SUPPORTING INFORMATION

The discovery of AZD4831, a mechanism-based irreversible inhibitor of myeloperoxidase, as a potential treatment for heart failure with preserved ejection fraction

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1. Synthesis and characterization of all compounds – general information

All solvents and reagents were purchased from commercial suppliers and used without further purification. ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on Bruker Avance spectrometers at proton frequencies of 400, 500 or 600 MHz at 25°C, frequency stated in each experiment. The chemical shifts (δ) are reported in parts per million (ppm), with the residual solvent signal used as a reference. Coupling constants (J) are reported as Hz. NMR abbreviations are used as follows:, s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, hept = heptet, m = multiplet. NMR peaks were assigned using MestReNova (14.2.0). All microwave-assisted synthesis was carried out in an initiator synthesizers single mode cavity instrument producing controlled irradiation at 2450 MHz (Biotage AB, Uppsala, Sweden). Analytic UHPLC/MS experiments were performed using a Waters Acquity UPLC system combined and a Waters SQD Mass Spectrometer. The UHPLC was equipped with both a BEH C18 column 1.7 µm 2.1 × 50 mm in combination with a 46 mM ammonium carbonate/NH_3 buffer at pH 10 and a HSS C18 column 1.8 µm 2.1× 50 mm in combination with 11 mM ammonium formate buffer at pH 3 with a flow rate of 1 mL/min. The mass spectrometer was operated with electrospray ionization (ESI) in both positive and negative mode. Preparative HPLC was performed by either a Waters Fraction Lynx with ZQ MS detector on either a Waters Xbridge C18 OBD 5 µm column (19 × 150 mm, flow rate 30 mL/min or 30×150 mm, flow rate 60 mL/min) using a gradient of 5–95% MeCN in 0.2% NH_3 (aq) at pH 10 or a Waters SunFire C18 OBD 5 µm column (19 × 150 mm, flow rate 30 mL/min or 30 × 150 mm, flow rate 60 mL/min) using a gradient of 5–95% MeCN in 0.1 M formic acid (aq) or by a Gilson Preparative HPLC with a UV/VIS detector 155 on a Kromasil C8 10 µm column (20 × 250 mm, flow rate 19 mL/min or 50 × 250 mm, flow rate 100 mL/min) using a varying gradient of MeCN in 0.1 M formic acid (aq) or by Gilson Preparative HPLC with a UV/VIS detector 155 on a Waters Xbridge C18 10 µm column (19 × 250 mm, flow rate 19 mL/min or 50 × 250 mm, flow rate 100 mL/min) using a varying gradient of MeCN in 0.2% NH3 (aq). Molecular mass (HR-ESI-MS) was determined on a mass spectrometer equipped with an electrospray ion source. Flash column chromatography was carried out on prepacked silica gel columns supplied by Biotage and using Biotage automated flash systems with UV detection or self-

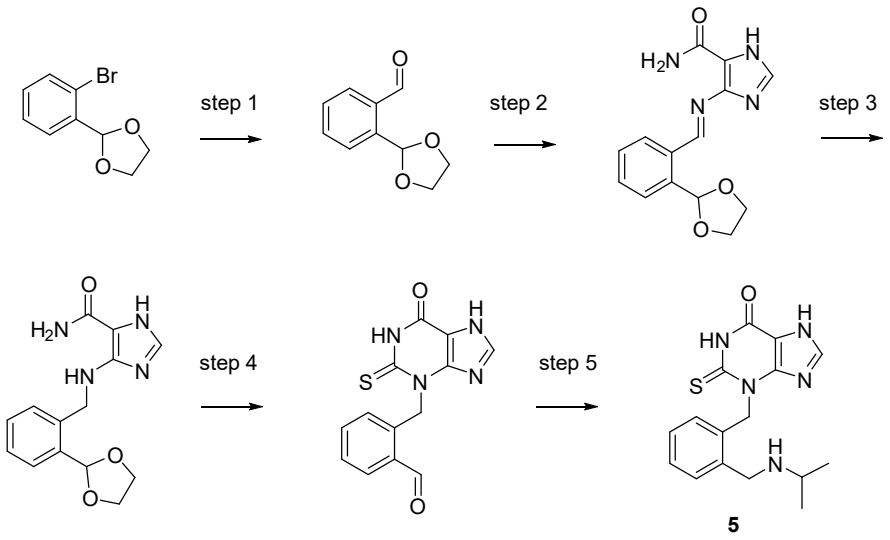
packed silica gel columns (PHA, 100–200 or 300–400 mesh). Vibrational circular dichroism (VCD) spectra were recorded on a BioTools ChiralIR system equipped with dual source and dual photoelastic modulators (PEM).

The samples were prepared by dissolving 10.0 or 20.0 mg of the material in 110 or 200 µl of $CDCl_3$. The solutions were then transferred to a 0.100 mm BaF_2 cell and the VCD spectra were acquired for seven hours. The resolution was 4 cm^{-1}. A Monte Carlo molecular mechanics search for low energy geometries was conducted for full structures of the two isomers, RS and SS. MacroModel within the Maestro graphical interface (Schrödinger Inc.) was used to generate starting coordinates for conformers. All conformers within 5 kcal/mol of the lowest energy conformer were used as starting points for density functional theory (DFT) minimizations within Gaussian09. Optimized structures, harmonic vibrational frequencies/intensities, VCD rotational strengths, and free energies at standard temperature and pressure (including zero-point energies) were determined for each conformer. In these calculations, the functional B3LYP and the basis set 6–31G* were used. Simulations of infrared and VCD spectra for each conformation were generated using an in-house built program to fit Lorentzian line shapes (12 cm^{-1} line width) to the computed spectra thereby allowing direct comparisons between simulated and experimental spectra.

All screening compounds had purity >95%.

2. Synthesis procedures for intermediates and final compounds

Synthesis of 1-(5-chloro-2-((methylamino)methyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one trifluoroacetate (5).



Step 1. 2-(1,3-Dioxolan-2-yl)benzaldehyde

2-(2-Bromophenyl)-1,3-dioxolane (9.90 g, 43 mmol) was dissolved in diethyl ether (100 mL) and cooled to -78°C. *n*-BuLi (1.6 M in hexanes) (27 mL, 43 mmol) was added dropwise and the reaction mixture was stirred for 15 min at -78°C. DMF (3.7 mL, 48 mmol) was added and the reaction mixture was stirred at -78°C for 15 min and then allowed to warm to rt overnight. The mixture was quenched by addition of sat. aq NH_4Cl solution and then extracted with diethyl ether (2 × 100 mL). The combined organics were washed with brine (100 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purified by flash chromatography, using a gradient of 0–50% EtOAc in heptane to obtain the title compound (4.32 g, 56%) as an oil.

Step 2. (E)-4-(2-(1,3-Dioxolan-2-yl)benzylideneamino)-1H-imidazole-5-carboxamide.

2-(1,3-Dioxolan-2-yl)benzaldehyde (3.70 g, 20.8 mmol) and 4-amino-1H-imidazole-5-carboxamide (2.62 g, 20.8 mmol) were dissolved in EtOH (100 mL) and heated to reflux under nitrogen for 24 h. The reaction mixture was allowed to cool to rt and a formed precipitate was collected by filtration

and washed with EtOH to give the title compound (4.25 g, 72%) as an off-white solid. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 12.95 (s, 1H), 9.51 (s, 1H), 8.08–8.17 (m, 1H), 7.88 (d, *J* = 3.0 Hz, 1H), 7.73 (s, 1H), 7.64–7.7 (m, 2H), 7.51–7.59 (m, 2H), 6.23 (s, 1H), 3.98–4.17 (m, 4H). LC-MS (ESI) *m/z*: 287.2 (calcd for $C_{14}H_{14}N_4O_3$ $[M+H]^+$, 287.1).

Step 3. 4-(2-(1,3-Dioxolan-2-yl)benzylamino)-1H-imidazole-5-carboxamide.

$NaBH_4$ (1.45 g, 38.4 mmol) was added portionwise to a solution of (E)-4-(2-(1,3-dioxolan-2-yl)benzylideneamino)-1H-imidazole-5-carboxamide (2.20 g, 7.68 mmol) in DMSO (20 mL) at rt The reaction mixture was stirred for 36 h at rt, diluted with EtOAc (100 mL) and washed with water (2 × 100 mL). The aq layers were further extracted with EtOAc (2 × 100 mL) and the organic layers were combined, dried (Na_2SO_4) and concentrated *in vacuo* to give the title compound (2.20 g, 99%) as yellow foam. LC-MS (ESI) *m/z*: 287.2 (calcd for $C_{14}H_{16}N_4O_3$ $[M-H]^-$, 287.1).

Step 4. 2-((6-Oxo-2-thioxo-1H-purin-3(2H,6H,7H)-yl)methyl)benzaldehyde.

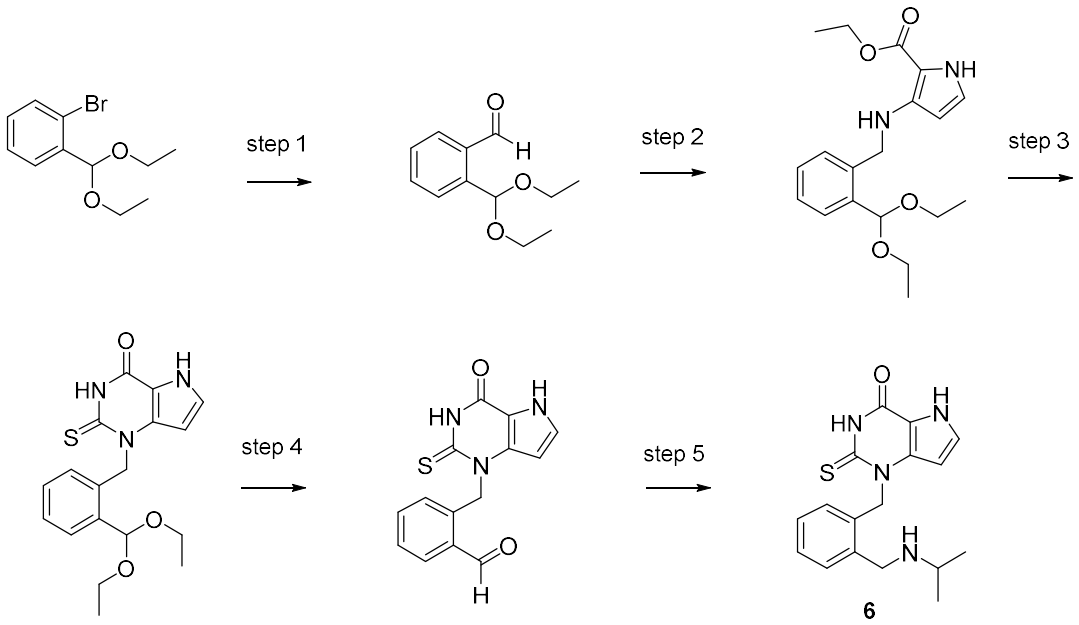
4-(2-(1,3-Dioxolan-2-yl)benzylamino)-1H-imidazole-5-carboxamide (2.20 g, 7.63 mmol) was dissolved in DCM (40 mL) and ethyl isothiocyanatoformate (1.35 mL, 11.4 mmol) was added. The reaction mixture was stirred at rt for 60 h, followed by solvent removal *in vacuo*. A 2M aq solution of NaOH (20 mL) was added and the mixture was heated to 80°C for 2 h. Cooled and acidified to pH 2 by cautious addition of conc. HCl. The formed precipitate was collected by filtration, washed with water (50 mL) and dried in vacuo to give the title compound (1.68 g, 77%) as a pale yellow solid. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 13.91 (s, 1H), 12.62 (s, 1H), 10.30 (s, 1H), 8.09 (s, 1H), 7.98–8.05 (m, 1H), 7.51–7.56 (m, 2H), 6.93–6.99 (m, 1H), 6.10 (s, 2H). LC-MS (ESI) *m/z*: 285.0 (calcd for $C_{13}H_{10}N_4O_2S$ $[M-H]^-$, 285.0).

Step 5. 1-(5-Chloro-2-((methylamino)methyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one trifluoroacetate (5).

2-((6-Oxo-2-thioxo-1H-purin-3(2H,6H,7H)-yl)methyl)benzaldehyde (150 mg, 0.52 mmol) was dissolved in NMP (4 mL) and propan-2-amine (0.070 mL, 0.79 mmol) was added. The reaction mixture was stirred at rt for 30 min, followed by addition of STAB (167 mg, 0.79 mmol). The reaction

mixture was stirred at rt for 20 h whereupon more propan-2-amine (0.020 mL) and STAB (65 mg) were added. Stirred at rt for 2 h. Quenched by addition of water (1 mL). Purified on a SunFire Prep C8 10um 30x100 OBD column using a gradient of 8–43% MeOH in 0.1% TFA (aq) giving a solid which was triturated in diethyl ether (1 mL) for 1 h and then filtered to obtain **5** (60 mg, 26%) as white a solid. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 7.26 (s, 1H), 6.67–6.73 (m, 1H), 6.61–6.66 (m, 1H), 6.53–6.59 (m, 2H), 5.16 (s, 2H), 3.73 (s, 2H), 2.87 (hept, *J* = 6.6 Hz, 1H), 0.70 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ 174.9, 152.8, 149.4, 141.6, 135.9, 131.2, 130.3, 129.8, 127.9, 127.2, 111.2, 50.8, 47.7, 45.1, 18.9. HRMS (ESI) m/z: 330.1401 (calcd for $C_{16}H_{19}N_5OS$ $[M+H]^+$, 330.1389).

Synthesis of 1-(2-((isopropylamino)methyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one trifluoroacetate (6).



Step 1. 2-(Diethoxymethyl)benzaldehyde.

1-Bromo-2-(diethoxymethyl)benzene (10.9 mL, 54.0 mmol) was dissolved in THF (120 mL) and the solution cooled to –70ºC under nitrogen. *n*-BuLi (2.5 M solution in hexanes) (23.8 mL, 59.4 mmol) was then added at such a rate as to ensure the internal temperature did not exceed –65ºC. Stirred for 20 min. Dry DMF (6.25 mL, 81.0 mmol) was then added at a rate such that the internal

temperature did not exceed –65°C. The cooling bath was removed and the reaction allowed to warm to rt The reaction was poured into water (250 mL) and extracted with diethyl ether (3 × 100 mL). The combined extracts were washed with water (2 × 100 mL), dried (Na_2SO_4) and concentrated *in vacuo* to afford 2-(diethoxymethyl)benzaldehyde (11.1 g, 99%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.50 (s, 1H), 7.92 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.65–7.72 (m, 1H), 7.58 (td, *J* = 7.5, 1.5 Hz, 1H), 7.48 (td, *J* = 7.8, 7.0, Hz, 1H), 5.96 (s, 1H), 3.65–3.73 (m, 2H), 3.52–3.63 (m, 2H), 1.23 (t, *J* = 7.0 Hz, 6H).

Step 2. Ethyl 3-(2-(diethoxymethyl)benzylamino)-1H-pyrrole-2-carboxylate.

A solution of ethyl 3-amino-1H-pyrrole-2-carboxylate hydrochloride (4.58 g, 24 mmol) in EtOH (50 mL) was treated with DIPEA (5.0 mL, 29 mmol) and stirred for 10 min before the addition of HOAc (3.30 mL, 57.6 mmol) and $NaBH_3CN$ (2.26 g, 36.0 mmol). A solution of 2-(diethoxymethyl)benzaldehyde (5.00 g, 24.0 mmol) in EtOH (30 mL) was then added dropwise over 15 min and the resultant suspension stirred at rt overnight. The reaction was concentrated *in vacuo* and the residue treated with water (200 mL) and extracted with DCM (3 × 150 mL). The organics were dried (Na_2SO_4) and concentrated *in vacuo* to afford crude ethyl 3-(2-(diethoxymethyl)benzylamino)-1H-pyrrole-2-carboxylate (8.02 g, 96%) as an orange oil. Used without purification in the next step.

Step 3. 1-(2-(diethoxymethyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one.

A solution of ethyl 3-(2-(diethoxymethyl)benzylamino)-1H-pyrrole-2-carboxylate (8.00 g, 23.1 mmol) in DCM (100 mL) was treated with benzoyl isothiocyanate (3.10 mL, 23.1 mmol) and stirred at rt for 1 h. The solvent was removed *in vacuo* and the residue dissolved in MeOH (100 mL), treated with sodium hydroxide (4.62 g, 115 mmol) and stirred under reflux for 3 h. The reaction was concentrated *in vacuo* and the residue dissolved in water (200 mL), neutralized with HOAc and extracted with DCM (3 × 150 mL). The organics were dried ($MgSO_4$), concentrated *in vacuo* and the residue triturated with diethyl ether to afford 1-(2-(diethoxymethyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (4.11 g, 49%) as a pale brown solid. ^{1}H NMR (400 MHz,

$(CD_3)_2SO$) δ 12.43 (s, 1H), 12.34 (s, 1H), 7.51 (dd, *J* = 7.4, 1.6 Hz, 1H), 7.17–7.32 (m, 3H), 6.74 (dd, *J* = 7.6, 1.5 Hz, 1H), 5.96 (d, *J* = 2.8 Hz, 1H), 5.83 (s, 2H), 5.72 (s, 1H), 3.67 (dq, *J* = 9.6, 7.0 Hz, 2H), 3.56 (dq, *J* = 9.5, 7.0 Hz, 2H), 1.21 (t, *J* = 7.0 Hz, 6H). LC-MS (ESI) m/z: 358.2 (calcd for $C_{18}H_{21}N_3O_3S$ $[M-H]^-$, 358.1).

Step 4. 2-((4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-1-yl)methyl)benzaldehyde.

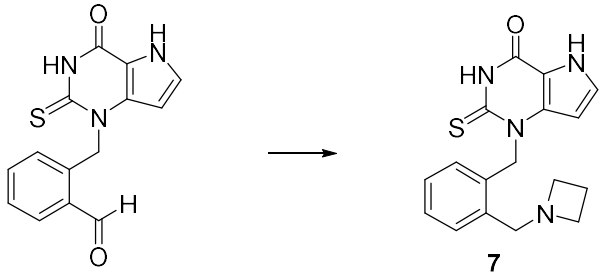
1-(2-(Diethoxymethyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (4.00 g, 11 mmol) was suspended in DCM (40 mL) and the suspension cooled to 5 °C. TFA (10 mL, 130 mmol) was added dropwise, the cooling bath removed, and the reaction stirred for 40 min. The solvents were removed *in vacuo* and the residue azeotroped with MeCN (2 × 30 mL). The residue was triturated with diethyl ether, filtered and dried *in vacuo* to afford 2-((4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-1-yl)methyl)benzaldehyde (2.76 g, 87%) as a pale brown solid. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 12.48 (s, 1H), 12.37 (s, 1H), 10.27 (s, 1H), 7.99–8.08 (m, 1H), 7.49–7.58 (m, 2H), 7.29 (t, *J* = 3.0 Hz, 1H), 6.78–6.9 (m, 1H), 6.07 (s, 2H), 6.04 (t, *J* = 2.5 Hz, 1H). LC-MS (ESI) m/z: 286.0 (calcd for $C_{14}H_{11}N_3O_2S$ $[M+H]^+$, 286.1).

Step 5. 1-(2-((isopropylamino)methyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one trifluoroacetate (6).

A solution of 2-((4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-1-yl)methyl)benzaldehyde (160 mg, 0.56 mmol) and propan-2-amine (0.446 mL, 5.61 mmol) in dry NMP (5 mL) was stirred for 1 h and then treated with $NaBH_4$ (15 mg, 0.39 mmol) and stirred at rt for 30 min. The solution was adsorbed by gravity onto a 10 g SCX column preswollen in NMP and then washed with MeOH (100 mL). The neutral washes were discarded and the crude product eluted using 3 N methanolic ammonia (50 mL). The solvent was removed *in vacuo* and the residue was purified on a Gemini-NX C18 5 um 110A 30x100 AXIA column eluting on a 13 to 48% gradient of MeOH in water (0.1% TFA). The solid was slurried in DCM (2 mL) and then collected and dried to give **6** (60 mg, 33%). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 12.55 (s, 1H), 12.43 (s, 1H), 8.88 (s, 2H), 7.56 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.33–7.39 (m, 2H), 7.29 (td, *J* = 7.6, 1.4 Hz, 1H), 6.75–6.81 (m, 1H),

6.05–6.09 (m, 1H), 5.80 (s, 2H), 4.34–4.44 (m, 2H), 3.49–3.6 (m, 1H), 1.37 (d, *J* = 6.5 Hz, 6H). ^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ 174.0, 153.1, 137.2, 134.9, 130.8, 130.5, 129.6, 128.5, 127.7, 125.8, 114.2, 97.5, 51.1, 50.7, 44.7, 19.2. HRMS (ESI) m/z: 329.1432 (calcd for $C_{17}H_{20}N_4OS$ $[M+H]^+$, 329.1436).

Synthesis of 1-(2-(azetidin-1-ylmethyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one trifluoroacetate (7).



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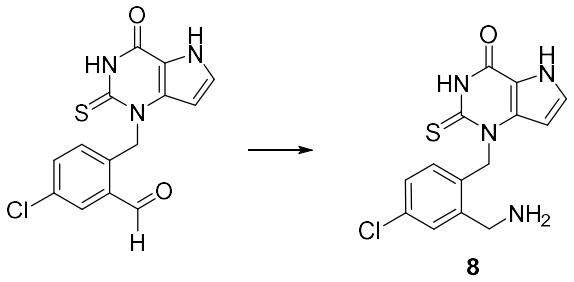
A solution of 2-((4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-1-yl)methyl)-benzaldehyde (100 mg, 0.35 mmol; see step 4, compound **6**), azetidine hydrochloride (66 mg, 0.70 mmol) and DIPEA (0.184 mL, 1.05 mmol) in dry MeCN (4 mL) was stirred for 1 h and then treated with STAB (223 mg, 1.05 mmol) and stirred at rt for 72 h. The reaction was concentrated *in vacuo* and the crude product purified on a SunFire Prep C8 10 um 30 × 100 OBD column eluting on a 5 to 40% gradient of MeOH in water (0.1%TFA). The resultant solid was slurried in DCM (2 ml) to afford **7** (111 mg, 72%). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 12.46–12.69 (m, 1H), 12.42 (s, 1H), 10.31 (s, 1H), 7.47 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.31–7.39 (m, 2H), 7.29 (td, *J* = 7.6, 1.5 Hz, 1H), 6.77 (dd, J = 7.7, 1.5 Hz, 1H), 6.10 (t, J = 2.5 Hz, 1H), 5.77 (s, 2H), 4.62 (s, 2H), 3.98–4.28 (m, 4H), 2.2–2.47 (m, 2H). ^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ 173.9, 153.1, 137.4, 135.2, 130.4, 129.8, 129.2, 128.6, 127.9, 126.0, 114.2, 97.35, 54.6, 54.3, 50.7 16.6. HRMS (ESI) m/z: 327.1270 (calcd for $C_{17}H_{18}N_4OS$ $[M+H]^+$, 327.1280).

Synthesis of 1-(2-(aminomethyl)-4-chlorobenzyl)-2-thioxo-1,2,3,5-tetrahydro-4H-pyrrolo[3,2-d]pyrimidin-4-one (8).



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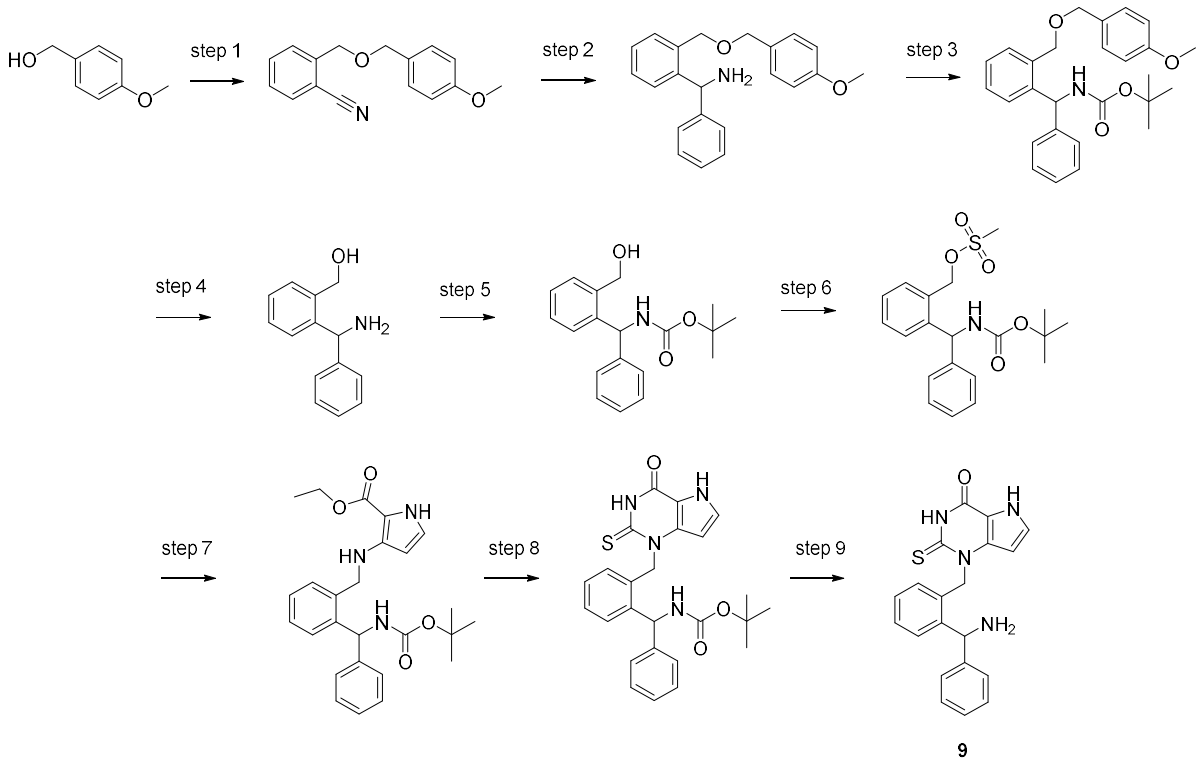
5-chloro-2-((4-oxo-2-thioxo-2,3,4,5-tetrahydro-1*H*-pyrrolo[3,2-d]pyrimidin-1-yl)methyl)benzaldehyde (see step 5, compound **14**) (194 mg, 0.61 mmol) was added to a mixture of hydroxylamine hydrochloride (46 mg, 0.67 mmol) and HOAc (80%, 4 mL). The mixture was stirred at rt for 5 h and then zinc (198 mg, 3.03 mmol) was added. The reaction mixture was stirred at 60° C for 2 h. An aq solution of NaOH (1 M) was added to adjust the pH to 12. The solvents were removed under reduced pressure and the residue was was purified by preparative HPLC (Kromasil C8, 10 µm, 50 × 250 ID mm, 5-45% MeCN in water/MeCN/FA, 95/5/0.2) to give (67 mg, 98%) of the title compound. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 7.50 (d, *J* = 2.3 Hz, 1H), 7.30 (d, *J* = 2.8 Hz, 1H), 7.14 (dd, *J* = 8.3, 2.4 Hz, 1H), 6.64 (d, *J* = 8.3 Hz, 1H), 6.09 (d, *J* = 2.8 Hz, 1H), 5.69 (s, 2H), 3.88 (s, 2H). ^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ 173.5, 152.6, 143.3, 136.8, 131.9, 131.4, 128.0, 127.4, 126.2, 126.1, 113.7, 96.9, 49.7, 42.4. HRMS (ESI) m/z 321.0572 (calcd for $C_{14}H_{13}ClN_4OS$ $[M+H]^+$ 321.0577).

Synthesis of 1-(2-(Amino(phenyl)methyl)benzyl)-2-thioxo-1,2,3,5-tetrahydro-4H-pyrrolo[3,2-d]pyrimidin-4-one (9).



Step 1. 2-(((4-Methoxybenzyl)oxy)methyl)benzonitrile.

Sodium hydride (0.629 g, 15.73 mmol) was added to a solution of (4-methoxyphenyl)methanol (1.96 mL, 15.73 mmol) in dry THF (50 mL) at 0°C under nitrogen. The reaction was stirred for 1 h, the cooling bath was removed and a solution of 2-(bromomethyl)benzonitrile (2.57 g, 13.11 mmol) in dry THF (10 mL) was added. The reaction was stirred at 60°C overnight, then cooled to rt and quenched with water (100 mL). The mixture was extracted with EtOAc (200 mL) and the organics washed with water (100 mL), and brine (100 mL), then dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash silica chromatography (100% DCM) to give (3.06 g, 92%) of the title compound as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (d, *J* = 7.7 Hz, 1H), 7.56–7.62 (m, 2H), 7.35–7.42 (m, 1H), 7.33 (d, *J* = 8.3 Hz, 2H), 6.90 (d, *J* = 8.3 Hz, 2H), 4.72 (s, 2H), 4.59 (s, 2H), 3.81 (s, 3H).

Step 2. (2-(((4-methoxybenzyl)oxy)methyl)phenyl)(phenyl)methanamine.

A solution of 2-(((4-methoxybenzyl)oxy)methyl)benzonitrile (3.00 g, 11.8 mmol) in dry THF (50 mL) was cooled to 0°C under nitrogen and treated dropwise with 2.8 M phenylmagnesium bromide in diethyl ether (8.46 mL, 23.69 mmol). The reaction was allowed to warm to rt and stirred under reflux for 2 h, then cooled to rt and poured into 1:1 (v/v) saturated NH_4Cl(aq) /NH_3(aq) (200 mL). The mixture was extracted with EtOAc (2 × 150 mL) and the organics dried ($MgSO_4$) and concentrated *in vacuo* to afford the crude imine. The imine was dissolved in MeOH (70 mL), cooled to 0°C and then treated portionwise with $NaBH_4$ (0.896 g, 23.69 mmol). The reaction was stirred at 0°C for 4 h, then concentrated *in vacuo* and the residue treated with water (100 mL) and extracted with DCM (2 × 100 mL). The organics were washed with brine (100 mL), then dried (Na_2SO_4) and concentrated *in vacuo* to afford a brown oil which was purified by flash chromatography (iso-cratic, 50% EtOAc in DCM) to give (2.96 g, 75%) of the title compound as an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (dd, *J* = 7.6, 1.4 Hz, 1H), 7.18–7.34 (m, 10H), 6.84–6.9 (m, 2H), 5.49 (s, 1H), 4.63 (d, *J* = 11.4 Hz, 1H), 4.36–4.51 (m, 3H), 3.80 (s, 3H), 1.76 (s, 2H). LC-MS (ESI) *m/z*: 334.2 (calcd for $C_{22}H_{23}NO_2$ $[M+H]^+$, 334.18).

Step 3. *tert*-Butyl ((2-(((4-methoxybenzyl)oxy)methyl)phenyl)(phenyl)methyl)carbamate.

Boc_2O (2.2 mL, 9.6 mmol) was added to a solution of (2-((4-methoxybenzyloxy)methyl)phenyl)(phenyl)methanamine (2.9 g, 8.70 mmol) and DIPEA (1.823 mL, 10.44 mmol) in DCM (30 mL) and the mixture was stirred at rt for 4 h. The reaction was diluted with further DCM (100 mL) and washed with water (100 mL) and brine (100 mL). The organics were dried ($MgSO_4$), and concentrated *in vacuo* to give (3.66 g, 97%) of the title compound as a yellow oil. Used without further purification in the next step. MS (ESI) *m/z*: 334.2 (calcd for $C_{27}H_{31}NO_4$ [M-Boc+H]$^+$, 334.18).

Step 4. (2-(Amino(phenyl)methyl)phenyl)methanol.

TFA (5 mL) was added to a solution of *tert*-butyl ((2-(((4-methoxybenzyl)oxy)methyl)phenyl)(phenyl)methyl)carbamate (100 mg, 0.23 mmol) in DCM (5 mL) and the reaction was stirred at rt overnight. The reaction was concentrated *in vacuo* and the residue

dissolved in MeOH (5 mL) and loaded on to a 10 g SCX cartridge. The impurities were washed through with MeOH (50 mL) and discarded. The product was eluted with 1 M methanolic ammonia (50 mL) and concentrated *in vacuo*. The crude product was purified by flash chromatography (100 % EtOAc) to give (44 mg, 89%) of the title compound as a colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16–7.44 (m, 8H), 6.96 (dd, *J* = 7.6, 1.4 Hz, 1H), 5.55 (s, 1H), 4.83 (d, *J* = 12.1 Hz, 1H), 4.43 (d, *J* = 12.1 Hz, 1H) (contains residual solvent, MeOH). LC-MS (ESI) *m/z*: 214.2 (calcd for $C_{14}H_{15}NO$ $[M+H]^+$, 214.12).

Step 5. *tert*-Butyl ((2-(hydroxymethyl)phenyl)(phenyl)methyl)carbamate.

Boc_2O (1.535 g, 7.03 mmol) was added to a solution of (2-(amino(phenyl)methyl)phenyl)methanol (1.5 g, 7.03 mmol) in DCM (20 mL) and the reaction was stirred at rt for 24 h. The reaction was washed with water (20 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford the crude title compound as a yellow oil (2.28 g). Used without purification. LC-MS (ESI) *m/z*: 214.2 (calcd for $C_{19}H_{23}NO_3$ $[M-Boc+H]^+$, 214.12).

Step 6. 2-(((*tert*-Butoxycarbonyl)amino)(phenyl)methyl)benzyl methanesulfonate.

Mesyl chloride (0.27 mL, 3.51 mmol) was added dropwise to a solution of *tert*-butyl ((2-(hydroxymethyl)phenyl)(phenyl)methyl)carbamate (1 g, 3.19 mmol) and DIPEA (0.669 mL, 3.83 mmol) in DCM (20 mL) at 0°C. The reaction was stirred for 2 h then diluted with further DCM (50 mL) and washed with water (50 mL). The organics were dried ($MgSO_4$), filtered through a short plug of silica and concentrated *in vacuo* to afford the title compound (1.03 g, 82 %). Used without purification.

Step 7. Ethyl 3-((2-(((*tert*-butoxycarbonyl)amino)(phenyl)methyl)benzyl)amino)-1H-pyrrole-2-carboxylate.

2-(((*tert*-Butoxycarbonyl)amino)(phenyl)methyl)benzyl methanesulfonate (1 g, 2.55 mmol), potassium iodide (0.28 g, 1.70 mmol), K_2CO_3 (0.94 g, 6.81 mmol) and ethyl 3-amino-1H-pyrrole-2-carboxylate hydrochloride (0.32 g, 1.70 mmol) in a mixture of heptane (15 mL) and water (15 mL) were stirred at 70°C overnight. The mixture was diluted with water (50 mL) and DCM (50 mL) and

the layers separated. The aq layer was extracted with DCM (50 mL) and the combined organics dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography (iso-cratic, 20% EtOAc in *iso*-hexane) to give the title compound (0.60 g, 78%). LC-MS showed the product to be ~2:1 mixture of mono-and di-alkylated products. Used without further purification.

Step 8. *tert*-Butyl ((2-((4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-1-yl)methyl)phenyl)(phenyl)methyl)carbamate.

