnel and washed with 1 M HCl, followed by brine and dried over $MgSO_4$. The material filtered, concentrated, and then was purified on a Combiflash Rf system (Hexanes/EtOAc, 0-100% EtOAc) to afford **F4** as a white solid (39.8 mg, 33%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.15 (s, 1H), 8.25 (t, *J* = 1.9 Hz, 1H), 7.85 – 7.80 (m, 1H), 7.62 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.44 (t, *J* = 7.9 Hz, 1H), 3.85 (s, 3H), 2.06 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.48, 165.99, 139.55, 129.93, 129.05, 123.47, 123.26, 119.28, 52.06, 23.90. HRMS (ESI-TOF) calculated for $C_{10}H_{11}NO_3H^+$ [M+H]$^+$: 194.0772, observed 194.0810.



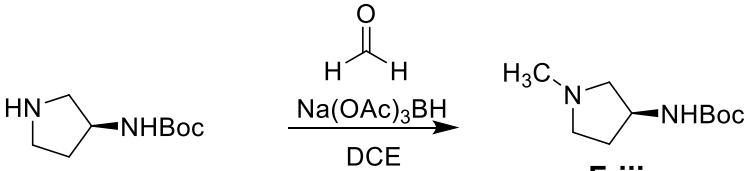
F-ii

(*S*)-1-boc-3-aminopyrrolidine (100 mg, 0.54 mmol) was dissolved in 3 mL of THF, followed by addition of triethylamine (90 µL, 0.65 mmol) and methyl 3-isocyanatobenzoate (114 mg, 0.65 mmol). The reaction was stirred at ambient temperature for 18 h, followed by removal of solvent and purification on a combiflash Rf system (hexanes/EtOAc, 0-100% EtOAc) to afford **F-ii** as a clear oil (96 mg, 49%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.59 (d, *J* = 3.3 Hz, 1H), 8.12 (s, 1H), 7.54 (dd, *J* = 8.1, 2.2 Hz, 1H), 7.52 – 7.47 (m, 1H), 7.36 (t, *J* = 7.9 Hz, 1H), 6.49 (t, *J* = 5.3 Hz, 1H), 4.14 (p, *J* = 5.6 Hz, 1H), 3.84 (s, 3H), 3.46 (ddd, *J* = 17.7, 10.8, 5.9 Hz, 1H), 3.30 (s, 1H: solvent obscuring second H), 3.09 (dd, *J* = 10.9, 4.3 Hz, 1H), 2.05 (tq, *J* = 13.3, 7.1, 5.2 Hz, 1H), 1.78 (dh, *J* = 12.7, 6.1 Hz, 1H), 1.40 (s, 9H). ^{13}C NMR, mixture of rotational isomers (126 MHz, DMSO-d_6) δ 166.16, 154.64, 153.45, 140.53, 129.95, 128.95, 122.09, 121.75, 117.97, 78.25, 51.99, 51.60, 51.15, 49.26, 48.48, 43.76, 43.54, 31.18, 30.21, 28.06. HRMS (ESI-TOF) calculated for $C_{18}H_{25}N_3O_5Na^+$ [M+Na]$^+$: 386.1692, observed 386.1683.



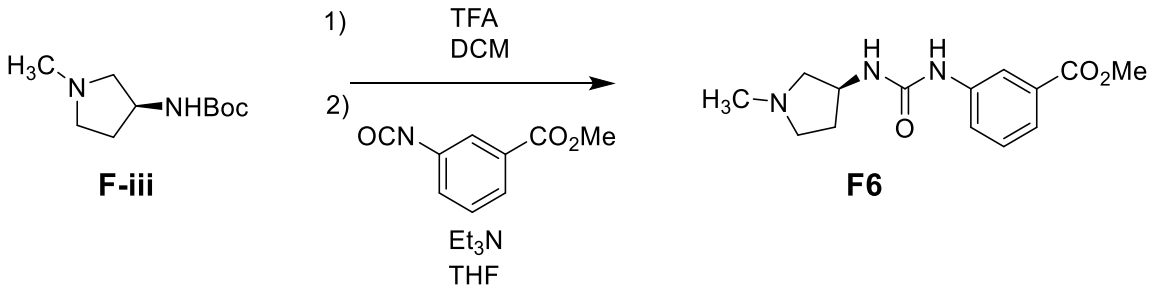
F-ii **F5**

F-ii (569.8 mg, 1.565 mmol) was dissolved in 3 mL of TFA and 1 mL of DCM. The reaction was stirred at ambient temperature for 1 h. After completion, the solvent was removed and the product was precipitated in diethyl ether. The solid was collected by filtration and was dried under vacuum to afford **F5** as a trifluoroacetate salt (503.7 mg, 85%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.31 (s, 1H), 9.03 – 8.86 (m, 2H), 8.18 (t, *J* = 2.0 Hz, 1H), 7.58 (dd, *J* = 8.1, 2.1 Hz, 1H), 7.50 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.37 (t, *J* = 7.9 Hz, 1H), 7.19 (d, *J* = 6.0 Hz, 1H), 4.26 (h, *J* = 6.1 Hz, 1H), 3.83 (s, 3H), 3.38 (dq, *J* = 11.9, 6.1 Hz, 1H), 3.31 (h, *J* = 5.9 Hz, 1H), 3.23 (hept, *J* = 6.1 Hz, 1H), 3.09 (dq, *J* = 11.2, 5.4 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.32, 155.08, 140.77, 130.07, 129.03, 122.33, 121.96, 118.22, 52.10, 49.79, 48.91, 43.83, 29.99. HRMS (ESI-TOF) calculated for $C_{13}H_{17}N_3O_3H^+$ [M+H]$^+$: 264.1303, observed 264.1341.



F-iii

(*S*)-3-(Boc-amino)pyrrolidine (312.7 mg, 1.679 mmol) was dissolved in 5 mL of 1,2-dichloroethane. Formaldehyde (185 µL of a 37% solution in H_2O/MeOH, 6.71 mmol) was added and the mixture was stirred at ambient temperature for 20 min. Sodium triacetoxyborohydride (725.0 mg, 3.421 mmol) was then added over 10 min and the mixture was stirred at ambient temperature for 18 h. The reaction was quenched with saturated NH_4Cl and diluted with H_2O at pH 5. The aqueous layer was washed 3 X with DCM and separated. The pH was adjusted to > 10 using 1M NaOH and extracted 3 X into DCM. The combined organic layers were washed with brine and dried over $MgSO_4$. After filtration and removal of solvent **F-iii** was isolated as a white solid (257.0 mg, 1.283 mmol, 77%). ^{1}H NMR (500 MHz, Methanol-d_4) δ 4.09 (q, *J* = 6.6, 5.8 Hz, 1H), 2.82 (dd, *J* = 10.0, 7.2 Hz, 1H), 2.67 (td, *J* = 8.8, 6.3 Hz, 1H), 2.54 (q, *J* = 8.0 Hz, 1H), 2.40 (dd, *J* = 10.1, 5.3 Hz, 1H), 2.36 (s, 3H), 2.24 (dtd, *J* = 14.1, 8.4, 6.2 Hz, 1H), 1.69 – 1.62 (m, 1H), 1.45 (s, 9H). ^{13}C NMR, mixture of rotational isomers (126 MHz, Chloroform-*d*) δ 155.40, 155.35, 79.08, 63.27, 63.25, 54.97, 50.35, 50.23, 41.89, 33.17, 33.13, 28.39. HRMS (ESI-TOF) calculated for $C_{10}H_{20}N_2O_2H^+$ [M+H]$^+$: 201.1558, observed 201.1626

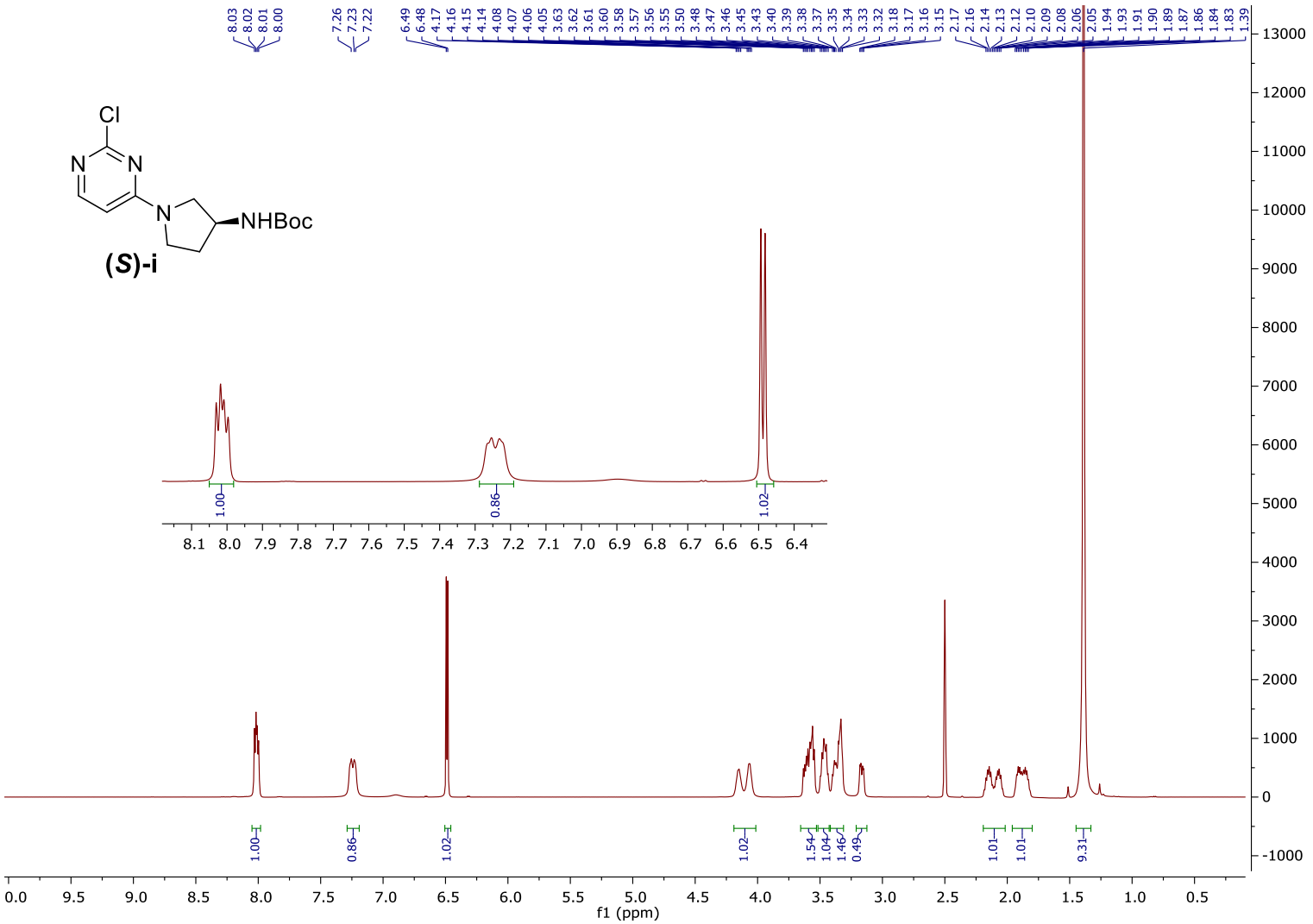


F-iii **F6**

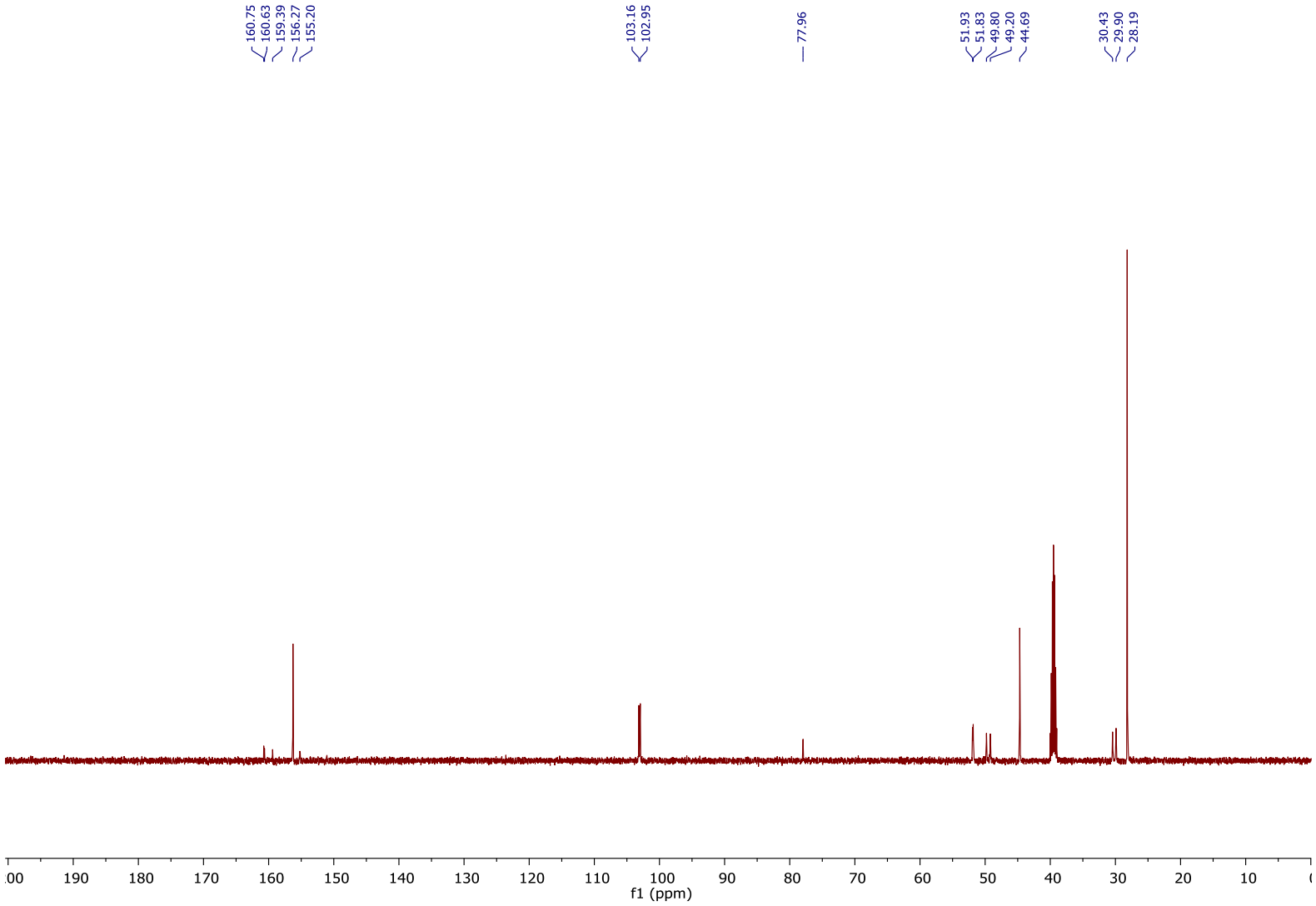
F-iii (257.0 mg, 1.283 mmol) was dissolved in 3 mL of TFA and 1 mL of DCM. The reaction was stirred at ambient temperature for 90 min and the solvent was removed. The crude material was then dissolved

in 3 mL of THF, and triethylamine (537 µL, 3.85 mmol) was added slowly and gas was observed. Methyl 3-isocyanatobenzoate (340 mg, 1.92 mmol) was then added and the reaction was stirred for 18 h. The mixture was then diluted with EtOAc and washed 3X with 10% Na_2CO_3 followed by brine and dried over $MgSO_4$. Following filtration, the solvent was removed and the material was purified on a Combiflash Rf system (EtOAc/MeOH, 0-10% MeOH) to afford **F6** as a white solid (177.9 mg, 0.6415 mmol, 50%, 2 steps). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.94 (d, *J* = 2.3 Hz, 1H), 7.65 (dd, *J* = 7.8, 1.7 Hz, 2H), 7.31 (t, *J* = 7.9 Hz, 1H), 6.34 (d, *J* = 7.8 Hz, 1H), 4.24 (s, 1H), 3.85 (s, 3H), 3.37 (s, 1H), 3.01 (t, *J* = 9.2 Hz, 1H), 2.85 (d, *J* = 10.3 Hz, 1H), 2.47 (dd, *J* = 10.2, 6.7 Hz, 1H), 2.39 (s, 3H), 2.32 (ddt, *J* = 12.4, 8.7, 4.4 Hz, 1H), 2.20 (q, *J* = 8.8 Hz, 1H), 1.86 – 1.75 (m, 1H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 167.04, 155.66, 139.84, 130.76, 129.04, 123.94, 123.67, 120.25, 62.94, 55.14, 52.12, 50.28, 41.59, 32.98. HRMS (ESI-TOF) calculated for $C_{14}H_{19}N_3O_3H^+$ $[M+H]^+$: 278.1460, observed 278.1495.

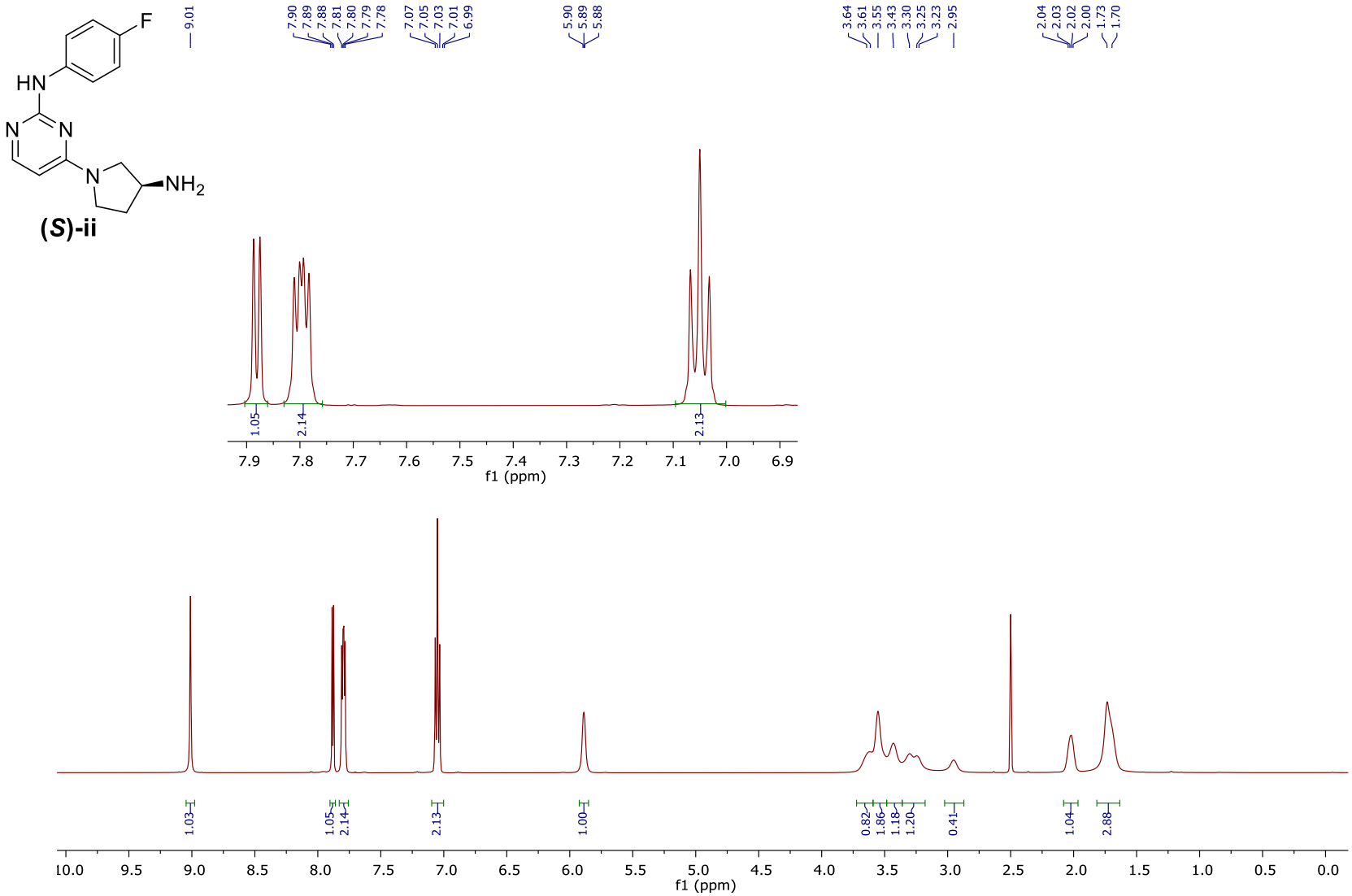
(*S*)-i, ^{1}H, 500 MHz, DMSO-d_6



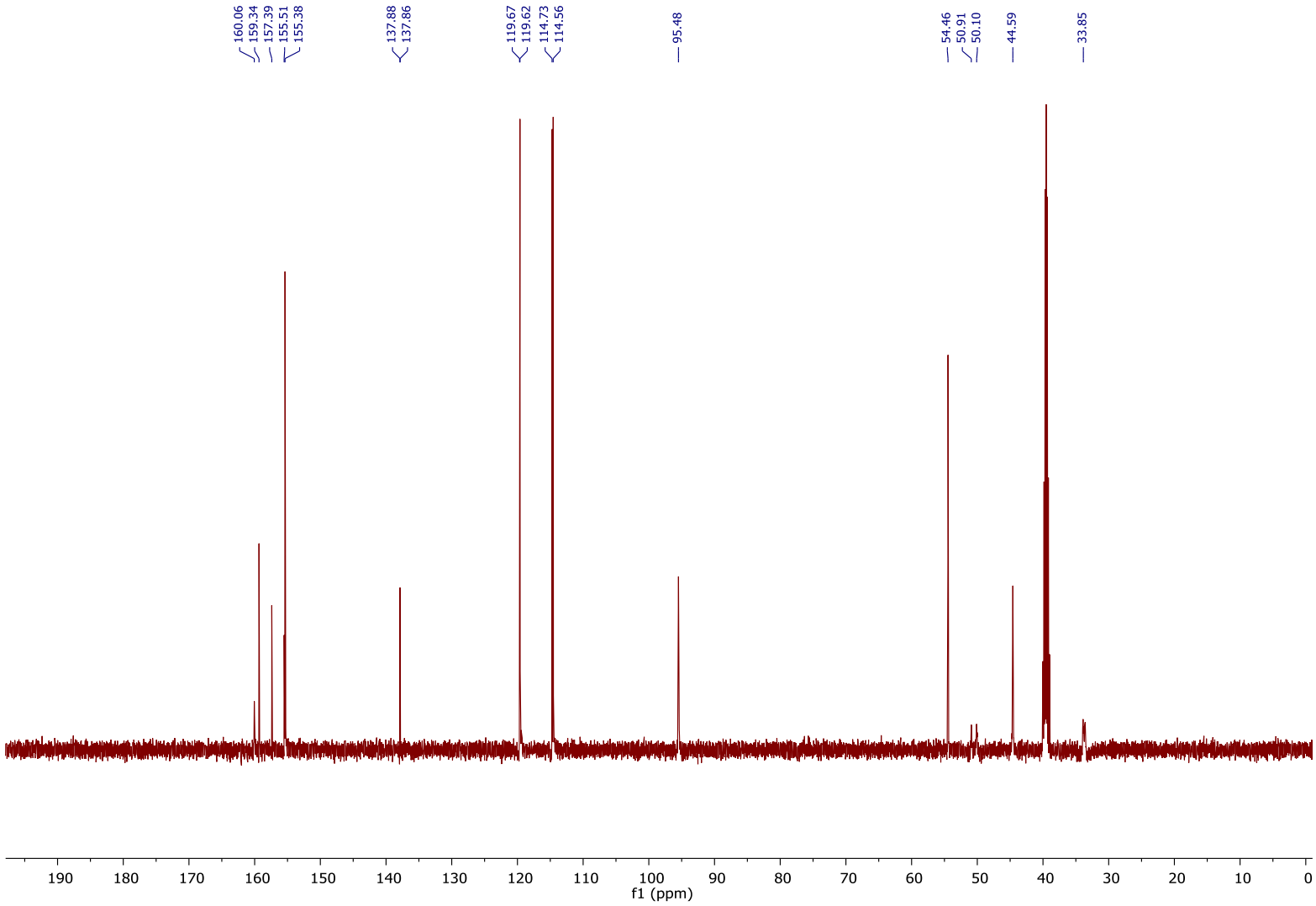
(*S*)-i, ^{13}C, 126 MHz, DMSO-d_6



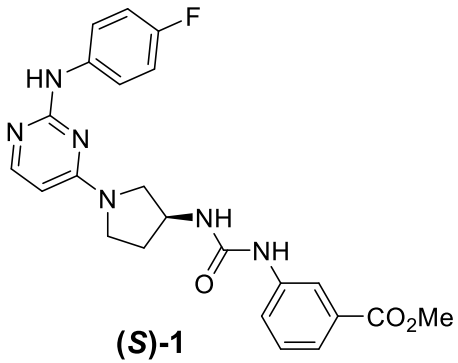
(*S*)-ii, ^{1}H, 500 MHz, DMSO-d_6



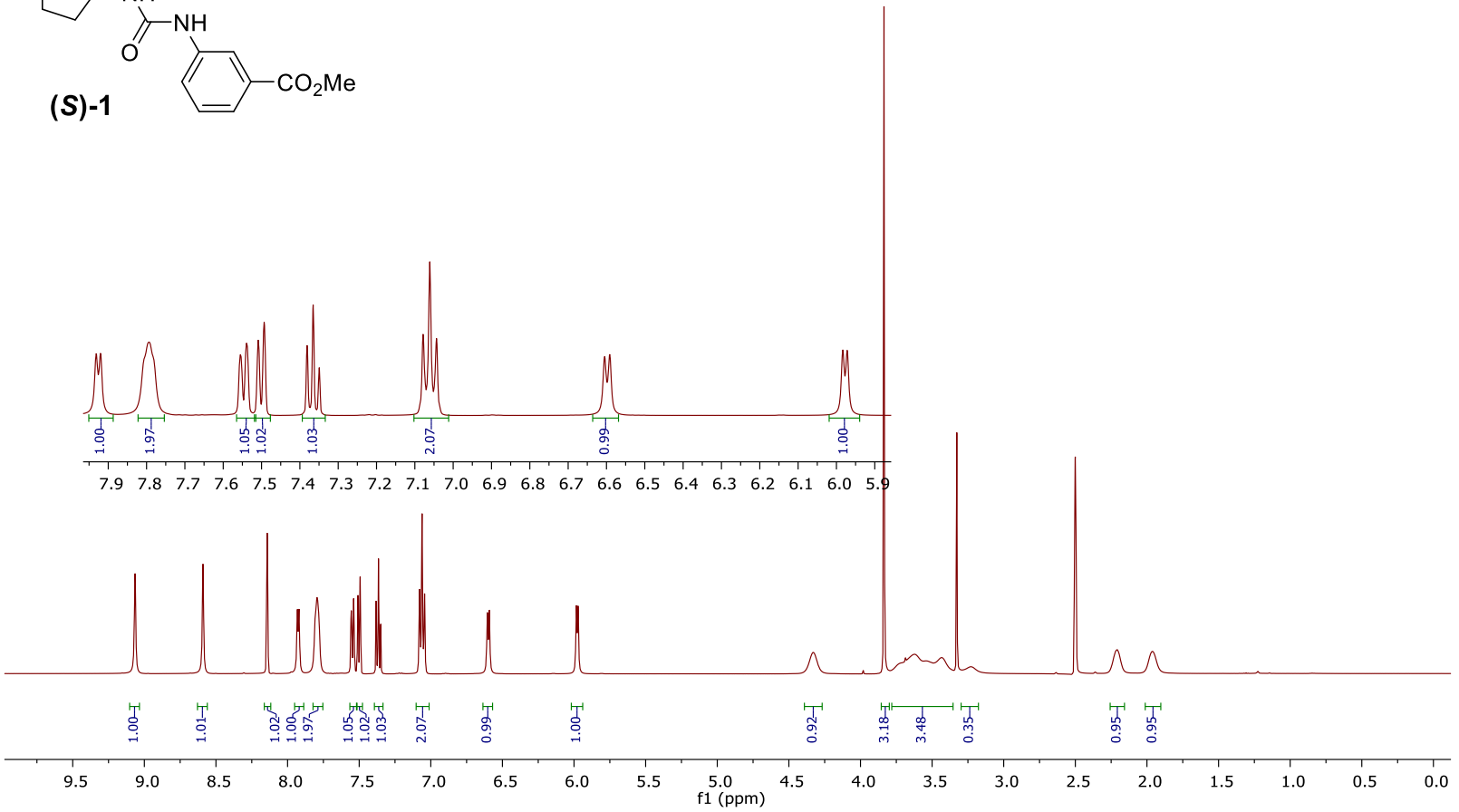
(*S*)-ii, ^{13}C, 126 MHz, DMSO-d_6



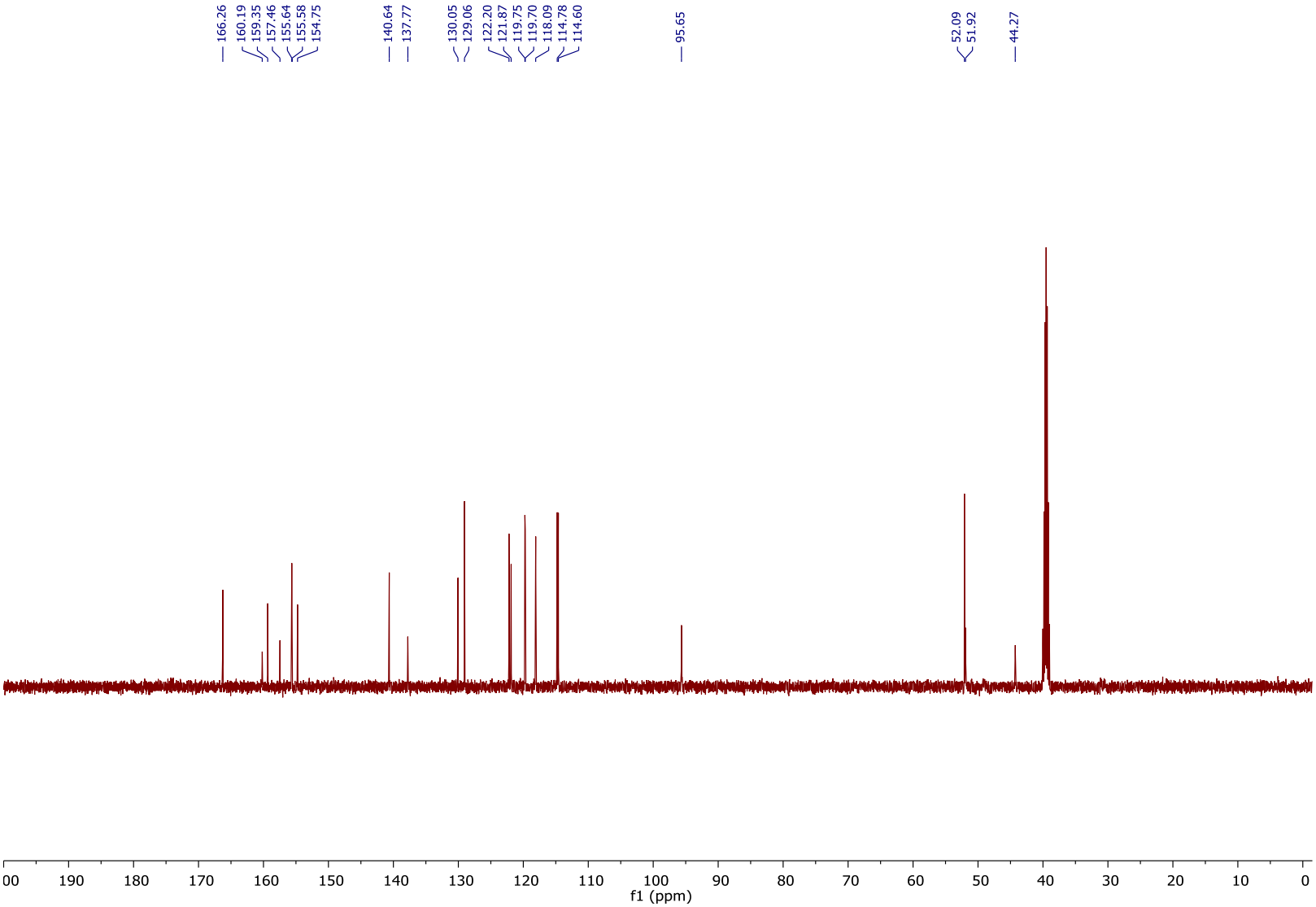
(*S*)-1, ^{1}H, 500 MHz, DMSO-d_6



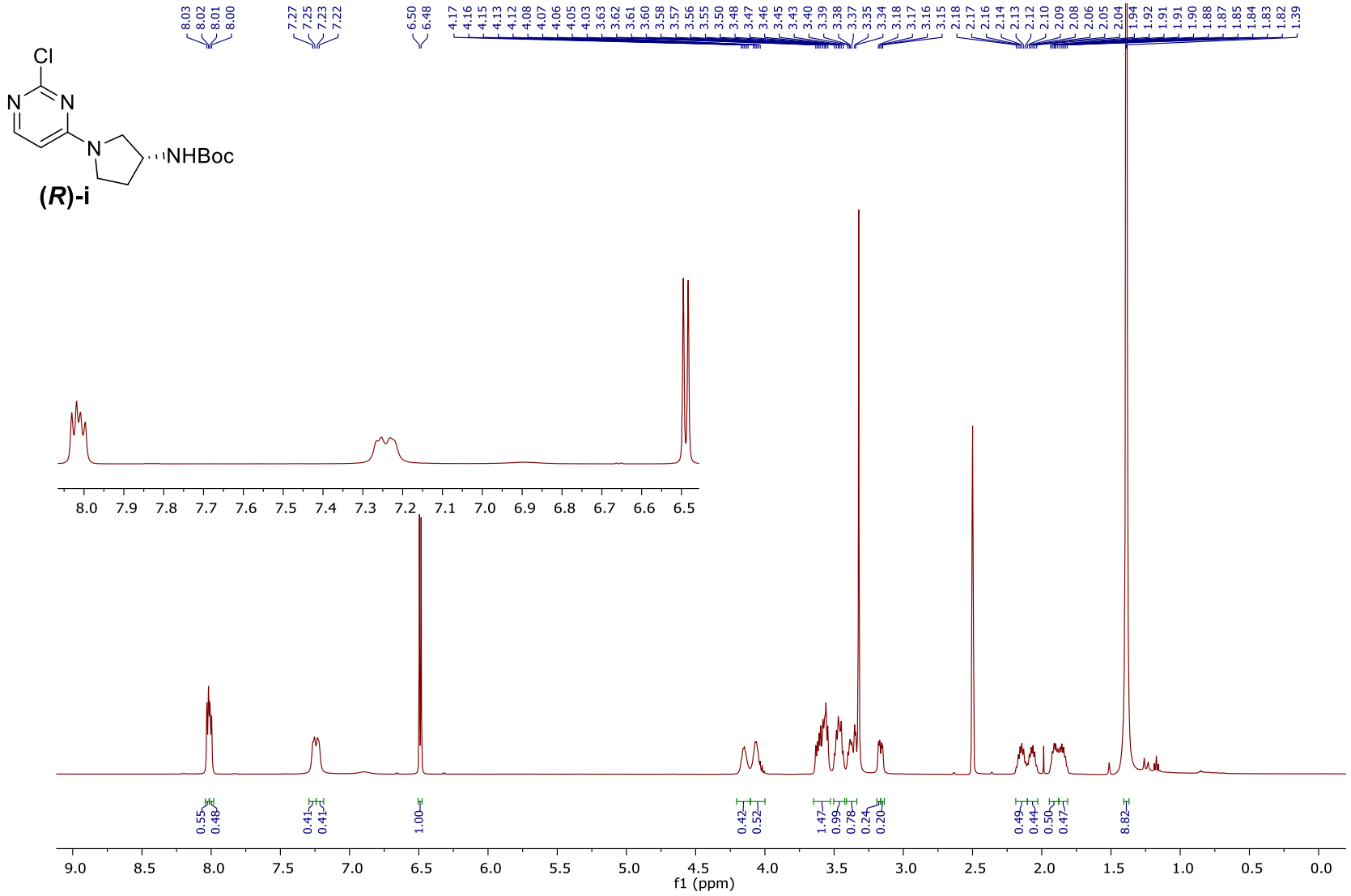
(*S*)-1



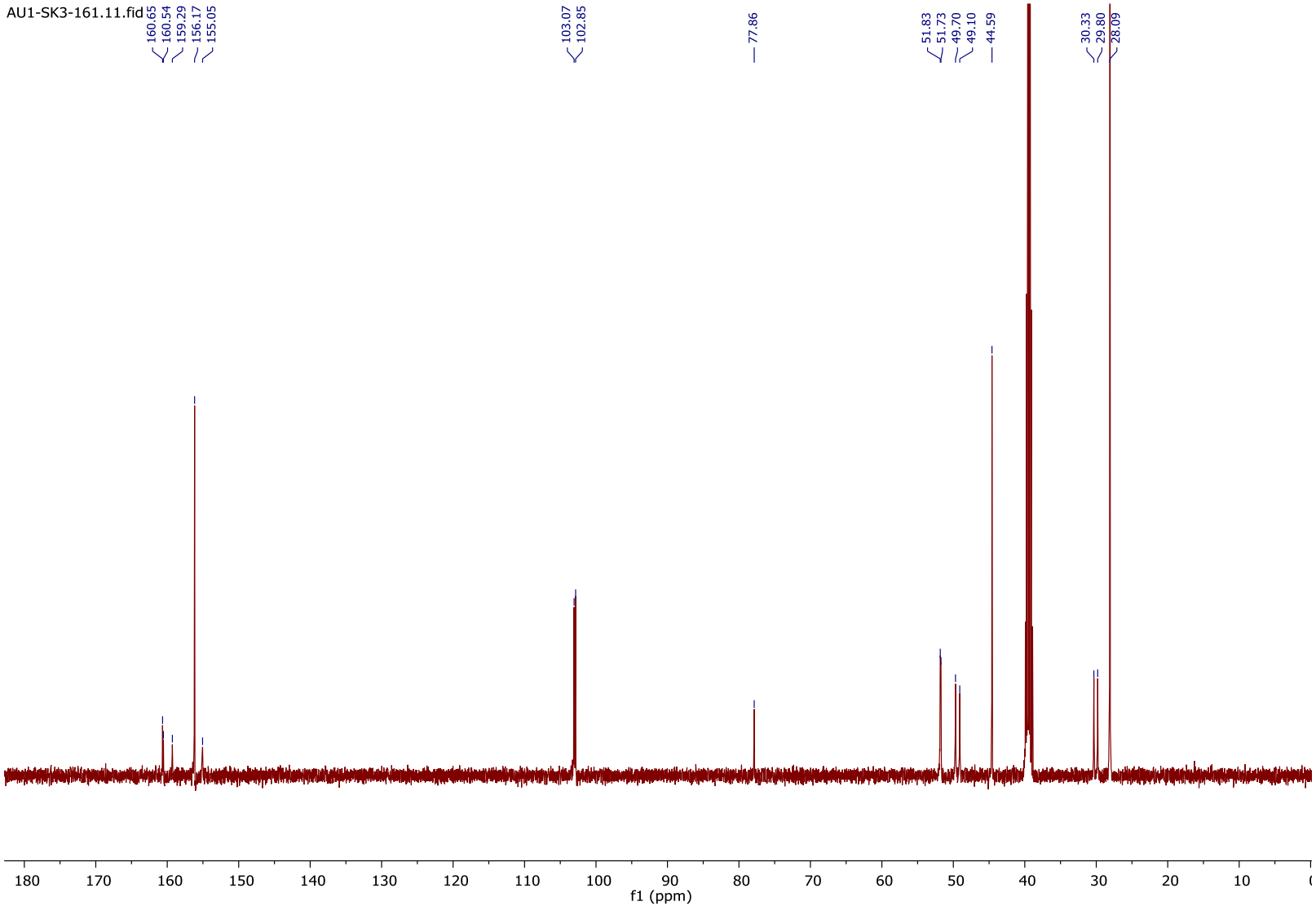
(*S*)-1, ^{13}C, 126 MHz, DMSO-d_6



(*R*)-i, ^{1}H, 500 MHz, DMSO-d_6



(*R*)-i, ^{13}C, 126 MHz, DMSO-d_6



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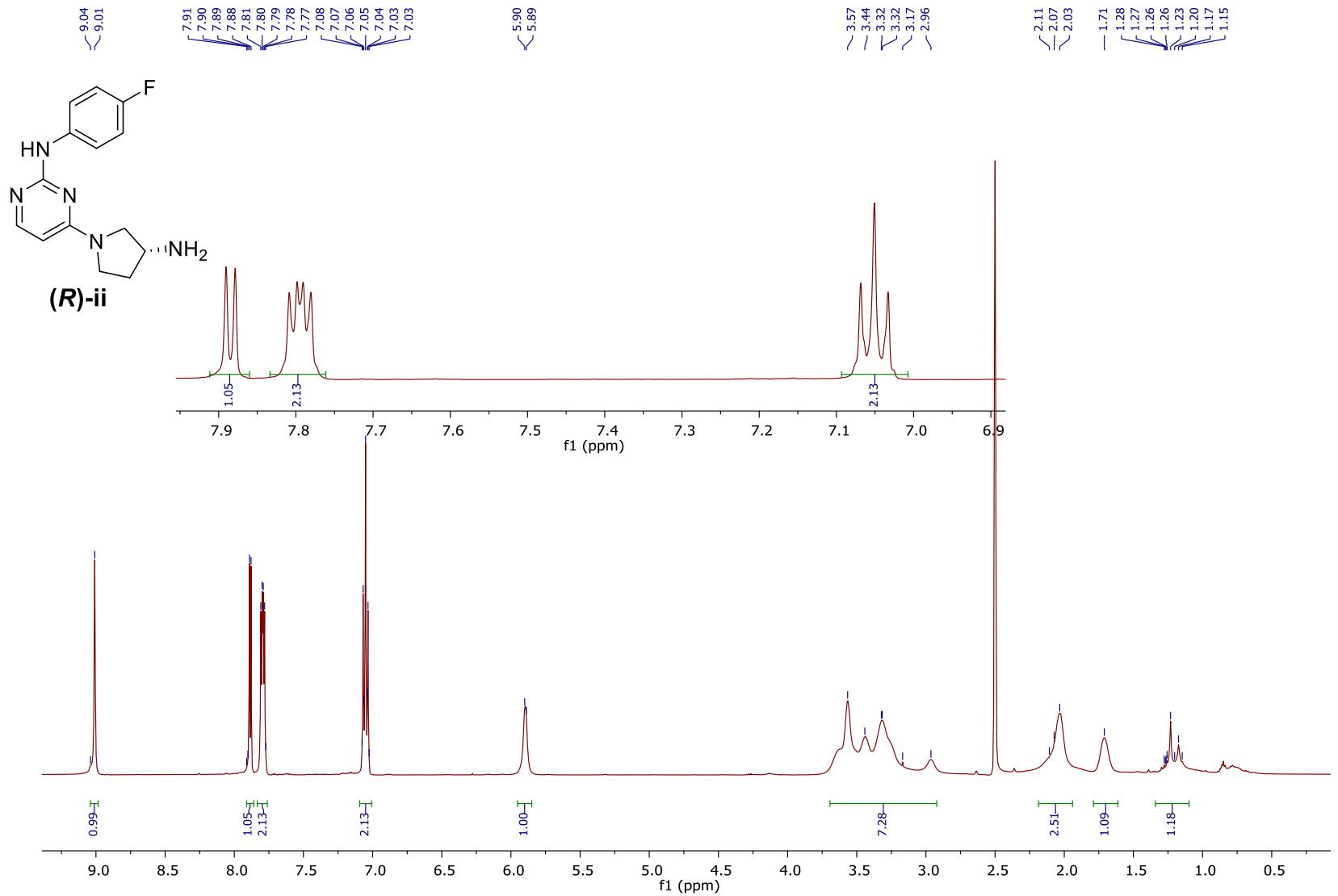
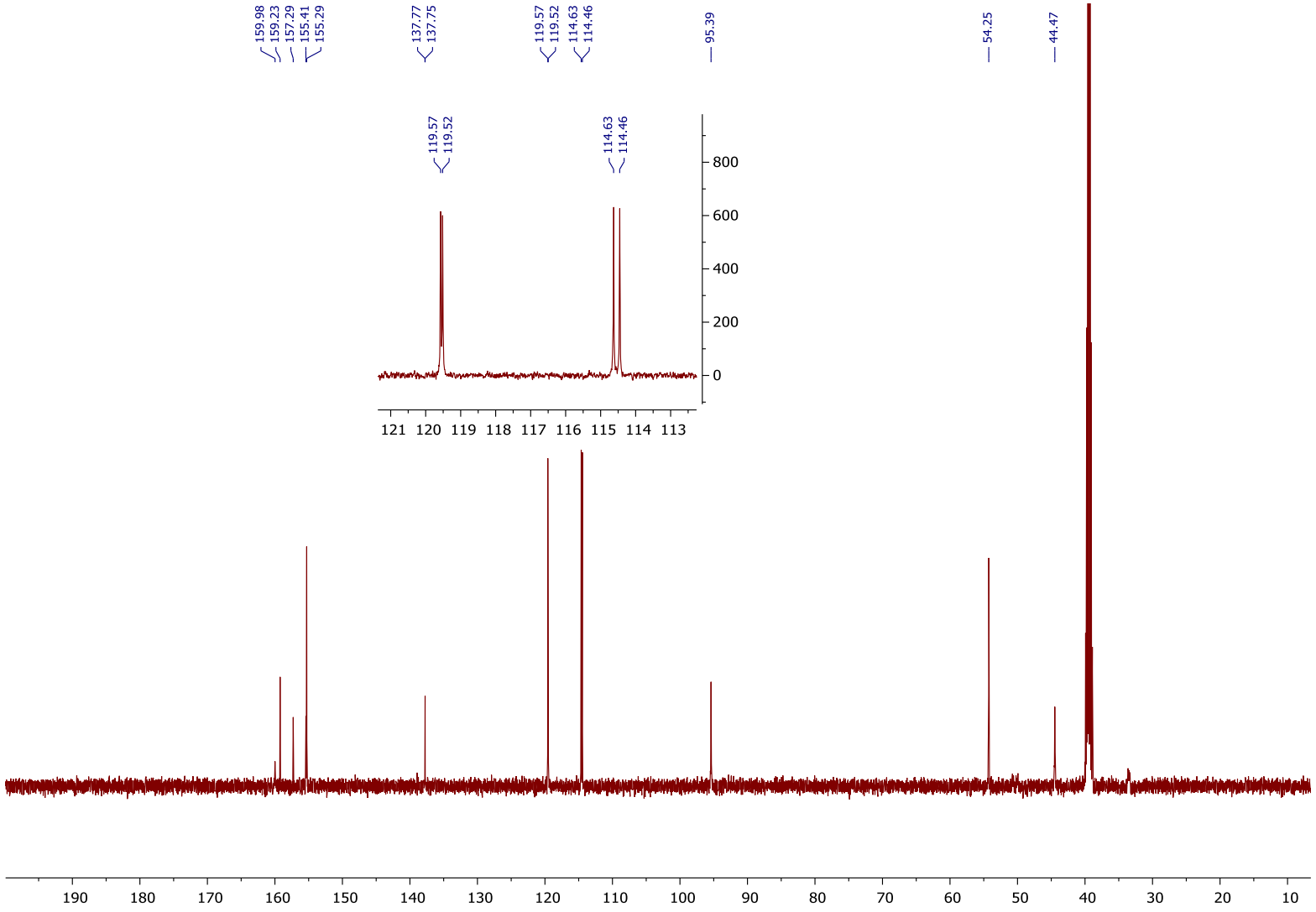
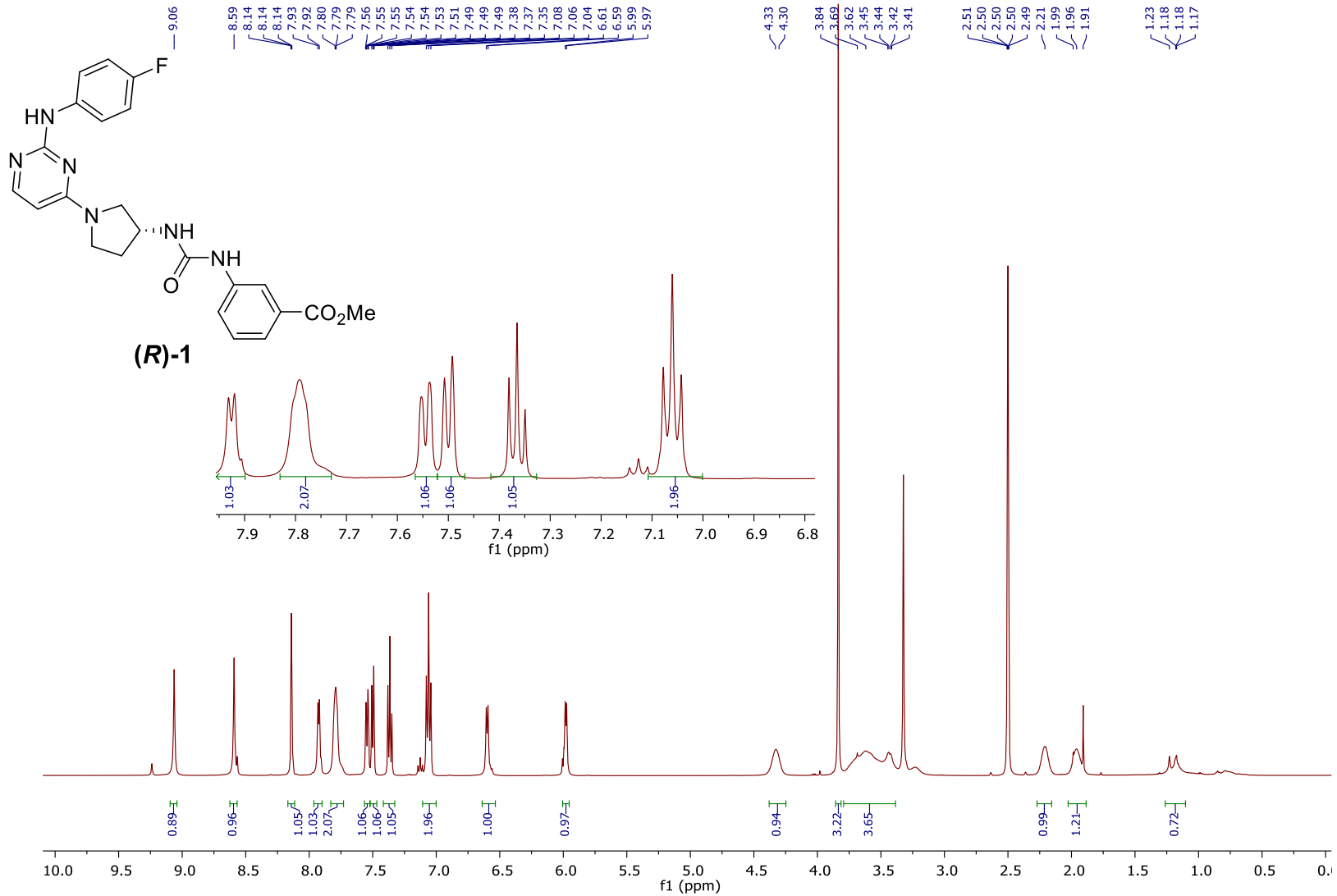


