Fragment-sized EthR inhibitors exhibit exceptionally strong ethionamide boosting effect in whole cell *Mycobacterium tuberculosis* assays

Supplementary Information

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Molecular Biology

The EthR gene was cloned into a pHAT5 vector[1] using BamHI and EcoRI restriction sites. *Escherichia coli* BL21 (DE3) (Novagen) strain was used for EthR expression. Fresh overnight starting culture (25 mL) grown in LB media overnight at 37 °C, 220 rpm was added to LB media (1L) and incubated at 37°C until the colony reached OD_{600} ~0.8. The culture was then induced with IPTG (1mM) and incubated at 37 °C, 220 rpm, for an additional 3 h. The cells were harvested by centrifugation (4200 *g* for 20 min at 4 °C) and re-suspended in lysis buffer [50 mM Hepes (pH 7.5) and 150 mM NaCl; 25 mL] supplemented with EDTA-free complete protease inhibitor cocktail (Roche). The cells were lysed by sonication (10 pulses of 30 s each) and debris was removed by centrifugation (35000 *g* for 1 h at 4 °C). The supernatant was loaded onto a 5 mL HiTrap IMAC Fast Flow column (GE Healthcare) charged with Ni^{2+} at 5 mL/min rate and washed with buffer (50 mM Hepes pH 7.5, 150 mM NaCl and 20 mM imidazole; 50 mL). After elution with elution buffer (50 mM Hepes pH 7.5, 150 mM NaCl and 250 mM imidazole), the protein was further purified by size exclusion chromatography (Superdex 200) and concentrated (4200 *g* at 4 °C) using 10 kDa Amicon® Ultra concentrators.

X-ray crystallography

Crystallisation of EthR was performed using the sitting-drop vapour diffusion method at 25 °C. A drop consisted of 1.0µL of reservoir solution (1.7–2.1 M ammonium sulphate, 0.1 M MES-Na (pH 6–7), 5-15% (v/v) glycerol and 7–12% (v/v) 1,4-dioxane) and 0.5–1.0 µL of protein solution (20 mg/mL EthR, 0.5M NaCl, 15mM Tris/HCl pH 8.0 and 10% (v/v) glycerol).[2] Compounds (100mM in DMSO) were mixed with mother liquor (1.9 M ammonium sulphate, 0.1 M MES-Na pH 6.5 and 12.5% (v/v) glycerol) to a final concentration of 1–10 mM. EthR crystals were washed free from 1,4-dioxane by placing them in 1,4-dioxane free mother liquor for a few hours. The washed EthR crystals were then transferred to the fragment-containing solutions and incubated for 1–16 h. Crystals were cryoprotected by passing them briefly through mother liquor containing 20% (v/v) of ethylene glycol and then flash-frozen in liquid nitrogen. X-ray crystallographic datasets were collected at the European Synchrotron Radiation Facility (Grenoble, France) and at the Diamond Light Source (Harwell, UK). X-ray datasets were indexed and integrated using autoPROC[3], XDS[4] and Mosfilm[5]. The scaling of datasets was carried out using SCALA/AIMLESS software.[6] Structures were solved using the molecular replacement method with PHASER[7] (PDB ID 1T56 was used as search probe). Structures were further refined with Refmac5[8] (part of CCP4[9] suite), PHENIX[10] or BUSTER[11] to satisfactory level of R/Rfree using maximum-likelihood restrained refinement. Ligand restrain files were prepared by Dundee PRODRG2 server,[12] libcheck[13] or PHENIX elbow software. Every structure was modelled manually in Coot[14] (including ligand and essential water molecules). Images of X-ray crystal structures in figures were prepared using PyMOL (http://www.pymol.org).

Figure S1 X-ray crystallography data collection and final refinement statistics

X-ray crystallography data collection and final refinement statistics
*Parameters shown in brackets are for the highest resolution shell

CMPD#	01	03	04	05	07	08	31	09	10
PDB ID	5F1J	5IOY	5IOZ	5J3L	5IPA	5J1R	5J1Y	5J1U	5IP6
Data collection*									
Space group	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$
Cell parameters:									
a [Å]	121.81	121.35	121.83	121.13	122.35	122.40	121.29	121.38	120.81
b [Å]	121.81	121.35	121.83	121.13	122.35	122.40	121.29	121.38	120.81
c [Å]	33.73	33.85	33.80	34.05	33.71	33.68	33.68	33.72	33.74
α=β=γ=90°									
Resolution range [Å]	43.07–1.63 (1.67–1.63)	42.90–1.77 (1.82–1.77)	40.61–2.02 (2.07–2.02)	60.56–1.66 (1.70–1.66)	86.63–1.73 (1.78–1.73)	122.29–1.84 (1.89–1.84)	85.90–1.79 (1.84–1.79)	85.84–1.79 (1.84–1.79)	85.73–1.91 (1.96–1.91)
No. of observations									
total	424257 (20912)	321676 (23225)	215947 (14901)	396935 (30008)	346609 (26308)	280831 (22092)	308594 (22106)	307350 (22075)	252285 (19291)
unique	32327 (2352)	25363 (1833)	17308 (1228)	30632 (2258)	24595 (1793)	19631 (1433)	22833 (1683)	24439 (1751)	18743 (1381)
R_{merge}	0.065(0.700)	0.075 (0.823)	0.129 (0.946)	0.051 (0.682)	0.064 (0.724)	0.112 (0.949)	0.127 (1.741)	0.069 (0.793)	0.100 (1.207)
I/σ(I)	23.0 (2.9)	24.7 (3.9)	18.2 (3.7)	29.7 (4.1)	17.0 (2.7)	13.8 (3.4)	11.7 (2.5)	22.5 (3.8)	14.7 (2.7)
Completeness [%]	99.8 (99.8)	99.9 (99.9)	99.8 (99.0)	99.9 (99.7)	99.9 (99.8)	100 (100)	100 (100)	99.9 (99.9)	99.7 (99.8)
Multiplicity	13.1 (8.9)	12.7 (12.7)	12.5 (12.1)	13.0 (13.3)	12.8 (13.0)	12.4 (13.1)	12.8 (12.8)	12.6 (12.6)	12.8 (13.0)
Refinement									
Refinement program	REFMAC	REFMAC	REFMAC	REFMAC	REFMAC	REFMAC	REFMAC	REFMAC	REFMAC
Resolution [Å]	33.78–1.63	42.90–1.77	31.46–2.02	54.17–1.66	26.59–1.78	29.69–1.92	29.45–1.81	26.51–1.80	29.45–1.93
No. reflections	32230	24017	16392	29039	24031	19181	22310	22819	18331
R_{work}/R_{free} [%]	19.7/22.8	18.4/21.7	19.6/23.5	20.2/22.2	18.5/20.4	19.0/21.8	21.3/25.2	19.8/23.4	21.3/23.8
RMS deviations									
Bonds [Å]	0.030	0.025	0.022	0.028	0.027	0.023	0.025	0.021	0.022
Angles [°]	2.27	2.47	1.92	2.36	2.47	2.01	2.15	1.93	2.07
Ramachandran									
Favoured [%]	98	99	98	97	99	99	98	99	100
Outliers [%]	1	0	0	0	0	0	0	0	0

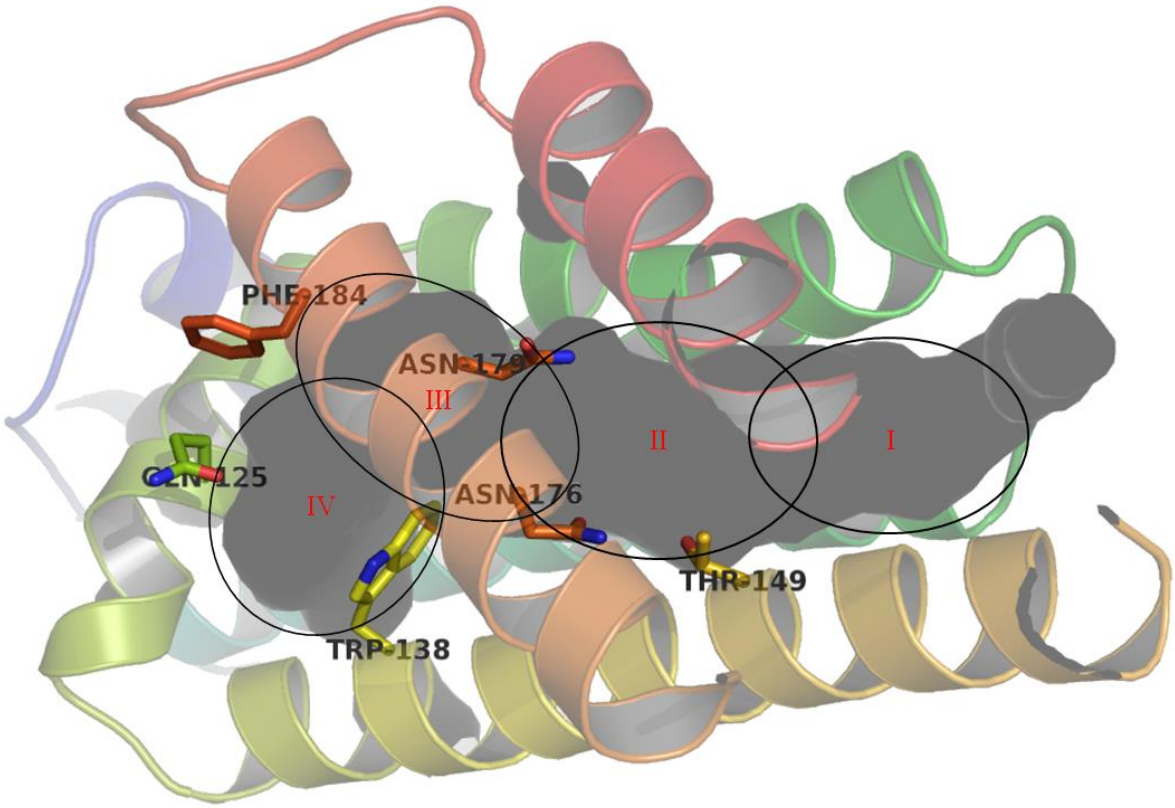


Figure S2 X-ray crystal structure of EthR monomer and schematic division of the EthR binding cavity into four sub-pockets denoted I, II, III and IV.