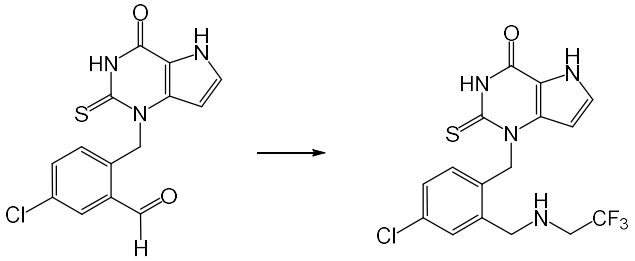
A solution of ethyl 3-(2-((*tert*-butoxycarbonylamino)(phenyl)methyl)benzylamino)-1H-pyrrole-2-carboxylate (600 mg, 1.33 mmol, assumed 100% pure) in DCM (10 mL) was treated with benzoyl isothiocyanate (0.179 mL, 1.33 mmol) and stirred at rt overnight. The solvent was removed *in vacuo* and the residue dissolved in MeOH (10 mL) and treated with NaOH (267 mg, 6.67 mmol) and stirred under reflux for 1 h. The solvent was removed *in vacuo* and the residue purified by flash chromatography (iso-cratic, 50% EtOAc in *iso*-hexane) to give the title compound (162 mg, 26%) as a tan solid. LC-MS (ESI) *m/z*: 463.2 (calcd for $C_{25}H_{26}N_4O_3S$ $[M+H]^+$, 463.18).

Step 9. 1-(2-(Amino(phenyl)methyl)benzyl)-2-thioxo-1,2,3,5-tetrahydro-4H-pyrrolo[3,2-d]pyrimidin-4-one (9).

A solution of *tert*-butyl ((2-((4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-1-yl)methyl)phenyl)(phenyl)methyl)carbamate (160 mg, 0.35 mmol) in DCM (5 mL) was treated with 4M HCl in 1,4-dioxan (3 mL, 12.00 mmol) and stirred at rt for 3 h. The solvent was removed *in vacuo* and the residue dissolved in MeOH (2 mL) and loaded on to a 10 g SCX cartridge. The impurities were washed through with MeOH (50 mL) and discarded. The product was eluted with 1 N methanolic ammonia (50 mL). The solvent was removed *in vacuo* and the residue was slurried in DCM (5 mL) for 72 h then filtered to afford the title compound (113 mg, 90%) as a tan solid. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 7.54 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.39–7.44 (m, 2H), 7.34 (t, *J* = 7.6 Hz, 2H), 7.24 (tq, *J* = 7.4, 1.5 Hz, 2H), 7.19 (d, *J* = 2.8 Hz, 1H), 7.09 (td, *J* = 7.6, 1.4 Hz, 1H), 6.60 (dd, *J* = 7.8, 1.3 Hz, 1H), 5.80 (d, *J* = 16.5 Hz, 1H), 5.64 (d, *J* = 16.5 Hz, 1H), 5.51 (d, *J* = 2.9 Hz, 1H), 5.46 (s, 1H). ^{13}C NMR (126 MHz, DMSO) δ 174.1, 153.0, 145.5, 143.6, 137.1, 132.7, 128.7, 128.3,

127.9, 127.8, 127.2, 127.1, 127.1, 124.9, 114.2, 97.0, 55.9, 50.9. HRMS (ESI) m/z 346.1015: (calcd for $C_{20}H_{18}N_4OS$ $[M+H]^+$, 346.1014).

Synthesis of 1-(4-Chloro-2-(((2,2,2-trifluoroethyl)amino)methyl)benzyl)-2-thioxo-1,2,3,5-tetrahydro-4H-pyrrolo[3,2-d]pyrimidin-4-one (10).



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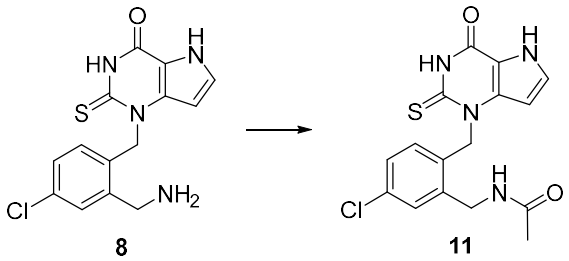
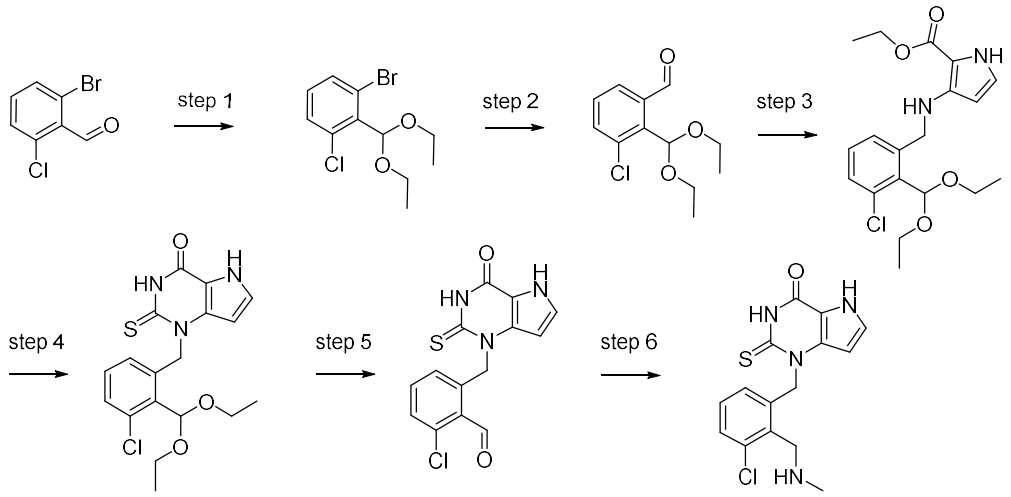
TEA (458 µl, 3.28 mmol) and $NaBH_3CN$ (791 mg, 12.59 mmol) were added to a suspension of 5-chloro-2-((4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-1-yl)methyl)benzaldehyde (175 mg, 0.55 mmol) (see step 5 compound **14**) and 2,2,2-trifluoroethaneamine hydrochloride (445 mg, 3.28 mmol) in MeOH. HOAc (2 mL) was added and stirring was continued overnight. The reaction was diluted with water and extracted with EtOAc. The product was purified by the prep team on a MeCN-based HPLC-system to afford the title compound (66 mg, 30%). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 12.44 (s, 2H), 7.46 (d, *J* = 2.3 Hz, 1H), 7.31 (d, *J* = 2.8 Hz, 1H), 7.21 (dd, *J* = 8.3, 2.3 Hz, 1H), 6.68 (d, *J* = 8.3 Hz, 1H), 6.05 (d, *J* = 2.8 Hz, 1H), 5.73 (s, 2H), 3.95 (d, *J* = 6.6 Hz, 2H), 3.31–3.4 (m, 2H, overlapping with the H_2O peak), 3.07 (p, *J* = 6.9 Hz, 1H). ^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ 174.0, 153.1, 140.0, 137.2, 133.3, 131.7, 130.1, 129.2, 128.5, 127.8, 127.4, 127.0, 125.6, 123.4, 114.2, 97.2, 50.3, 50.2, 49.9, 49.6, 49.4, 49.1. ^{19}F NMR (471 MHz, $(CD_3)_2SO$) δ -70.12. HRMS (ESI) m/z 403.0613: (calcd for $C_{16}H_{14}ClF_3N_4OS$ $[M+H]^+$, 403.0607).

Synthesis of *N*-(5-chloro-2-((4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-1-yl)methyl)benzyl)acetamid (11).



Acetic anhydride (0.5 mL, 5.30 mmol) was added to **8** (75 mg, 0.23 mmol). The mixture was stirred at rt for 1.5 h. The compound was purified by preparative HPLC on a (Kromasil C8, 10 µm, 50 × 250 ID mm, 0-50% MeCN in water/MeCN/FA, 95/5/0.2) to give the title compound (54 mg, 64%). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 12.45 (s, 2H), 8.48 (t, *J* = 5.8 Hz, 1H), 7.34 (dd, *J* = 6.2, 2.6 Hz, 2H), 7.20 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.66 (d, *J* = 8.4 Hz, 1H), 6.07 (d, *J* = 2.9 Hz, 1H), 5.67 (s, 2H), 4.41 (d, *J* = 5.8 Hz, 2H), 1.93 (s, 3H). ^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ 174.0, 169.8, 153.1, 139.4, 137.2, 132.5, 131.9, 128.6, 128.5, 127.4, 127.0, 114.2, 97.2, 50.2, 39.7 23.0. (one carbon (CH2-NH2) is overlapping with the solvent peak, HSQC used to determine the chemical shift). HRMS (ESI) m/z 363.0668: (calcd for $C_{16}H_{15}ClN_4O_2S$ $[M+H]^+$, 363.0682).

Synthesis of 1-(3-chloro-2-((methylamino)methyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one trifluoroacetate (12).



Step 1. 1-Bromo-3-chloro-2-(diethoxymethyl)benzene.

2-Bromo-6-chlorobenzaldehyde (4.0 g, 18 mmol) was dissolved in EtOH (200 mL) and treated with triethyl orthoformate (6.1 mL, 36 mmol) and tetra-n-butylammonium tribromide (0.26 g, 0.55 mmol), then stirred under gentle reflux for 24 h. The reaction was cooled, evaporated to dryness and the residue azeotroped with MeCN (15 mL). The crude product was purified by flash silica chromatography eluting with 10% EtOAc in isohexane. Pure fractions were concentrated *in vacuo* to afford 1-bromo-3-chloro-2-(diethoxymethyl)benzene (5.3 g, 99 %) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.34 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.08 (t, *J* = 8.0 Hz, 1H), 6.01 (s, 1H), 3.79 (dq, *J* = 9.5, 7.1 Hz, 2H), 3.53 (dq, *J* = 9.5, 7.0 Hz, 2H), 1.27 (t, *J* = 7.0 Hz, 6H).

Step 2. 3-Chloro-2-(diethoxymethyl)benzaldehyde.

1-Bromo-3-chloro-2-(diethoxymethyl)benzene (5.3 g, 18 mmol) was dissolved in THF (50 mL) and the solution cooled to –70°C. A 1.6 M solution of *n*-BuLi in hexanes (12 mL, 20 mmol) was added dropwise ensuring the internal temperature did not rise above –65°C. The reaction was stirred for 75 min at –70°C. N-formylmorpholine (3.6 mL, 36 mmol) was then added dropwise, keeping the temperature below –65°C and the reaction was allowed to stir to rt overnight. Quenched with water (50 mL) and extracted with diethyl ether (100 mL). The organic layer was separated, dried ($MgSO_4$),

concentrated *in vacuo* and the residue purified by flash silica chromatography eluting with DCM. Pure fractions were evaporated to dryness to afford 3-chloro-2-(diethoxymethyl)benzaldehyde (3.1 g, 71%) as a colorless oil which crystallized on standing. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.78 (s, 1H), 7.86 (dd, *J* = 7.7, 1.3 Hz, 1H), 7.53 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.36 (t, *J* = 7.9 Hz, 1H), 6.05 (s, 1H), 3.73–3.98 (m, 2H), 3.52–3.6 (m, 2H), 1.23 (t, *J* = 7.0 Hz, 6H).

Step 3. Ethyl 3-(3-chloro-2-(diethoxymethyl)benzylamino)-1H-pyrrole-2-carboxylate.

A solution of ethyl 3-amino-1H-pyrrole-2-carboxylate hydrochloride (1.6 g, 8.2 mmol) in EtOH (25 mL) was treated with DIPEA (1.7 mL, 9.9 mmol) and stirred for 10 min before the addition of HOAc (1.1 mL, 20 mmol) and $NaBH_3CN$ (1.6 g, 25 mmol). A solution of 3-chloro-2-(diethoxymethyl)benzaldehyde (2.0 g, 8.2 mmol) in EtOH (10 mL) was then added dropwise over 15 min and the resultant suspension stirred at rt overnight. The reaction was concentrated *in vacuo* and the residue treated with water (100 mL) and extracted with DCM (2 × 100 mL). The organics were dried (Na_2SO_4), concentrated *in vacuo* and the residue purified by flash silica chromatography eluting with 20% EtOAc in isohexane to afford ethyl 3-(3-chloro-2-(diethoxymethyl)benzylamino)-1H-pyrrole-2-carboxylate (2.1 g, 67%) as a colorless oil.

Step 4. 1-(3-Chloro-2-(diethoxymethyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one.

A solution of ethyl 3-(3-chloro-2-(diethoxymethyl)benzylamino)-1H-pyrrole-2-carboxylate (2.1 g, 5.5 mmol) in DCM (30 mL) was treated with benzoyl isothiocyanate (0.74 mL, 5.5 mmol) and stirred overnight. The solvent was removed *in vacuo* and the residue dissolved in MeOH (30 mL) then treated with sodium hydroxide (1.10 g, 28 mmol) and stirred under reflux for 2 h. The solvent was removed *in vacuo* and the residue treated with water (100 mL), acidified to pH 5 with HOAc and extracted with DCM (2 × 100 mL). The organics were dried ($MgSO_4$), concentrated *in vacuo* and the residue purified by flash silica chromatography eluting with 40% EtOAc in isohexane to afford 1-(3-chloro-2-(diethoxymethyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (1.54 g, 71%) as a yellow solid. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 12.45 (s, 1H), 12.39 (s, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.2–7.27 (m, 2H), 6.78 (d, *J* = 7.8 Hz, 1H), 5.84–6.28 (m, 4H), 3.8–3.9 (m, 2H), 3.55–

3.69 (m, 2H), 1.23 (t, *J* = 7.0 Hz, 6H). LC-MS (ESI) m/z: 392.0 (calcd for $C_{18}H_{20}ClN_3O_3S$ $[M-H]^-$, 392.1).

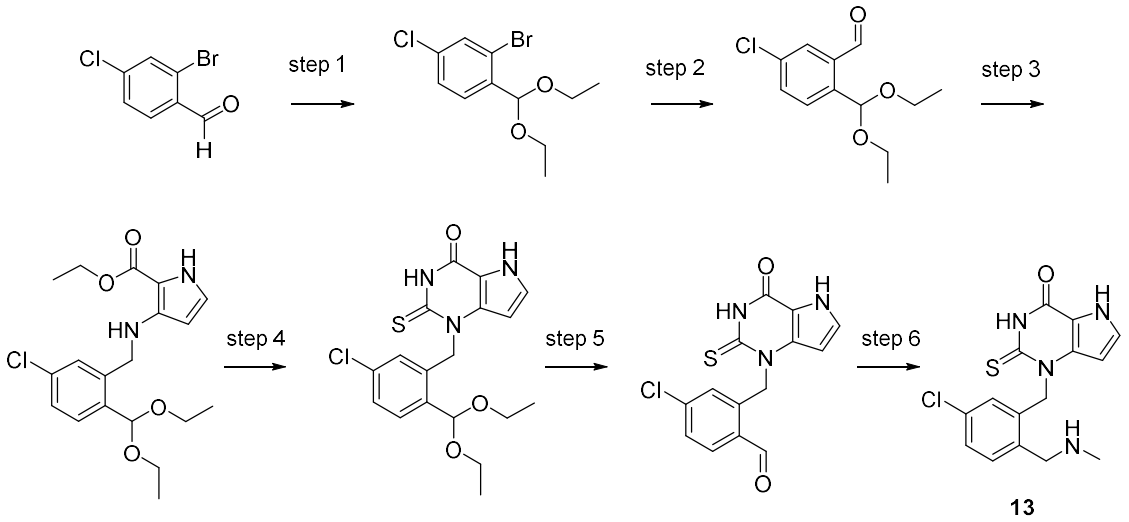
Step 5. 2-Chloro-6-((4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-1-yl)methyl)benzaldehyde.

A solution of 1-(3-chloro-2-(diethoxymethyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (1.53 g, 3.9 mmol) in THF (20 mL) was treated with 2N hydrochloric acid (20 mL) and stirred at 50°C for 2 h. The reaction was concentrated *in vacuo* and the residue suspended in water (50 mL), filtered and the solid dried *in vacuo* at 70°C to afford 2-chloro-6-((4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-1-yl)methyl)benzaldehyde (1.20 g, 97%) as an off-white solid. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 12.50 (s, 1H), 12.40 (s, 1H), 10.59 (s, 1H), 7.54–7.58 (m, 1H), 7.51 (t, *J* = 7.9 Hz, 1H), 7.30 (t, *J* = 3.0 Hz, 1H), 6.81 (d, *J* = 7.7 Hz, 1H), 6.11 (t, *J* = 2.4 Hz, 1H), 5.90 (s, 2H). LC-MS (ESI) m/z: 318.0 (calcd for $C_{14}H_{10}ClN_3O_2S$ $[M-H]^-$, 318.0).

Step 6. 1-(3-Chloro-2-((methylamino)methyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one trifluoroacetate (12).

2-Chloro-6-((4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-1-yl)methyl)benzaldehyde (0.144 g, 0.45 mmol) was suspended in MeCN (15 mL) and the solution treated with methanamine (0.56 mL, 4.50 mmol). Stirred at rt for 4 h. $NaBH_3CN$ (0.057 g, 0.90 mmol) was added and the reaction stirred at rt for 18 h. The solvent was removed *in vacuo* and the residue dissolved in DMSO/MeOH and purified by preparative HPLC using a gradient of MeOH in water (0.1% TFA). Pure fractions were concentrated to afford **12** (43 mg). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 12.57 (s, 1H), 12.47 (s, 1H), 8.81 (s, 2H), 7.53 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.28–7.43 (m, 2H), 6.75 (dd, *J* = 8.0, 1.2 Hz, 1H), 6.13 (t, *J* = 2.3 Hz, 1H), 5.87 (s, 2H), 4.50 (s, 2H), 2.79 (s, 3H). ^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ 174.0, 153.1, 138.8, 137.2, 136.3, 131.9, 129.0, 128.6, 127.8, 125.0, 114.2, 97.4, 51.2, 46.0, 34.0. HRMS (ESI) m/z: 335.0748 (calcd for $C_{15}H_{15}ClN_4OS$ $[M+H]^+$, 335.0733).

Synthesis of 1-(5-chloro-2-((methylamino)methyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (13).



Step 1. 2-Bromo-4-chloro-1-(diethoxymethyl)benzene.

2-Bromo-4-chlorobenzaldehyde (10 g, 46 mmol) was dissolved in EtOH (99.5%, 40 mL). Triethylorthoformate (15 mL, 91 mmol) was added followed by HCl in dioxane (4 M, 5.0 mL, 20 mmol). The reaction mixture was refluxed overnight. The solvents were removed *in vacuo* and co-evaporated with EtOH several times to remove excess triethylorthoformate. Finally co-evaporation with DCM left a light yellow oil of the title compound (13.6 g, quantitative yield). Used as such in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54–7.61 (m, 2H), 7.31 (dd, *J* = 8.4, 2.1 Hz, 1H), 5.60 (s, 1H), 3.52–3.71 (m, 4H), 1.24 (t, *J* = 7.1 Hz, 6H).

Step 2. 5-chloro-2-(diethoxymethyl)benzaldehyde.

2-Bromo-4-chloro-1-(diethoxymethyl)benzene (13 g, 44 mmol) was dissolved in 2-MeTHF (150 mL) and cooled to -78°C. *n*-BuLi (2.5 M in hexane) (19 mL, 46 mmol) was added dropwise over 3 min. Followed by dropwise addition of DMF (3.8 mL, 49 mmol) at -78°C over 5 min. The reaction was allowed to reach 0°C and left for 1 h at this temperature. Water (75 mL) was slowly added followed by brine. The organic layer was washed with brine, then filtered through a phase separator and concentrated *in vacuo*. Co-evaporation with DCM gave the title compound (10.4 g, 97%). Used as such in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.46 (s, 1H), 7.88 (d, *J* = 2.3 Hz, 1H), 7.62 (d, *J*

= 8.2 Hz, 1H), 7.53 (dd, *J* = 8.2, 2.3 Hz, 1H), 3.63–3.73 (m, 2H), 3.51–3.6 (m, 2H), 1.22 (t, *J* = 7.1 Hz, 6H).

Step 3. Ethyl 3-(5-chloro-2-(diethoxymethyl)benzylamino)-1H-pyrrole-2-carboxylate.

Ethyl 3-amino-1H-pyrrole-2-carboxylate hydrochloride (2.4 g, 13 mmol), DIPEA (2.2 mL, 13 mmol), 5-chloro-2-(diethoxymethyl)benzaldehyde (2.8 g, 12 mmol) and HOAc (1.4 mL, 23 mmol) were dissolved in EtOH (99.5%, 100 mL). After 1 h $NaBH_3CN$ (0.9 g, 14 mmol) was added. The reaction was stirred at rt for 3 h. The reaction was quenched with water and concentrated *in vacuo*. The crude product was taken up in a mixture of DCM and water. The DCM was removed *in vacuo*. The residue was purified by automated flash at 50% EtOAc in heptane to yield the title compound (3.4 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.2 Hz, 1H), 7.45 (d, *J* = 2.2 Hz, 1H), 7.22 (dd, *J* = 8.3, 2.2 Hz, 1H), 6.69 (s, 1H), 5.63 (t, *J* = 2.8 Hz, 1H), 5.59 (s, 1H), 4.48 (s, 2H), 4.31 (q, *J* = 7.1 Hz, 2H), 3.47–3.66 (m, 4H), 1.34 (t, *J* = 7.1 Hz, 3H), 1.23 (t, *J* = 7.1 Hz, 6H).

Step 4. 1-(5-chloro-2-(diethoxymethyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one.

Ethyl 3-(5-chloro-2-(diethoxymethyl)benzylamino)-1H-pyrrole-2-carboxylate (3.4 g, 8.8 mmol) was dissolved in MeOH (40 mL). Benzoyl isothiocyanate (1.2 mL, 8.8 mmol) was added and the reaction mixture was stirred at rt for 1 h. Cs_2CO_3 (6.2 g, 19 mmol) was added and the reaction mixture was stirred at 60°C for 3 h. The solvent was removed under reduced pressure. Water and DCM were added. The layers were separated and the aq layer was extracted with DCM (3x). The organic layers were combined and the solvent was removed under reduced pressure. The crude product was re-dissolved in EtOH (200 mL) and the formed precipitate was filtered off and dried under vacuum to yield 0.82 g of crude product. Purified by automated flash chromatography using a gradient of 50–100% EtOAc in heptane to yield the title compound (756 mg, 22%) as solid. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 12.44 (d, *J* = 35.8 Hz, 2H), 7.55 (d, *J* = 8.2 Hz, 1H), 7.34 (dd, *J* = 8.3, 2.2 Hz, 1H), 7.30 (d, *J* = 2.8 Hz, 1H), 6.70 (d, *J* = 2.1 Hz, 1H), 6.00 (d, *J* = 2.9 Hz, 1H), 5.78 (d, *J* = 19.4 Hz, 3H), 3.5–3.7 (m, 4H), 1.20 (t, *J* = 7.0 Hz, 6H).

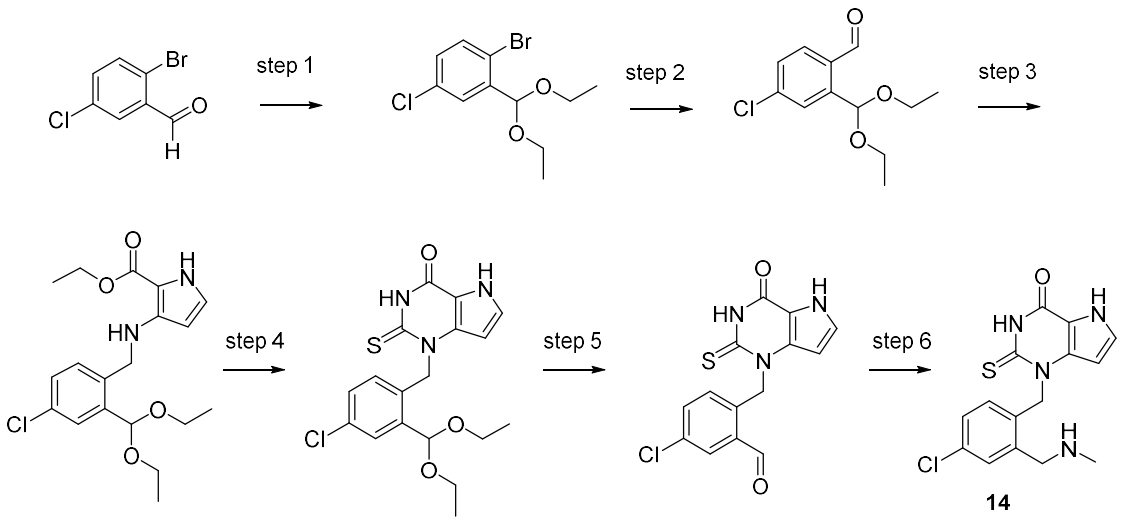
Step 5. 4-Chloro-2-((4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-1-yl)methyl)benzaldehyde.

1-(5-Chloro-2-(diethoxymethyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (0.75 g, 1.90 mmol) was suspended in DCM (20 mL) and TFA (1.4 mL, 19 mmol) was added. The reaction mixture was stirred at rt for 3h. The formed precipitate was filtered off, washed with DCM and dried under vacuum to yield the title compound (0.53 g, 87%). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 12.53 (s, 1H), 12.41 (s, 1H), 10.25 (s, 1H), 8.06 (d, *J* = 8.2 Hz, 1H), 7.63 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.32 (t, *J* = 3.0 Hz, 1H), 6.84 (s, 1H), 6.02–6.14 (m, 3H). LC-MS (ESI) *m/z*: 318.1 (calcd for $C_{14}H_{10}ClN_3O_2S$ $[M-H]^-$, 318.0).

Step 6. 1-(5-Chloro-2-((methylamino)methyl)benzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (13).

A microwave vial was charged with 4-chloro-2-((4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-1-yl)methyl)benzaldehyde (100 mg, 0.31 mmol) and methanamine (2 mL, 4.0 mmol, 2M in MeOH). The reaction was heated at 100°C for 15 min in the microwave to form the imine. THF (3 mL) was added followed by portionwise addition of $NaBH_4$ (95 mg, 2.5 mmol). The reaction mixture was stirred at rt for 30 min. The reaction was quenched with water (2 mL), and concentrated *in vacuo* to give 120 mg of crude product. Purified by preparative HPLC on a XBridge C18 column (10 µm 250x50 ID mm) using a gradient of 0-45% MeCN in H_2O/MeCN/NH_3 (95/5/0.2) buffer over 15 min with a flow of 100 mL/min to yield **13** (20 mg, 19%). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.38 (d, *J* = 8.1 Hz, 1H), 7.30 (d, *J* = 2.8 Hz, 1H), 7.27 (dd, *J* = 8.1, 2.2 Hz, 1H), 6.64 (d, *J* = 2.2 Hz, 1H), 6.09 (d, *J* = 2.9 Hz, 1H), 5.78 (s, 2H), 3.77 (s, 2H), 2.34 (s, 3H). ^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ 173.7, 152.7, 136.9, 136.7, 136.5, 131.5, 131.3, 128.2, 126.5, 124.3, 113.8, 96.8, 52.5, 49.9, 36.0. HRMS (ESI) m/z: 335.0738 (calcd for $C_{15}H_{15}ClN_4OS$ $[M+H]^+$, 335.0733).

Synthesis of 1-(4-Chloro-2-((methylamino)methyl)benzyl)-2-thioxo-1,2,3,5-tetrahydro-4H-pyrrolo[3,2-d]pyrimidin-4-one (14).



Step 1. 1-Bromo-4-chloro-2-(diethoxymethyl)benzene.

2-Bromo-5-chlorobenzaldehyde (250 g, 1139 mmol) was added in portions during 50 min to triethoxymethane (379 mL, 2278 mmol) in a reactor (5 L) under a nitrogen atmosphere under stirring. The reactor was cooled during the addition to keep the reaction temperature below 21°C. The mixture was stirred at 17°C for 3 h, another portion of triethoxymethane (100 mL, 601 mmol) was added and stirring was then continued for additional 3.5 h. Heptane (300 mL) was added and the mixture was filtered through celite. The filter cake was washed with heptane (200 mL) and the combined solutions were concentrated *in vacuo*. The residue was co-evaporated four times with heptane (200 mL) and then purified in portions by flash chromatography (gradient, 0%-50% EtOAc in hexane) to give 280 g (84%) of the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55–7.62 (m, 2H), 7.32 (dd, *J* = 8.4, 2.1 Hz, 1H), 5.61 (s, 1H), 3.53–3.72 (m, 4H), 1.25 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 139.8, 134.3, 132.4, 130.1, 127.7, 120.4, 100.0, 62.0, 15.0.

Step 2. 4-Chloro-2-(diethoxymethyl)benzaldehyde.

n-BuLi (2.5 M in hexane, 342 mL, 855 mmol) was added via tubing to a cold (–60°C) solution of l-bromo-4-chloro-2-(diethoxymethyl)benzene (251 g, 855 mmol) in 2-MeTHF (3 L) under a nitrogen atmosphere in a reactor (5 L). After stirring for 40 min, DMF (73 mL, 940 mmol) was added over a

period of 12 min. The temperature of the mixture rose to –49°C during the addition. The mixture was stirred for 40 min and then the temperature was increased to 0°C. After additional 30 min stirring, water (300 mL) was added for a period of 5 min followed by a half sat. solution of brine (1.5 L). The layers were separated and the water phase was extracted with 2-MeTHF (1 L). The combined organic phase was washed with brine, dried ($MgSO_4$) and concentrated *in vacuo* overnight at 30°C to give the title compound (193 g, 87%) as a light brown oil, which was used as such without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.41 (s, 1H), 7.84 (d, *J* = 8.2 Hz, 1H), 7.68 (d, *J* = 2.1 Hz, 1H), 7.42 (dd, *J* = 8.2, 2.1, 1H), 5.93 (s, 1H), 3.5–3.72 (m, 4H), 1.22 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.7, 142.5, 139.6, 132.1, 130.9, 128.7, 127.5, 99.2, 62.2, 14.9.

Step 3. Ethyl 3-((4-chloro-2-(diethoxymethyl)benzyl)amino)-1*H*-pyrrole-2-carboxylate.

DIPEA (92 g, 714 mmol) dissolved in MeOH (100 mL) was added to a solution of 4-chloro-2-(diethoxymethyl)benzaldehyde (181 g, 649 mmol) and MeOH (1 L) in a reactor (10 L). Ethyl 3-amino-1*H*-pyrrole-2-carboxylate hydrochloride (132 g, 648 mmol) was added together with MeOH (700 mL). The mixture was stirred at 20°C overnight and then HOAc (78 g, 1298 mmol) was added. $NaBH_3CN$ (41 g, 649 mmol) was added in several portions under a period of 7 min with stirring and cooling, so that the temperature did not rise above 27°C. The solution was stirred for 40 min and then water (1.8 L) was added. The mixture was extracted twice with DCM (1 L) and the combined organic solutions were dried (K_2CO_3) and concentrated to a volume of 1300 mL. After 4 days the mixture was concentrated in the presence of MeOH to give a MeOH solution (approximately 1 L) of the title compound. The material was used in the next step without further purification.

Step 4. 1-(4-Chloro-2-(diethoxymethyl)benzyl)-2-thioxo-1,2,3,5-tetrahydro-4*H*-pyrrolo[3,2-d]pyrimidin-4-one.

Benzoylisothiocyanate (89 g, 677 mmol) was added to the solution from the step 3 of ethyl 3-((4-chloro-2-(diethoxymethyl)benzyl)amino)-1*H*-pyrrole-2-carboxylate (550 mmol) in MeOH (approximately 1 L) so that the reaction temperature was maintained between 17°C and 22°C. The mixture was stirred for 15 min and another portion of benzoyl isothiocyanate (13.5 g, 82.5 mmol) was added and after additional stirring for 50 min, still another portion of benzoyl isothiocyanate (8

g, 50 mmol) was added. The mixture was stirred for 30 min and then Cs_2CO_3 (383 g, 1177 mmol) was added during a period of 15 min. The temperature was increased to 30°C for 30 min and then to 40°C for 20 min. The mixture was then stirred at 50°C for 4 h and then at 10°C overnight. HOAc (140 mL) was added at 10°C during a period of 20 min and then the temperature of the mixture was increased to 19°C. Water (1.4 L) was slowly added to the formed precipitate and the solid material was isolated by filtration. The filter cake was washed with toluene (2 L) and then dried *in vacuo* for 3 days to give (201 g, 80%, two steps) of the title compound. ^{1}H NMR (600 MHz, $(CD_3)_2SO$) δ 12.34 (s, 2H), 7.54 (d, *J* = 2.3 Hz, 1H), 7.26–7.32 (m, 2H), 6.75 (d, *J* = 8.4 Hz, 1H), 5.98 (d, *J* = 2.9 Hz, 1H), 5.78 (s, 3H), 3.54–3.68 (m, 4H), 1.21 (t, *J* = 7.0 Hz, 6H).

Step 5. 5-Chloro-2-((4-oxo-2-thioxo-2,3,4,5-tetrahydro-1*H*-pyrrolo[3,2-d]pyrimidin-1-yl)methyl)benzaldehyde trifluoroacetate.

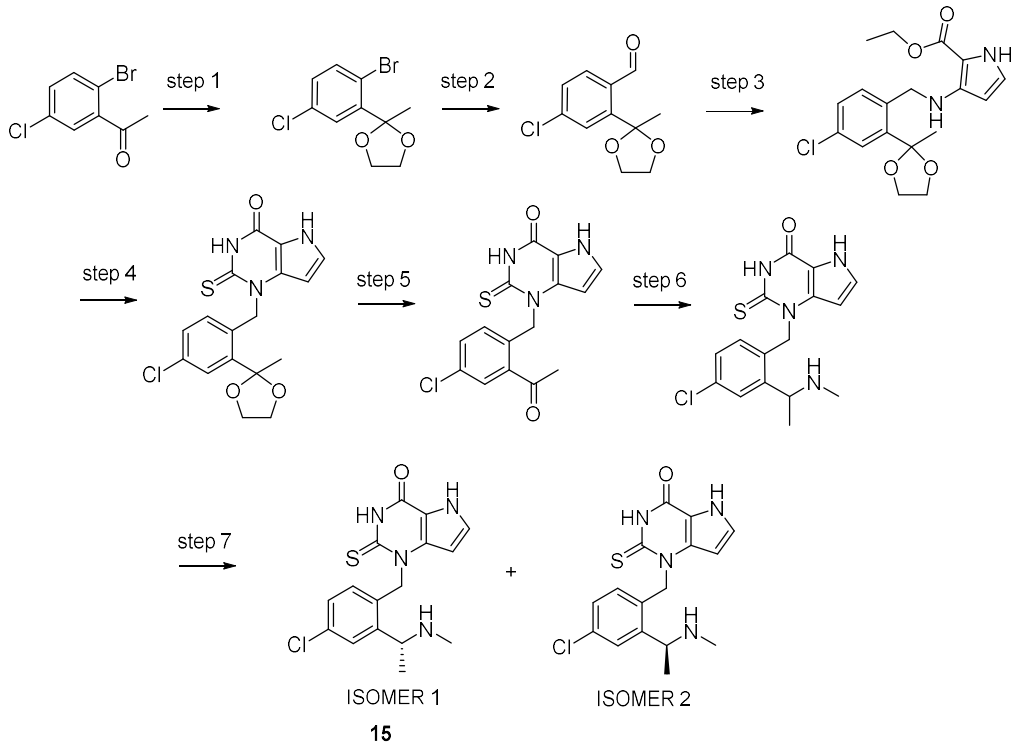
1-(4-Chloro-2-(diethoxymethyl)benzyl)-2-thioxo-1,2,3,5-tetrahydro-4*H*-pyrrolo[3,2-d]pyrimidin-4-one (1 g, 2.54 mmol) was added to a cooled (ice-bath) mixture of TFA (1.89 mL, 25.39 mmol) and DCM (8 mL). The ice-bath was removed and the mixture was stirred at rt for 2.5 h. The formed precipitate was isolated by filtration and washed with DCM to give the title compound (0.69 g, 85%) as a solid. ^{1}H NMR (600 MHz, $(CD_3)_2SO$) δ 12.46–12.53 (m, 1H), 12.40 (s, 1H), 10.24 (s, 1H), 8.08 (d, J = 2.4 Hz, 1H), 7.59 (dd, J = 8.4, 2.4 Hz, 1H), 7.30 (t, J = 3.0 Hz, 1H), 6.88 (d, J = 8.4 Hz, 1H), 6.09 (t, J = 2.5 Hz, 1H), 6.03 (s, 2H). ^{13}C NMR (151 MHz, $(CD_3)_2SO$) δ 192.98, 192.92, 173.7, 152.8, 136.9, 136.0, 135.0, 133.6, 133.2, 132.4, 128.2, 128.0, 113.9, 96.8, 50.66. LC-MS (ESI) *m/z*: 319.9 (calcd for $C_{14}H_{10}ClN_3O_2S$ $[M+H]^+$, 320.0).