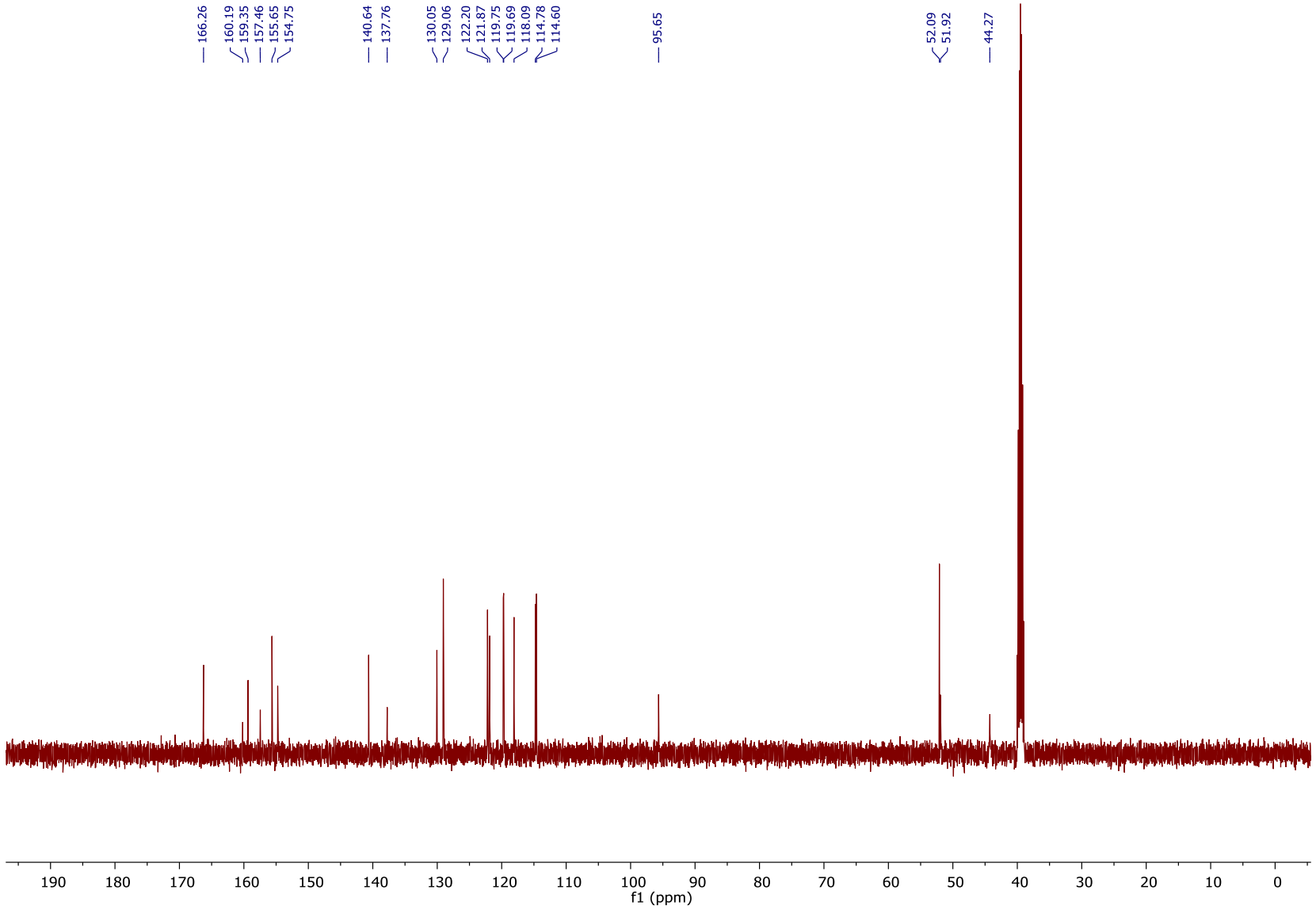
(*R*)-ii, ^{13}C, 126 MHz, DMSO-d_6



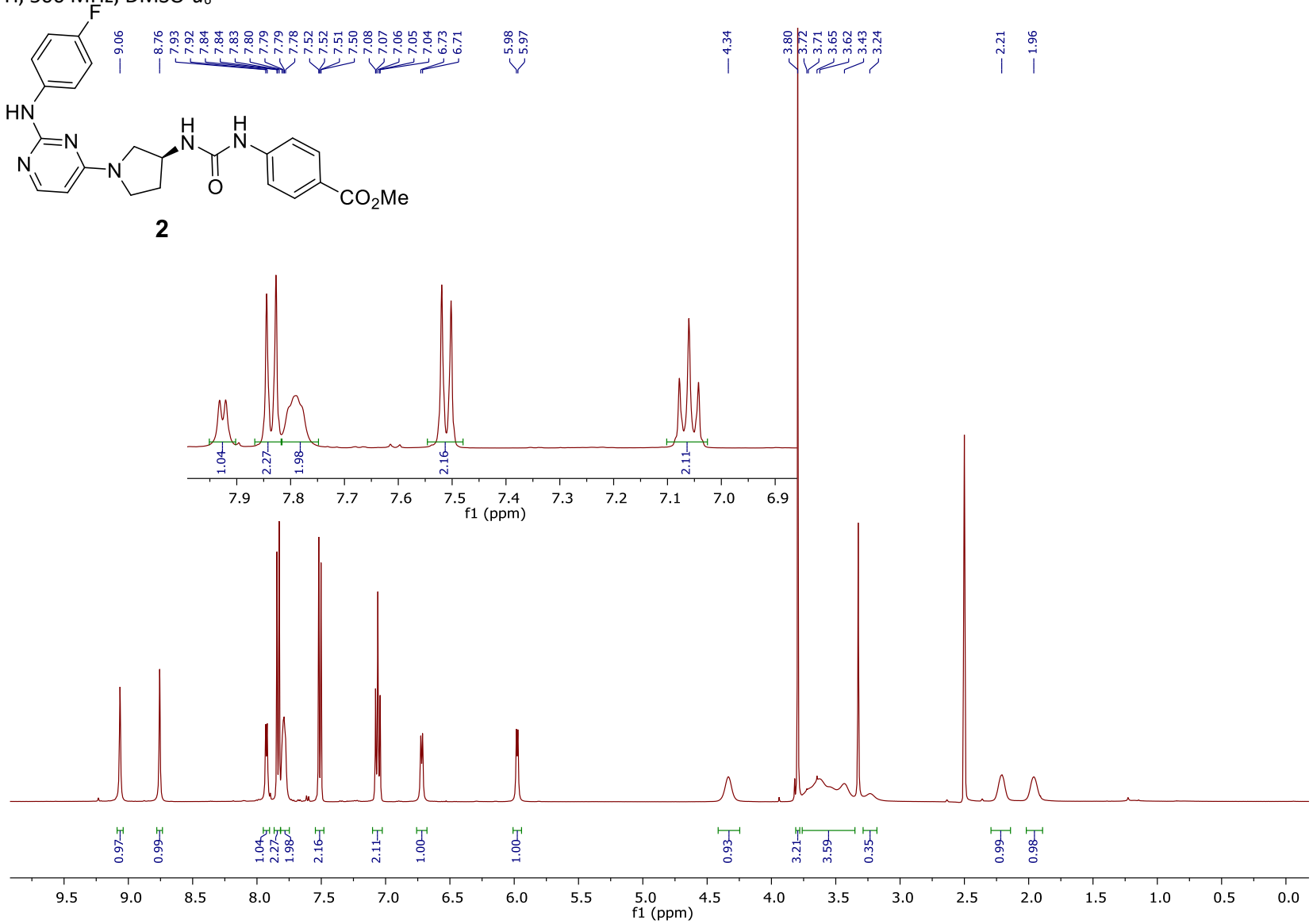
(*R*)-1, ^{1}H, 500 MHz, DMSO-d_6



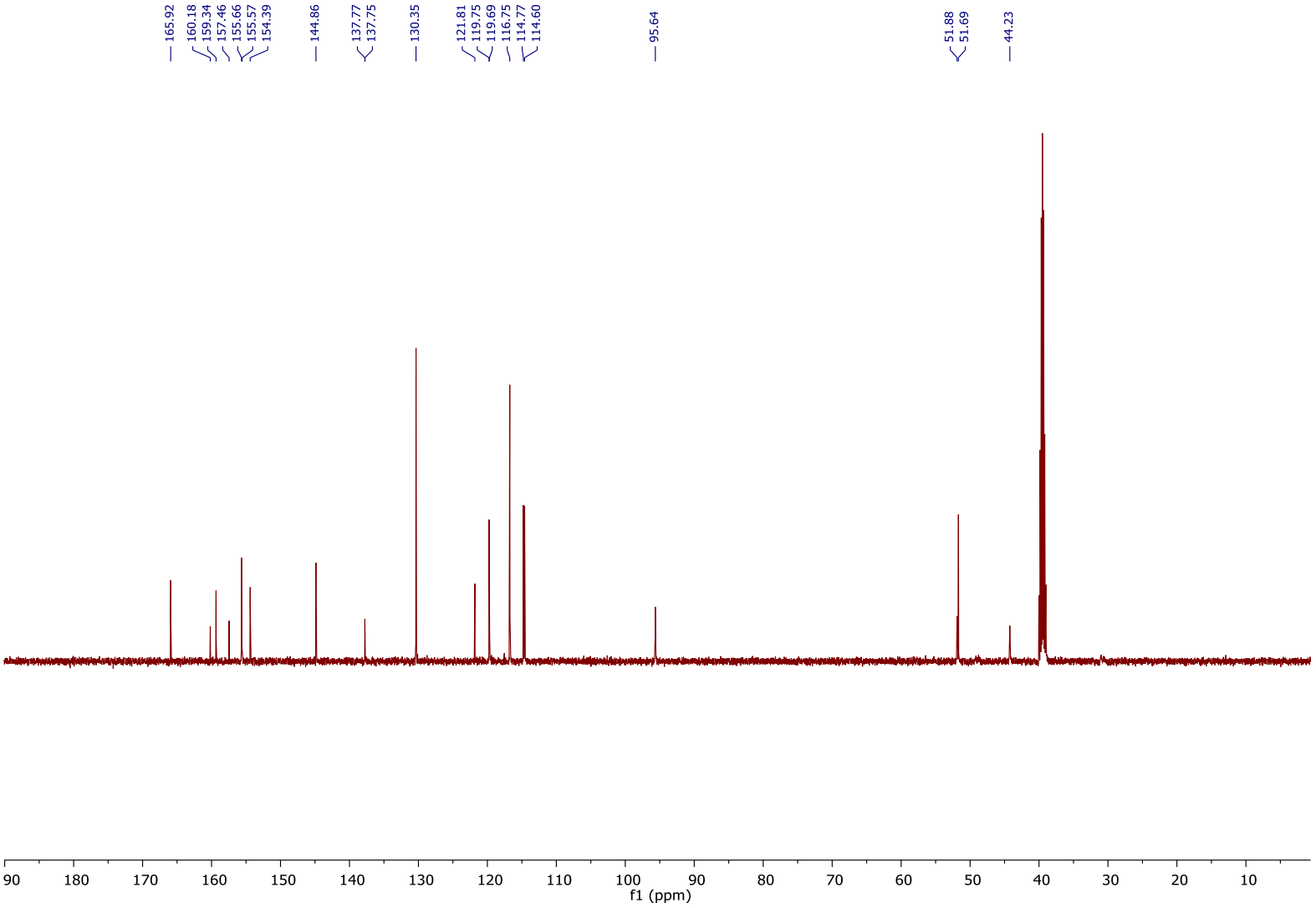
(*R*)-1, ^{13}C, 126 MHz, DMSO-d_6



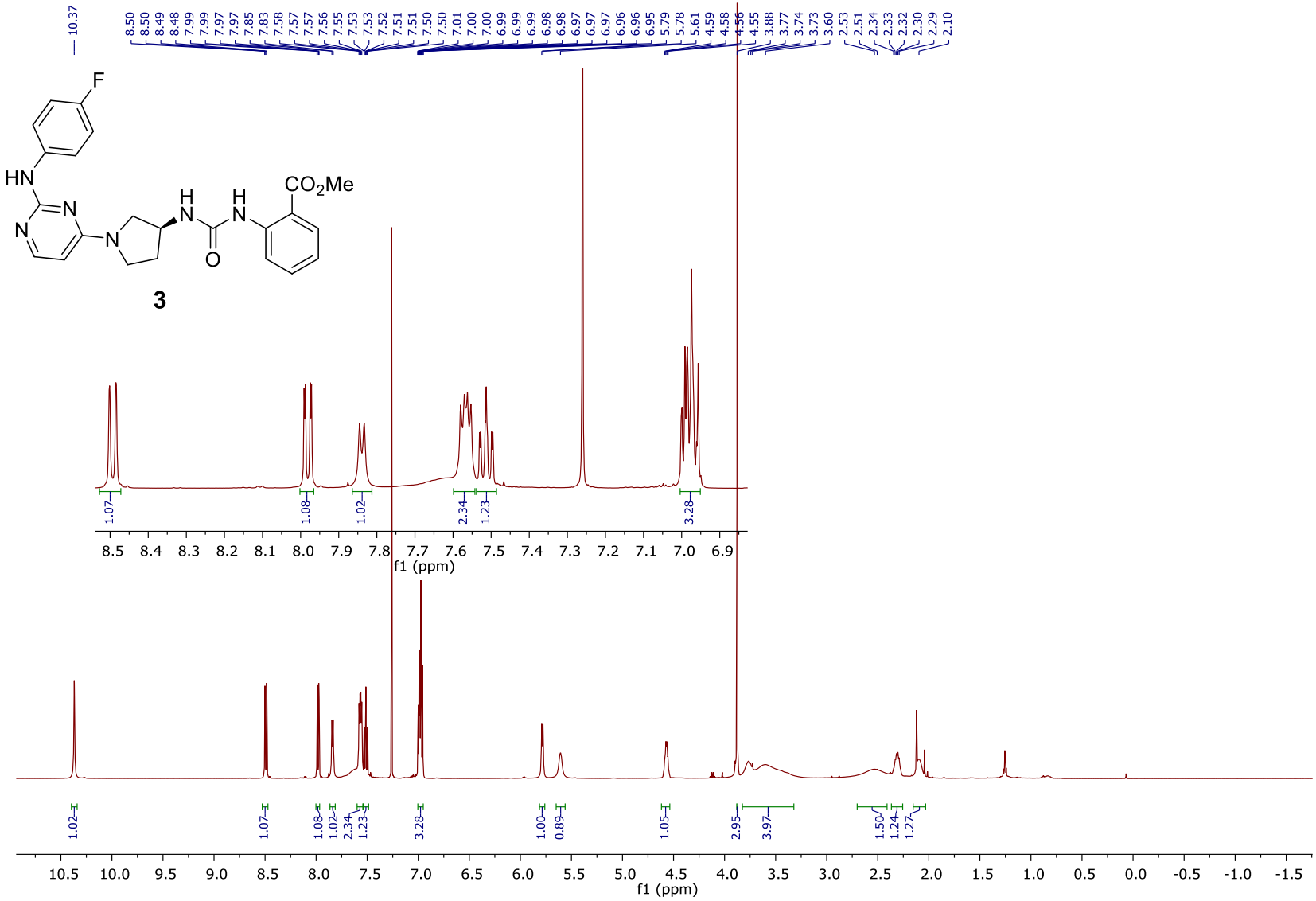
2, ^{1}H, 500 MHz, DMSO-d_6



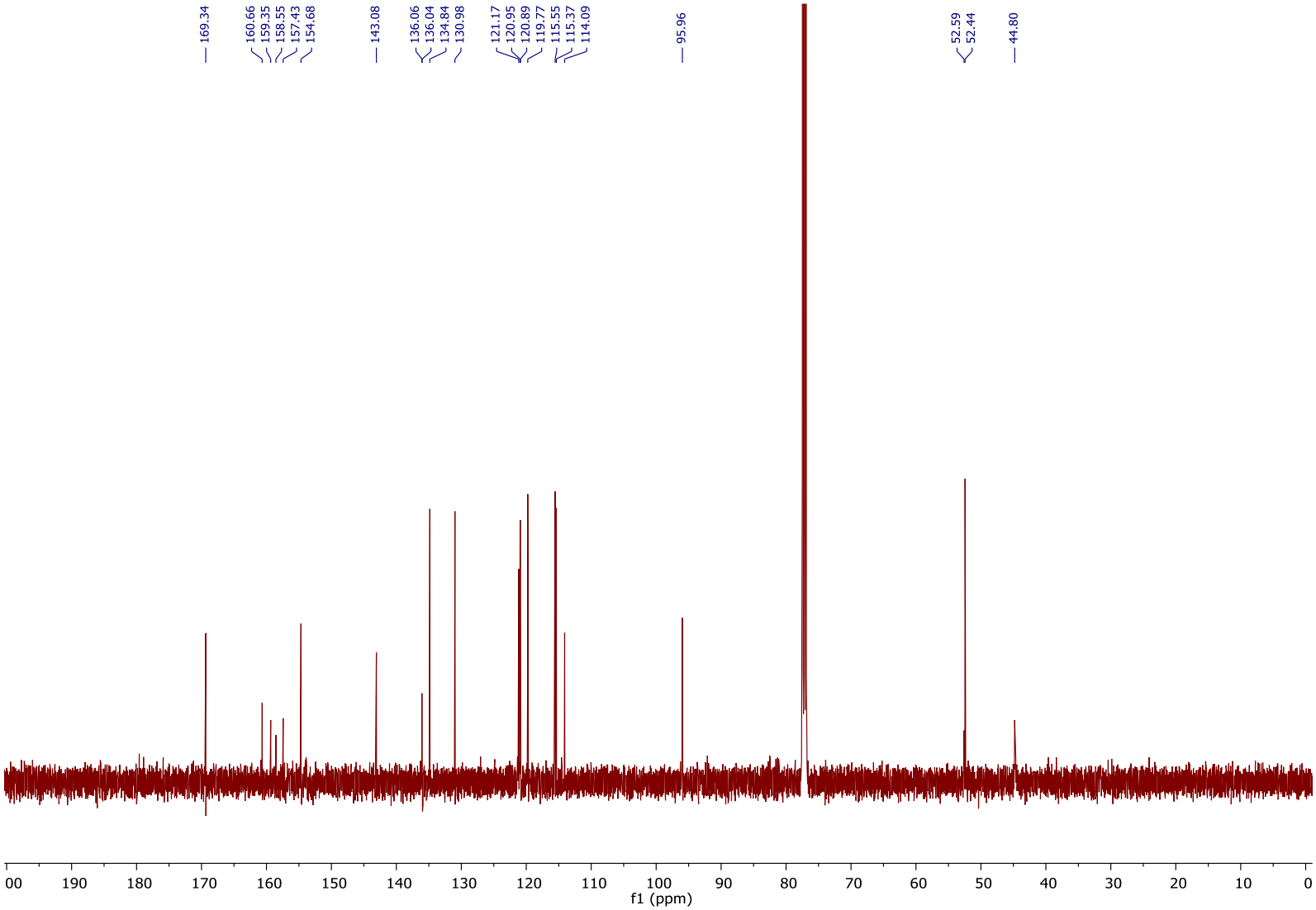
2, ^{13}C, 126 MHz, DMSO-d_6



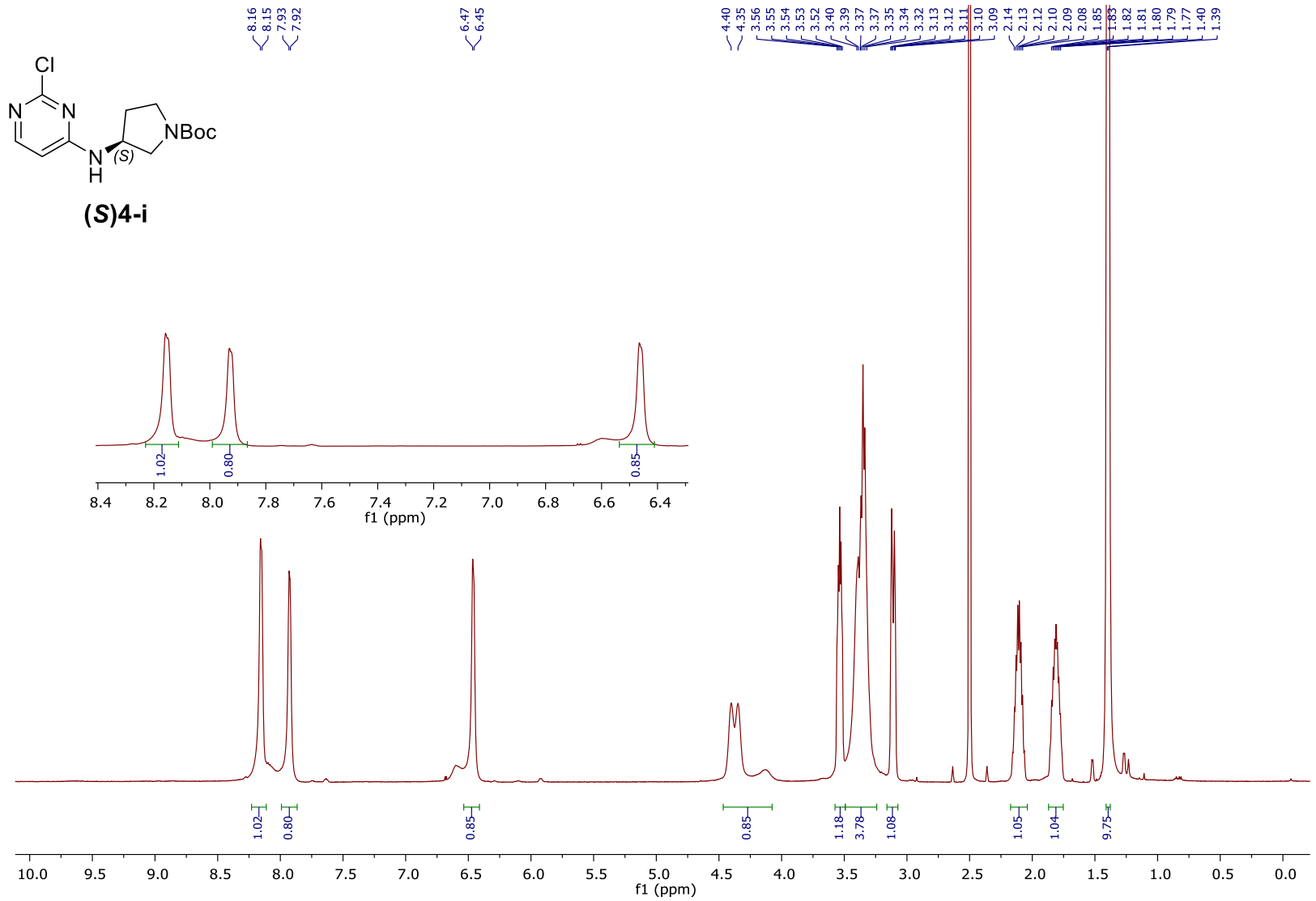
3, ^{1}H, 500 MHz, $CDCl_3$



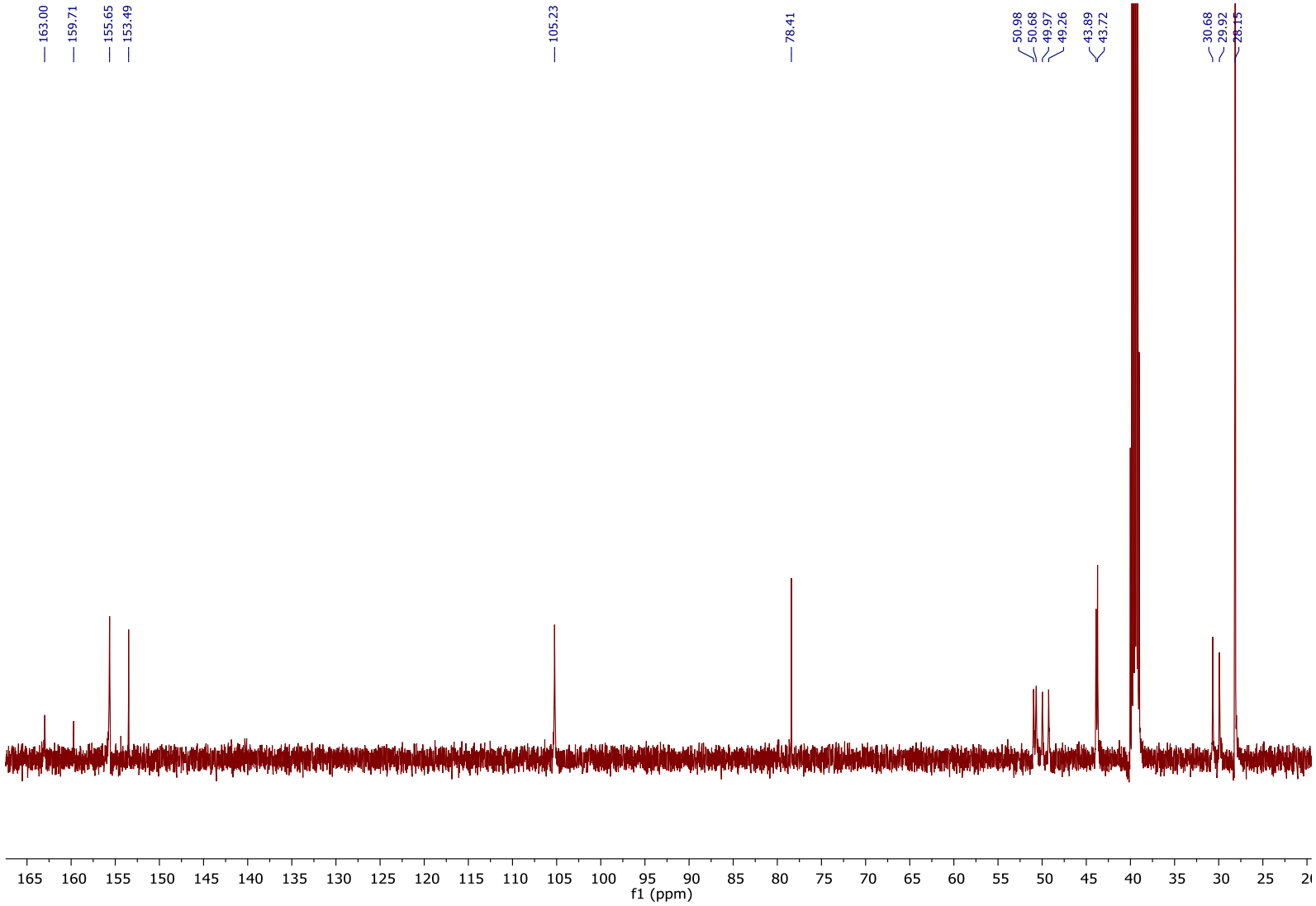
3, ^{13}C, 126 MHz, $CDCl_3$



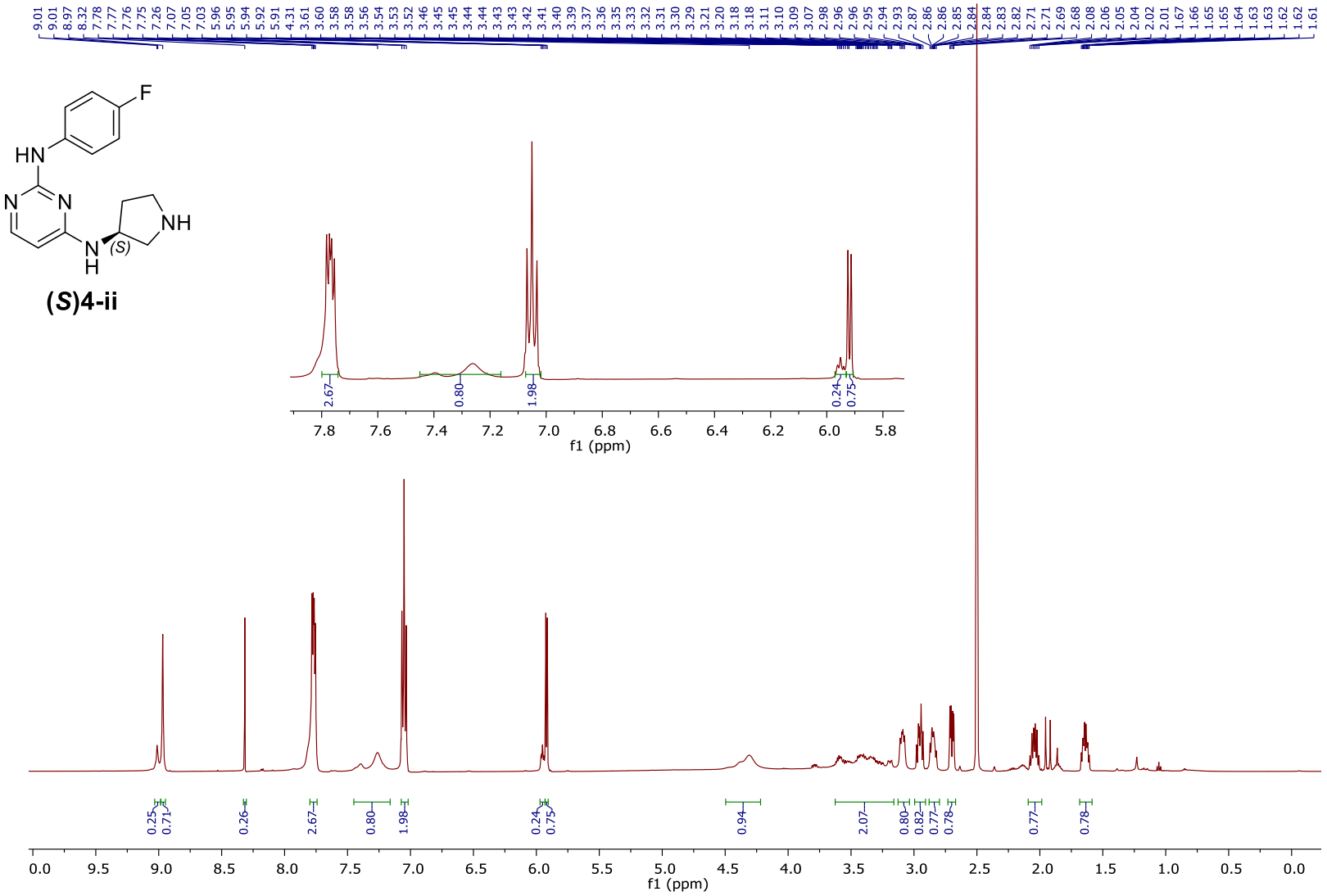
(*S*)4-i, ^{1}H, 500 MHz, DMSO-d_6



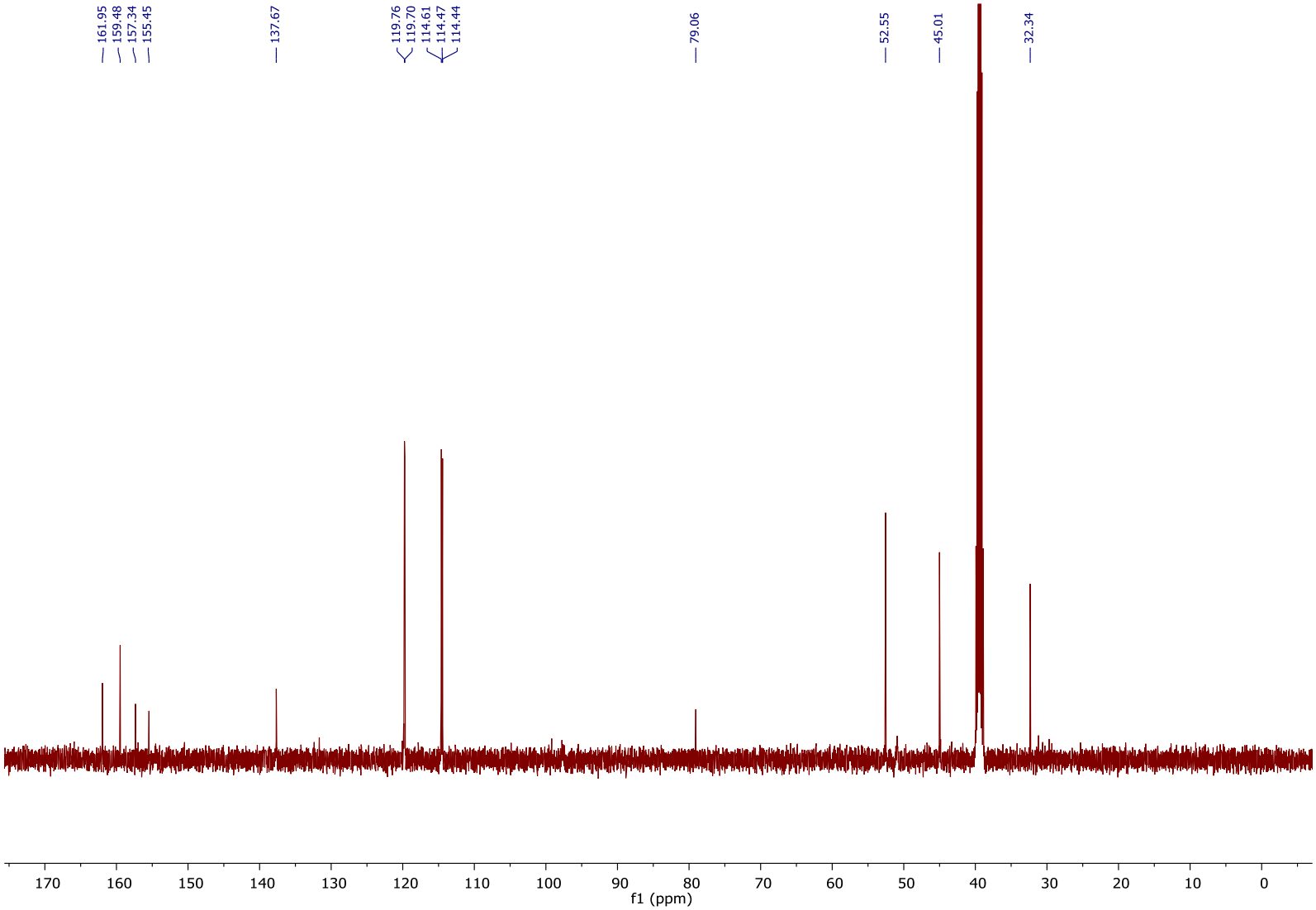
(*S*)4-i, ^{13}C, 126 MHz, DMSO-d_6



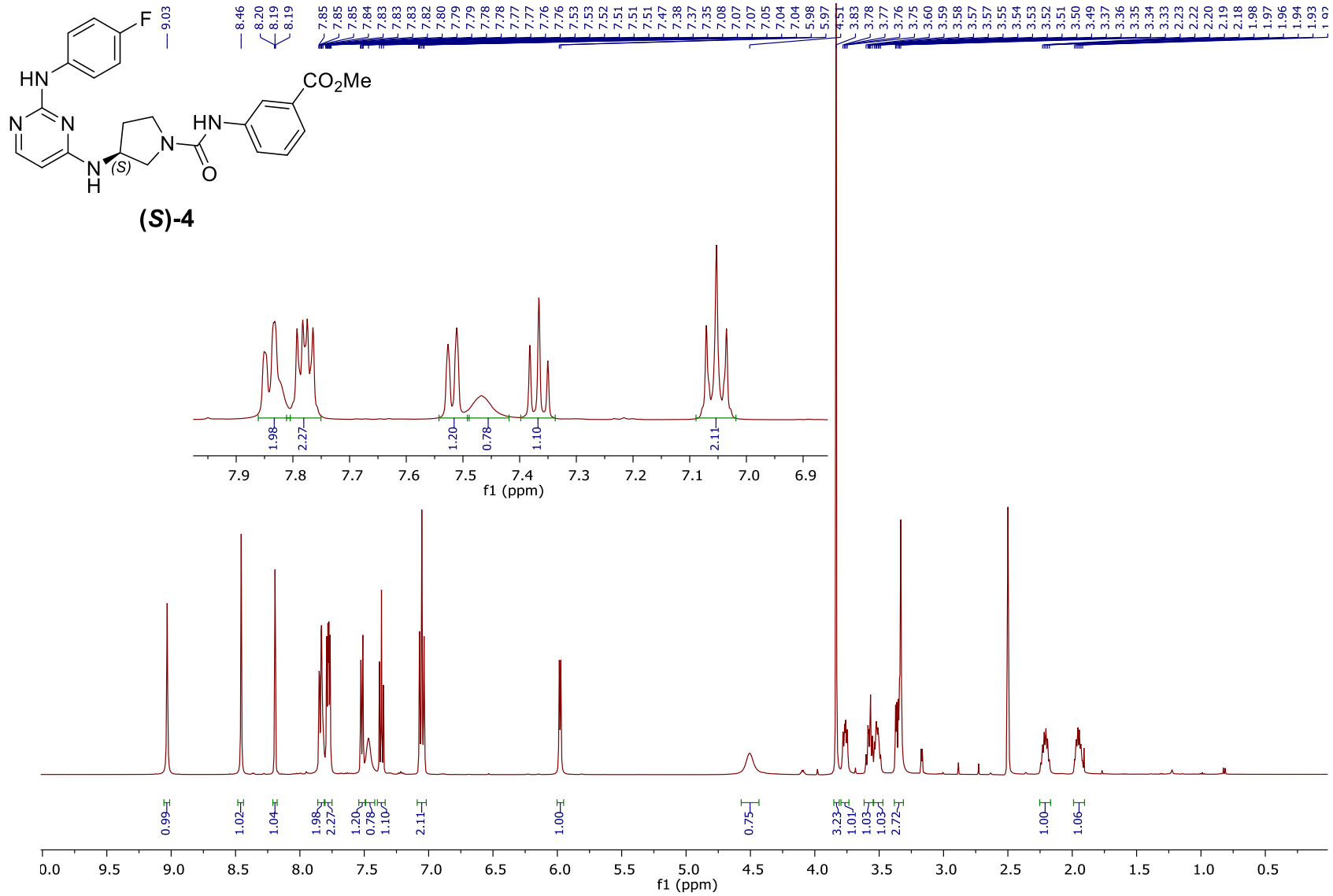
(*S*)4-ii, ^{1}H, 500 MHz, DMSO-d_6



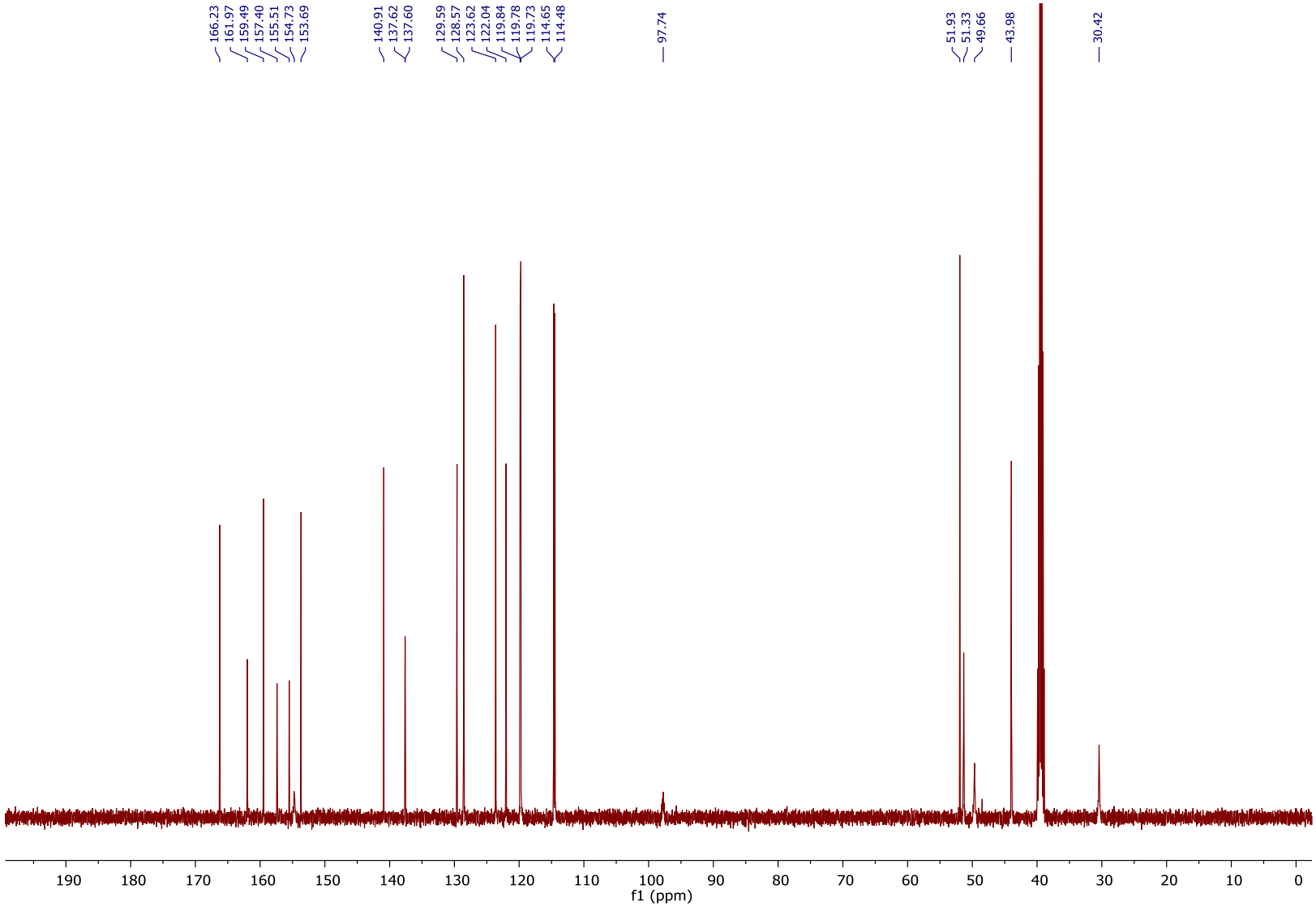
(*S*)4-ii, ^{13}C, 126 MHz, DMSO-d_6



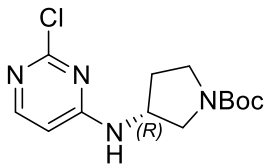
(*S*)-4, ^{1}H, 500 MHz, DMSO-d_6



(*S*)-4, ^{13}C, 126 MHz, DMSO-d_6

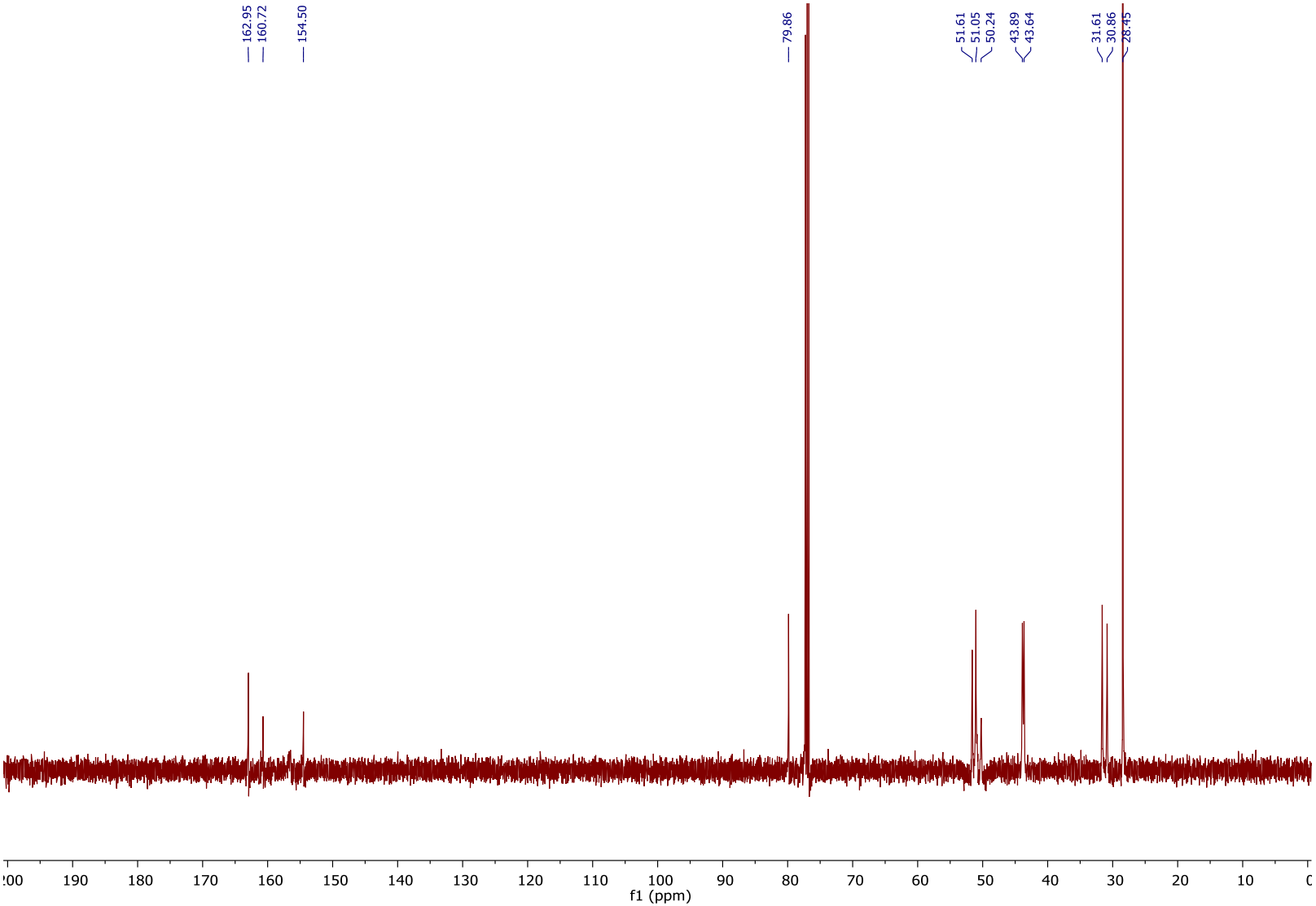


(*R*)4-i, ^{1}H, 500 MHz, $CDCl_3$

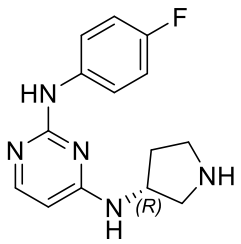


(*R*)4-i

(*R*)4-i, ^{13}C, 126 MHz, $CDCl_3$

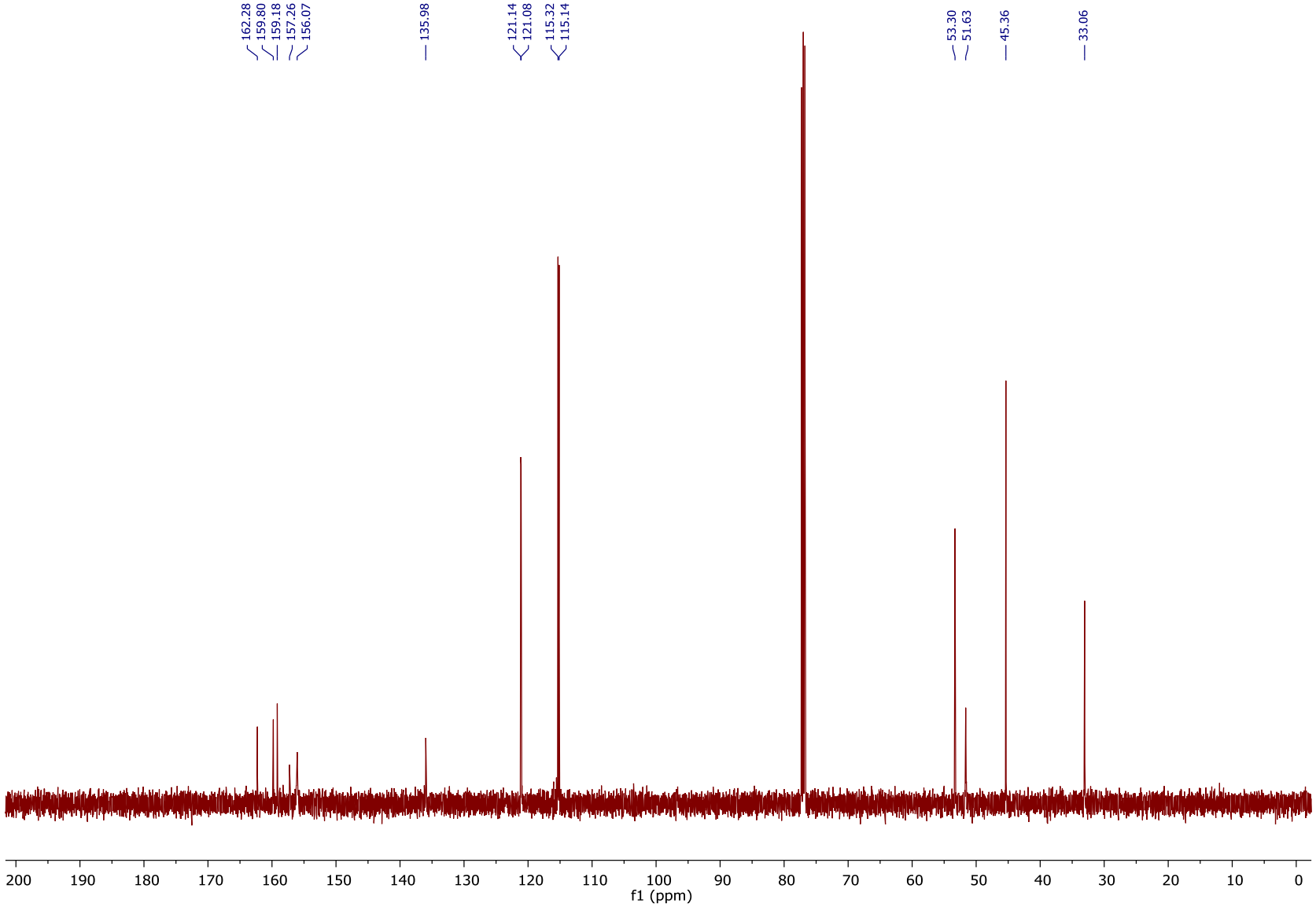


(*R*)4-ii, ^{1}H, 500 MHz, $CDCl_3$

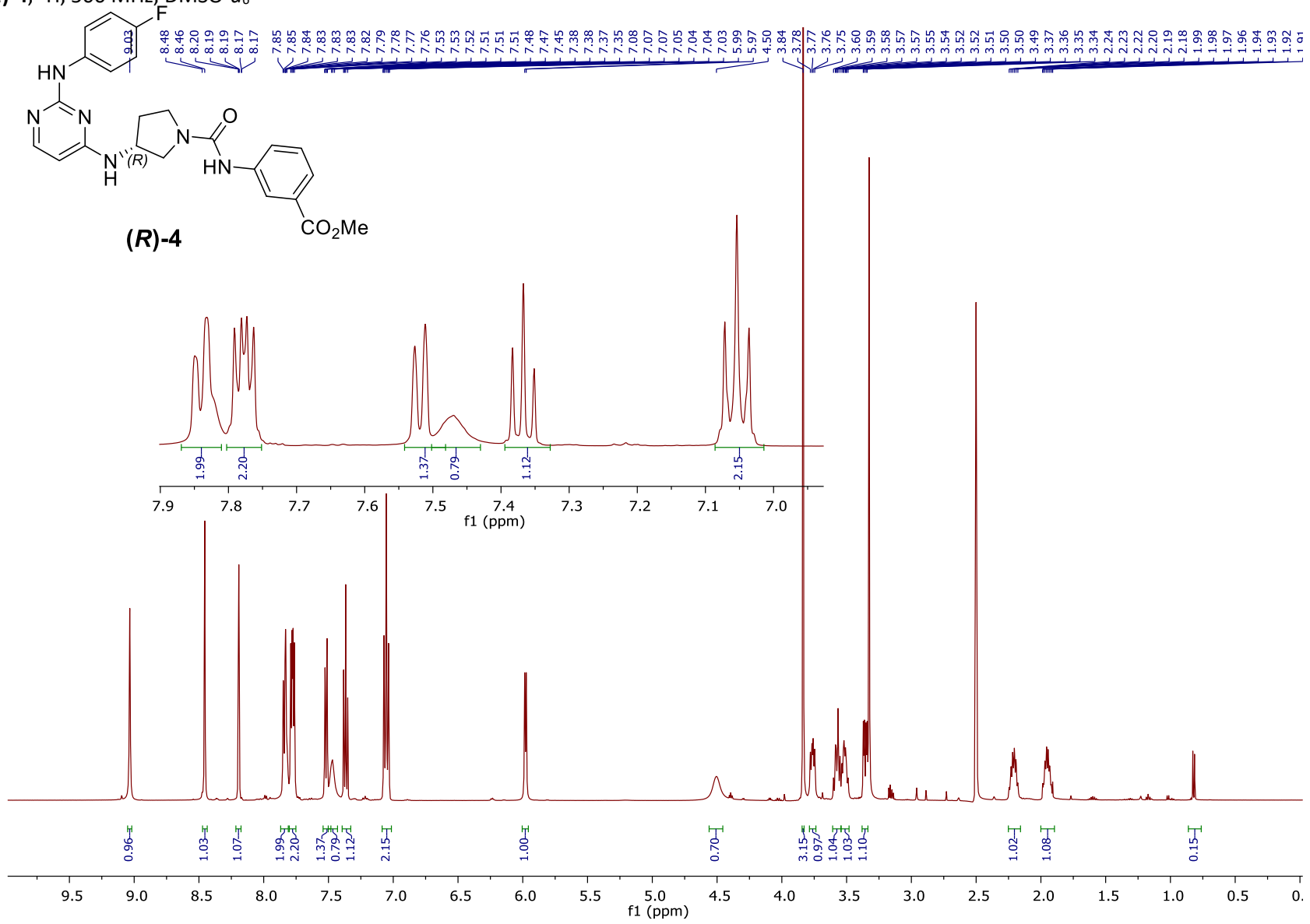


(*R*)4-ii

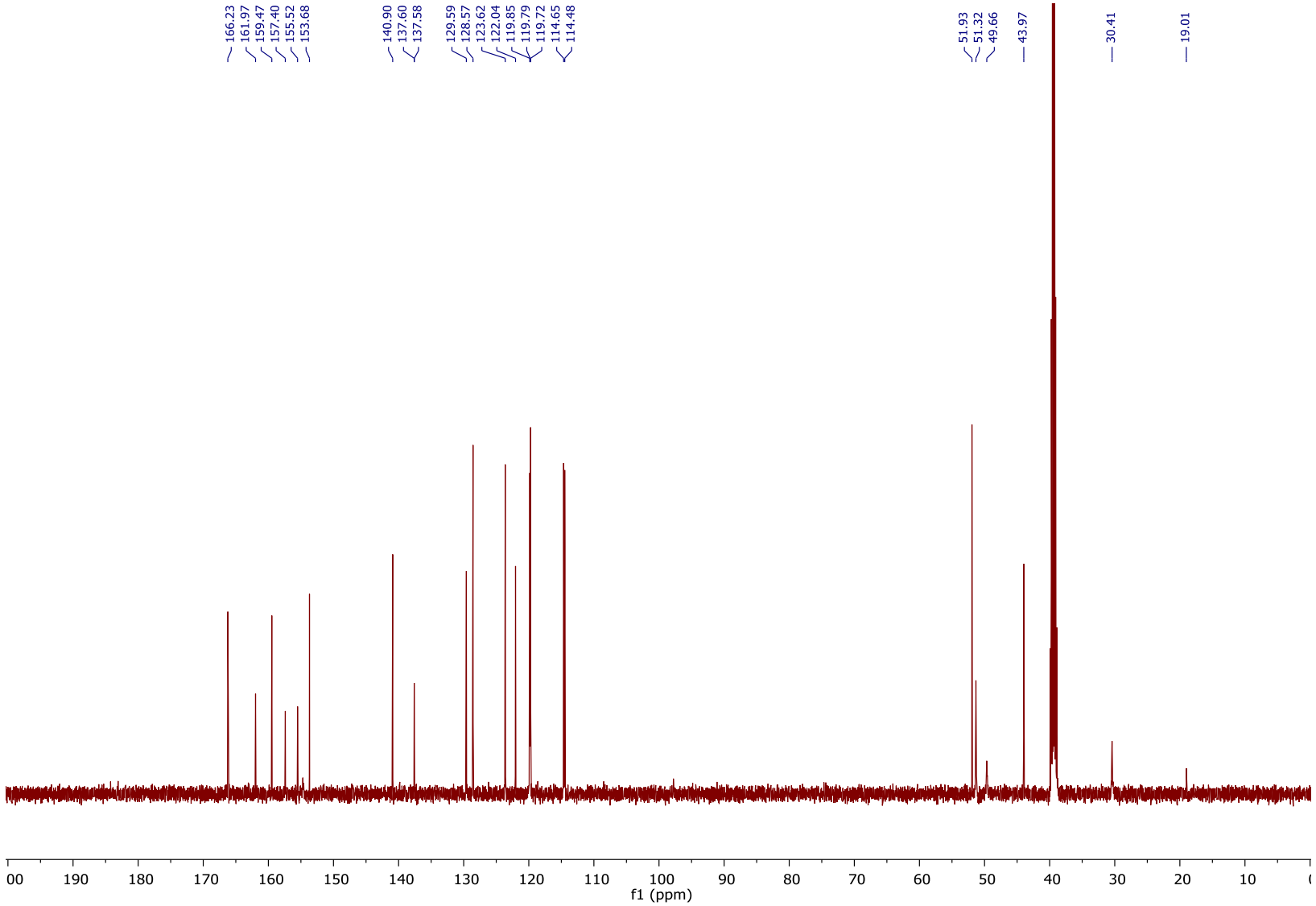
(*R*)4-ii, ^{13}C, 126 MHz, $CDCl_3$



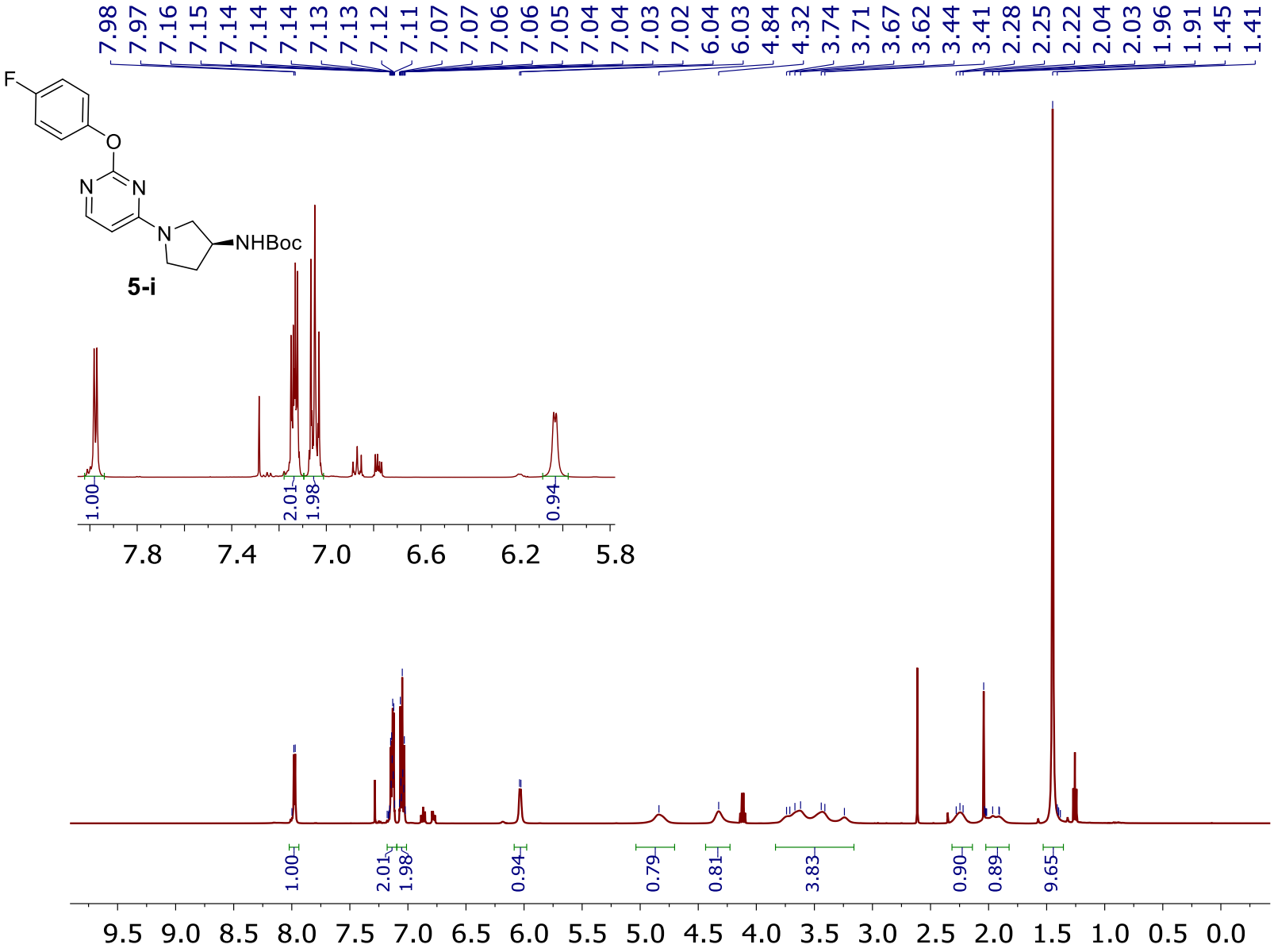
(*R*)-4, ^{1}H, 500 MHz, DMSO-d_6



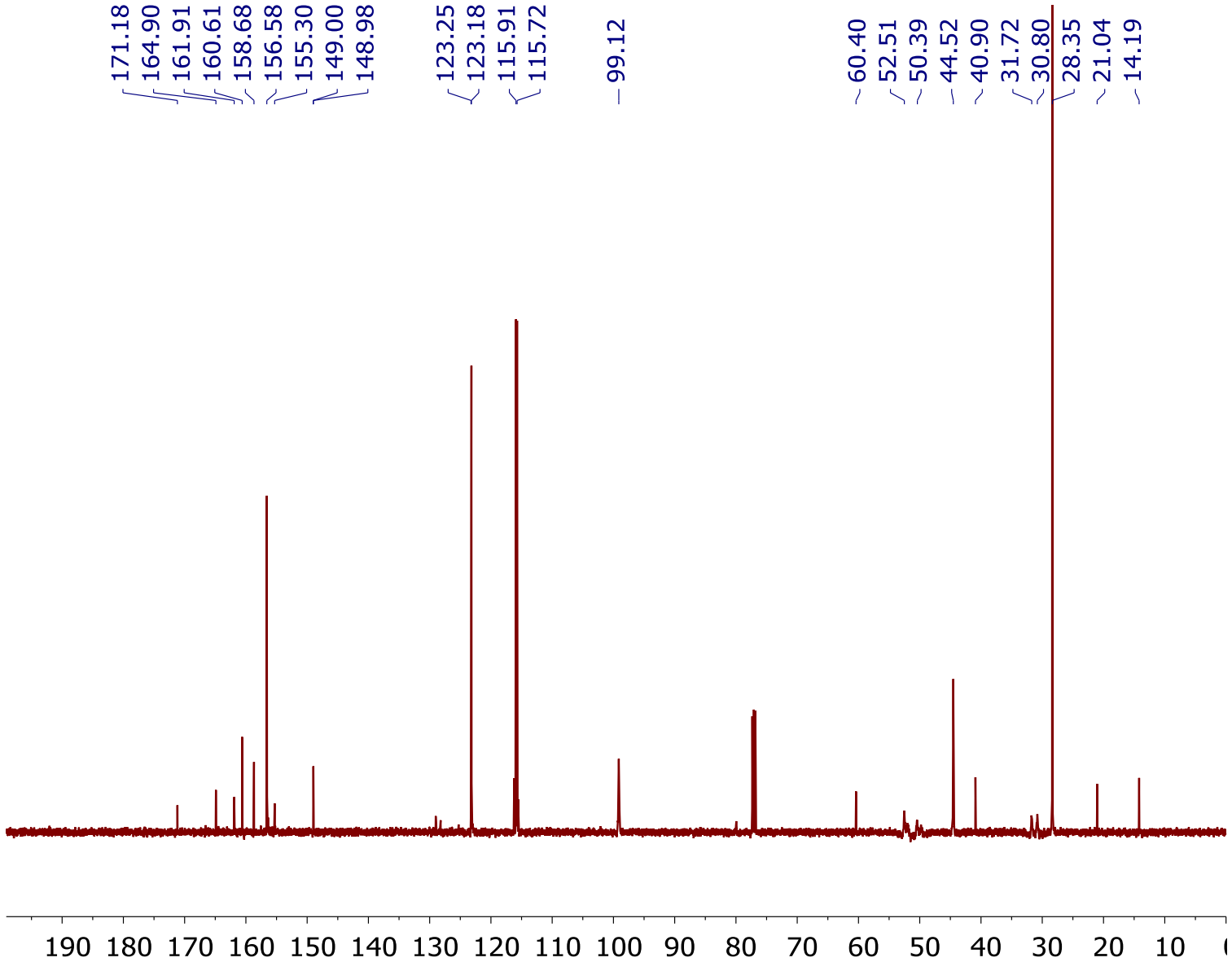