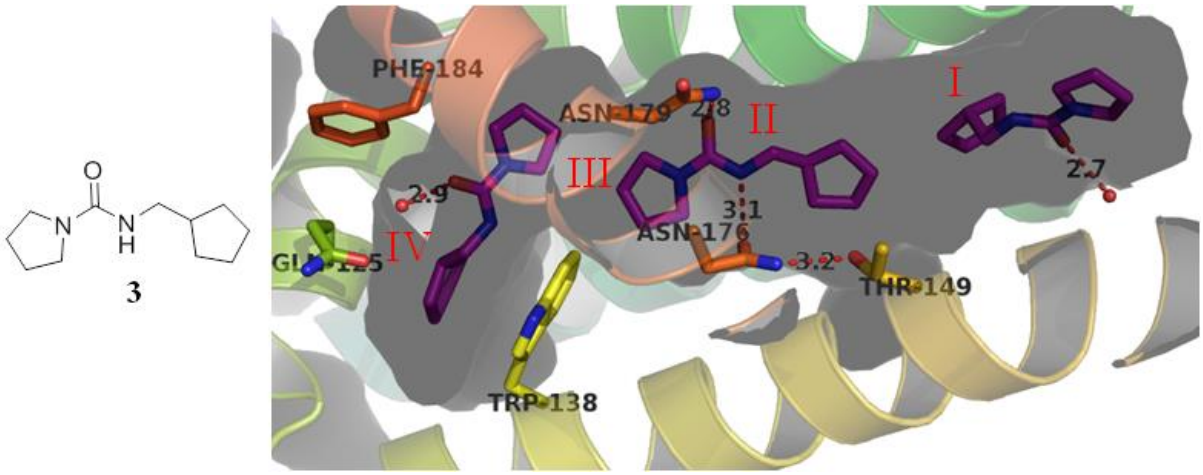


Figure S3 X-ray crystal structure of ligand *N*-(cyclopentylmethyl)pyrrolidine-1-carboxamide (**3**) bound to EthR. Unusually, three molecules of urea **3** are accommodated in the EthR binding cavity. (PDB code 5IOY)

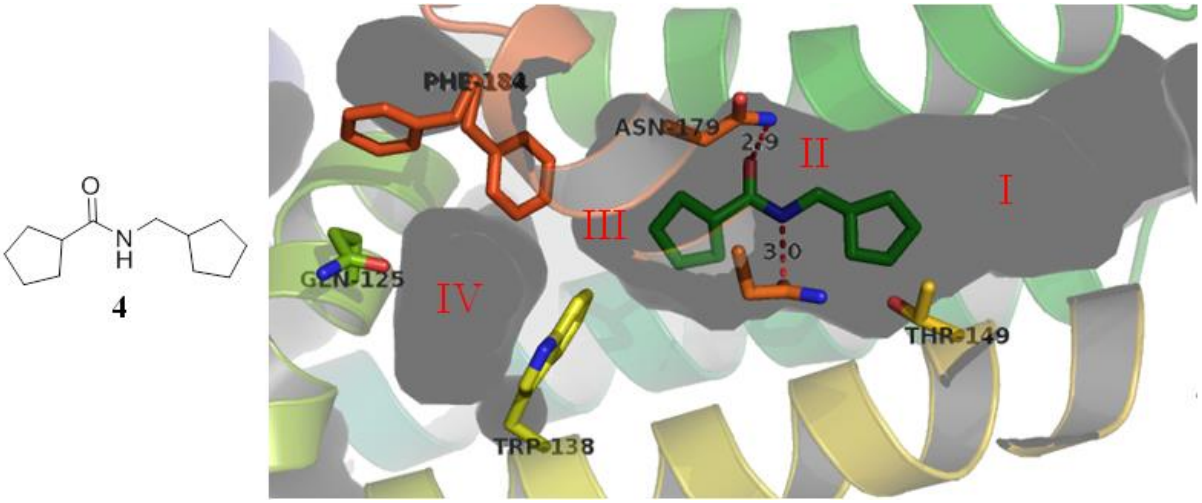


Figure S4 X-ray crystal structure of ligand *N*-(cyclopentylmethyl)cyclopentanecarboxamide (**4**) bound to EthR. (PDB code 5IOZ)

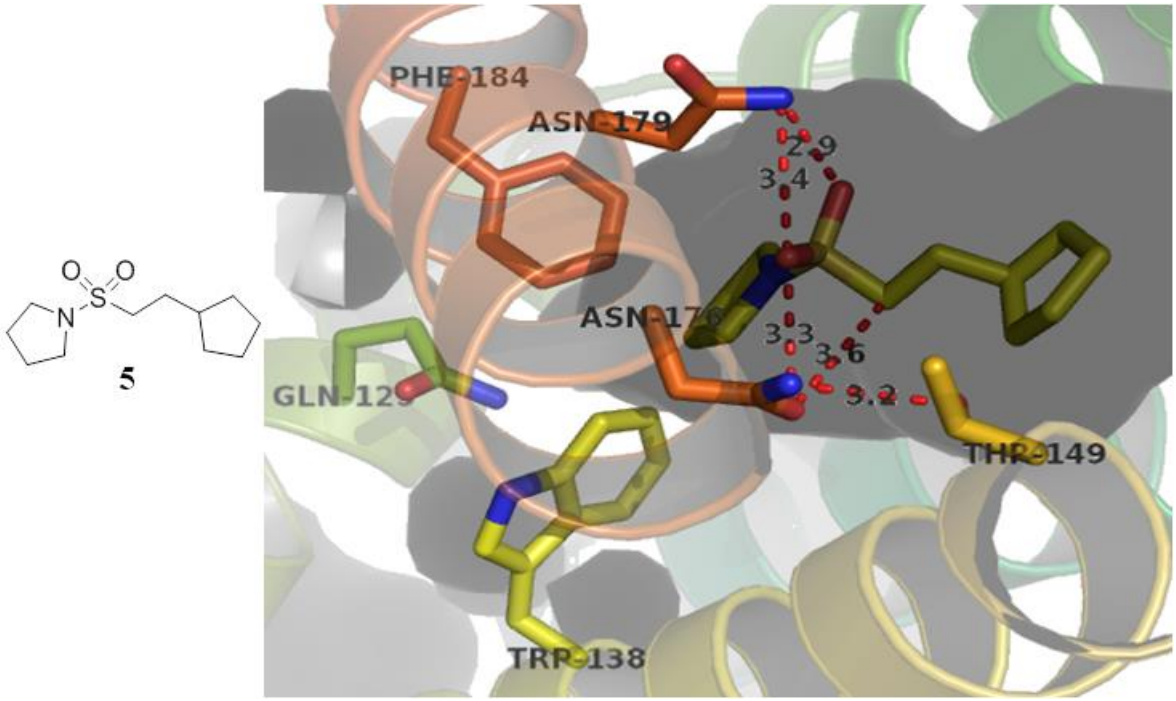


Figure S5 X-ray crystal structure of ligand 1-((2-cyclopentylethyl)sulfonyl)pyrrolidine (**5**) bound to EthR. (PDB code 5J3L)

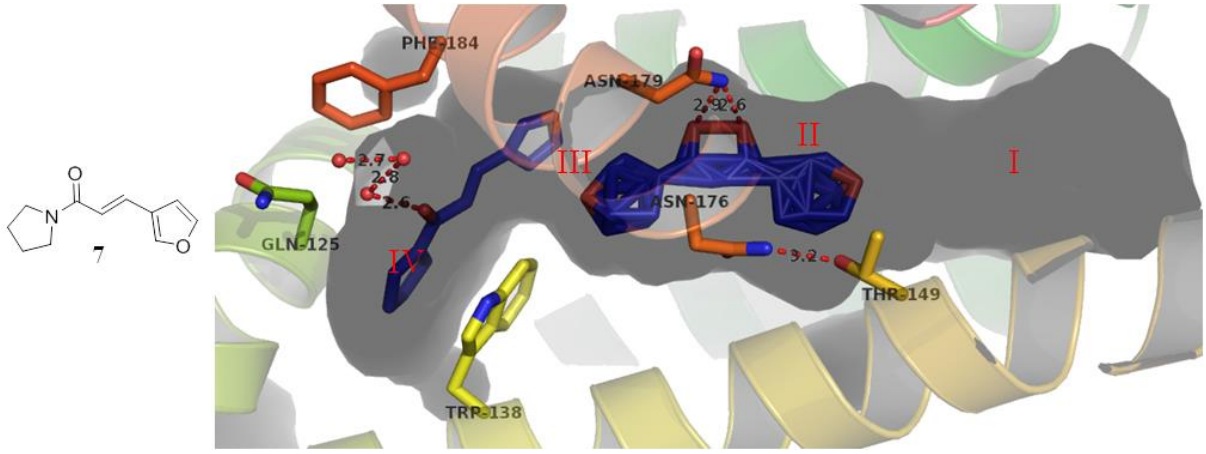


Figure S6 X-ray crystal structure of ligand (*E*)-3-(Furan-3-yl)-1-(pyrrolidin-1-yl)prop-2-en-1-one (**7**) bound to EthR. (PDB code 5IPA)

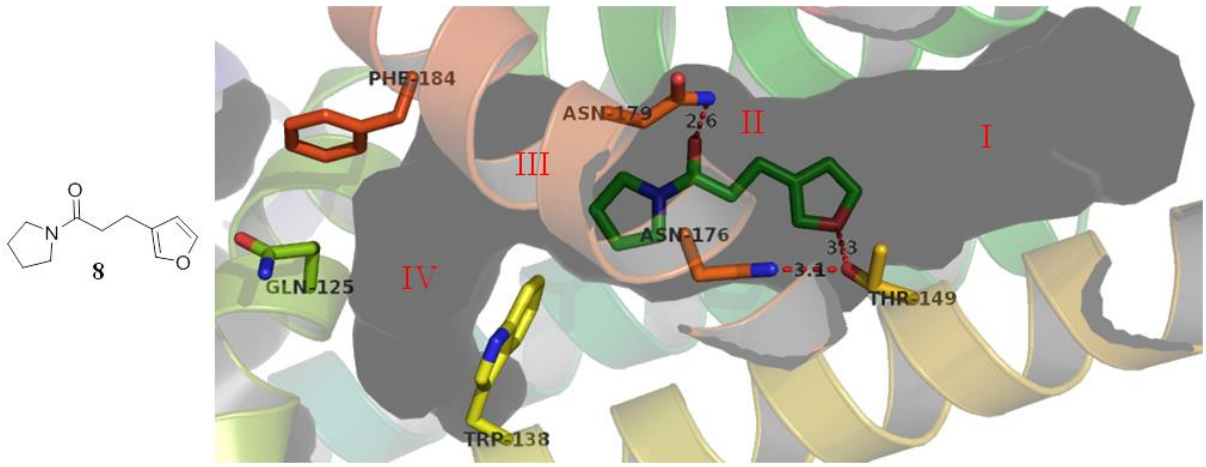


Figure S7 X-ray crystal structure of ligand 3-(furan-3-yl)-1-(pyrrolidin-1-yl)propan-1-one (**8**) bound to EthR. (PDB code 5J1R)