Step 6. 1-(4-Chloro-2-((methylamino)methyl)benzyl)-2-thioxo-1,2,3,5-tetrahydro-4*H*-pyrrolo[3,2-d]pyrimidin-4-one. (14)

A mixture of 5-chloro-2-((4-oxo-2-thioxo-2,3,4,5-tetrahydro-1*H*-pyrrolo[3,2-d]pyrimidin-1-yl)methyl)benzaldehyde (1.00 g, 3.13 mmol) and methanamine (20 mL, 40.00 mmol) was heated in a microwave oven at 100°C for 20 min. The vial was opened and the mixture was diluted with MeOH and stirred at rt whereupon $NaBH_4$ (0.95 g, 25 mmol) was added in portions during 5 min. After stirring at rt for 1 h, the mixture was heated to reflux for 2 h. Another portion of $NaBH_4$ (0.47 g,

12.5 mmol) was added and the mixture was refluxed for an additional 15 min. The solvent was removed *in vacuo* and water (20 mL) was added to the residue followed by an aq solution of HCl (1 M, 10 mL) to adjust the pH to 1. The mixture was cooled with an ice-bath and the formed precipitate was filtered off and washed with water (100 mL). The filtrate was cooled with an ice-bath and the pH adjusted to pH 9 using an aq NH_3 solution (12%, 6 mL). A precipitate was isolated by filtration and drying under vacuum gave **14** (700 mg, 67%). ^{1}H NMR (600 MHz, CD_3COOD) δ 11.63 (s, 4H), 7.61 (d, J = 2.2 Hz, 1H), 7.43 (d, J = 2.9 Hz, 1H), 7.31 (dd, J = 8.5, 2.2 Hz, 1H), 6.93 (d, J = 8.5 Hz, 1H), 6.20 (d, J = 2.9 Hz, 1H), 5.78 (s, 2H), 4.55 (s, 2H), 2.91 (s, 3H). ^{13}C NMR (151 MHz, CD_3COOD) δ 174.4, 155.3, 140.2, 134.5, 134.3, 132.2, 132.0, 131.0, 130.9, 128.9, 114.4, 98.1, 51.6, 49.6, 33.8, 26.3. HRMS (ESI) m/z 335.0746 : (calcd for $C_{15}H_{15}ClN_4OS$ $[M+H]^+$, 335.0733).

Synthesis of (R or S)-1-(4-chloro-2-(1-(methylamino)ethyl)benzyl)-2-thioxo-1,2,3,5-tetrahydro-4*H*-pyrrolo[3,2-d]pyrimidin-4-one (15).



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Step 1. 2-(2-Bromo-5-chlorophenyl)-2-methyl-1,3-dioxolane.

Ethane-1,2-diol (5.96 mL, 106.51 mmol) and 4-methylbenzenesulfonic acid (0.67 g, 3 .91 mmol) were added to a solution of 1-(2-bromo-5-chlorophenyl)ethan-1-one (8.29 g, 35.50 mmol) in toluene (180 mL) in a round bottomed flask fitted with a Dean-Stark trap and the reaction mixture was heated at reflux for 3.5 h. The reaction mixture was cooled to rt and an aq solution of K_2CO_3 (1 M) was added and the layers were separated. The aq phase was extracted with toluene and the combined organic layers were washed with water and brine. The solution was dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure to give (9.29 g, 94%) of the title compound. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, J = 2.6 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.13 (dd, J = 8.4, 2.6 Hz, 1H), 4.01–4.11 (m, 2H), 3.72–3.84 (m, 2H), 1.80 (s, 3H).^{13}C NMR (126 MHz, $CDCl_3$) δ 143.1, 136.1, 133.4, 129.4, 128.1, 118.4, 108.2, 64.4, 25.0.

Step 2. 4-Chloro-2-(2-methyl-1,3-dioxolan-2-yl)benzaldehyde.

n-BuLi (2.5 M in hexane, 14.73 mL, 36.82 mmol) was added drop wise to a solution of 2-(2-bromo-5-chlorophenyl)-2-methyl-l,3-dioxolane (9.29 g, 33.47 mmol) in THF (100 mL) during 30 min at under a nitrogen atmosphere and at –78°C. The resulting solution was stirred at –78°C for 30 min. DMF (3.87 mL, 50.21 mmol) was added dropwise at –78°C. After the addition was complete, the reaction was allowed to warm to rt and stirring was continued for 10 min. The reaction mixture was quenched with an aq solution of NH_4Cl (sat.) and the phases were separated. The aq phase was extracted twice with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$) and the solvents were removed under reduced pressure. The product was purified by flash chromatography (gradient, 10–20% EtOAc in hexane) to give the title compound (5.95, 78%) as transparent colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.53 (d, J = 0.8 Hz, 1H), 7.76 (d, J = 8.3 Hz, 1H), 7.54 (d, J = 2.1 Hz, 1H), 7.26 (ddd, J = 8.3, 2.1, 0.8 Hz, 1H), 3.94–3.98 (m, 2H), 3.63–3.68 (m, 3H), 1.68 (s, 3H).

Step 3. Ethyl 3-((4-chloro-2-(2-methyl-1,3-dioxolan-2-yl)benzyl)amino)-1*H*-pyrrole-2-carboxylate.

DIPEA (3.34 mL, 19.17 mmol) followed by HOAc (1.99 mL, 34.85 mmol) were added to a solution of ethyl 3-amino-1*H*-pyrrole-2-carboxylate hydrochloride (3.65 g, 19.17 mmol) in EtOH (99.5%, 100 mL) and to the solution were added. The reaction mixture was cooled to 10°C and then 4-chloro-2-(2-methyl-l,3-dioxolan-2-yl)benzaldehyde (3.95 g, 17.43 mmol) dissolved in EtOH (99.5%, 10 mL) was added. The reaction mixture was allowed to reach rt for 1 h. $NaBH_3CN$ (1.31 g, 20.91 mmol) was added and the mixture was stirred at rt for 18 h. The reaction mixture was quenched with water (50 mL) and the pH was adjusted to 11 with NaOH. The mixture was extracted trice with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$) and the solvent was removed under reduced pressure. The product was purified by flash chromatography (isocratic, 10% EtOAc in hexane) to give the title compound (4.61 g, 72%) as a colorless gum. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 2.3 Hz, 1H), 7.42 (d, J = 8.3 Hz, 1H), 7.22 (dd, J = 8.2, 2.4 Hz, 1H), 6.70 (s, 1H), 5.68 (t, J = 2.7 Hz, 1H), 4.54 (d, J = 6.3 Hz, 2H), 4.24–4.36 (m, 2H), 4.04–4.11 (m, 2H), 3.78–3.84 (m, 2H), 1.71 (s, 3H), 1.34 (t, J = 7.1 Hz, 3H).

Step 4. 1-(4-Chloro-2-(2-methyl-1,3-dioxolan-2-yl)benzyl)-2-thioxo-1,2,3,5-tetrahydro-4*H*-pyrrolo[3,2-d]pyrimidin-4-one.

Benzoyl isothiocyanate (1.70 mL, 12.64 mmol) was added dropwise to a solution of ethyl 3-((4-chloro-2-(2-methyl-1,3-dioxolan-2-yl)benzyl)amino)-1*H*-pyrrole-2-carboxylate**.** (4.61 g, 12.64 mmol) in MeOH (14 mL) and the reaction mixture was stirred at rt for 10 min. A further portion of benzoyl isothiocyanate (0.17 mL, 1.26 mmol) was added and the reaction was stirred for 30 min. Cs_2CO_3 (8.85 g, 27.17 mmol) was added and the mixture was stirred at 60°C for 1.5 h. The solvent was removed under reduced pressure. Water (30 mL) and EtOAc (70 mL) were added and a precipitate formed in the organic phase was isolated by filtration. After washing the solid with Et_2O gave (4.71 g, 99%) of the title compound as a white solid. LC-MS (ESI) *m/z*: 378.2 (calcd for $C_{17}H_{16}ClN_3O_3S$ $[M+H]^+$, 378.07).

Step 5. 1-(2-Acetyl-4-chlorobenzyl)-2-thioxo-1,2,3,5-tetrahydro-4*H*-pyrrolo[3,2-d]pyrimidin-4-one.

To a suspension of 1-(4-chloro-2-(2-methyl-1,3-dioxolan-2-yl)benzyl)-2-thioxo-1,2,3,5-tetrahydro-4*H*-pyrrolo[3,2-d]pyrimidin-4-one (4.71 g, 12.47 mmol) in DCM (25 mL) was added TFA (9.26 mL, 124.65 mmol). The reaction mixture was stirred at rt for 2 h and then an aq solution of NaOH (3.8 M) was added until a pH of approximately 11 was reached. The formed solid was isolated by filtration and the product was washed with DCM and then with Et_2O to give (3.49 g, 84%) of the title compound as a white solid. ^{1}H NMR (500 MHz, , $(CD_3)_2SO$)) δ 12.48 (s, 1H), 12.35 (s, 1H), 8.07 (d, *J* = 2.3 Hz, 1H), 7.50 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.30 (t, *J* = 3.0 Hz, 1H), 6.84 (d, *J* = 8.5 Hz, 1H), 6.08 (t, *J* = 2.5 Hz, 1H), 5.79 (s, 2H), 2.70 (s, 3H).). LC-MS (ESI) *m/z*: 334.1 (calcd for $C_{15}H_{12}ClN_3O_2S$ $[M+H]^+$, 334.04).

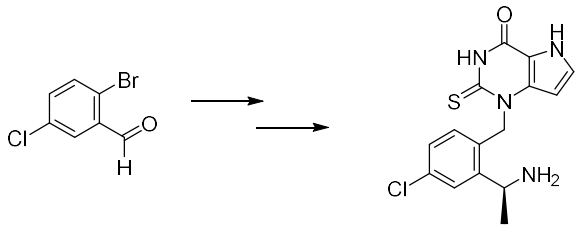
Step 6. 1-(4-Chloro-2-(1-(methylamino)ethyl)benzyl)-2-thioxo-1,2,3,5-tetrahydro-4*H*-pyrrolo[3,2-d]pyrimidin-4-one formate.

$Ti(O^iPr)_4$ (0.52 mL, 1.79 mmol) and 2 M methanamine in THF (2.68 mL, 5.36 mmol) were added to a suspension of 1-(2-Acetyl-4-chlorobenzyl)-2-thioxo-1,2,3,5-tetrahydro-4*H*-pyrrolo[3,2-d]pyrimidin-4-one (0.29 g, 0.88 mmol) in EtOH (99.5%, 2.0 mL). The mixture was stirred at rt for 4 h and then $NaBH_4$ (66.9 mg, 1.77 mmol) was added. After stirring at rt for 30 min, water was added followed by 2 M NH_3 in MeOH to adjust the pH to 11. The suspension was stirred at rt for 30 min and then the precipitate was filtered off and washed with MeOH and EtOAc. The filtrate was removed under reduced pressure and the product was purified by preparative HPLC (XBridge C18, 10 µm, 50 × 250 mm, 0-30% MeCN in water/MeCN/FA, 95/5/0.2) to give the title compound (0.13 g, 43%). ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 7.60 (d, *J* = 2.3 Hz, 1H), 7.32 (d, *J* = 2.8 Hz, 1H), 7.18 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.65 (d, *J* = 8.4 Hz, 1H), 6.00 (d, *J* = 2.9 Hz, 1H), 5.73 (q, *J* = 16.5 Hz, 2H), 4.14 (q, *J* = 6.5 Hz, 1H), 2.29 (s, 3H), 1.35 (d, *J* = 6.5 Hz, 3H). LC-MS (ESI) *m/z*: 349.0 (calcd for $C_{16}H_{17}ClN_4OS$ $[M+H]^+$, 349.09).

Step 7. (R or S)-1-(4-chloro-2-(1-(methylamino)ethyl)benzyl)-2-thioxo-1,2,3,5-tetrahydro-4H-pyrrolo[3,2-d]pyrimidin-4-one (15).

The enantiomers of 1-(4-chloro-2-(1-(methylamino)ethyl)benzyl)-2-thioxo-1,2,3,5-tetrahydro-4H-pyrrolo[3,2-d]pyrimidin-4-one (130 mg, 0.37 mmol) were separated using SFC chromatography on a ChiralPak AD column (5 µm, 20 × 250 mm), eluted with 35% EtOH/DEA 100/0.5 in CO_2, 175 bar at 40°C, at a flow of 60 mL/min and detected at 260 nm. The first eluted compound was collected and evaporated to give (58 mg, 45%, 99.9% ee) of the title compound. ^{1}H NMR (500 MHz, $(CD_3)_2SO$)) δ 7.58 (d, *J* = 2.3 Hz, 1H), 7.33 (d, *J* = 2.8 Hz, 1H), 7.15 (dd, *J* = 8.4, 2.3 Hz, 1H), 6.64 (d, *J* = 8.4 Hz, 1H), 5.99 (d, *J* = 2.8 Hz, 1H), 5.64–5.88 (m, 2H), 4.04 (q, *J* = 6.5 Hz, 1H), 2.24 (s, 3H), 1.31 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (126 MHz, $(CD_3)_2SO$)) δ 173.9, 153.1, 145.6, 137.3, 132.5, 132.2, 128.6, 127.2, 126.7, 126.3, 114.2, 97.2, 54.9, 50.3, 34.4, 22.7. $[a]^{20}_D$ = + 50 (c= 0.3, $(CD_3)_2SO$). HRMS (ESI) m/z 349.0897: (calcd for $C_{16}H_{17}ClN_4OS$ $[M+H]^+$, 349.0890). ee = 99.9 % (ChiralPak AD column (5 µm, 4.6 × 250 mm), eluted with 35% EtOH/DEA 100/0.5 in CO_2, 150 bar at 40°C, at a flow of 1 mL/min and detected at 260 nm).

Synthesis of (S)-1-(2-(1-aminoethyl)-4-chlorobenzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one acetate (17).

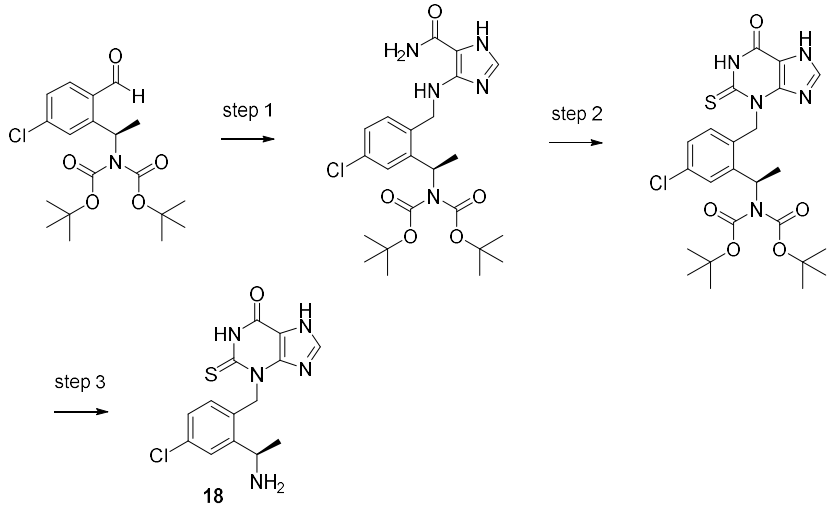


17

Starting with 2.0 g of 2-bromo-5-chlorobenzaldehyde and using a similar protocol as described for compound **16** (Scheme 2) but employing the *R*-enantiomer of 2-methylpropane-2-sulfinamide rather than the *S*-enantiomer afforded 0.40 g of the title compound as the acetate salt with an enantiomeric excess of 95.1%. $[\alpha]_D^{20}$ = -55° (c=0.5, MeOH). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.66 (d, *J* = 2.3 Hz, 1H), 7.31 (d, *J* = 2.8 Hz, 1H), 7.13 (dd, *J* = 8.3, 2.4 Hz, 1H), 6.60 (d, *J* = 8.3 Hz, 1H),

6.04 (d, *J* = 2.8 Hz, 1H), 5.62–5.86 (m, 2H), 4.41 (q, *J* = 6.5 Hz, 1H), 1.34 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ 174.0, 153.1, 147.7, 137.3, 132.3, 131.3, 128.6, 126.9, 126.8, 126.0, 114.2, 97.4, 50.3, 46.5, 24.7. HRMS (ESI) m/z: 335.0712 (calcd for $C_{15}H_{15}ClN_4OS$ $[M+H]^+$, 335.0733).

Synthesis (R)-3-(2-(1-aminoethyl)-4-chlorobenzyl)-2-thioxo-2,3-dihydro-1H-purin-6(7H)-one acetate (18).



Step 1. *tert*-Butyl N-tert-butoxycarbonyl-N-[(1R)-1-[2-[[(5-carbamoyl-1H-imidazol-4-yl)amino]methyl]-5-chloro-phenyl]ethyl]carbamate.

tert-Butyl N-tert-butoxycarbonyl-N-[(1*R*)-1-(5-chloro-2-formyl-phenyl)ethyl]carbamate (2.30 g, 5.70 mmol) was dissolved in EtOH (99.5%, 50 mL). HOAc (0.13 mL, 2.29 mmol) and 4-amino-1H-imidazole-5-carboxamide (0.79 g, 6.29 mmol) were added. The mixture was stirred at rt for 26 h. $NaBH_4$ (0.24 g, 6.29 mmol) was added. The mixture was stirred at rt for 3.5 h whereupon more $NaBH_4$ (0.24 g, 6.29 mmol) was added. The reaction mixture was stirred for 1 h. Water (20 mL) was added and some of the EtOH was removed *in vacuo*. Added brine (20 mL) and extracted with EtOAc (×2). Dried (Na_2SO_4) and filtered to give the title compound (3.01 g, quantitative yield) as grey solid. Used as such in the next step. LC-MS (ESI) *m/z*: 492.4 (calcd for $C_{23}H_{32}ClN_5O_5$ $[M-H]^-$, 492.2).

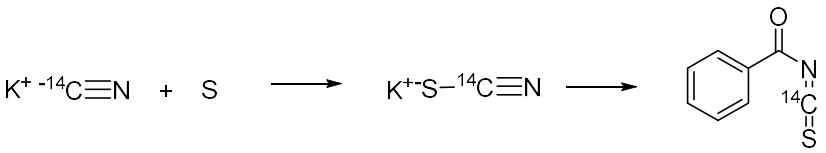
Step 2. *tert*-Butyl N-tert-butoxycarbonyl-N-[(1R)-1-[5-chloro-2-[(6-oxo-2-thioxo-7H-purin-3-yl)methyl]phenyl]ethyl]carbamate.

tert-Butyl N-tert-butoxycarbonyl-N-[(1R)-1-[2-[[(5-carbamoyl-1H-imidazol-4-yl)amino]methyl]-5-chloro-phenyl]ethyl]carbamate (2.90 g, 6.00 mmol) was dissolved in EtOH (99.5%, 13 mL) and benzoyl isothiocyanate (0.96 mL, 7.14 mmol) was added. The reaction mixture was stirred at rt overnight whereupon more benzoyl isothiocyanate (0.4 mL, 0.5eq) was added. Stirred at rt for 1 h. Added more benzoyl isothiocyanate (0.4 mL, 0.5eq) and stirred for 1 h. Added 1M aq NaOH solution (17.8 mL, 17.8 mmol) and heated in an oil-bath at 78°C for 6 h. Extracted with EtOAc. The organic phase was washed with 10% aq citric acid solution and several times with water. Purified by automated flash chromatography using a gradient of 0–75% DCM/MeOH (93/7) in DCM to yield the title compound (1.04 g, 33%). LC-MS (ESI) *m/z*: 534.4 (calcd for $C_{24}H_{30}ClN_5O_5S$ $[M-H]^-$, 534.2).

Step 3. (R)-3-(2-(1-aminoethyl)-4-chlorobenzyl)-2-thioxo-2,3-dihydro-1H-purin-6(7H)-one acetate (18).

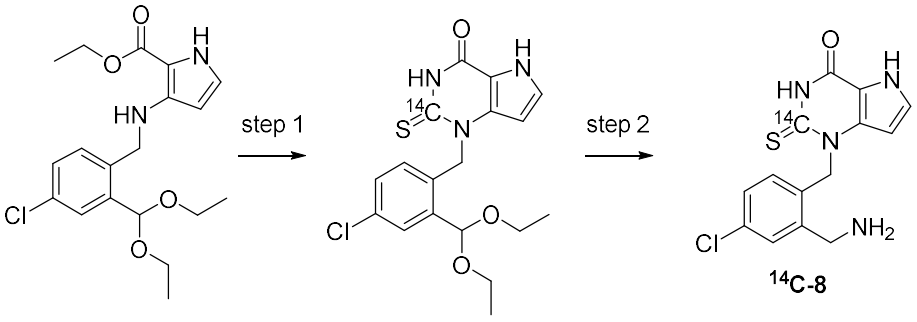
tert-Butyl N-*tert*-butoxycarbonyl-N-[(1R)-1-[5-chloro-2-[(6-oxo-2-thioxo-7H-purin-3-yl)methyl]phenyl]ethyl]carbamate (1.04 g, 1.94 mmol) was treated with a MeOH solution of HCl (1.25 M, 10.8 mL, 13.6 mmol). The mixture was stirred at 50°C for 1 h. Cooled in an ice-bath and water (3.7 mL) was added. Followed by slow addition of 25% NH_3 to pH 8. Filtered off a brown precipitate, 0.231 g. Purified by preparative HPLC on a Kromasil C8 column (10 µm 250x50 ID mm) using a gradient of 0–17% MeCN in H_2O/MeCN/HOAc 95/5/0.2 buffer over 20 min with a flow of 100 mL/min (the run started with a 'trappingstep' at 0% over 7 min) to yield **18** (0.069 g, 9%) as a white solid. $[\alpha]_D^{20}$: +188.8 (c 0.5, MeOH). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.59 (s, 1H), 7.53 (s, 1H), 7.26 (d, *J* = 8.4 Hz, 1H), 7.12 (d, *J* = 8.5 Hz, 1H), 5.79 (dd, *J* = 183.7, 15.4 Hz, 2H), 4.85 (q, *J* = 6.6 Hz, 1H), 1.51 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ 172.7, 154.8, 149.7, 144.8, 141.8, 133.5, 132.1, 128.5, 127.8, 125.7, 116.6, 47.0, 45.3, 21.2. HRMS (ESI) m/z: 336.0671 (calcd for $C_{14}H_{14}ClN_5OS$ $[M+H]^+$, 336.0686).

Synthesis of ^{14}C-labelled benzoyl isothiocyanate.



$K^{14}CN$ (0.035 g, 0.52 mmol, 1000 MBq) and sulfur (0.018 g, 0.57 mmol) were suspended in acetone (2 mL). The mixture was heated at 110°C for 90 min in a microwave reactor. The reaction mixture was concentrated under a stream of nitrogen. The crude product was dissolved in MeCN (3 mL) and benzoyl chloride (0.054 mL, 0.46 mmol) was added. Heated at 70°C for 1 h in a microwave reactor. The crude reaction mixture was used in the next step.

Synthesis of ^{14}C-labeled 1-(2-(aminomethyl)-4-chlorobenzyl)-2-thioxo-1,2,3,5-tetrahydro-4H-pyrrolo[3,2-d]pyrimidin-4-one (^{14}C-8).



Step 1. ^{14}C-labeled 1-(4-chloro-2-(diethoxymethyl)benzyl)-2-thioxo-1,2,3,5-tetrahydro-4*H*-pyrrolo[3,2-d]pyrimidin-4-one.

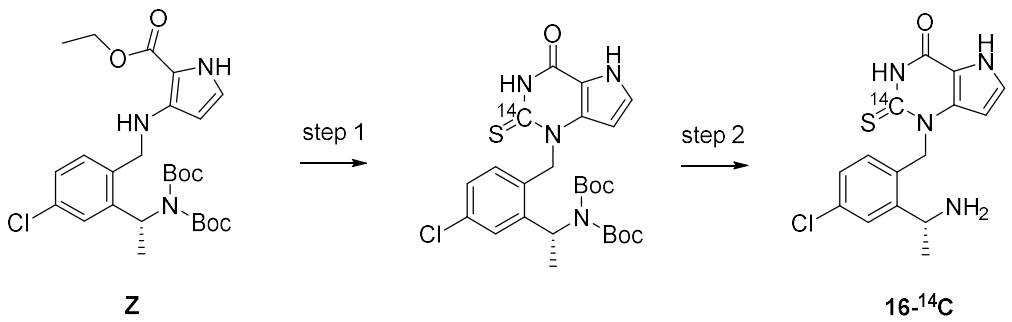
A solution of ethyl 3-((4-chloro-2-(diethoxymethyl)benzyl)amino)-1*H*-pyrrole-2-carboxylate (0.425 g, 1.12 mmol) (see step 3, compound **14**) in MeCN (2 mL) was added to a solution of ^{14}C benzoyl isothiocyanate (0.93 mmol) dissolved in 2 mL MeCN. The resulting mixture was stirred at ambient temperature for 2 h. The solvents were removed under a stream of nitrogen and the residue was purified by flash chromatography (0% to 100% of EtOAc in heptane). The residue was dissolved in MeOH (10 mL) and cesium carbonate (0.761 g, 2.33 mmol) was added, stirred at 50°C for 8 h. The reaction mixture was concentrated under a stream of nitrogen, the residue was diluted with water

and extracted with DCM. The aqueous phase was neutralized with HOAc and extracted further with DCM. The combined organic solutions were dried using a phase separator and the solvents were removed under reduced pressure. The residue was purified by flash chromatography (0–75% EtOAc in heptane) to give the title compound (288 mg, 0.728 mmol, 1560 MBq, 78%). Identity was confirmed by coelution with reference substance. LC-MS (ESI) m/z: 394.0 (calcd for [^{14}C]C17H20ClN3O3S [M-H]$^-$ 394.9).

Step 2. ^{14}C-labeled 1-(2-(aminomethyl)-4-chlorobenzyl)-2-thioxo-1,2,3,5-tetrahydro-4H-pyrrolo[3,2-d]pyrimidin-4-one (^{14}C-8).

TFA (0.049 mL, 0.65 mmol) was added to a solution of 1-(4-chloro-2-(diethoxymethyl)benzyl)-2-thioxo-1,2,3,5-tetrahydro-4H-pyrrolo[3,2-d]pyrimidin-4-one (0.026 g, 0.07 mmol, 140MBq) in DCM (2 mL). Stirred at ambient temperature for 10 min. The reaction mixture was concentrated under a stream of nitrogen and the residue was dissolved in HOAc (1 mL) and NMP (2 mL). Hydroxylamine hydrochloride (11 µL, 0.26 mmol) and zinc (0.043 g, 0.65 mmol) were added and the reaction mixture was stirred at 50°C for 15 h. Zinc (0.086 g, 1.3 mmol) was added and the reaction mixture was stirred at 50°C for 80 h. The reaction mixture was filtered, the solvents were removed under reduced pressure and the residue was purified by preparative HPLC (Kromasil C8, 10 µm 100x20 ID mm, 0-25% MeCN in water/MeCN/HOAc 95/5/0.5 buffer) to give the title compound (8 mg, 0.025 mmol, 56 MBq, 40%). Identity was confirmed by coelution with reference substance. LC-MS (ESI) m/z: 321.1 (calcd for [^{14}C]$C_{13}H_{13}ClN_4OS$ [M-H]$^-$ 321.8).

Synthesis of ^{14}C-labeled (R)-1-(2-(1-aminoethyl)-4-chlorobenzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (^{14}C-16).



Z 16-^{14}C

Step 1. ^{14}C labeled di-tert-butyl [(1R)-1-{5-chloro-2-[(4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-1-yl)methyl]phenyl}ethyl]imidodicarbonate.

A solution of ethyl 3-[(2-{(1R)-1-[bis(tert-butoxycarbonyl)amino]ethyl}-4-chlorobenzyl)amino]-1H-pyrrole-2-carboxylate (intermediate **Z**, see Scheme 2 in the main text) (0.312 g, 0.60 mmol) in MeCN (1 mL) was added to a solution of ^{14}C benzoyl isothiocyanate (0.46 mmol) dissolved in 3 mL MeCN. The mixture was stirred at ambient temperature overnight. The solvents were removed under reduced pressure and the residue was purified by flash chromatography (gradient, 0–3% MeOH in DCM). The residue was dissolved in MeOH (10 mL). Cesium carbonate (300 mg, 0.92 mmol) was added and the reaction mixture was heated to 65°C for 1 h in a microwave reactor. The reaction mixture was concentrated under a stream of nitrogen and the residue was diluted with water and acidified with HOAc (100 µL) and extracted with DCM. The solvents were removed under reduced pressure and the residue was purified by preparative HPLC (Kromasil C8,10 µm, 250x20 ID mm, 10–90% MeCN in water/MeCN/HOAc 95/5/0.5) to give the title compound (155 mg, 0.29 mmol, 555 MBq, 62%). Identity was confirmed by coelution with reference substance. LC-MS (ESI) *m/z*: 535.3 (calcd for $[^{14}C]C_{24}H_{31}ClN_4O_5S$ $[M-H]^-$, 536.0).

Step 2. ^{14}C labeled (R)-1-(2-(1-aminoethyl)-4-chlorobenzyl)-2-thioxo-2,3-dihydro-1H-pyrrolo[3,2-d]pyrimidin-4(5H)-one (^{14}C-16).

^{14}C labeled di-tert-butyl [(1R)-1-{5-chloro-2-[(4-oxo-2-thioxo-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-1-yl)methyl]phenyl}ethyl]imidodicarbonate (155 mg, 0.29 mmol, 555 MBq) was dissolved in HCl (1.25M) in MeOH (10 mL). The resulting mixture was stirred at rt for 2 days, then heated at 60°C for 30 min. The solvent was removed under a stream of nitrogen and the residue was purified by preparative HPLC (XBridge C18, 10 µm, 100x19 ID mm, 0-20% MeCN in water/MeCN/HOAc 95/5/0.5) to give the title compound (48 mg, 0.14 mmol, 275 MBq, 50%). LC-MS (ESI) *m/z*: 337.1 (calcd for $[^{14}C]C_{14}H_{15}ClN_4OS$ $[M+H]^+$ 337.8).

3. Physicochemical assays

Octanol/water partitioning

Partitioning of compounds between 1-octanol and 0.1 M phosphate buffer, pH 7.4, at 20°C were determined using a modified version of the shake-flask method described by Leo *et al.*[1] Compounds were dissolved, in a 96-well plate, in 400 µL octanol, and 400 µL of buffer was added to each well. The plate was vigorously stirred for 5 min and then put on an Edmund Bühler shaker (Edmund Bühler GmbH, Hechingen, Germany) for 18 h at 20°C. Aliquots of 5 µL octanol were transferred and diluted with 495 µL MeCN/H_2O (1/1) and, to avoid contamination of the buffer, the rest of the octanol was removed before 150 µL of buffer samples were transferred. Octanol and buffer samples were diluted with MeCN/H_2O (1/1) in four steps of 10 times to yield octanol samples diluted 100 to 1000000 times and buffer samples diluted 1 to 10000 times. LC/MS/MS was used for analysis, and distribution coefficient at pH 7.4 ($LogD_{7.4}$) was calculated from the integrated peak areas of the samples in the linear MS response range. $LogD_{7.4}$ results were reported as median values. CIR was 1.41 (on the original non-log scale) based on the mean of compounds evaluated at three occasions and the MDD was 0.21.

Solubility

This method measures the thermodynamic solubility of research compounds based on a shake-flask approach.[2] The nature of the solid state was unknown in these experiments and may be subject to variability. Test compounds dissolved in DMSO, 30 µL 10 mM, in glass vials were dried using a Genevac vacuum evaporator (Genevac Ltd, Ipswich, UK). When samples were dry, 300 µL 0.1 M phosphate buffer, pH 7.4, was added to the glass vials. The vials were put on an Edmund Bühler shaker (Edmund Bühler GmbH, Hechingen, Germany) for 18 h at 20°C. Samples were filtered through a Whatman GF/B 96-well filter and 20 µL of filtrated samples were transferred to separate wells in a plate containing 180 µL MeCN/H_2O (1/1). Standards were prepared by diluting the 10 mM compound solutions with MeCN/H_2O (1/1) to 200 µM. Three further dilution steps of 10

times were applied to both the samples and standards and they were all analyzed by LC-MS/MS. Solubility was determined using the integrated peak areas of the samples in the linear MS response range.

Single crystal X-ray structure of the mesylate salt of compound 16 (AZD4831)

Single, clear colorless rod-shaped crystals of a mono-mesylate salt of compound **16** (AZD4831) were re-crystallized from a mixture of ethanol and DCM by slow evaporation. A suitable crystal (0.21×0.03×0.01) was selected and mounted on a MITIGEN holder in perfluoro ether oil on a Rigaku AFC11 007-HF diffractometer. The crystal was kept at T = 100(2) K during data collection. Using Olex2[3] the structure was solved with the ShelXS structure solution program,[4] using the Direct Methods solution method. The model was refined with version of ShelXL[4] using least-squares minimization.