(*R*)-4, ^{13}C, 126 MHz, DMSO-d_6



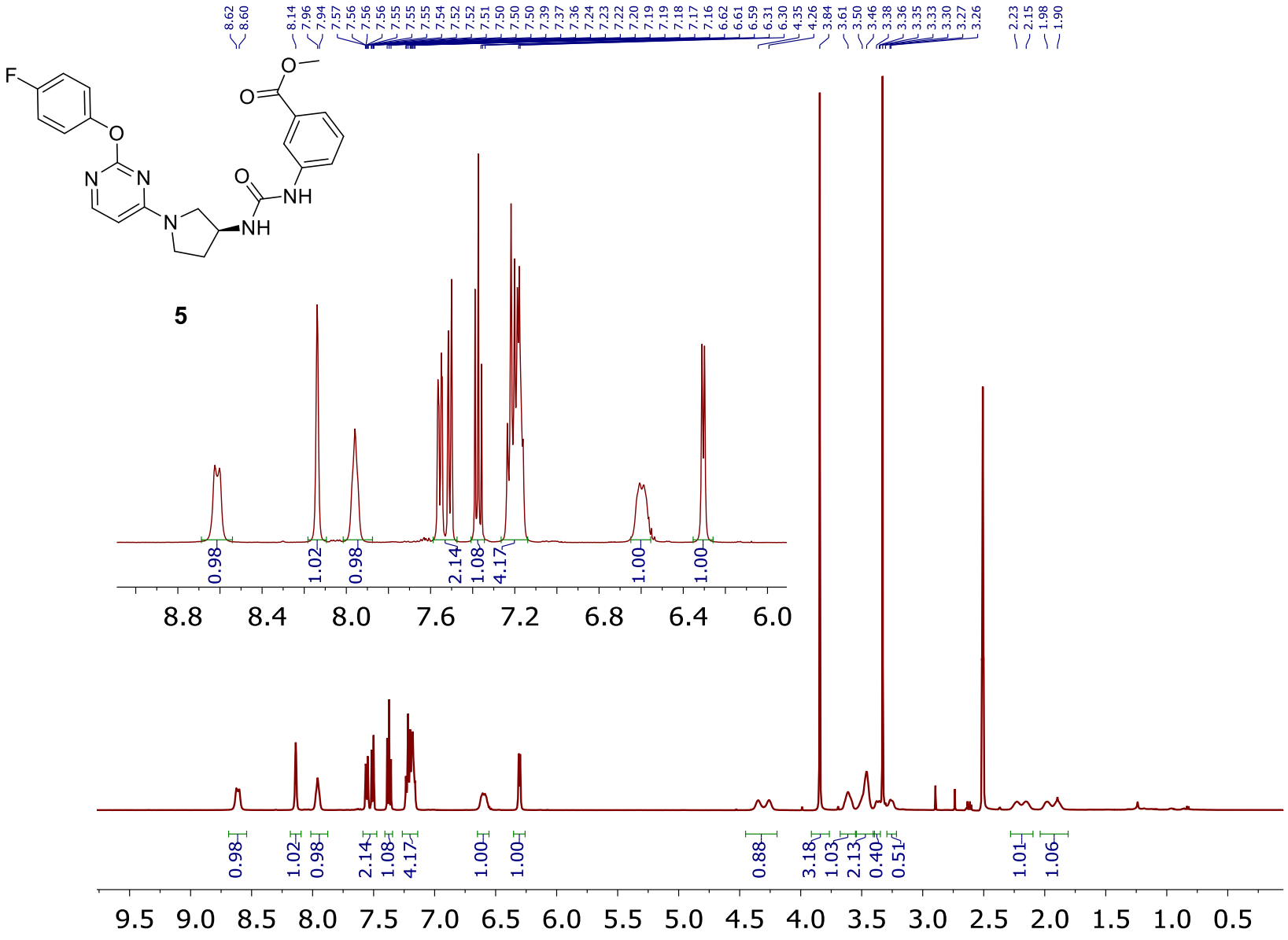
5-i, ^{1}H, 500 MHz, $CDCl_3$



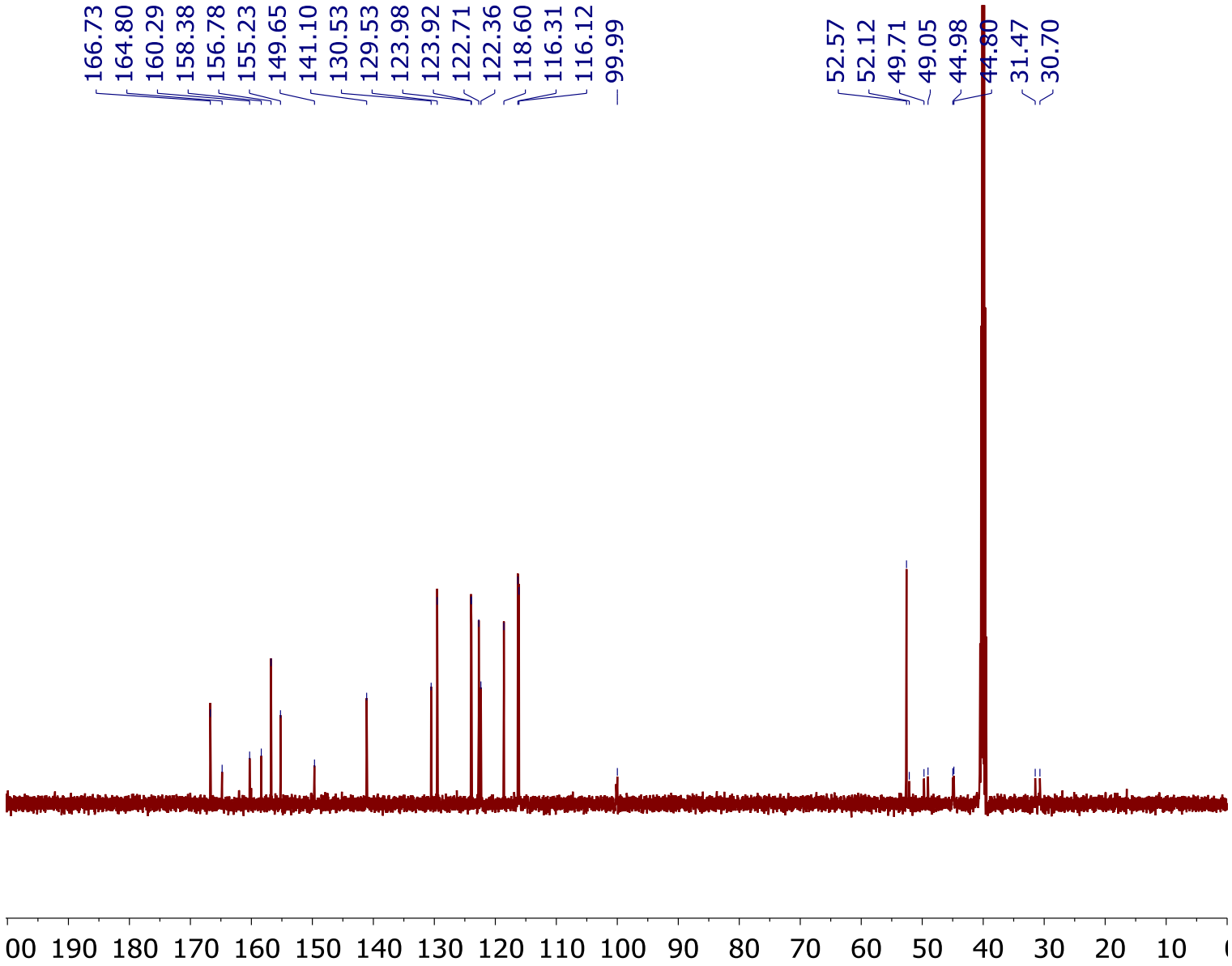
5-i, ^{13}C, 126 MHz, $CDCl_3$



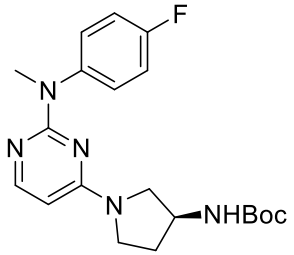
5, ^{1}H, 500 MHz, DMSO-d_6



5, ^{13}C, 126 MHz, DMSO-d_6

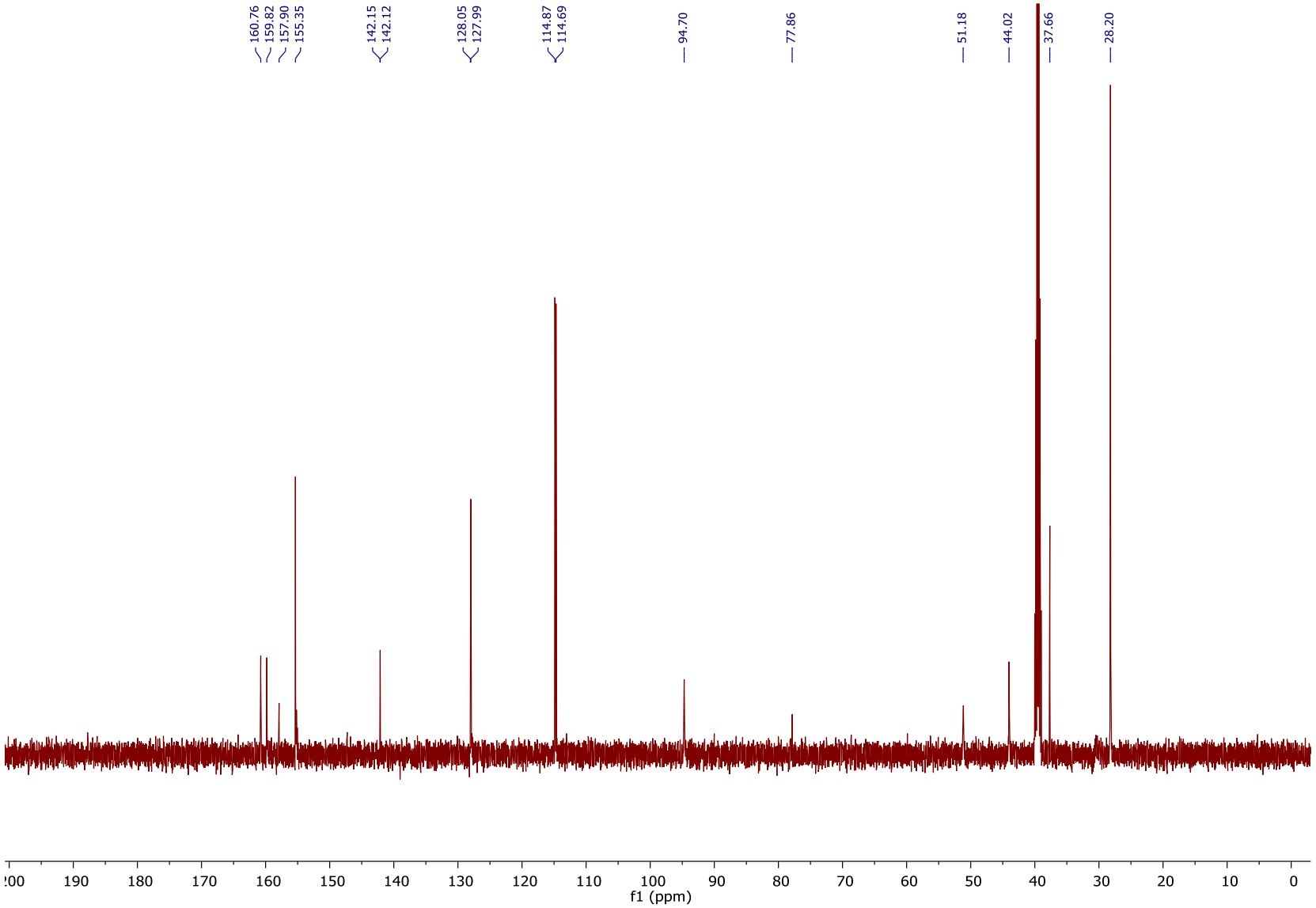


6-i, ^{1}H, 500 MHz, DMSO-d_6

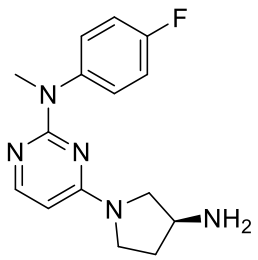


6-i

6-i, ^{13}C, 126 MHz, DMSO-d_6

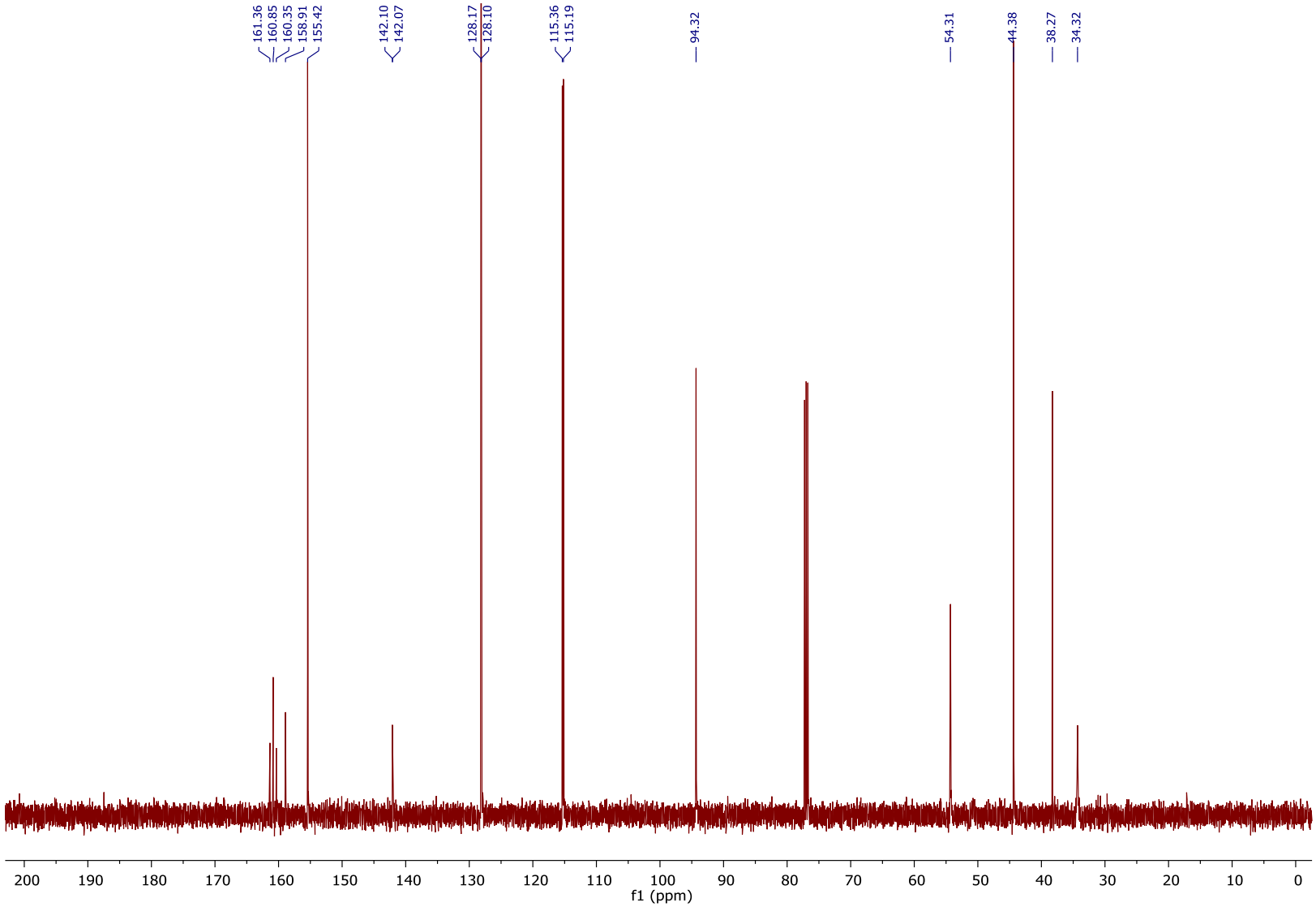


6-ii, ^{1}H, 500 MHz, $CDCl_3$

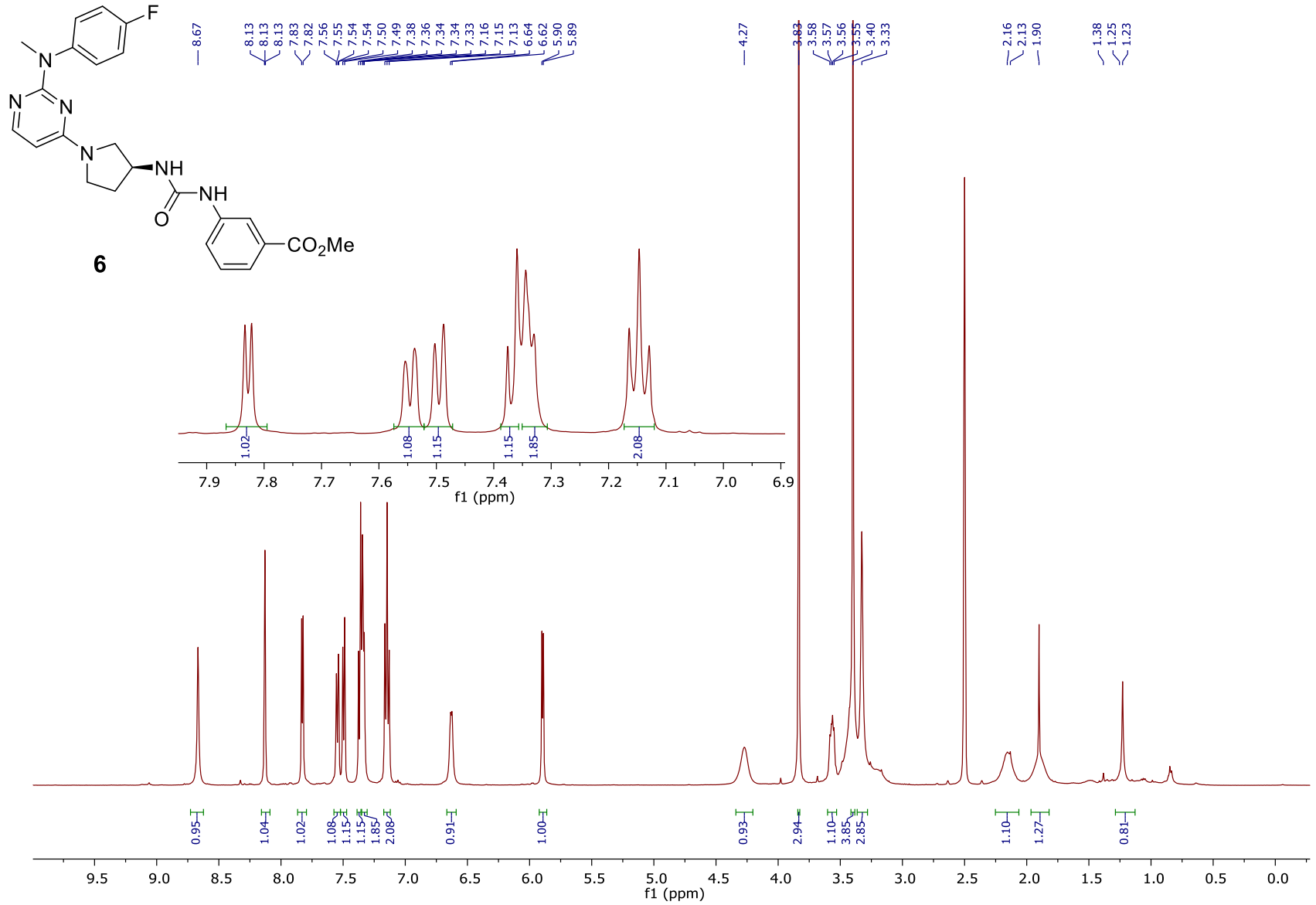


6-ii

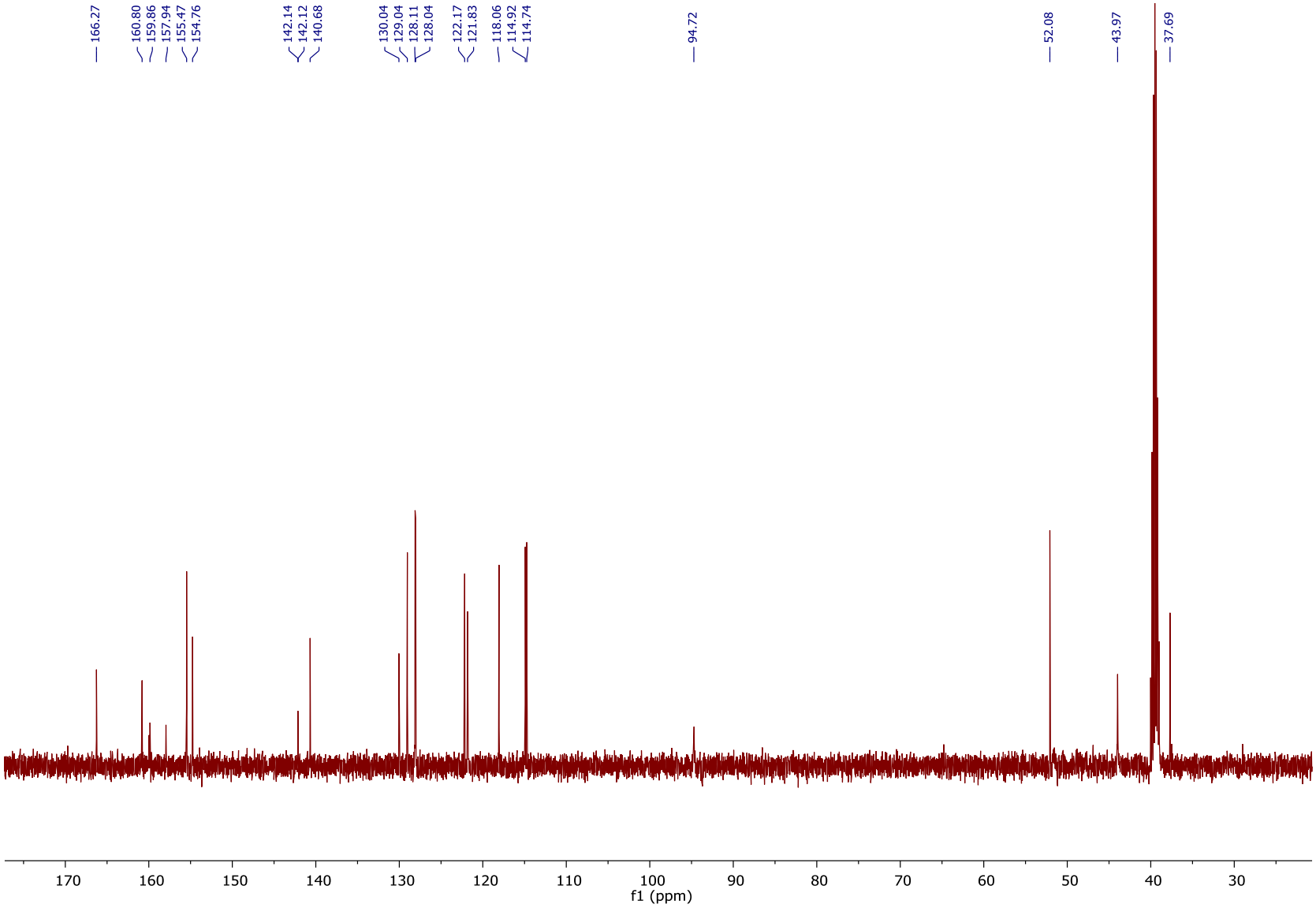
6-ii, ^{13}C, 126 MHz, $CDCl_3$



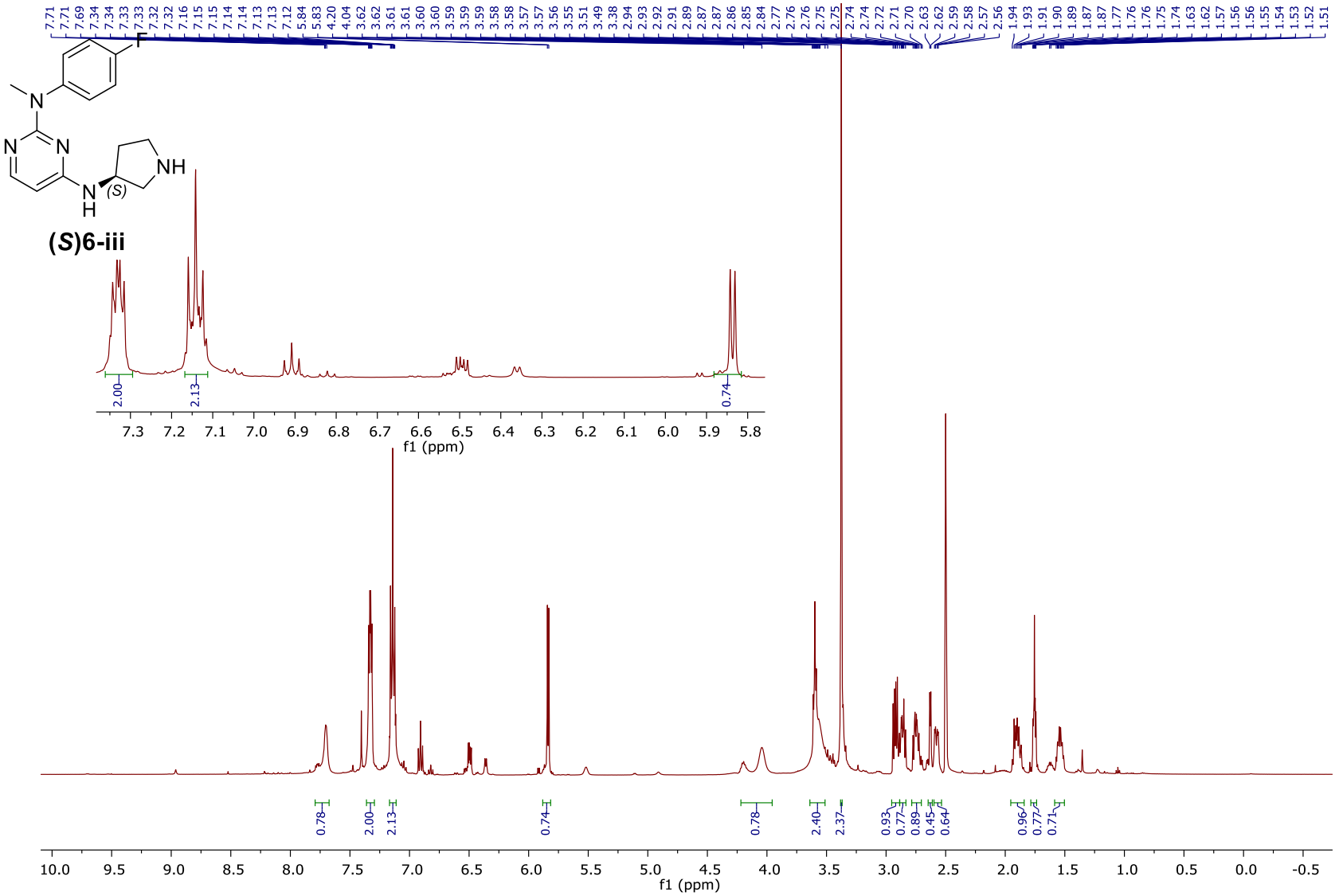
6, ^{1}H, 500 MHz, DMSO-d_6



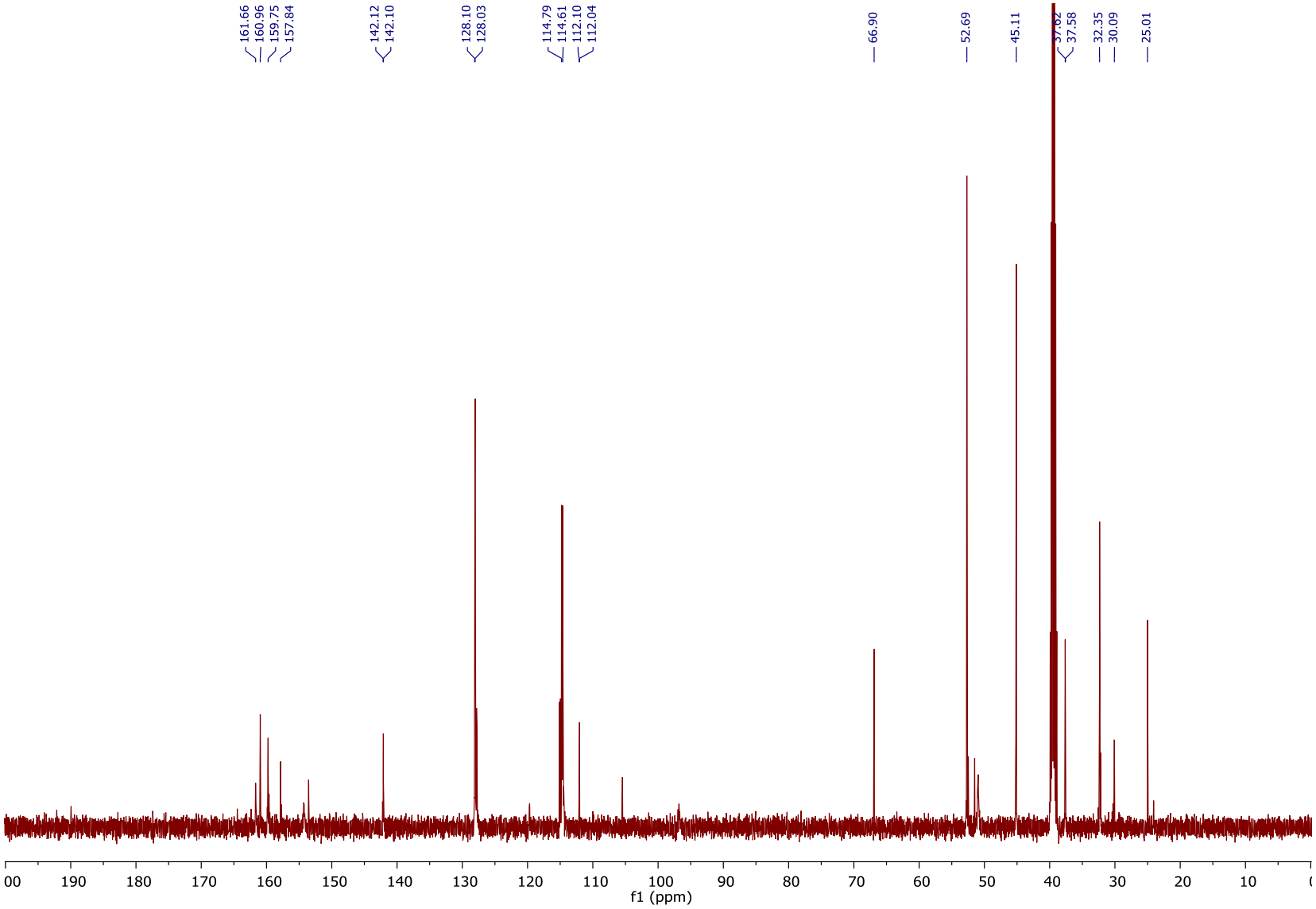
6, ^{13}C, 126 MHz, DMSO-d_6



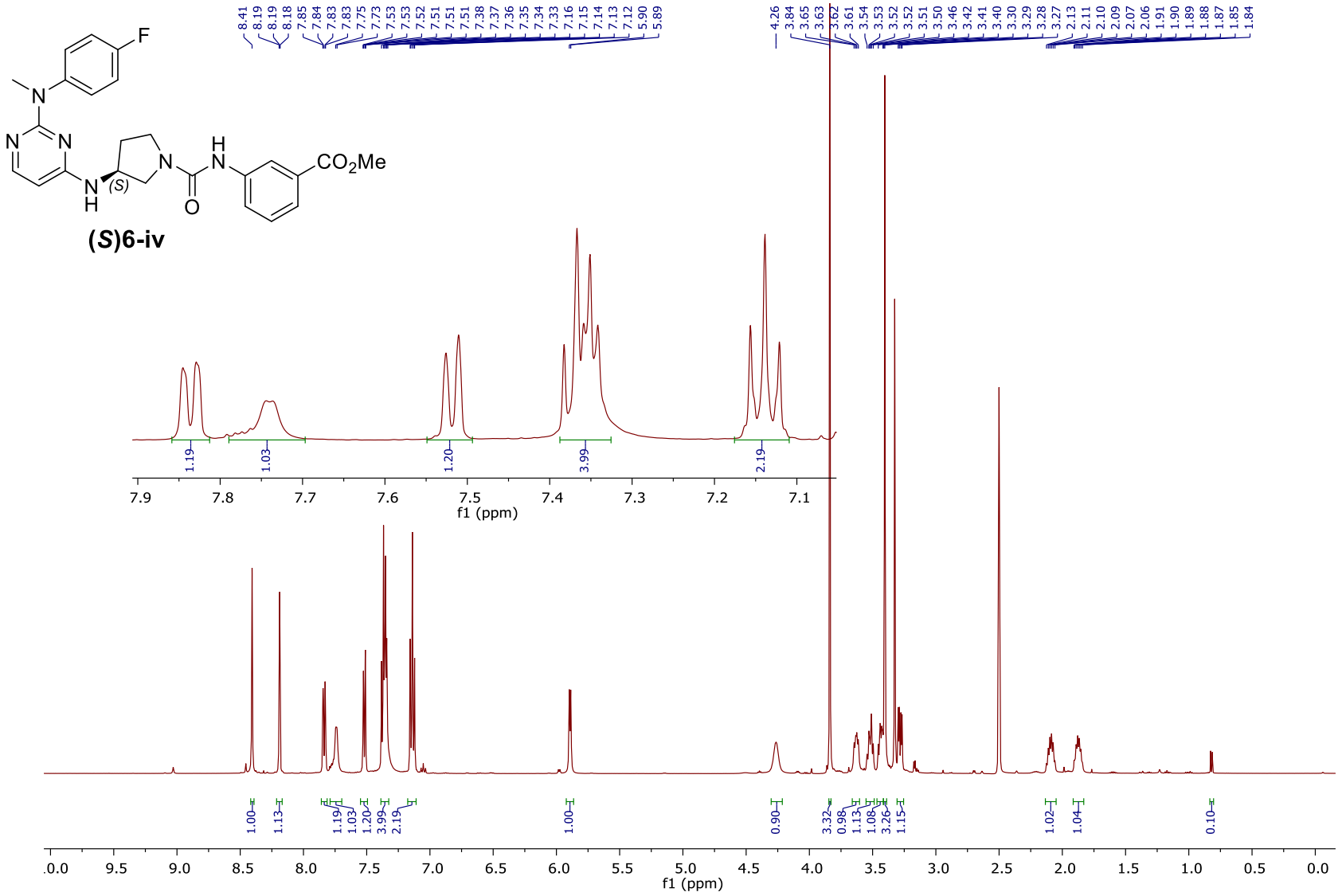
(*S*)6-iii, ^{1}H, 500 MHz, DMSO-d_6



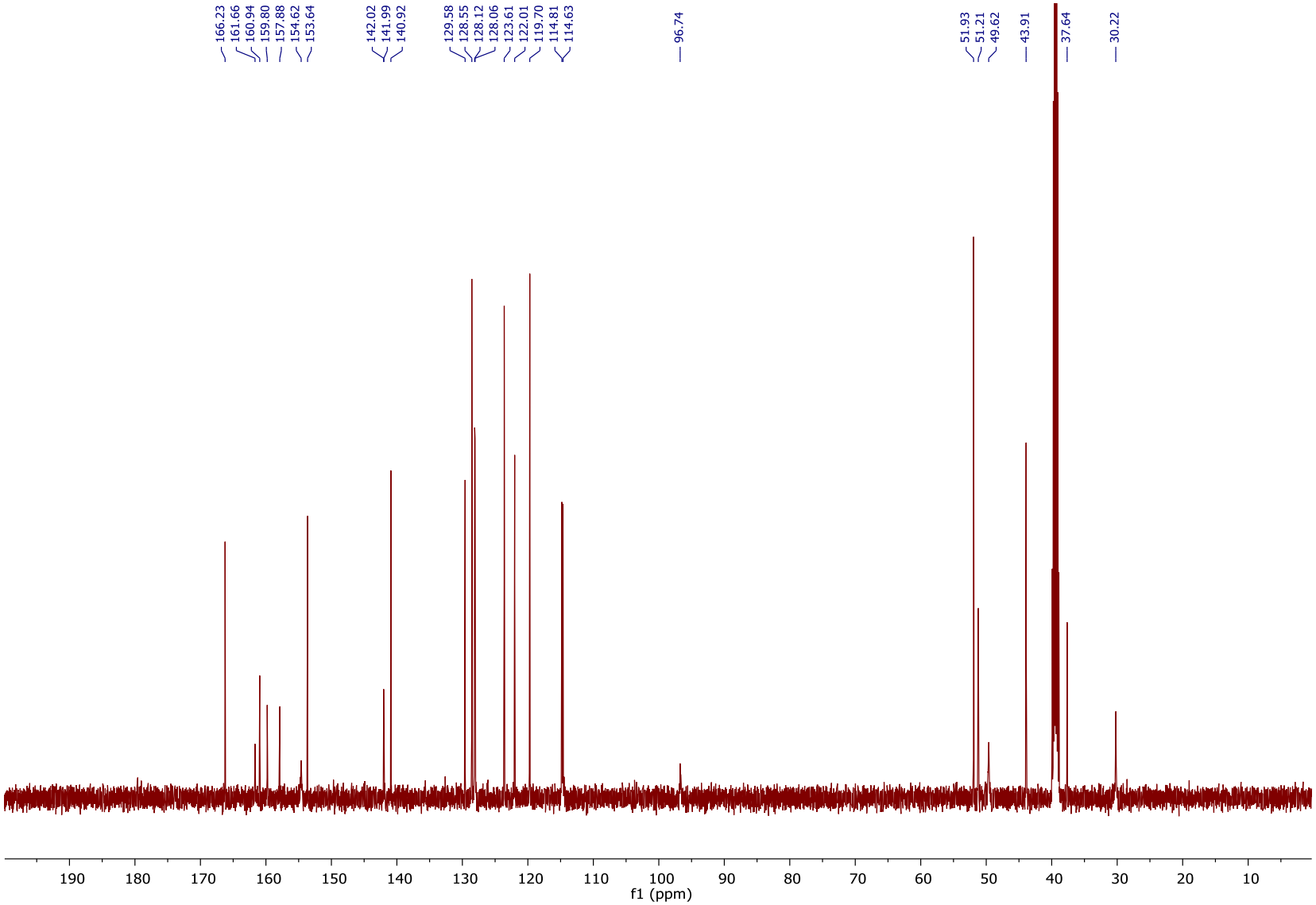
(*S*)6-iii, ^{13}C, 126 MHz, DMSO-d_6



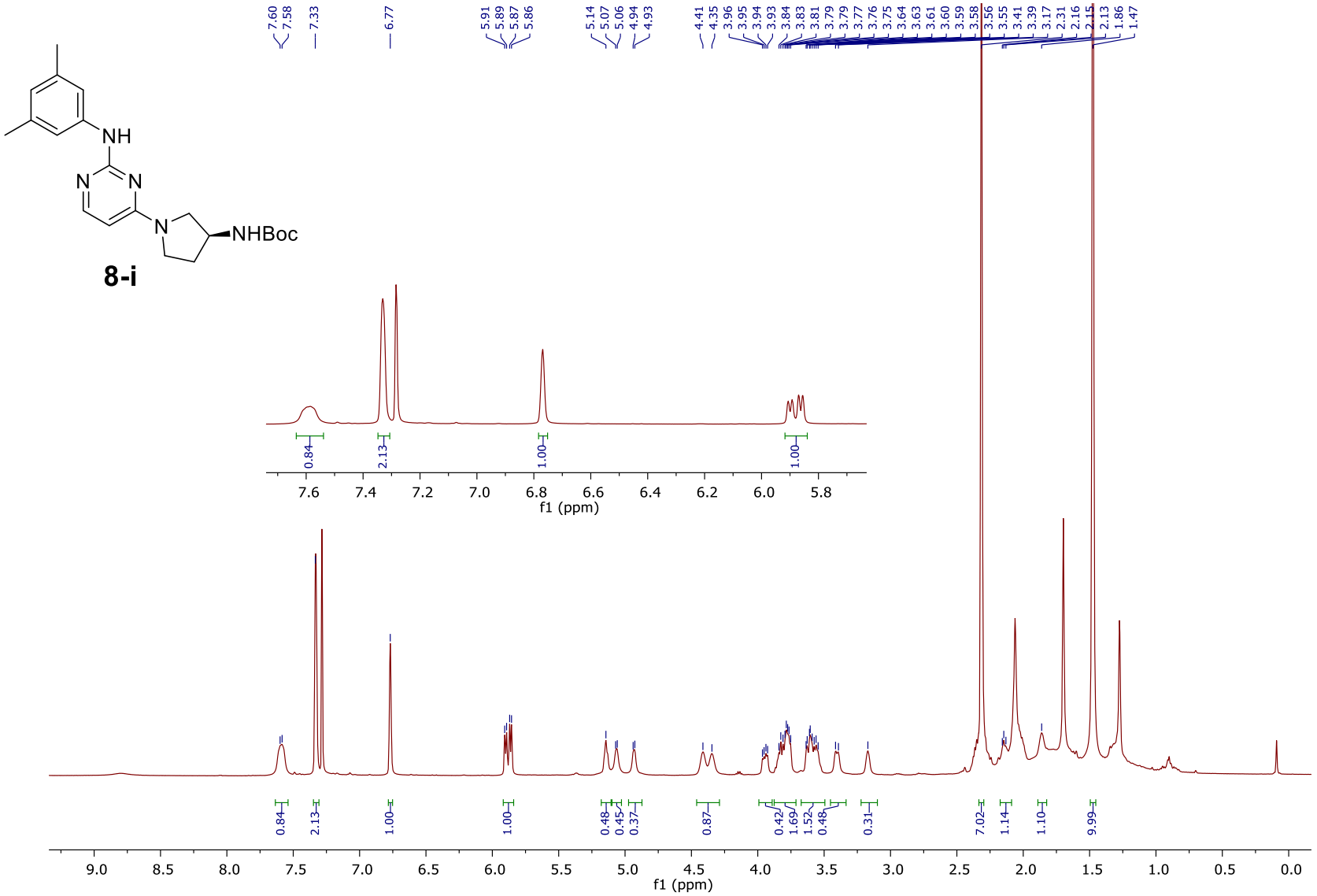
(*S*)6-iv, ^{1}H, 500 MHz, DMSO-d_6



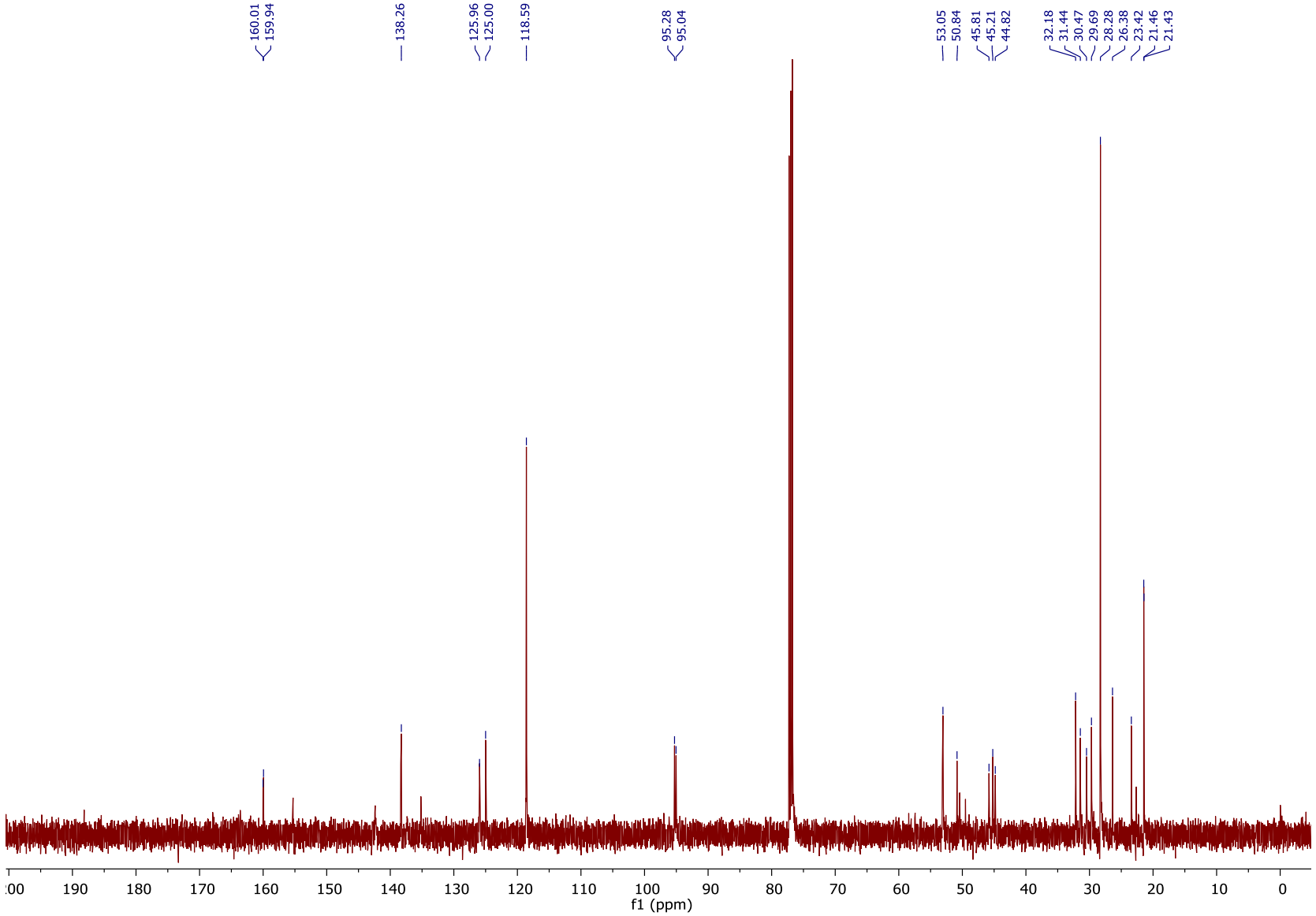
(*S*)6-iv, ^{13}C, 126 MHz, DMSO-d_6



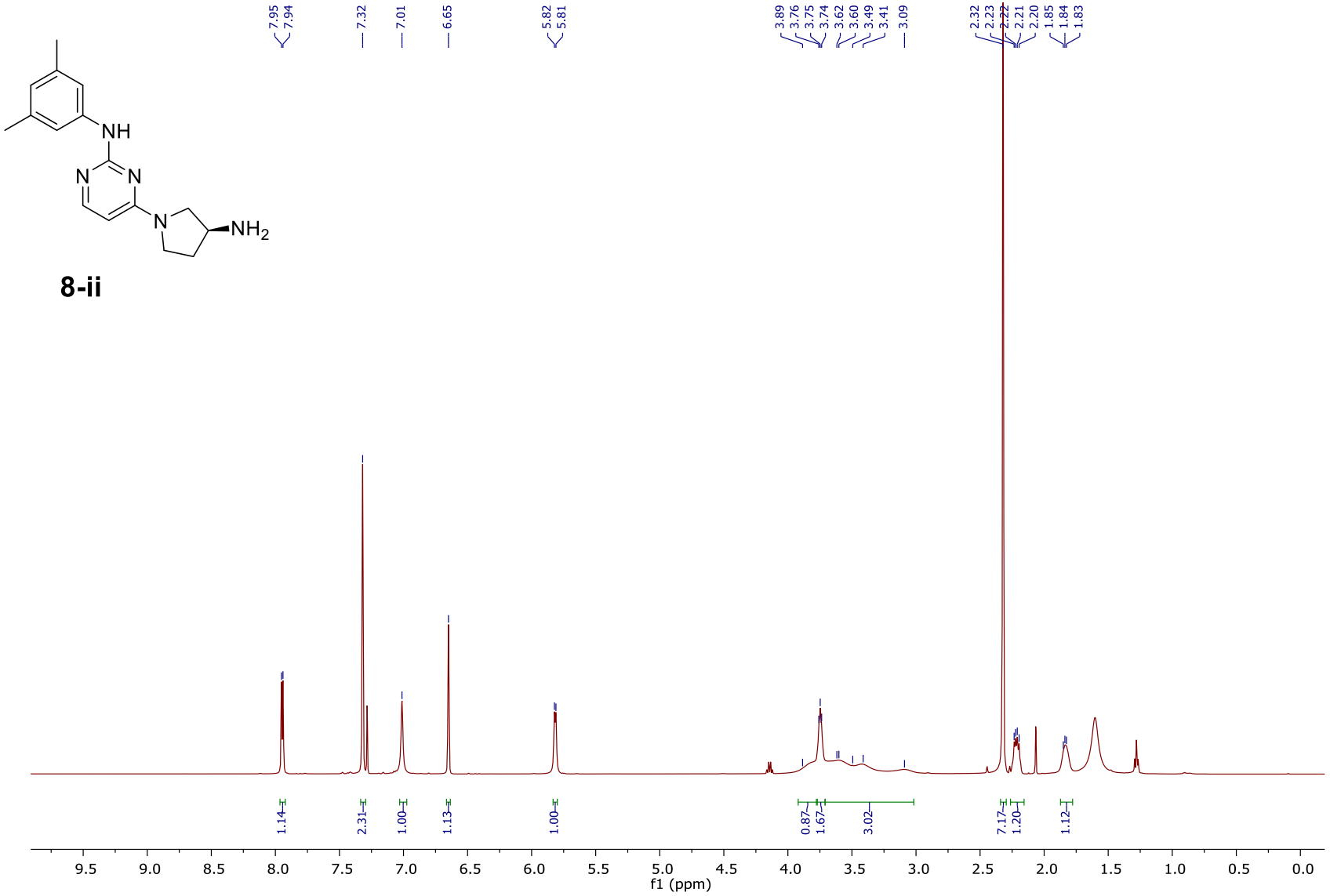
8-i, ^{1}H, 500 MHz, $CDCl_3$



8-i, ^{13}C, 126 MHz, $CDCl_3$

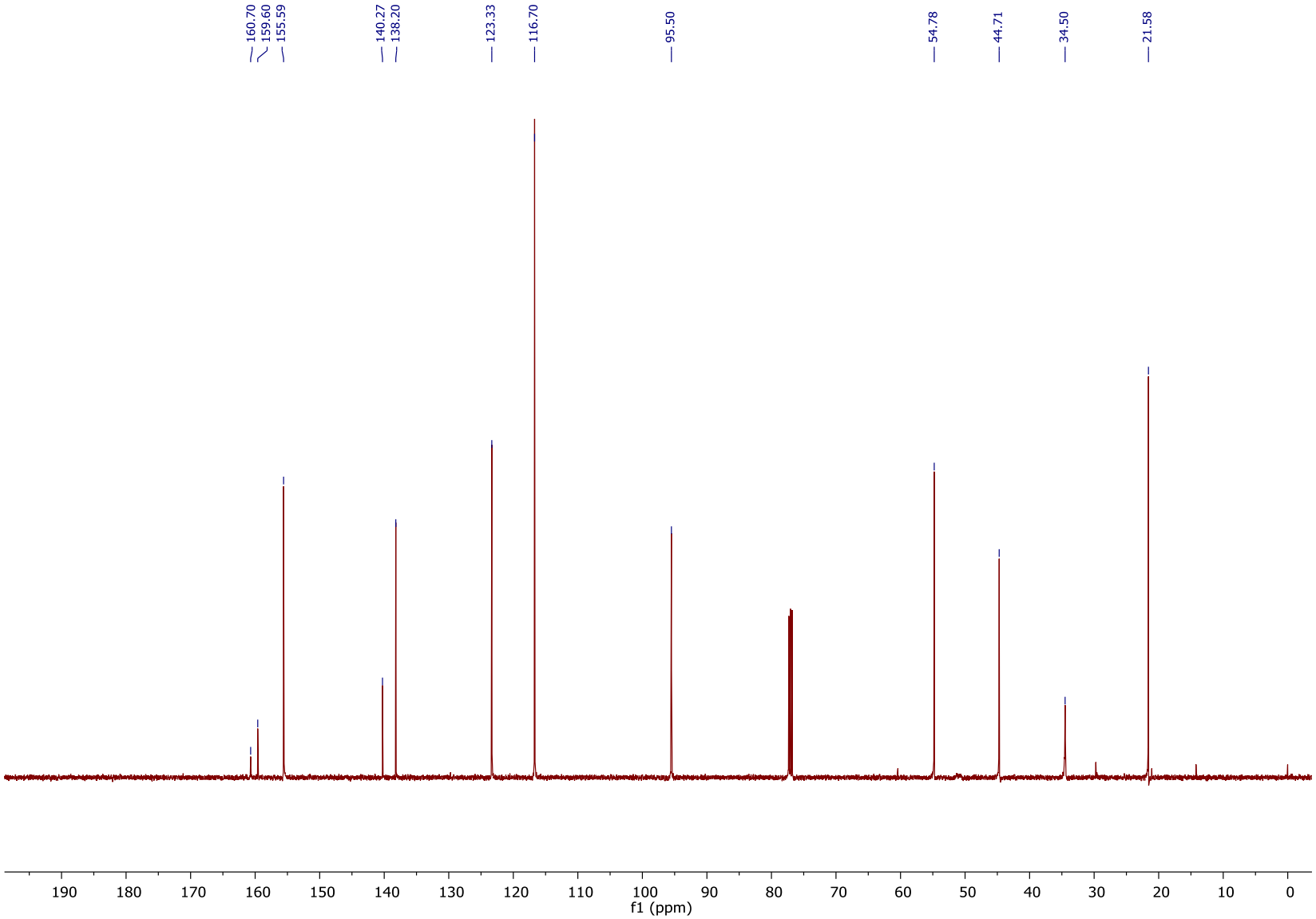


8-ii, ^{1}H, 500 MHz, $CDCl_3$

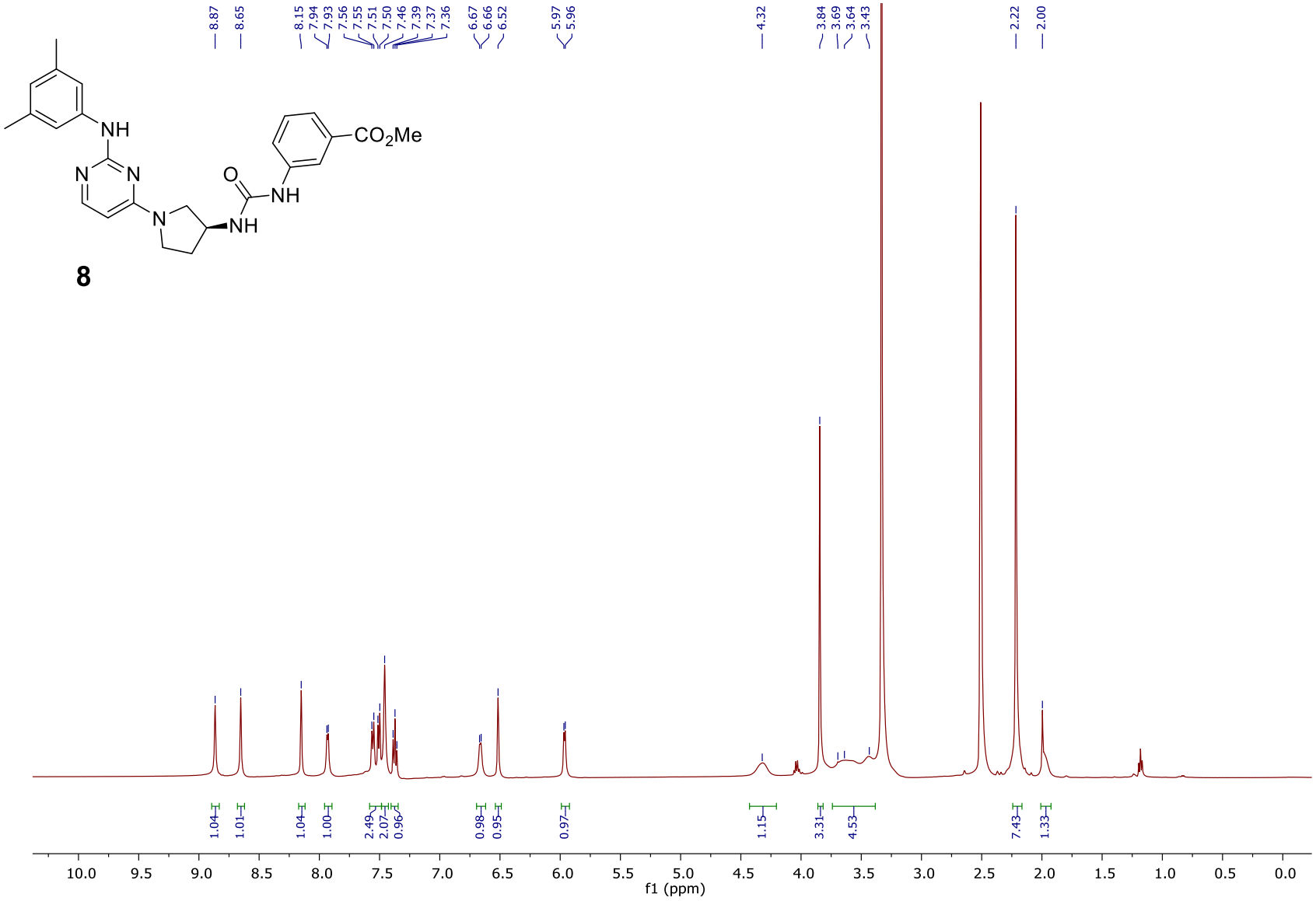


8-ii

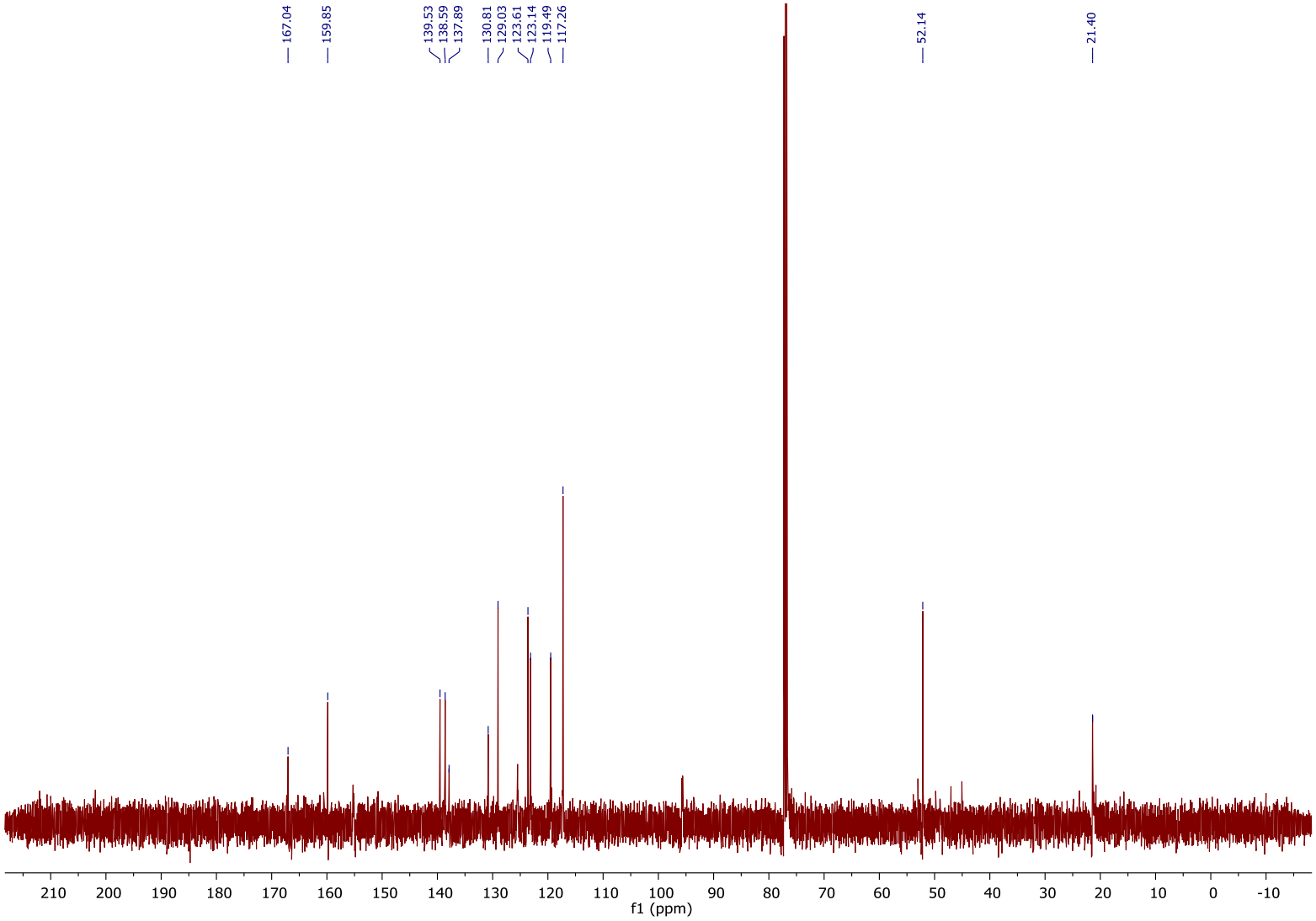
8-ii, ^{13}C, 126 MHz, $CDCl_3$



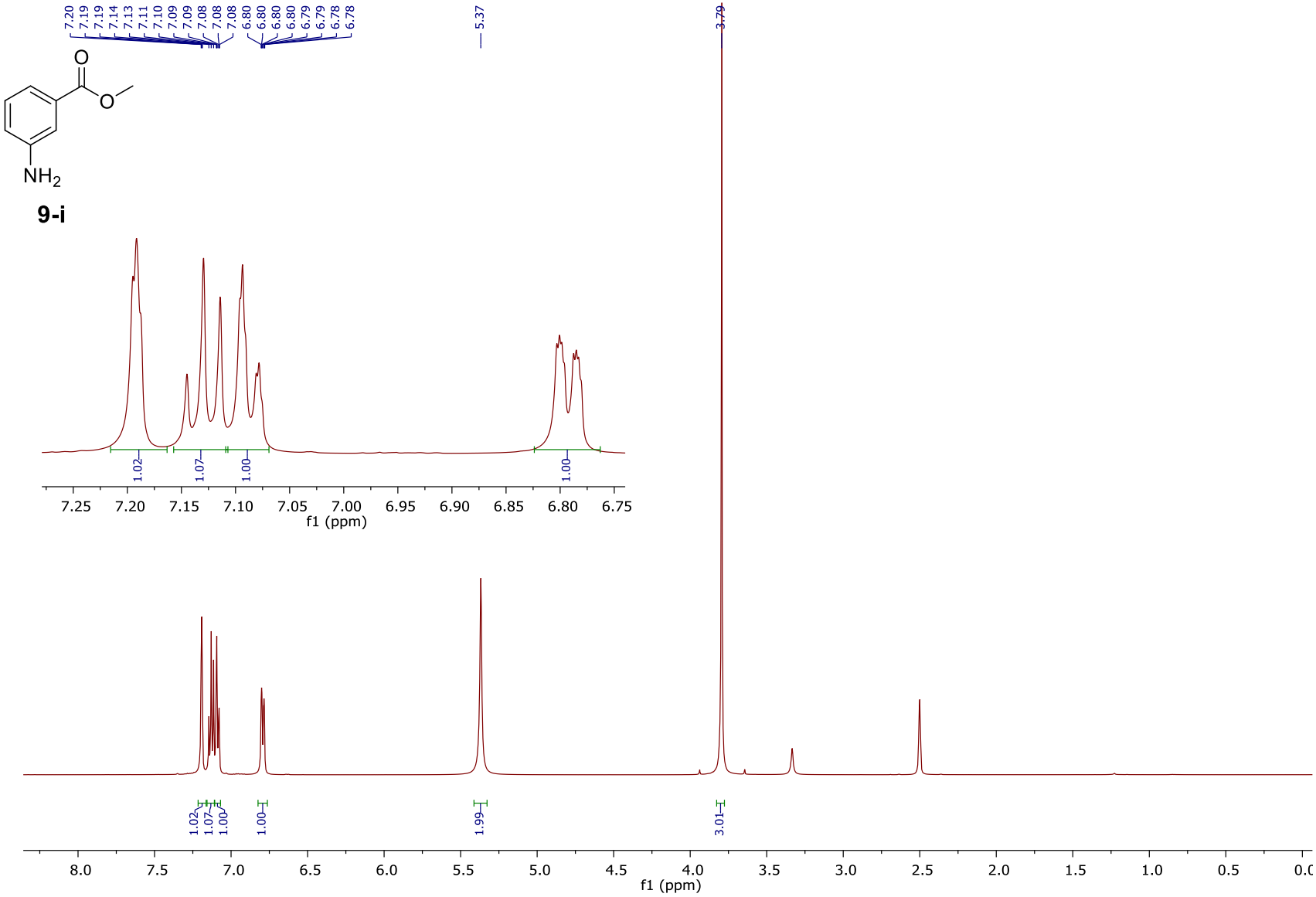
8, ^{1}H, 500 MHz, DMSO-d_6