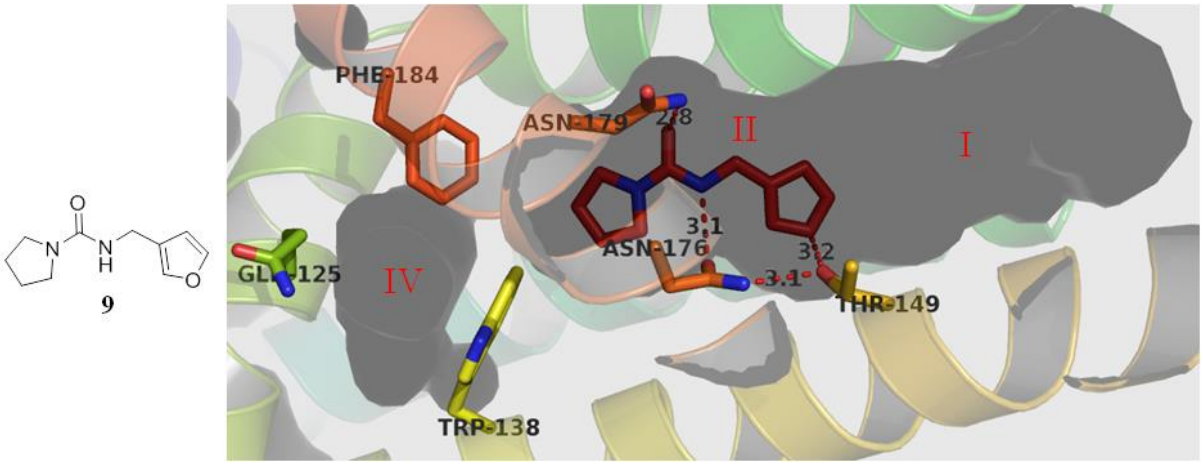


Figure S8 X-ray crystal structure of ligand *N*-(furan-3-ylmethyl)pyrrolidine-1-carboxamide (**9**) bound to EthR. (PDB code 5J1U)

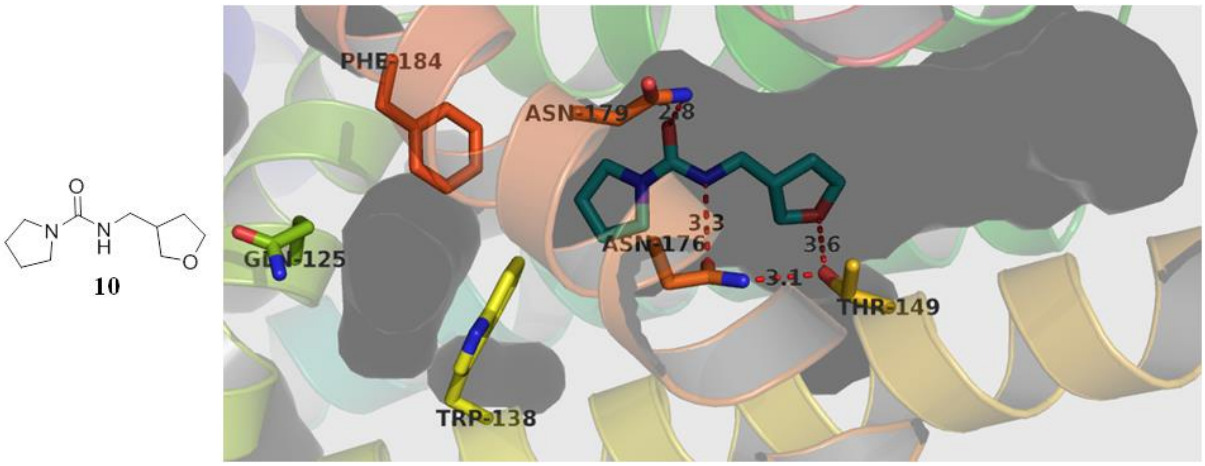


Figure S9 X-ray crystal structure of ligand *N*-((tetrahydrofuran-3-yl)methyl)pyrrolidine-1-carboxamide (**10**) bound to EthR. (PDB code 5IP6)

Biophysical assays

Isothermal titration calorimetry (ITC)

An aqueous solution of the fragment to be tested (1 mM) was prepared containing NaCl (300 mM), Tris.HCl (20 mM, pH = 8.0), d_6-DMSO (10% v/v) and glycerol (to match the 10% v/v glycerol content in the EthR stock solution). A separate aqueous solution of EthR (50 µM) containing NaCl (300 mM), Tris.HCl (20 mM, pH = 8.0) and DMSO-d_6 (10% v/v) was prepared and placed in the sample cell of a MicroCal iTC_{200} microcalorimeter (GE Healthcare). The fragment solution was then titrated to the EthR solution over 19 or 39 injections (first injection of 0.4 µl and subsequent injections of 2.0 µL or 1.0 µL). Data was fitted to a one site binding model using Origin software.

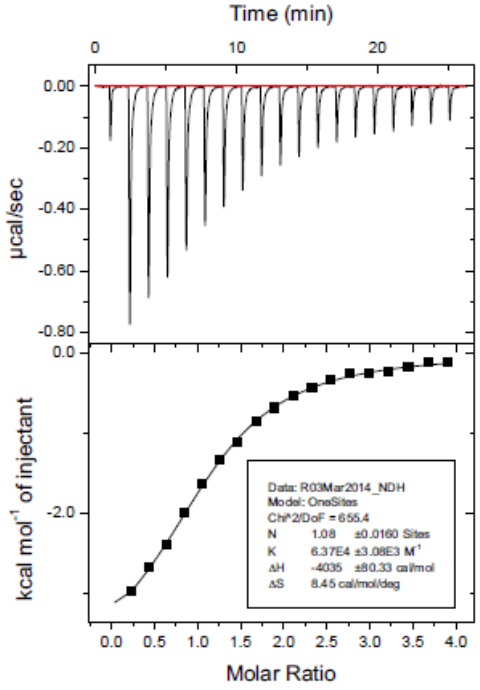


Figure S10 ITC trace for cyclopentylmethyl pyrrolidine-1-carboxylate **2** binding to EthR.

Determination of minimum effective concentration (MEC) values for ethionamide boosting of EthR fragments

Regular 7H9-based medium was prepared by adding 4.7 g Middlebrook 7H9 broth to double distilled water (900 mL) containing glycerol (2 mL, added via sterile syringe) and 0.5 mL Tween 80 (1 mL, added via sterile syringe). The mixture was stirred until all solids dissolved and passed through a 0.2 µM filter. ADC (100 mL) was aseptically added to the above 7H9-based medium. [ADC was prepared by dissolving BSA fraction V (50 g), glucose (20 g) and sodium chloride (8.1 g) in water to make up a total volume of 1L. The ADC solution was filter sterilised and stored at 4 °C.]

1) Isolated *M. tuberculosis* cells (ATCC 27294) were grown to an OD 0.2 – 0.3 in 7H9/ADC/Tween and diluted by a factor of 1000 in the required medium.
2) 50 µL of required medium was added to all wells of a 96-well clear round bottom plate, except the first row.
3) In the first row add 100 µL of ethionamide booster compound diluted in medium at twice the initial desired concentration (initial desired concentration is 50 µM). Using multichannel pipettor, transfer 50 µL to each next row starting with row 1 and ending with row 12, discarding 50 µL after row 12.
4) Isoniazid and DMSO only were used as positive and negative controls respectively.
5) To each well add 50 µL of the 1:1000 culture dilution (prepared in step 1 above, i.e. approximating 10,000 bacteria per well). For ethionamide synergy, add 50 µL of cell dilution (prepared in step 1 above) containing 0.2ug/mL ethionamide.
6) Plates were incubated for a total of two weeks at 37 °C in zip-lock bags.
7) Plates were read after 1 and 2 weeks with inverted enlarging mirror plate reader and graded as either growth or no growth. MEC is the lowest concentration of EthR ligand that completely inhibits growth. Photos are taken of the plates at both time points.

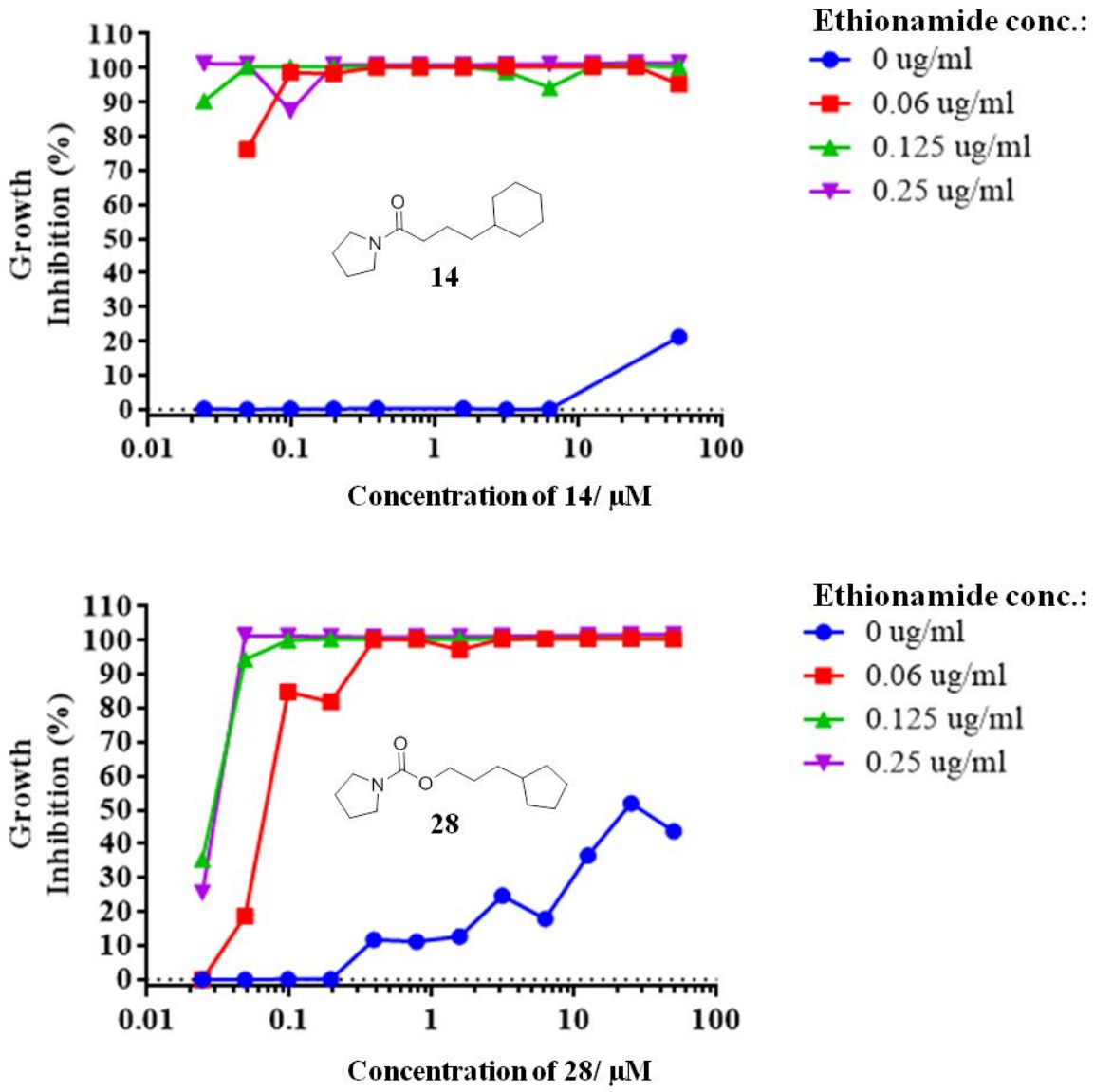


Figure S11 Investigation of the effect of compounds **14** and **28** on the growth of *M. tuberculosis* at different sub-MIC concentrations of ethionamide.