All non-hydrogen atoms were refined anisotropically. Hydrogen atom positions were calculated geometrically and refined using the riding model. N-H hydrogens were clearly identified from peaks in the difference map and subsequently placed in calculated positions and refined using a riding model. The NH_3 hydrogens were clearly identified for N101 from the difference map and subsequently placed in calculated positions and refined using a riding model, the difference peaks around N201 were less clear, but this was treated in the same way. The partial occupancy of the major DCM component was freely refined and then fixed at the refined value of 0.6.Remaining electron density peaks around the DCM were fitted with two additional DCM fragments with occupancies fixed to 0.1. These were treated as rigid bodies and thermal parameters of all DCM atoms were constrained to be the same (**Figure S1**).

There are two molecules of interest in the asymmetric unit; both have chirality R at C102 and C202. As refined in P2(1) the crystal has a pseudo center of inversion, however, this is broken by a) the

chiral center and b) the orientation of the 2-amino ethyl group with respect to the phenyl ring (which is slightly different in the two molecules).

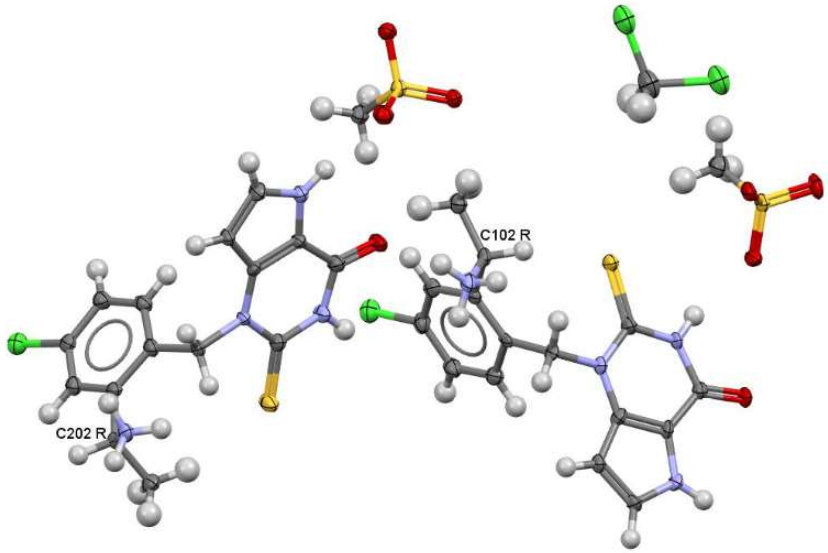


Figure S1. Thermal ellipsoid drawing of compound 16 (AZD4831) drawn at 50% probability level.

The disorder of the minor DCM components has been omitted for clarity.

Crystal Data

C16.4H19.8Cl1.8N4O4S2, Mr = 464.89, monoclinic, P21 (No. 4), a = 12.9240(17) Å, b = 8.8090(9) Å, c = 19.1706(19) Å, b = 105.8300(10)°, a = g = 90°, V = 2099.8(4) Å3, T = 100(2) K, Z = 4, Z' = 2, m(CuKa) = 4.680, 28662 reflections measured, 6384 unique (Rint = 0.0899) which were used in all calculations. The final wR2 was 0.1340 (all data) and R1 was 0.0539 (I > 2(I)) and GooF 1.026.

The crystal structures have been deposited in the Cambridge Structural Database, including structure files and fractional atomic coordinates, isotropic and anisotropic displacement parameters, bond lengths and bond angles (CBD references 2127753–2127754).

4. *In vitro* pharmacology

Assessment of inhibition (IC_{50})

Experiments were performed in phosphate-buffered saline (PBS), pH7.4. A 10 mM luminol (A4695, Sigma Aldrich, St Louis, MO, USA) stock was prepared in water and diluted in PBS to a final concentration of 100 µM. H_2O_2 was prepared as 1 mM stock in PBS, yielding a final concentration of 50 µM after addition into the assay. Myeloperoxidase (MPO) inhibitors were serially diluted in DMSO in a separate plate as a 100x stock solution. MPO (purified from HL60 cells), eosinophil peroxidase (EPO; Cell Sciences, Newburyport, MA, USA) and thyroid peroxidase (TPO; produced in insect cells; RSR Ltd, Cardiff, UK), were diluted to yield approximately 5600, 2300 and 9300 light counts per seconds (LCPS) upon incubation with luminol, respectively

The experiment was run by pipetting 2 µL 100x stock solution of inhibitor and 200 µL diluted enzyme in PBS into wells in a 96-well Optiplate (6005290, Perkin Elmer /Thermo Fischer, Waltham, MS, USA), followed by addition of 10 µL H_2O_2 containing PBS. Chemiluminescence measurement (Perkin Elmer Wallac Microbeta Trilux 1450-029 (12-detector), Turkuu, Finland) was started directly and recorded after 2, 10 and 15 minutes. Chemiluminescence recorded after 15 minutes was used to calculate the IC_{50} values.

Assessment of irreversible binding

Anti-MPO coated ELISA strips (BioLegend, San Diego, CA, USA; 440007) were spray painted white on the outside to avoid cross-well light contamination. Purified MPO (350 ng/mL) was incubated in Biolegend assay buffer B in the painted ELISA strips for 1 hour at room temperature. The strips were then washed three times using 300 µL MEBSS buffer and serially diluted MPO inhibitor was added as a 100× stock in DMSO. MPO was activated with H_2O_2 (50 µmol/L) in luminol-containing MEBSS buffer and chemiluminescence was measured after 2 min. The strips then were washed four times with 300 µL MEBSS buffer. New luminol-MEBSS buffer was subsequently added and the MPO was reactivated by addition of 50 µmol/L H_2O_2, and the chemiluminescence measured after an

additional 2 min. Irreversibility of EPO inhibtion was assessed similarly, but using 0.8 µg/mL EPO immobilized on anti-EPO ELISA strips (Biomatik, Kitchener, ON, Canada; EKE54700-96T). Results with MPO are shown in **Figure S2**.

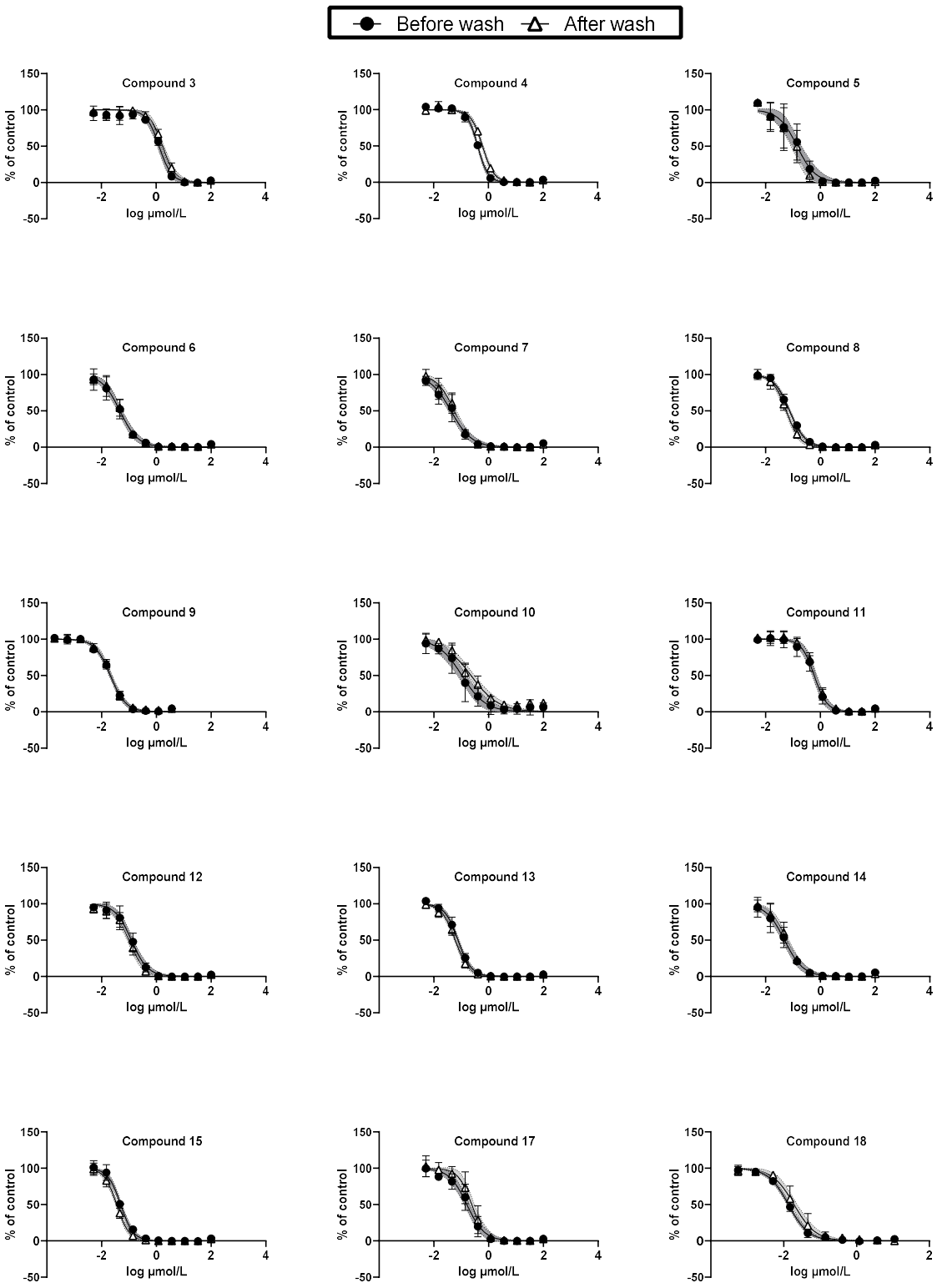


Figure S2: Assessment of irreversible binding of the additional compounds in Table 1.

The figure shows the concentration–response curves achieved on immobilized myeloperoxidase in the presence of the indicated concentration of the inhibitor (black circles) and after repeated washing and reactivation without addition of new inhibitor (white triangles). Each data point represents the mean ± SD of triplicate wells and the shaded areas represent the 95% confidence intervals for the fitted curves.

Assessment of cell intragranular potency

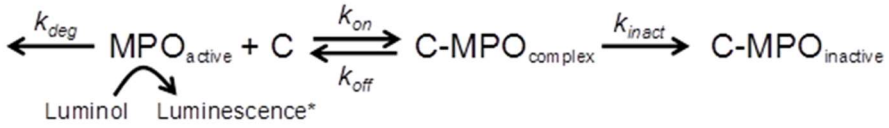
The promyeloid HL60 cell line (ATCC, Manassas, VA, USA) was differentiated into neutrophils using 1% DMSO in RPMI 1640 medium supplemented with 10% fetal calf serum for 6 days.[5] The cells were washed with Krebs-Ringer buffer supplemented with glucose and seeded at 2×10^5 cells/well in 96-well Optiplate plates. Cells were stimulated in the same buffer supplemented with 100 µmol/L luminol by addition of 2 mg/mL zymosan (Z4250, Sigma Aldrich, St Louis, MO, USA) and titrated concentrations of MPO inhibitors. Chemiluminescence was monitored over time using the microbeta counter described above and the potency of the inhibition was calculated from the 1-h value (at which the chemiluminescence peaked, data not shown).

Determination of rate constants

To determine the binding and inactivation rate constants, 0.16 nM human MPO monomer was co-incubated with titrated concentrations of compound **16** (AZD4831) as described above and the chemiluminescence data was recorded every 30 s. In addition, compound **16** was also co-incubated with a higher human MPO monomer concentration of 2.4 nM. The rate constants k_{on}, k_{off}, and k_{inact} were determined by simultaneously fitting a receptor kinetics model including an irreversible inactivation of the drug–enzyme complex to the cumulative chemiluminescence data generated for compound **16** (Equation 1).

When no drug was added to the incubations, a slight reduction in the MPO activity was still observed over time. It was anticipated that the oxidative environment per se influenced the enzyme activity. The reduced MPO activity was accounted for in the model by including a degradation rate constant k_{deg}. The relation between active MPO and the chemiluminescence produced was assumed to be linear. The receptor kinetics modeling was made in Phoenix 6.4 (Pharsight, CA) using the naïve pool approach.

Equation 1.



The reversible dissociation constant K_d was calculated by Equation 2.

Equation 2.

$$K_d = \frac{k_{off}}{k_{on}}$$

5. Estimation of therapeutic dose of compound 16 (AZD4831)

A serial dilution of compound **16** was spiked into the MPO enzyme incubations at final concentrations of 1000 to 0.017 nM (threefold dilution steps). Two studies were performed at different MPO concentrations (0.1 nM and 1.5 nM). The monomeric MPO enzyme were activated by addition of H_2O_2 and the drug induced suppression of the luminol-induced luminescence was monitored every 20th second for 915 s.

An enzyme kinetics model with a reversable binding of **16** to MPO and with an irreversible inactivation of the drug–enzyme complex (Equation 1) were simultaneously fit to the cumulative luminescence generated in the two studies (Equations 3–6).

Equations 3–6.

$$\frac{dMPO_{active}}{dt} = -k_{deg} \cdot MPO_{active} - k_{on} \cdot MPO_{active} + k_{off} \cdot C\text{-}MPO_{complex}$$

$$\frac{dC\text{-}MPO_{complex}}{dt} = k_{on} \cdot MPO_{active} - \left(k_{off} + k_{inact}\right) \cdot C\text{-}MPO_{complex}$$

$$\frac{dC\text{-}MPO_{inactive}}{dt} = k_{inact} \cdot C\text{-}MPO_{complex}$$

$$\frac{dC}{dt} = -k_{on} \cdot C + k_{off} \cdot C\text{-}MPO_{complex}$$

k_{on} is the second order binding rate constant, k_{off} is the first order dissociation rate constant and k_{inact} is the first order inactivation rate constant. The monomeric myeloperoxidase and drug concentrations added to the incubations were used as initial conditions for MPO_{active} and C, respectively. MPO, myeloperoxidase.

Without the inhibitor present, a slight decrease in the cumulative luminescence was observed over time. As the assay was run using a great access of H_2O_2 and luminol compared with MPO, substrate depletion is not expected. The high H_2O_2 concentration may though generate radicals that influence the enzyme activity. The reduced MPO activity was accounted for in the model by including a degradation rate constant k_{deg} which affected the active MPO concentration.

The receptor kinetics modeling was performed in Phoenix 6.2 (Centara, CA) using a naïve pool approach with a log-additive error model for the residual. The model fit and the calculated parameter estimates are shown in Figure S3 and Table S1, respectively.

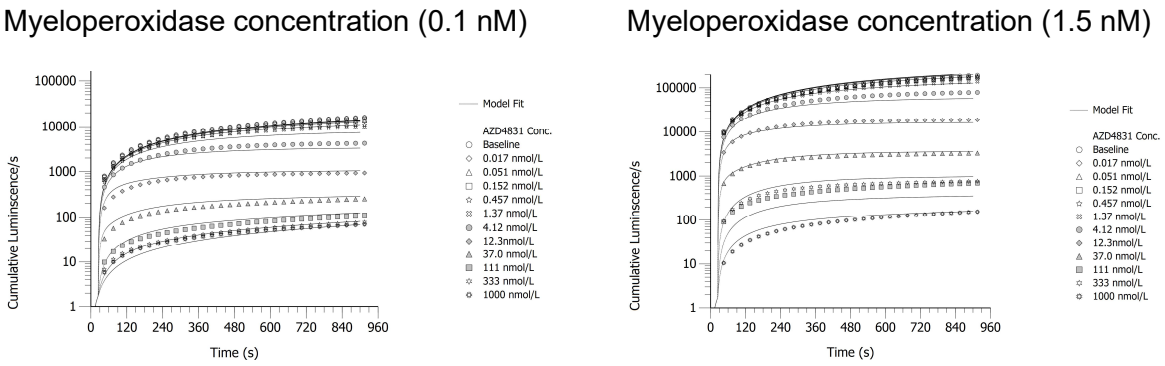


Figure S3. Model fit to observed cumulative luminescence.

Parameter	Estimate	CV%	Comment
k_{on} (nM^{-1} h^{-1})	7.8	3	Binding rate of AZD4831 to active MPO
k_{off} (h^{-1})	11	7	Dissociation rate or inhibitor from active MPO
k_{inact} (h^{-1})	17	7	Inactivation rate of drug-MPO complex
k_{deg} (h^{-1})	3.9	9	Degradation rate of MPO in assay
k_{lum} (h^{-1})	920000	3	Rate of MPO catalyzed luminol metabolism
back (nM^{-1} h^{-1})	260	2	Background luminescence
Eps	0.21	2	Residual error – Log additive weight

Table S1. Parameter estimates

MPO, myeloperoxidase

Binding and inactivation rate constants were determined by fitting a kinetic model to the chemiluminescence data. The dissociation constant ($K_d = k_{off}/k_{on}$) for compound **16** was estimated to be 1.4 nM and the inactivation and degradation rate constants were estimated to be 17 h^{-1} and 3.9 h^{-1}, respectively.

Furthermore we assumed that the assay conditions mimic local extracellular MPO activity in inflamed tissues and that the unbound drug concentration in plasma is in rapid equilibrium at the site of inflammation. The unbound concentration of AZD4831 (**16**) required to generate 80% inhibition *in vivo* ($IC_{80,unbound}$) was calculated according to Equation 7.

Equation 7.

$$IC_{80,unbound} = \frac{k_{out}}{k_{irr}} \cdot \left(\frac{1}{0.2} - 1\right)$$

where k_{out} is the estimated fractional turnover rate of active MPO *in vivo,* 0.2 is the fraction of remaining MPO activity after inhibition, and k_{irr} is a composite parameter determined by k_{on}, k_{off}, k_{inact} and k_{out} according to the relationship in Equation 8.

Equation 8.

$$k_{irr} = k_{on} \cdot \left(\frac{k_{off}}{k_{off} + k_{out} + k_{inact}} \right)$$

Of these parameters, k_{out} was associated with the greatest uncertainty. Building on the assumption that *in vitro* assay conditions mimic those *in vivo,* modeling of k_{out} (data not shown) and the estimated degradation rate constant in the assay (k_{deg} = 0.065 min^{-1}); k_{out} *in vivo* was estimated to 0.27 min$^{-1.}$ As a result, $IC_{80,unbound}$ was estimated to be 11 nM,. This corresponds to a total plasma concentration of 31 nM ($f_{u,human}$ = 34.9%) which was used as targeted trough concentration for once-daily dosing in humans.

Using different scaling approaches, we next predicted the human dose based on the target trough concentration. For volume prediction (V), the average value (5.9 L/kg) of the Øie-Tozer model[6] and allometric scaling was used. For plasma clearance (CL_p) prediction, allometric scaling predicted a lower clearance than *in vitro-in vivo* extrapolation[7] and the hepatic liver blood flow methods (2.9 vs 5.0 and 6.1 mL/min/kg, respectively).[8] Because the slope of the allometric plot for clearance was shallower than normal (0.55 vs 0.7), we opted to use the more conservative hepatic blood flow method for clearance prediction to avoid underestimation.[9] We simulated the human concentration–time profile of AZD4831 (**16**) using a one-compartment method, where the absorption rate constant (k_a) was set to 1 h^{-1} and an oral bioavailability (F) of 69% was calculated as the product of fraction absorbed (f_a = 0.9) and the first pass extraction through the liver ($F = f_a \cdot (1-Cl_b/Q_H)$). Human blood clearance (CL_b) was calculated by correcting the predicted plasma clearance for the blood/plasma partition ratio (C_b/C_p = 1.32) and setting the hepatic blood flow (Q_H) was to 20 mL/min/kg.[10]

A once-daily dose of 20 mg was predicted to generate a total plasma drug concentration of 31 nM at the end of each dosing interval (i.e. unbound drug concentrations of 11 nM, $IC_{80,unbound}$). Based on the longer half-life observed in humans, these concentrations were reached at lower doses, with 2.5

mg in patients with HFpEF and 5 mg in healthy volunteers yielding approximately 50% MPO inhibition in an ex vivo neutrophil activation assay. [11-13]

6. *In vivo* pharmacology

Zymosan-induced peritonitis in the mouse

Female BALB/c mice (BALB/cAnNCr, Charles River Labs, Sulzfeld, Germany) weighing 18–20 g, were allocated to Macrolon L3 cages with wood shavings, n=7/cage, and housed in a 12-h light/dark cycle at 50% humidity and 21°C at AstraZeneca Gothenburg, Sweden. The experiment was initiated following acclimatization for a week by intraperitoneal injection of 1 mL 4% (w/v) thioglycollate broth (B2551, Sigma Aldrich, St Louis, MO, USA), followed by an intraperitoneal injection with 7 mg/kg zymosan (Z4250, Sigma Aldrich, St Louis, MO, USA) approximately 20 h later. The mice were per orally gavaged with vehicle (0.5% hydroxypropylmethylcellulose 15000 cps in purified water, Shin-Etsu chemical company, Houston, TX, USA) or with formulations of compound **6** (AZD4831) at 0.01, 0.1, 1 or 10 µmol/kg, 2 h before the administration of zymosan and terminated 2 h after zymosan injection.

Mice were anesthetized with isoflurane and blood was collected by cardiac puncture using 1 mL syringes with 23G needles preaspirated in PBS in heparin and transferred to Heparin tubes (450478, Greiner, Kremsmunster Oberösterreich, Austria) that were centrifuged, 2800 rpm for 10 min at +8 degrees to separate out the plasma. The peritoneal cavity was rinsed with 2 mL of ice-cold PBS using a plastic Pasteur pipette, and 1.5 mL lavage was removed and centrifuged at 3500 rpm at rt for 5 min to separate cells from the lavage. Hemolysis in samples was determined visually and hemolytic samples excluded as the hemolysis results in false positive luminescence due to a reaction with hemoglobin.

The peroxidase activity in the centrifuged lavage was determined by mixing 15 µL lavage with 10 µL 1.5 mmol/L H_2O_2 and 200 µL MEBSS buffer containing 100 µmol/L luminol. Luminescence was read 4 times every 4 min for 12 s in a microbeta counter. The data from the 12 min time point (cycle 3)

were used in the current experiments as this time point reflects maximum chemiluminescence. Data are expressed as LPS (light per second).

To quantitate compound **16** (AZD4831) 20 µL plasma samples were treated with acetonitrile to precipitate protein followed by ultra performance liquid chromatography (UPLC) and tandem mass spectrometry (UPLC-MS/MS). Precipitation was obtained by addition of 180 µL acetonitrile (containing internal standard) followed by mixing and centrifugation. The supernatant was diluted 1:1 with 0.2% formic acid and injected on an ACQUITY UPLC HSS T3 1.8 µm 2.1x50 mm column and eluted using a gradient mobile-phase profile (mobile phase A: 0.2% formic acid in water, mobile phase B: 0.2% formic acid in acetonitrile) and analytes were detected by multiple reaction monitoring (MRM). The lower limit of quantification (LLOQ) was 1 nM. Results are shown in **Table S2**.

The animal experiment was approved by the local ethics committee in Gothenburg (ethical application 74-2011).

Table S2. AZD4831 plasma concentration after peroral gavage in mice

Dose of AZD4831 (µmol/kg)	n	AZD4831 total plasma concentration 4h after peroral gavage (nM, mean±SD)
0.01	7	<LLOQ
0.1	7	1.7±0.57
1	7	15±3.5
10	7	176±44

LLOQ, lower limit of quantification

7. K_d measurement by NMR

One-dimensional competition-based ligand observed ^{1}H NMR experiments was used to determine the affinity of thioxanthine compounds to native MPO. A weak MPO ligand, BHA (benzhydroxamic

acid), was used as a reporter.[14] In the presence of the protein (2–3.5 μM), the signal from the reporter is reduced owing to chemical exchange between the free and protein bound form. The concentration of the compound of interest, binding to the same site, is then gradually increased resulting in displacement of the reporter and recovery of its signal. The K_d for BHA has previously been reported as 5 mM.[15] The K_d of the compound of interest is determined by an equilibrium analysis. A TECAN Genesis (TECAN INC, Zurich, Switzerland) liquid handling robot, connected via a Bruker SampleRail (Bruker Biospin AG, Switzerland) system to the spectrometer, was used to prepare samples just prior to the NMR measurements. An aq buffer with 50 mM sodium phosphate, pH 7.5, 100 mM sodium chloride and 10% D_2O was used. The reporter concentration was 50 μM and the protein concentration was chosen to give a 40% decrease of the reporter signal. The thioxanthine compounds, dissolved to 10 mM in DMSO, were titrated to the NMR sample in 6 steps, starting from 1 μM and ending at 225 μM. One-dimensional ^{1}H Carr-Purcell-Meiboom-Gill (CPMG) NMR spectra were acquired at 298 K with 256 scans and 3 s relaxation delay on a Bruker DRX 800 MHz spectrometer with a triple resonance inverse cryoprobe. The CPMG spin lock time was 200 ms and the water and DMSO signals were suppressed by excitation sculpting with 3 ms biselective sinc-shaped 180 degree flip back pulses. The NMR data was processed, and baseline corrected and the integral of the benzene reporter peak at 7.71 ppm was obtained, using the Topspin 3.5 software (Bruker Biospin AG, Switzerland). The recovery of the reporter signal, assumed to correlate linearly with the fraction of reporter bound to the native MPO, was analyzed according to Dalvit.[14] K_ds of the thioxanthine compounds were obtained using Excel and the Solver add-in.

8. X-ray crystal structures of myeloperoxidase ground state complexes

MPO was crystallized by mixing 1 μL of a 10 mg/ml protein solution with an equal volume of a well solution containing 18% PEG3350 and 0.1 M sodium chloride. The drop was allowed to equilibrate over a reservoir containing well solution. Crystals formed within a day. Compounds were introduced by transferring the crystal to a synthetic mother liquor consisting of the protein buffer components and mother liquor supplemented with 2 mM compound from a DMSO stock. The final DMSO

concentration was less than 2%. Prior to data collection the drops containing crystals were supplied with glycerol as a cryoprotectant. The crystals were then quickly removed from the drop and flash-cooled in liquid nitrogen. Data were collected at beam line ID23-1 and ID23-2, at ESRF, Grenoble, France. The data were processed using autoPROC.[16] Initial phasing was done by molecular replacement using a high resolution ligand free structure of MPO (PDB id code 1cxp) as a starting model.[17] Model rebuilding was performed within Coot and refinement was performed using autoBUSTER.[18, 19] For statistics and details about the structure refinement see **Table S3**. The coordinates have been deposited at the PDB (7NI1 and 7NI3) and will be made accessible upon publication of this manuscript.

Table S3. Data collection and refinement statistics.

	MPO in complex with **3** PDB: 7NI3	MPO in complex with **9** PDB: 7NI1
Data collection		
Space group	$P2_1$	$P2_1$
Cell dimensions		
a,*b*, *c* (Å)	92.66, 63.64, 111.103	93.26, 63.84, 111.39
α,β,γ (°)	90.00, 97.15, 90.00	90.00, 97.25, 90.00
Resolution (Å)	37.26-2.1 (2.15-2.1)**	48.8-2.1 (2.16-2.1)
R_{merge}	10.1(39.9)	17.0(55.2)
$I/\sigma I$	9.8 (2.9)	9.4(2.7)
Completeness (%)	83.4(98.6)	98.2 (92.0)
Redundancy	2.6 (2.2)	4.7 (4.4)73796
Refinement		
Resolution (Å)	30.61-2.10	46.24-2.11
No. reflections	56547	73796
R_{work}/ R_{free}	17.5 (22.4)	21.7 (25.6)
No. atoms		
Protein	9100	9096
Heterogen	317	359
Water	796	644
B-factors		
Protein	16.3	23.6
Ligand	38.3	28.8
Water	20.2	23.9
R.m.s deviations		
Bond lengths (Å)	0.010	0.010
Bond angles (°)	1.04	1.04

**Highest resolution shell is shown in parenthesis. MPO, myeloperoxidase.

9. Drug metabolism and pharmacokinetic assays

Human liver microsome metabolic stability

Human liver microsomes were defrosted on ice and diluted to 1 mg/mL of microsomal protein in 0.1 M phosphate buffer, pH 7.4. Compounds at 1 µM were incubated with the liver microsome suspensions and 1 mM NADPH at 37°C in a 96-well plate. At 0.5, 5, 10, 15, 20 and 30 min, aliquots of 30 µL were transferred to a 96-well plate containing 120 µL acetonitrile. This plate was centrifuged for 20 min and supernatant was removed and diluted (1/1) with water before analysis by LC-MS/MS. Peak areas were determined from extracted ion chromatograms, and the *in vitro* intrinsic clearance (*in vitro* CL_{int}, in µL/min/mg microsomal protein) of parent compound was calculated from the slope in the regression analysis of the natural logarithm of parent concentration vs time curve.

Cyanide trapping of reactive metabolites

The experimental setup for cyanide trapping of reactive metabolites in human liver microsomes (0.5 mg protein/mL) was the same as for metabolic stability with the following modifications. The final concentration of the tested compound was 10 µM, the potassium cyanide concentration was 3.3 mM and the NADPH concentration was 0.66 mM. All incubations were performed in 0.1 M potassium phosphate buffer pH 7.4 at 37°C for 30 min. The incubations were terminated by the addition of two volumes of cold acetonitrile (containing 0.2% formic acid) to the incubation mixture. The samples were centrifuged (Eppendorf Centrifuge 5810R) for 10 minutes at 4°C at 2900 *g*. The samples were analyzed by ultra-performance liquid chromatography (Waters ACQUITY UPLC, Milford, MA, USA) coupled to a Waters Quattro Premier TOF instrument (Wythenshawe, UK) equipped with an electrospray interface. The software used to process the data was MetaboLynx (Waters). Product ion spectra of major metabolites were acquired to allow interpretation and structural assignments.

Metabolic stability in human and male Han Wistar rat hepatocytes

Hepatocyte metabolic stability was determined in accordance with the method described by Jacobson *et al.*[20] Cryopreserved hepatocytes at a concentration of 10^6 viable cells/mL were used. After thawing, hepatocytes were incubated for 10 min to warm to 37°C and test compounds, dissolved in acetonitrile, were added to give a final concentration of 1 µM. At 0.5, 5, 15, 30, 45, 60, 80, 100 and 120 min, the incubation system was mixed and 20 µL aliquots were transferred at each time point to wells in a separate plate filled with 80 µL MeCN to stop the reaction. The quenching plate was then vortexed followed by centrifugation, and supernatants were analyzed by LC-MS/MS. Peak areas were determined from extracted ion chromatograms, and the *in vitro* intrinsic clearance (*in vitro* CL_{int}, in µL/min/10^6 cells) of parent compound was calculated from the slope in the regression analysis of the natural logarithm of parent concentration vs time curve.

Fraction unbound in rat hepatocyte incubation

Equilibrium dialysis of compounds was used to assess the fraction unbound in rat hepatocyte incubations. Hepatocytes at a concentration of 10^6 viable cells/mL were incubated at 37°C for 1 h with the cytochrome P450 inhibitor 1-aminobenzotriazole (1-ABT) at 1 mM. salicylamide was added to yield a final concentration of 1.5 mM and the solution was incubated for another 5 min. Then, test compounds were added to give a concentration of 1 µM and, after mixing, the solution was transferred to the equilibrium dialysis device (RED, Thermo Fischer Scientific Inc., Rockford, USA) and dialyzed against media buffer for 4 h at 37°C. Calibration curves ranging from 1 to 1000 nM were made using aliquots of the 1 µM spiked hepatocyte suspensions that were transferred to a new plate followed by serial dilutions with blank hepatocyte suspensions. After dialysis, samples from the dialysis cells containing hepatocyte suspension were diluted with media buffer and the media buffer samples were diluted with blank hepatocyte suspension. Calibration curve samples and dialysis samples were precipitated with MeCN and after centrifugation, supernatants were removed and analyzed using LC/MS/MS. Fraction unbound in the incubation was calculated as the

concentration in the media buffer sample divided by the concentration in the hepatocyte suspension sample.

Fraction unbound in human plasma

Equilibrium dialysis of compounds was used to assess the fraction unbound in human plasma. Stock solutions of compounds dissolved in DMSO were pooled into cassettes with up to ten compounds in each cassette with a concentration of 1 mM. The pooled stock solutions were diluted in plasma to a compound concentration of 5 µM. Warfarin, propranolol and metoprolol were used as reference compounds in each run. The equilibrium dialysis device (RED, Thermo Fischer Scientific Inc., Rockford, USA) was used for dialysis against phosphate buffer pH 7.4 for 18 h at 37°C. Calibration curves ranging from 0.001 to 7 µM were made using aliquots of the 7 µM spiked plasma pools that were transferred to a new plate followed by serial dilutions with blank plasma. Calibration curve samples and dialysis samples were precipitated with MeCN and after centrifugation, supernatants were removed and analyzed using LC/MS/MS (Waters Xevo-TQS). Fraction unbound in the incubation was calculated as the concentration in the media buffer sample divided by the concentration in the plasma sample. The Fu is reported as the median. CIR was 1.29 for the human Fu assay with a MDR of 1.44 for compounds tested on three occasions.

Intrinsic Caco-2 cell permeability

As reported previously, a monolayer of Caco-2 cells, cultured on semi-permeable polycarbonate surfaces, was used to study the permeability in the apical to basolateral direction. HTS Transwell-24 well permeable supports from Corning Corporation (Cambridge, MA, USA) were used.[21] HBSS buffer (25 mM Hepes, pH 7.4, containing 50 µM quinidine, 30 µM benzbromarone and 20 µM sulfasalazine) was dispensed to the basal side of the monolayer. The assay was initiated by adding the test substrate at 10 µM in HBSS buffer (25 mM HEPES, pH 6.5, containing 50 µM quinidine, 30 µM benzbromarone and 20 µM sulfasalazine) to the apical side of the monolayer. Samples were withdrawn from the donor and acceptor sides before the addition of the test substrate and at 45 and 120 min post addition of the test substrate. The Transwell-24 plates were incubated in a shaking

incubator at 37°C between sampling. All samples were analyzed by LC/MS/MS using ultra-performance liquid chromatography (Waters ACQUITY UPLC, Milford, MA, USA) coupled to a Waters Xevo TQ-S instrument (Waters, Manchester, UK). The apparent permeability (P_{app}) was calculated from the compound concentration on the donor and receiver sides.

Cytochrome P450 inhibition

A fluorescence-based method in 96-well format was used to determine the inhibition of five different CYPs (1A2, 2C9, 2D6, 3A4 and 2C19).[22] The recombinant human enzymes used were prepared in house, except for CYP2D6 (Cypex Ltd, Dundee, UK). Different coumarin substrates, biotransformed into fluorescent metabolites, were used as probes for each individual CYP. A fluorescence plate reader (SpectraMax GeminiXS, Molecular Devices, Sunnyvale, California, USA) was used to measure the levels of metabolites formed. A dilution series of the test substrates was prepared at eight different concentrations. For each CYP, a mixture of the enzyme, corresponding coumarin substrate, potassium phosphate buffer pH 7.4 and water (concentrations and volumes were CYP-dependent) were added to each well in a black 96-well plate. The test substrates at different concentrations were added. After 10 min pre-incubation, the co-factor NADPH was added to initiate the reaction. After 20–50 min (CYP and substrate-dependent) the reaction was terminated by addition of trisbase/MeCN (20:80). The plates were transferred to the fluorescence plate reader where the wavelengths were set individually for the different coumarin substrates and their respective fluorescent metabolite. The responses were exported to Excel where the IC_{50} curves were plotted (percent inhibition versus concentration) and IC_{50} values calculated for each test substrate and enzyme using XL-fit.