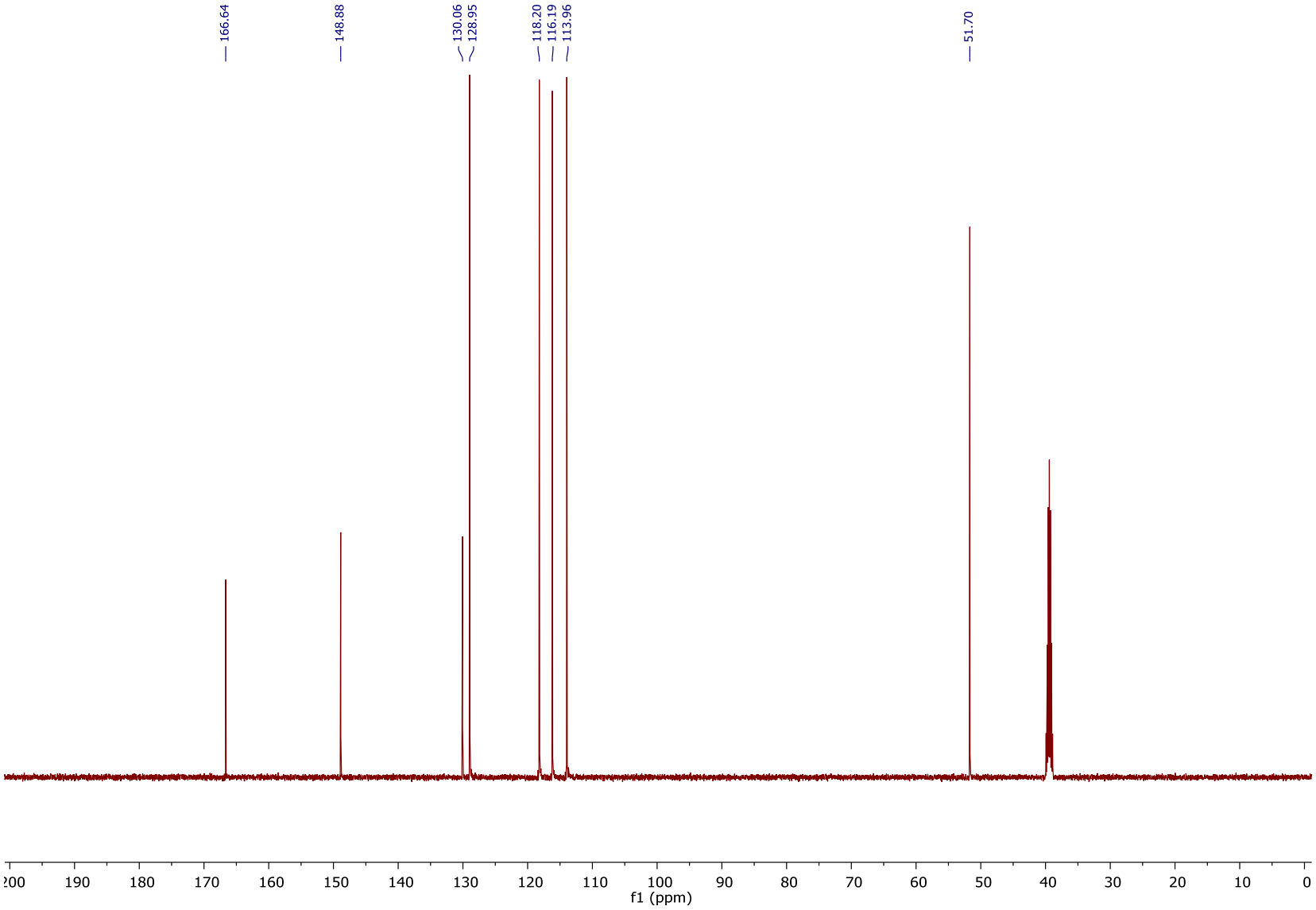
8, ^{13}C, 126 MHz, DMSO-d_6



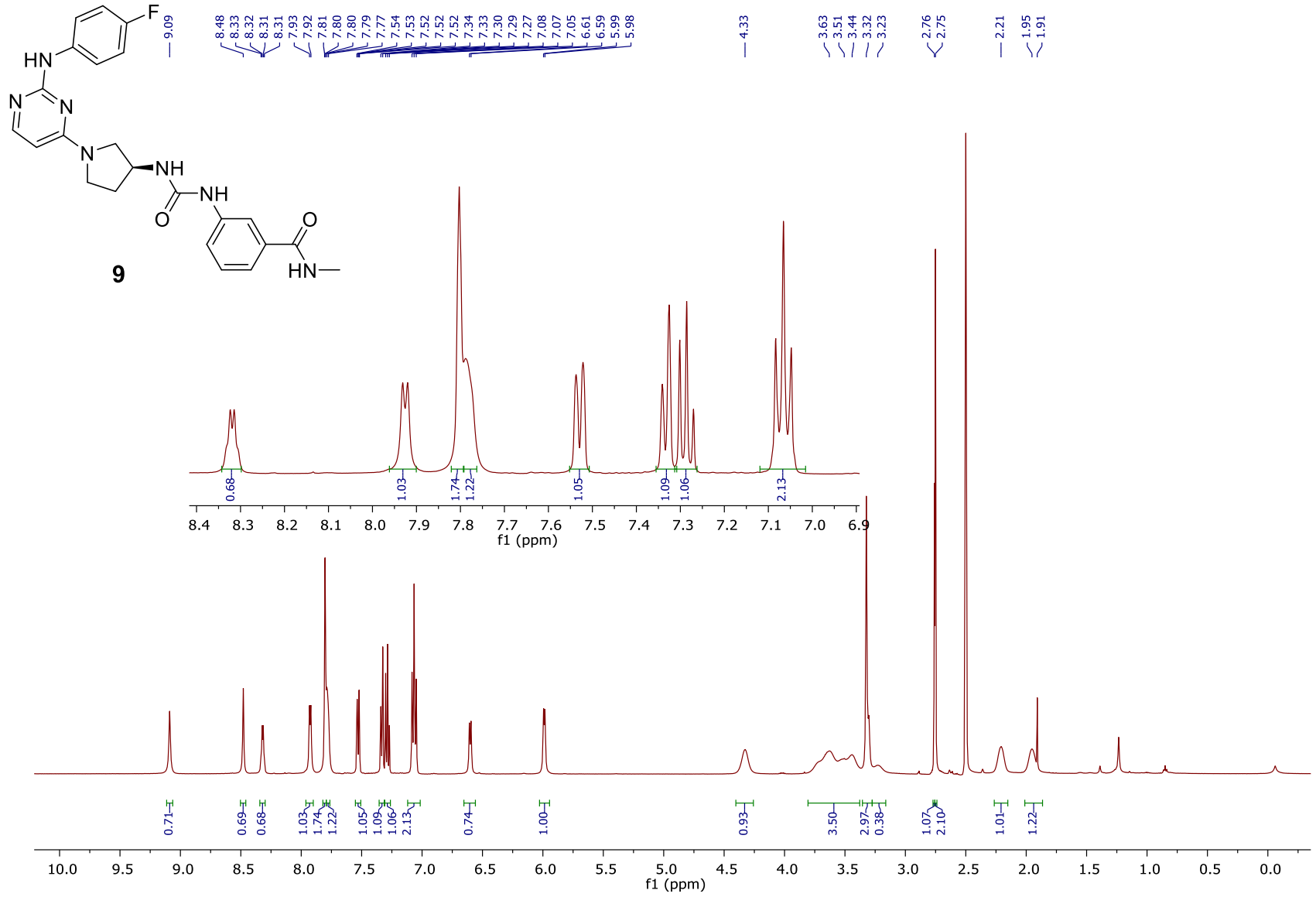
9-i, ^{1}H, 500 MHz, DMSO-d_6



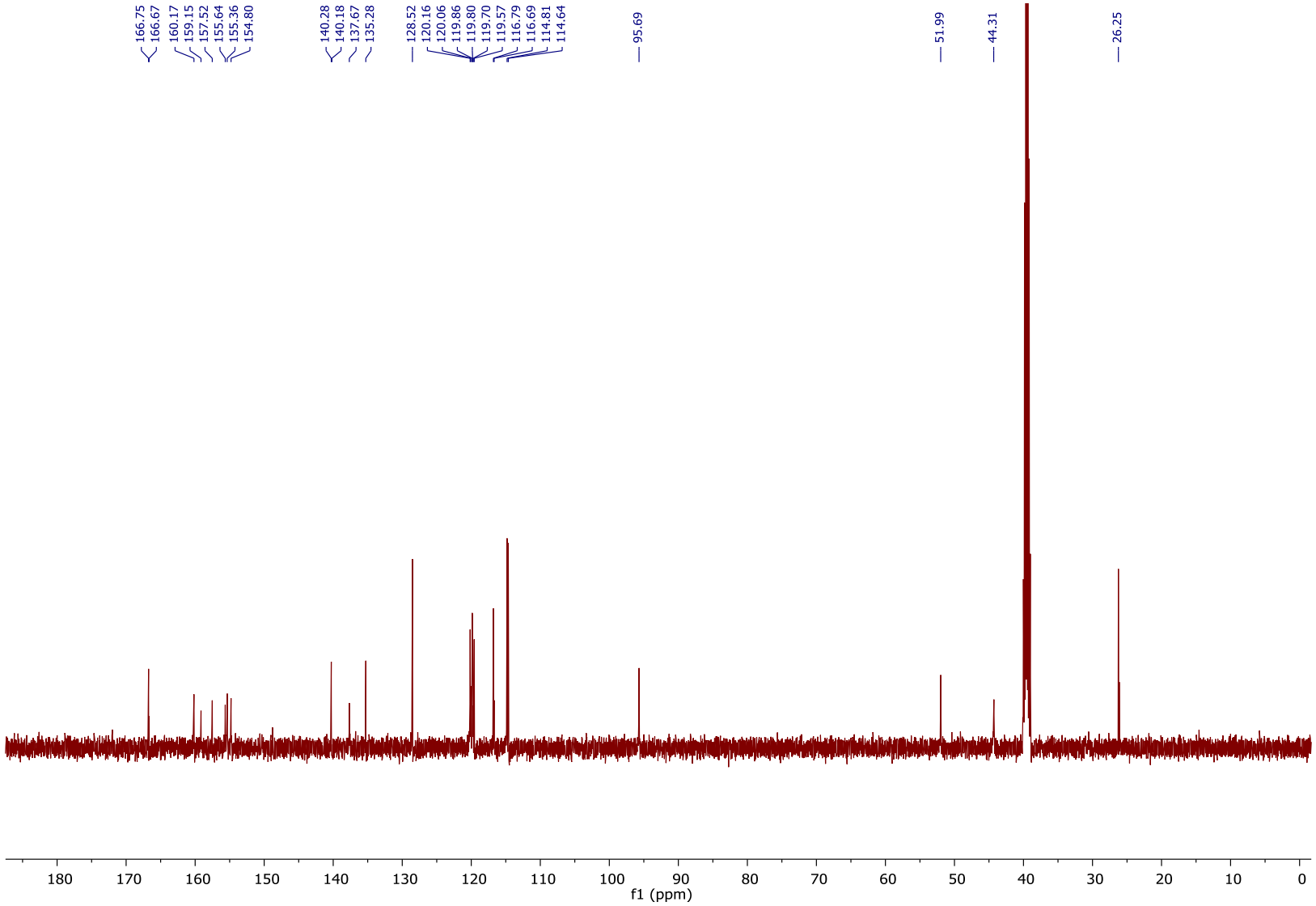
9-i, ^{13}C, 126 MHz, DMSO-d_6



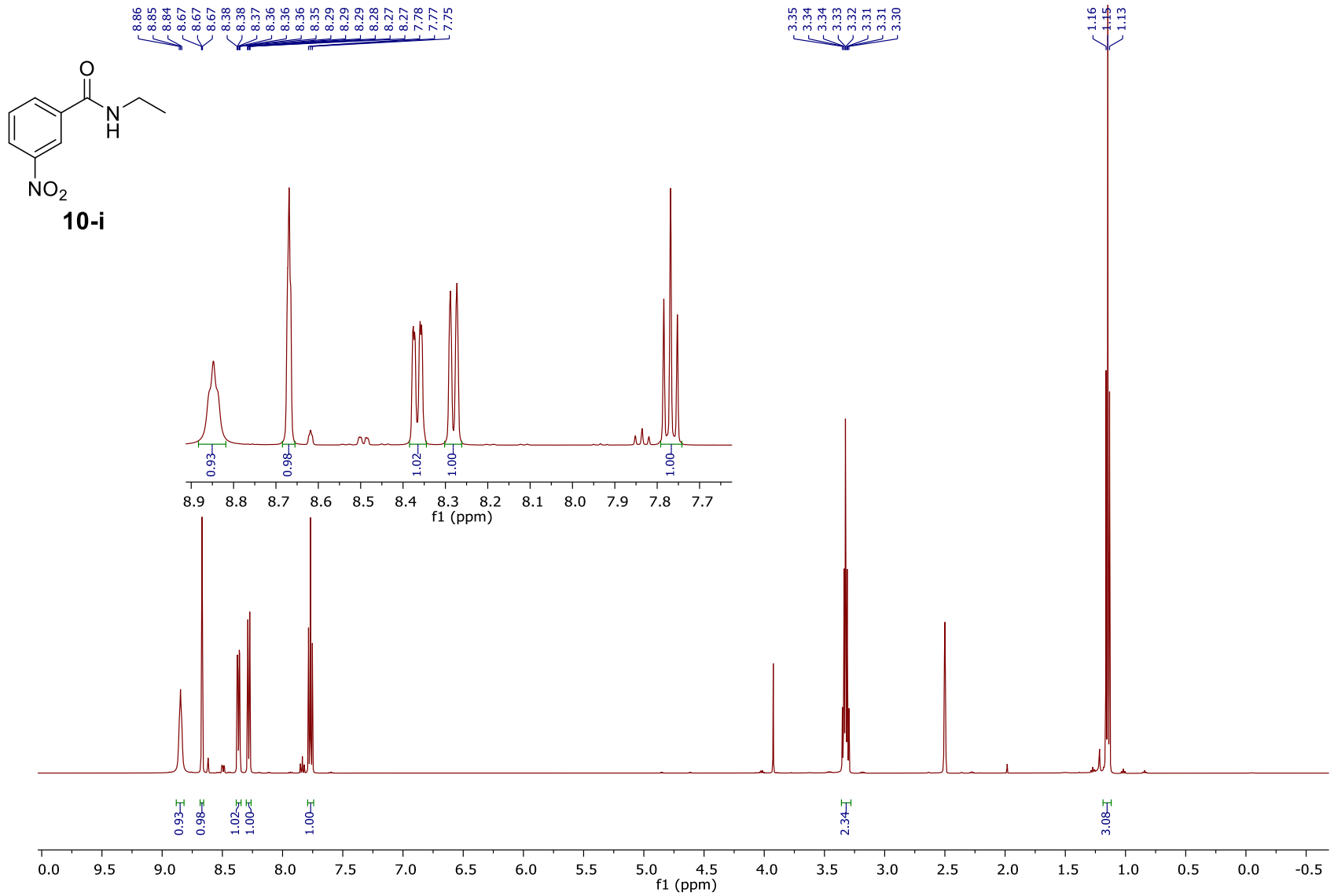
9, ^{1}H, 500 MHz, DMSO-d_6



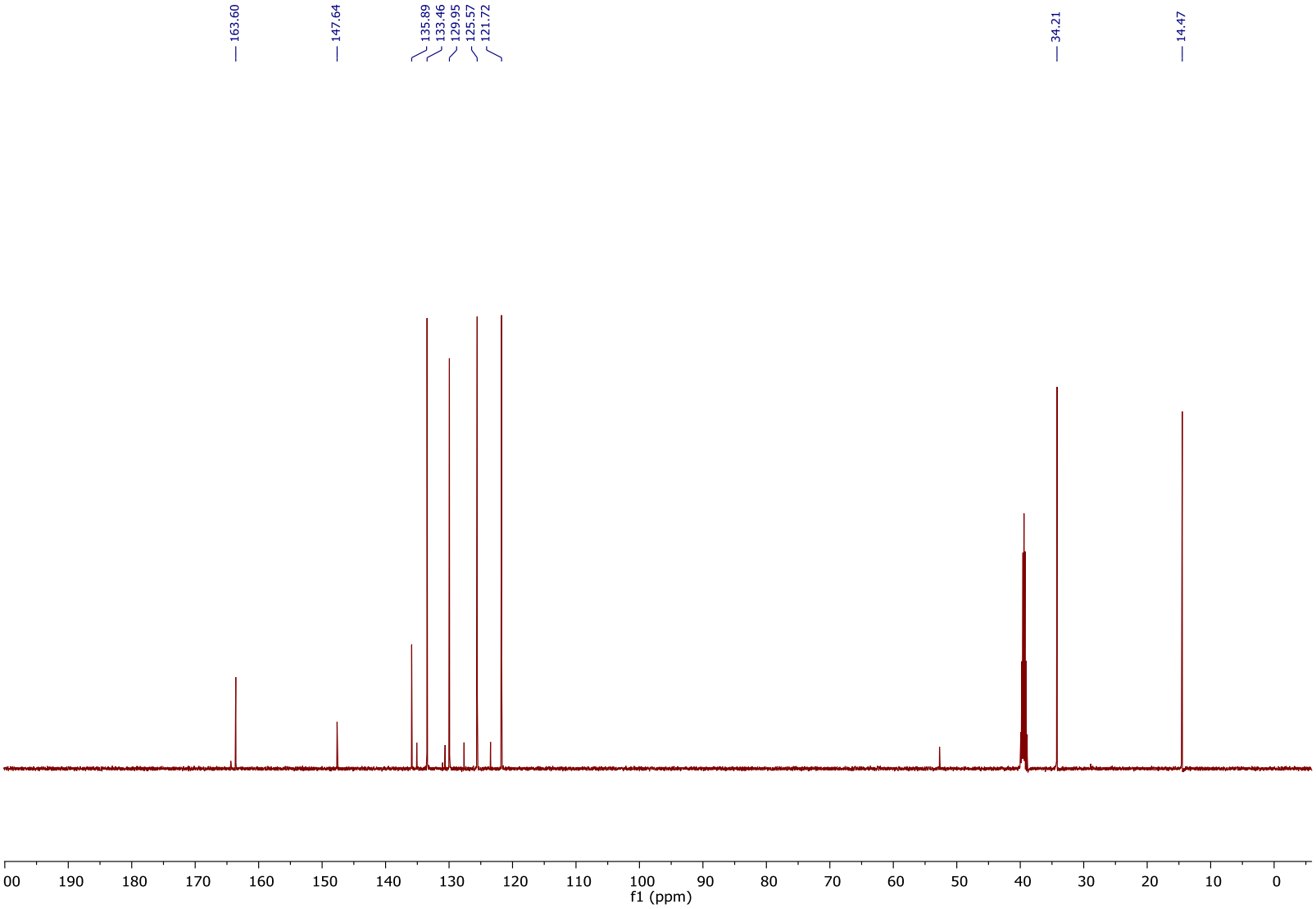
9, ^{13}C, 126 MHz, DMSO-d_6



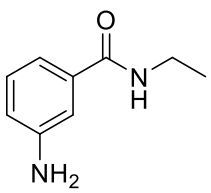
10-i, ^{1}H, 500 MHz, DMSO-d_6



10-i, ^{13}C, 126 MHz, DMSO-d_6

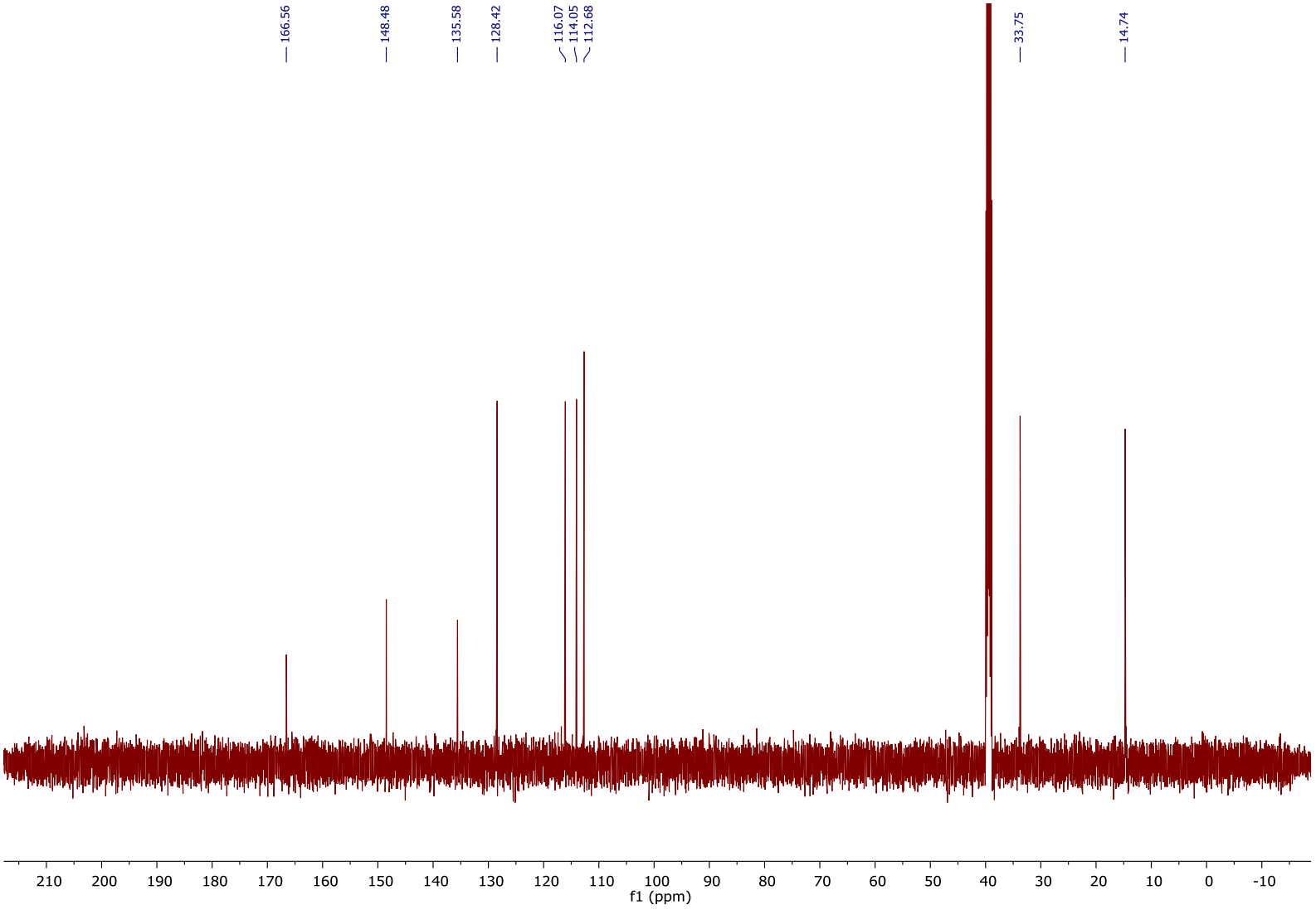


10-ii, ^{1}H, 500 MHz, DMSO-d_6

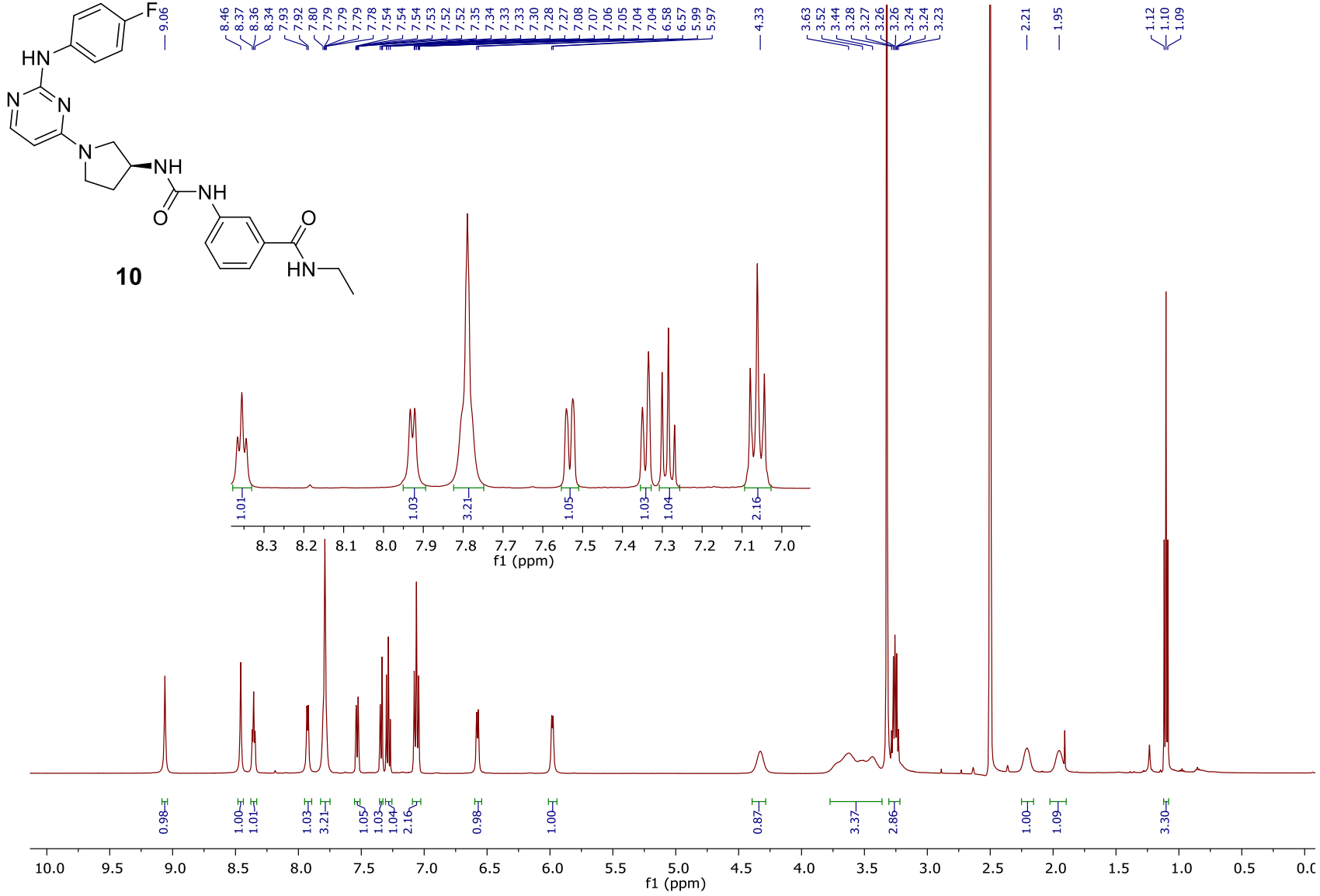


10-ii

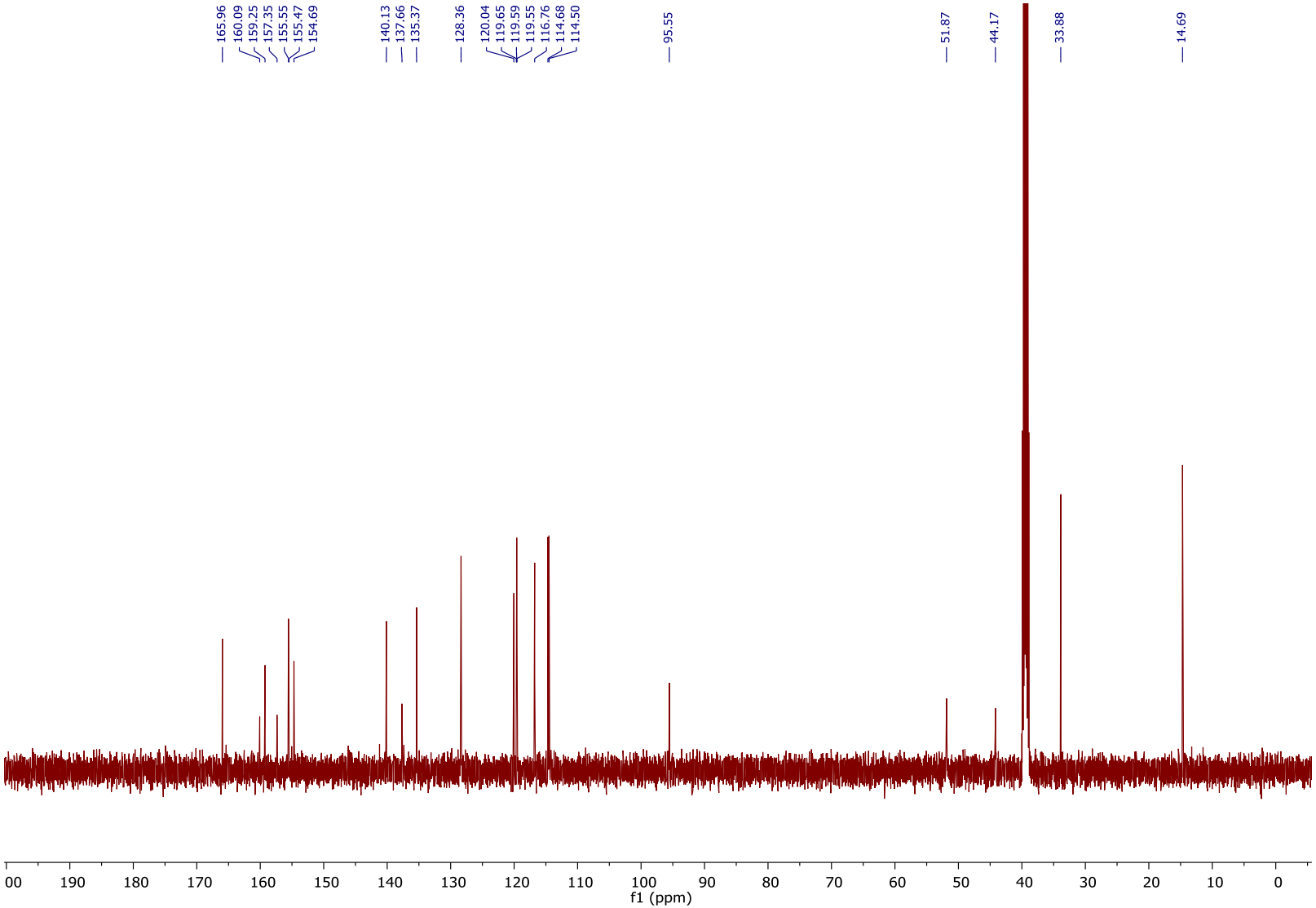
10-ii, ^{13}C, 126 MHz, DMSO-d_6



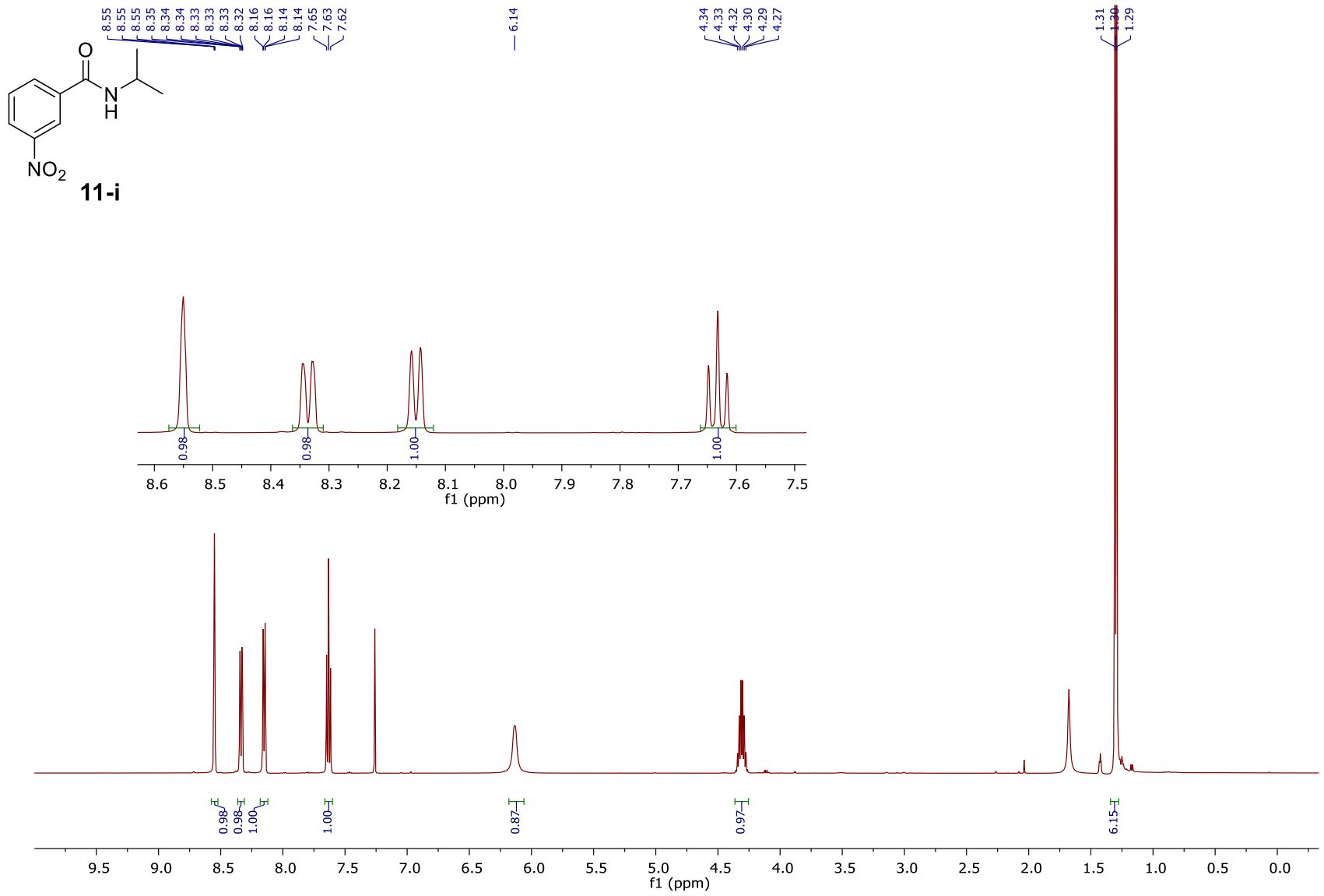
10, ^{1}H, 500 MHz, DMSO-d_6



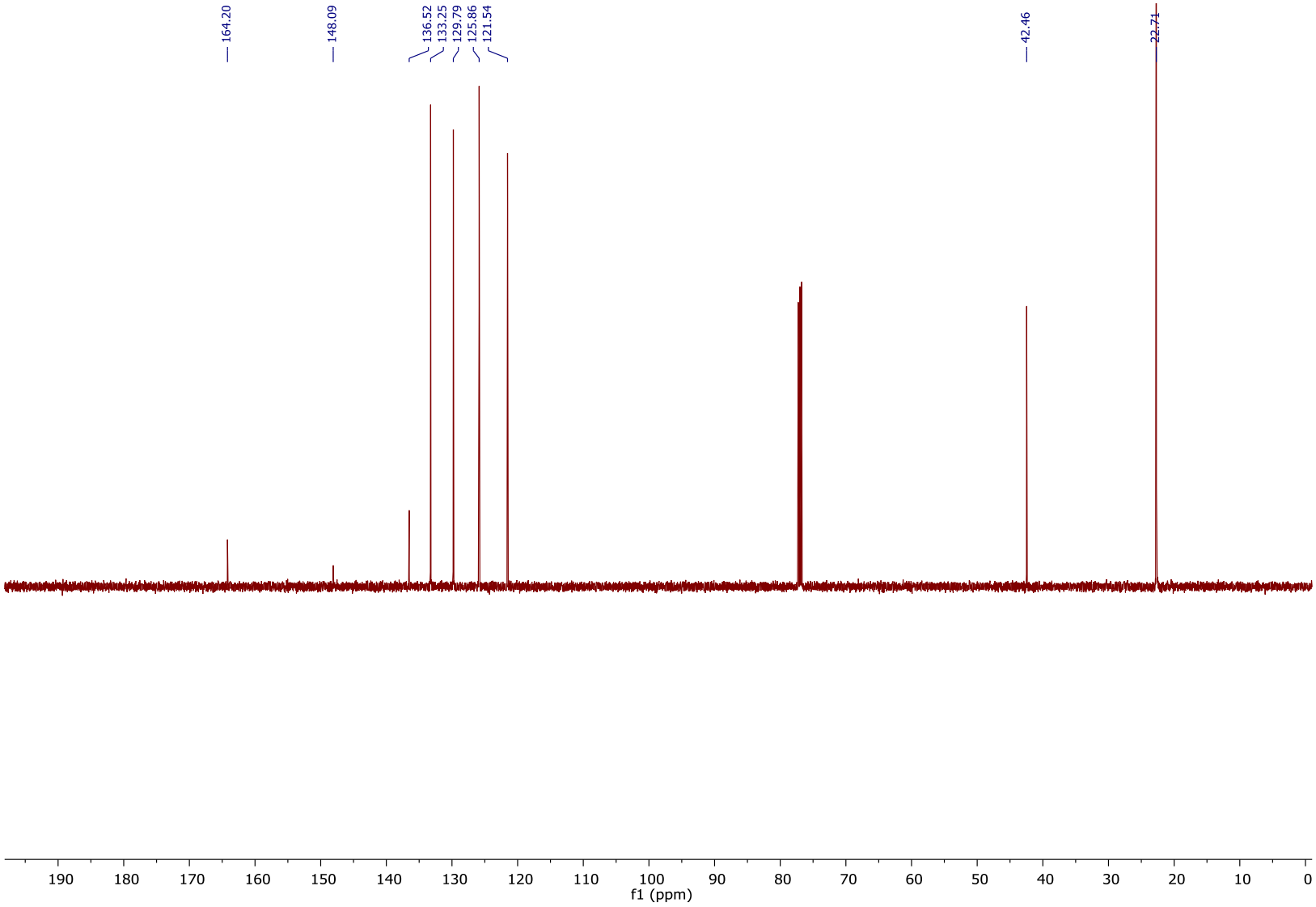
10, ^{13}C, 126 MHz, DMSO-d_6



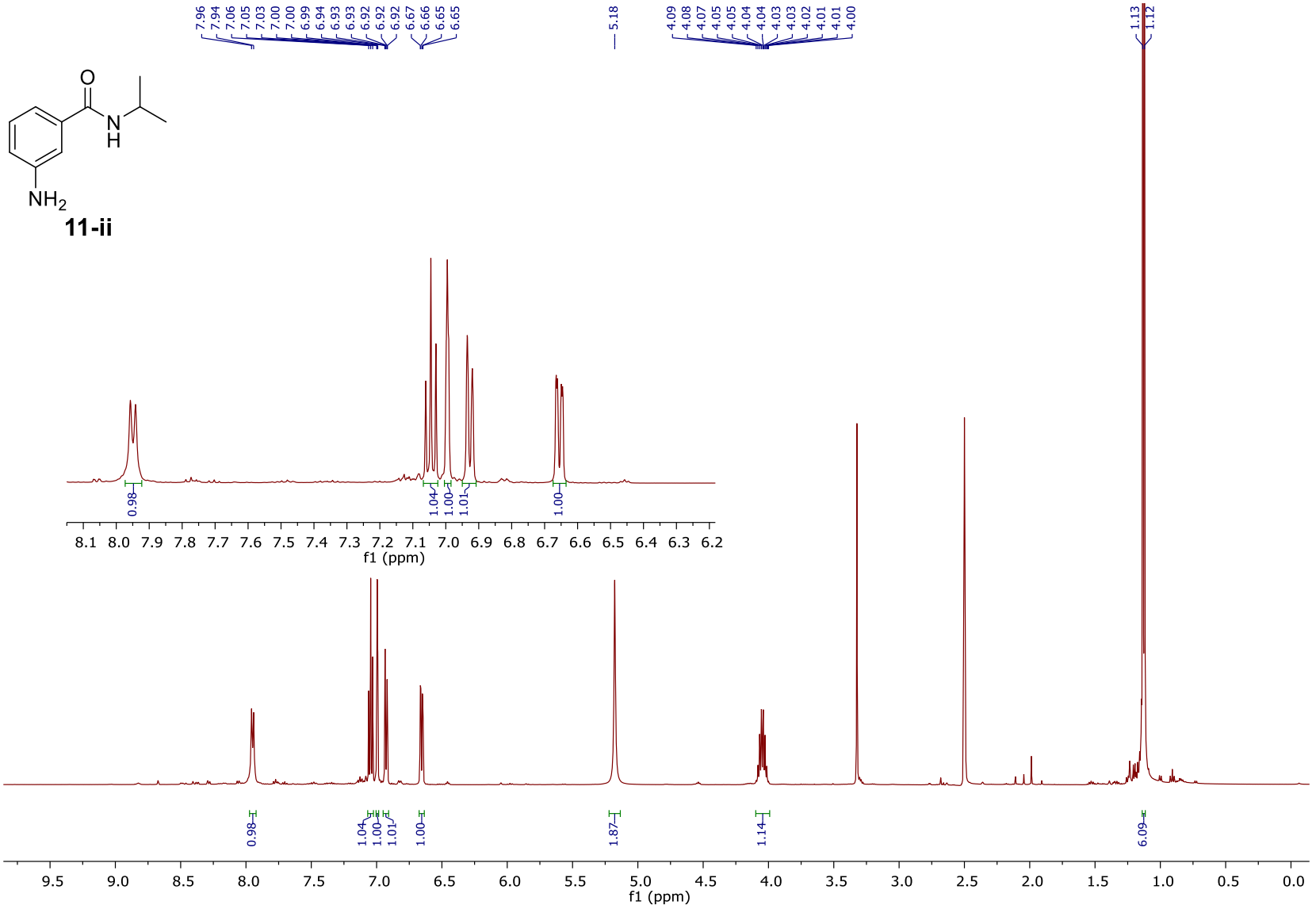
11-i, ^{1}H, 500 MHz, $CDCl_3$



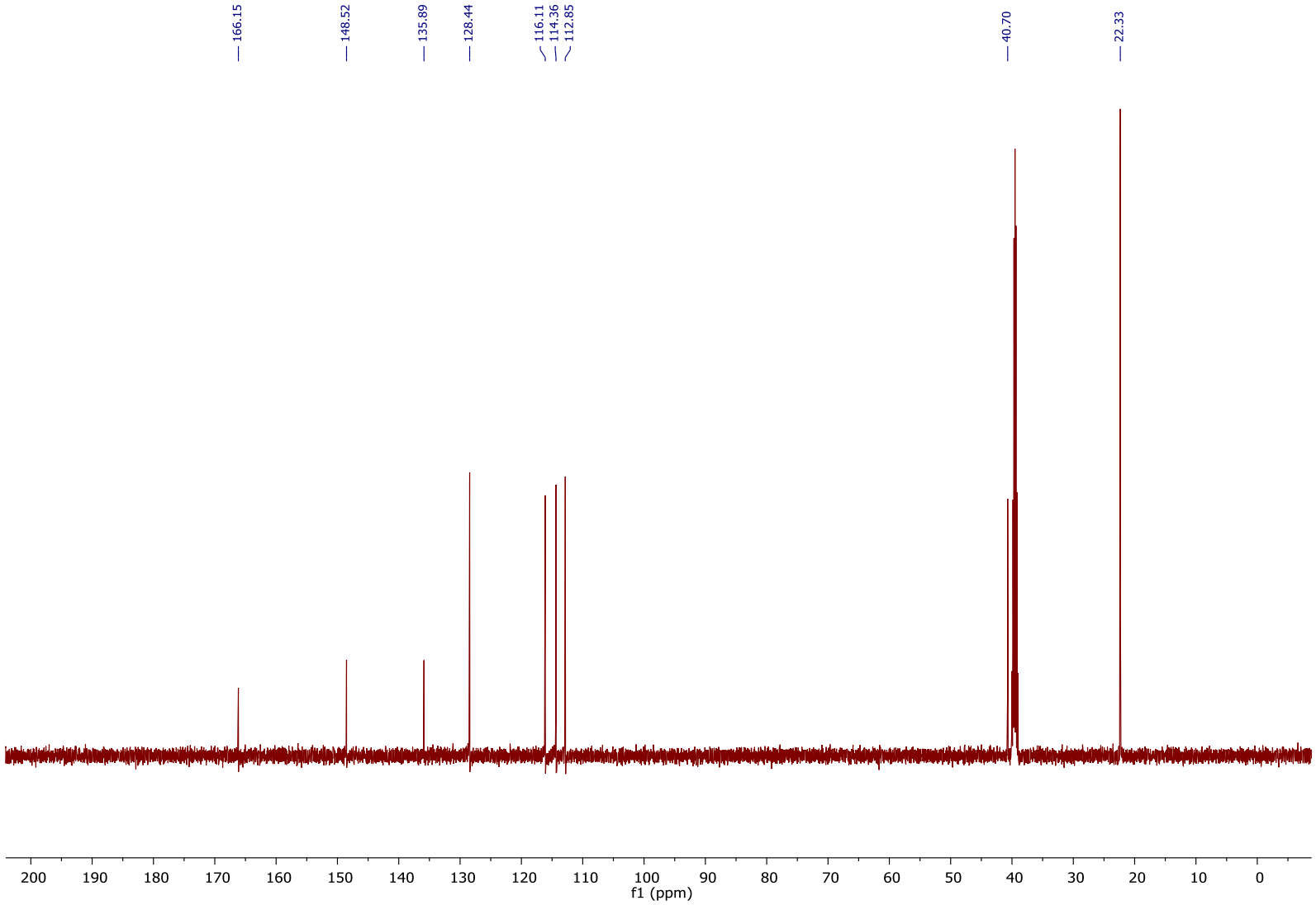
11-i, ^{13}C, 126 MHz, $CDCl_3$



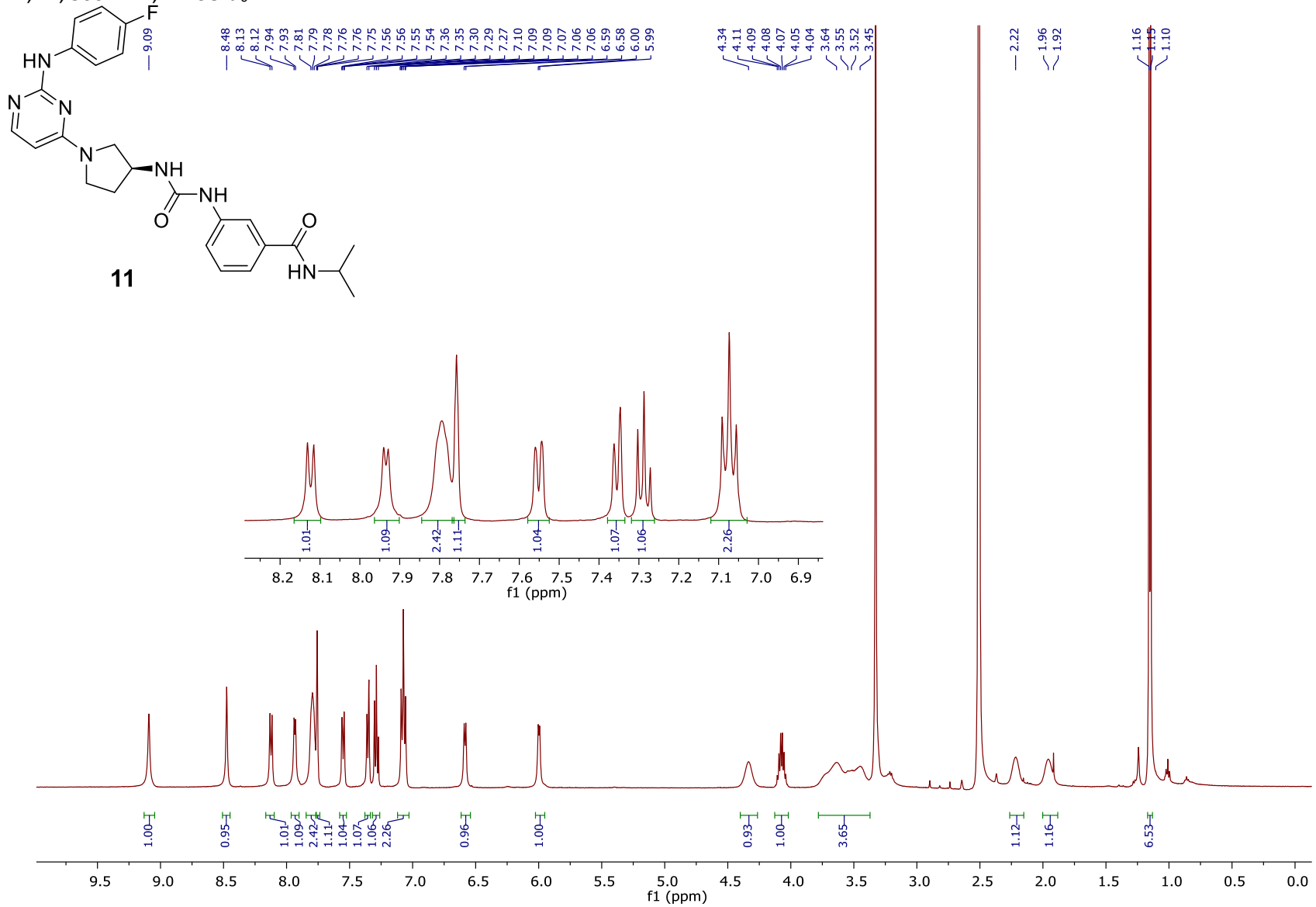
11-ii, ^{1}H, 500 MHz, DMSO-d_6



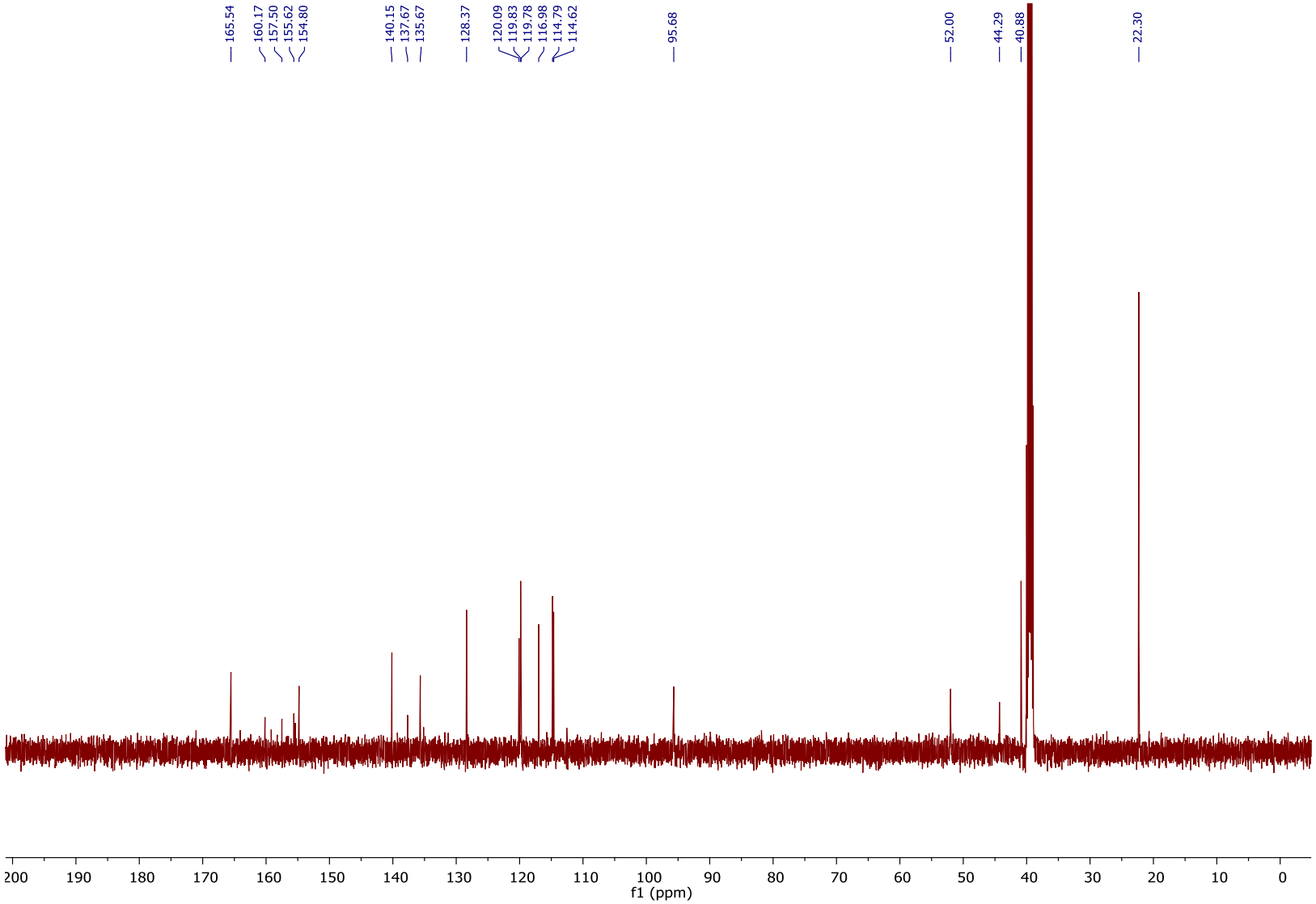
11-ii, ^{13}C, 126 MHz, DMSO-d_6



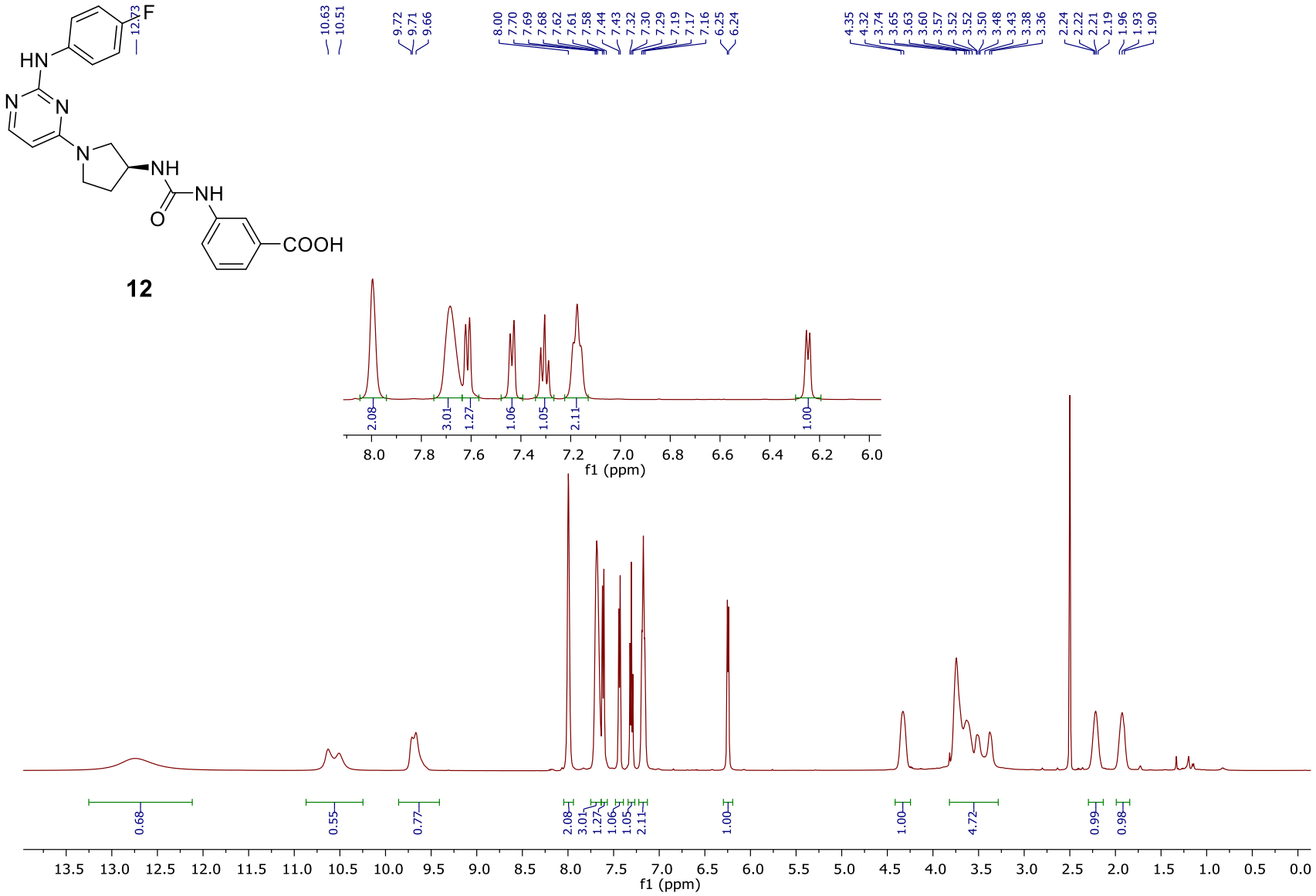
11, ^{1}H, 500 MHz, DMSO-d_6



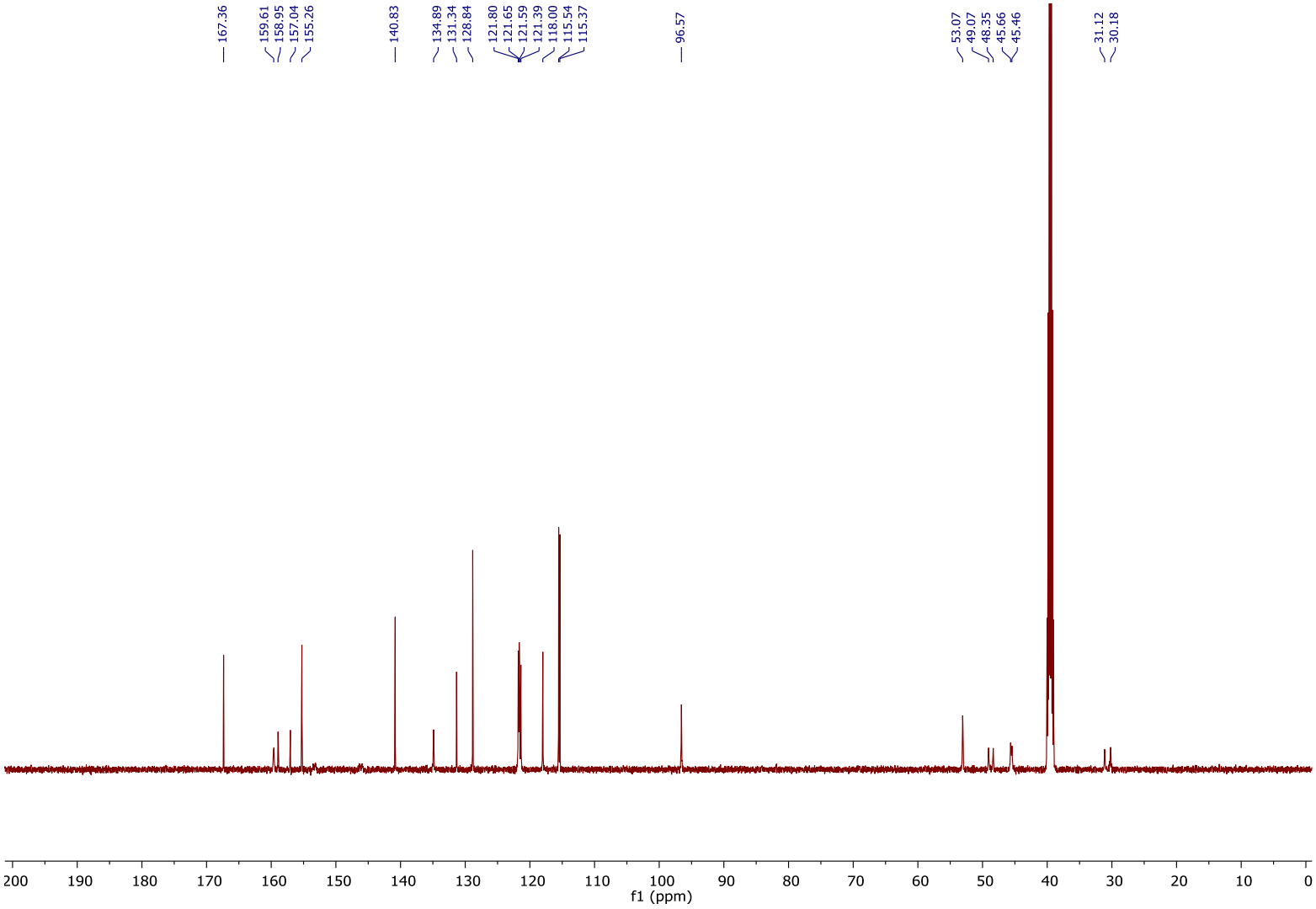
11, ^{13}C, 126 MHz, DMSO-d_6



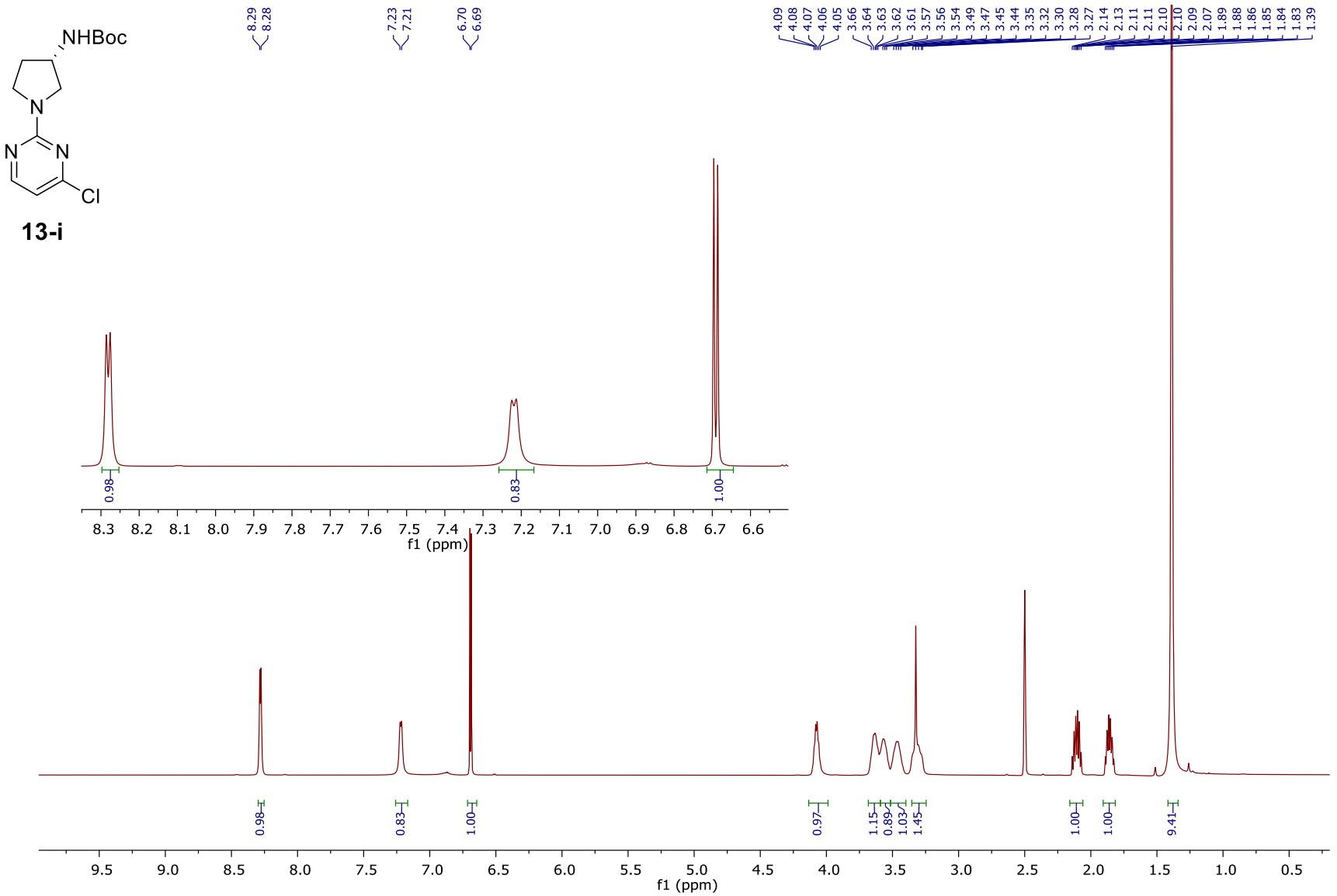
12, ^{1}H, 500 MHz, DMSO-d_6



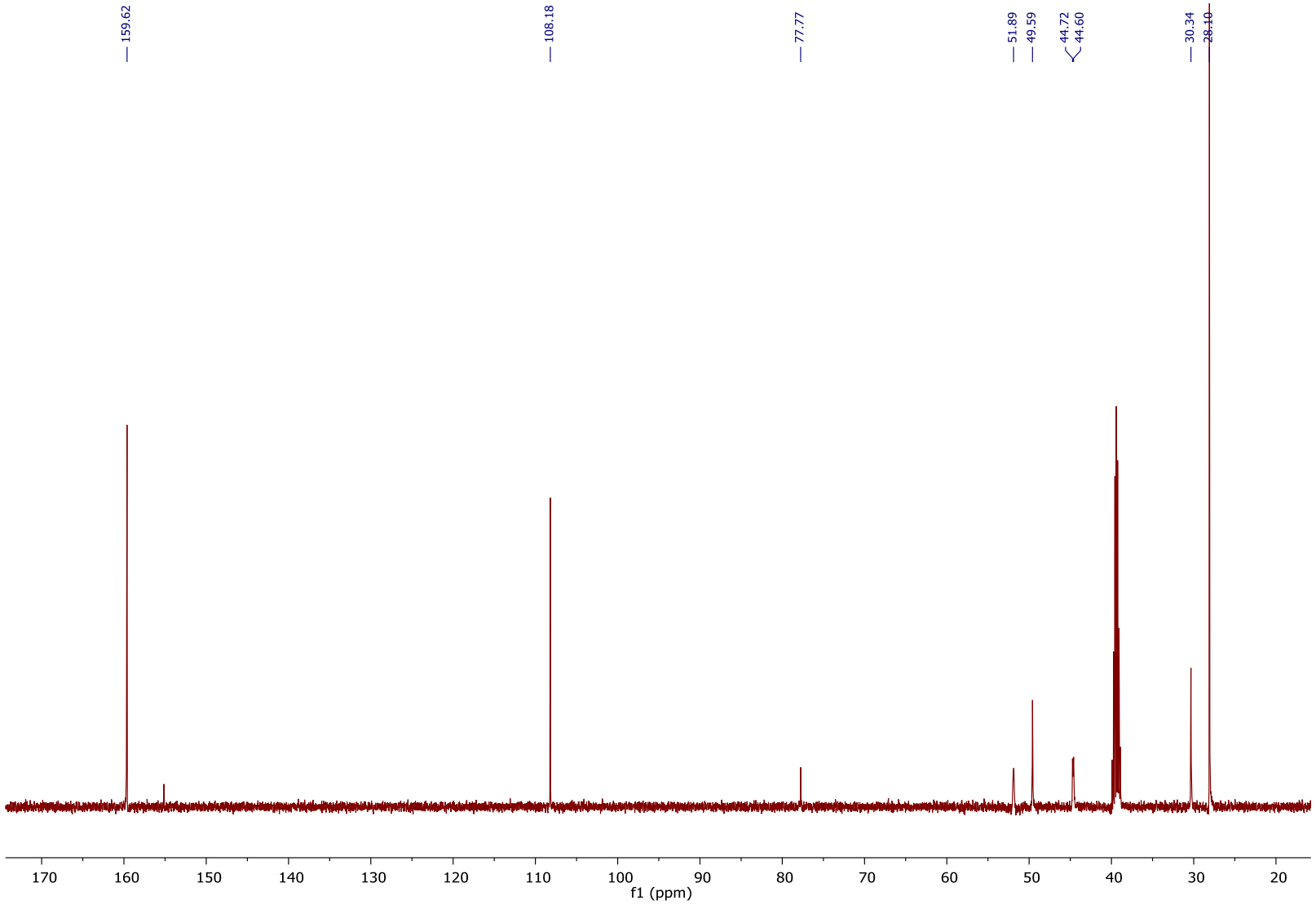
12, ^{13}C, 126 MHz, DMSO-d_6



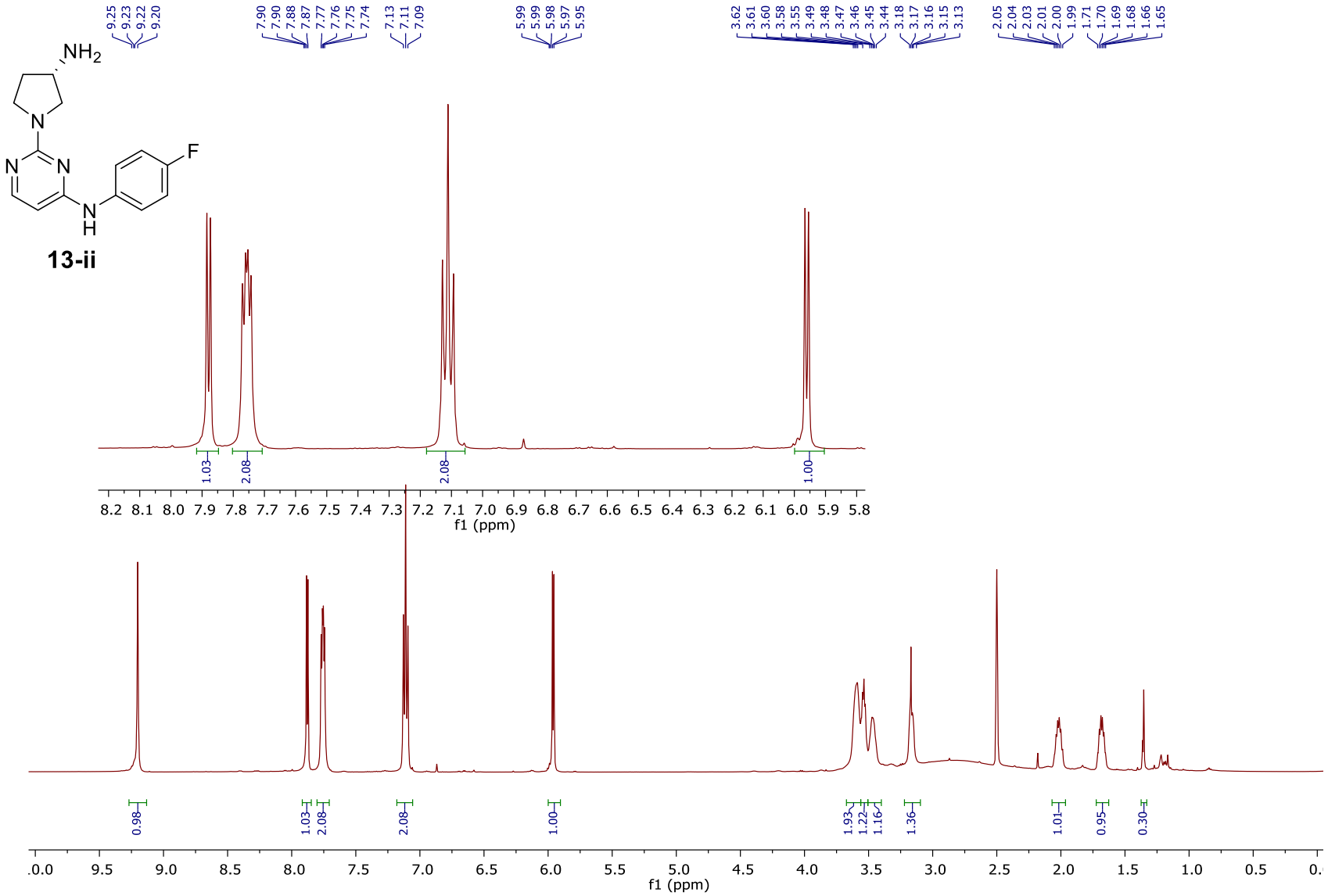
13-i, ^{1}H, 500 MHz, DMSO-d_6