Macrophage (intracellular) assay

Raw264.7 macrophages (10^8 cells) were infected with H37Rv-GFP suspension at a multiplicity of infection (MOI) of 1:1 in 300 mL for 2 h at 37 °C with shaking (100 rpm). After two washes by centrifugation at 1100 rpm for 5 min, the remaining extracellular bacilli from the infected cells suspension were killed by a 1 h amikacin (20 μM, Sigma) treatment. After a final centrifugation step, 40 μL of M. tuberculosis H37Rv-GFP colonised macrophages were dispensed with the Wellmate (Matrix) into 384-well Evotec plates pre-plated with 10 μL of compound mixture diluted in cell medium and incubated for 5 days at 37 °C, 5% CO_2. Macrophages were then stained with SYTO 60 (Invitrogen, S11342) for 1 h followed by plate sealing. Confocal images were recorded on an automated fluorescent ultrahigh-throughput microscope Opera (Evotec). This microscope is based on an inverted microscope architecture that allows imaging of cells cultivated in 96- or 384-well microplates (Evotec). Images were acquired with a 20x water immersion objective (NA 0.70). A double laser excitation (488 and 635 nm) and dedicated dichroic mirrors were used to record green fluorescence of mycobacteria and red fluorescence of the macrophages on two different cameras, respectively. A series of four pictures at the centre of each well were taken, and each image was then processed using dedicated image analysis.[17-19] The percent of infected cells, and the number of cells are the two parameters extracted from images analysis as previously reported.[18] Data of two replicates are average.

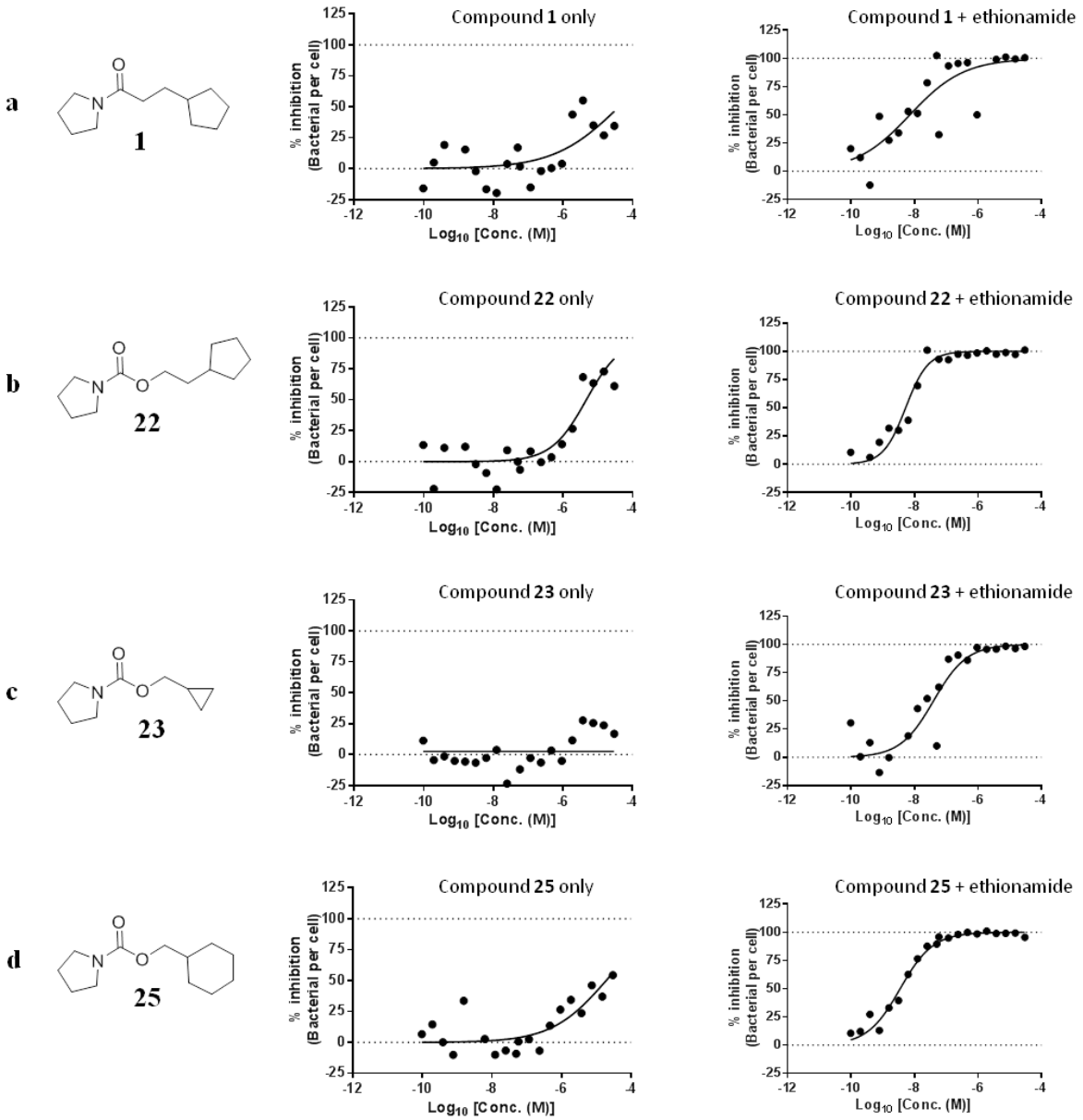


Figure S12 First screen of compounds **1**, **22**, **23** and **25** in the macrophage assay (n=1). Inhibition of bacterial replication in macrophages for compounds **1**, **22**, **23** and **25** respectively alone and in the presence of ethionamide (normal MIC/10). The parameter used as a read-out was the area of bacteria present per infected cell (expressed in px). Normalizations were performed based on the average values obtained for the negative (DMSO 1%) and positive (isoniazid 1 µg/ml) controls. Percentage of inhibition is plotted against the $\log_{10}$ of the compounds concentration, determined in the absence or in the presence of ethionamide at 1/10 of its MIC for the macrophage assay (0.033 µg/mL). Fitting was performed by Prism software using the sigmoidal dose-response (variable slope) model, constraining top and bottom values of the curve to 100% and 0% respectively.

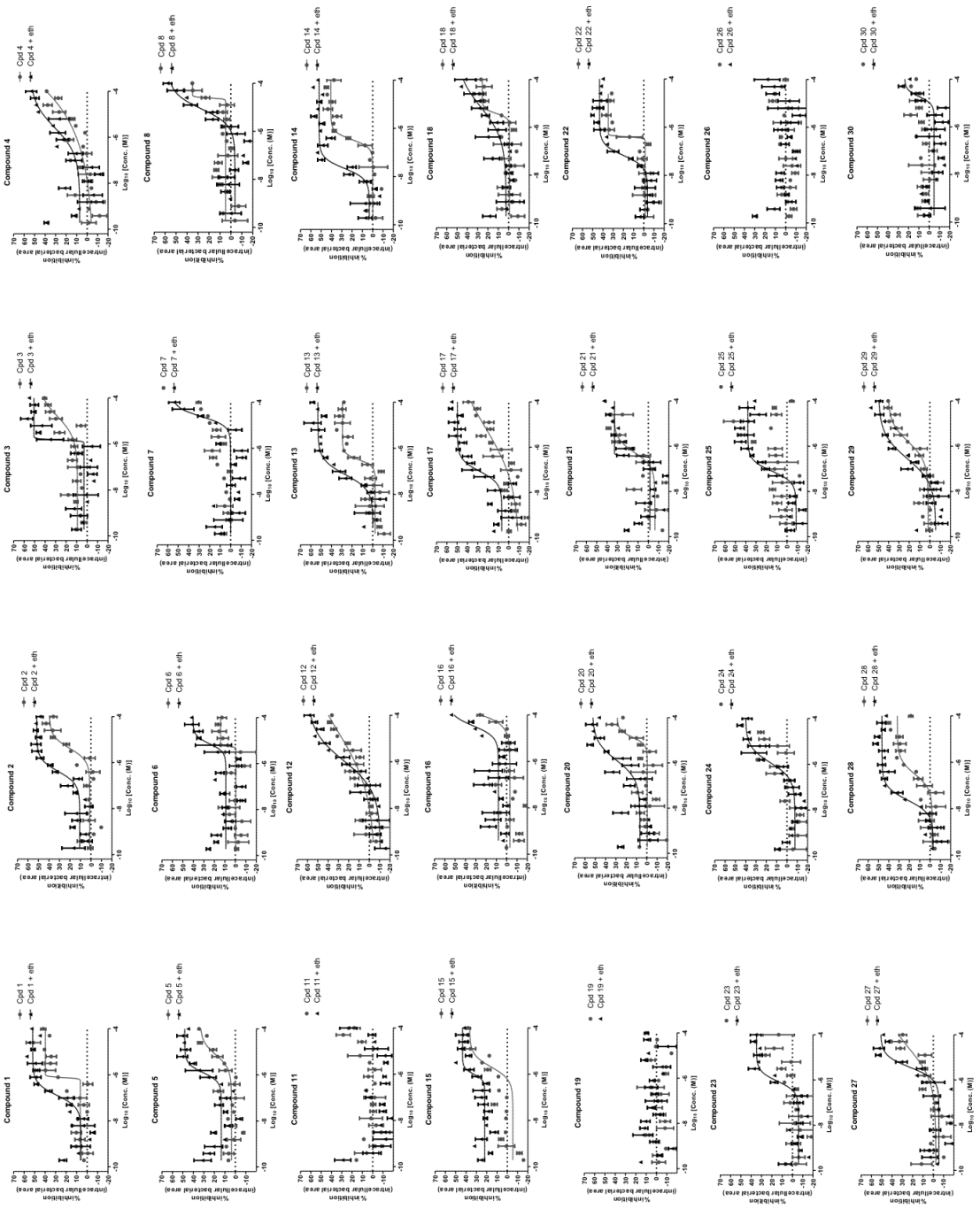


Figure S13: Screening data of compounds in the macrophage assay (not normalised). Percentage of inhibition is plotted against the $\log_{10}$ of the compounds concentration, determined in the absence or in the presence of ethionamide at 1/10 of its MIC for the macrophage assay (0.033 µg/mL). Fitting was performed by Prism software using the sigmoidal dose-response (variable slope) model.