Rat pharmacokinetics *in vivo*

Two days prior to dosing, male Han Wistar rats were prepared by cannulation of the left carotid artery for blood sampling and by cannulation of the right jugular vein for intravenous administration. The catheters were filled with heparin (100 IU mL^{-1}), exteriorized at the nape of the neck and sealed. The surgery was performed under isoflurane (Forene®, Abbott) anesthesia. After surgery

the rats were housed individually and had free access to food and water. About 16 h prior to dosing the animals were deprived of food and fasted until 4 h after dosing. The rats had free access to drinking water during the experiment. On the experiment day, the test item formulation was administered orally by gavage or intravenously in the jugular vein. At pre-defined time points, blood samples of about 0.150 mL were withdrawn from the carotid artery up to 24 h after dosing. A total of 10 samples were withdrawn. The blood samples were collected in heparinized plastic tubes and centrifuged, within 30 min, for five min at 10 000 *g* and 4°C. The plasma was transferred to a 96-well plate and stored at –20°C until analysis by LC-MS/MS.

Transport across the blood-brain barrier in rats

Three female Sprague-Dawley rats with intravenous catheters placed in the *vena jugularis* were given infusions of the test compound at a concentration of 1 µmol/kg/h for 4 hours. Terminal blood samples were taken by heart puncture and collected in tubes containing heparin, and the plasma was stored. Before collection of the brain, the heart was removed to minimize the contamination of the brain tissue. The rat was decapitated and the brain was removed from the cranium and divided along the central line. One of the pieces was transferred into a tared plastic tube and 3 mL water per gram of brain tissue was added. The brain was completely homogenized by sonification. Following protein precipitation, the plasma and the brain homogenates were analyzed by LC/MS/MS and brain/plasma ratios (Cbr/Cpl) were calculated. The fraction unbound in plasma and brain was determined by equilibrium dialysis for 18h of plasma and brain homogenates spiked with the test compound. This allowed for calculation of unbound brain/plasma ratios (Cubr/Cupl)

Dog pharmacokinetics *in vivo*

Male and female Beagle dogs were used. The dogs had free access to water and were fed once daily, not earlier than 4 hours after dosing. On the experiment day, the test item formulation was administered orally by gavage or intravenously in a superficial front leg vein (this vein was not used for blood sampling). The sond used for gavage was rinsed with about 20 mL of water and emptied with 20 mL of air before it was taken out. The compounds were dosed to 2 dogs per administration

route. At pre-defined time points, blood samples of about 0.5 mL were withdrawn from a superficial front leg vein up to 24 h after dosing. A total of 10 samples were withdrawn. The blood samples were collected in heparinized plastic tubes and centrifuged, within 30 minutes, for five minutes at 10 000 *g* and 4°C. The plasma was transferred to a 96 well plate and stored at –20°C until analysis by LC-MS/MS.

Early dose to man (eD2M) predictions

To aid rapid prioritization of compounds, we used an 'early dose to human' (eD2H) prediction (Equation 9) based on a composite of MPO IC_{50} and metabolic stability in human hepatocytes.[23] A free steady state plasma level of 10 times the MPO enzyme IC_{50} was assumed as a requirement for functional efficacy *in vivo*.

Equation 9.

$$\mathrm{eD2H} = \frac{\mathrm{IC50} \cdot \mathrm{X} \cdot \mathrm{CL} \cdot \mathrm{T}}{\mathrm{F}} \quad \text{wherein } \mathrm{CL} = \frac{\mathrm{Qh} \cdot \mathrm{CLint}}{\mathrm{Qh} + \mathrm{CLint}}$$

eD2H = early dose to human. IC50 = myeloperoxidase enzyme IC_{50}. X = offset factor between *in vivo* and *in vitro* IC_{50}, assumed to be 10 for these eD2H predictions. CL = predicted human hepatic clearance. T = dosing interval. F = bioavailability. CL_{int} = intrinsic clearance in human hepatocytes. Q_h = hepatic blood flow.

Human clearance (CL, Equation 1) was predicted by the established well-stirred model[24] based on human hepatocyte (hHep) CL_{int} values rather than human liver microsome CL_{int}. Hepatocytes represent a more complete *in vitro* system regarding drug metabolizing enzymes compared to HLM. The assumption was that the compounds were eliminated mainly via hepatic metabolism.

Covalent binding to liver microsomes

Human liver microsomes (1 mg protein/mL) were incubated with 10 µM [^{14}C]-substrate for 60 min at 37ºC in the presence or absence of 1 mM NADPH in 100 mM phosphate buffer (pH 7.4). The reactions were initiated by the addition of [^{14}C]-substrate after a 5 min pre-incubation period at 37°C.

Duplicate incubations were performed. Aliquots for estimation of covalent bindning and LCMS analysis were collected after 0 and 2 hours. For estimation of the covalent binding, protein was precipitated by addition of 4 volumes of ice-cold acetone. Samples were stored at 4ºC for 60 minutes and centrifuged at 45 *g* (4ºC, 5 min), followed by a gentle vortex. Samples were harvested and counted according to the method described by Thompson *et al.*[25] The levels of covalent binding indicated by non-extractable radioactivity were expressed as picomole equivalents per milligram protein (pmol eq/mg protein) using the specific activity of the test compound and the protein levels for each sample.[26] To account for any nonspecific binding, the average covalent binding for the 0 h samples was subtracted from the average covalent binding for the incubated samples at different time points. The same protocol was applied for incubations in the presence of 1 mM formaldehyde and with human liver microsomes that had been heat inactivated at 53°C.

Rat quantitative whole body autoradiography

Lister Hooded male rats were given a single dose of [^{14}C]-**16** (AZD4831) 10 MBq/kg (4.55 µmol/kg) and sacrificed at 1 h, 12 h, 24 h, 7 days and 21 days following oral dosing and at 24 h following intravenous dosing. Autoradiograms were generated before and after extraction of tissue slices. The oral dose was administered by metal gavage at a target dose of 4.55 µmol/kg and a target dose volume of 4 mL/kg. The intravenous dose was administered via the tail vein at a target dose level of 4.55 µmol/kg and a target dose volume of 1 mL/kg. Following oral administration, one male rat was humanely killed by CO_2 narcosis at each of 1 h, 12 h, 24 h, 7 days and 21 days post-dose. The animal dosed intravenously was humanely killed by CO_2 narcosis at 24 h post dose. At the time of sacrifice from 24 h post-dose onwards, each animal was weighed and the weight recorded. Immediately following sacrifice the right eye was removed and weighed into a tared Combustocone (Perkin-Elmer). The carcass of each animal was then frozen by immersion in a mixture of solid CO_2 and hexane for circa 30 min. The frozen carcass was embedded in a block of carboxymethylcellulose which was frozen in the same way. After equilibration at circa –20°C, sagittal sections (20 µm thick) were taken through each animal using a whole-body cryomicrotome (CM3600, Leica GmbH, Germany) and freeze-dried. For animals sacrificed at 1 h and 24 h after oral

administration and the animal sacrificed at 24 h after intravenous administration, additional selected sections were washed sequentially with water, 5% trichloroacetic acid, 50% ethanol, 99.5% ethanol, 99.5% ethanol, 50% ethanol and running water. For washes 1–6, sections were placed in a suitable volume of the appropriate solvent and gently agitated for 4 min. Wash 7 was performed for 10 mins. After the wash procedure the sections were left to air dry overnight before exposure on storage phosphor screens. The radioactivity present in various organs and tissues in whole body sections and extracted whole body sections was determined by quantitative whole-body autoradiography using a Typhoon FLA7000 scanner and AIDA image analysis software (version 4.06, Raytest Isotopenmeβgeräte GmbH, Germany).

For analysis, representative whole body sections were placed into close contact with phosphor screens and left for a period of 7 days. On each phosphor screen a set of external standards was also exposed. These standards were prepared from blood spiked with a serial dilution of a ^{14}C-labelled reference solution, which was dispensed into holes drilled into a block of carboxymethylcellulose, frozen and then sectioned in the same way as the animal samples. After the phosphor screen was scanned, an image of the radioactivity in the sample was stored digitally. For quantitative analysis, six background areas were defined on each storage phosphor screen image. The software automatically calculated the mean background and subsequently subtracted this from all standards and tissues analysed. A regression coefficient was derived by comparing the response of each standard with the nominal concentration over the range of radioactive concentrations used and forcing the response curve through the origin. The concentrations of the standards used were in the range of 2 to 13 904 ng equiv/g and 2 to 13 920 ng equiv/g for oral and intravenous dosing, respectively. The response curve is linear over these concentrations and is assumed to be linear to the limit of reliable determination. Each organ or tissue of interest was then identified and integrated and the software automatically calculated the concentration (ng equiv/g) using the regression equation derived from the standards. The limit of reliable measurement for each storage screen was calculated from the assessment of the mean background of the plate and

defined as 3 times the standard deviation of the mean above background. At the specific activity used in this study, the limit of reliable measurement was in the range of 1 to 3 ng equiv/g.

10. Notes

All procedures performed on animals were in accordance with regulations and established guidelines and were reviewed and approved by the Göteborg Animal Research Ethical Board.

11. Data tables

Table S4. Potency and selectivity of myeloperoxidase inhibitors

Compound	MPO IC_{50} (nM)	MPO IC_{50} SD (nM)	TPO IC_{50} (μM)	TPO IC_{50} SD (μM)	HL cell IC_{50} (μM)	HL cell IC_{50} SD (μM)
2	187.0	13.8	0.99	0.33	35.5	1.2
3	279.1	96.0	2.40	0.73	29.0	1.3
4	32.0	4.0	0.59	0.02	No data	
5	38.6	1.5	4.20	0.28	80.0	7.7
6	30.9	10.5	8.10	0.53	13.0	0.8
7	15.1	8.0	1.50	0.09	6.6	0.4
8	15.0	3.4	1.30	0.43	11.0	0.6
9	5.0	0.8	1.39	0.30	No data	
10	35.0	7.7	3.40	2.18	1.3	0.1
11	140.3	31.6	1.40	0.33	16.0	1.2
12	39.3	11.4	4.20	0.10	9.4	0.7
13	27.1	3.3	1.90	0.24	6.1	0.4
14	15.9	3.6	2.10	0.29	6.6	0.3
15	12.7	1.7	1.10	0.29	4.6	0.5
16	1.5	0.4	0.69	0.08	0.8	0.1
17	26.4	4.5	1.10	0.33	20.0	4.0
18	3.6	1.0	0.77	0.11	3.1	0.3

HL, human liver; MPO, myeloperoxidase; TPO, thyroid peroxidase.

Table S5. Physicochemical and *in vitro* drug metabolism and pharmacokinetic properties of compounds 5–18.

Compound	LogD n=3 (SD)	Solubility at pH7.4 (μM) n=3 (SD)	Human liver microsome intrinsic clearance (μL/min/mg) n=3 (SD)	Rat hepatocyte intrinsic clearance (μL/min/10^6 cells) n=3 (SD)	Human hepatocyte intrinsic clearance (μL/min/10^6 cells) n=3 (SD)	Caco-2 cell intrinsic permeability (10^6.cm/s) n=2 (SD)	Early dose to human prediction (mg/day)*
5	0.3 (0.1)	18 (11)	< 3	< 1	< 1	0.89 (0.14)	150
6	0.9 (0.1)	140 (37)	4.5 (1.4)	11 (1.8)	< 1	12 (0.07)	110
7	1.0 (0.1)	930 (130)	< 3	19 (2.4)	< 1	19 (0.50)	71
8	1.2 (0.1)	42 (15)	< 3	3.2 (1.4)	3.2 (0.74)	6.0 (1.8)	180
9	1.7 (0.1)	8.3 (3) (n = 2)	39 (16)	20 (4.4)	1.9 (0.90)	18 (3.3)	14
10	3.3 (0.1)	0.37 (0.21)	8.2 (1.5)	30 (4.6)	4.3 (0.75)	49 (6.9)	1000
11	2.0 (0.1)	3.3 (1.5)	< 3	< 1	< 1	11 (0.14)	630
12	1.3 (0.1)	150 (4.0)	11 (2.2)	15 (1.7)	< 1	19 (0.57)	290
13	1.1 (0.2)	210 (92)	3.3 (0.55)	21 (1.8)	< 1	9.6 (0.81)	120
14	1.3 (0.1)	160 (94)	< 3	2.4 (0.16)	< 1	17 (0.21)	68
15	1.5 (0.1)	190 (57)	5.5 (2.5)	5.6 (3.7)	< 1	19 (2.8)	49
16	1.2 (0.0)	270 (300)	< 3	1.8 (0.98)	< 1	11 (6.4)	8
17	1.4 (0.3)	850 (120)	< 3	<1	< 1	10 (2.7)	97
18	0.7 (0.1)	880 (130)	< 3	< 1	< 1	1.3 (0.94)	15

*Early dose to human predictions were based on a composite of myeloperoxidase (MPO) IC_{50} and metabolic stability in human hepatocytes. A free steady state plasma level of 10 times MPO IC_{50} was assumed as a requirement for functional efficacy *in vivo* (see Supporting Information).

Table S6: Safety and pharmacokinetic parameters of compounds 8, 14, 16 and 18

Compound	hERG IC_{50} (µM) n = 3 (SD)	CYP3A4 IC_{50} (µM) n = 3 (SD)	Rat clearance (mL/min/kg) n = 2 (SD)	Rat F (%) n = 2 (SD)	Dog clearance (mL/min/kg) n = 2 (SD)	Dog F (%) n = 2 (SD)
8	> 40	19 (9.6)	32[a] (1.5)	68 (19)	19 (4.5)	45 (2.7)
14	10 (1.7)	> 30	22 (1.4)	60 (5.7)	23 (0.0)	72 (8.4)
16	21 (3.7)	6.0 (1.6)	19[a] (5.8)	61 (11)	5.7 0.69	52 (2.5)
18	23 (8.3)	8.8 (3.9)	20 (2.1)	7.4 (1.6)	No data	No data

CYP3A4, cytochrome P45 3A4; F, bioavailability; hERG, human ether-à-go-go-related gene (potassium voltage-gated channel subfamily H member 2 isoform a).

[a]n = 4

12. Abbreviations

aq, aqueous; Boc_2O, di-*tert*-butyl dicarbonate; brine, saturated aqeous sodium chloride solution; *n*-BuLi, *n*-butyllithium, cataCXium® A, Di(1-adamantyl)-n-butylphosphine; DCM, dichloromethane; DEA, diethylamine; DIPEA, *N*-ethyl-*N*-*iso*propylamine; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; Et, Ethyl; EtOAc, ethylacetate; EtOH, ethanol; FA, formic acid; h, hours; HOAc, acetic acid; iPr, isopropyl, Me, methyl, MeCN, acetonitrile, MeOH, methanol; 2-MeTHF, 2-methyltetrahydrofuran; N, Normal; NMR, nuclear magnetic resonance; NMP, N-methylpyrrolidone; OAc, acetate; rt, room temperature; sat., saturated; STAB, sodium triacetoxyborohydride; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran

13. References

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14. HPLC purity analyses, NMR spectra and VCD spectra

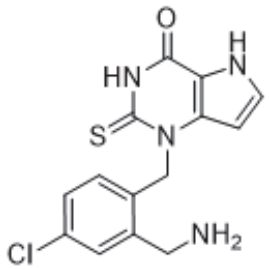
Purity Analysis Compound 8

Sample was 99% pure (UV 230 nm)
Instrumental setup

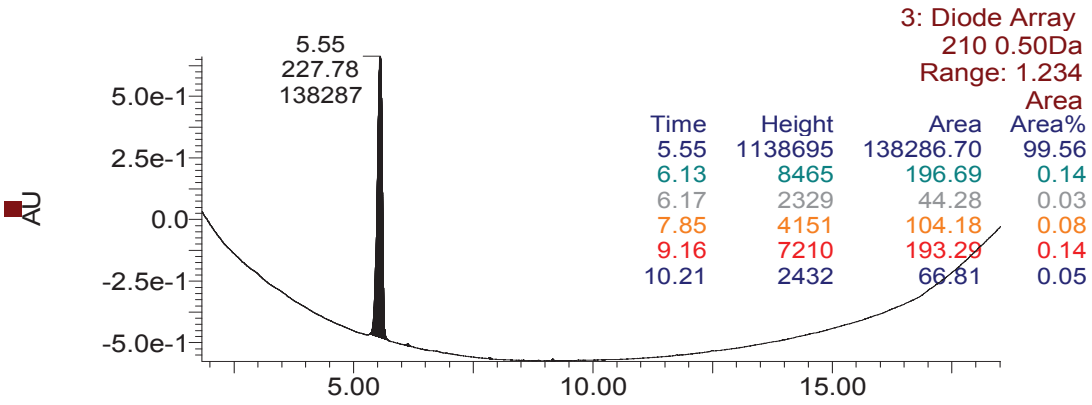
Basic conditions
Column Acquity UPLC BEH C18, 2.1mm×150mm, 1.7µm particles
Temperature 60ºC
Gradient 5% ACN to 95% ACN in 18 minutes; 47mM NH_4, 6.5mM NH_4HCO_3, pH 10
Flow 0.6 mL/min

Acidic conditions
Column Acquity UPLC CSH C18, 2.1mm×150mm, 1.7µm particles
Temperature 60ºC
Gradient 5% ACN to 95% ACN in 18 minutes; 1mM ammonium formate, 10 mM formic acid, pH 3
Flow 0.6 mL/min

Xevo
pH10, 210nm, 20min **60C**

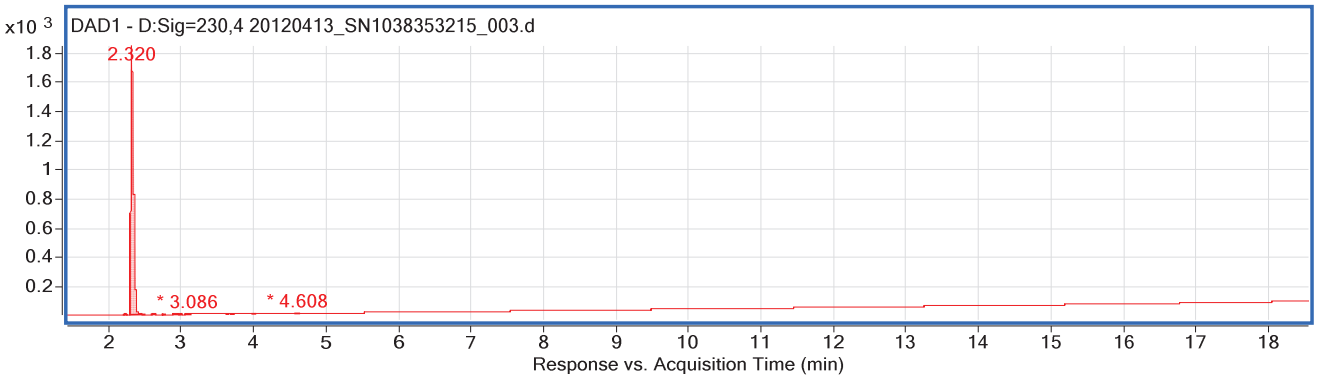


8



RT	Height	Area	Area%
5.554	1138695	138286.703	99.6
6.133	8465	196.687	0.14
6.173	2329	44.278	0.03
7.845	4151	104.182	0.08
9.158	7210	193.295	0.14
10.208	2432	66.814	0.05
		138891.959	

pH3 20min 230nm



RT	Area	Height	Area Sum %
2.23	7.72	3.83	0.15
2.32	5000.32	1841.81	99.2
2.623	8.92	3.7	0.18
2.753	2.22	1.33	0.04
2.93	6.75	3.35	0.13
2.999	1.41	1.19	0.03
3.086	8.72	4.91	0.17
3.638	1.12	0.77	0.02
3.708	1.12	0.49	0.02
4	2.67	1.9	0.05
4.608	1.78	0.9	0.04

LC Purity of Compound 14

LC purity is 98.2% at 230 nm (Figure 1)

Column: Acquity UPLC CSH C18, 2.1mm×100mm, 1.7µm particles

Temperature: 30ºC

LC gradient: 2% ACN to 95% ACN in 18 minutes.; 10 mM ammonium formate, pH 7

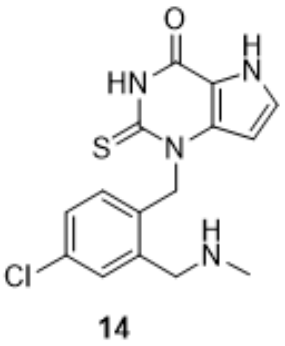
Flow: 0.4 mL/min

Injection volume: 1.4 µL

Sample preparation: 0.2 mg/mL in DMSO:MeCN:aq (10:40:50) solution

UV response at 230 nm

The sample was also analysed by an additional method (Acquity UPLC CSH C18 column at pH3) (data not shown).



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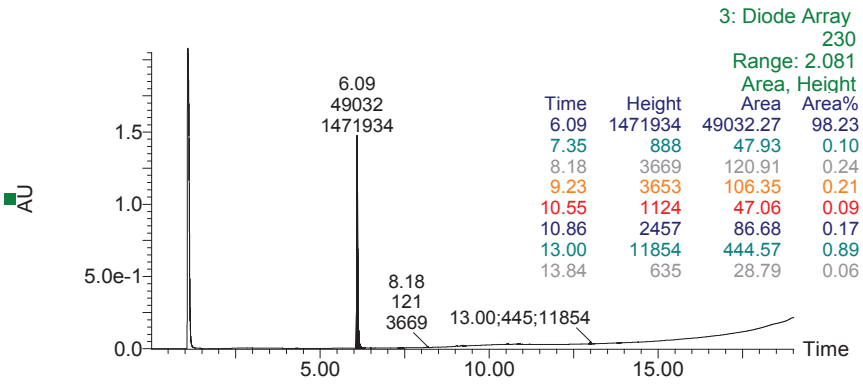


Figure 1. LC chromatogram of 14 @ 230 nm

Table 1. Integration peak list

UV 230nm

RT	Area Sum%	
6.09	98.2	
7.35	0.10	
8.18	0.24	
9.23	0.21	
10.55	0.09	
10.86	0.17	
13.00	0.89	
13.84	0.06	

Compound 16 - Purity analysis

Instrumental setup: 10min pH3 HSS C18 Xevo

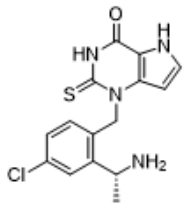
Column	Acquity UPLC HSS C18 100mm×2.1mm, 1.7µm particles
Temperature	60ºC
Gradient	2% ACN to 95% ACN in 9 minutes; 1mM ammonium formate, 10 mM formic acid, pH 3
Flow	1 mL/min
Purity	Relative Absorbance at 230 nm

Additional Method: 10min pH10 BEH Xevo

Column	Acquity UPLC BEH C18 100mm×2.1mm, 1.7µm particles
Temperature	60ºC
Gradient	2% ACN to 95% ACN in 9 minutes; 47mM NH4, 6.5mM NH4HCO3, pH 10
Flow	1 mL/min
Purity	Relative Absorbance at 210 nm

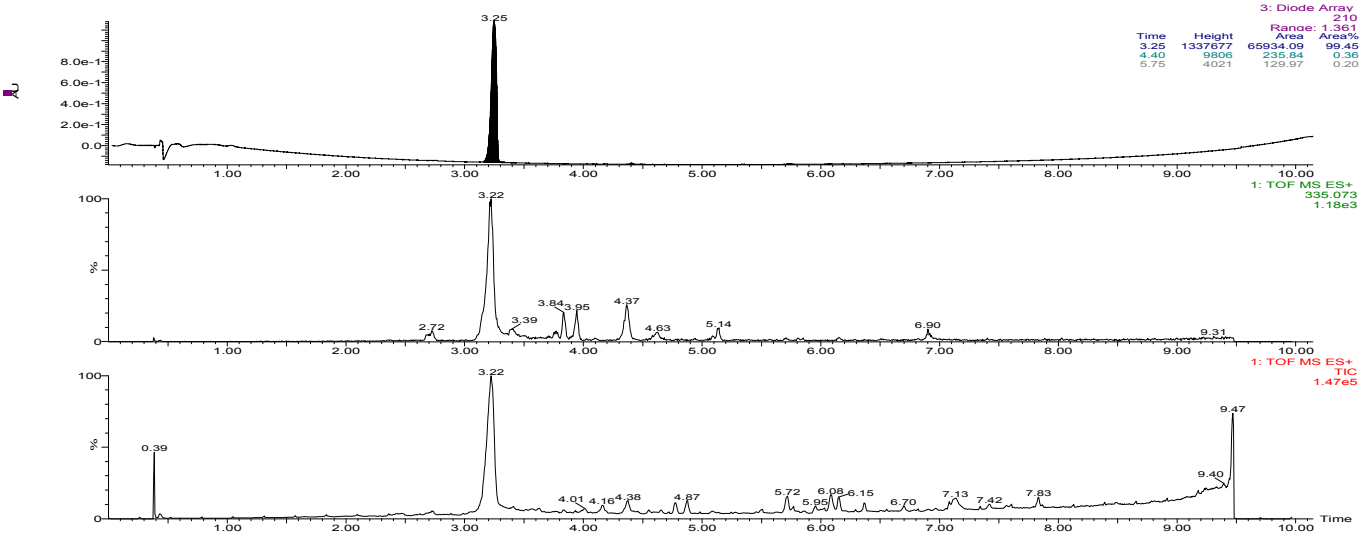
Purity (%)

99



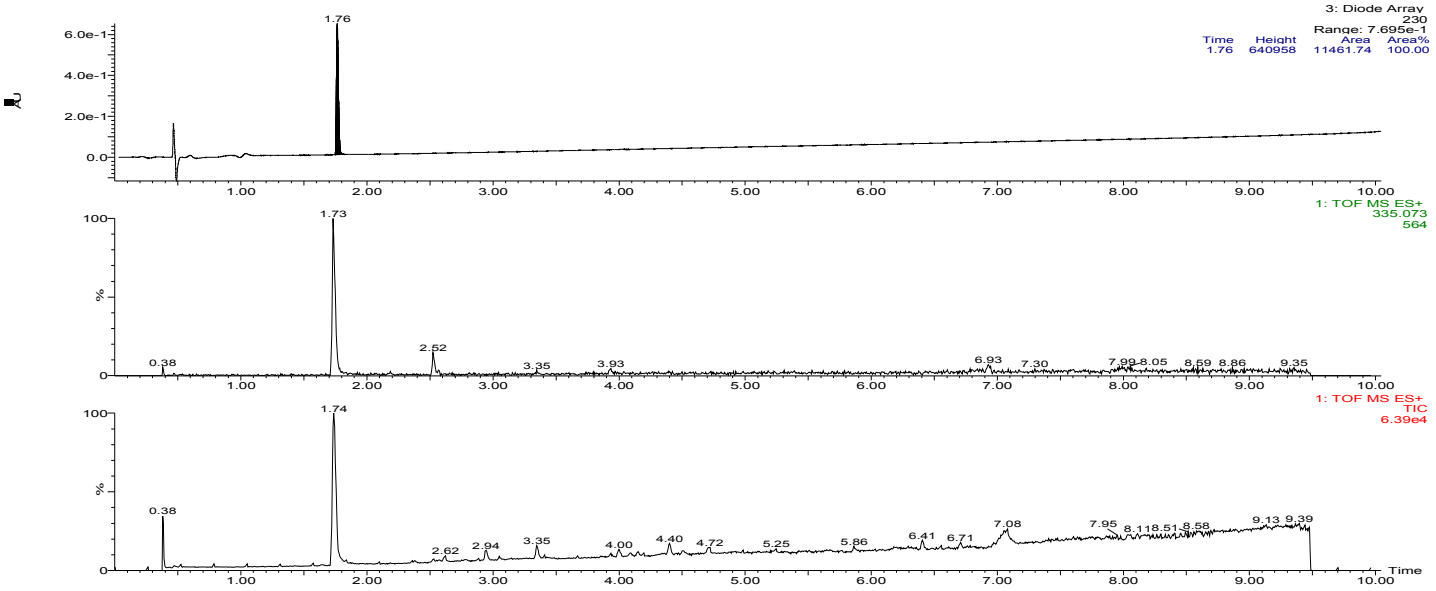
16

pH10, 210nm



rt	height	area	area%
3.248	1337677	65934.1	99.45
4.404	9806	235.837	0.36
5.746	4021	129.974	0.20
		66299.9	

pH3, 230nm



rt	height	area	area%
1.764	640958	11461.7	100

Compound 18 - LC/MS

Openlynx Report walk-up LC/MS

UserName:kemikw (Westerlund

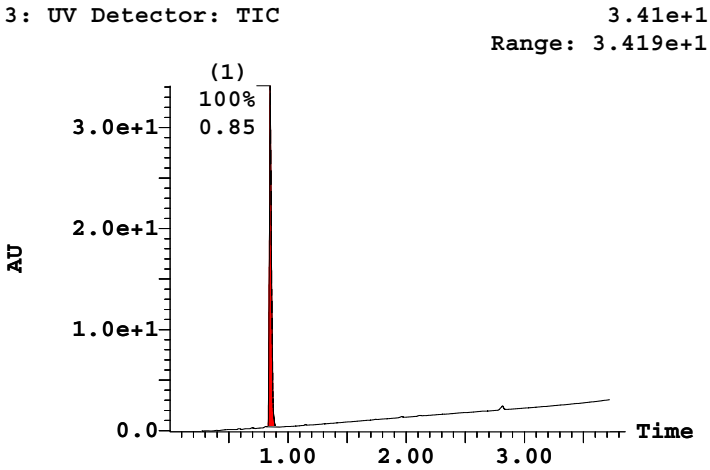
Method:c:\MassLynx\COL2_RM_PH3_HSSC18_4MIN.OLP

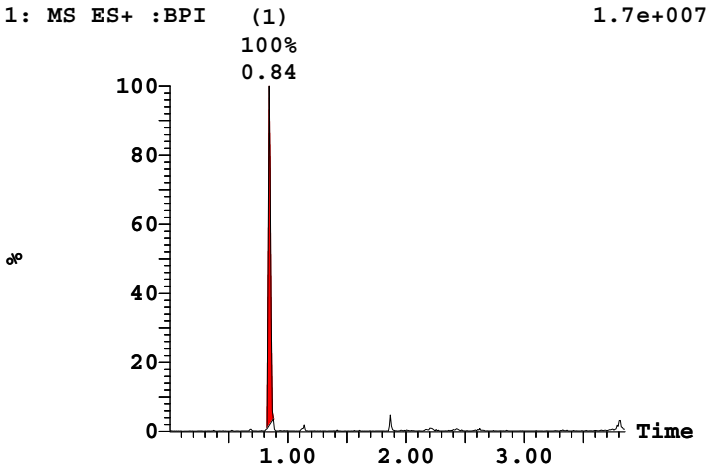
Printed: Mon Jan 13 08:40:44 2014

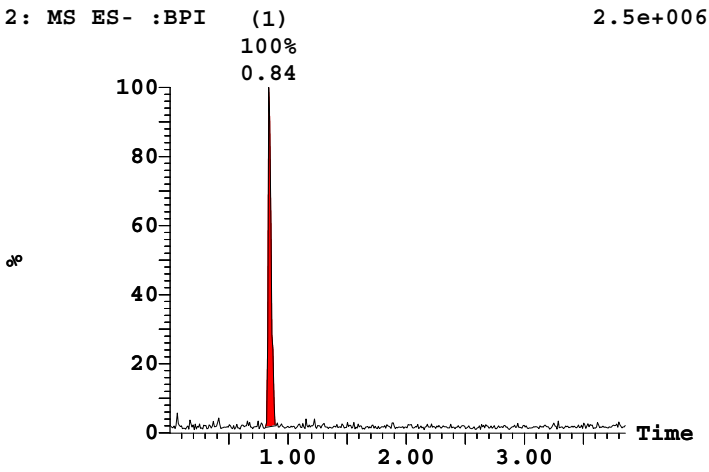
Instrument:ACQ-SQD2#LCA098

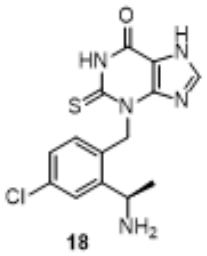
Column: 1.8µ 2.1x50 mm

Sample Report:

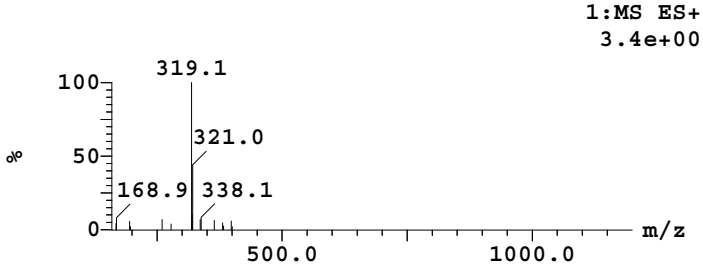








Peak ID	Time	Mass Found
1	0.84	



Peak ID	Time	Mass Found
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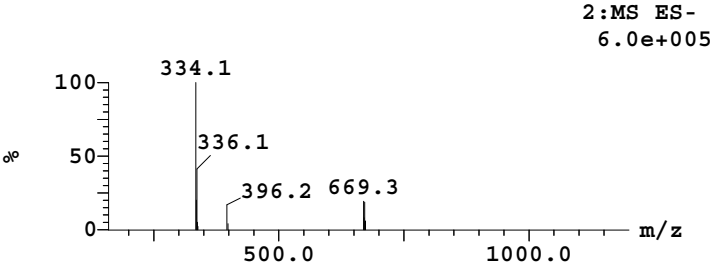

UserName:kemikw (Westerlund

Instrument:ACQ-SQD2#LCA098

Method:c:\MassLynx\COL2_RM_PH3_HSSC18_4MIN.OLP

Column: 1.8µ 2.1x50 mm

Printed: Mon Jan 13 08:40:44 2014

Sample Report (continued):

Peak ID	Time	Mass Found
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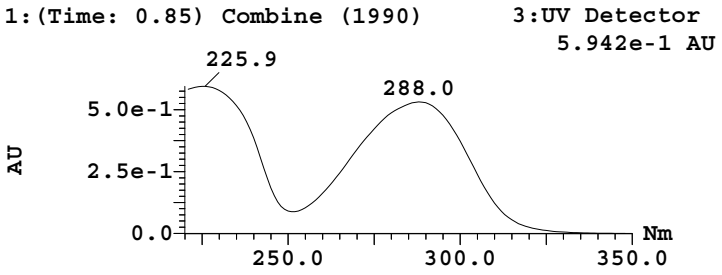

Header:
Acquired Name: EN07008-43-INSTR27-G
Acquired Date: 13-Jan-2014
Acquired Time: 08:35:54
User Name: kemikw (Westerlund
Instrument: ACQ-SQD2#LCA098
Sample Description: HPLC
SampleID: EN07008-43
Bottle Number: 1:28