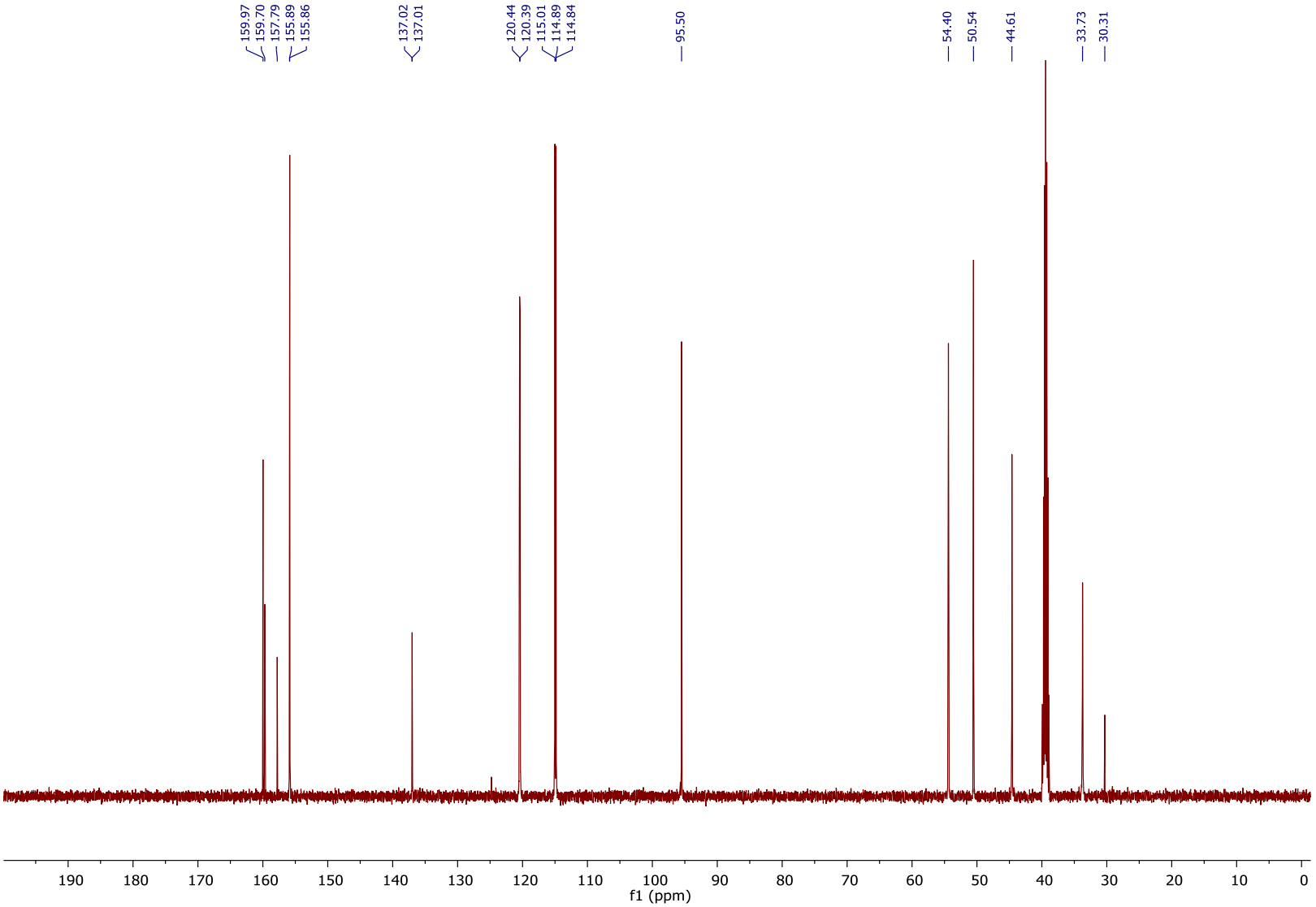
13-i, ^{13}C, 126 MHz, DMSO-d_6



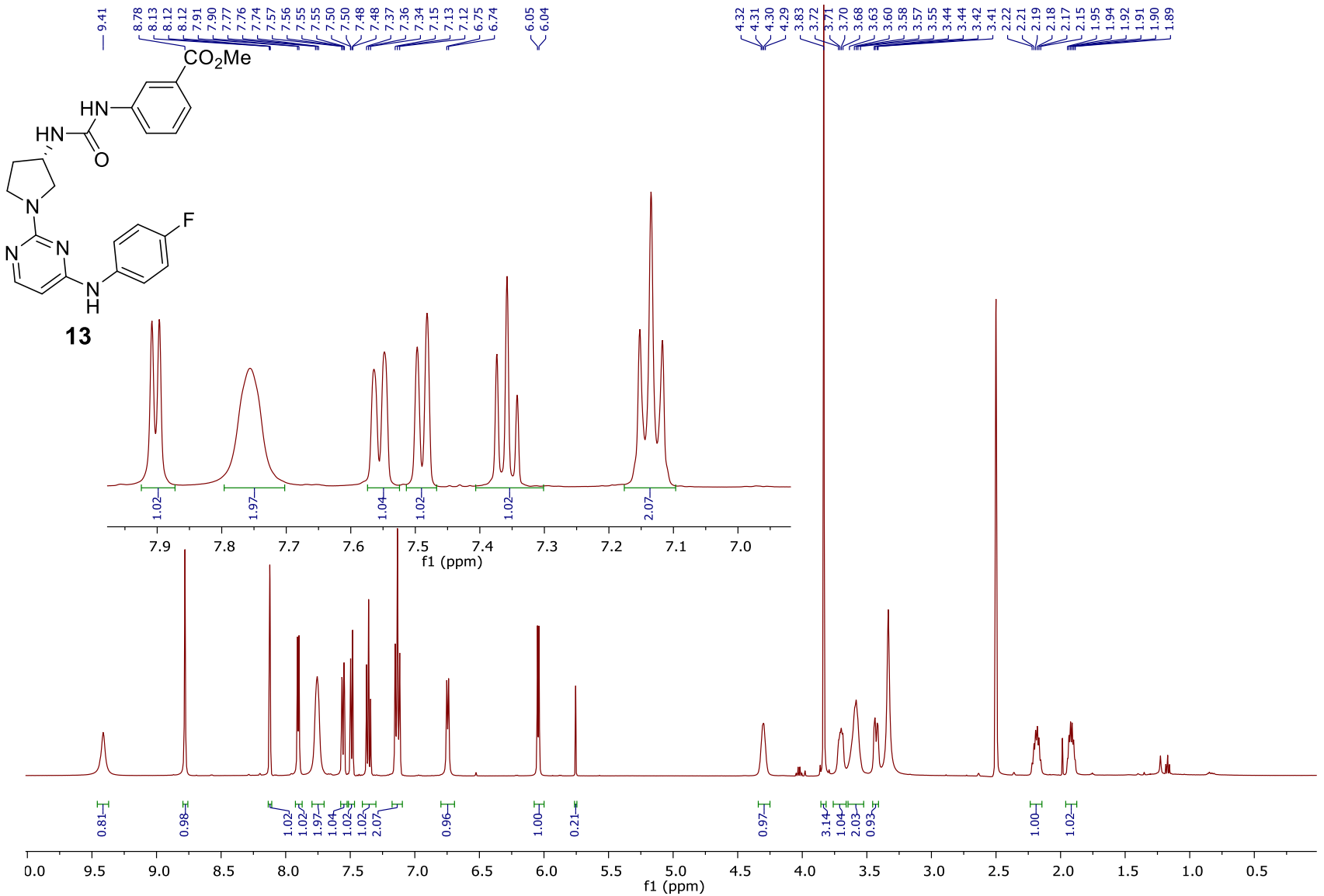
13-ii, ^{1}H, 500 MHz, DMSO-d_6



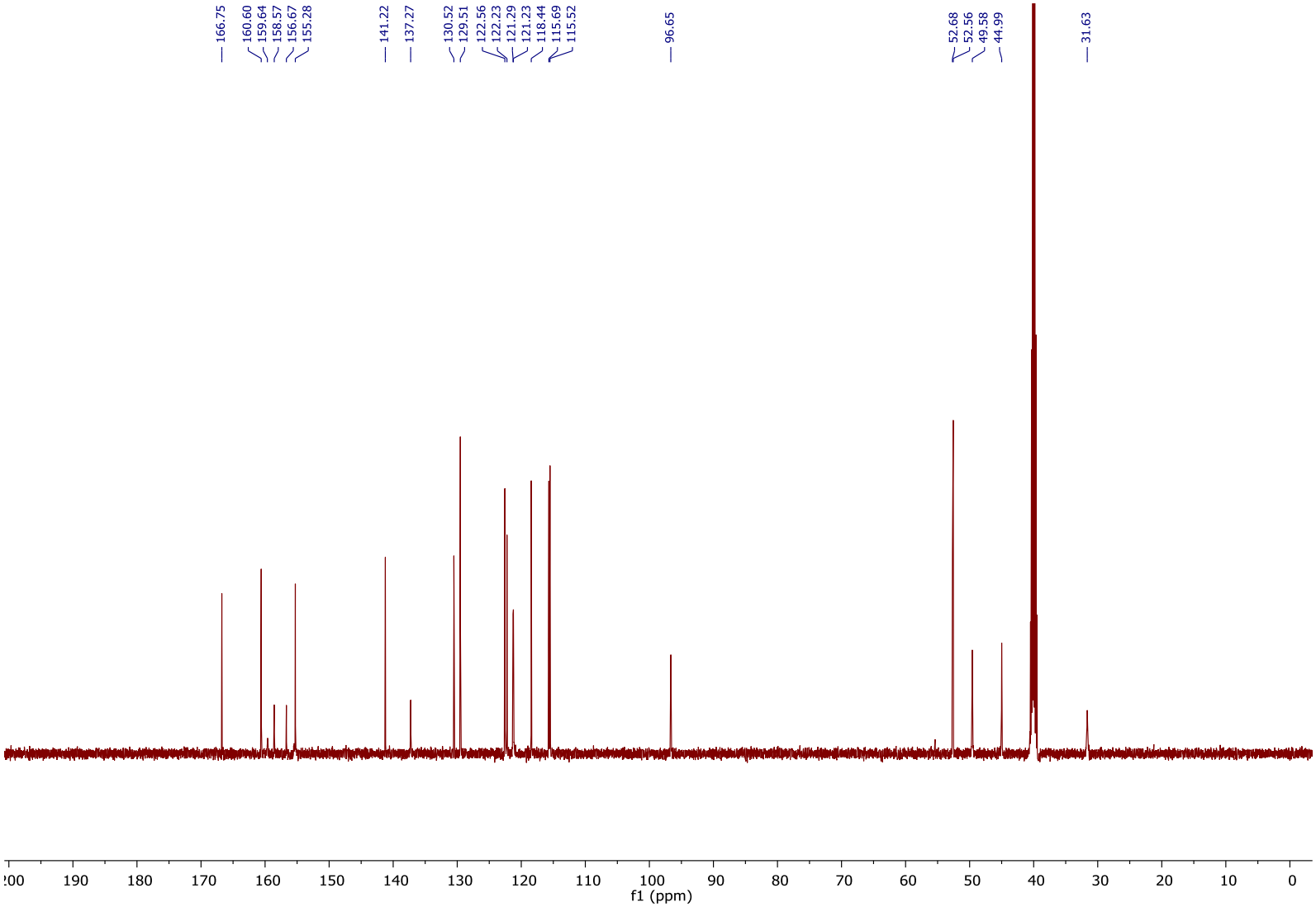
13-ii, ^{13}C, 126 MHz, DMSO-d_6



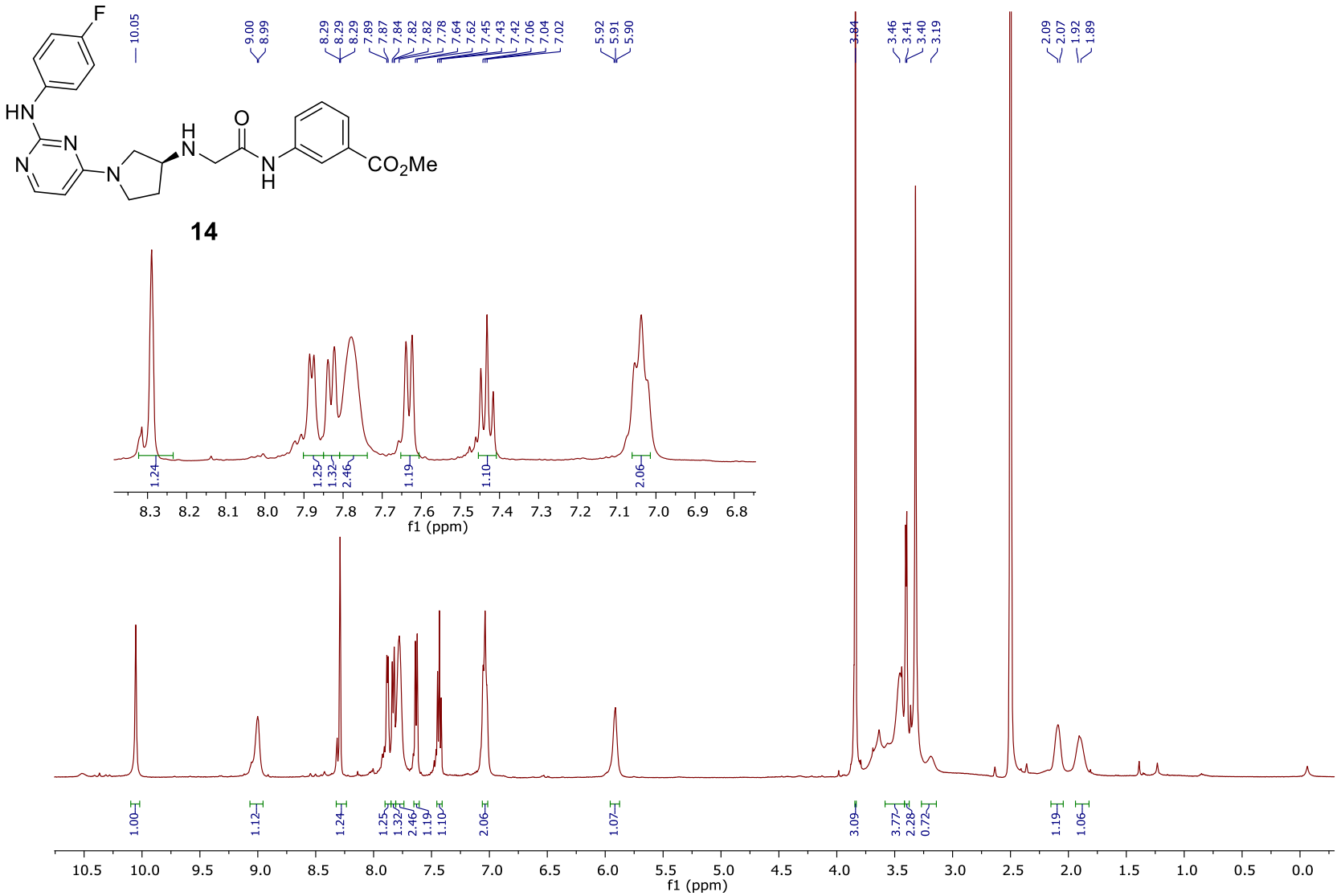
13, ^{1}H, 500 MHz, DMSO-d_6



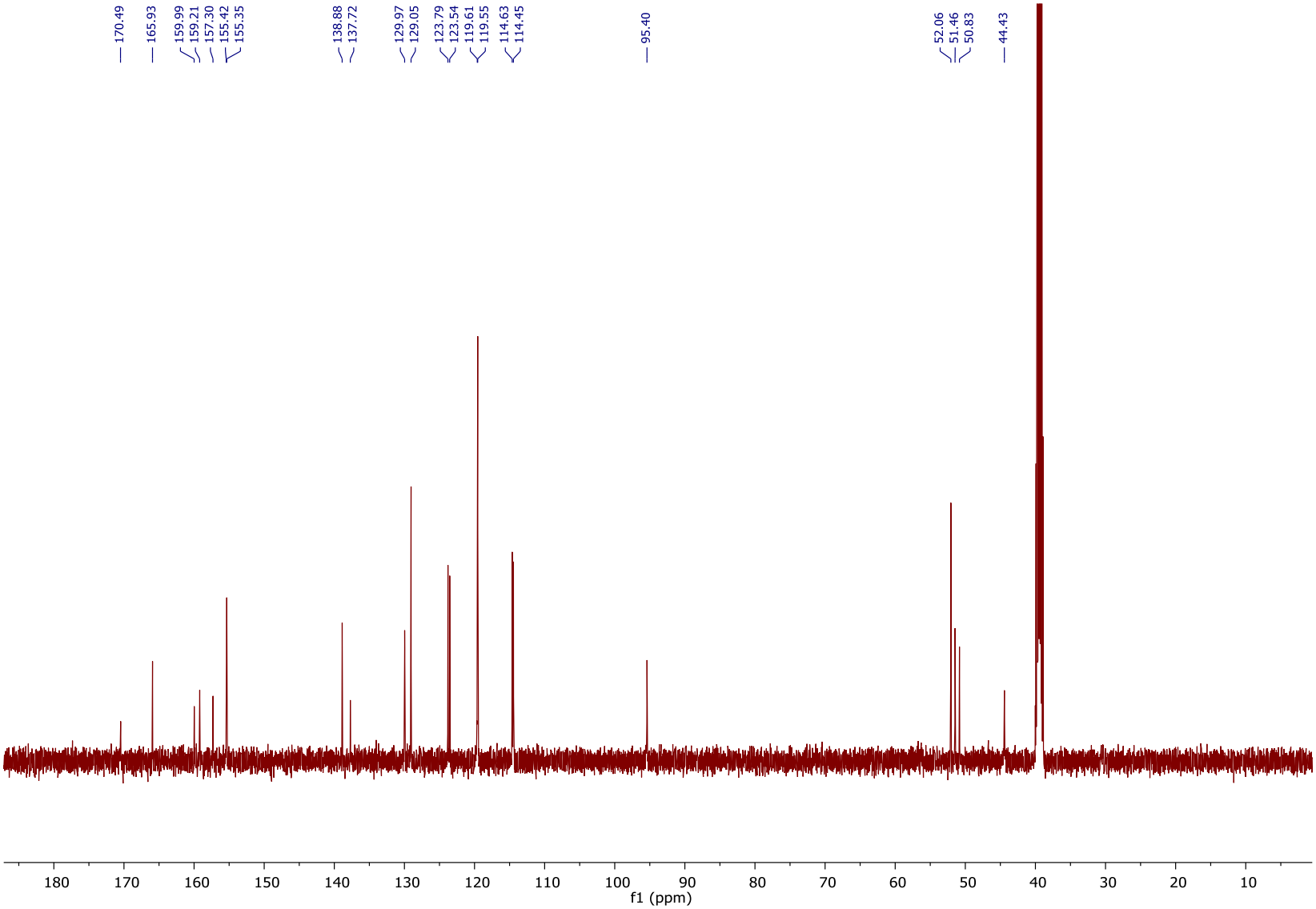
13, ^{13}C, 126 MHz, DMSO-d_6



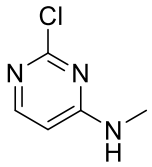
14, ^{1}H, 500 MHz, DMSO-d_6



14, ^{13}C, 126 MHz, DMSO-d_6

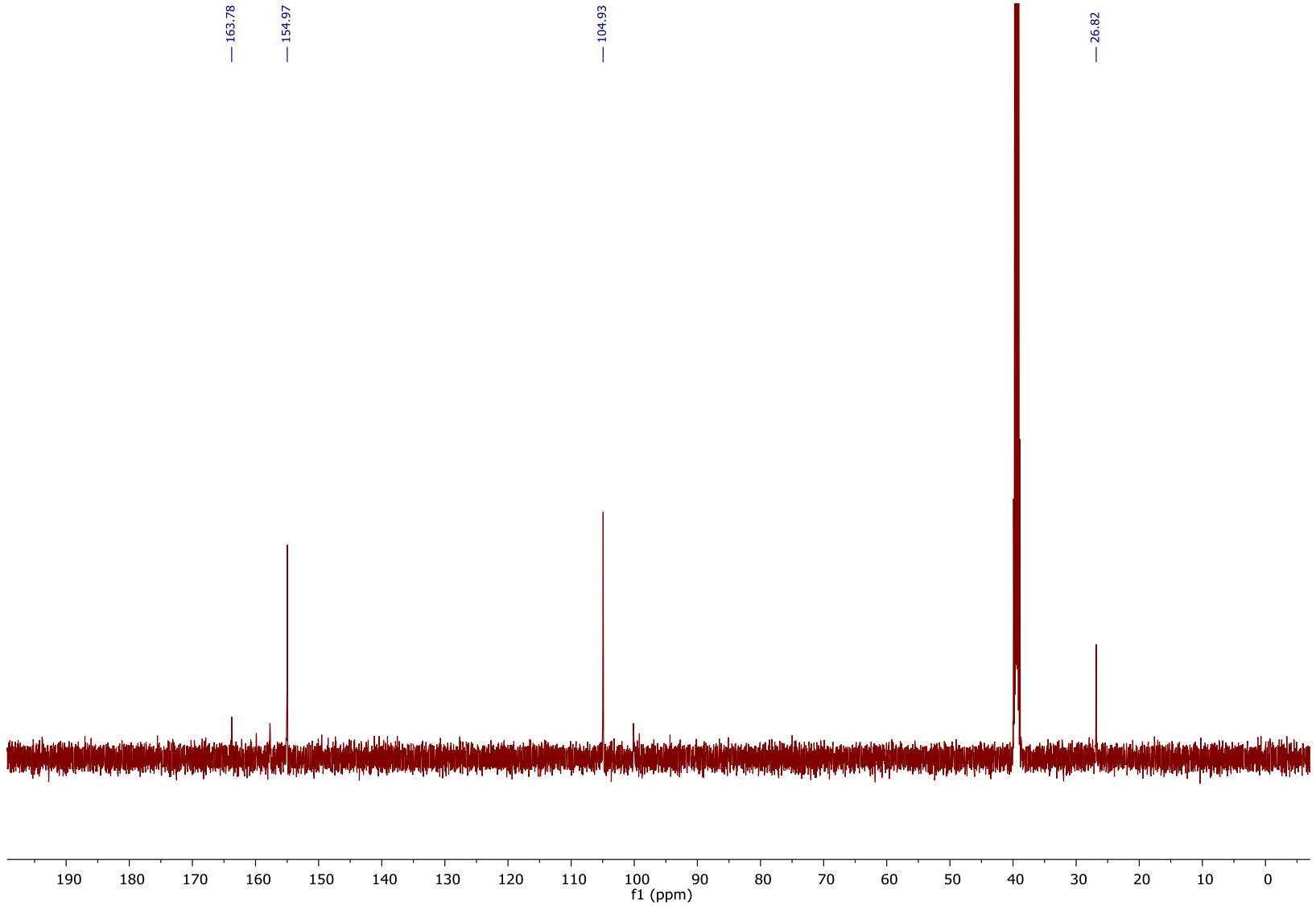


F-i, ^{1}H, 500 MHz, DMSO-d_6

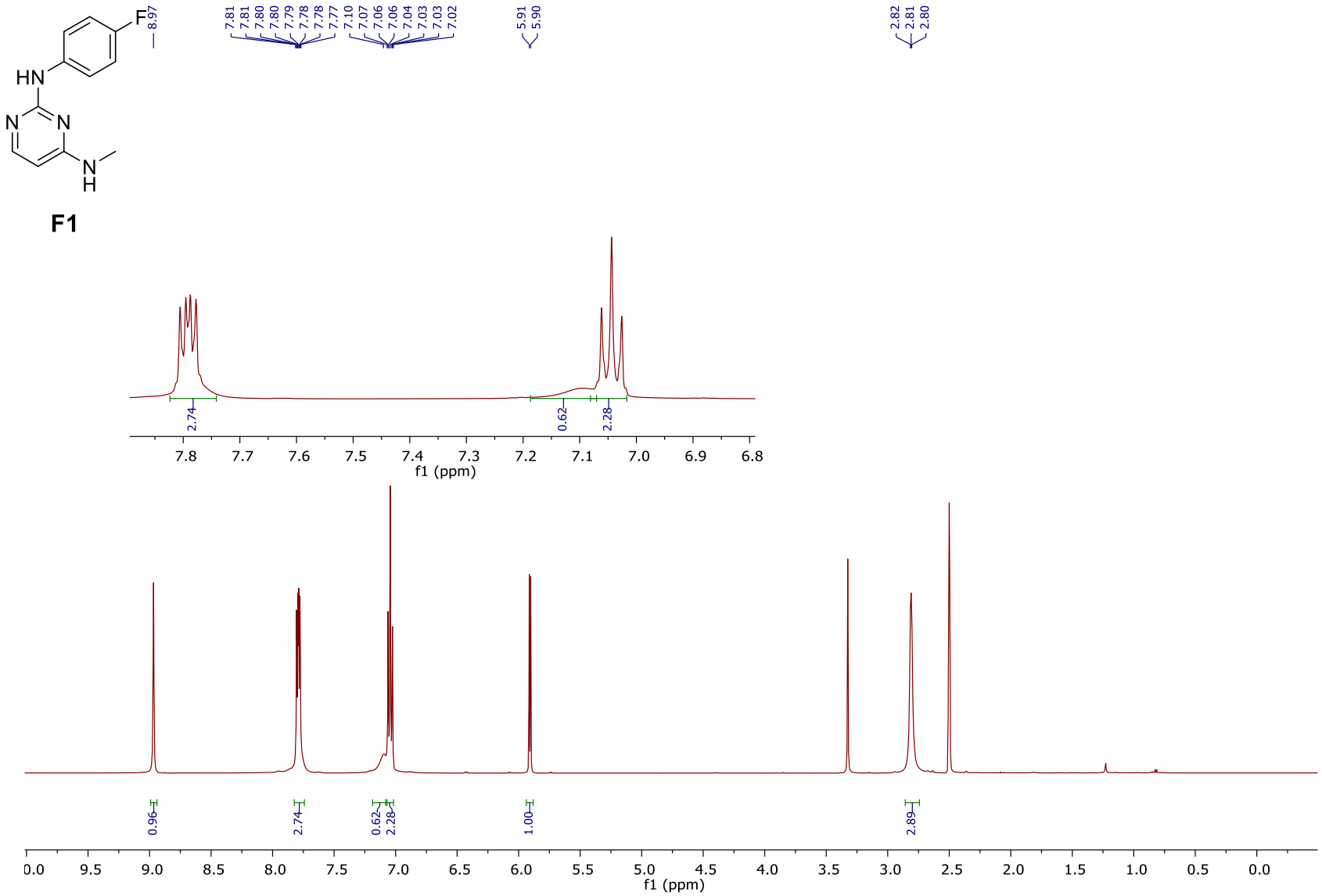


F-i

F-i, ^{13}C, 126 MHz, DMSO-d_6

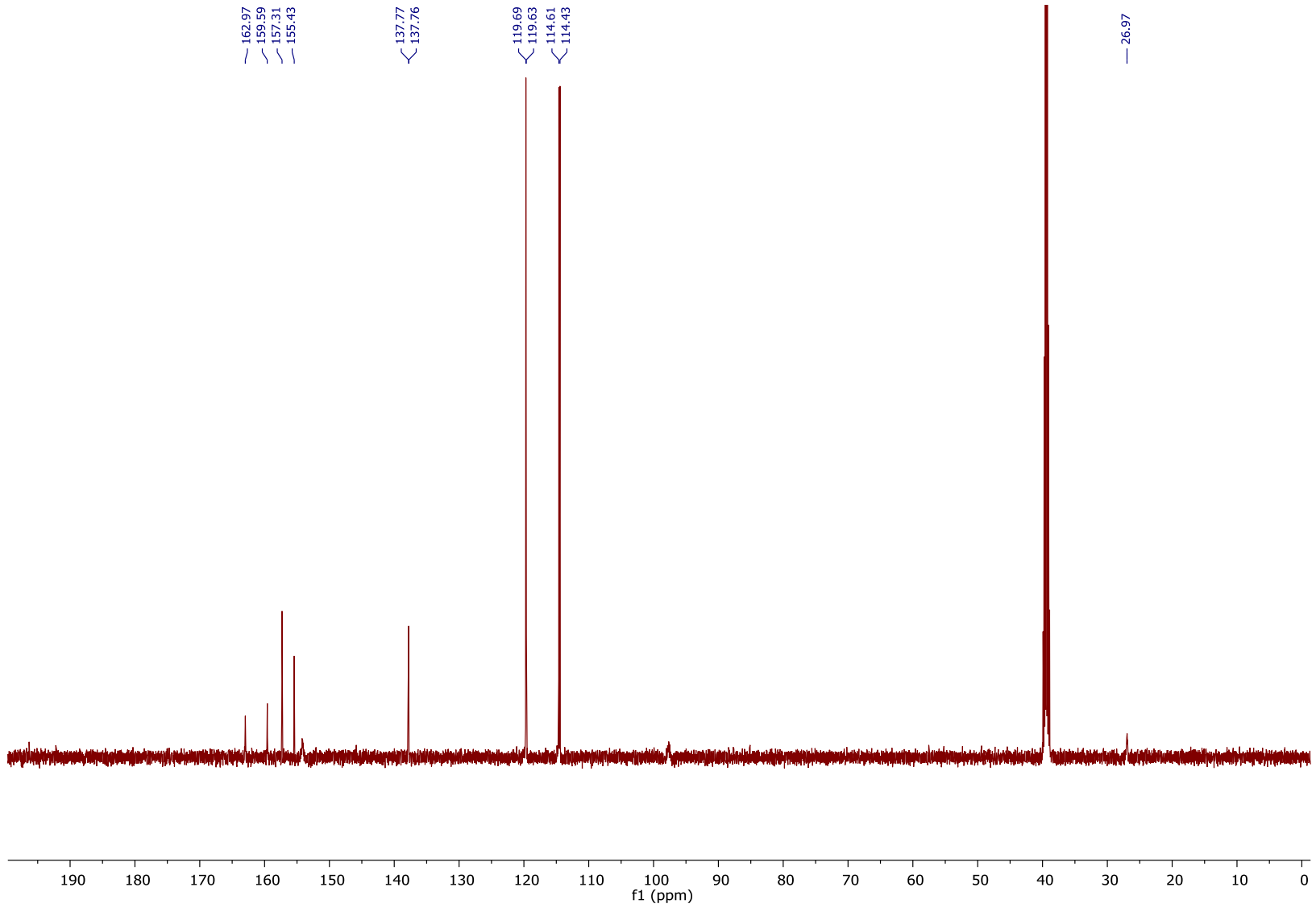


F1, ^{1}H, 500 MHz, DMSO-d_6

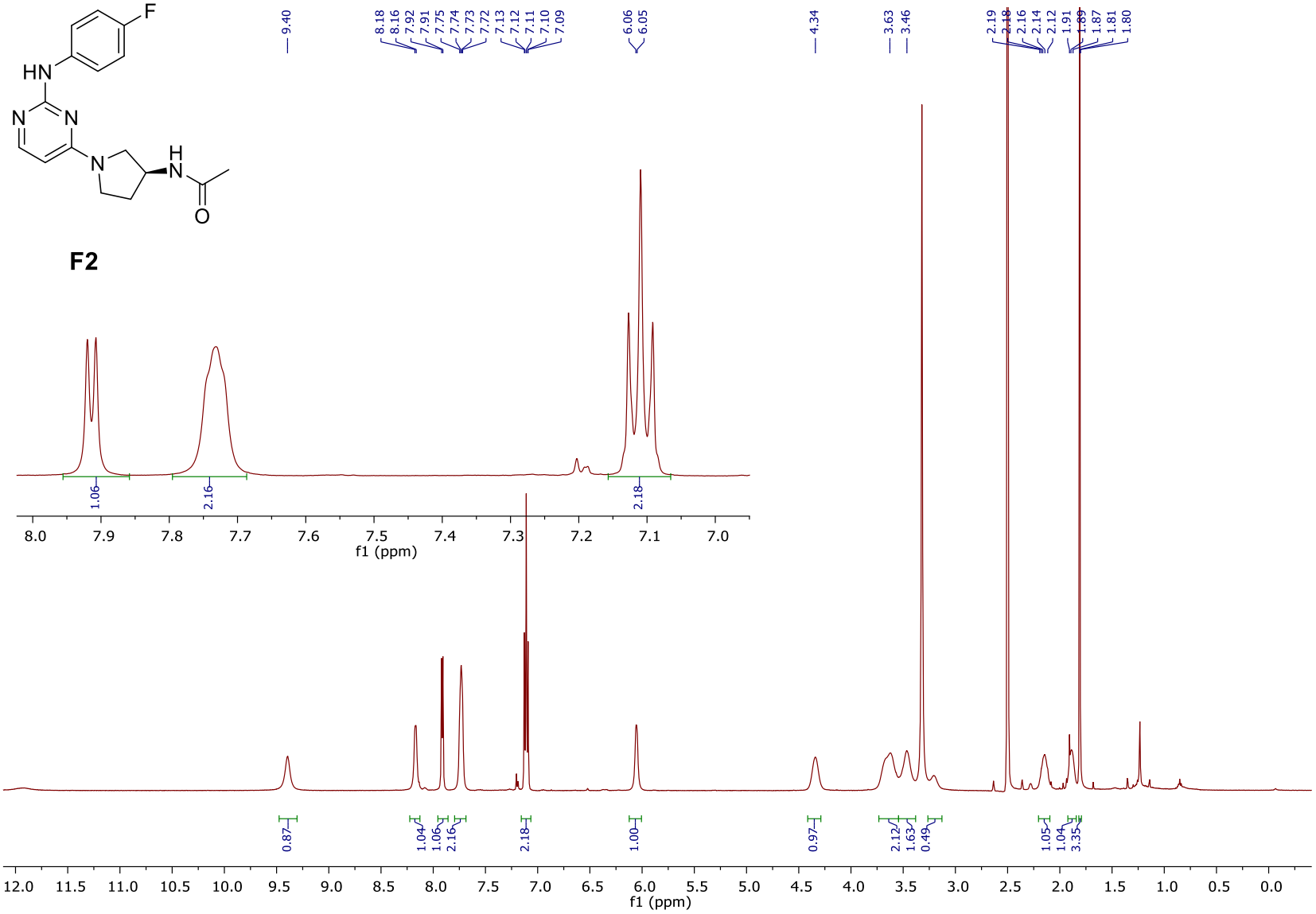


F1

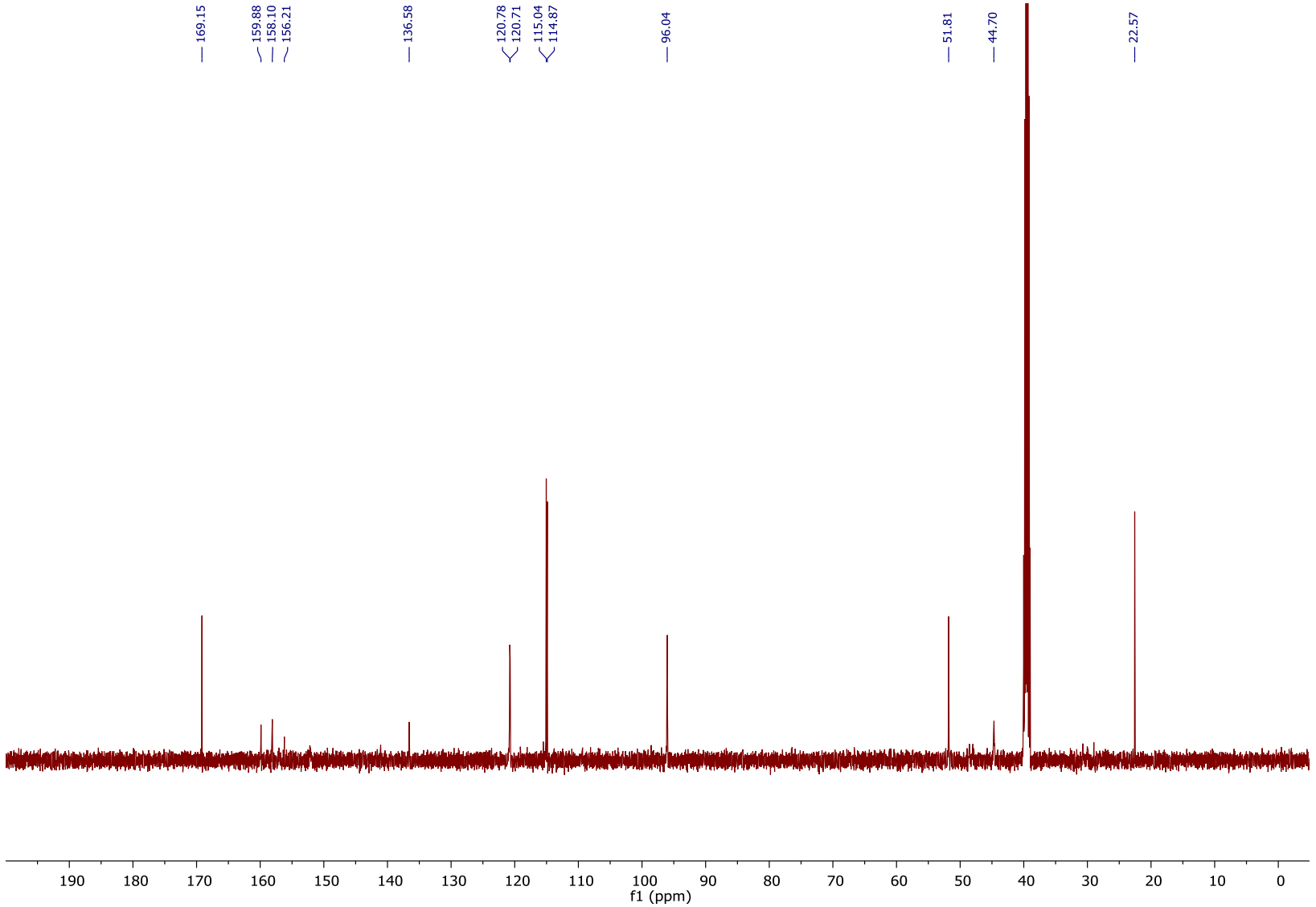
F1, ^{13}C, 126 MHz, DMSO-d_6



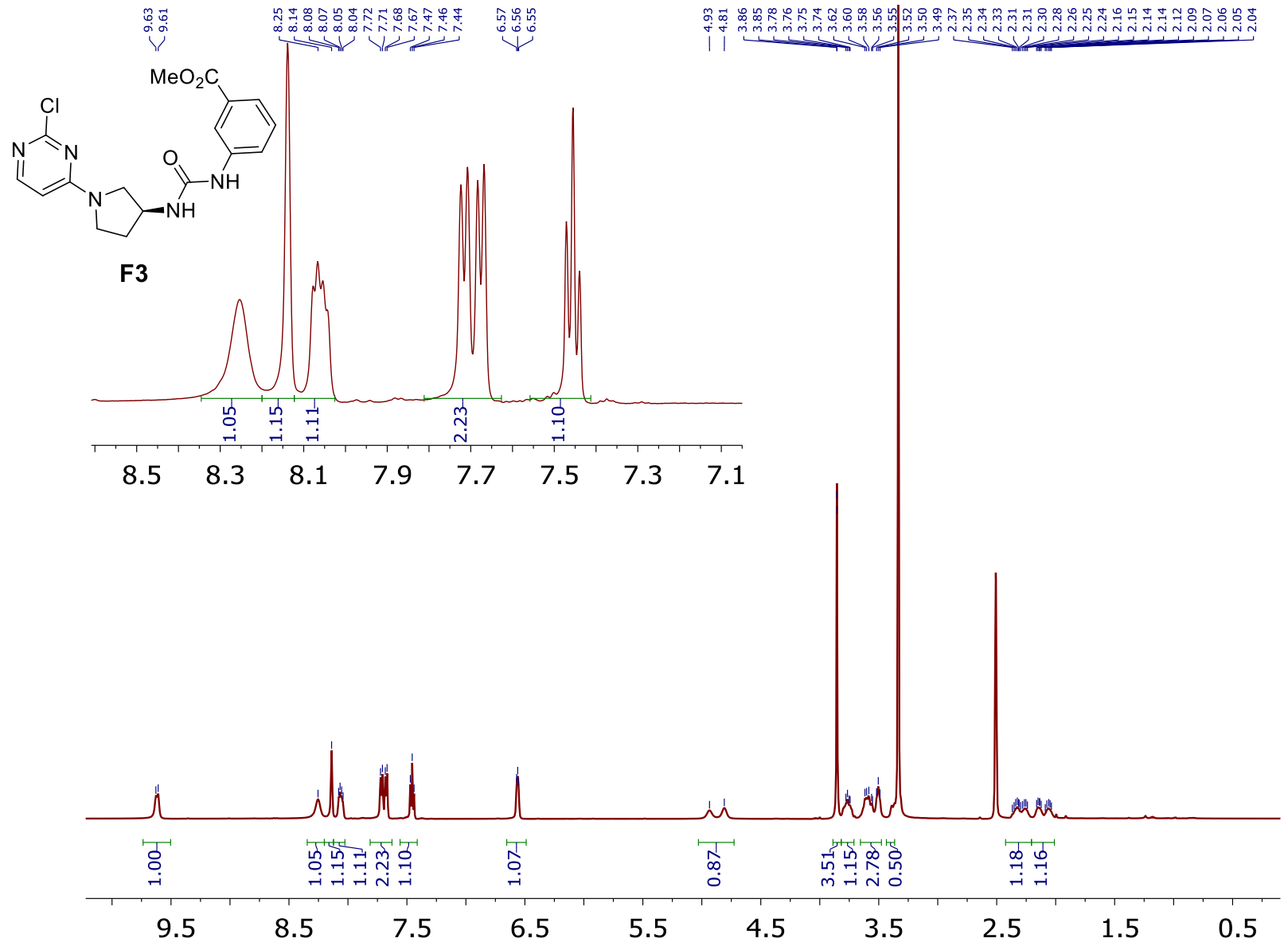
F2, ^{1}H, 500 MHz, DMSO-d_6



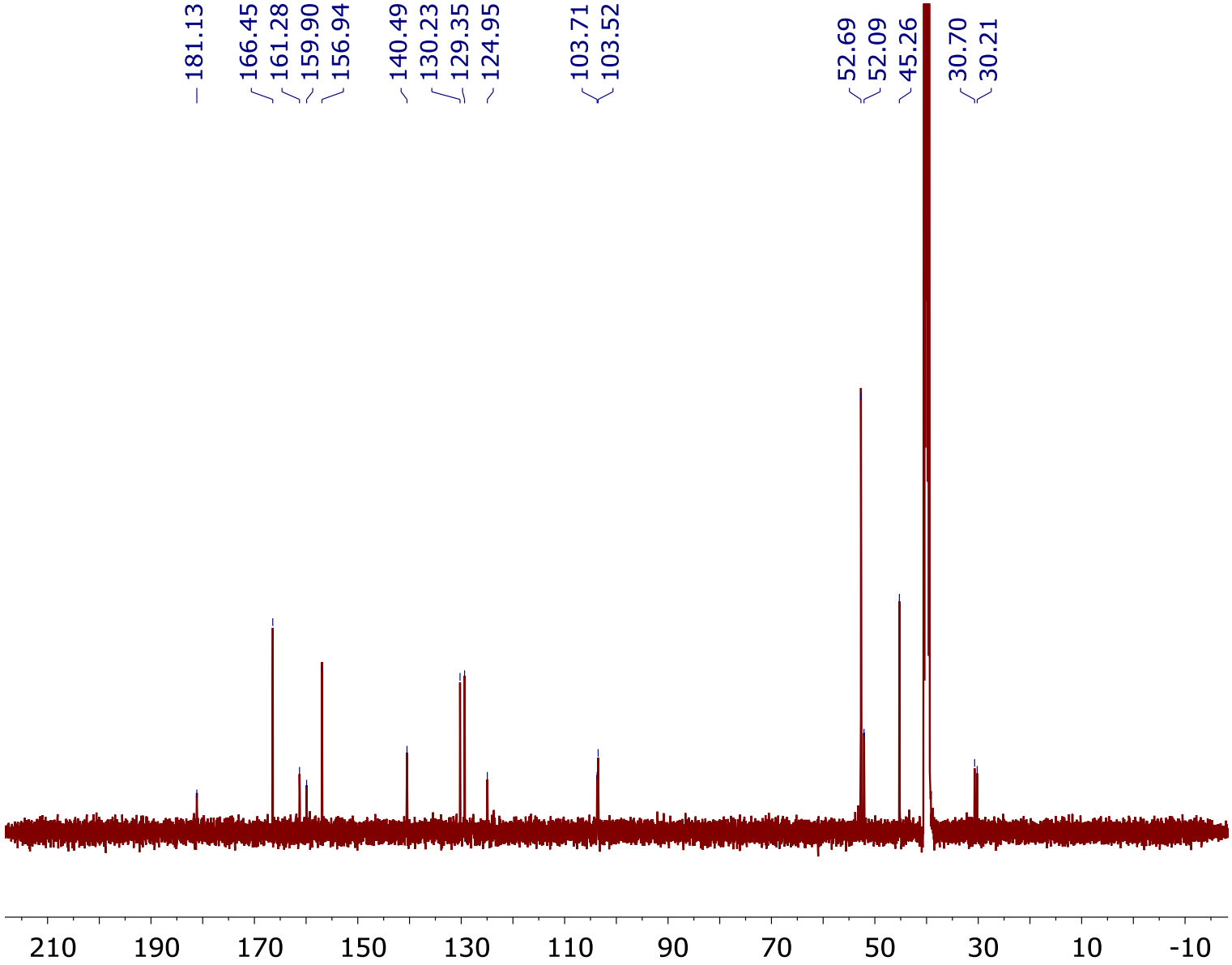
F2, ^{13}C, 126 MHz, DMSO-d_6



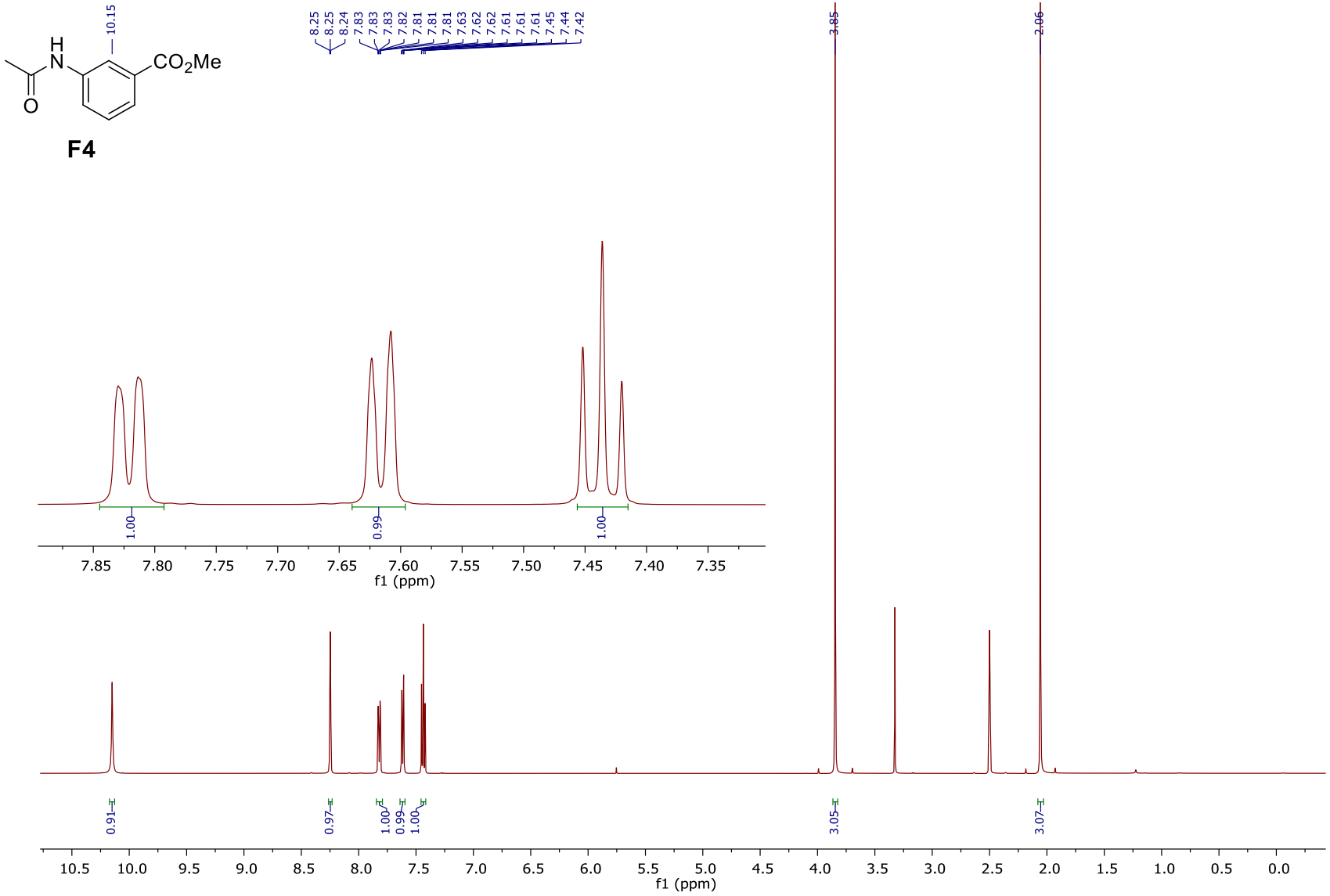
F3, ^{1}H, 500 MHz, DMSO-d_6



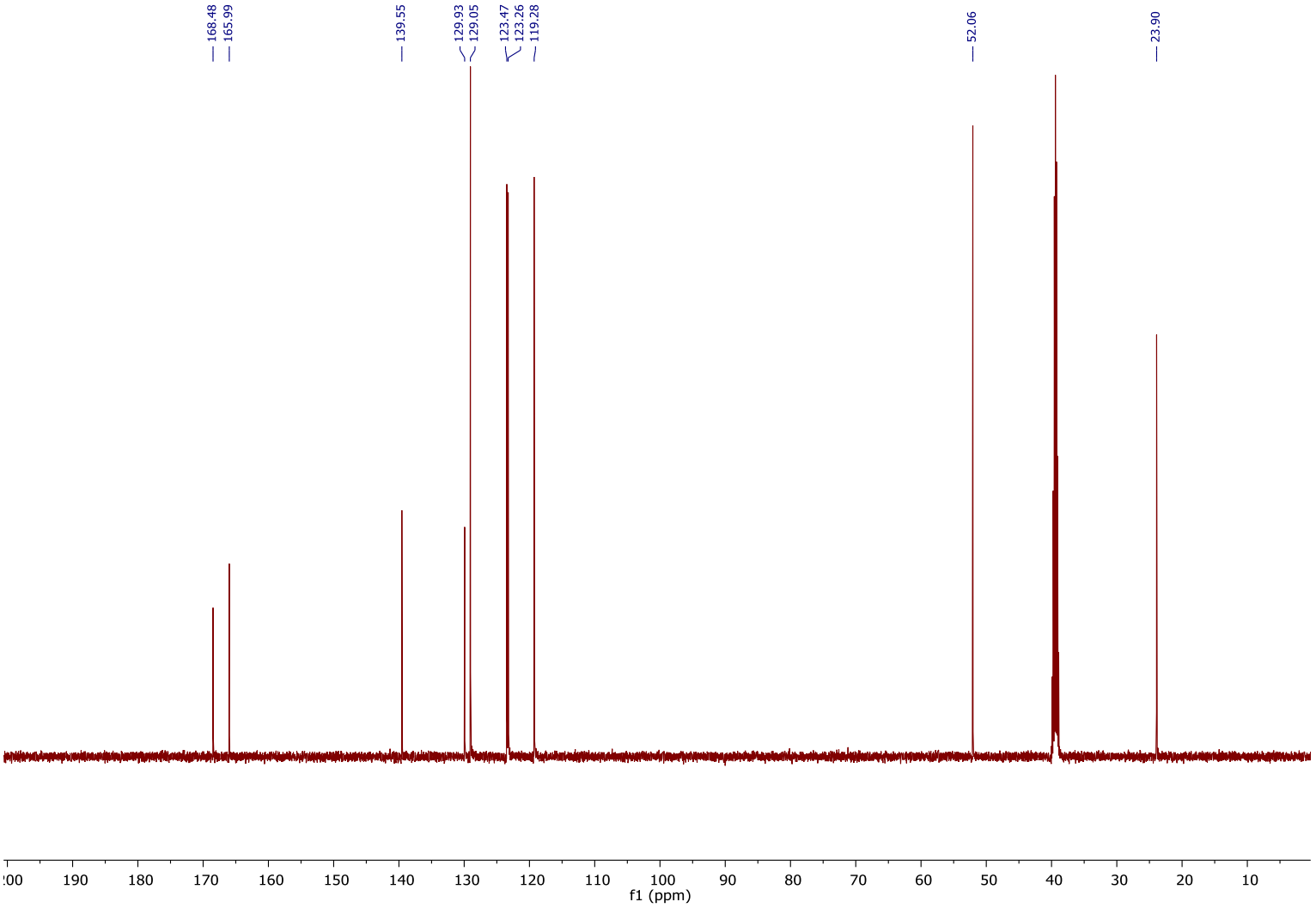
F3, ^{13}C, 126 MHz, DMSO-d_6



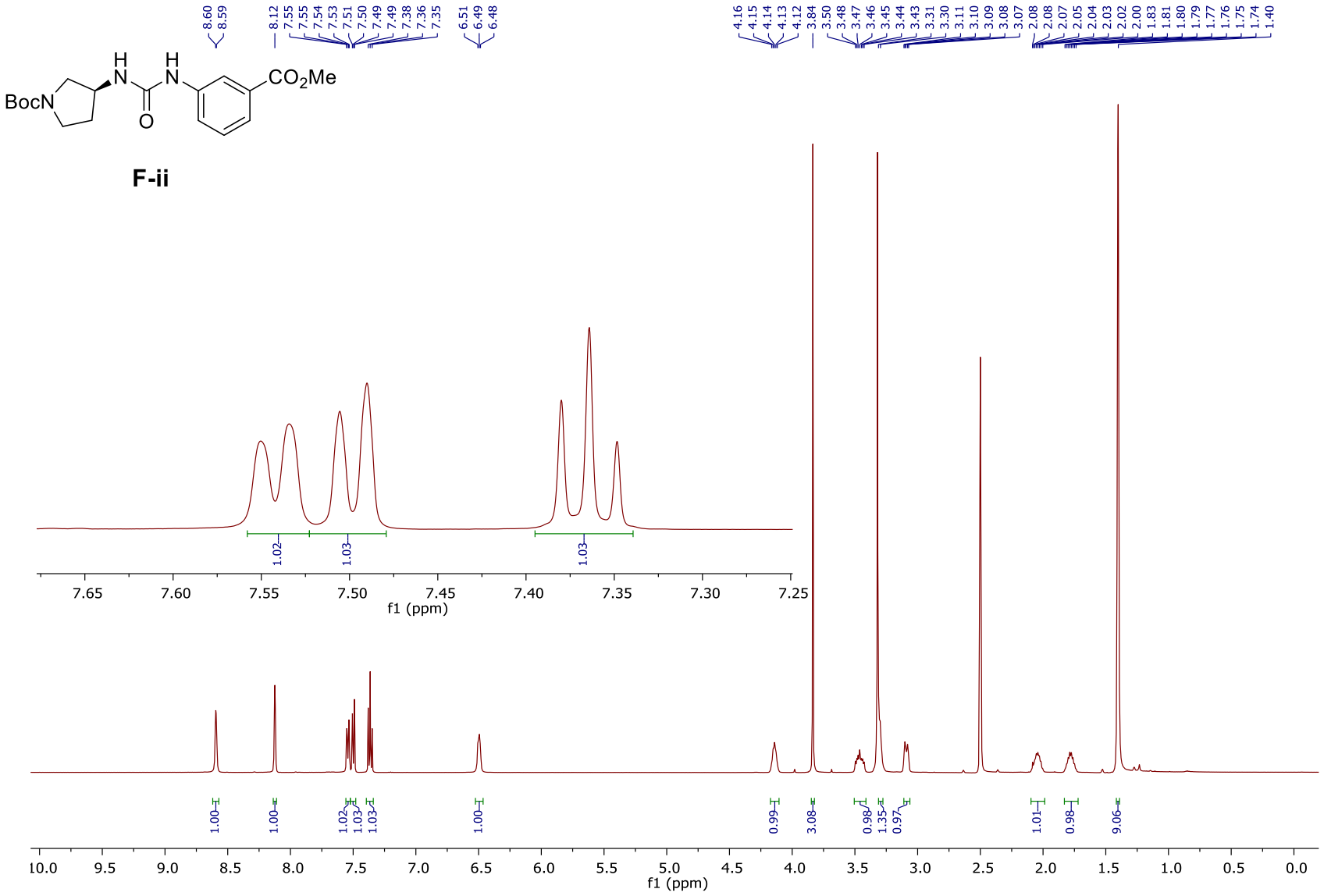
F4, ^{1}H, 500 MHz, DMSO-d_6



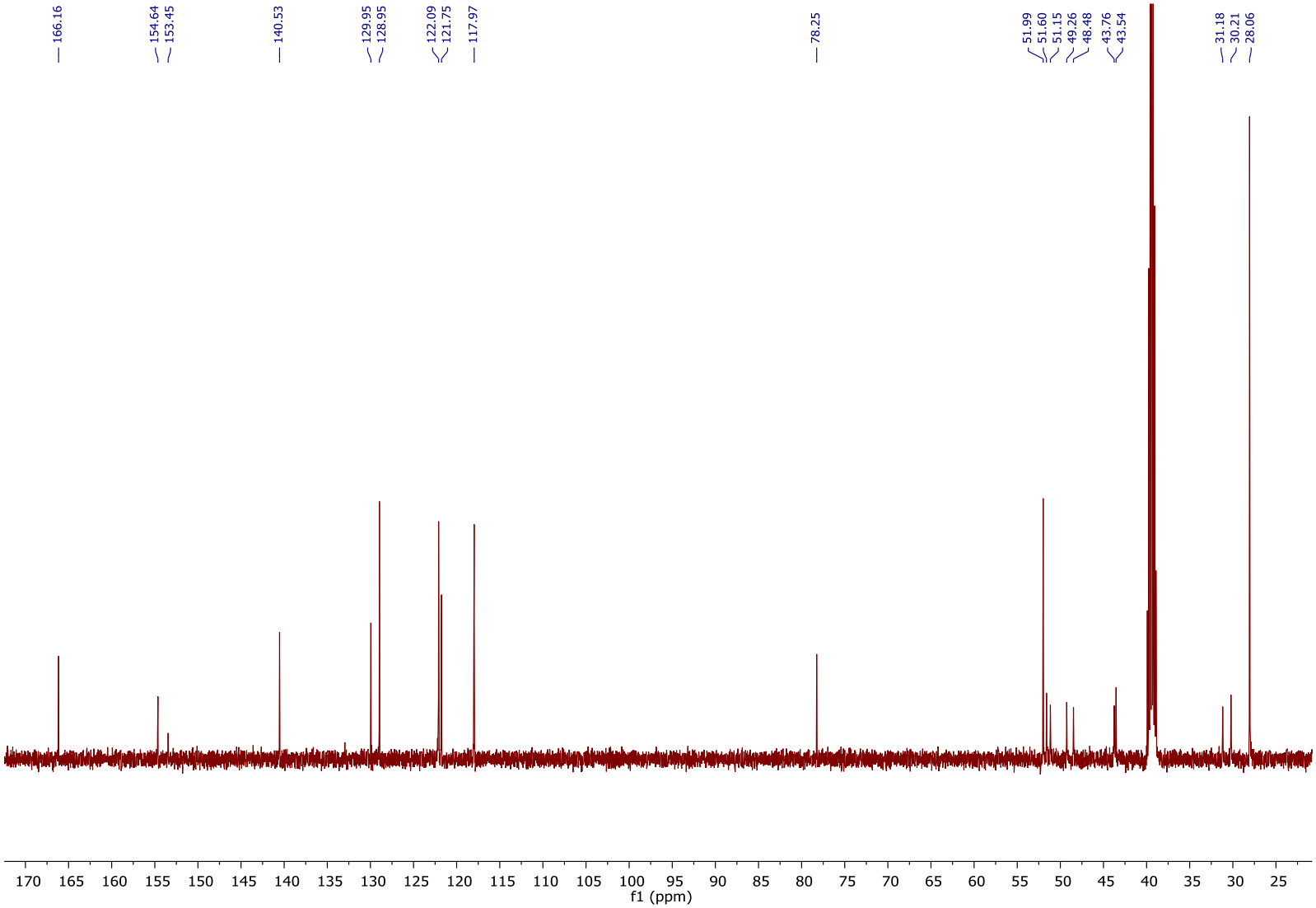
F4, ^{13}C, 126 MHz, DMSO-d_6



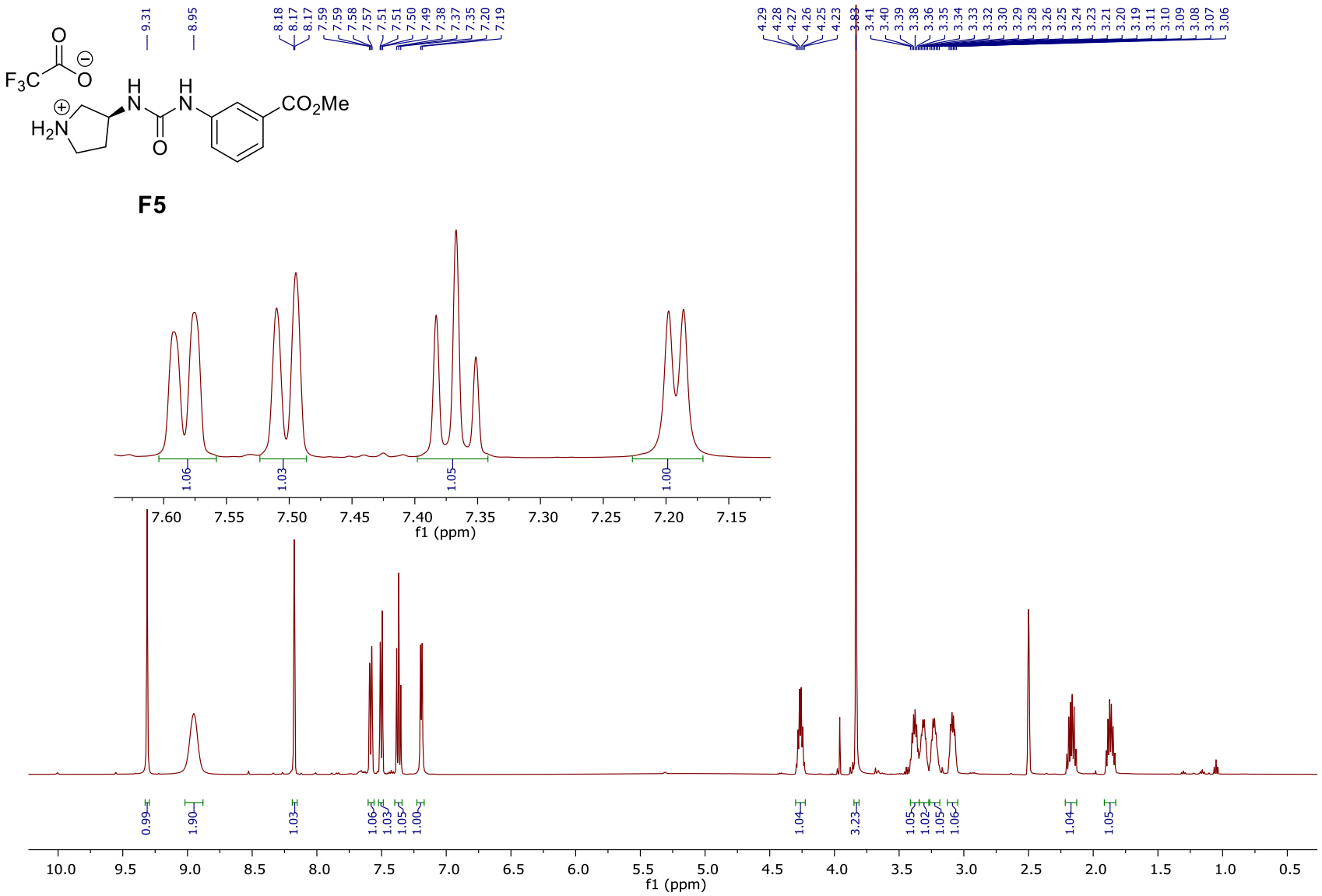
F-ii, ^{1}H, 500 MHz, DMSO-d_6



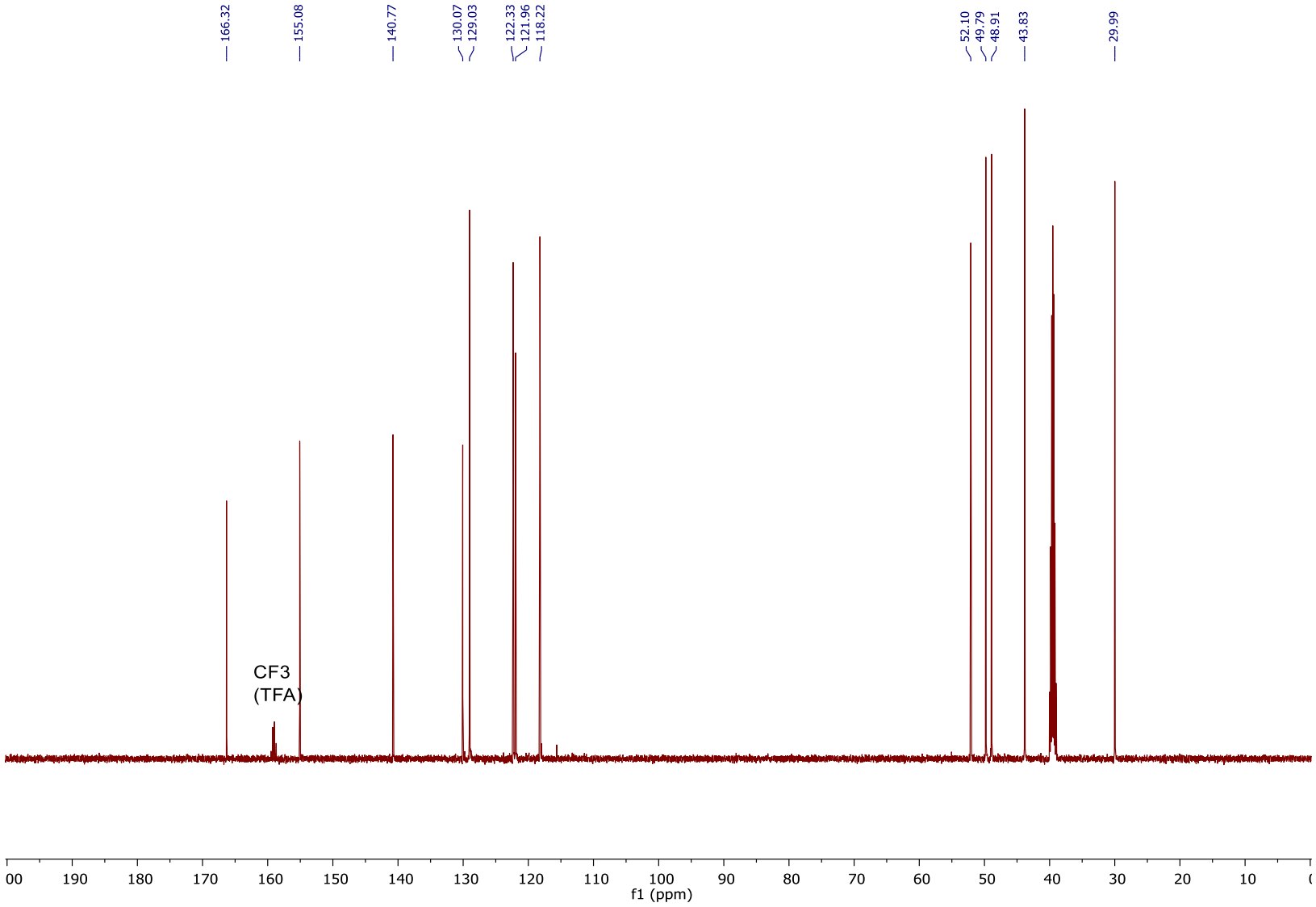
F-ii, ^{13}C, 126 MHz, DMSO-d_6



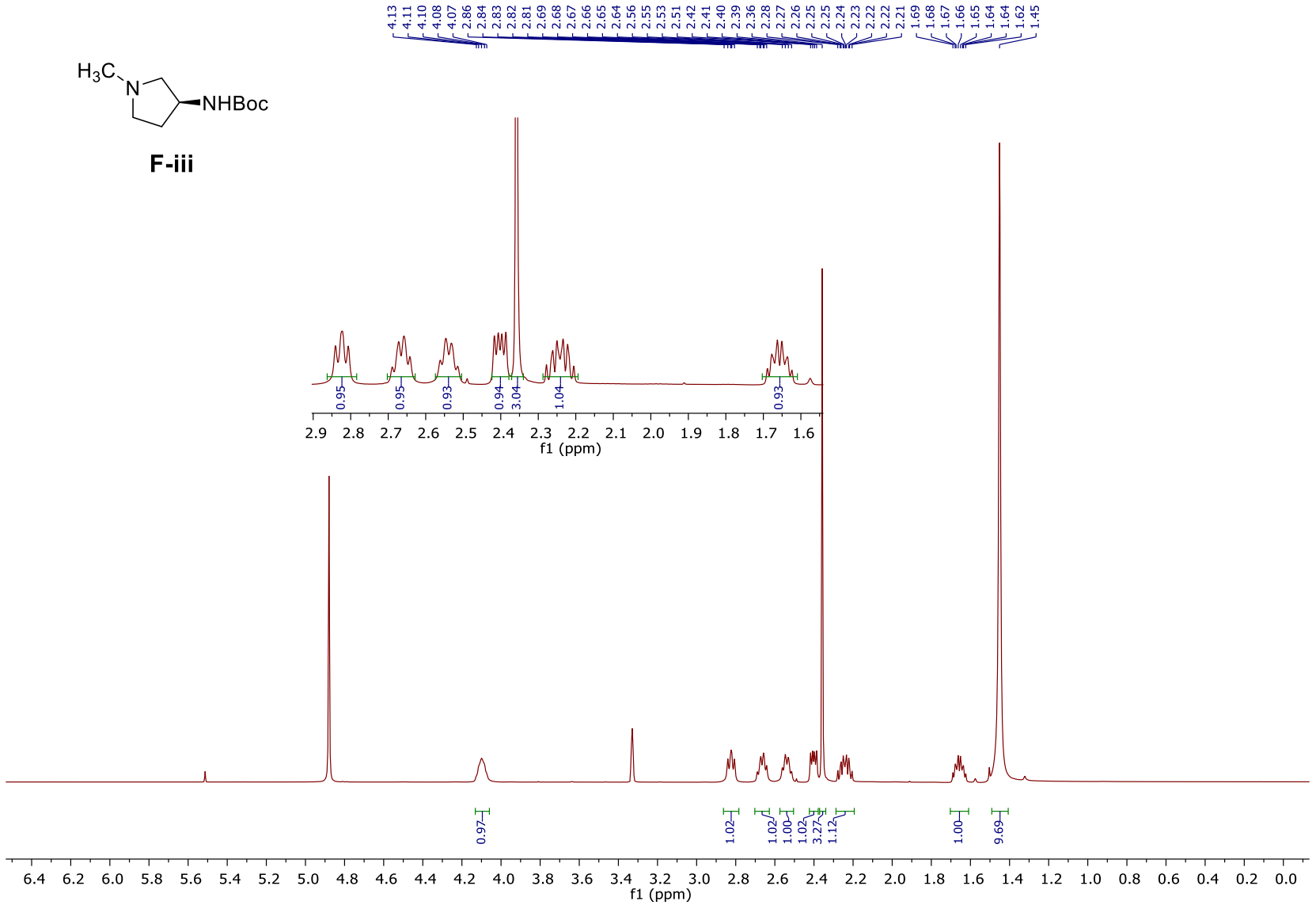