Chemistry

General Information

^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker DPX-400 or Bruker DPX-500 NMR spectrometers. Chemical shifts are given in parts per million (ppm). All ^{13}C NMR spectra are proton decoupled. Coupling constants are reported in Hz where interpretable and the conventional abbreviations for assigning peak multiplicity are used as follows: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad.

High resolution mass spectrometry (HRMS) was performed using a Waters LCT Premier high-resolution spectrometer in electrospray ionisation (ESI) mode.

LCMS spectra were recorded using a Waters HClass UPLC system coupled to a Waters single quad detector eluting at a constant flow rate of 0.8 mL/ min using a constant gradient of 5 – 100% acetonitrile in 0.1% v/v aqueous formic acid.

Infrared spectrometry was performed using a Perkin-Elmer One FTIR Spectrometer with attenuated transmittance reflectance (ATR). The abbreviations (w) and (br) have been used to describe weak and broad IR absorbances respectively.

All commercially available reagents were used as purchased without further purification. All organic solvents used were either freshly distilled or purchased as anhydrous. Purification of intermediates and final compounds was carried out by automated flash column chromatography using Biotage SNAP Kp-Sil pre-packed columns run on either Biotage Isolera One or Biotage Isolera Four instruments.

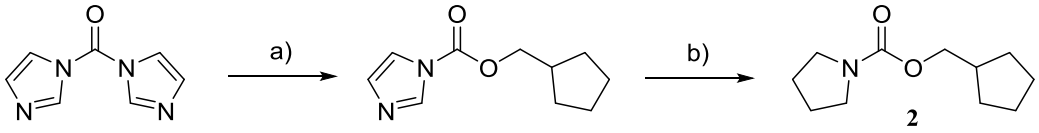
Microwave reactions were performed using a Biotage Initiator system under reaction conditions as indicated for each individual reaction.

Following aqueous work-up, organic solutions of intermediates and final compounds were dried using Isolute ® phase separators from Biotage (referred to as hydrophobic frits).

The purity of the compounds was measured by LC-MS with UV-Vis detection and all compounds were of a purity of > 95% unless otherwise stated.

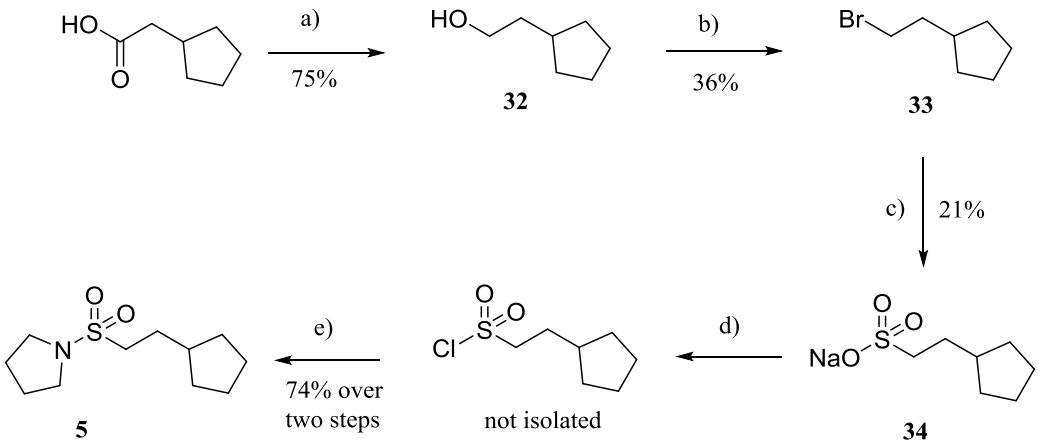
Synthetic Schemes

Figure S13 Synthesis of cyclopentylmethyl pyrrolidine-1-carboxylate **(2)**



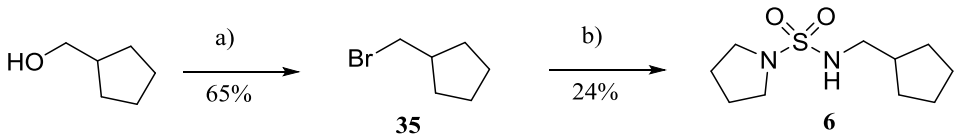
a) i) DCM, DIPEA, 0 °C, ii) cyclopentane methanol; 0 → 22 °C, o/n; b) pyrrolidine, 22 °C, o/n.

Figure S14 Synthesis of 1-((2-cyclopentylethyl)sulfonyl)pyrrolidine **(5)**



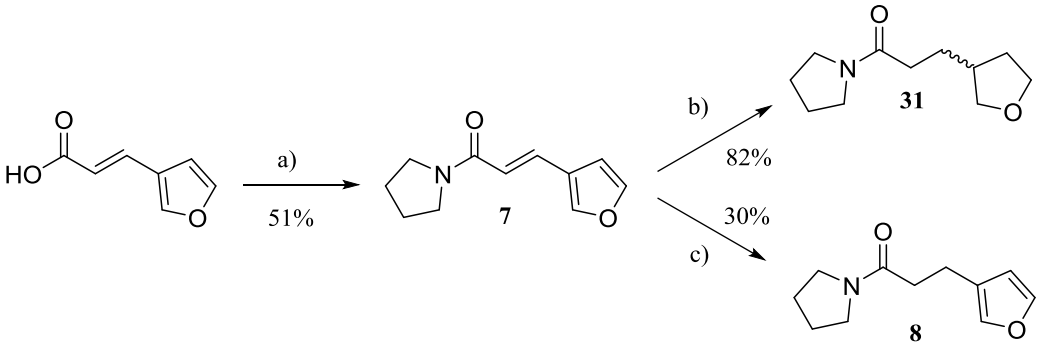
a) $LiAlH_4$, dry THF; b) i) PPh_3, anhydrous DCM; 0 °C, ii) NBS, portion-wise addition; 0 → 22 °C, o/n; c) Na_2SO_3, 18-crown-6, H_2O; reflux, 36 h; d) $SOCl_2$, toluene; 80 °C; e) pyrrolidine; 80 °C, 2 h.

Figure S15 Synthesis of *N*-(cyclopentylmethyl)pyrrolidine-1-sulfonamide **(6)**



a) i) PPh_3, DMF; 0 °C; ii) Br_2 (added over 10 min), 0 → 22 °C, o/n; b) pyrrolidine-1-sulphonamide (**36)**, Cs_2CO_3, anhydrous DMF; 90 °C, o/n.

Figure S16 Synthesis of (*E*)-3-(furan-3-yl)-1-(pyrrolidin-1-yl)prop-2-en-1-one **(7)**, 3-(furan-3-yl)-1-(pyrrolidin-1-yl)propan-1-one **(8)** and 1-(pyrrolidin-1-yl)-3-(tetrahydrofuran-3-yl)propan-1-one **(31)**



a) i) pyrrolidine, DIPEA, DCM ii) COMU, 22 °C; b) H_2 (1 atm.), 30% wt/wt Pd/C, EtOAc, 22 °C, 72 h; c) H_2 (1 atm.), 10% wt/wt Pd/C, EtOAc, 22 °C, 30 min.

General method A[20]

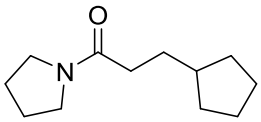
Amine (1 equivalent), carboxylic acid (1 equivalent) and diisopropylethylamine (5 equivalents) were dissolved in anhydrous DCM (2 mL). COMU[20] (1.1 eq.) was added and the reaction mixture was stirred at room temperature for 16 – 24 h. The solvent was evaporated *in vacuo*. The residue was dissolved in EtOAc (10 mL) and washed with water (2 x 10 mL). The organic layer was concentrated *in vacuo* and the crude material was purified by automated flash chromatography.

General method B[21]

To a suspension of 1, 1'-carbonyldiimidazole (1.1 eq.) in anhydrous THF (10 mL/g of alcohol) was added slowly the alcohol (1 eq.) and the reaction mixture was left stirring at 22 °C overnight. Pyrrolidine (1 eq.) was added and the reaction mixture was stirred 22 °C for five hours. The volatile organics were removed *in vacuo* and the crude residue was purified by flash chromatography eluting with a gradient of 0-33% of ethyl acetate in petroleum ether (40-60).

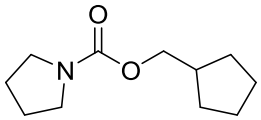
Synthesis

3-Cyclopentyl-1-(pyrrolidin-1-yl)propan-1-one (1)[22]



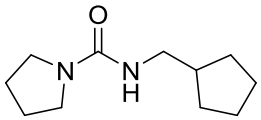
Prepared according to **general method A** using pyrrolidine (58.5 μL, 0.7 mmol), cyclopentylpropionic acid (100 μL, 0.7 mmol), DCM (5 mL), DIPEA (610 μL, 3.5 mmol) and COMU (330 mg, 0.77 mmol). Purification by automated flash chromatography (0 – 100% EtOAc in pet. ether 40/ 60) afforded the amide **1** as a yellow oil (75 mg, 55%); TLC (EtOAc): R_f = 0.24; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.53 – 3.39 (m, 4H), 2.33 – 2.24 (m, 2H), 2.03 – 1.92 (m, 2H), 1.92 – 1.73 (m, 6H), 1.73 – 1.47 (m, 5H), 1.22 – 1.04 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.0, 46.6, 45.6, 39.9, 34.1, 32.5, 31.2, 26.1, 25.2, 24.4; IR, ν_{max} (ATR): 2946, 2866, 1635, 1424 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{12}H_{22}NO$, 196.1701; found, 196.1693.

Cyclopentylmethyl pyrrolidine-1-carboxylate (2)



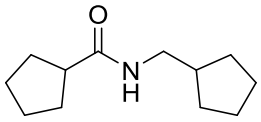
Prepared according to **general method B** using 1,1'-carbonyldiimidazole (0.89 g, 5.5 mmol), anhydrous THF (5.0 mL), cyclopentanemethanol (0.54 mL, 5.0 mmol) and pyrrolidine (0.42 mL, 5.0 mmol). The *carbamate* **2** was isolated as a colourless liquid (0.82 g, 83%). TLC (EtOAc/petroleum ether (40-60), 1:2, v/v): R_f = 0.57; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.02 – 3.92 (m, 2H), 3.46 – 3.28 (m, 4H), 2.31 – 2.12 (m, 1H), 1.95 – 1.82 (m, 4H), 1.82 – 1.69 (m, 2H), 1.69 – 1.49 (m, 4H), 1.38 – 1.21 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.4, 68.8, 46.0, 45.7, 38.9, 29.2, 25.7, 25.3, 25.0; IR, ν_{max} (ATR): 2949, 2870, 1698 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{11}H_{20}NO_2$, 198.1489; found, 198.1487.