MS Method:
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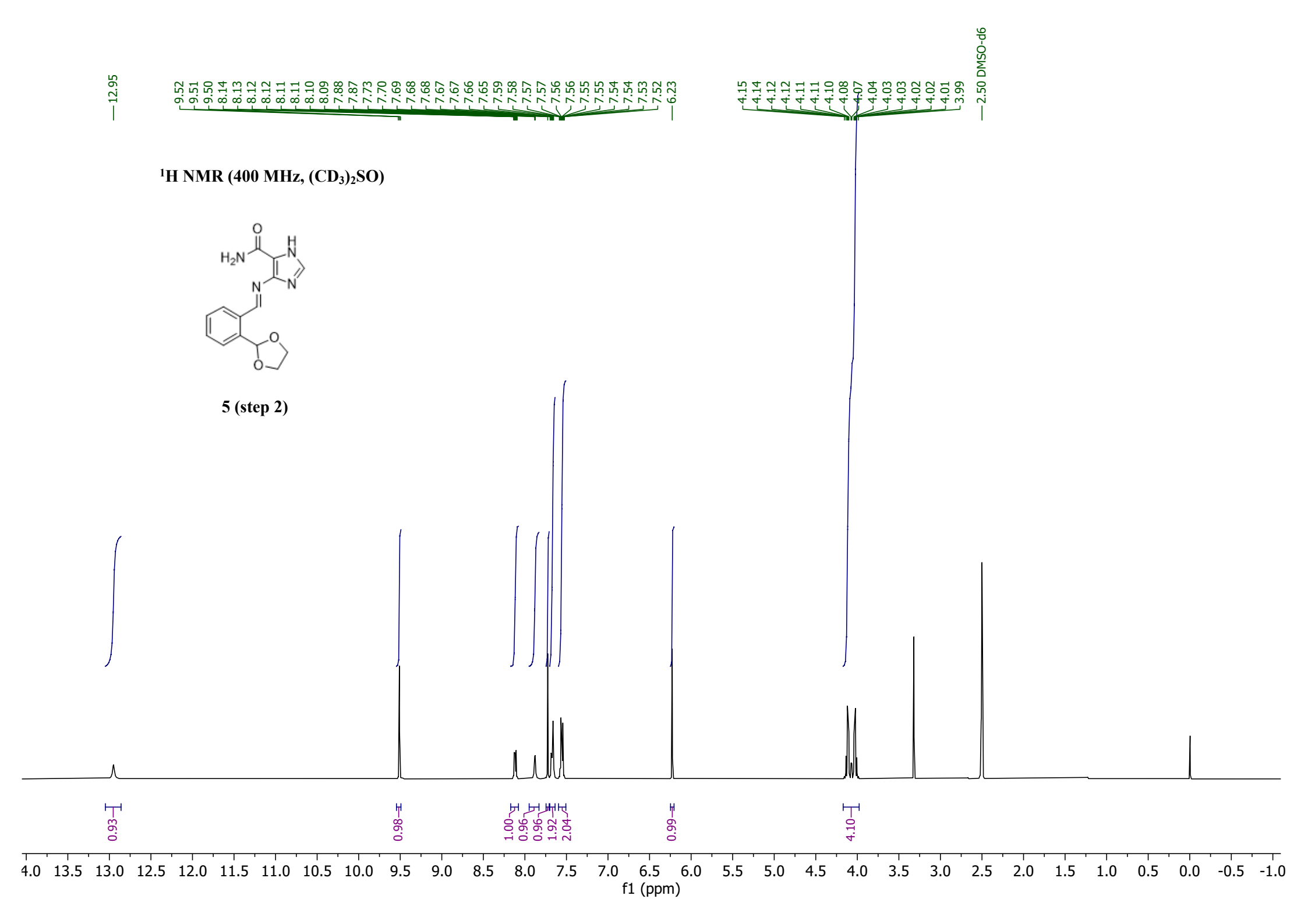
Parameter File - C:\MassLynx\OA2014_KH461.PRO\ACQUDB\Standard OA.IPR
Polarity ES+
Capillary (kV) 3.50
Cone (V) 55.00
Source Temperature (?C) 150
Desolvation Temperature (?C) 400
Cone Gas Flow (L/Hr) 50
Desolvation Gas Flow (L/Hr) 1200
LM 1 Resolution 13.00
HM 1 Resolution 14.60
Ion Energy 1 0.50
Cone Energy Ramp :Disabled
Probe Temperature Ramp :Disabled

Polarity ES-
Capillary (kV) 3.50
Cone (V) 30.00
LM 1 Resolution 11.40
HM 1 Resolution 14.60
Ion Energy 1 0.50
Cone Energy Ramp :Disabled
Probe Temperature Ramp :Disabled

Run method parameters:
Waters Acquity SDS
Run Time: 4.00 min
Solvent Name A: Water
Solvent Name B: Acetonitrile
[Gradient Table]
Time(min) Flow Rate %A %B Curve
1. Initial 1.000 90.0 10.0 Initial
2. 0.20 1.000 90.0 10.0 6
3. 3.70 1.000 1.0 99.0 6
4. 3.80 1.000 1.0 99.0 6
5. 3.81 1.000 90.0 10.0 6
Run Events: Yes
Gradient Start (Relative to Injection): 0 uL
Participate in pre-analysis: No

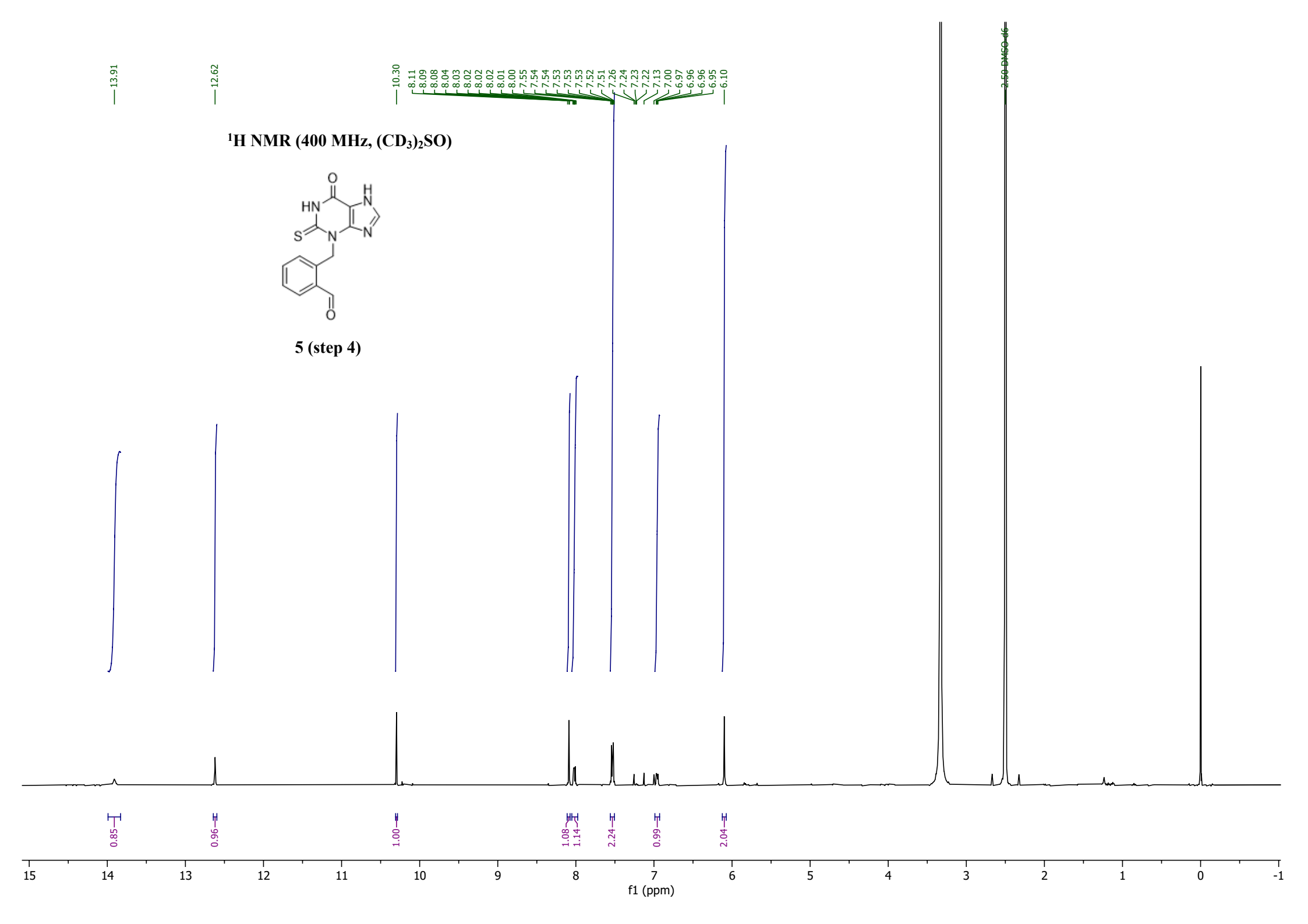
Waters Acquity PDA
Range: 220 - 350

Injection Volume (ul) - 2.00



^{1}H NMR (400 MHz, $(CD_3)_2SO$)

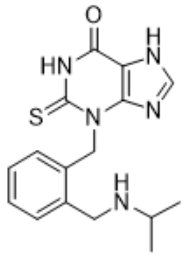
5 (step 2)



^{1}H NMR (400 MHz, $(CD_3)_2SO$)

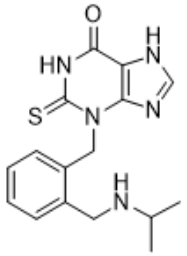
5 (step 4)

^{1}H NMR (500 MHz, $(CD_3)_2SO$)

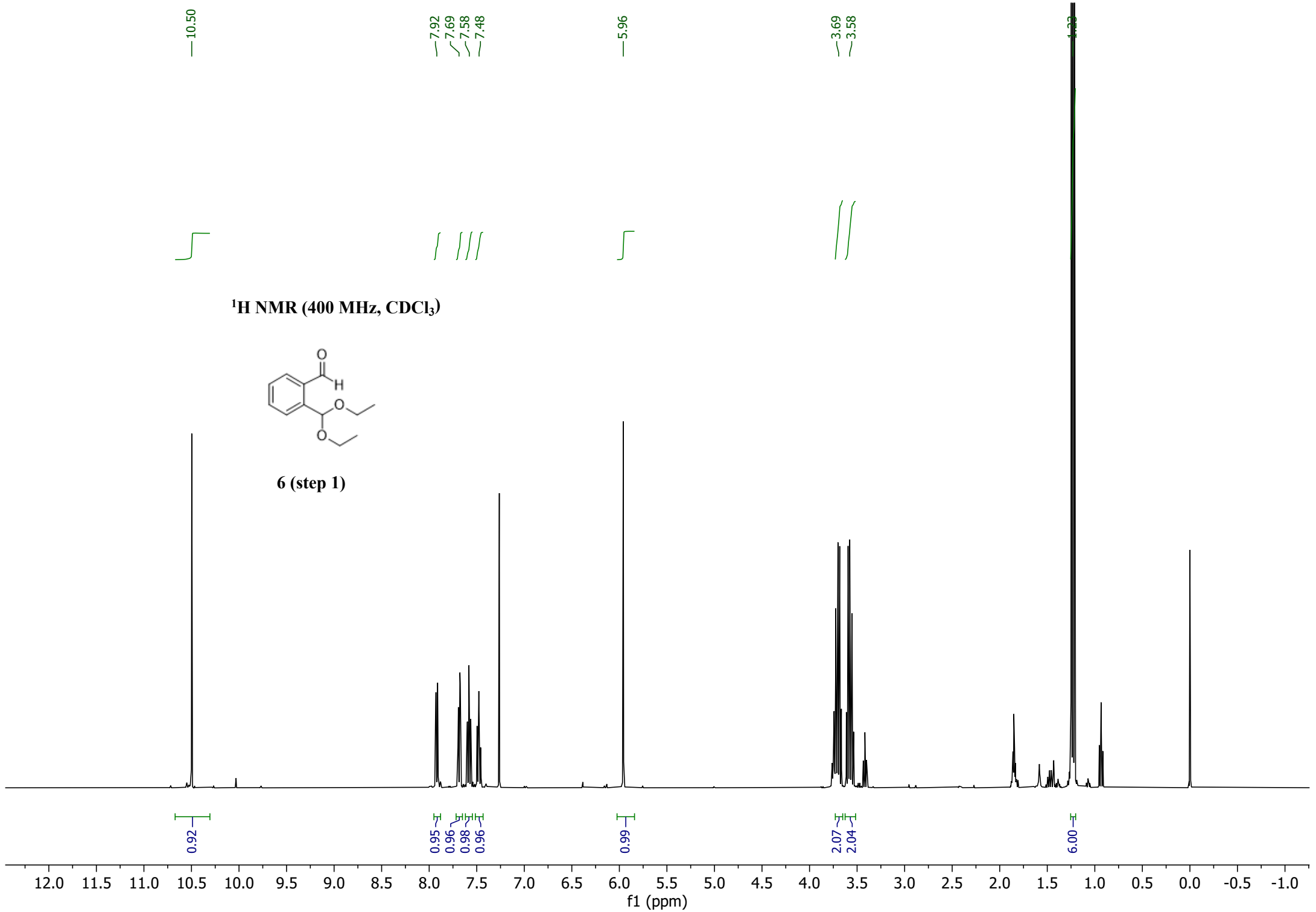


5

^{13}C NMR (126 MHz, $(CD_3)_2SO$)

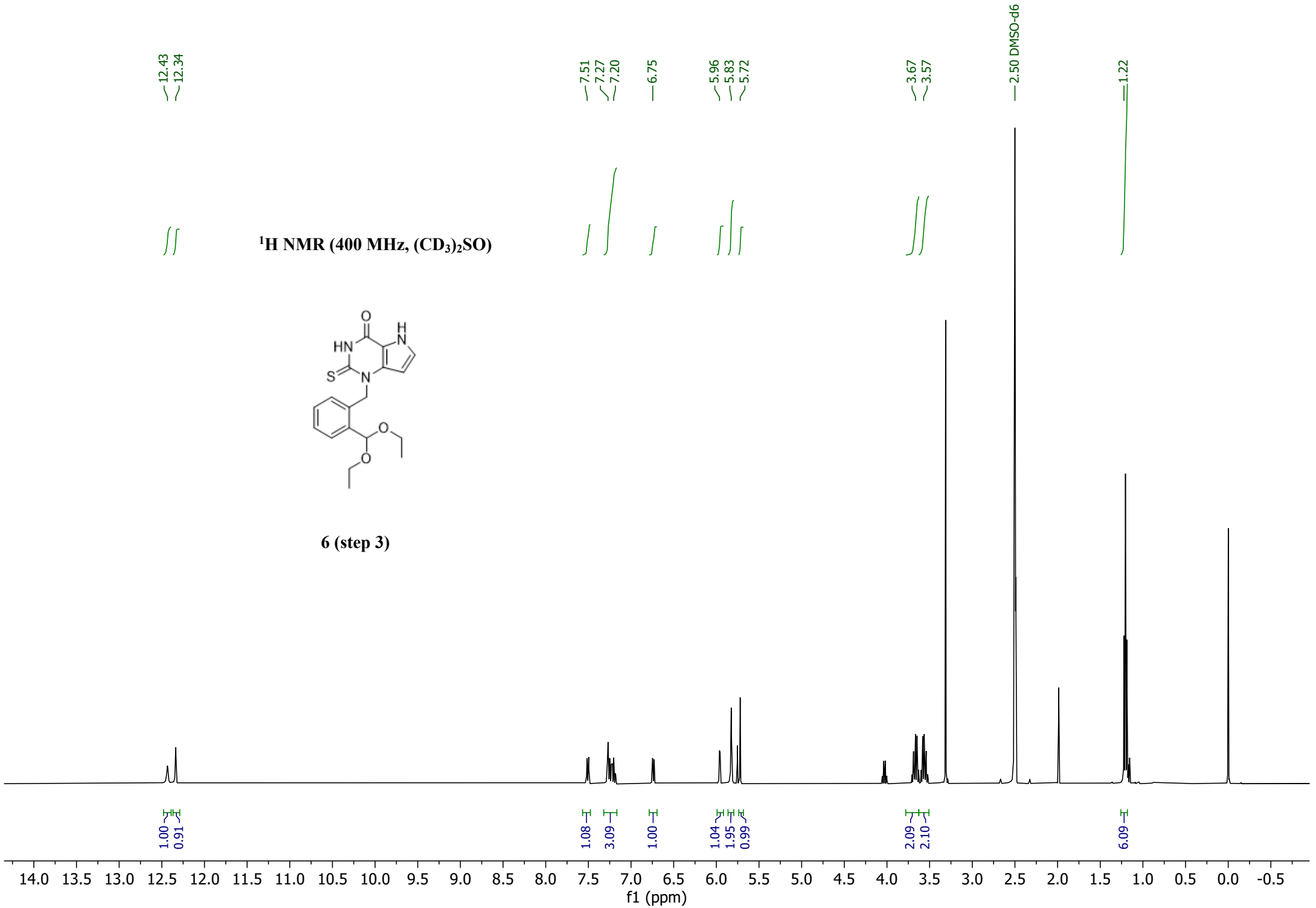


5



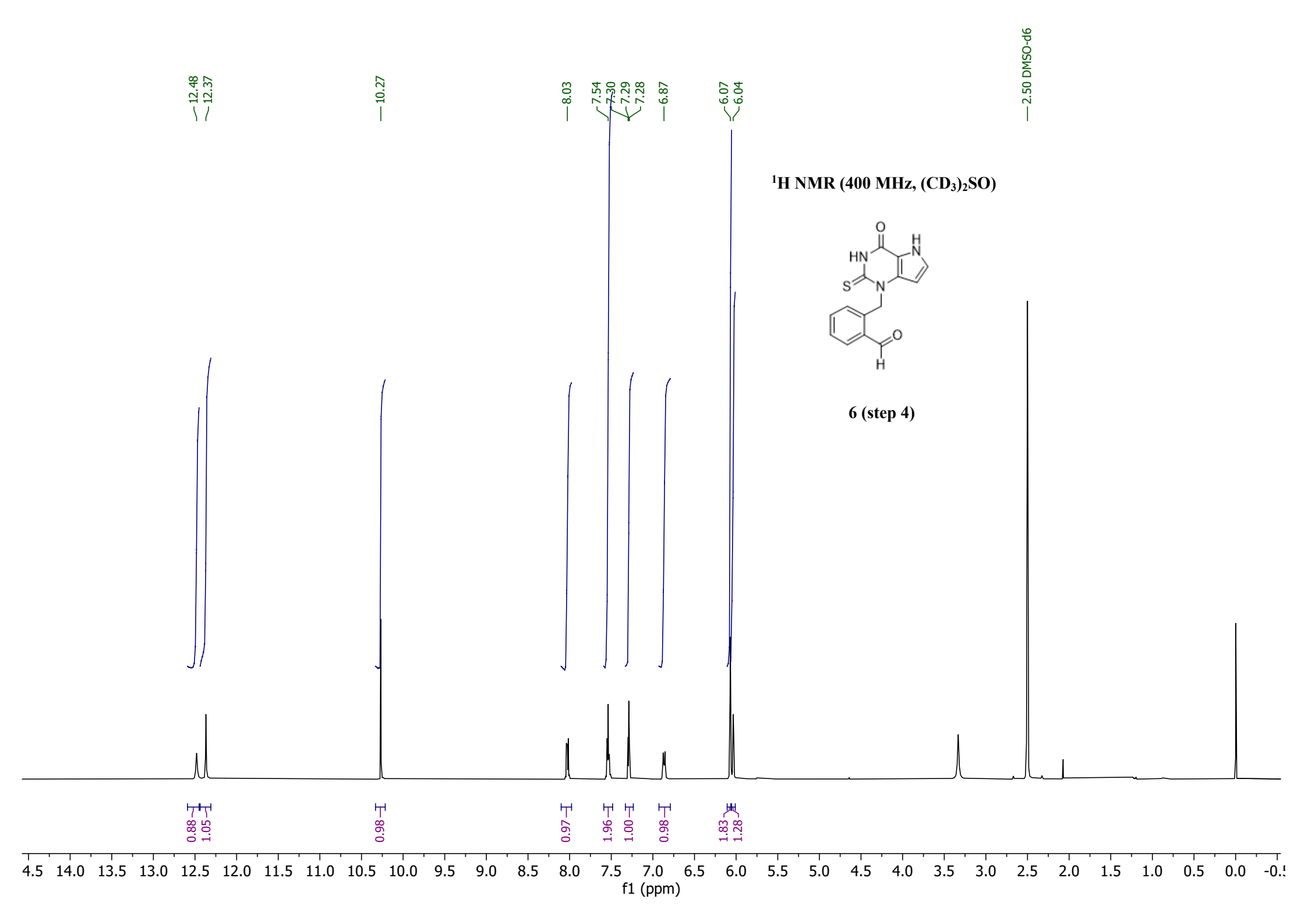
^{1}H NMR (400 MHz, $CDCl_3$)

6 (step 1)



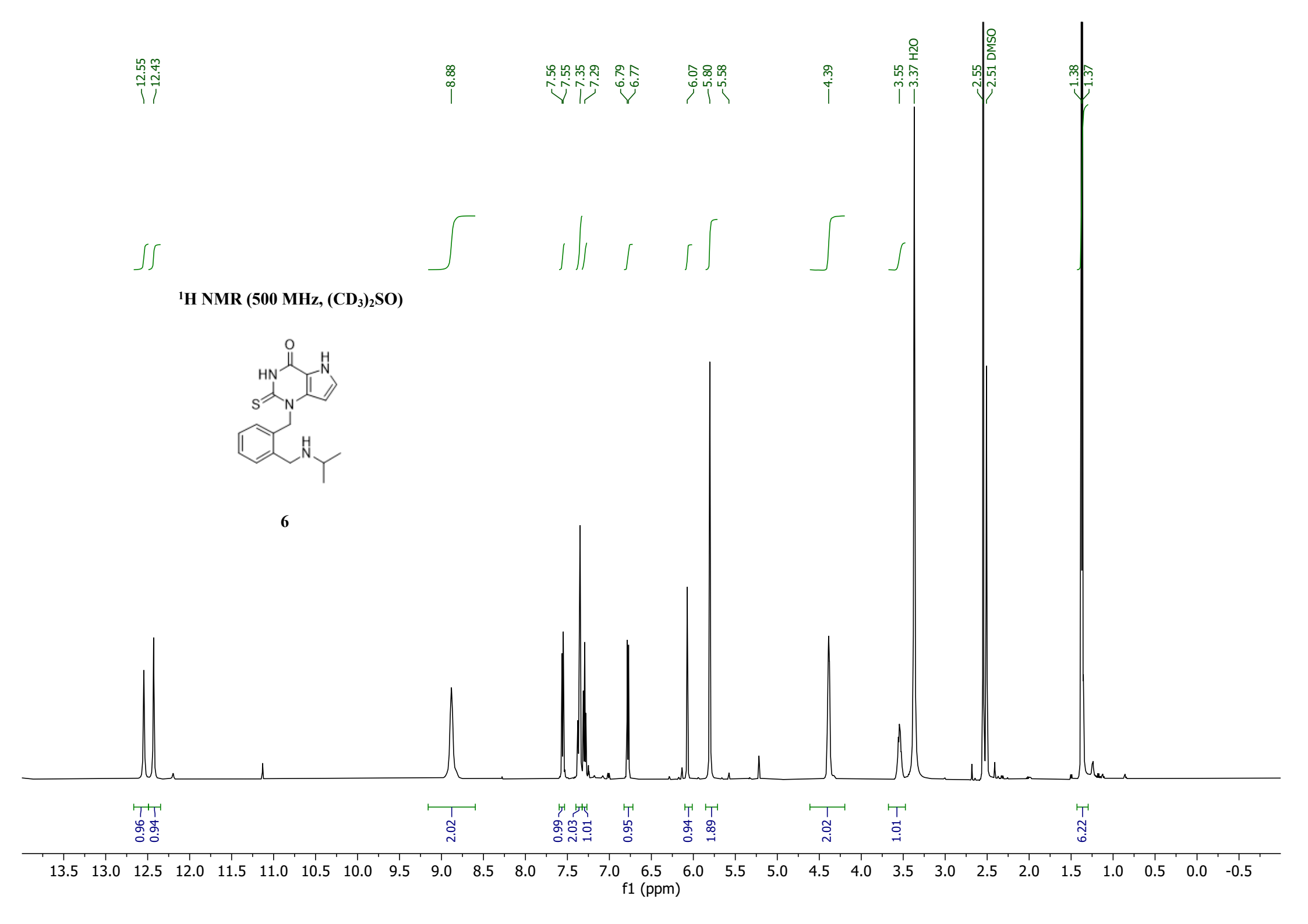
^{1}H NMR (400 MHz, $(CD_3)_2SO$)

6 (step 3)



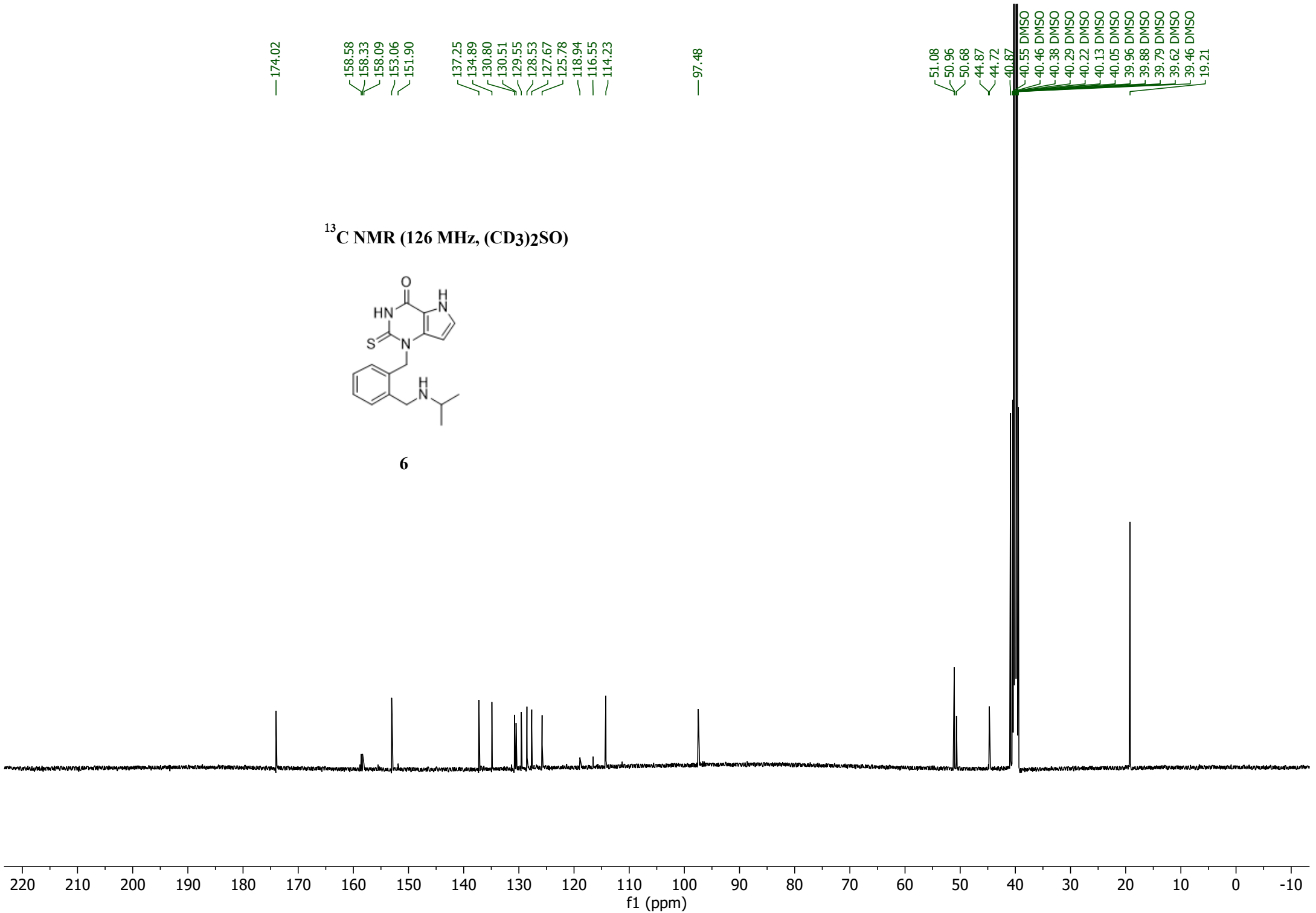
^{1}H NMR (400 MHz, $(CD_3)_2SO$)

6 (step 4)



^{1}H NMR (500 MHz, $(CD_3)_2SO$)

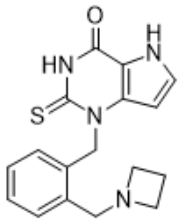
6



^{13}C NMR (126 MHz, $(CD_3)_2SO$)

6

^{1}H NMR (500 MHz, $(CD_3)_2SO$)



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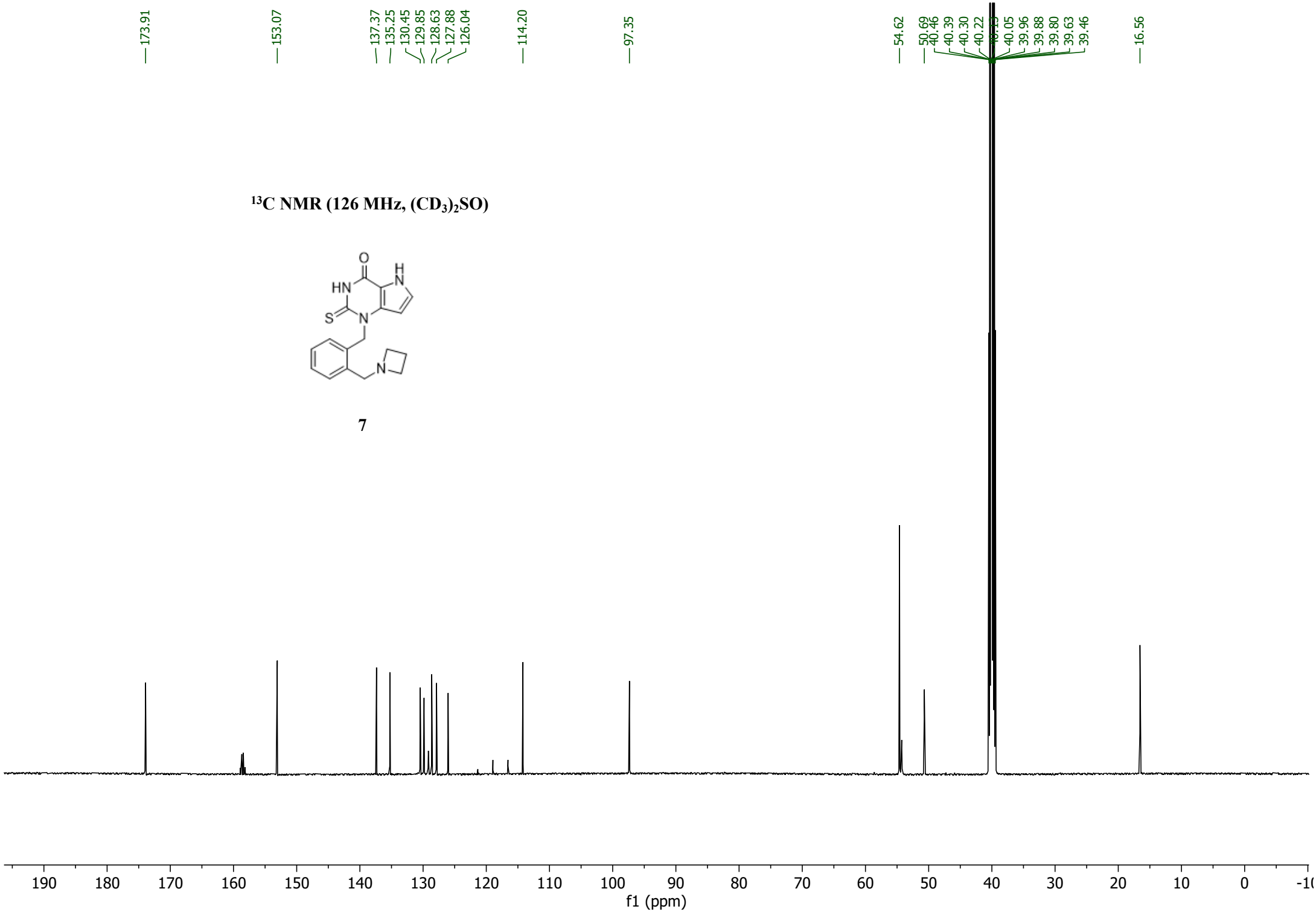
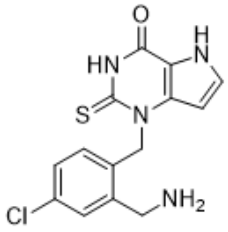


^{13}C NMR (126 MHz, $(CD_3)_2SO$)

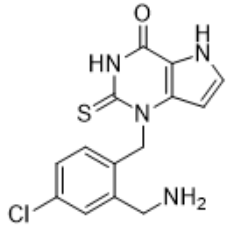
7

^{1}H NMR (500 MHz, $(CD_3)_2SO$)



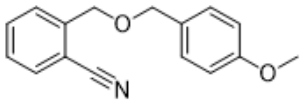
8

^{13}C NMR (126 MHz, $(CD_3)_2SO$)



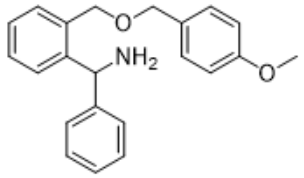
8

^{1}H NMR (500 MHz, $CDCl_3$)