F5, ^{1}H, 500 MHz, DMSO-d_6



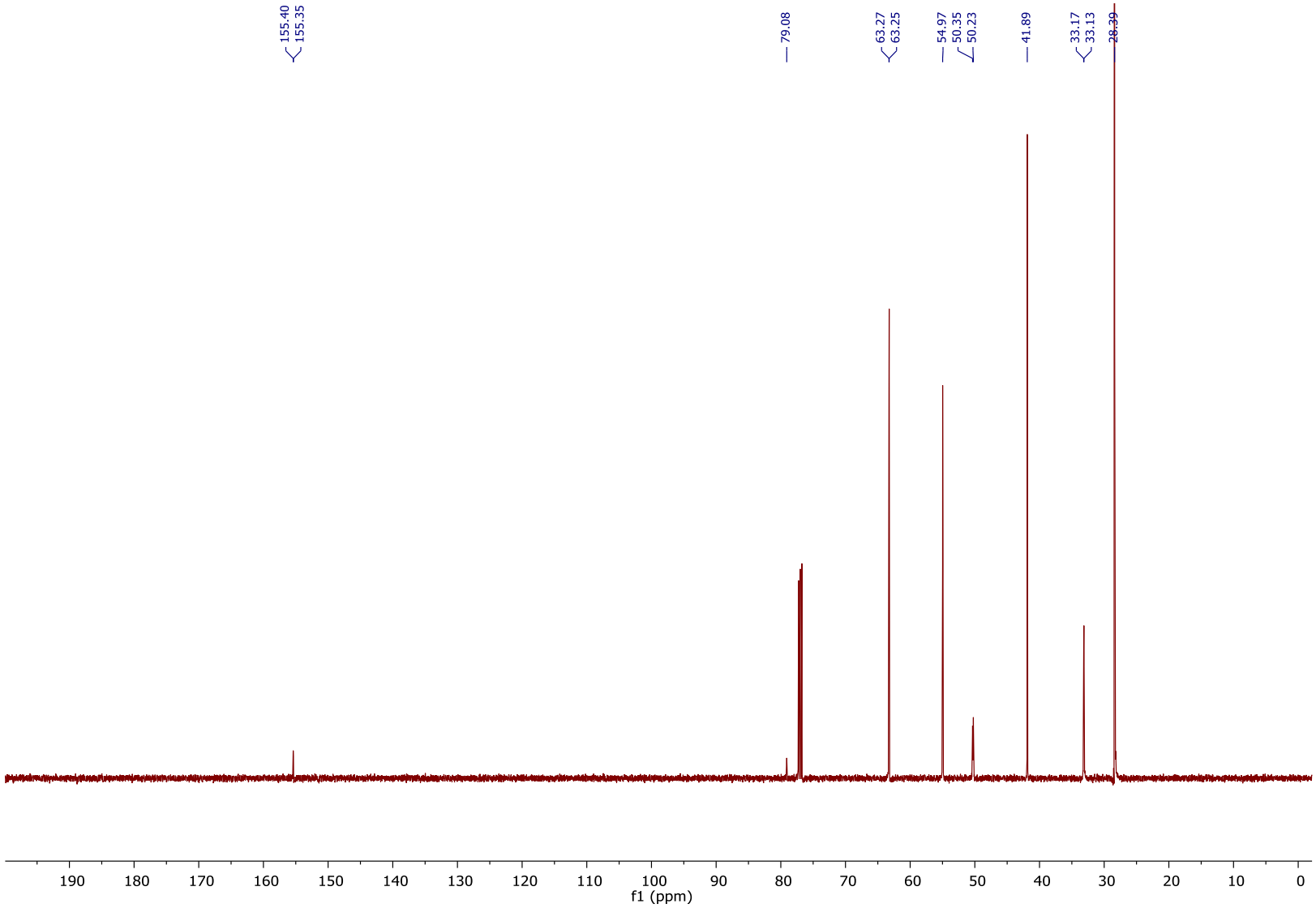
F5, ^{13}C, 126 MHz, DMSO-d_6



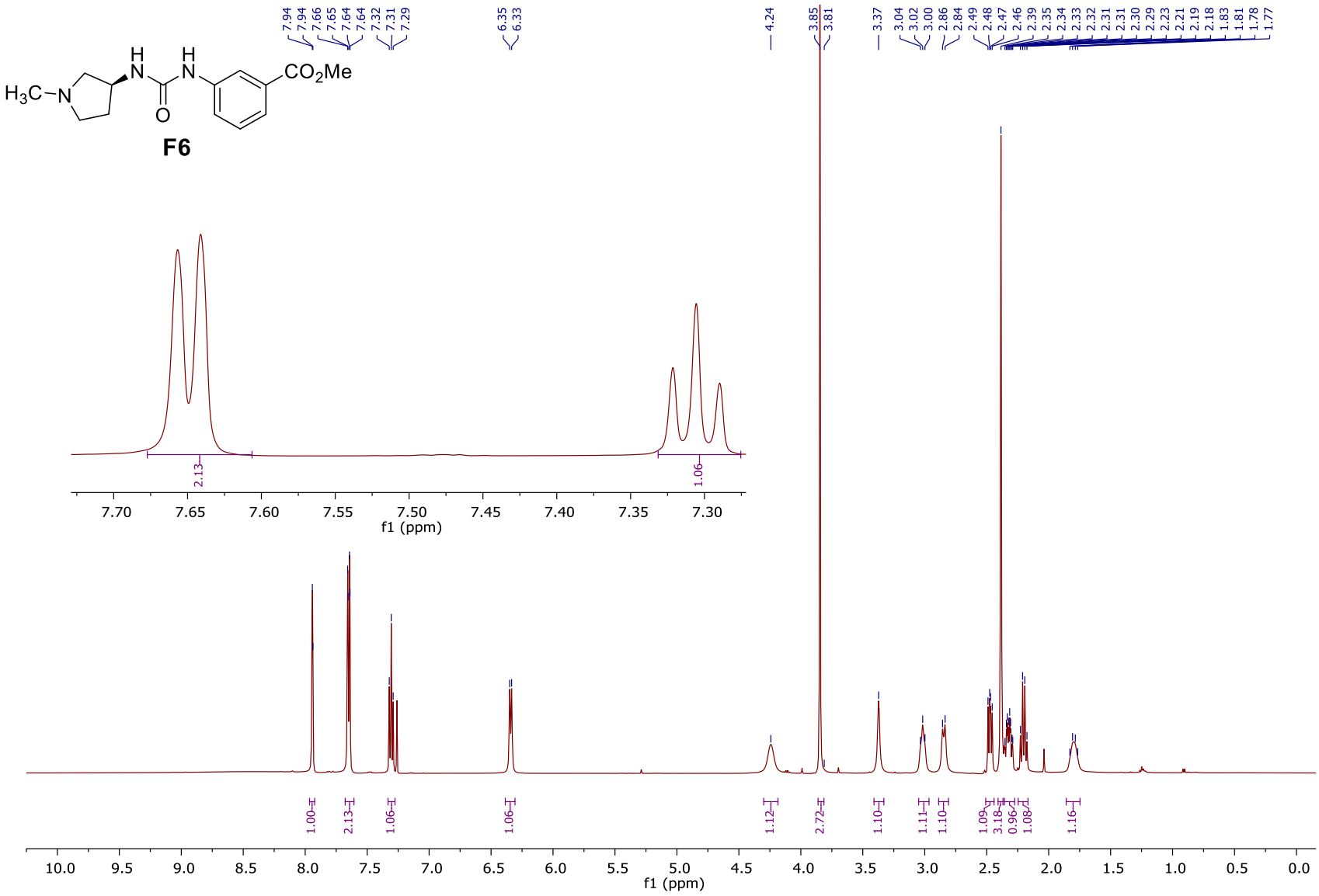
F-iii, ^{1}H, 500 MHz, MeOD



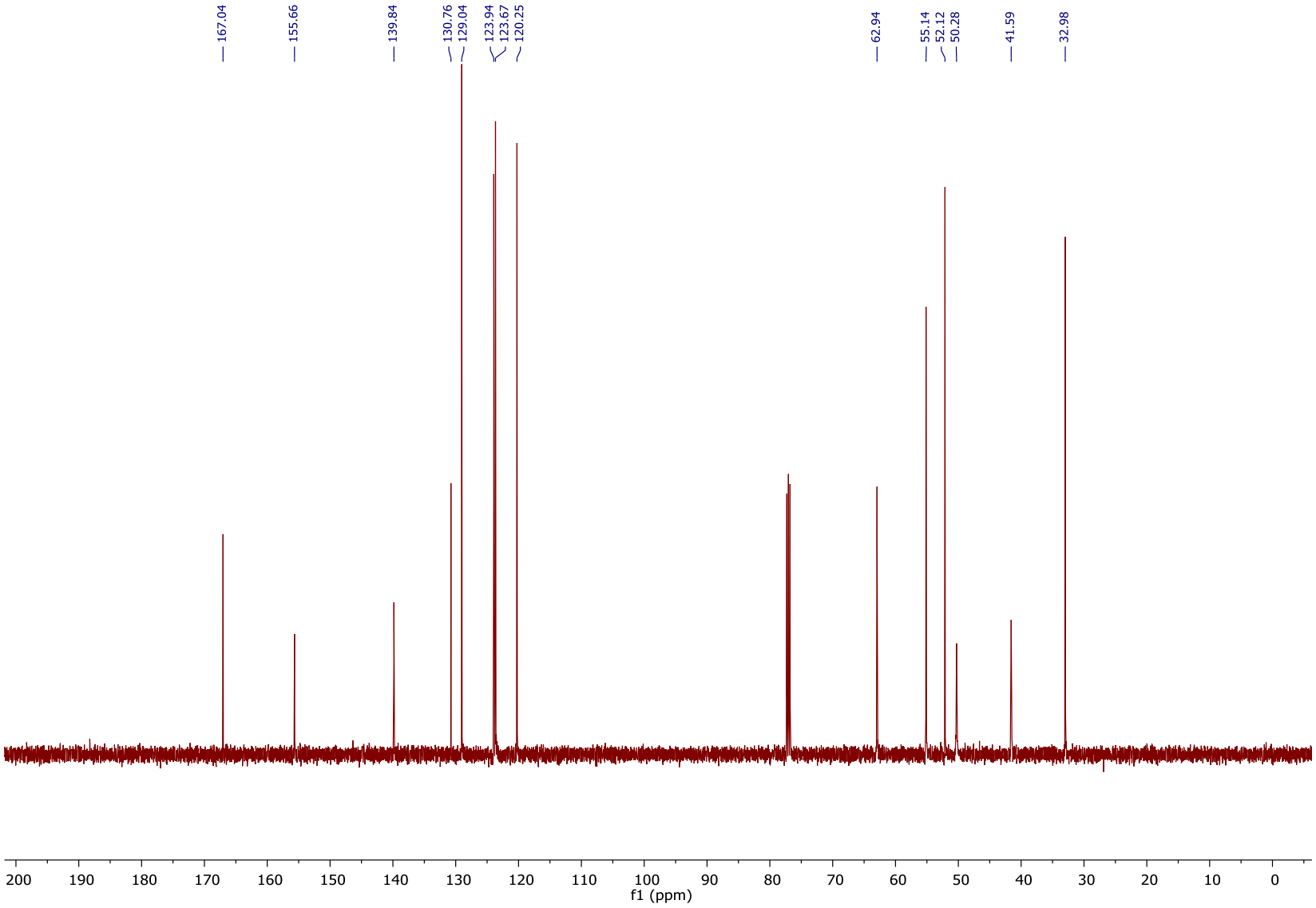
F-iii, ^{13}C, 126 MHz, $CDCl_3$



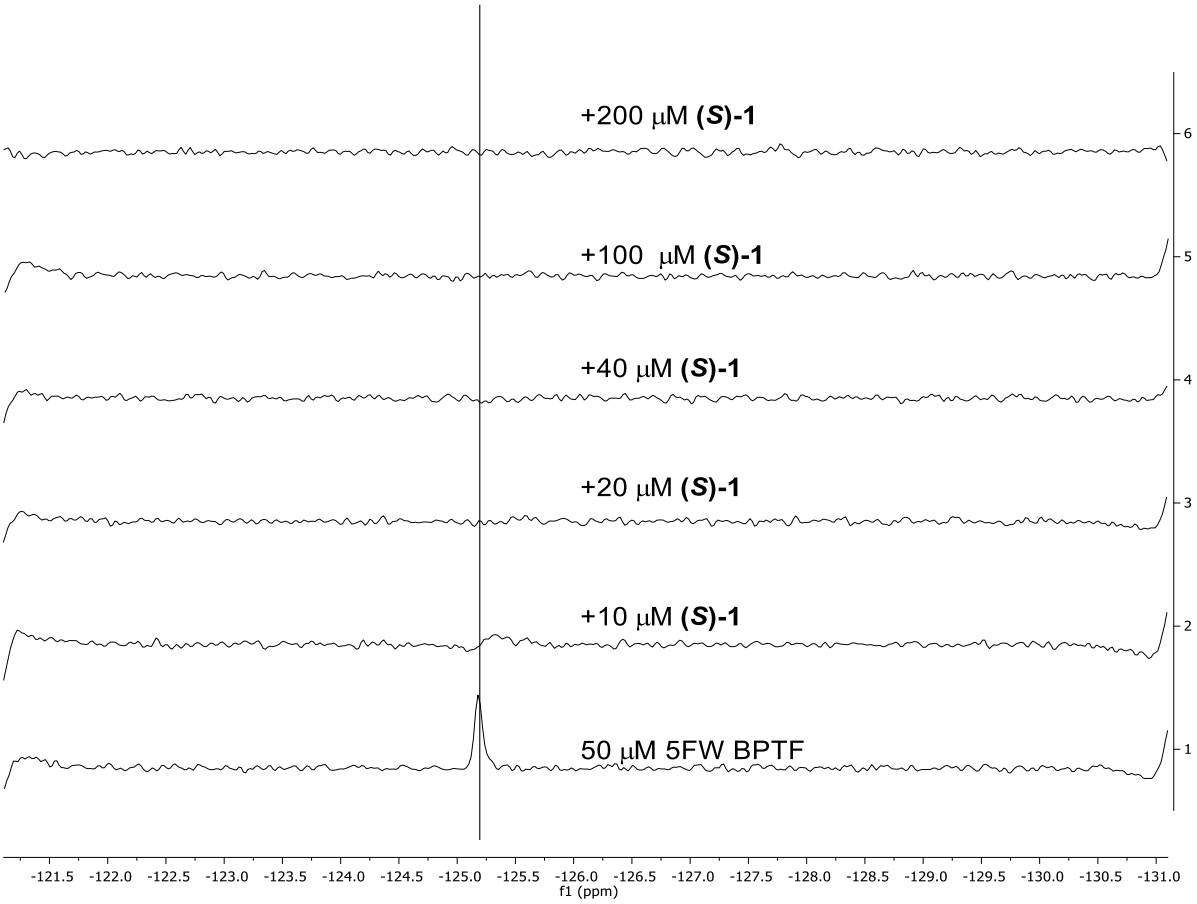
F6, ^{1}H, 500 MHz, $CDCl_3$

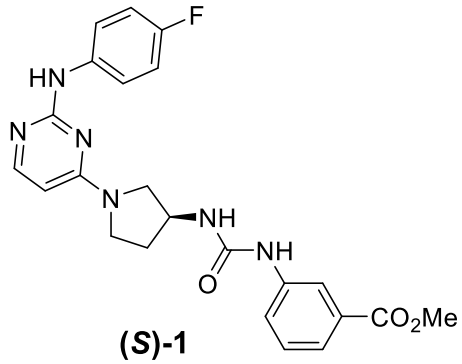


F6, ^{13}C, 126 MHz, $CDCl_3$



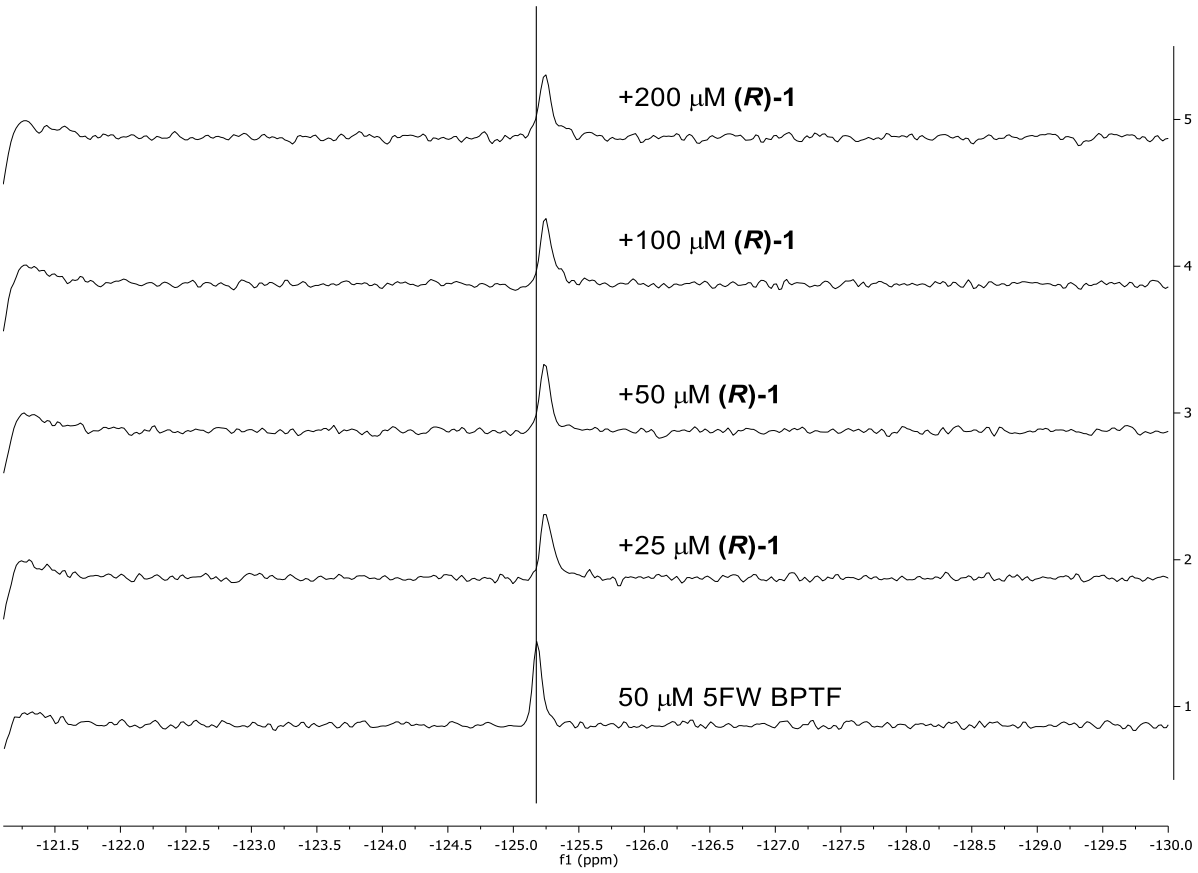
PrOF NMR titrations

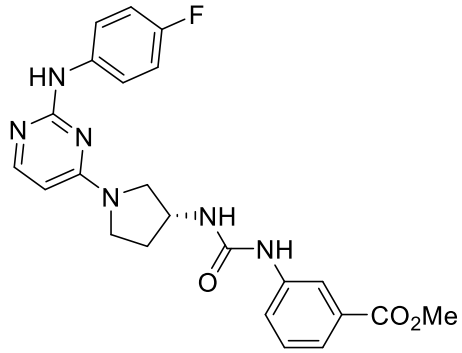




(*S*)-1

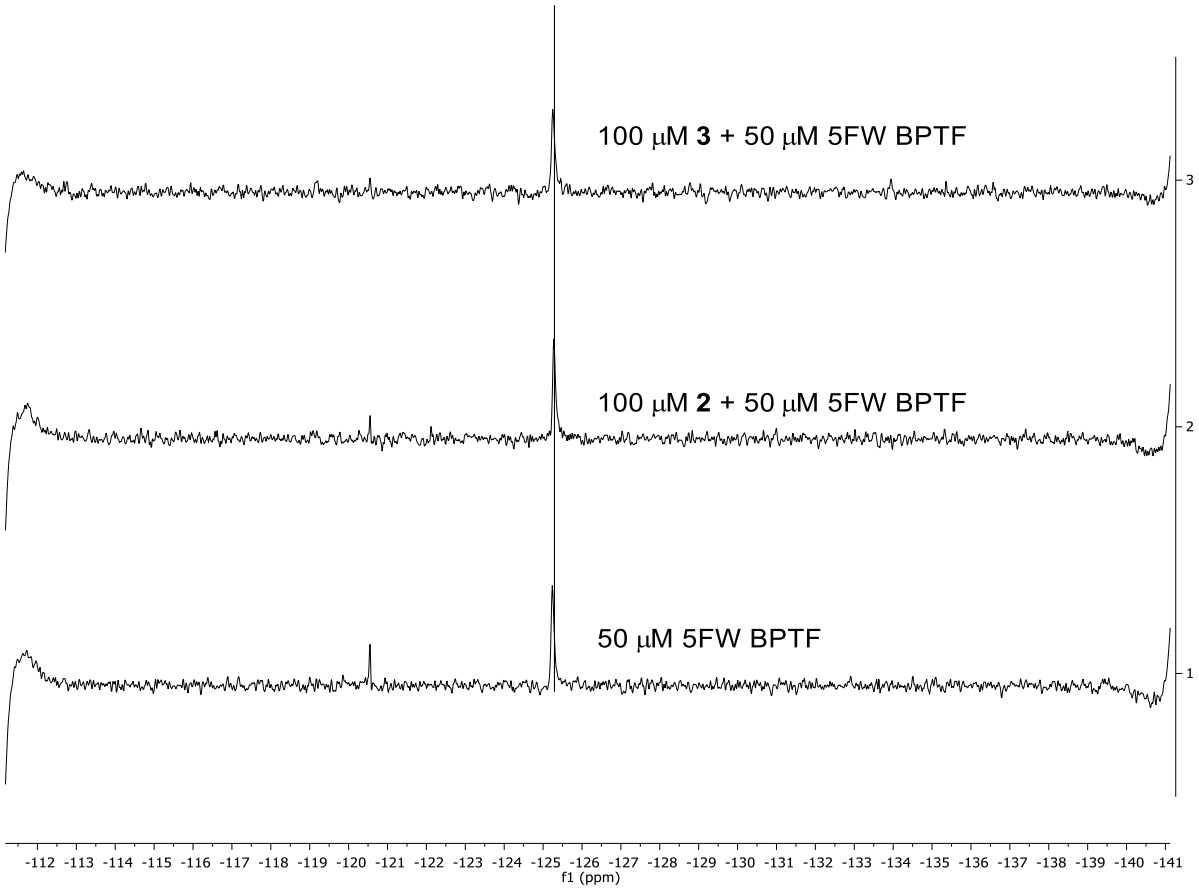
Figure S6: PrOF NMR titration of **(*S*)-1** with 5FW BPTF. Resonance broadens into baseline, no resonance is recovered.

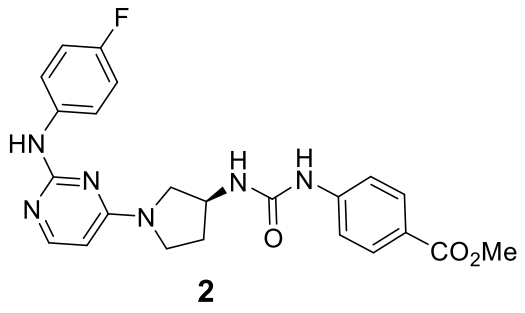




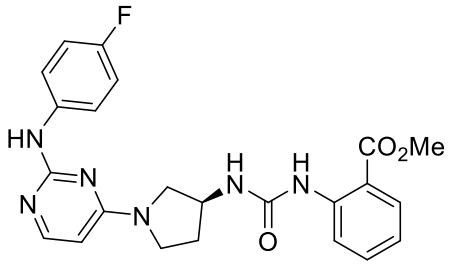
(*R*)-1

Figure S7: PrOF NMR titration of **(*R*)-1** with 5FW BPTF. No dose-response observed, slight movement (0.06 ppm change) seen at all concentrations



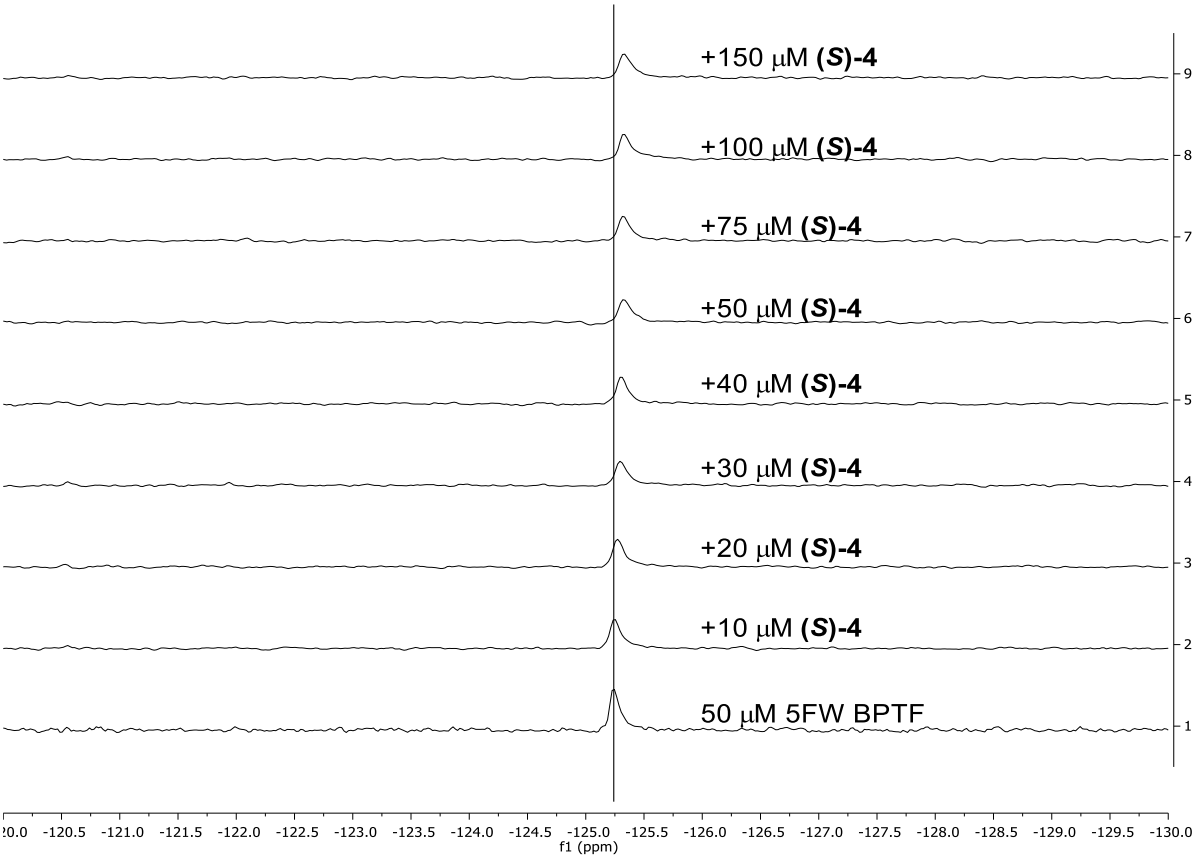


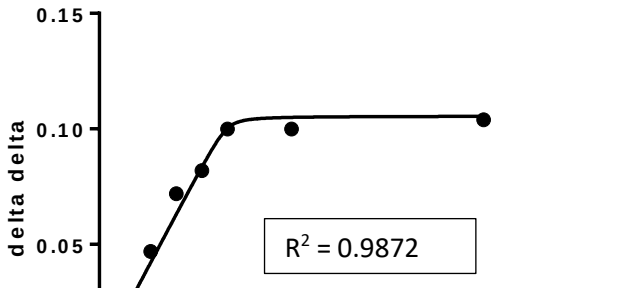
2

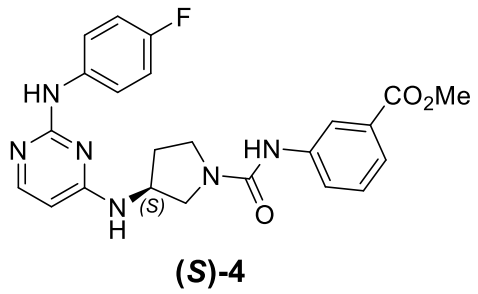


3

Figure S8: Single-point PrOF NMR analysis of **2** and **3** with 5FW BPTF. No resonance movement or broadening observed, compounds do not bind



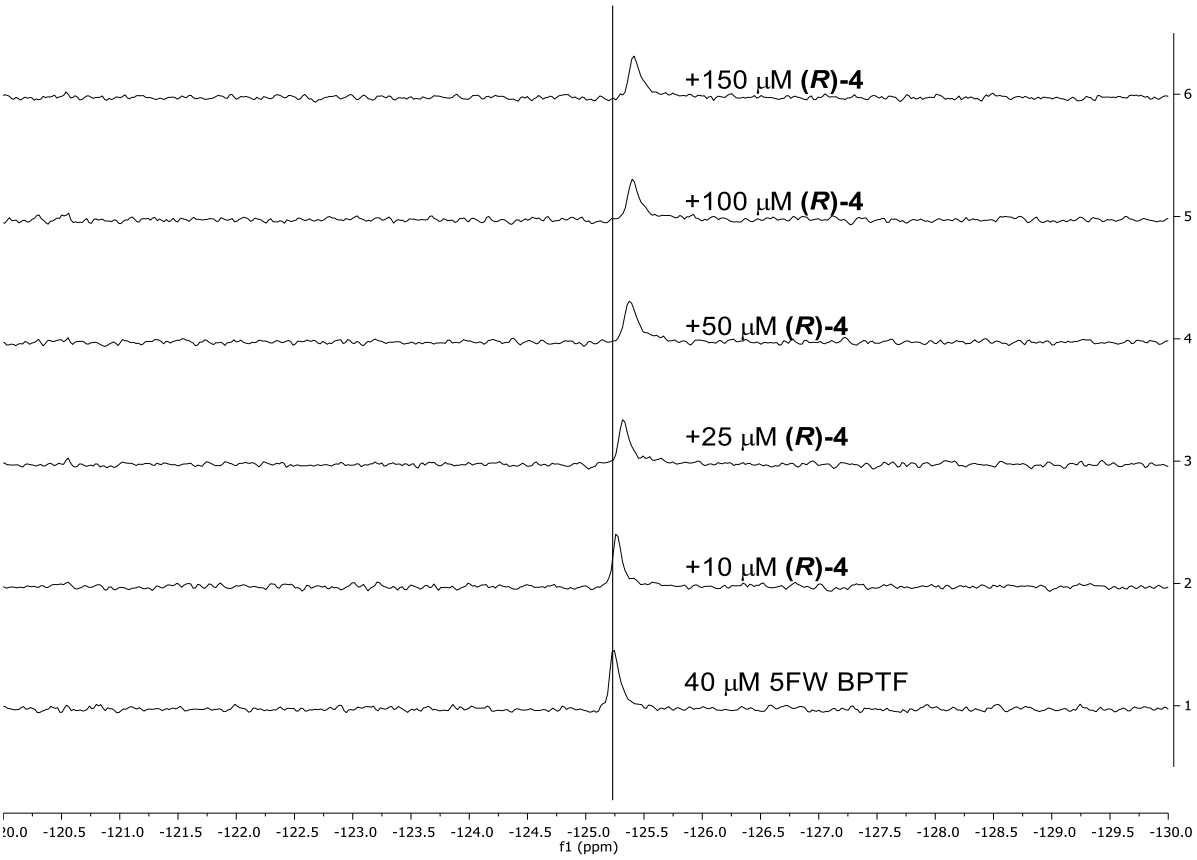


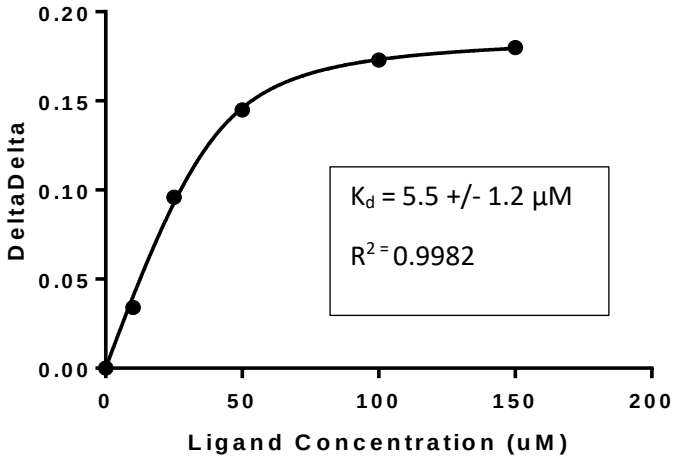


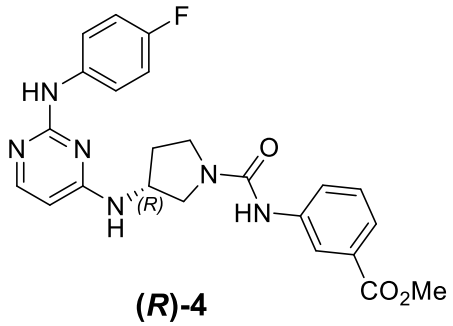
(*S*)-4

Apparent stoichiometric binding prevented K_d determination, followed up with alpha screen

Figure S9: PrOF NMR titration of **(*S*)-4** with 5FW BPTF.