***N*-(Cyclopentylmethyl)pyrrolidine-1-carboxamide (3)**



To a suspension of 1,1'-carbonyldiimidazole (95.3 mg, 0.6 mmol) in anhydrous THF (0.5 mL) was added a solution of cyclopentylmethanamine in diethyl ether (53% wt/wt, 100 mg, 0.5 mmol) and the reaction mixture was stirred at ambient temperature for 16 h. Pyrrolidine (45 μL, 0.5 mmol) was added and the mixture stirred at 22 °C for a further 24 h. The organics were removed *in vacuo* and the crude product was purified by flash chromatography eluting with a gradient of 20-100% of ethyl acetate in petroleum ether (40-60). The *urea* **3** was isolated as a white solid (56 mg, 53%). TLC (EtOAc): R_f = 0.20; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.20 (s, 1H), 3.42 – 3.28 (m, 4H), 3.28 – 3.10 (m, 2H), 2.13 – 1.99 (m, 1H), 1.97 – 1.87 (m, 4H), 1.82 – 1.70 (m, 2H), 1.70 – 1.49 (m, 8H), 1.30 – 1.15 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.9, 45.6, 45.5, 40.3, 30.2, 25.6, 25.2; IR, ν_{max} (ATR): 3351 (NH), 2948, 2863, 1715, 1616, 1525 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{11}H_{21}N_2O$, 197.1648; found, 197.1645.

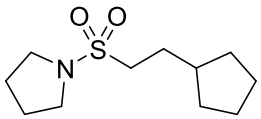
***N*-(Cyclopentylmethyl)cyclopentanecarboxamide (4)**



Cyclopentanecarboxylic acid (100 μL, 0.92 mmol), diisopropylethylamine (0.8 mL, 4.6 mmol) and cyclopentylmethanamine in diethyl ether (53% w/w, 173 mg, 0.92 mmol) were dissolved in anhydrous DCM (5.0 mL). COMU (0.43 g, 1.0 mmol) was added and the reaction mixture was stirred at 22 °C overnight. The volatile organics were then removed *in vacuo* to afford dark green oil, which was re-dissolved in EtOAc (15 mL) and washed with water (2 x 10 mL). The organic phase was concentrated *in vacuo* and the crude residue was purified by flash chromatography eluting with a gradient of 0-100% ethyl acetate in petroleum ether 40-60. The desired *amide* **4** was isolated as a pale yellow amorphous solid (123 mg, 68%). TLC (EtOAc): R_f = 0.58; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.44 (s, 1H), 3.28 – 3.15 (m, 2H), 2.58 – 2.42 (m, 1H), 2.14 – 1.95 (m, 1H), 1.94 – 1.70 (m, 6H), 1.70 – 1.48 (m, 9H), 1.30 – 1.09 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.1, 46.1, 44.4, 39.80, 30.5, 30.3,

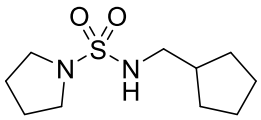
25.9, 25.2; IR, ν_{max} (ATR): 3299 (NH), 2948, 2867, 1635, 1554, 1450 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{12}H_{22}NO$, 196.1701; found, 196.1708.

1-((2-Cyclopentylethyl)sulfonyl)pyrrolidine (5)



Sodium 2-cyclopentylethane-1-sulfonate **34** (50 mg, 0.25 mmol) was suspended in anhydrous toluene (0.5 mL). Thionyl chloride (37 µL, 0.50 mmol) and 18-crown-6 (66 mg, 0.25 mmol) were added and the reaction mixture was heated to 80 °C with stirring for 4 h. After cooling to ambient temperature an additional portion of thionyl chloride (20 µL, 0.25 mmol) was added and the mixture was heated again to 80 °C and stirred at this temperature overnight. Pyrrolidine (2.5 mL) was added at ambient temperature and the reaction mixture was heated to 80 °C for an additional 2 h. After cooling to ambient temperature, the solvents were removed *in vacuo* and the crude residue was purified by flash chromatography eluting with a gradient of 20-100% of ethyl acetate in petroleum ether 40-60. The desired *sulfonamide* **5** was isolated as a white amorphous solid (43 mg, 74%). TLC (EtOAc): R_f = 0.67; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.43 – 3.33 (m, 4H), 3.04 – 2.94 (m, 2H), 2.01 – 1.91 (m, 5H), 1.91 – 1.74 (m, 4H), 1.73 – 1.49 (m, 4H), 1.21 – 1.07 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 49.1, 47.7, 39.0, 32.4, 29.3, 25.9, 25.1; IR, ν_{max} (ATR): 2942, 2870, 1457 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{11}H_{22}NO_2S$, 232.1366; found, 232.1361.

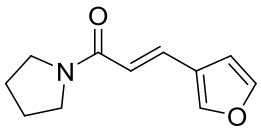
***N*-(Cyclopentylmethyl)pyrrolidine-1-sulfonamide (6)**



Pyrrolidine-1-sulfonamide **36** (120 mg, 0.8 mmol) was dissolved in anhydrous DMF (6.0 mL). Cesium carbonate (0.521 g, 1.6 mmol) and a solution of (bromomethyl)cyclopentane **35** in DMF (31% wt/wt, 0.355 g) were added. The reaction mixture was heated up to 90 °C and stirred at this temperature overnight. After cooling to 22 °C, the solids were removed by filtration and the filtrate was diluted with water (30 mL). The aqueous phase was extracted with ethyl acetate (30 mL). The organic phase was dried and concentrated *in vacuo*. The

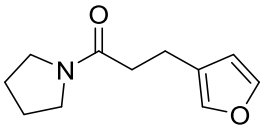
crude residue was purified by flash chromatography eluting with a gradient of 0-30% of ethyl acetate in petroleum ether (40-60). The desired *sulphonamide* **6** was isolated as a white solid (38 mg, 24%). TLC (EtOAc): R_f = 0.74; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.11 (br m, 1H), 3.40 – 3.25 (m, 4H), 3.06 – 2.94 (m, 2H), 2.15 – 2.00 (m, 1H), 2.00 – 1.88 (m, 4H), 1.88 – 1.74 (m, 2H), 1.72 – 1.51 (m, 4H), 1.32 – 1.09 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 48.6, 48.1, 39.7, 30.3, 25.6, 25.2; IR, ν_{max} (ATR): 3274 (NH), 2949, 2865, 1432 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{10}H_{21}N_2O_2S$, 233.1324; found, 233.1330.

(*E*)-3-(Furan-3-yl)-1-(pyrrolidin-1-yl)prop-2-en-1-one (7)



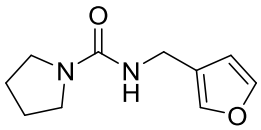
Trans-3-furanacrylic acid (200 mg, 1.4 mmol) was suspended in dichloromethane (10 mL). Diisopropylethylamine (1.3 mL, 7.0 mmol) and pyrrolidine (121 µL, 1.4 mmol) were added, followed by COMU (0.68 g, 1.5 mmol). The reaction mixture was stirred at ambient temperature for 2 h. The volatile organics were removed *in vacuo*. The crude orange-red product was re-dissolved in ethyl acetate (10 mL) and washed with water (2 x 10 mL). The organics were concentrated *in vacuo*. The desired *amide* **7** was purified by flash chromatography and isolated as an off-white solid (140 mg, 51%). TLC (EtOAc): R_f = 0.28; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 – 8.02 (m, 1H), 7.79 – 7.70 (m, 1H), 7.42 (d, *J* = 15.3 Hz, 1H), 7.03 – 6.93 (m, 1H), 6.73 (d, *J* = 15.3 Hz, 1H), 3.65 – 3.58 (m, 2H), 3.44 – 3.38 (m, 2H), 1.99 – 1.89 (m, 2H), 1.89 – 1.79 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.7, 144.8, 144.7, 130.7, 123.1, 119.5, 108.2, 46.1, 45.6, 25.7, 23.9; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{11}H_{14}NO_2$, 192.1025; found, 192.1025; LCMS (ESI) $[M + H]^+$ *m/z*: 192.2, retention time = 3.24 min.

3-(Furan-3-yl)-1-(pyrrolidin-1-yl)propan-1-one (8)



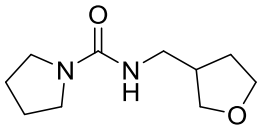
A mixture of (*E*)-3-(furan-3-yl)-1-(pyrrolidin-1-yl)prop-2-en-1-one **7** (20 mg, 0.1 mmol) and 30% wt/wt Pd/C (3 mg) in ethyl acetate (2.0 ml) was thoroughly deoxygenated, put under hydrogen atmosphere and stirred vigorously at ambient temperature for 30 min. The catalyst was removed by filtration through celite. The solvent was removed *in vacuo* and the crude product was purified by flash chromatography. The desired *amide* **8** was isolated as white crystalline solid (9 mg, 30%). TLC (EtOAc): R_f = 0.27; ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.51 – 7.44 (m, 1H), 7.43 – 7.34 (m, 1H), 6.49 – 6.35 (m, 1H), 3.50 – 3.42 (m, 2H), 3.42 – 3.34 (m, 2H), 2.78 – 2.70 (m, 2H), 2.56 – 2.48 (m, 2H), 2.01 – 1.89 (m, 2H), 1.90 – 1.78 (m, 2H); ^{13}C NMR (125 MHz, acetone-d_6) δ 170.8, 143.9, 140.4, 126.0, 112.4, 47.2, 46.4, 35.9, 27.1, 25.4, 21.1.

***N*-(Furan-3-ylmethyl)pyrrolidine-1-carboxamide (9)**



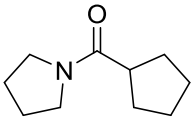
A solution of 3-furylmethylamine (100 mg, 1.0 mmol) and *N*,*N*-diisopropylethylamine (179 µL, 1.0 mmol) in anhydrous DCM (2.0 mL) was added to a solution of 1,1'-carbonyldiimidazole (175 mg, 1.1 mmol) in DCM (2.0 mL) cooled in ice/ water. After the addition was complete, the reaction mixture was allowed to warm up to 22 °C and was stirred at this temperature for 72 h. Pyrrolidine (86 µL, 1.0 mmol) was added and the reaction mixture was stirred for a further 12 h at ambient temperature. The volatile organics were evaporated *in vacuo* and the crude was purified by flash chromatography eluting with a gradient of 0-10% MeOH in DCM. The desired *urea* **9** was isolated as an off-white solid (141 mg, 71%). TLC (DCM/MeOH, 9:1, v/v): R_f = 0.41; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.33 (m, 2H), 6.43 (s, 1H), 4.29 (s, 2H), 3.43 – 3.26 (m, 4H), 1.99 – 1.85 (m, 4H), 1.67 (s, 1H); ^{13}C NMR (125 MHz, methanol-d_4) δ 159.0, 144.0, 140.8, 125.3, 111.1, 46.5, 36.0, 26.3; IR, ν_{max} (ATR): 3283 (NH), 2963, 2931, 2879, 1614, 1536 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{10}H_{15}N_2O_2$, 195.1128; found, 195.1124.