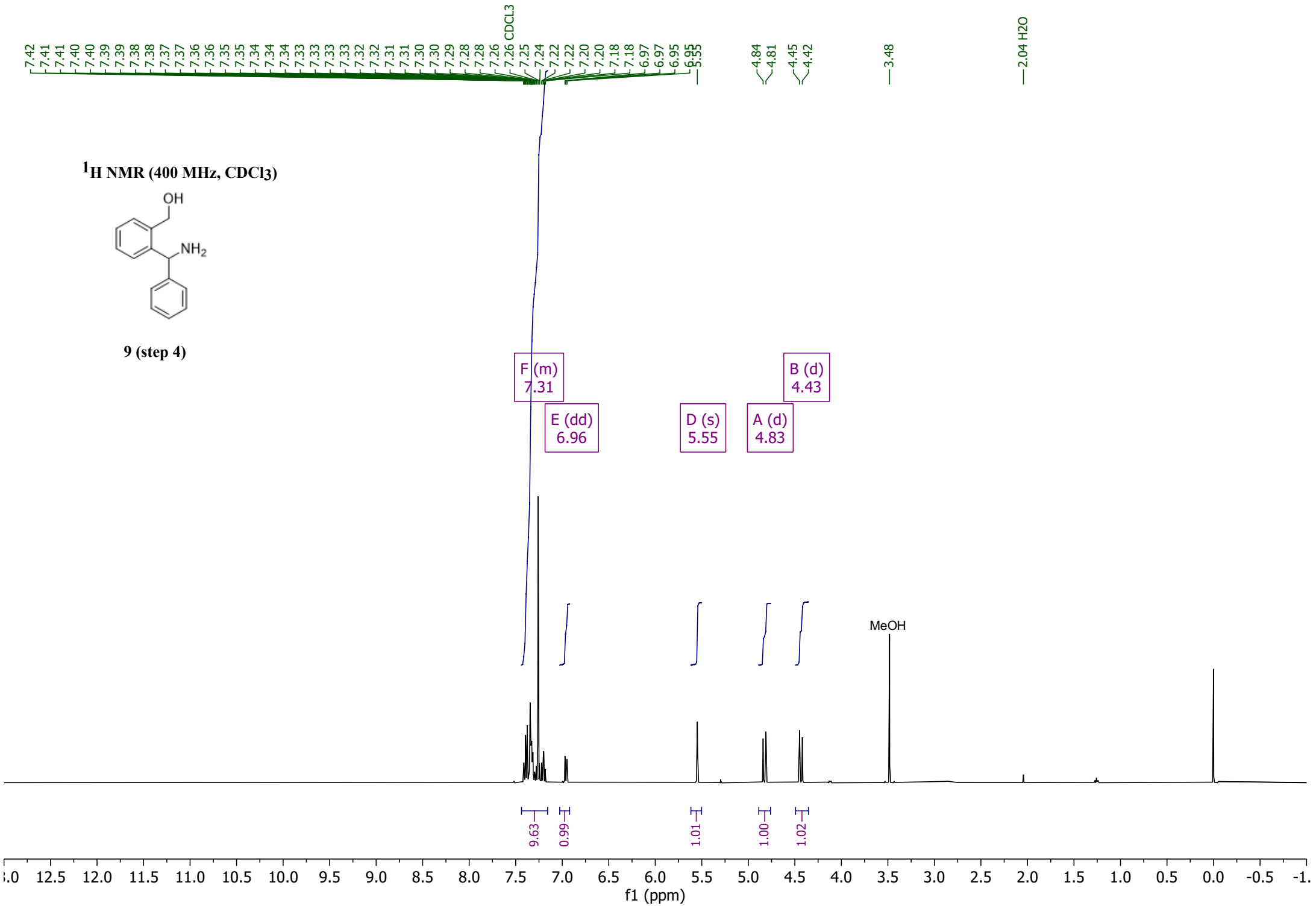


9 (step 1)

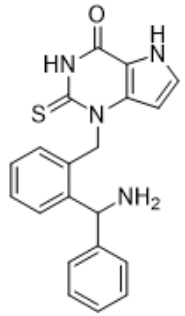
^{1}H NMR (400 MHz, $CDCl_3$)



9 (step 2)

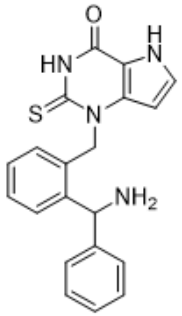

7.42 7.41 7.41 7.40 7.40 7.39 7.39 7.38 7.38 7.37 7.37 7.36 7.36 7.35 7.35 7.34 7.34 7.34 7.33 7.33 7.33 7.33 7.32 7.32 7.31 7.31 7.30 7.30 7.29 7.28 7.28 7.26 7.26 CDCL3 7.25 7.24 7.22 7.22 7.20 7.20 7.18 7.18 6.97 6.97 6.95 6.95 5.55
4.84 4.81 4.45 4.42
3.48
2.04 H2O
1H NMR (400 MHz, CDCl3)
OH
NH2
9 (step 4)
F (m) 7.31
E (dd) 6.96
D (s) 5.55
A (d) 4.83
B (d) 4.43
MeOH
9.63
0.99
1.01
1.00
1.02
f1 (ppm)

^{1}H NMR (500 MHz, $(CD_3)_2SO$)



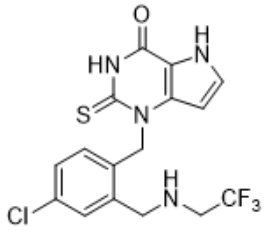
9

^{13}C NMR (126 MHz, $(CD_3)_2SO$)



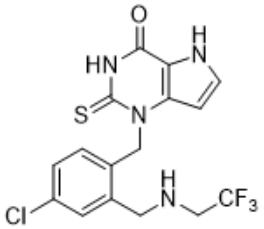
9

^{1}H NMR (500 MHz, $(CD_3)_2SO$)



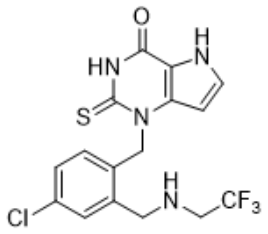
10

^{13}C NMR (126 MHz, $(CD_3)_2SO$)



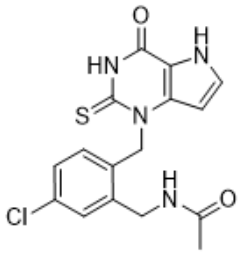
10

^{19}F NMR (471 MHz, $(CD_3)_2SO$)



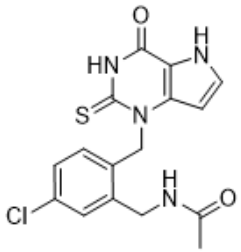
10

^{1}H NMR (500 MHz, $(CD_3)_2SO$)

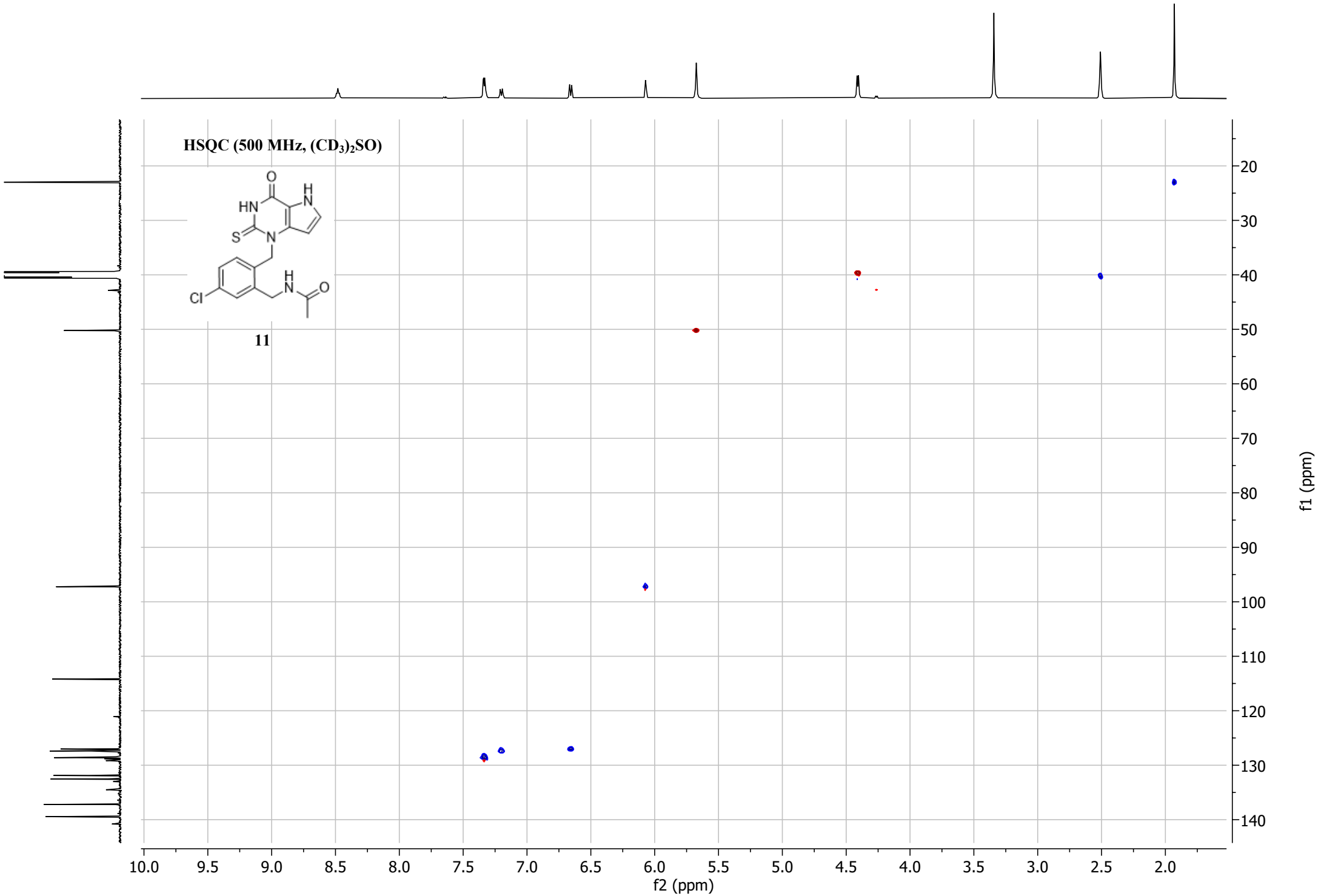


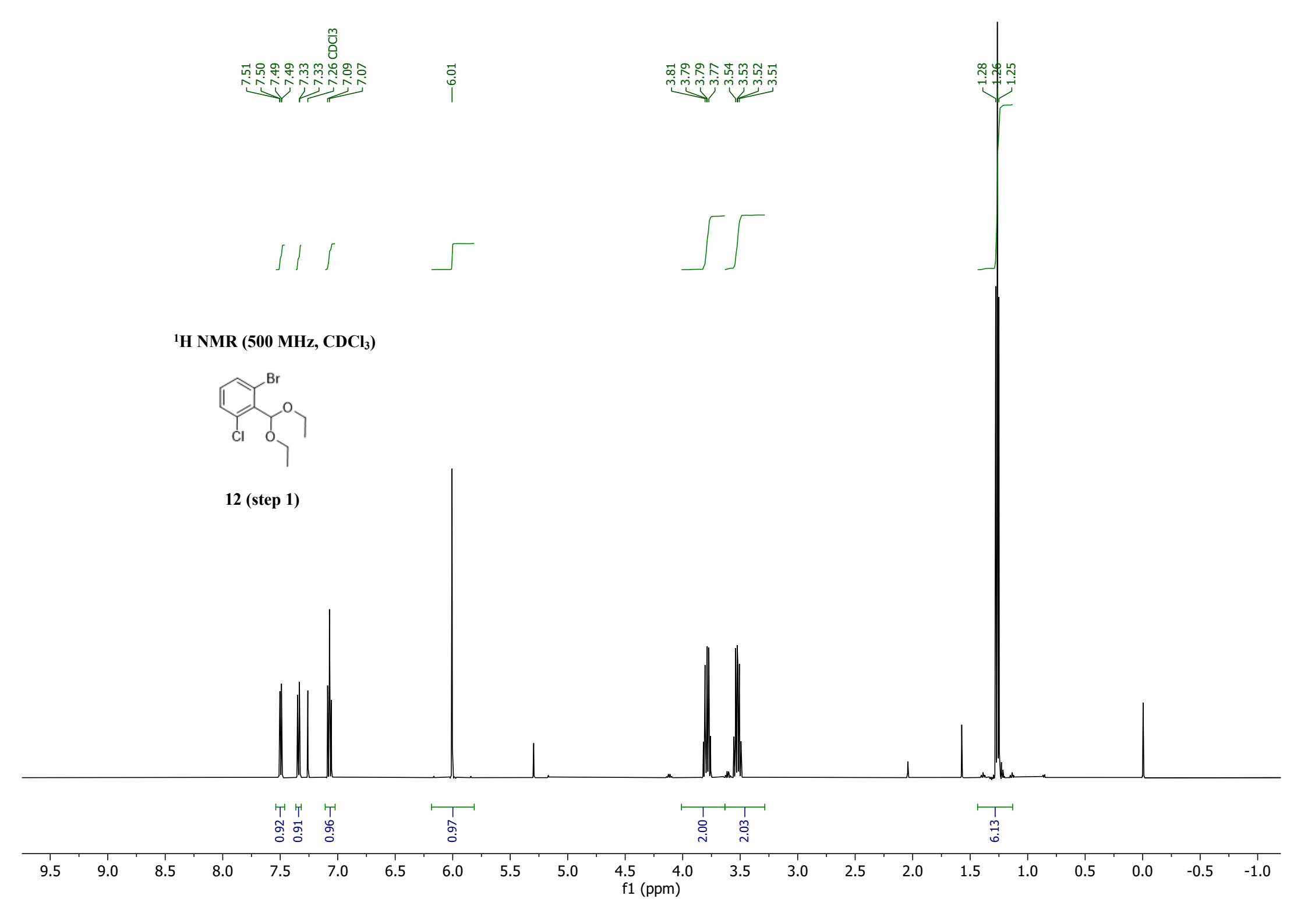
11

13C NMR (126 MHz, (CD3)2SO)



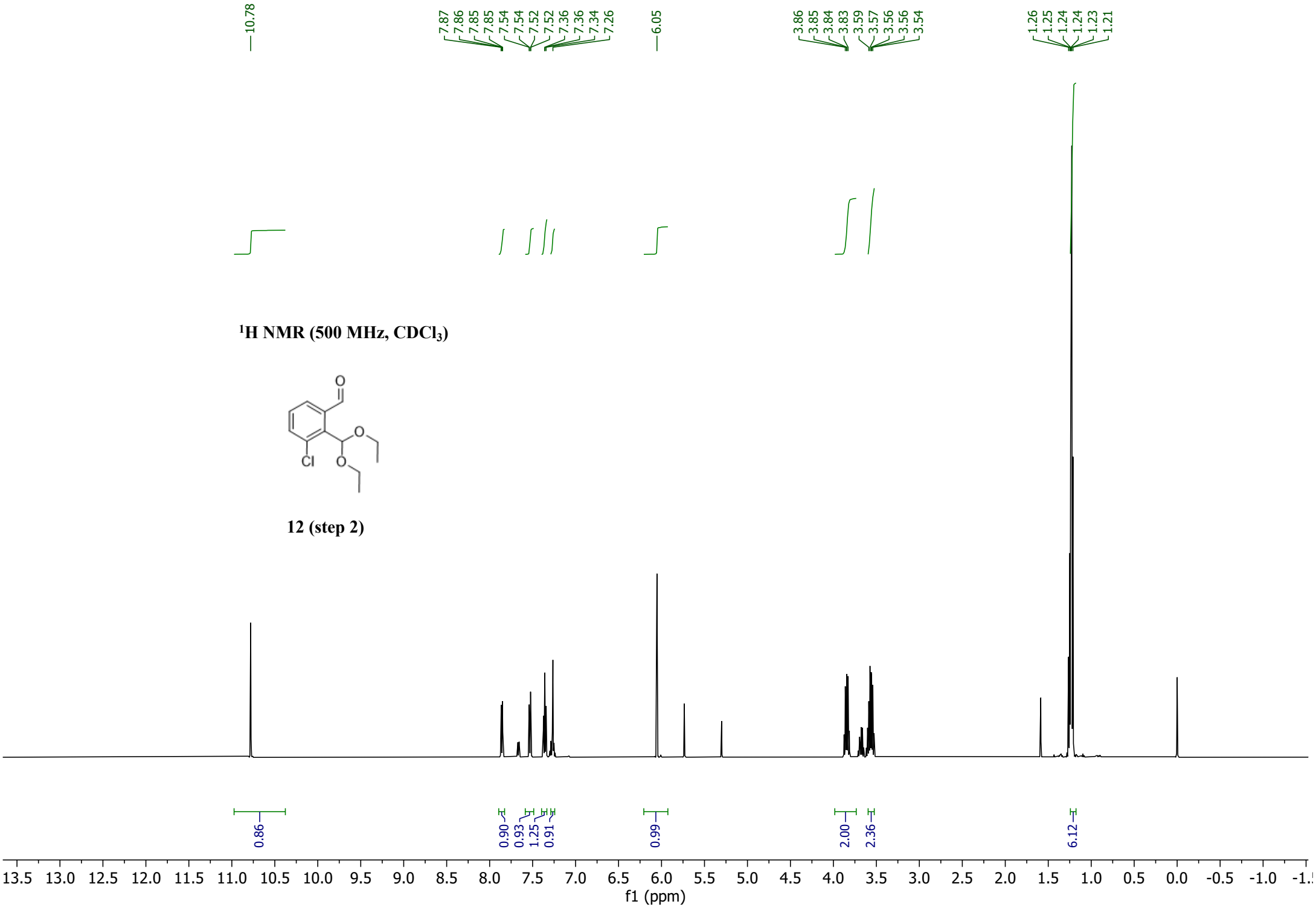
11


HSQC (500 MHz, (CD3)2SO)
O
HN
H
N
S
N
Cl
H
N
O
11
f2 (ppm)
f1 (ppm)
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
20
30
40
50
60
70
80
90
100
110
120
130
140



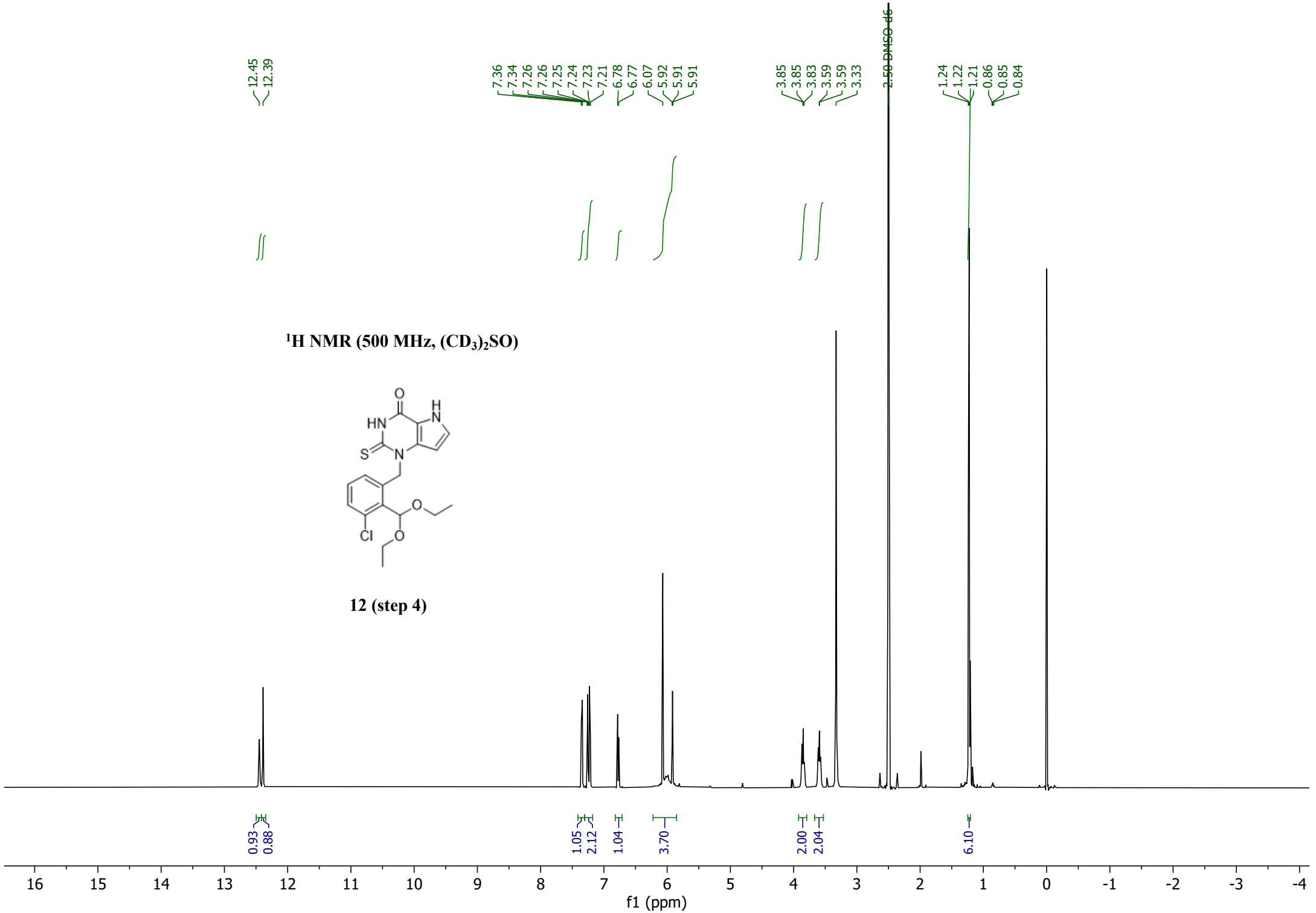
^{1}H NMR (500 MHz, $CDCl_3$)

12 (step 1)



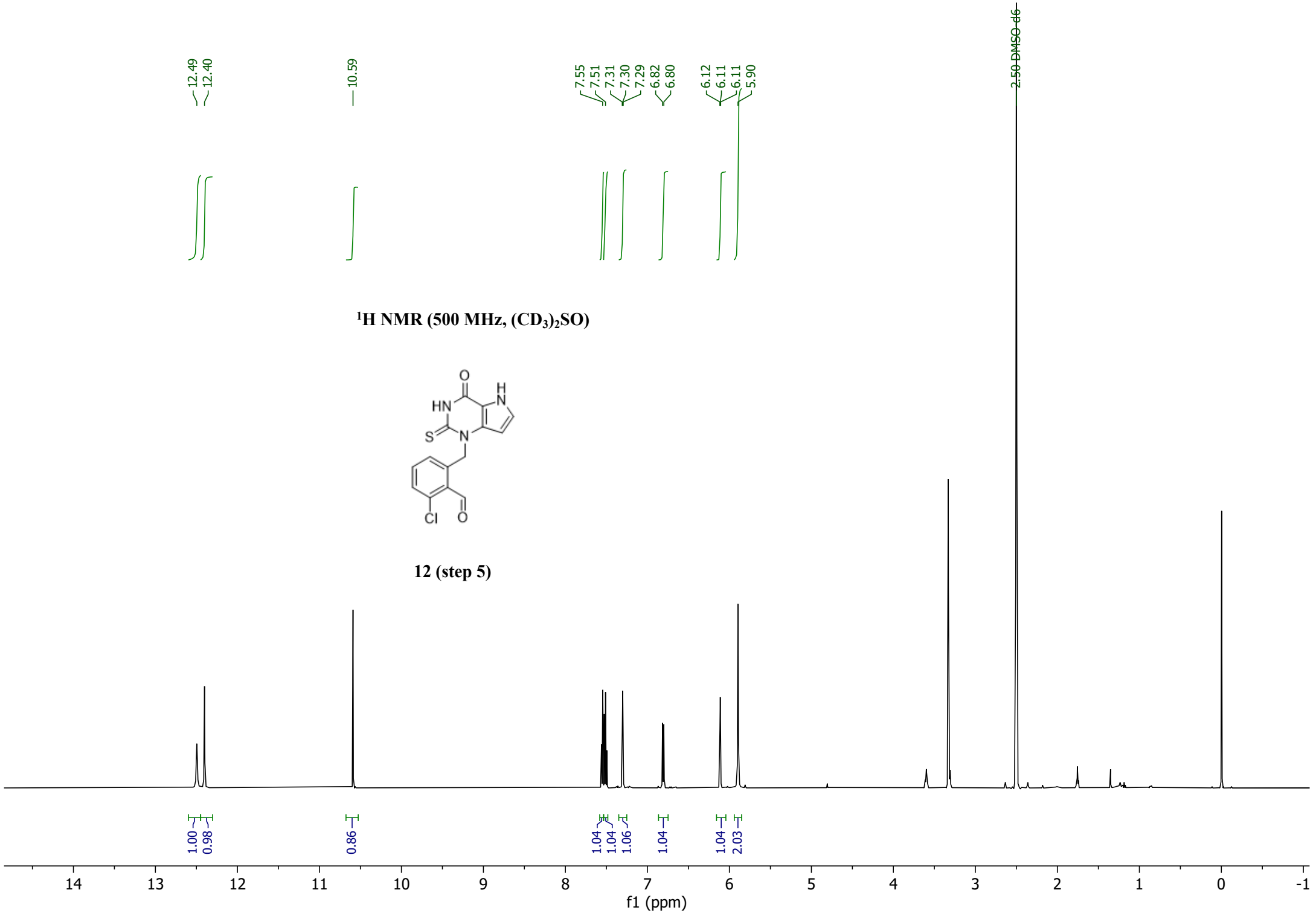
^{1}H NMR (500 MHz, $CDCl_3$)

12 (step 2)



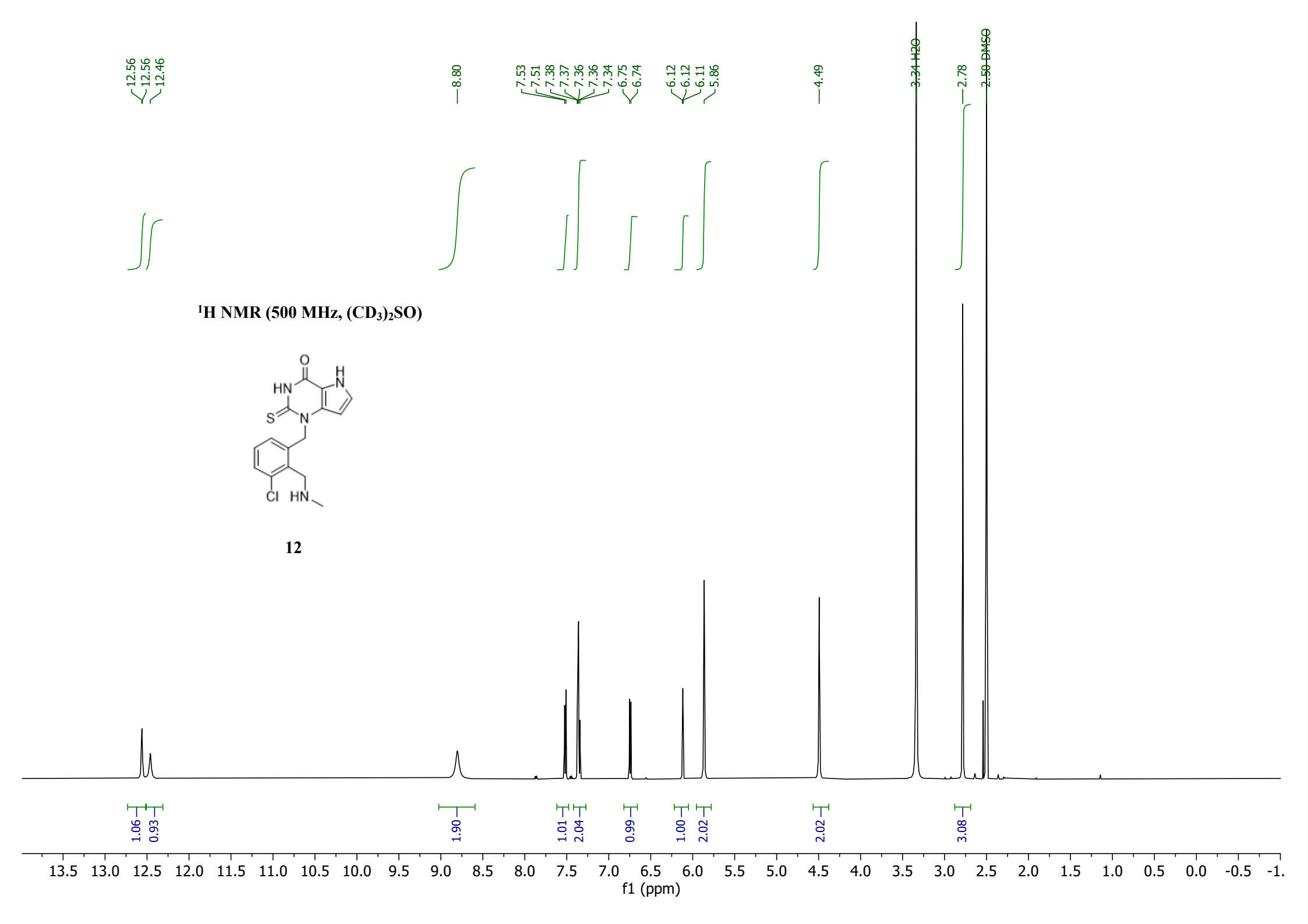
^{1}H NMR (500 MHz, $(CD_3)_2SO$)

12 (step 4)



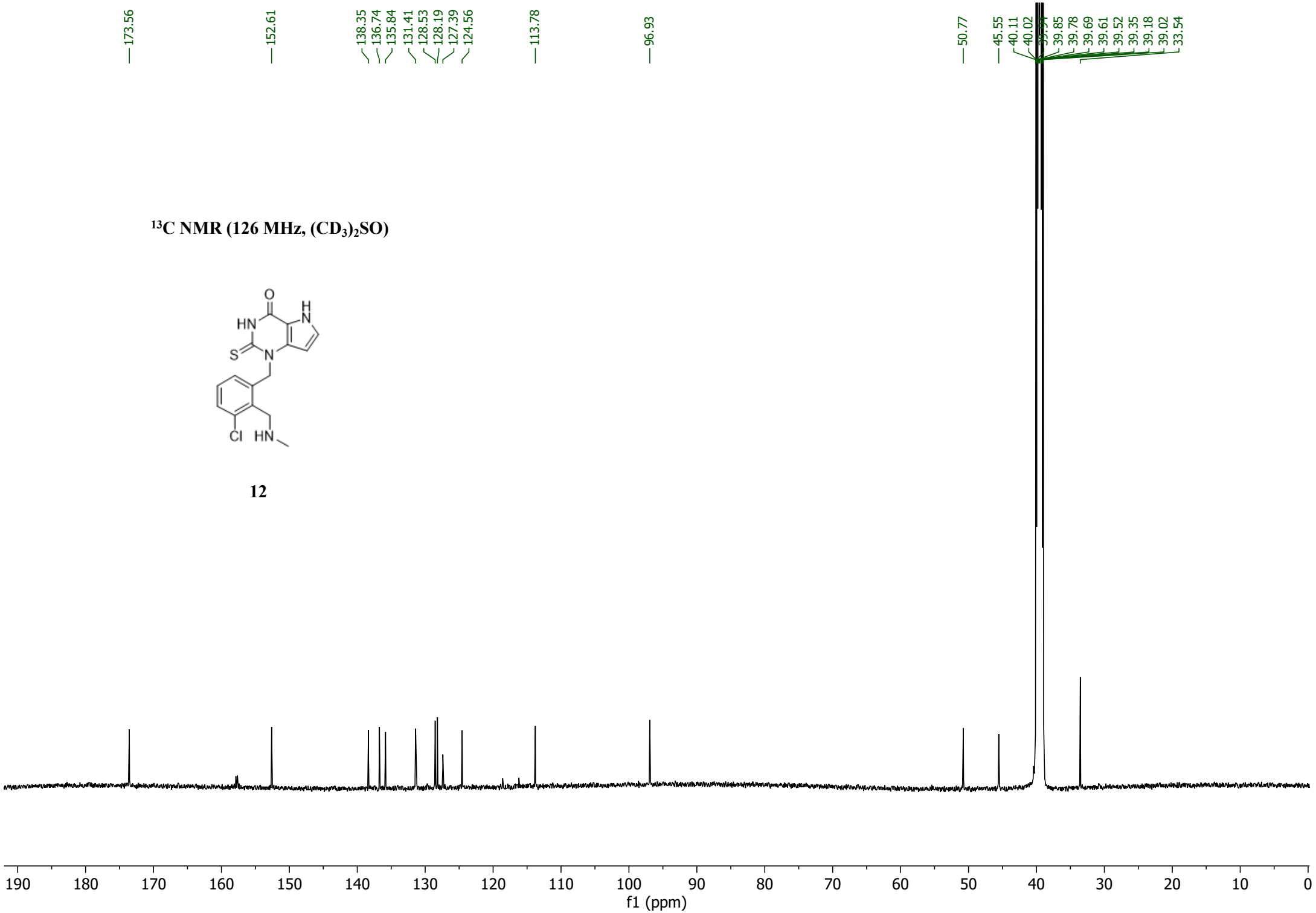
^{1}H NMR (500 MHz, $(CD_3)_2SO$)

12 (step 5)



^{1}H NMR (500 MHz, $(CD_3)_2SO$)

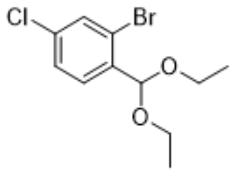
12



^{13}C NMR (126 MHz, $(CD_3)_2SO$)

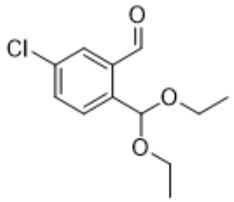
12

^{1}H NMR (400 MHz, $CDCl_3$)



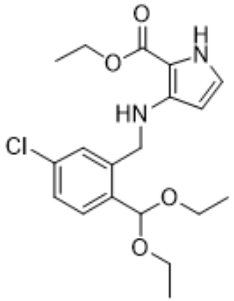
13 (step 1)

^{1}H NMR (400 MHz, $CDCl_3$)



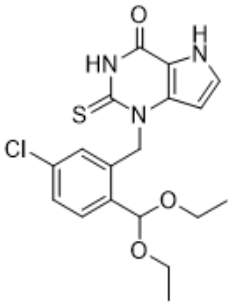
13 (step 2)

^{1}H NMR (400 MHz, $CDCl_3$)