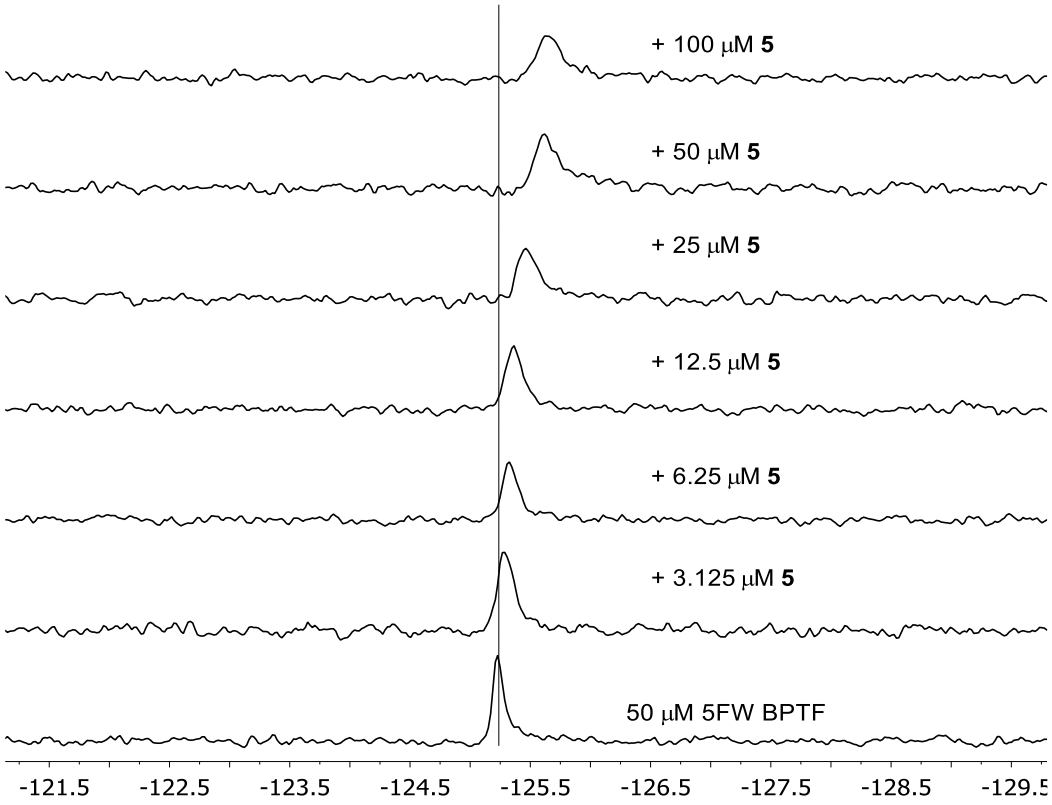


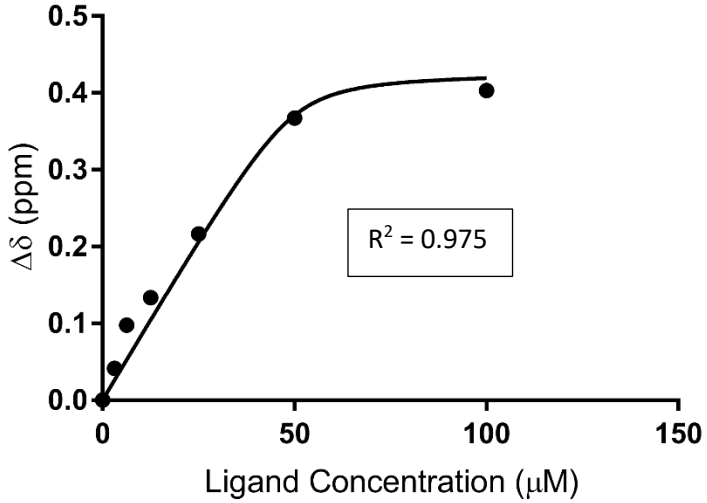


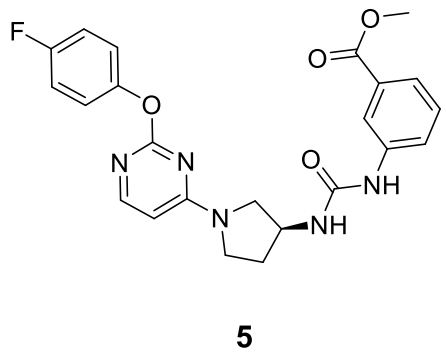


(*R*)-4

Figure S10: PrOF NMR titration of **(*R*)-1** with 5FW BPTF.



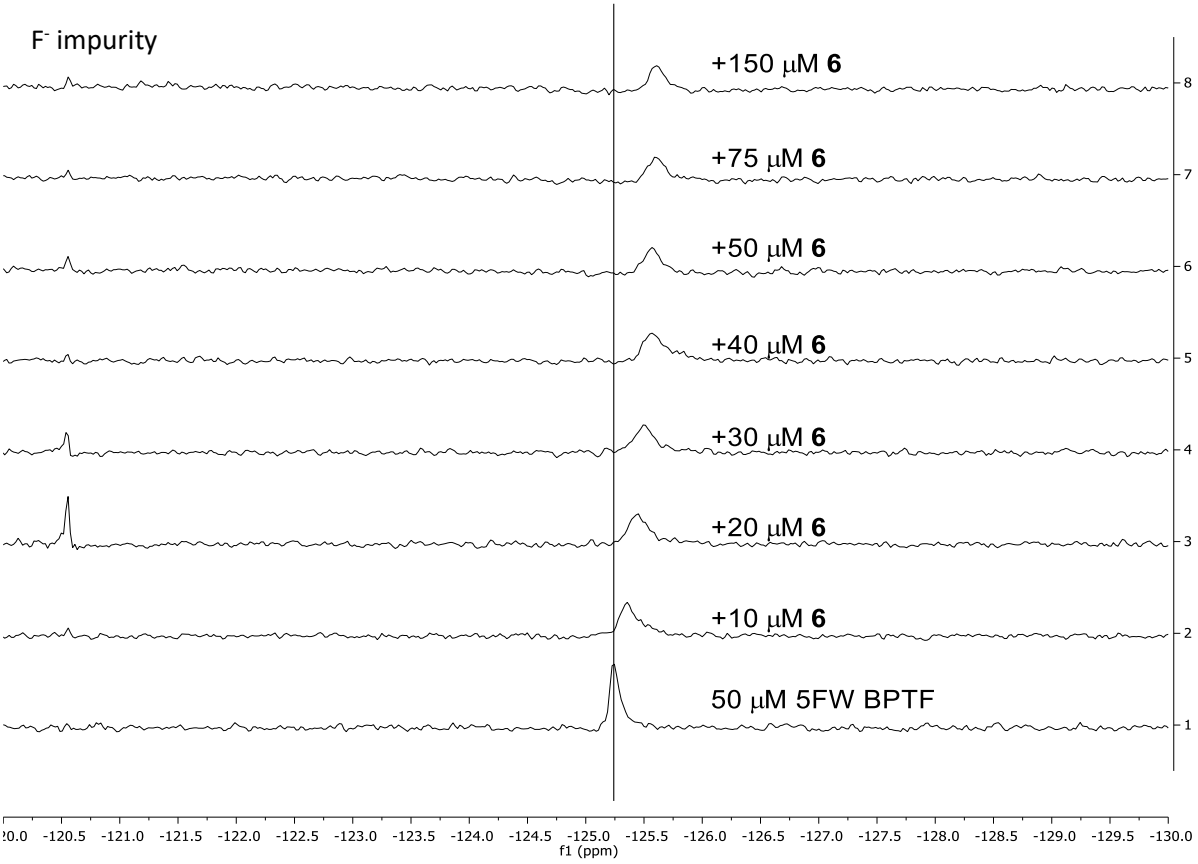


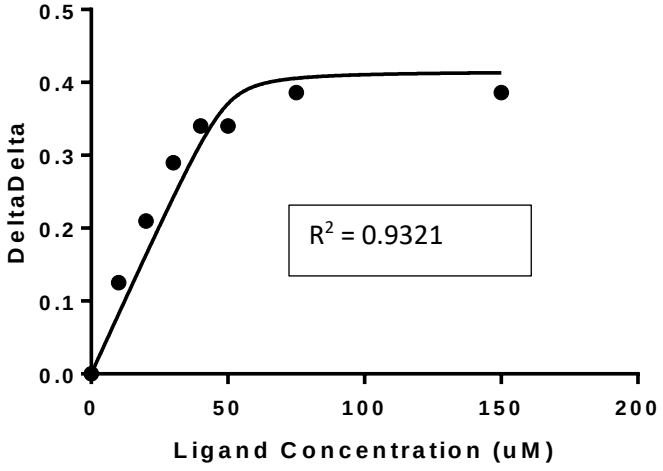


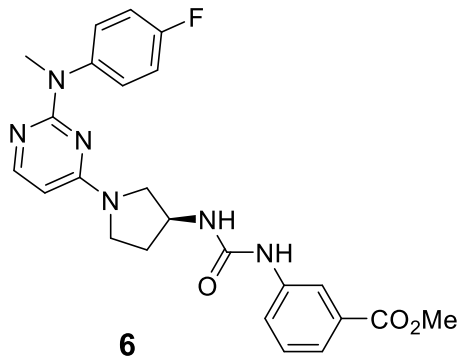
5

Apparent stoichiometric binding prevents accurate K_d determination

Figure S11: PrOF NMR titration of **5** with 5FW BPTF.







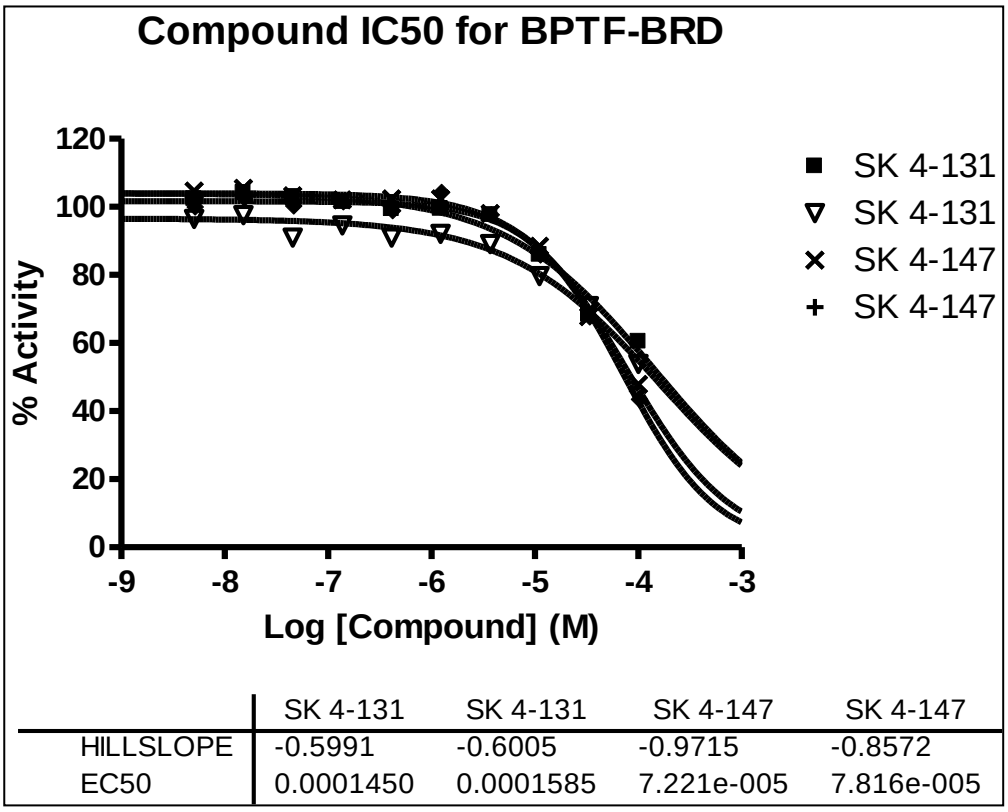
6

Apparent stoichiometric binding prevented determination of a $K_{d,}$

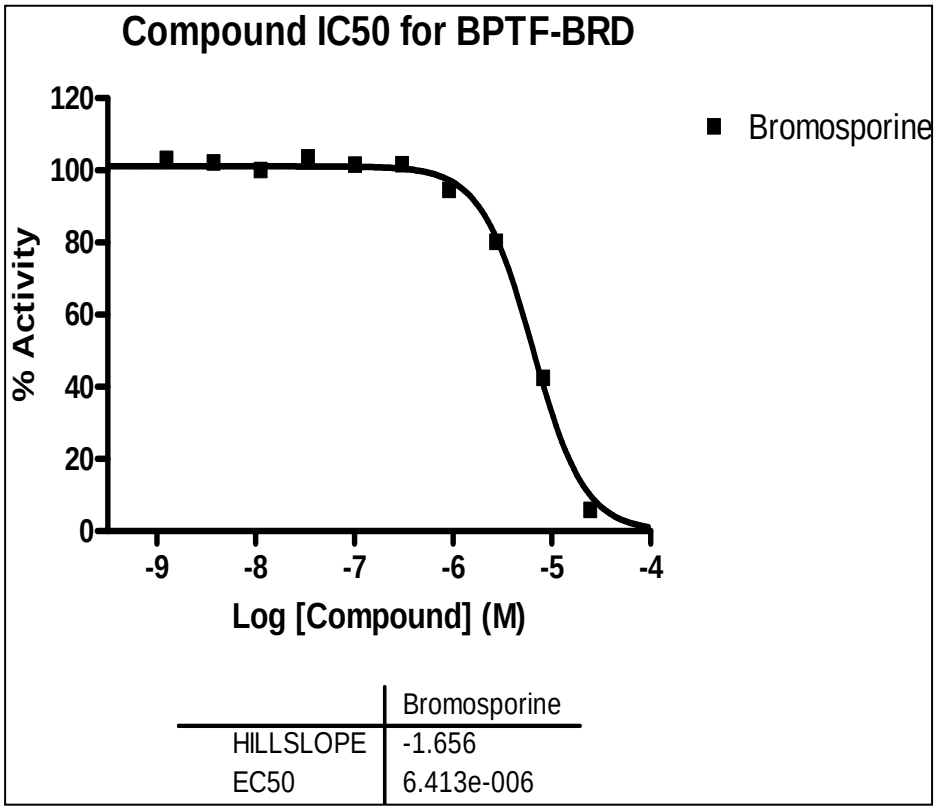
Followed up with alphascreen assay (next page)

Figure S12: PrOF NMR titration of **6** with 5FW BPTF.

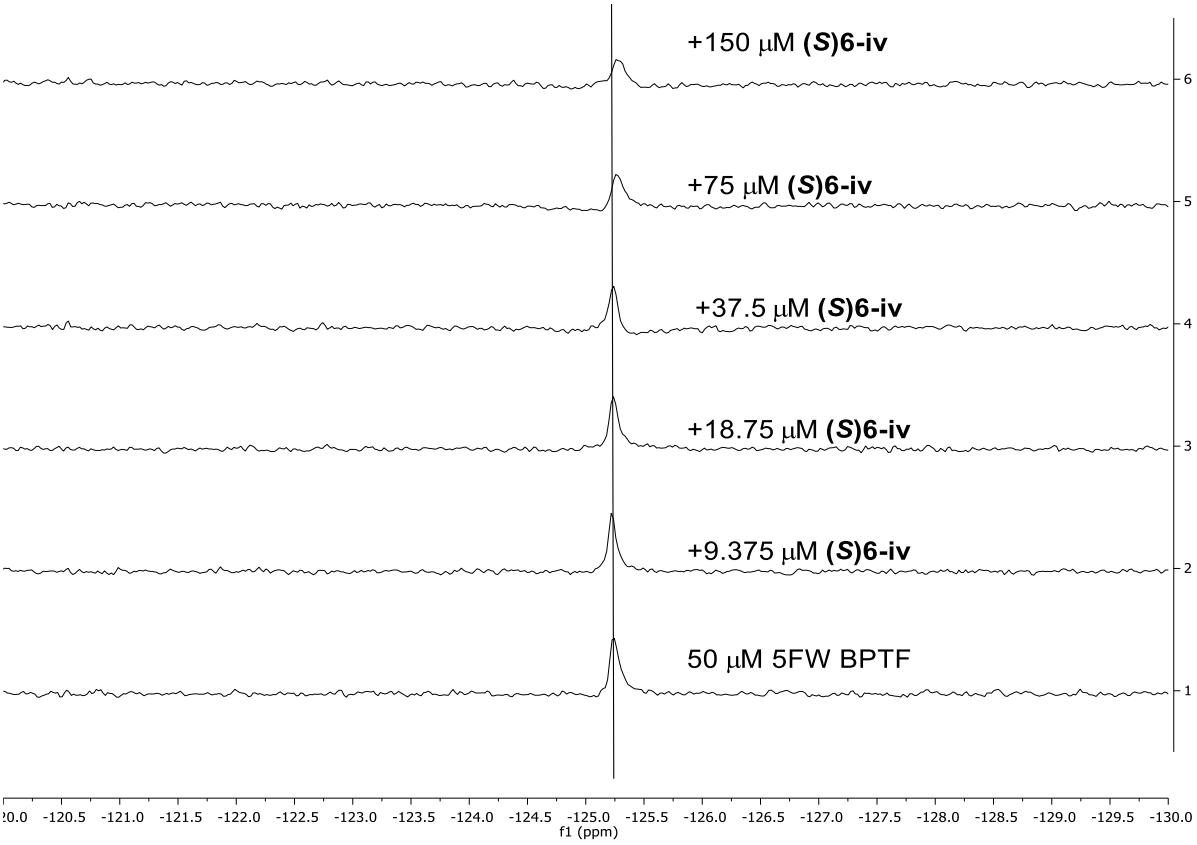
Figure S13: Alpha Screen data for **(*S*)-4**, **6,** and known pan-bromodomain inhibitor, bromosporine



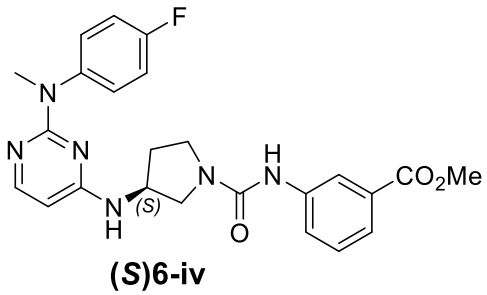
	SK 4-131	SK 4-131	SK 4-147	SK 4-147
HILLSLOPE	-0.5991	-0.6005	-0.9715	-0.8572
EC50	0.0001450	0.0001585	7.221e-005	7.816e-005



	Bromosporine
HILLSLOPE	-1.656
EC50	6.413e-006

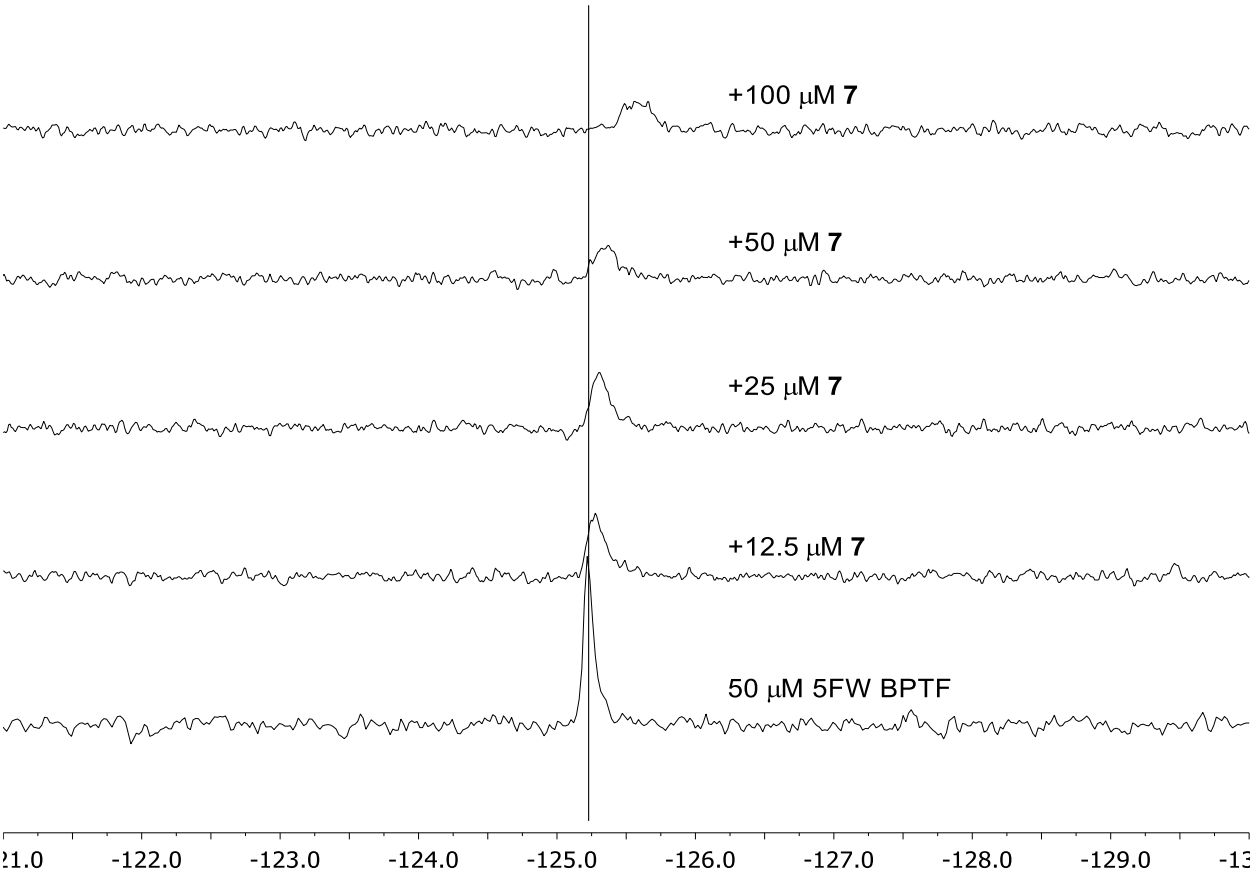


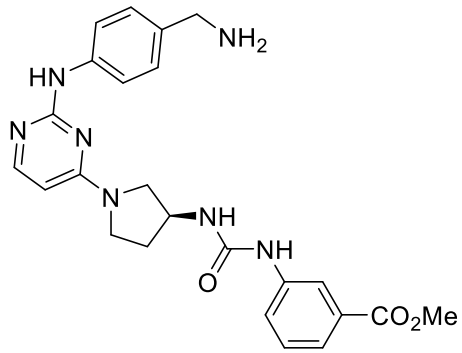
Slight broadening, can not fit to curve



(*S*)6-iv

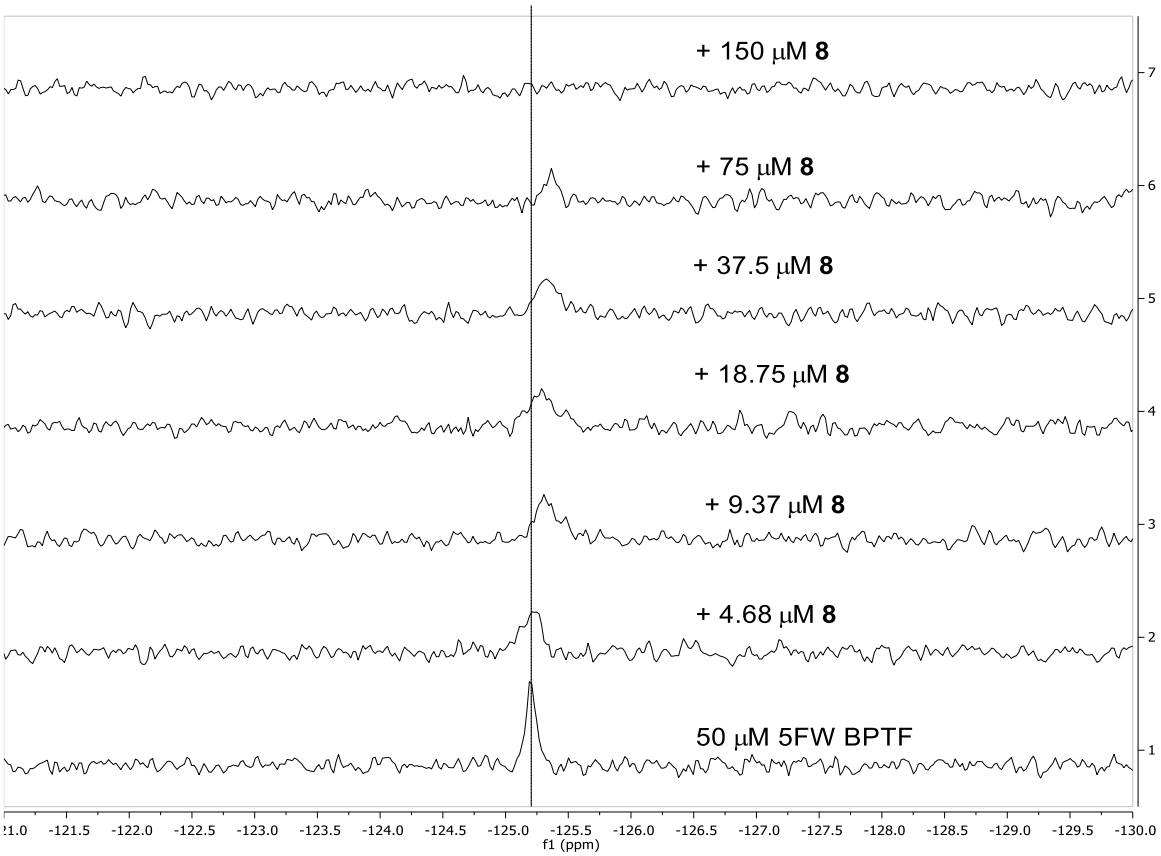
Figure S14: PrOF NMR titration of **(*S*)6-iv** with 5FW BPTF.

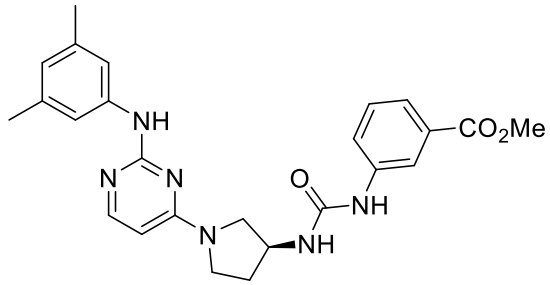




7

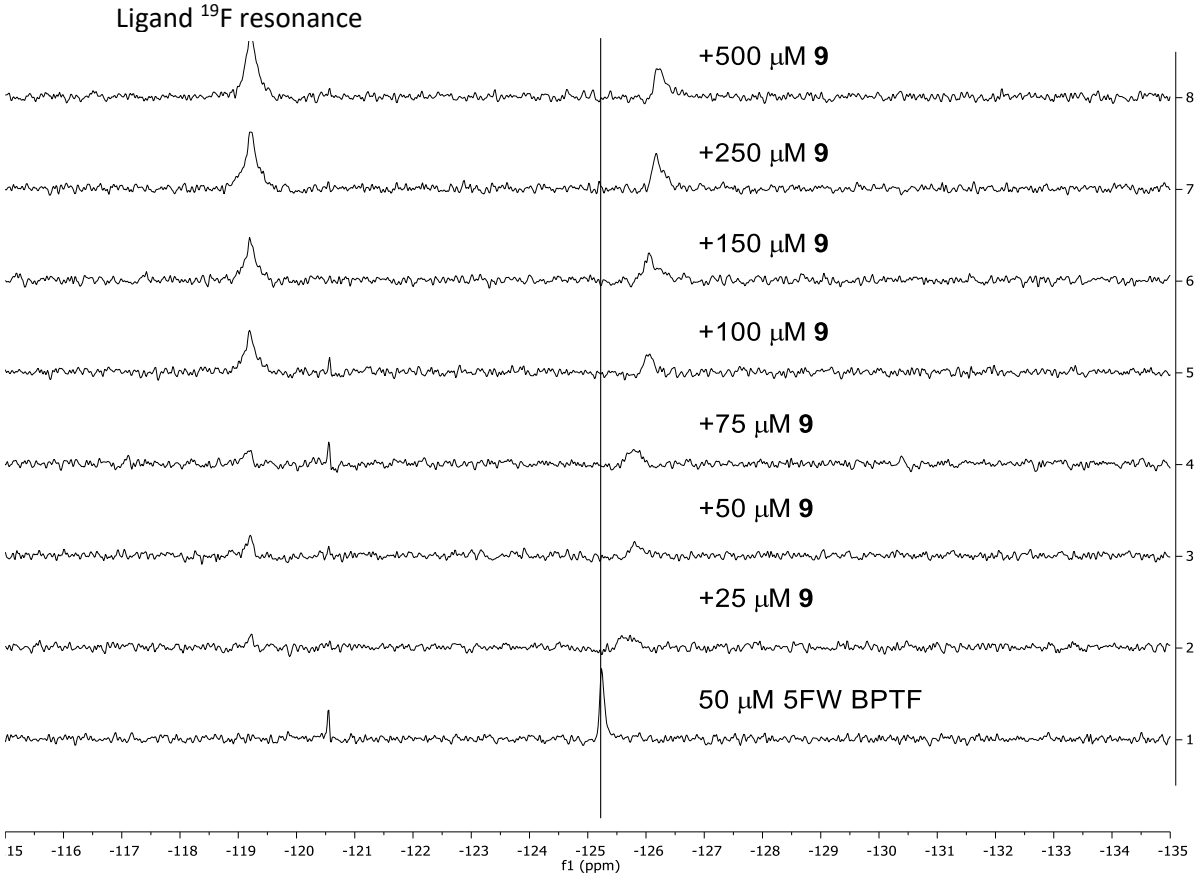
Figure S15: PrOF NMR titration of **7** with 5FW BPTF.





8

Figure S16: PrOF NMR titration of **8** with 5FW BPTF. Broadening and eventual disappearance into baseline, can not fit curve



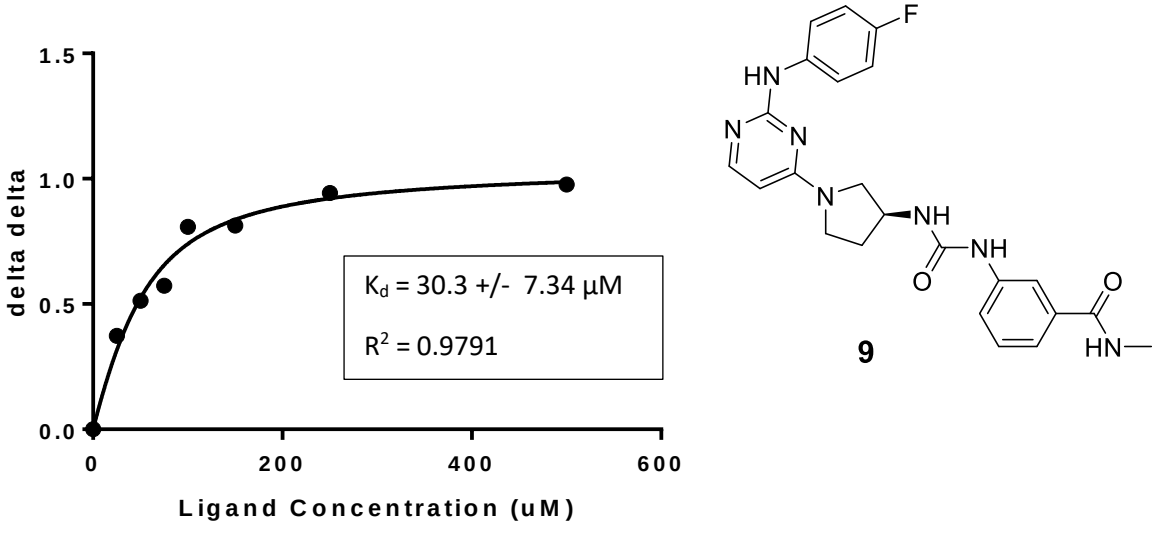
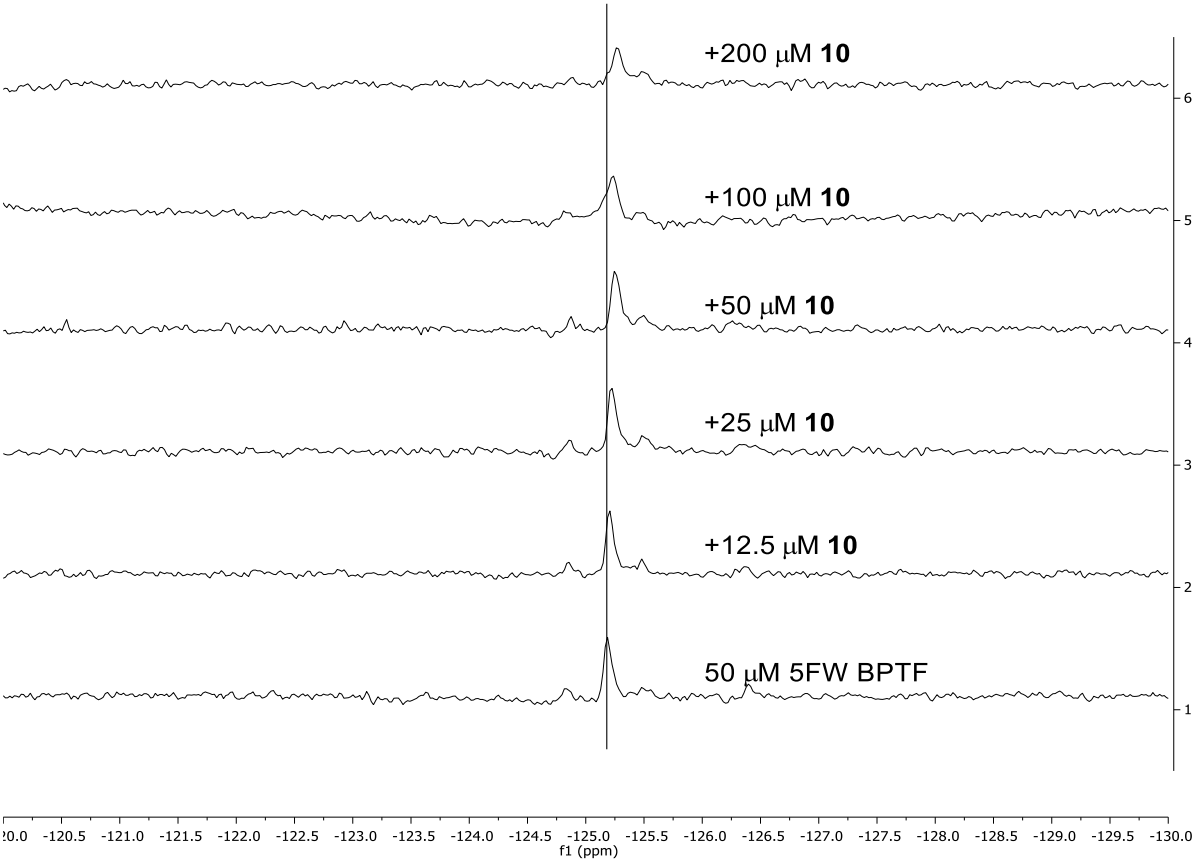
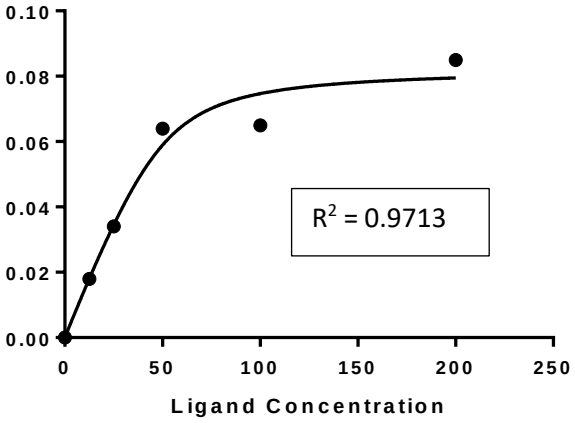
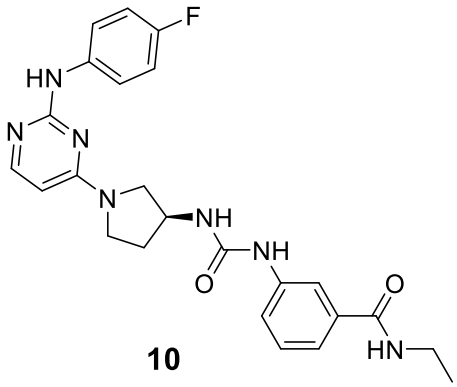


Figure S17: PrOF NMR titration of **9** with 5FW BPTF.



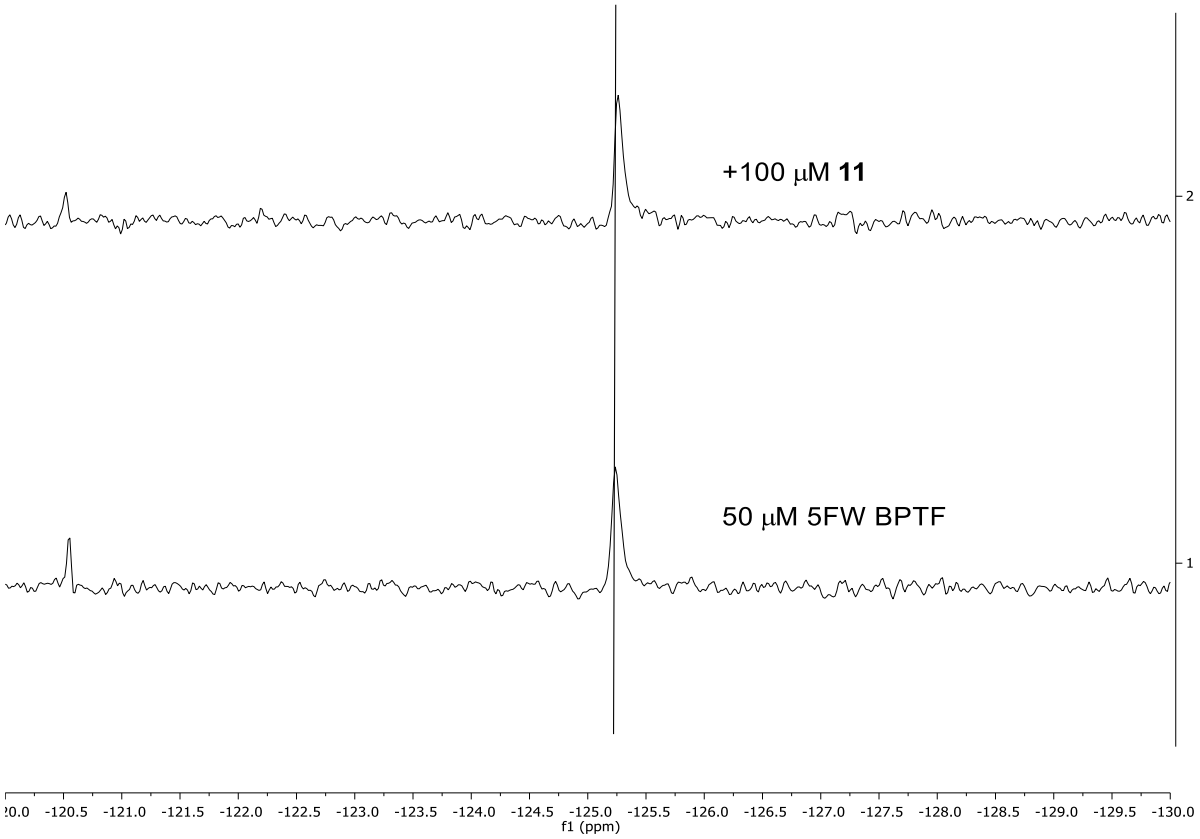


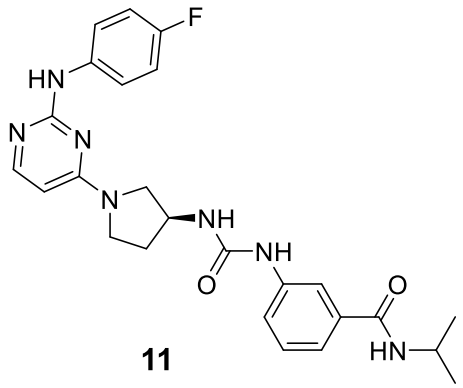


10

Small dynamic range prevents reliable K_d determination

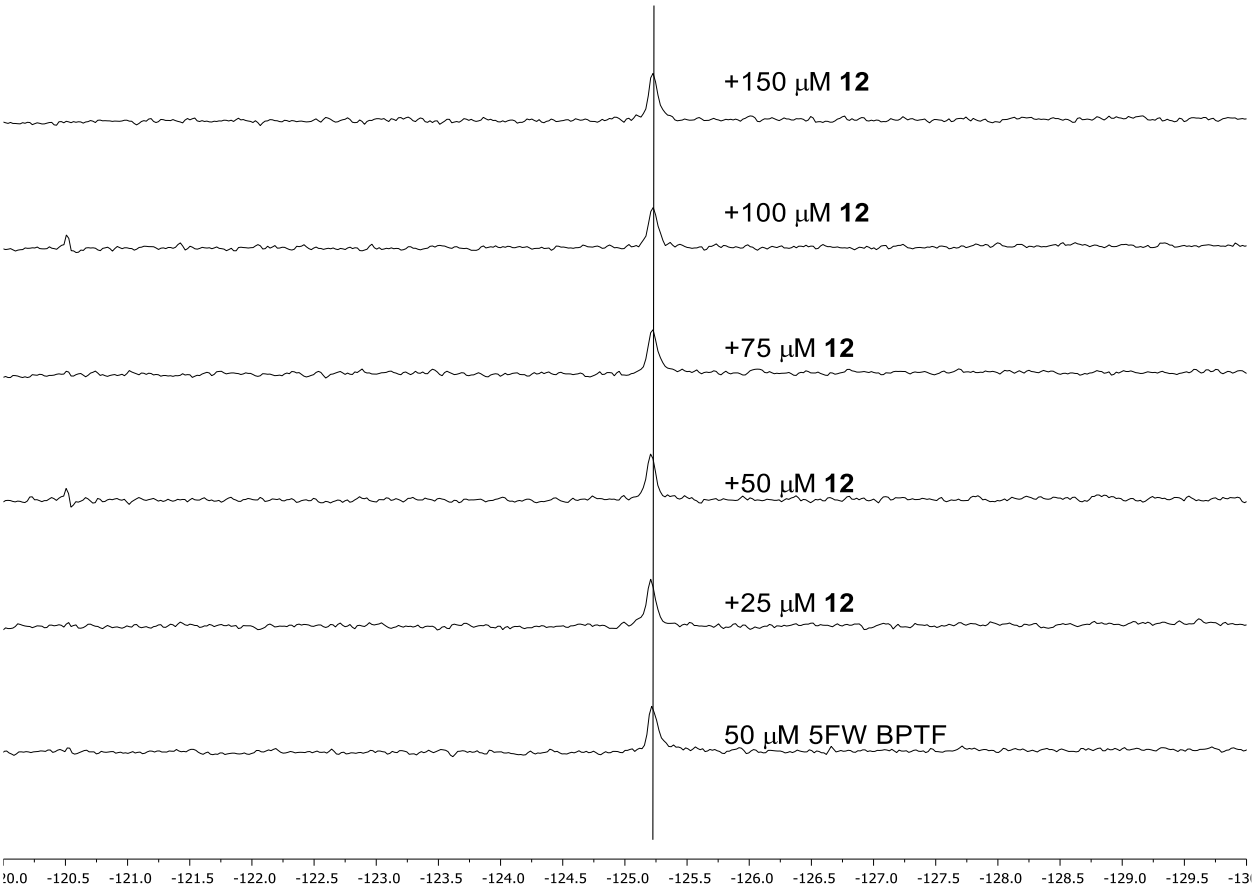
Figure S18: PrOF NMR titration of **10** with 5FW BPTF.

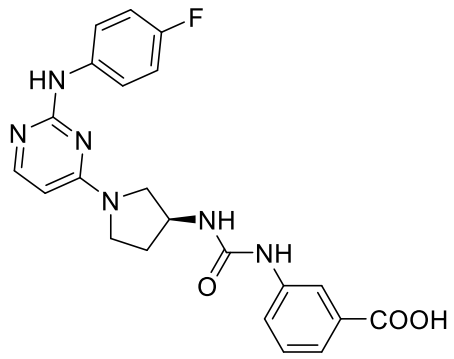




11

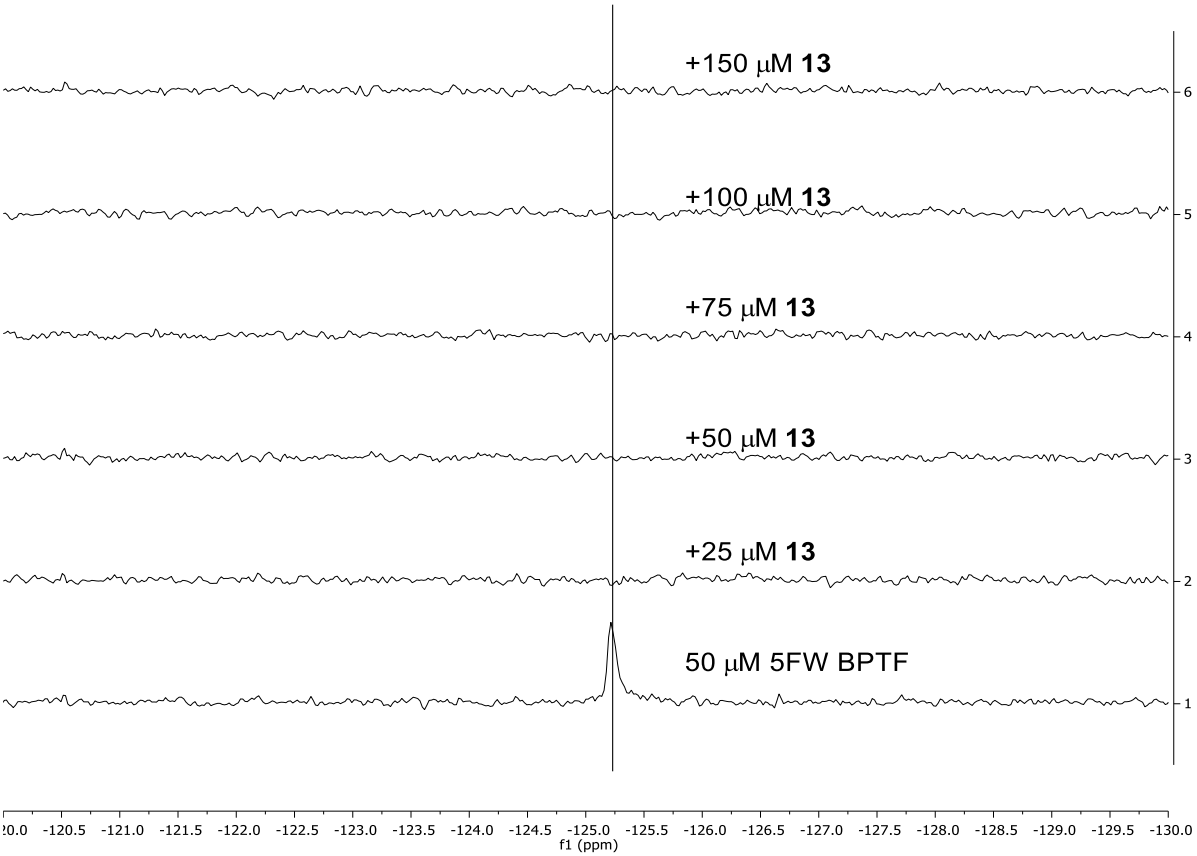
Figure S19: Single point PrOF NMR analysis of **8** with 5FW BPTF. No binding observed

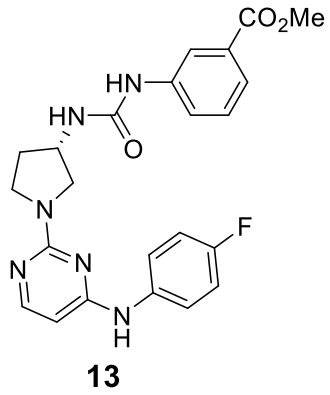




12

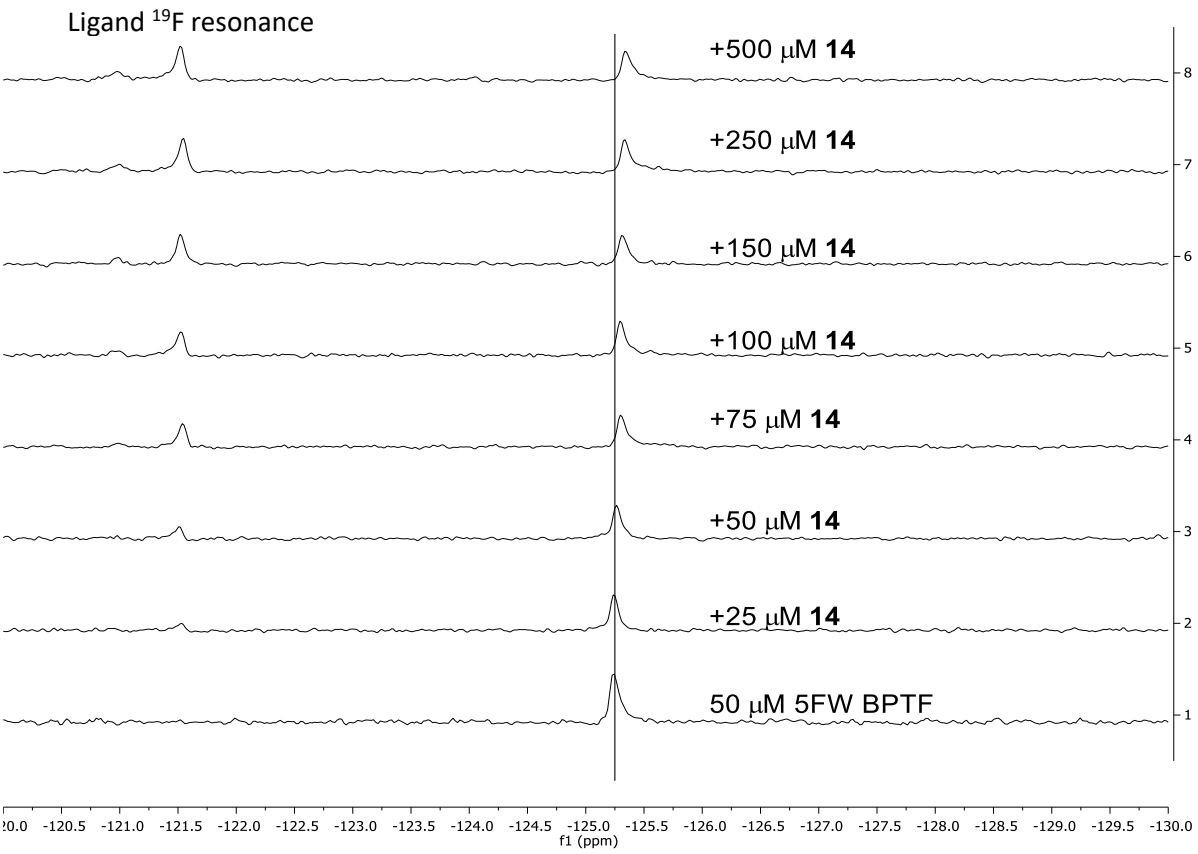
Figure S20: PrOF NMR titration of **12** with 5FW BPTF. No binding observed





13

Figure S21: PrOF NMR titration of **13** with 5FW BPTF. Broadens into baseline and does not return, no quantitative data possible



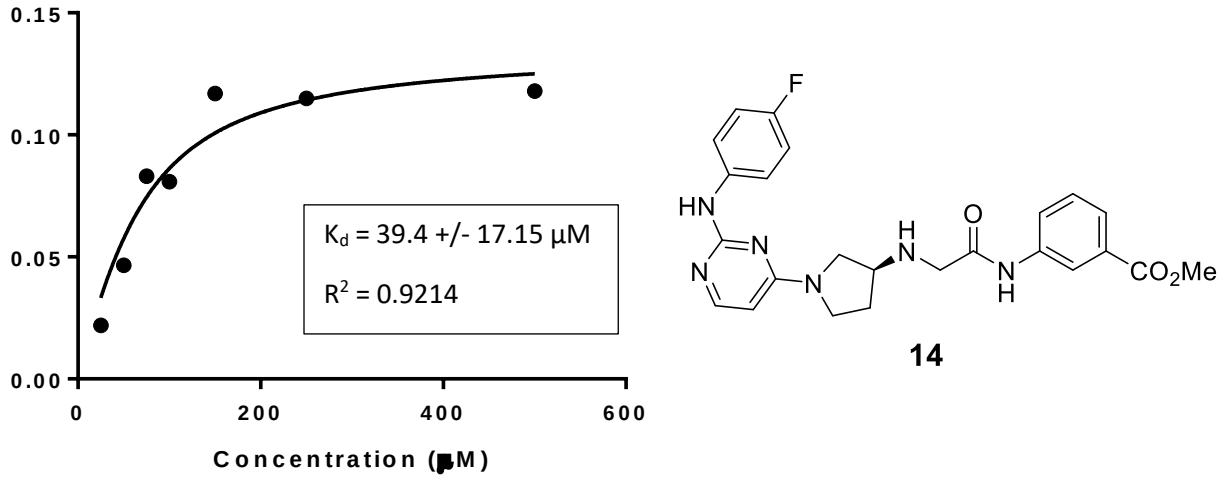
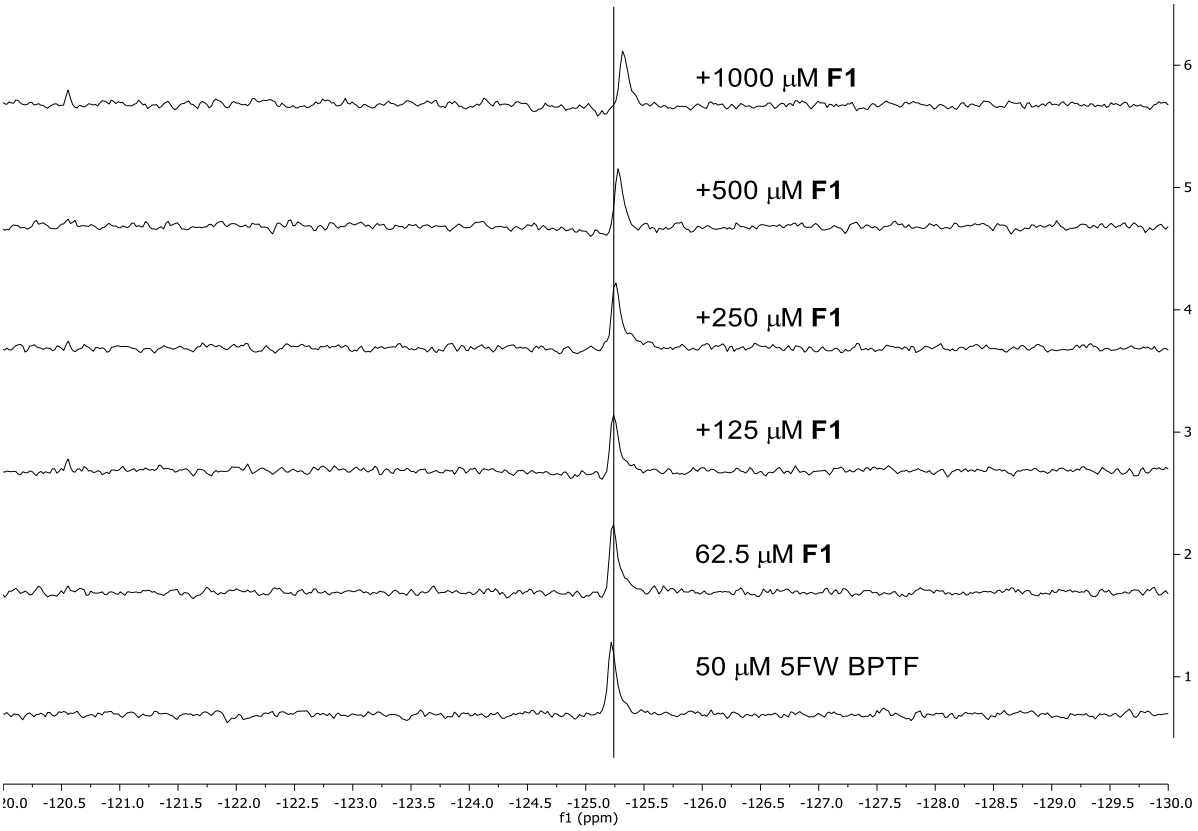
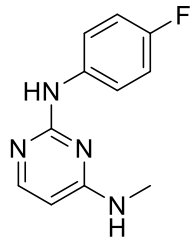


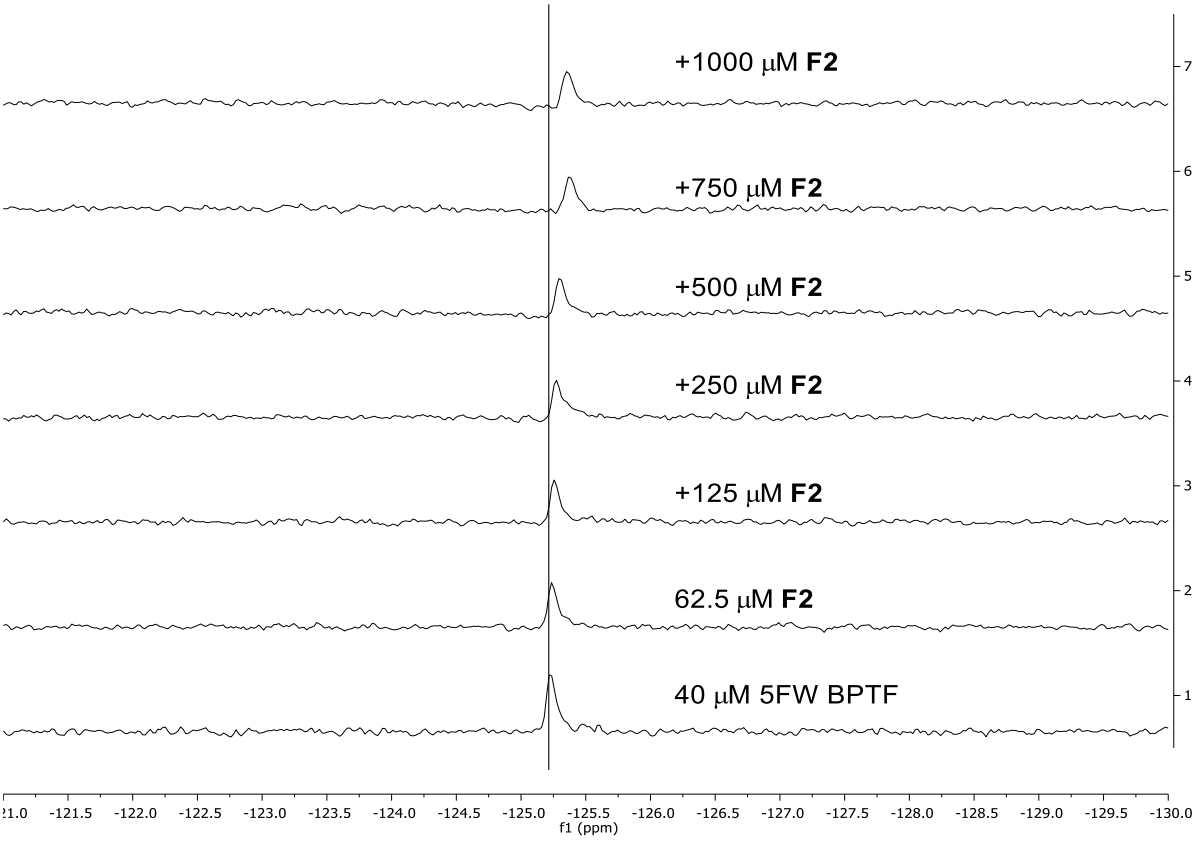
Figure S22: PrOF NMR titration of **14** with 5FW BPTF.