_N_-((Tetrahydrofuran-3-yl)methyl)pyrrolidine-1-carboxamide (10)



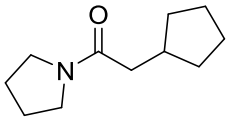
_N_-(Furan-3-ylmethyl)pyrrolidine-1-carboxamide (24.0 mg, 0.12 mmol) was dissolved in ethyl acetate (2.0 ml) and 10% wt/wt Pd/C (3.0 mg) was added. The reaction mixture was thoroughly deoxygenated by performing a vacuum/ nitrogen cycle three times. The reaction vessel was then put under vacuum, filled with hydrogen gas using a balloon and stirred under a hydrogen atmosphere at 22 °C overnight. The catalyst was removed by filtration through celite and the solvent was evaporated *in vacuo*. The crude residue was purified by flash chromatography eluting with a gradient of 0-10% MeOH in DCM. The desired *tetrahydrofuran* **10** was isolated as an off-white amorphous solid (16.6 mg, 68%). TLC (DCM/MeOH, 9:1, v/v): R_f = 0.31; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.39 (s, 1H), 3.95 – 3.69 (m, 3H), 3.64 – 3.54 (m, 1H), 3.42 – 3.17 (m, 6H), 2.59 – 2.45 (m, 1H), 2.12 – 1.98 (m, 1H), 1.98 – 1.86 (m, 4H), 1.72 – 1.58 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.8, 71.4, 67.8, 45.5, 43.5, 39.7, 29.8, 25.6; IR, ν_{max} (ATR): 3307 (NH), 2937, 2870, 2849, 1620, 1532 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{10}H_{19}N_2O_2$, 199.1447; found, 199.1450.

Cyclopentyl(pyrrolidin-1-yl)methanone (11)[23]



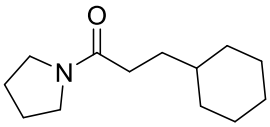
Prepared according to **general method A** using pyrrolidine (60 μL, 0.72 mmol), cyclopentanecarboxylic acid (78 μL, 0.72 mmol), DCM (5 mL), DIPEA (627 μL, 3.6 mmol) and COMU (340 mg, 0.79 mmol). Purification by automated flash chromatography (0 – 100 % EtOAc in pet. ether 40/ 60) afforded the amide **11** as a pale yellow oil (39 mg, 32 %). TLC (EtOAc): R_f = 0.28; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.52 – 3.40 (m, 4H), 2.83 – 2.71 (m, 1H), 2.01 – 1.90 (m, 2H), 1.90 – 1.69 (m, 8H), 1.64 – 1.48 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.9, 46.4, 45.7, 43.0, 29.8, 26.1, 24.3; IR, ν_{max} (ATR): 3479 (w, br), 2948, 2867, 1628, 1426 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{10}H_{18}NO$, 168.1383; found, 168.1383.

2-Cyclopentyl-1-(pyrrolidin-1-yl)ethan-1-one (12)



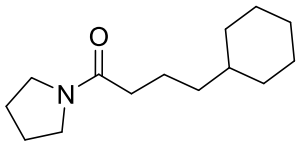
Prepared according to **general method A** using pyrrolidine (60 μL, 0.72 mmol), cyclopentaneacetic acid (90 μL, 0.72 mmol), DCM (5 mL), DIPEA (627 μL, 3.6 mmol) and COMU (340 mg, 0.79 mmol). Purification by automated flash chromatography (0 – 100 % EtOAc in pet. ether 40/ 60) afforded the *amide* **12** as a pale yellow oil (91 mg, 70 %). TLC (EtOAc): R_f = 0.22; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.55 – 3.38 (m, 4H), 2.38 – 2.26 (m, 3H), 2.02 – 1.80 (m, 6H), 1.70 – 1.48 (m, 4H), 1.24 – 1.09 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.6, 46.7, 45.5, 40.8, 36.5, 32.7, 26.1, 25.0, 24.4; IR, ν_{max} (ATR): 3472 (w, br), 2947, 2867, 1627, 1424 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{11}H_{20}NO$, 182.1545; found, 182.1537.

3-Cyclohexyl-1-(pyrrolidin-1-yl)propan-1-one (13)



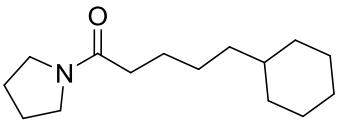
Prepared according to **general method A** using pyrrolidine (60 μL, 0.72 mmol), cyclohexanepropionic acid (123 μL, 0.72 mmol), DCM (5 mL), DIPEA (627 μL, 3.6 mmol) and COMU (340 mg, 0.79 mmol). Purification by automated flash chromatography (0 – 100 % EtOAc in pet. ether 40/ 60) afforded the *amide* **13** as a pale yellow oil (115 mg, 76 %). TLC (EtOAc): R_f = 0.26; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.52 – 3.39 (m, 4H), 2.33 – 2.23 (m, 2H), 2.02 – 1.92 (m, 2H), 1.92 – 1.82 (m, 2H), 1.82 – 1.62 (m, 5H), 1.61 – 1.51 (m, 2H), 1.34 – 1.13 (m, 4H), 1.00 – 0.86 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.3, 46.6, 45.7, 37.5, 33.1, 32.3, 26.6, 26.3, 26.1, 24.4; ν_{max} (ATR): 3467 (w, br), 2919, 2849, 1635, 1422 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{13}H_{24}NO$, 210.1852; found, 210.1849.

4-Cyclohexyl-1-(pyrrolidin-1-yl)butan-1-one (14)



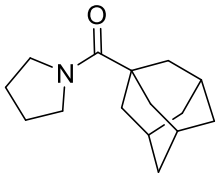
Prepared according to **general method A** using pyrrolidine (60 μL, 0.72 mmol), cyclohexanebutyric acid (123 mg, 0.72 mmol), DCM (5 mL), DIPEA (627 μL, 3.6 mmol) and COMU (340 mg, 0.79 mmol). Purification by automated flash chromatography (0 – 100 % EtOAc in pet. ether 40/ 60) afforded the *amide* **14** as a pale yellow oil (118 mg, 73 %). TLC (EtOAc): R_f = 0.28; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.53 – 3.35 (m, 4H), 2.31 – 2.18 (m, 2H), 2.02 – 1.92 (m, 2H), 1.92 – 1.82 (m, 2H), 1.82 – 1.58 (m, 7H), 1.30 – 1.11 (m, 6H), 0.97 – 0.80 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.0, 46.6, 45.6, 37.6, 37.3, 35.2, 33.3, 26.7, 26.4, 26.1, 24.4, 22.3; ν_{max} (ATR): 3476 (w, br), 2919, 2849, 1636, 1425 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{14}H_{26}NO$, 224.2014; found, 224.2008.

5-Cyclohexyl-1-(pyrrolidin-1-yl)pentan-1-one (15)



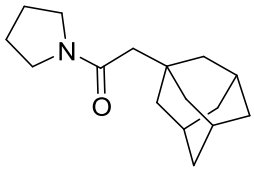
Prepared according to **general method A** using pyrrolidine (60 μL, 0.72 mmol), cyclohexanepentanoic acid (133 mg, 0.72 mmol), DCM (5 mL), DIPEA (627 μL, 3.6 mmol) and COMU (340 mg, 0.79 mmol). Purification by automated flash chromatography (0 – 100 % EtOAc in pet. ether 40/ 60) afforded the *amide* **15** as a pale yellow oil (116 mg, 68 %). TLC (EtOAc): R_f = 0.30; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.53 – 3.37 (m, 4H), 2.31 – 2.22 (m, 2H), 2.03 – 1.91 (m, 2H), 1.91 – 1.81 (m, 2H), 1.78 – 1.57 (m, 7H), 1.42 – 1.30 (m, 2H), 1.30 – 1.10 (m, 6H), 0.95 – 0.78 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 46.6, 45.6, 37.5, 37.3, 34.9, 33.39, 26.8, 26.7, 26.4, 26.1, 25.2, 24.4; ν_{max} (ATR): 2919, 2849, 1638, 1425 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{15}H_{28}NO$, 238.2171; found, 238.2169.

((1s,3s)-Adamantan-1-yl)(pyrrolidin-1-yl)methanone (16)[24,25]



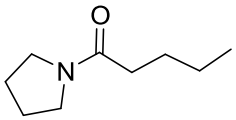
Prepared according to **general method A** using pyrrolidine (60 μL, 0.72 mmol), adamantanecarboxylic acid acid (130 mg, 0.72 mmol), DCM (5 mlL), DIPEA (627 μL, 3.6 mmol) and COMU (340 mg, 0.79 mmol). Purification by automated flash chromatography (0 – 100 % EtOAc in pet. ether 40/ 60) afforded the amide **16** as a pale orange gum (130 mg, 77%). TLC (EtOAc): R_f = 0.38; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.60 (s, 4H), 2.10 – 1.97 (m, 8H), 1.91 – 1.81 (m, 4H), 1.81 – 1.69 (m, 5H), 1.66 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.9, 48.0 (br), 41.7, 38.2, 36.7, 28.4; ν_{max} (ATR): 2977, 2903, 2847, 1723 (w), 1601 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{15}H_{24}NO$, 234.1852; found, 234.1847.

2-((1s,3s)-Adamantan-1-yl)-1-(pyrrolidin-1-yl)ethan-1-one (17)[25]



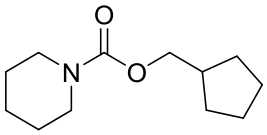
Prepared according to **general method A** using pyrrolidine (60 μL, 0.72 mmol), adamantaneacetic acid (140 mg, 0.72 mmol), DCM (5 mL), DIPEA (627 μL, 3.6 mmol) and COMU (340 mg, 0.79 mmol). Purification by automated flash chromatography (0 – 100% EtOAc in pet. ether 40/ 60) afforded the amide **17** as a pale yellow gum (114 mg, 64%). TLC (EtOAc): R_f = 0.29; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.54 – 3.41 (m, 4H), 2.09 (s, 2H), 2.01 – 1.91 (m, 5H), 1.90 – 1.82 (m, 2H), 1.76 – 1.67 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 48.2, 47.7, 45.5, 42.7, 36.8, 33.7, 28.7, 26.3, 24.4; ν_{max} (ATR): 2897, 2845, 1628, 1448, 1414 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{16}H_{26}NO$, 248.2014; found, 248.2005.