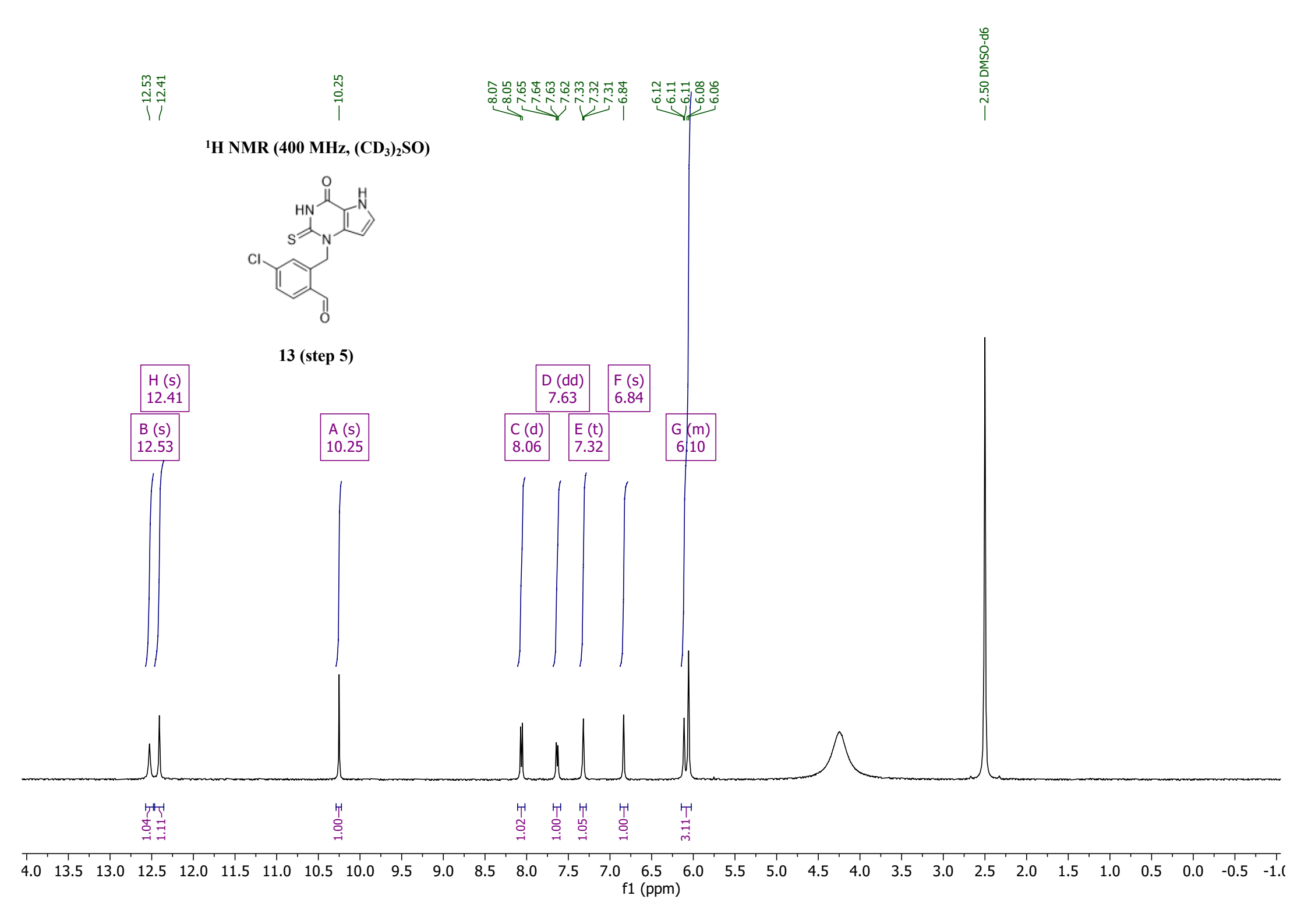


13 (step 3)

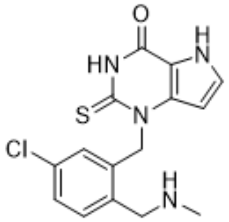
^{1}H NMR (400 MHz, $(CD_3)_2SO$)



13 (step 4)

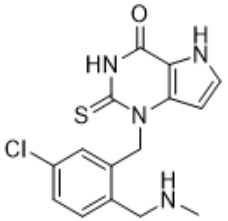

12.53
12.41
10.25
8.07
8.05
7.65
7.64
7.63
7.62
7.33
7.32
7.31
6.84
6.12
6.11
6.11
6.08
6.06
2.50 DMSO-d6
^{1}H NMR (400 MHz, $(CD_3)_2SO$)
O
HN
H
N
S
N
Cl
O
13 (step 5)
H (s)
12.41
B (s)
12.53
A (s)
10.25
D (dd)
7.63
F (s)
6.84
C (d)
8.06
E (t)
7.32
G (m)
6.10
1.04
1.11
1.00
1.02
1.00
1.05
1.00
3.11
4.0 13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

^{1}H NMR (400 MHz, $(CD_3)_2SO$)



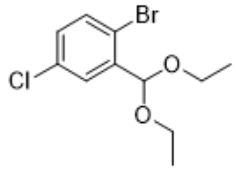
13

^{13}C NMR (126 MHz, $(CD_3)_2SO$)

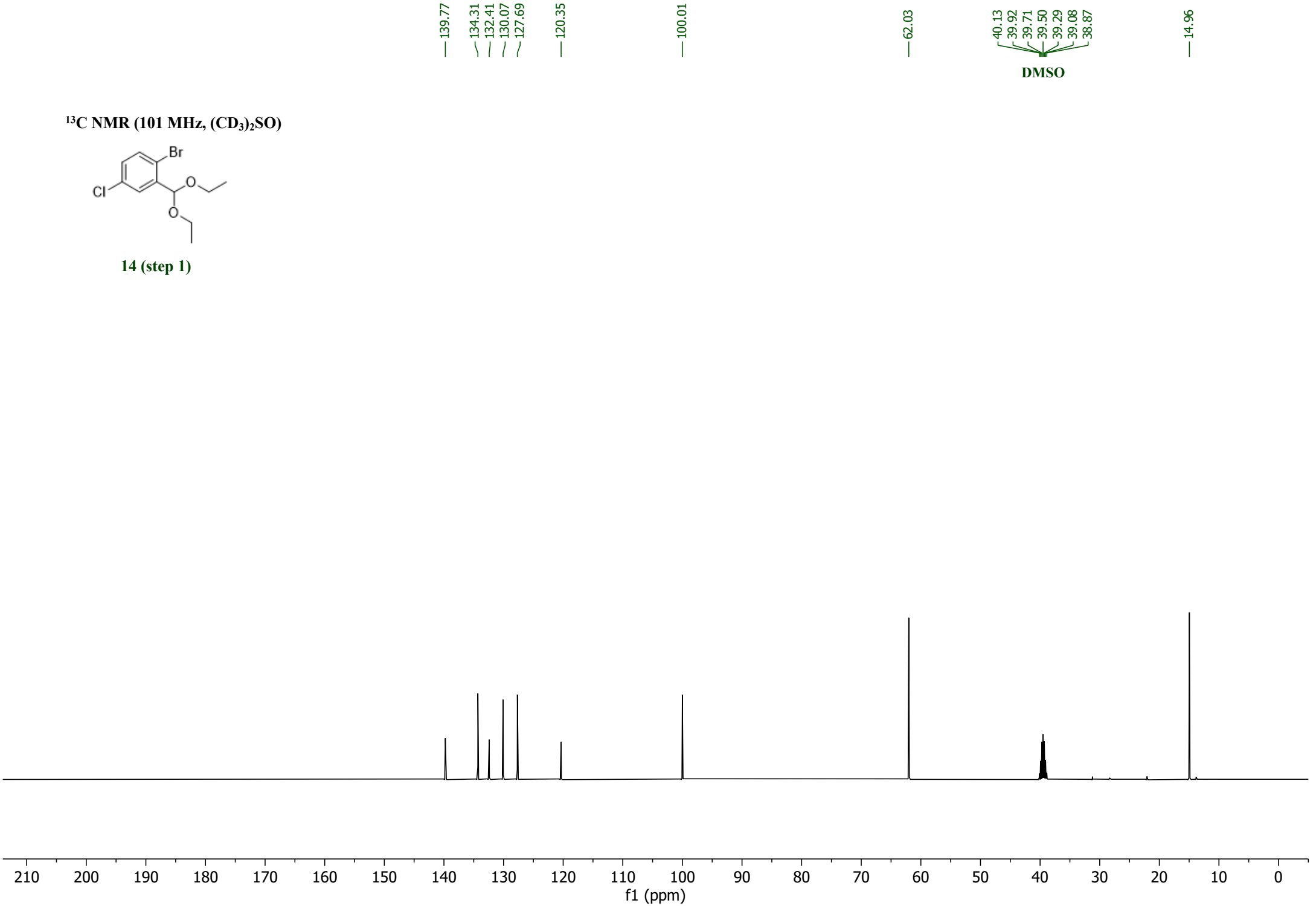


13

^{1}H NMR (400 MHz, $CDCl_3$)



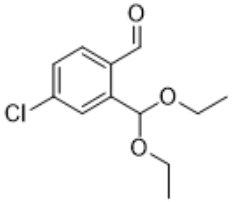
14 (step 1)



^{13}C NMR (101 MHz, $(CD_3)_2SO$)

14 (step 1)

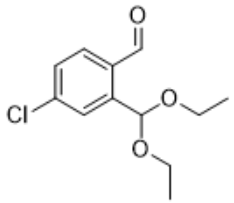
^{1}H NMR (400 MHz, $CDCl_3$)



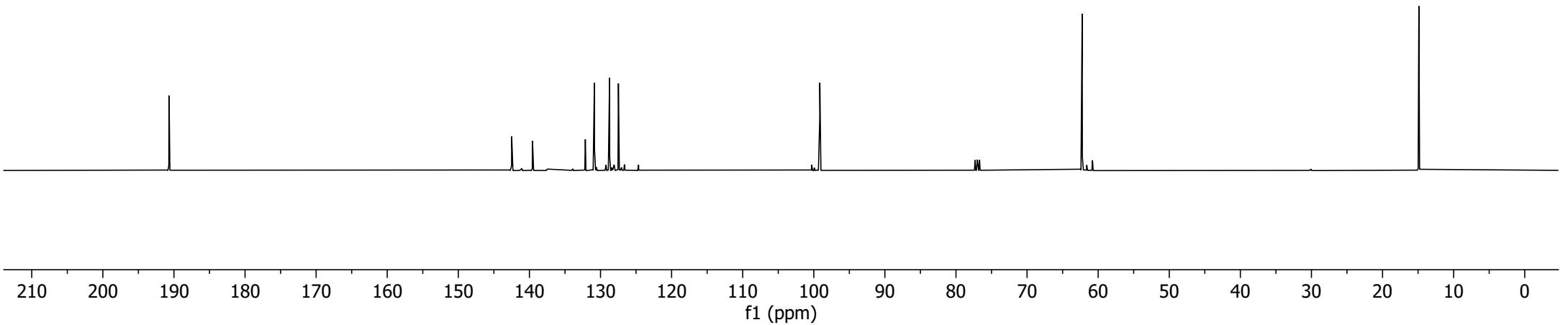
14 (step 2)

190.69
142.49
139.56
132.14
130.86
128.72
127.48
99.15
77.32
77.00
76.68
62.25
14.89
$CDCl_3$

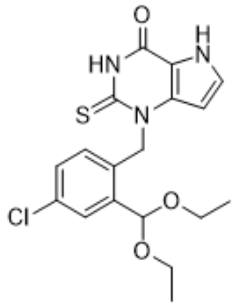
^{13}C NMR (101 MHz, $CDCl_3$)



14 (step 2)

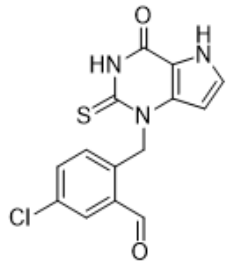


^{1}H NMR (600 MHz, $(CD_3)_2SO$)



14 (step 4)

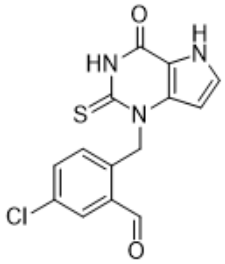
^{1}H NMR (600 MHz, $(CD_3)_2SO$)



14 (step 5)

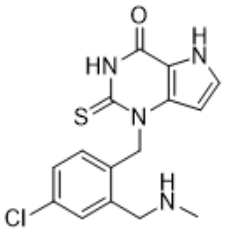
192.98
192.92
173.66
152.78
136.89
136.01
135.04
133.59
133.20
132.38
128.22
128.01
113.89
96.79
50.66
39.92 DMSO
39.78 DMSO
39.64 DMSO
39.50 DMSO
39.36 DMSO
39.22 DMSO
39.08 DMSO

^{13}C NMR (151 MHz, $(CD_3)_2SO$)

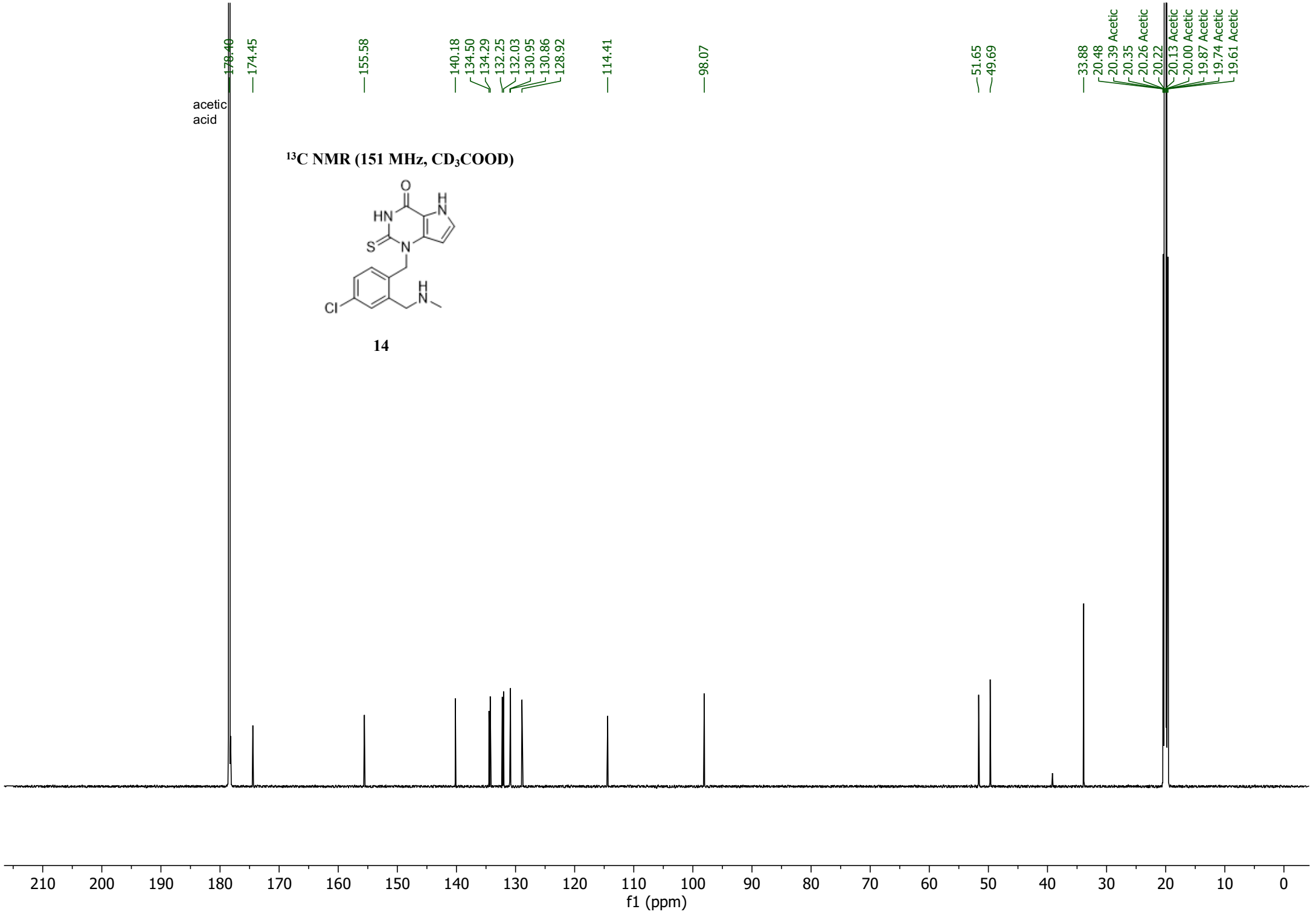


14 (step 5)

^{1}H NMR (600 MHz, CD_3COOD)



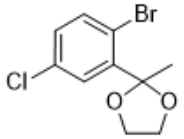
14



^{13}C NMR (151 MHz, CD_3COOD)

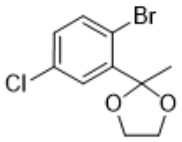
14

^{1}H NMR (500 MHz, $CDCl_3$)



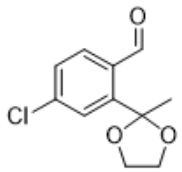
15 (step 1)

^{13}C NMR (126 MHz, $CDCl_3$)



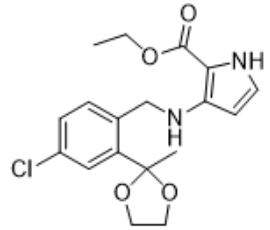
15 (step 1)

^{1}H NMR (500 MHz, $CDCl_3$)



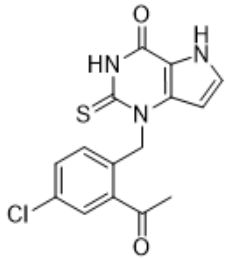
15 (step 2)

^{1}H NMR (400 MHz, $CDCl_3$)



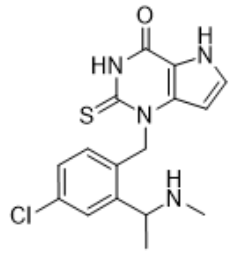
15 (step 3)

^{1}H NMR (500 MHz, , $(CD_3)_2SO$)



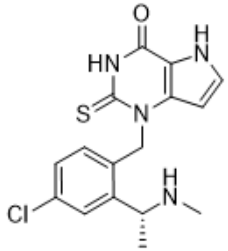
15 (step 5)

^{1}H NMR (500 MHz, $(CD_3)_2SO$)



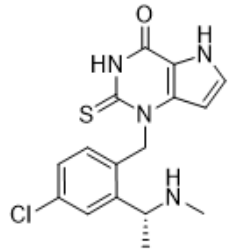
15 (step 6)

^{1}H NMR (500 MHz, $(CD_3)_2SO$)



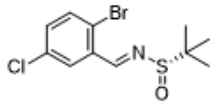
15

^{13}C NMR (126 MHz, $(CD_3)_2SO$)



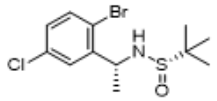
15

^{1}H NMR (400 MHz, $(CD_3)_2SO$)



16 (X1)

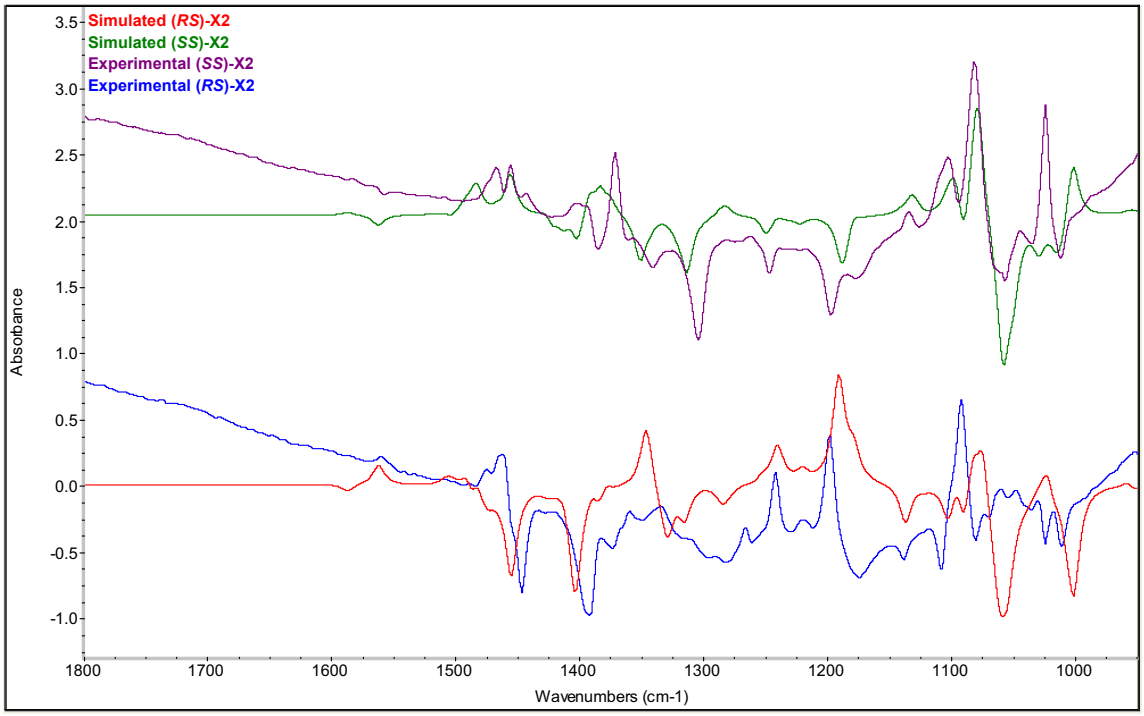
^{1}H NMR (400 MHz, $(CD_3)_2SO$)

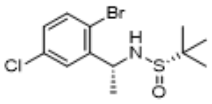


16 (X2)

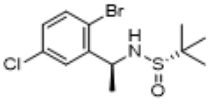
Compound 16 (X2) - Vibrational Circular Dichroism (VCD)

Experimental spectra of (*SS*)-X2 (purple) and (*RS*)-X2 (blue) and simulated spectra of the (*RS*)-X2 (red) and (*SS*)-X2 (green).



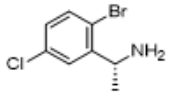


(*RS*)-X2



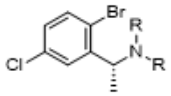
(*SS*)-X2

^{1}H NMR (400 MHz, $CDCl_3$)



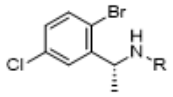
16 (Y)

^{1}H NMR (400 MHz, $(CD_3)_2SO$)



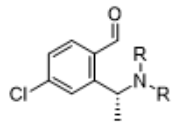
16 (Y1 (R=Boc))

^{1}H NMR (400 MHz, $CDCl_3$)



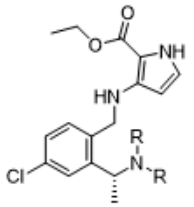
16 (Y1 (R=Boc))

^{1}H NMR (400 MHz, $(CD_3)_2SO$)



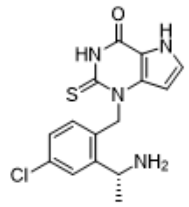
16 (Y2 (R=Boc))

^{1}H NMR (400 MHz, $(CD_3)_2SO$)



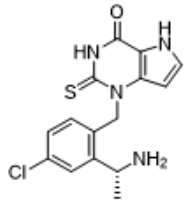
16 (Z (R=Boc))

^{1}H NMR (400 MHz, $(CD_3)_2SO$)



16

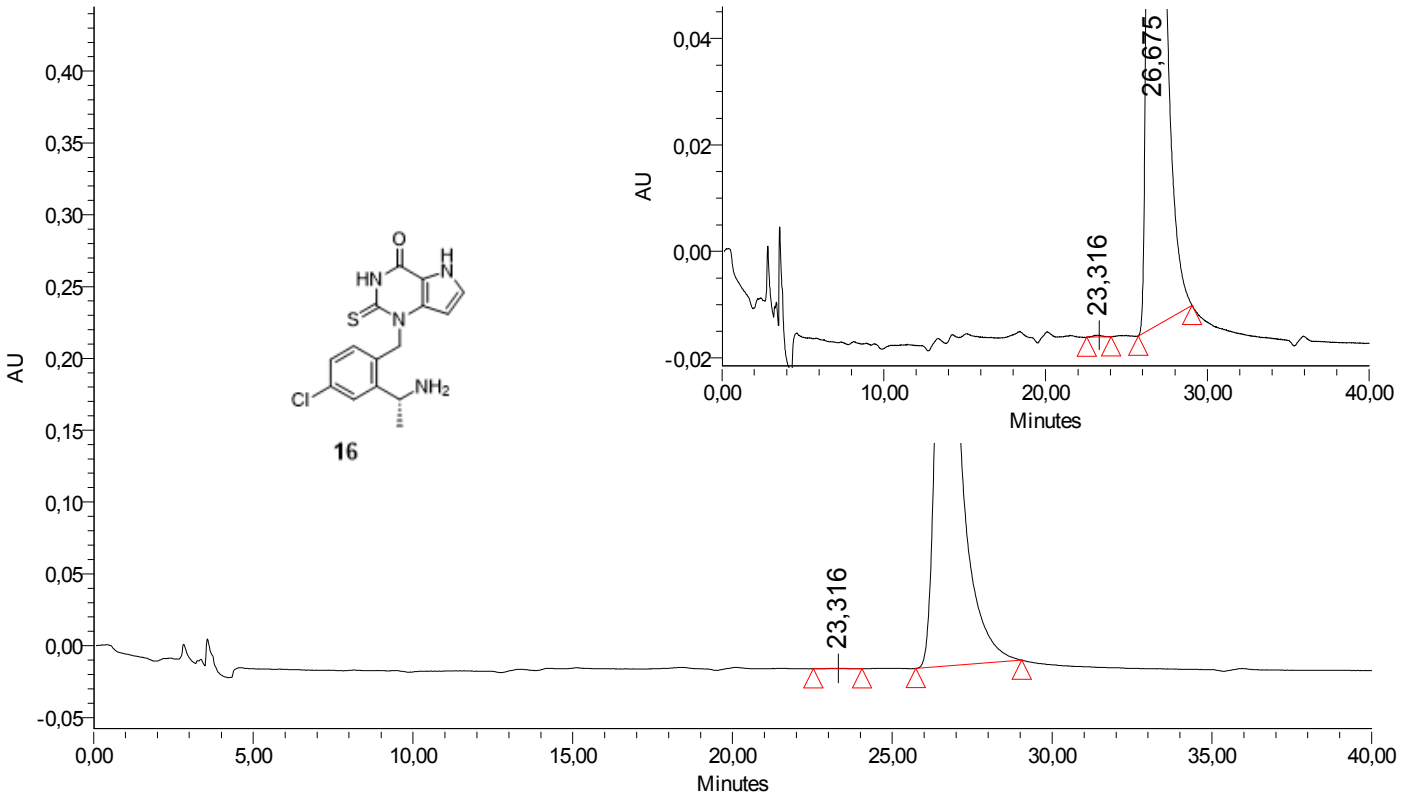
^{13}C NMR (151 MHz, $(CD_3)_2SO$)



16

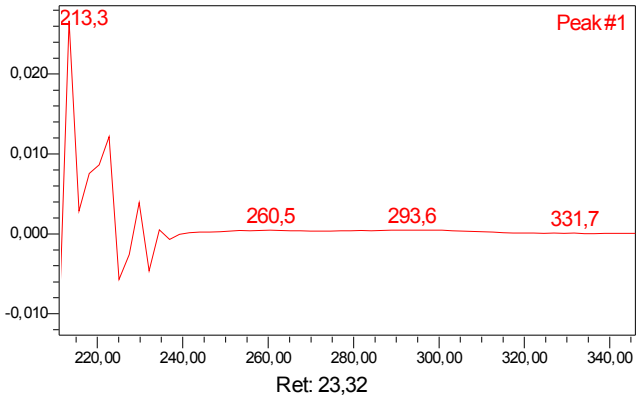
Compound 16 - ee and optical rotation

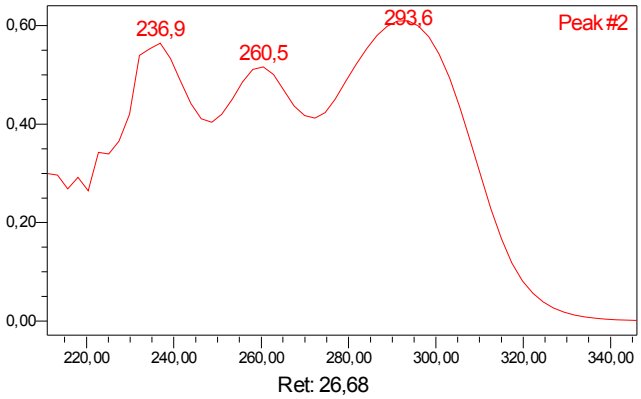
Column:	Chiralpak IA	**Column ID:**	x
Mobile Phase A:	Hep/(DCM/MeOH 95/5)/TEA 60/40/0.1	**Column Dimension:**	250 * 4.6
Mobile Phase B:		**Particle Size:**	5
Gradient:		**Injection volume:**	5,00 ul
		Flow:	1 ml/min
Temperature:	RT	**Wavelength:**	PDA 270,0 nm
Sample Concentration:	sample in MeOH	**Vial:**	81



	Retention Time	Area	% Area	k'	N	USP Resolution
1	23,316	12389	0,06	0,000	7165,4	
2	26,675	19998868	99,94	0,144	8257,9	3,303

ee = 99,9

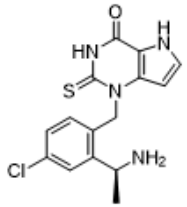




Characterization

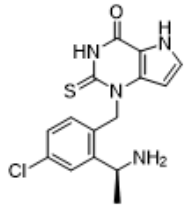
Sample Id	Optical Rotation	Wavelength (nm)	Solvent	Conc. (g/100ml)	Temp. (C)
Compound 16	+76.8	589	MeOH	0.3	20

^{1}H NMR (400 MHz, $(CD_3)_2SO$)



17

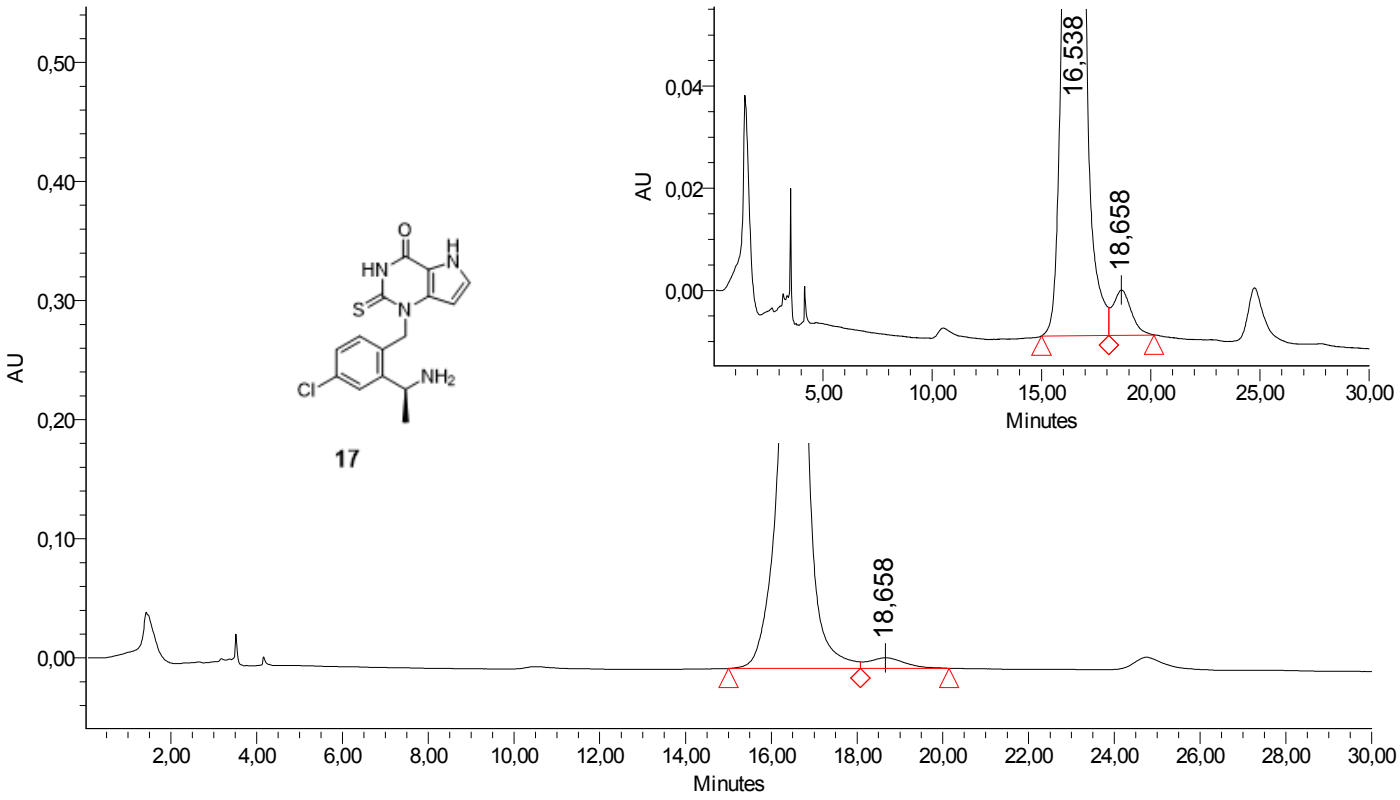
^{13}C NMR (126 MHz, $(CD_3)_2SO$)



17

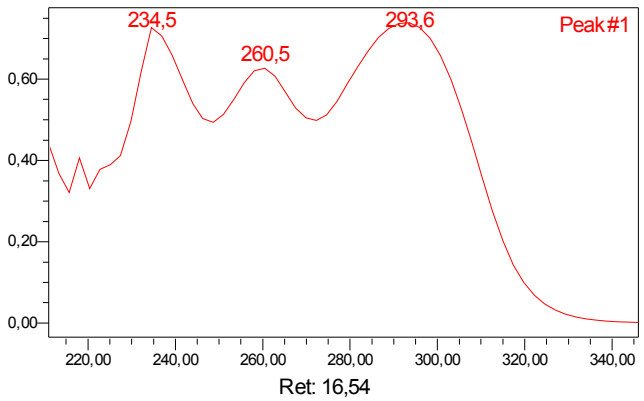
Compound 17 - ee and optical rotation

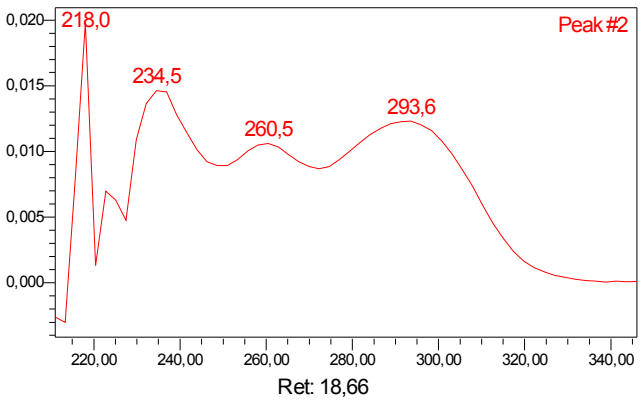
Column:	Chiralpak IA	**Column ID:**	x
Mobile Phase A:	Hep/(DCM/MeOH 95/5)/TEA 60/40/0.1	**Column Dimension:**	250 * 4.6
Mobile Phase B:		**Particle Size:**	5
Gradient:		**Injection volume:**	10,00 ul
		Flow:	1 ml/min
Temperature:	rt	**Wavelength:**	PDA 270,0 nm
Sample Concentration:	sample in MeOH/ACN 1/1	**Vial:**	80



	Retention Time	Area	% Area	k'	N	USP Resolution
1	16,538	20518416	97,53	0,000	4449,7	
2	18,658	520093	2,47	0,128		

ee = 95,1

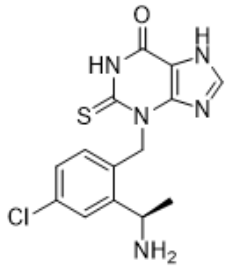




Characterization

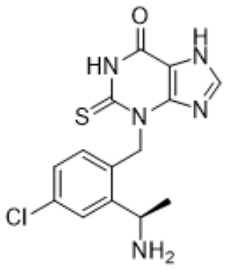
Sample Id	Optical Rotation	Wavelength (nm)	Solvent	Conc. (g/100ml)	Temp. (C)
Compound 17	-54.8	589	MeOH	0.5	20

^{1}H NMR (400 MHz, $(CD_3)_2SO$)



18

^{13}C NMR (126 MHz, $(CD_3)_2SO$)



18