F1

Figure S23: PrOF NMR titration of **F1** with 5FW BPTF. Chemical shift movement only observed at high concentrations, potentially due to non-specific binding.



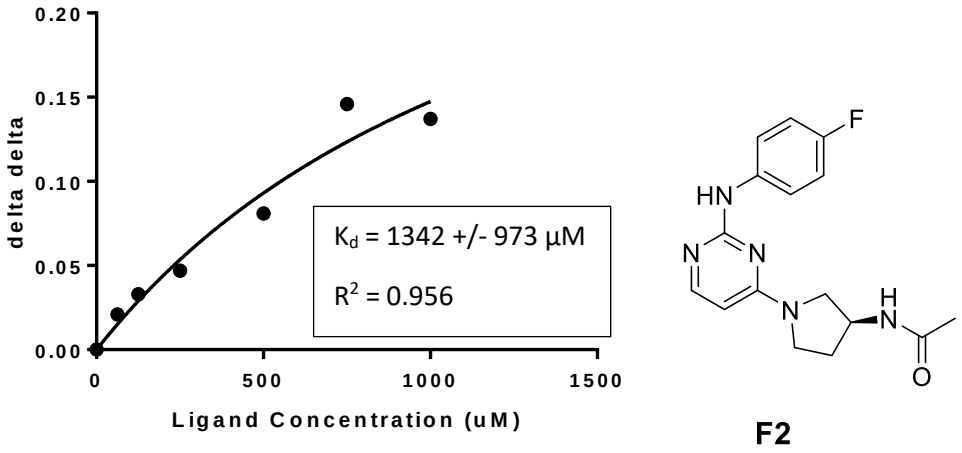
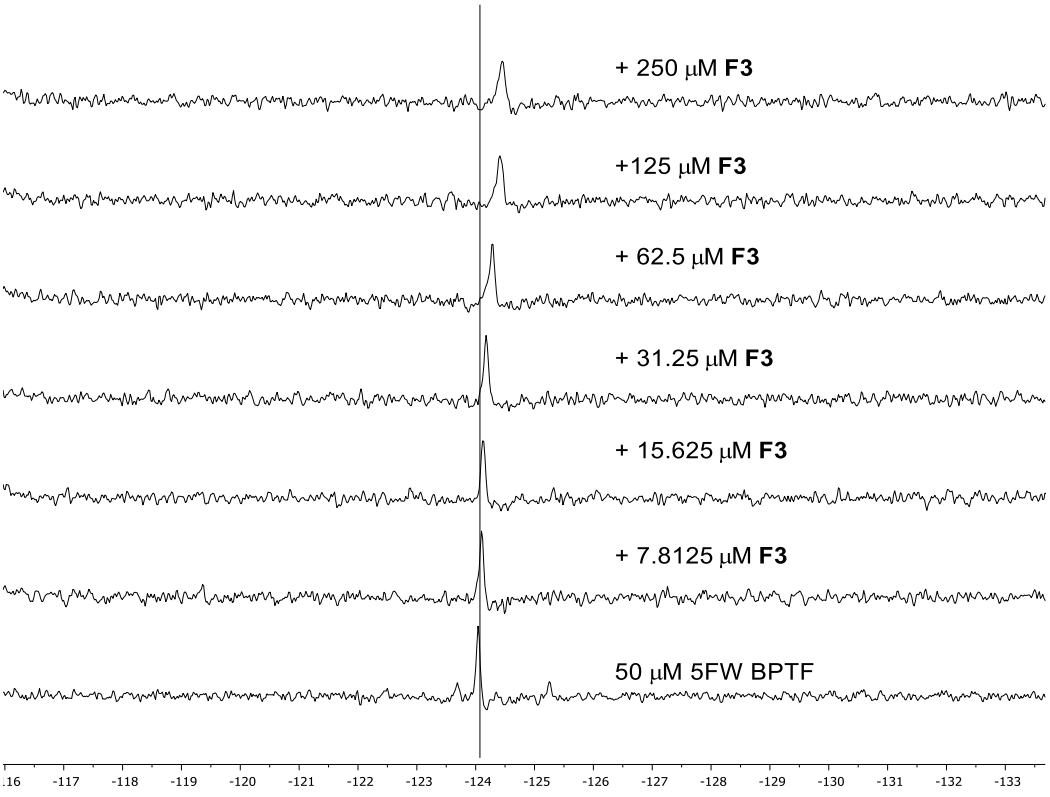
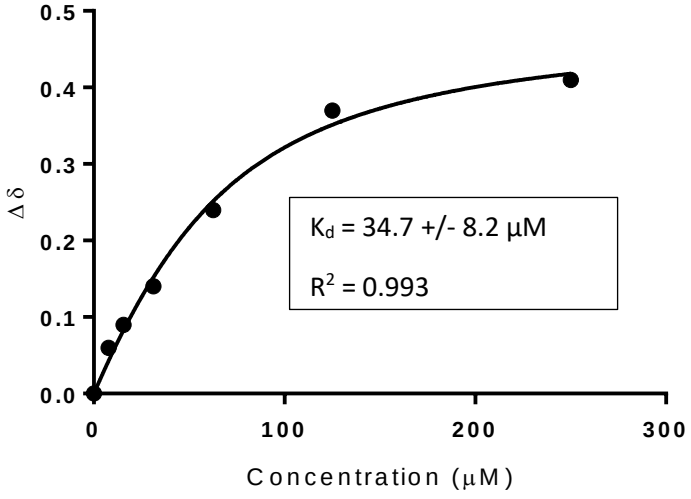
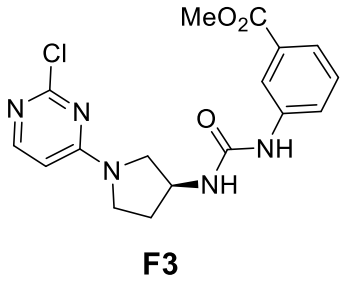


Figure S24: PrOF NMR titration of **F2** with 5FW BPTF.

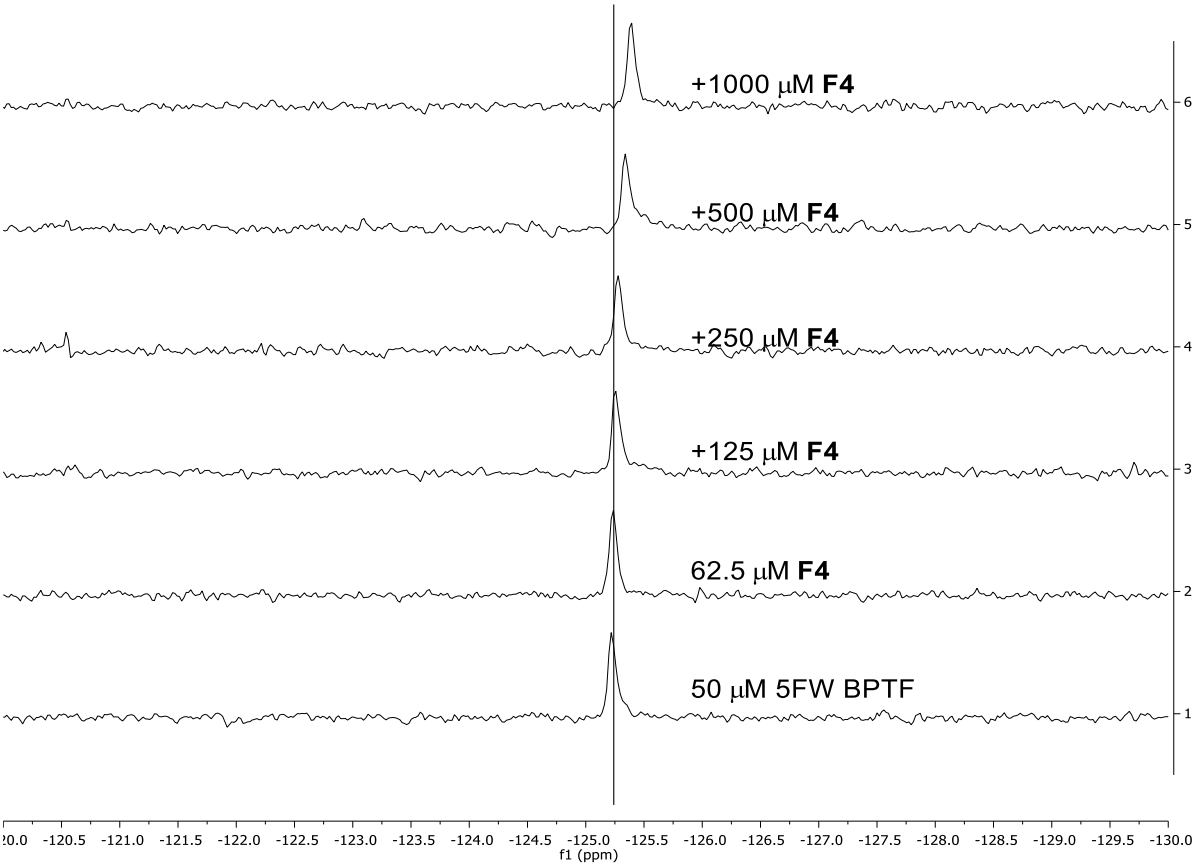


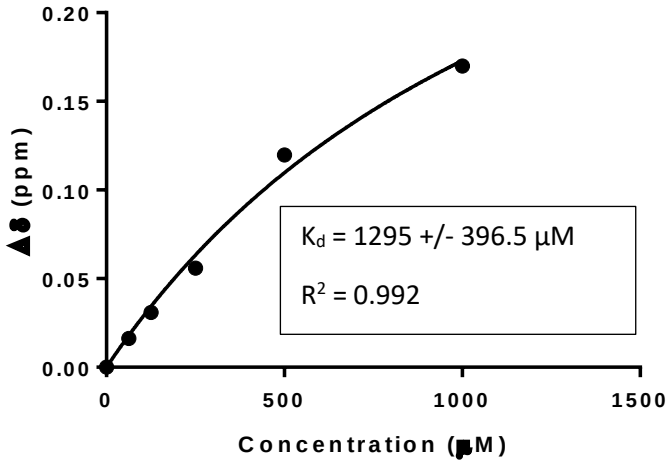


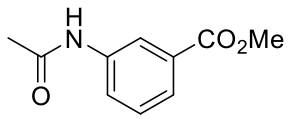


F3

Figure S25: PrOF NMR titration of **F3** with 5FW BPTF.

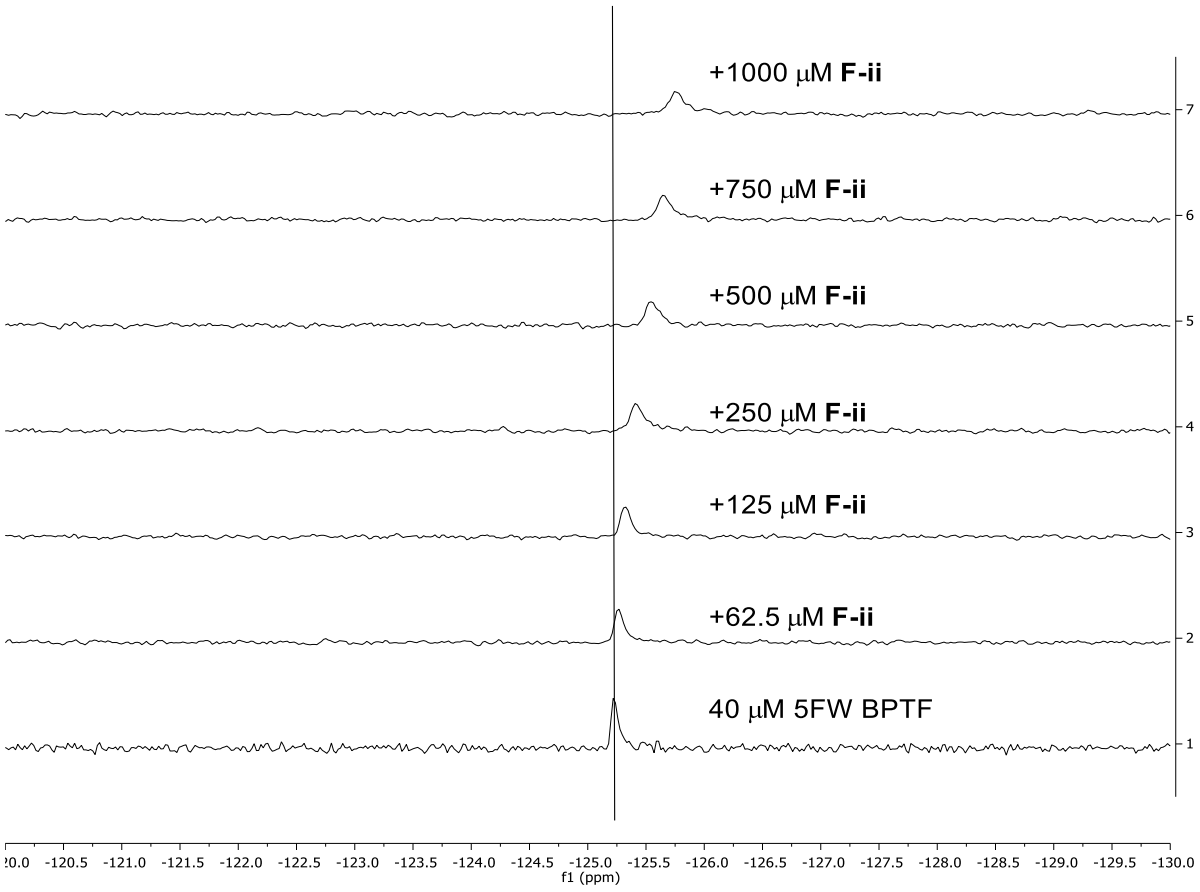


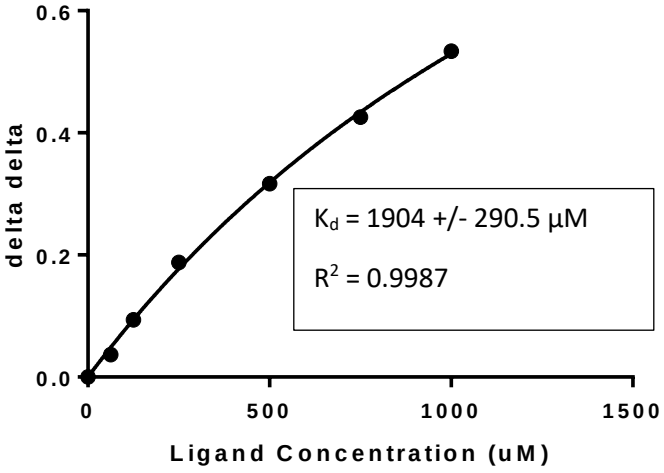


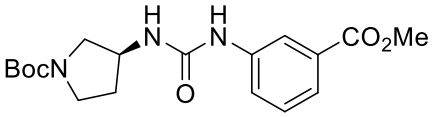


F4

Figure S26: PrOF NMR titration of **F4** with 5FW BPTF.

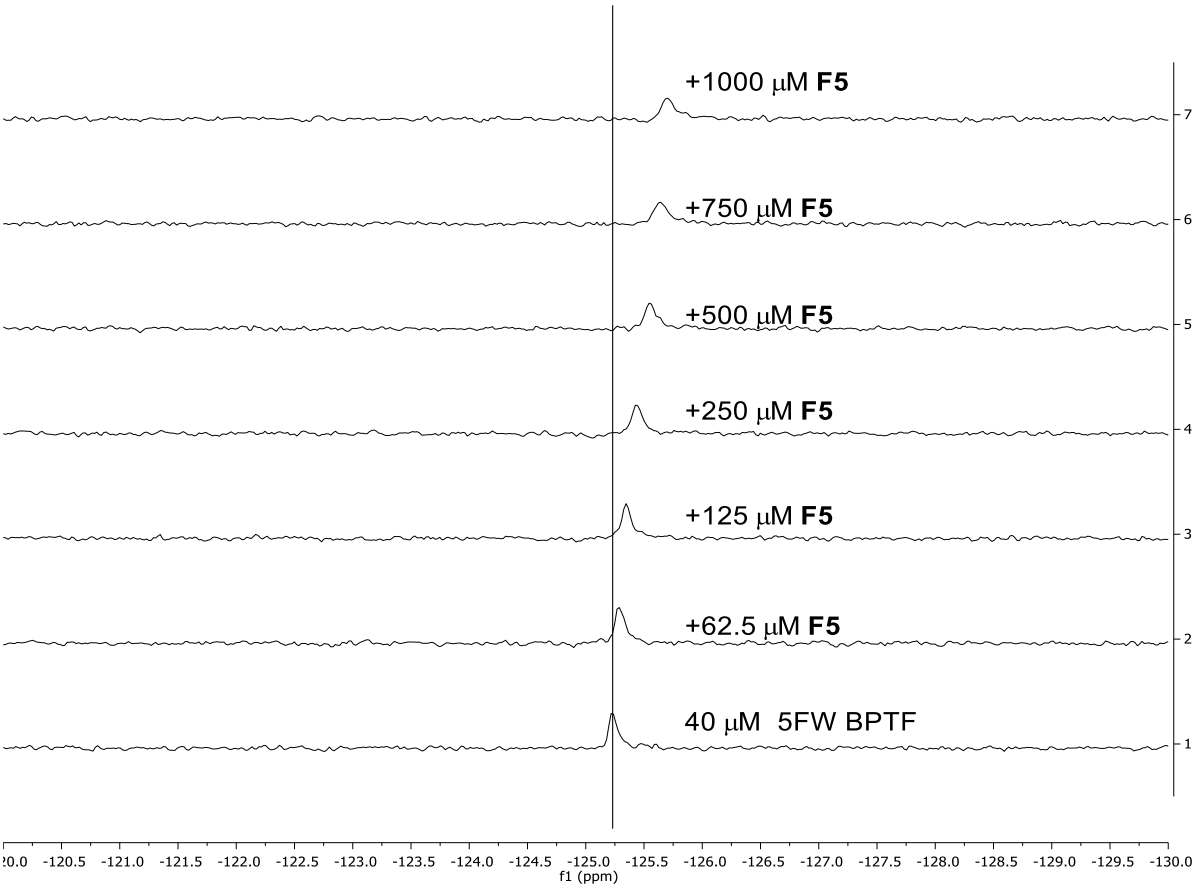


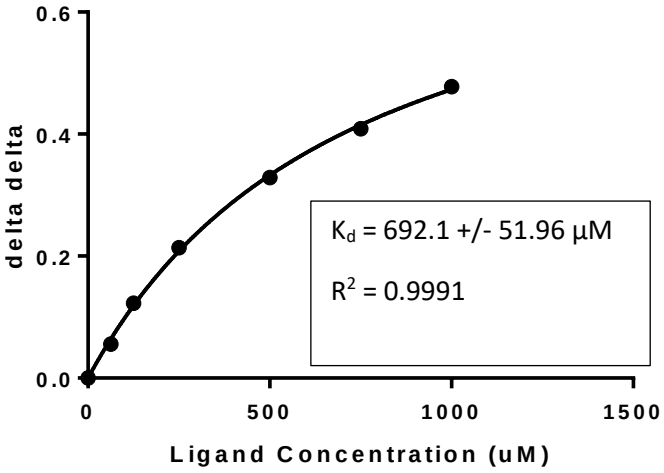




F-ii

Figure S27: PrOF NMR titration of **F-ii** with 5FW BPTF.





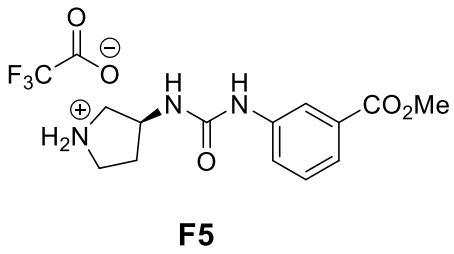
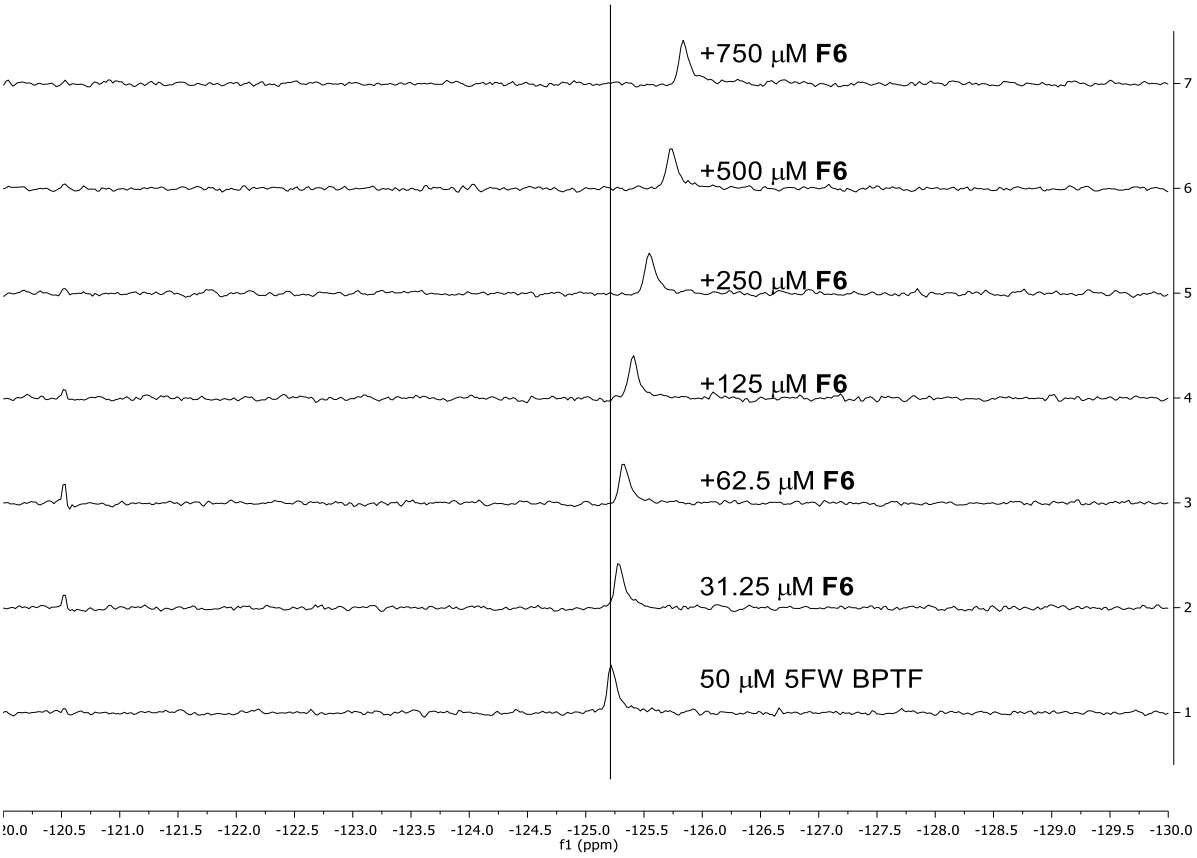
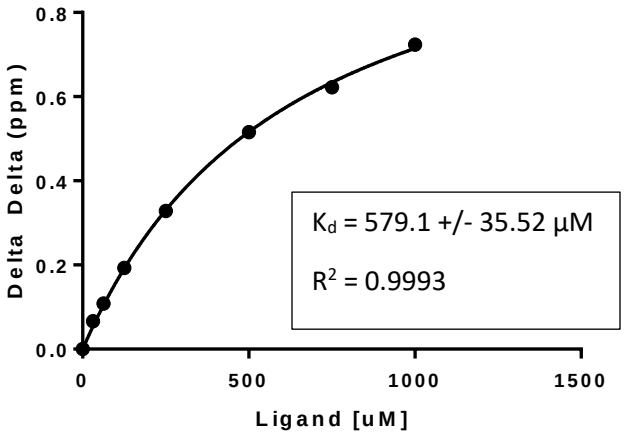
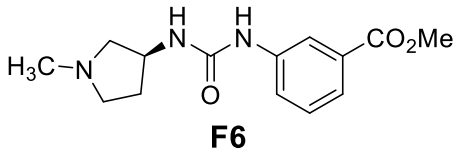


Figure S28: PrOF NMR titration of **F5** with 5FW BPTF.

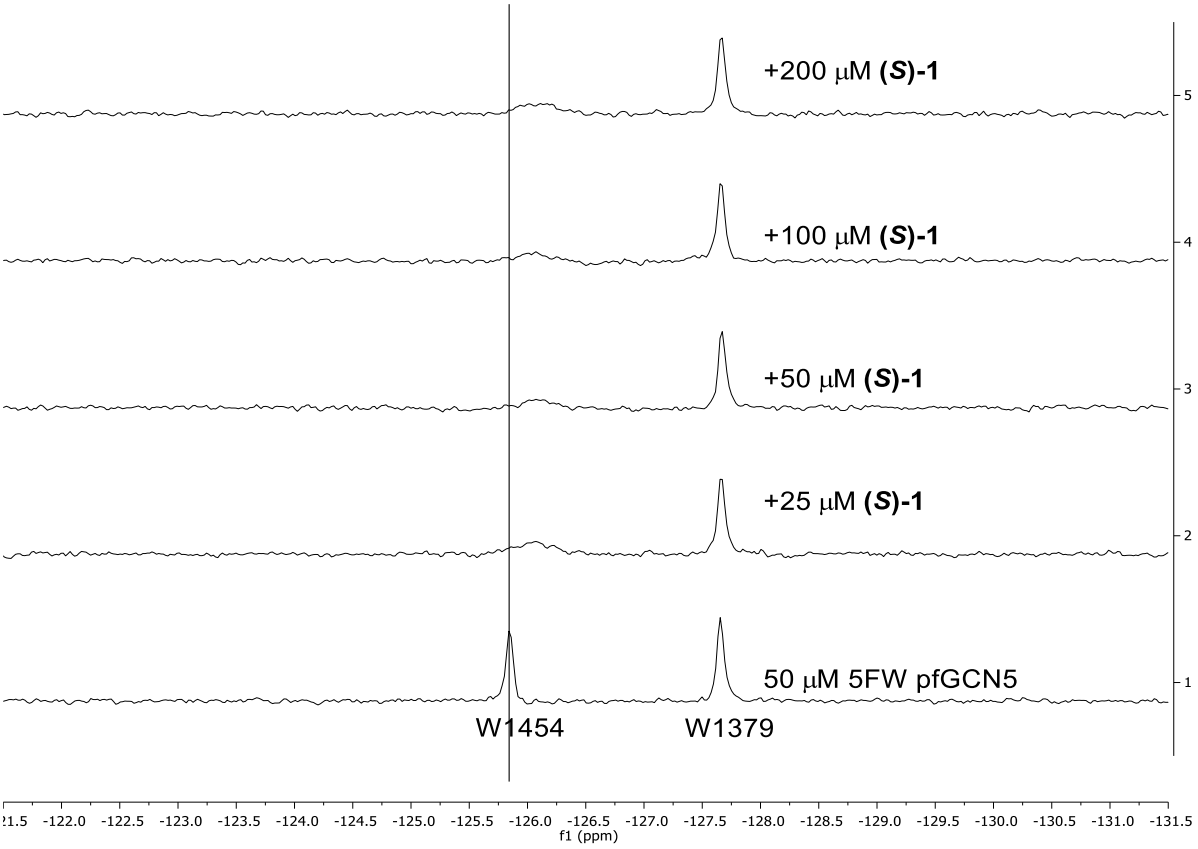


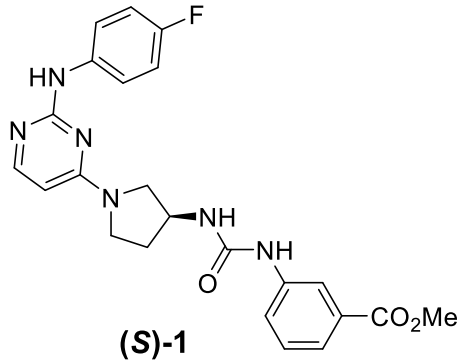




F6

Figure S29: PrOF NMR titration of **F6** with 5FW BPTF.





(*S*)-1

Figure S30: PrOF NMR titration of **(*S*)-1** with *Pf*GCN5. *Pf* GCN5 possesses two tryptophan residues, **(*S*)-1** impacts only W1454.

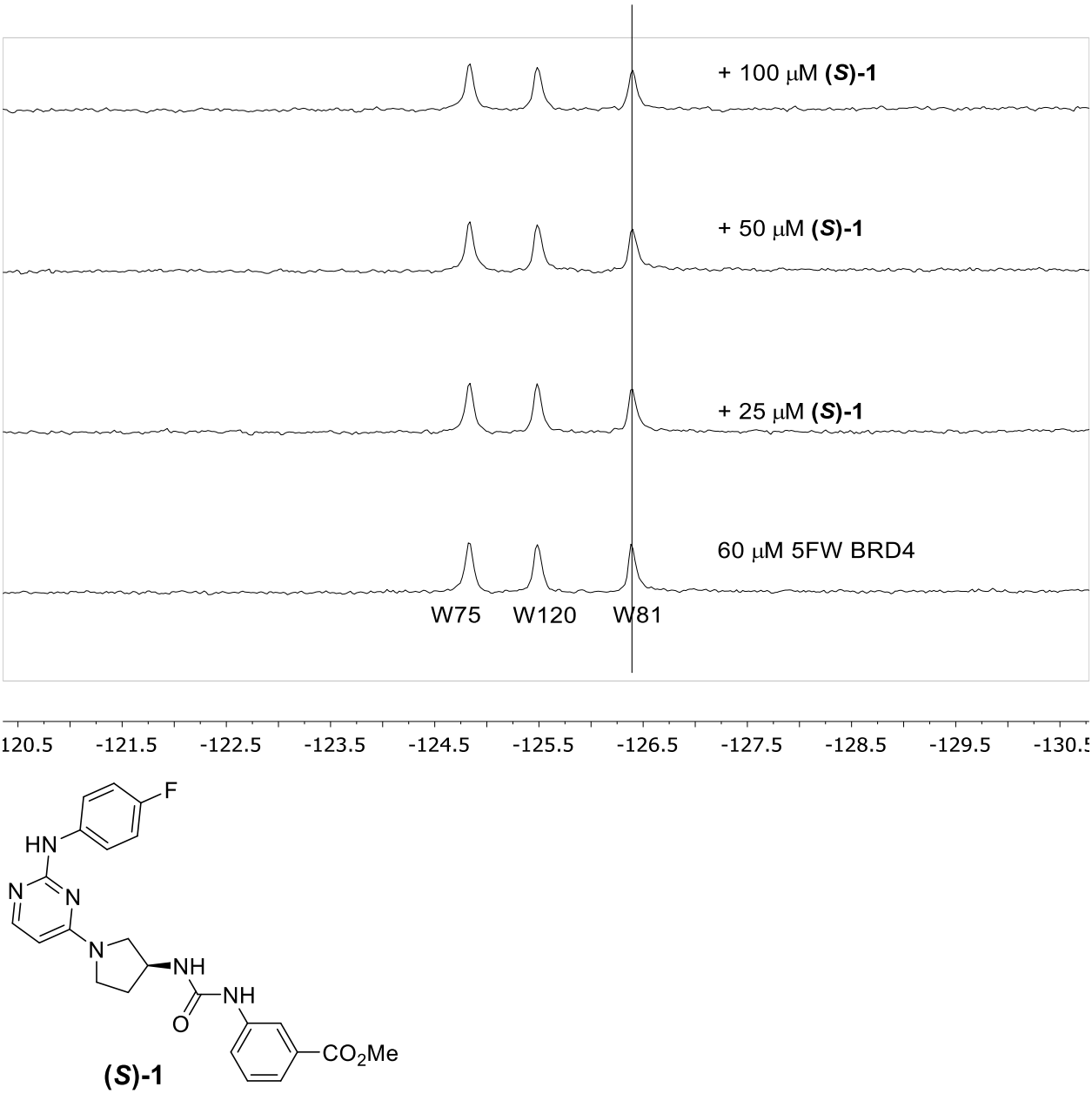


Figure S31: PrOF NMR titration of **(*S*)-1** with 5FW BRD4(BD1). No binding is observed

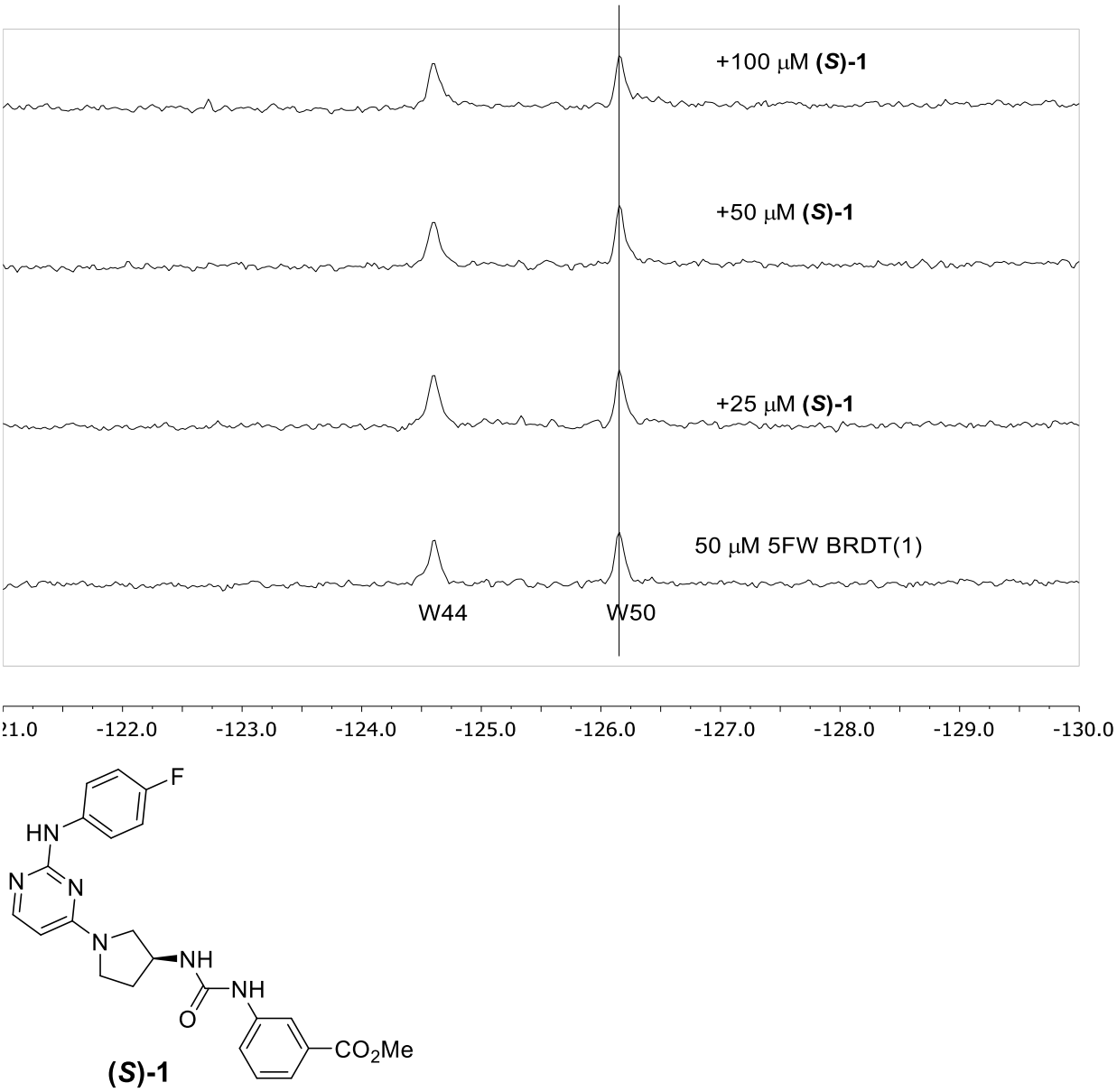
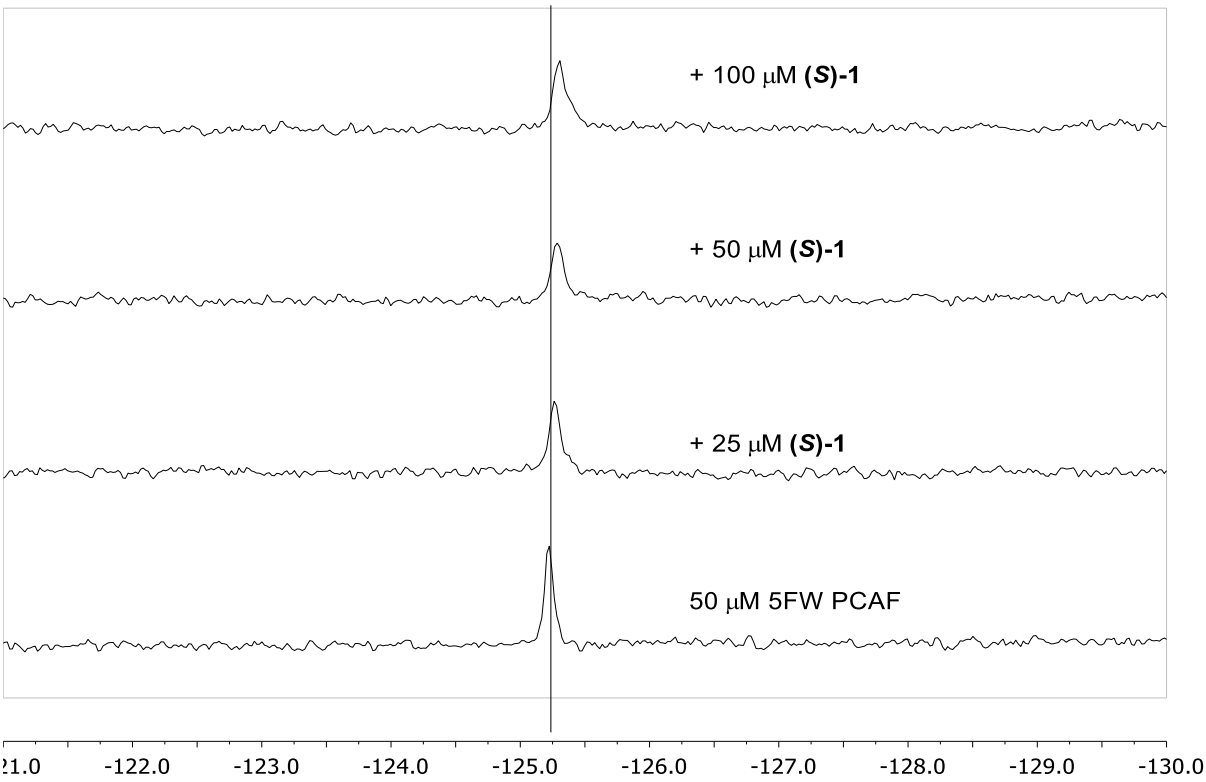
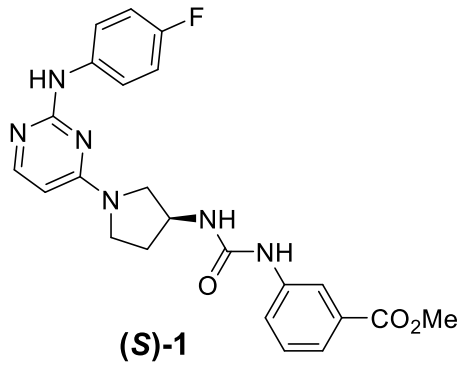


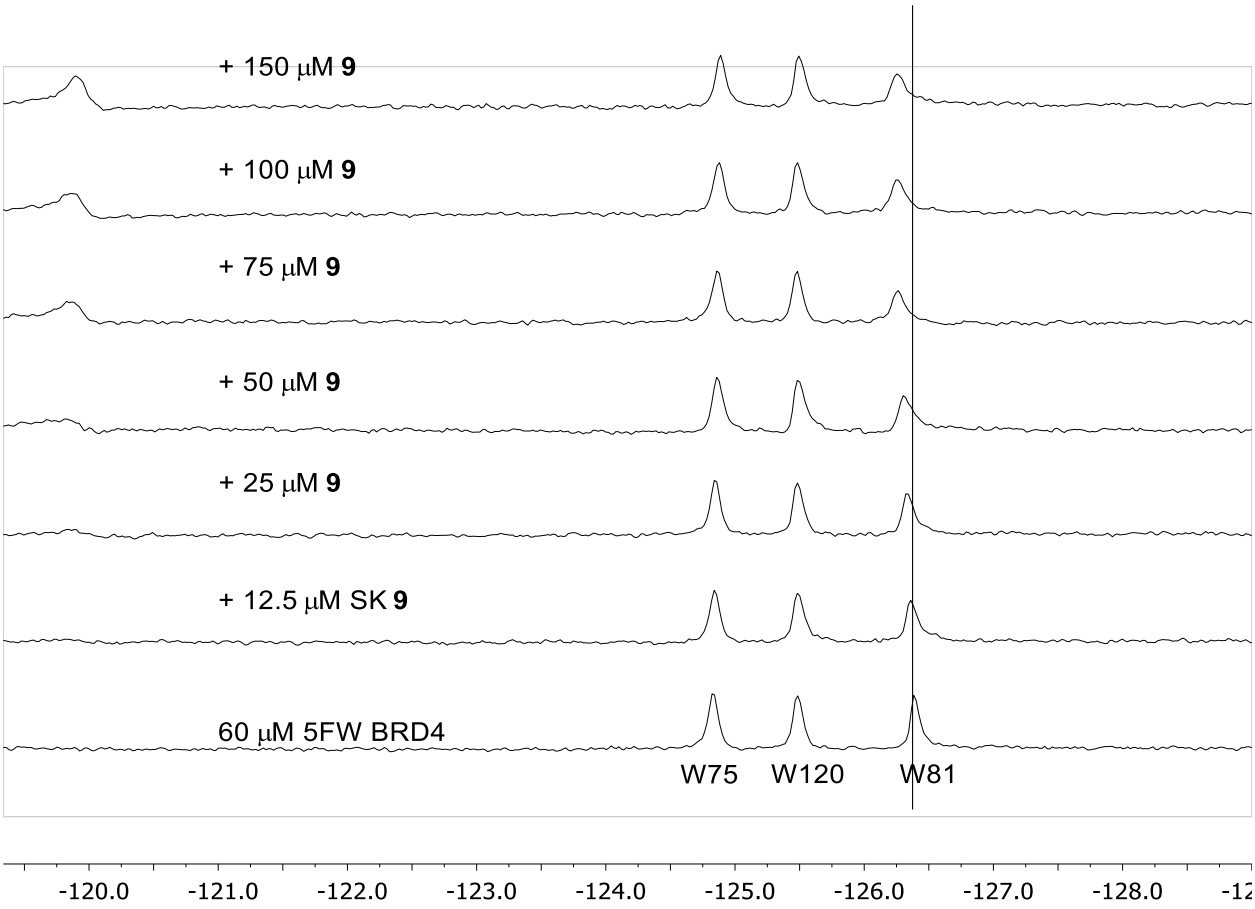
Figure S32: PrOF NMR titration of **(*S*)-1** with 5FW BRDT(BD1). No binding is observed

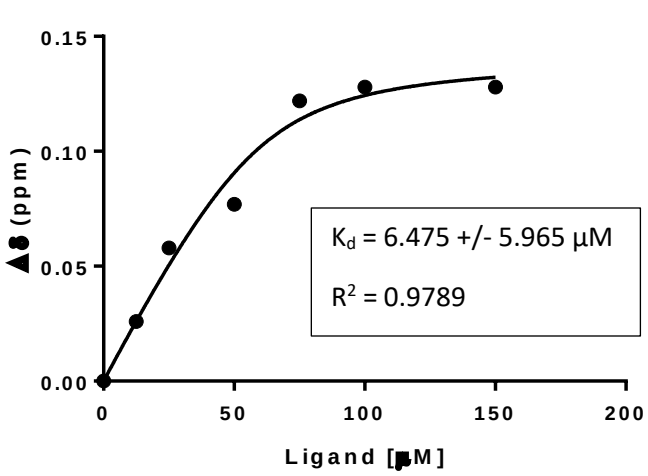




(*S*)-1

Figure S33: PrOF NMR titration of **(*S*)-1** with 5FW PCAF. Broadening and minor shift is observed, binding is ambiguous





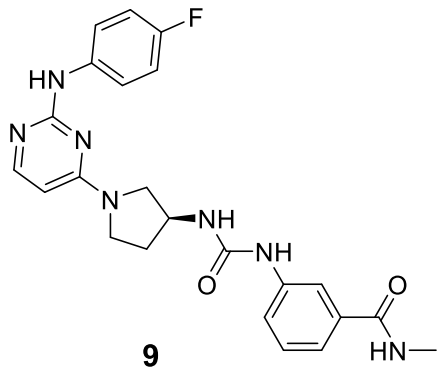


Figure S34: PrOF NMR titration of **9** with 5FW BRD4(BD1).

Crystallization conditions and X-ray data collection methods

Unlabeled BPTF (445 µM, in 50 mM Tris, 100 mM NaCl, 10% (v/v) glycerol) was crystalized using the sitting drop method with 25% (w/v) polyethylene glycol 1500 and 10% (w/v) proprionate-cacodylate-bis tris propane. Crystals were harvested, cryoprotected with ethylene glycol and flash frozen. A RigakuMSC Micromax 007 X-ray generator with Saturn 944+ CCD Camera was used for data collection. The structure was solved using molecular replacement with Phaser-MR[17] and the PDB structure 3QZS. Phenix[18] refine and Coot were used for model structure refinement.

Unlabeled BPTF sequence:
MSTEDAMTVLTPLTEKDYEGLKRVLRSLQAHKMAWPFLEPVDPNDAPDYYGVIKEPMDLATMEERVQRRY

5FW BPTF (247 µM, in 50 mM Tris, 100 mM NaCl, 10% (v/v) ethylene glycol) was crystalized at 20˚C using the hanging drop method with 100 mM $MgCl_2$ and 20% (v/v) polyethylene glycol 3350. Crystals were harvested, cryoprotected with ethylene glycol and flash frozen. A RigakuMSC Micromax 007 X-ray generator with Saturn 944+ CCD Camera was used for data collection. The structure was solved using molecular replacement with Phaser-MR[17] and PDB from the unlabeled BPTF. Phenix[18] refine and Coot were used for model structure refinement. Upon refinement there was insufficient electron density to model the N-terminal residues MST.

5FW BPTF Sequences:
EDAMTVLTPLTEKDYEGLKRVLRSLQAHKMAXPFLEPVDPNDAPDYYGVIKEPMDLATMEERVQRRY

Table S2. Unlabeled BPTF Data collection and refinement statistics.	
Wavelength	
Resolution range	37.94 - 1.59 (1.647 - 1.59)
Space group	P 1 21 1
Unit cell	27.323 66.802 39.274 90 104.949 90
Total reflections	66630 (6749)
Unique reflections	18193 (1782)
Multiplicity	3.7 (3.7)
Completeness (%)	97.23 (96.69)
Mean I/sigma(I)	12.06 (2.78)
Wilson B-factor	15.33
R-merge	0.0975 (0.6611)
R-meas	0.114 (0.776)
R-pim	0.05836 (0.4006)
CC1/2	0.996 (0.528)
CC*	0.999 (0.832)
Reflections used in refinement	17872 (1782)
Reflections used for R-free	1788 (178)
R-work	0.1629 (0.2174)
R-free	0.1980 (0.2391)
CC(work)	0.968 (0.880)
CC(free)	0.949 (0.855)
Number of non-hydrogen atoms	1187
macromolecules	1017
ligands	16
solvent	154
Protein residues	122
RMS(bonds)	0.007
RMS(angles)	0.87
Ramachandran favored (%)	100
Ramachandran allowed (%)	0
Ramachandran outliers (%)	0
Rotamer outliers (%)	0
Clashscore	2.93
Average B-factor	21.05
macromolecules	19.27
ligands	40.86
solvent	30.73

Statistics for the highest-resolution shell are shown in parentheses.

Table S3: 5FW BPTF Data collection and refinement statistics.	
Wavelength	
Resolution range	26.4 - 2.065 (2.139 - 2.065)
Space group	P 1 21 1
Unit cell	27.335 66.763 39.26 90 105.016 90
Total reflections	14605 (761)
Unique reflections	7452 (395)
Multiplicity	2.0 (1.9)
Completeness (%)	88.59 (47.88)
Mean I/sigma(I)	19.77 (9.55)
Wilson B-factor	11.57
R-merge	0.03296 (0.09002)
R-meas	0.04661 (0.1273)
R-pim	0.03296 (0.09002)
CC1/2	0.998 (0.98)
CC*	0.999 (0.995)
Reflections used in refinement	7450 (395)
Reflections used for R-free	389 (17)
R-work	0.1535 (0.1522)
R-free	0.2148 (0.3176)
CC(work)	0.965 (0.955)
CC(free)	0.938 (0.673)
Number of non-hydrogen atoms	1168
macromolecules	987
ligands	5
solvent	176
Protein residues	119
RMS(bonds)	0.014
RMS(angles)	1.47
Ramachandran favored (%)	98.25
Ramachandran allowed (%)	1.75
Ramachandran outliers (%)	0
Rotamer outliers (%)	0
Clashscore	6.7
Average B-factor	17.89
macromolecules	16.61
ligands	16.58
solvent	25.08
Statistics for the highest-resolution shell are shown in parentheses.	

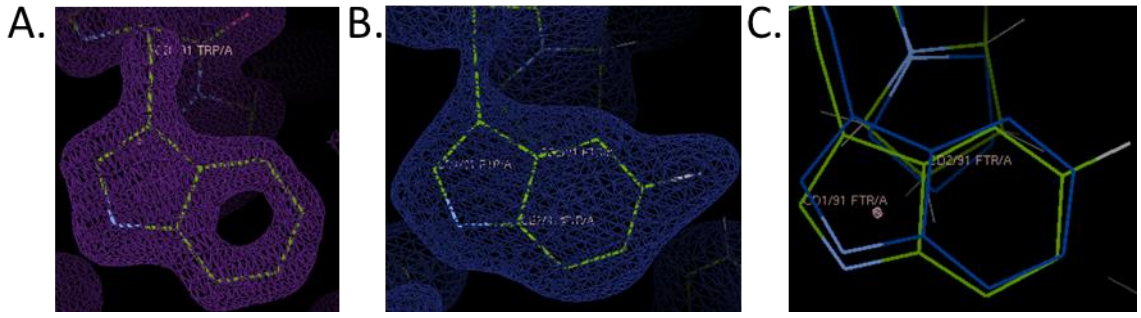


Figure S35: A. Electron density and models W residue for unlabeled BPTF structure. B) Electron density and modeled 5FW residue for the 5FW BPTF structure. C) Overlay of the modeled W (blue) and 5FW (green) residues from the unlabeled and 5FW BPTF structures.

RMSD alignment of 5FW and Unlabeled BPTF

RMSD alignment was done using secondary structure matching (SSM).

Moving (Unlabeled BPTF):
MSTEDAMTVLTPLTEKDYEGLKRVLRSLQAHKMAWPFLEPVDPNDAPDYYGVIKEPMDLATMEERVQRRY

Target (5FW BPTF):
EDAMTVLTPLTEKDYEGLKRVLRSLQAHKMAXPFLEPVDPNDAPDYYGVIKEPMDLATMEERVQRRY

Rotation - euler (alpha,beta,gamma) 7.2267 179.7618 7.3379

Translation - Angstroms 6.6968 -4.2895 75.8852

INFO: core rmsd achieved: 0.1548 Angstroms

number of residues in reference structure: 119

number of residues in moving structure: 122

number of residues in aligned sections (reference): 119

number of residues in aligned sections (moving): 122

number of aligned residues: 116

number of gaps: 0

number of misdirections: 0

number of SSE combinations: 5.0000

sequence identity: 99.1379%

Table S4: RMSD values between Unlabeled BPTF (moving) and 5FW BPTF (Reference)

Moving Reference Distance(Å)	Moving Reference Distance(Å)
A 63 <---> A 63 : 0.1642	A 87 <---> A 87 : 0.1051
A 64 <---> A 64 : 0.1265	A 88 <---> A 88 : 0.1744
A 65 <---> A 65 : 0.1667	A 89 <---> A 89 : 0.1191
A 66 <---> A 66 : 0.1048	A 90 <---> A 90 : 0.1005
A 67 <---> A 67 : 0.0880	A 91 <---> A 91 : 0.3516
A 68 <---> A 68 : 0.1451	A 92 <---> A 92 : 0.1880
A 69 <---> A 69 : 0.1458	A 93 <---> A 93 : 0.1720
A 70 <---> A 70 : 0.0596	A 94 <---> A 94 : 0.0843
A 71 <---> A 71 : 0.1132	A 95 <---> A 95 : 0.1040
A 72 <---> A 72 : 0.2768	A 96 <---> A 96 : 0.1194
A 73 <---> A 73 : 0.0703	A 97 <---> A 97 : 0.1019
A 74 <---> A 74 : 0.0846	A 98 <---> A 98 : 0.1082
A 75 <---> A 75 : 0.1051	A 99 <---> A 99 : 0.1454
A 76 <---> A 76 : 0.1141	A 100 <---> A 100 : 0.1224
A 77 <---> A 77 : 0.0625	A 101 <---> A 101 : 0.2340
A 78 <---> A 78 : 0.0609	A 102 <---> A 102 : 0.0264
A 79 <---> A 79 : 0.0577	A 103 <---> A 103 : 0.8093
A 80 <---> A 80 : 0.0525	A 104 <---> A 104 : 0.6393
A 81 <---> A 81 : 0.1388	A 105 <---> A 105 : 0.0656
A 82 <---> A 82 : 0.0905	A 106 <---> A 106 : 0.0509
A 83 <---> A 83 : 0.1282	A 107 <---> A 107 : 0.1190
A 84 <---> A 84 : 0.0782	A 108 <---> A 108 : 0.1670
A 85 <---> A 85 : 0.0528	A 109 <---> A 109 : 0.1060
A 86 <---> A 86 : 0.0905	A 110 <---> A 110 : 0.1957

Moving	Reference	Distance(Å)
A 111	A 111	0.1312
A 112	A 112	0.1246
A 113	A 113	0.0562
A 114	A 114	0.0640
A 115	A 115	0.0450
A 116	A 116	0.0267
A 117	A 117	0.0703
A 118	A 118	0.0569
A 119	A 119	0.0368
A 120	A 120	0.015
A 121	A 121	0.0701
A 122	A 122	0.0763
A 123	A 123	0.0910
A 124	A 124	0.1337
A 125	A 125	0.1118
A 126	A 126	0.0812
A 127	A 127	0.0404
A 128	A 128	0.0887
A 129	A 129	0.0566
A 130	A 130	0.0505
A 131	A 131	0.0686
A 132	A 132	0.1039
A 133	A 133	0.0669
A 134	A 134	0.0680
A 135	A 135	0.0527
A 136	A 136	0.0622
A 137	A 137	0.0533
A 138	A 138	0.0389
A 139	A 139	0.0821
A 140	A 140	0.0450
A 141	A 141	0.0785
A 142	A 142	0.0668
A 143	A 143	0.0401
A 144	A 144	0.0711
A 145	A 145	0.0979
A 146	A 146	0.0130
A 147	A 147	0.0300
A 148	A 148	0.0198
A 149	A 149	0.1103
A 150	A 150	0.3136
A 151	A 151	0.1244
A 152	A 152	0.1499
A 153	A 153	0.1176
A 154	A 154	0.0437
A 155	A 155	0.0465
A 156	A 156	0.0582
A 157	A 157	0.0507
A 158	A 158	0.0629
A 159	A 159	0.0737
A 160	A 160	0.0671
A 161	A 161	0.0948
A 162	A 162	0.0849
A 163	A 163	0.0788
A 164	A 164	0.0741
A 165	A 165	0.0412
A 166	A 166	0.0706
A 167	A 167	0.0464
A 168	A 168	0.0335
A 169	A 169	0.0832
A 170	A 170	0.0986
A 171	A 171	0.1001
A 172	A 172	0.1088

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