1-(Pyrrolidin-1-yl)pentan-1-one (18)



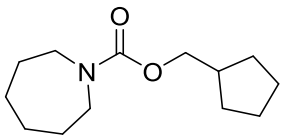
Prepared according to **general method A** using pyrrolidine (60 μL, 0.72 mmol), valeric acid (78 μL, 0.72 mmol), DCM (5 mL), DIPEA (627 μL, 3.6 mmol) and COMU (340 mg, 0.79 mmol). Purification by automated flash chromatography (0 – 100% EtOAc in pet. ether 40/ 60) afforded the *amide* **18** as a pale yellow liquid (57 mg, 51%). TLC (EtOAc): R_f = 0.21; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.55 – 3.36 (m, 4H), 2.36 – 2.20 (m, 2H), 2.01 – 1.91 (m, 2H), 1.91 – 1.81 (m, 2H), 1.70 – 1.60 (m, 2H), 1.44 – 1.33 (m, 2H), 0.98 – 0.90 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 46.6, 45.6, 34.6, 27.1, 26.1, 24.4, 22.6, 13.9; ν_{max} (ATR): 3470 (w, br), 2955, 2871, 1631, 1425 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_9H_{18}NO$, 156.1388; found, 156.1383.

Cyclopentylmethyl piperidine-1-carboxylate (20)



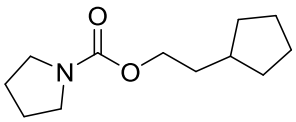
Prepared according to **general method B** using 1,1'-carbonyldiimidazole (0.18 g, 1.1 mmol), anhydrous THF (1.0 mL), cyclopentanemethanol (0.1 g, 1.0 mmol) and piperidine (99 μL, 1.0 mmol). The *carbamate* **20** was isolated as a colourless liquid (180 mg, 85%). TLC (EtOAc/petroleum ether (40-60), 1:1, v/v): R_f = 0.73; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.01 – 3.91 (m, 2H), 3.49 – 3.35 (m, 4H), 2.28 – 2.13 (m, 1H), 1.83 – 1.68 (m, 2H), 1.68 – 1.44 (m, 10H), 1.36 – 1.21 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.7, 69.1, 44.7, 38.9, 29.2, 25.7, 25.3, 24.4; ν_{max} (ATR): 2937, 2857, 1694, 1426 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{12}H_{22}NO_2$, 212.1651; found, 212.1651.

Cyclopentylmethyl azepane-1-carboxylate (21)



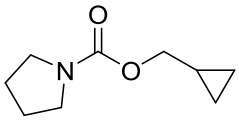
Prepared according to **general method B** using 1,1'-carbonyldiimidazole (0.18 g, 1.1 mmol), anhydrous THF (1.0 mL), cyclopentanemethanol (0.1 g, 1.0 mmol) and hexamethyleneimine (113 μL, 1.0 mmol). The *carbamate* **21** was isolated as a colourless liquid (166 mg, 74%). TLC (EtOAc/petroleum ether (40-60), 1:1, v/v): R_f = 0.68; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.05 – 3.92 (m, 2H), 3.52 – 3.32 (m, 4H), 2.31 – 2.14 (m, 1H), 1.85 – 1.49 (m, 14H), 1.36 – 1.22 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.6, 69.0, 46.9, 46.5, 38.9, 29.3, 28.6, 28.4, 27.4, 26.9, 25.4; ν_{max} (ATR): 2928, 2864, 1693 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{13}H_{24}NO_2$, 226.1807; found, 226.1815.

2-Cyclopentylethyl pyrrolidine-1-carboxylate (22)



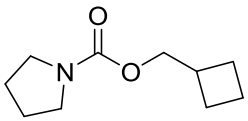
Prepared according to **general method B** using 1,1'-carbonyldiimidazole (0.18 g, 1.1 mmol), anhydrous THF (1.0 mL), 2-cyclopentylethanol (115 μL, 1.0 mmol) and pyrrolidine (84 μL, 1.0 mmol). The *carbamate* **22** was isolated as a colourless liquid (198 mg, 93%). TLC (EtOAc/petroleum ether (40-60), 1:1, v/v): R_f = 0.58; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.15 – 4.04 (m, 2H), 3.44 – 3.29 (m, 4H), 1.94 – 1.74 (m, 7H), 1.74 – 1.46 (m, 6H), 1.22 – 1.05 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.3, 64.6, 46.0, 37.0, 35.3, 32.7, 25.7, 25.0; ν_{max} (ATR): 2950, 2870, 1697 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{12}H_{22}NO_2$, 212.1651; found, 212.1656.

Cyclopropylmethyl pyrrolidine-1-carboxylate (23)



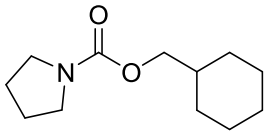
Prepared according to **general method B** using 1,1'-carbonyldiimidazole (0.18 g, 1.1 mmol), anhydrous THF (1.0 mL), cyclopropanemethanol (82 μL, 1.0 mmol) and pyrrolidine (84 μL, 1.0 mmol). The *carbamate* **23** was isolated as a colourless liquid (107 mg, 62%). TLC (EtOAc/petroleum ether (40-60), 4:1, v/v): R_f = 0.28; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.97 – 3.86 (m, 2H), 3.39 (s, 4H), 1.96 – 1.79 (m, 4H), 1.25 – 1.06 (m, 1H), 0.63 – 0.46 (m, 2H), 0.38 – 0.22 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.4, 69.5, 45.8, 25.7, 25.0, 10.3, 3.0; ν_{max} (ATR): 2954, 2875, 1693 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_9H_{16}NO_2$, 170.1181; found, 170.1176.

Cyclobutylmethyl pyrrolidine-1-carboxylate (24)



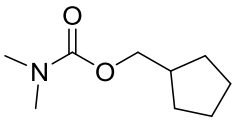
Prepared according to **general method B** using 1,1'-carbonyldiimidazole (0.18 g, 1.1 mmol), anhydrous THF (1.0 mL), cyclobutanemethanol (95 μL, 1.0 mmol) and pyrrolidine (84 μL, 1.0 mmol). The *carbamate* **24** was isolated as a colourless liquid (141 mg, 76%). TLC (EtOAc/petroleum ether (40-60), 1:1, v/v): R_f = 0.53; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.10 – 4.02 (m, 2H), 3.46 – 3.29 (m, 4H), 2.72 – 2.54 (m, 1H), 2.13 – 2.00 (m, 2H), 1.99 – 1.69 (m, 8H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.4, 68.6, 45.7, 34.5, 25.7 (br), 25.1 (br), 24.6, 18.4; ν_{max} (ATR): 2940, 2873, 1697 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{10}H_{18}NO_2$, 184.1338; found, 184.1341.

Cyclohexylmethyl pyrrolidine-1-carboxylate (25)



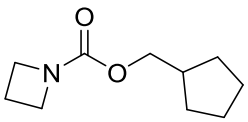
Prepared according to **general method B** using 1,1'-carbonyldiimidazole (0.18 g, 1.1 mmol), anhydrous THF (1.0 mL), cyclohexanemethanol (124 μL, 1.0 mmol) and pyrrolidine (84 μL, 1.0 mmol). The *carbamate* **25** was isolated as a colourless liquid (171 mg, 80%). TLC (EtOAc/petroleum ether (40-60), 1:1, v/v): R_f = 0.60; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.94 – 3.84 (m, 2H), 3.36 (s, 4H), 1.95 – 1.81 (m, 4H), 1.82 – 1.52 (m, 5H), 1.35 – 1.08 (m, 4H), 1.08 – 0.90 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.4, 70.1, 45.7, 37.5, 29.7, 26.5, 25.7, 25.0; ν_{max} (ATR): 2924, 2852, 1698 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{12}H_{22}NO_2$, 212.1651; found, 212.1658.

Cyclopentylmethyl dimethylcarbamate (26)



Prepared according to **general method B** using 1,1'-carbonyldiimidazole (0.18 g, 1.1 mmol), anhydrous THF (1.0 mL), cyclopentanemethanol (108 μL, 1.0 mmol), 33% w/w dimethylamine (137 μL, 1.0 mmol). The *carbamate* **26** was isolated as a colourless liquid (129 mg, 75%). TLC (EtOAc/petroleum ether (40-60), 1:1, v/v): R_f = 0.57; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.00 – 3.92 (m, 2H), 2.92 (s, 6H), 2.31 – 2.13 (m, 1H), 1.83 – 1.70 (m, 2H), 1.70 – 1.50 (m, 4H), 1.38 – 1.19 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.9, 69.3, 38.9, 36.3, 35.8, 29.2, 25.3; ν_{max} (ATR): 2948, 2869, 1699 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_9H_{18}NO_2$, 172.1338; found, 172.1337.

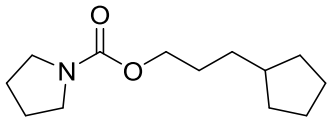
Cyclopentylmethyl azetidine-1-carboxylate (27)



Prepared according to **general method B** using 1,1'-carbonyldiimidazole (0.18 g, 1.1 mmol), anhydrous THF (1.0 mL), cyclopentanemethanol (108 μL, 1.0 mmol), azetidine

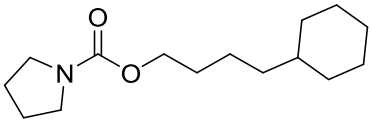
hydrochloride (93.4 mg, 1.0 mmol) and triethylamine (2 mmol). The *carbamate* **27** was isolated as a colourless liquid (89 mg, 49%). TLC (EtOAc/petroleum ether (40-60), 1:1, v/v): R_f = 0.54; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.07 – 3.98 (m, 4H), 3.98 – 3.90 (m, 2H), 2.32 – 2.11 (m, 3H), 1.82 – 1.68 (m, 2H), 1.68 – 1.48 (m, 4H), 1.34 – 1.19 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.0, 68.8, 49.3, 38.9, 29.2, 25.3, 15.7; ν_{max} (ATR): 2949, 2887, 1702 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{10}H_{18}NO_2$, 184.1338; found, 184.1333.

3-Cyclopentylpropyl pyrrolidine-1-carboxylate (28)



Prepared according to **general method B** using 1,1'-carbonyldiimidazole (0.18 g, 1.1 mmol), anhydrous THF (1.0 mL), 3-cyclopentan-1-propanol (142 μL, 1.0 mmol) and pyrrolidine (84 μL, 1.0 mmol). The *carbamate* **28** was isolated as a colourless liquid (156 mg, 69%). TLC (EtOAc/petroleum ether (40-60), 4:1, v/v): R_f = 0.35; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.14 – 4.02 (m, 2H), 3.49 – 3.26 (m, 4H), 1.96 – 1.72 (m, 7H), 1.72 – 1.46 (m, 6H), 1.45 – 1.31 (m, 2H), 1.18 – 1.01 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.4, 65.3, 46.1, 45.7, 39.8, 32.7, 32.3, 28.4, 25.7, 25.2, 25.0; ν_{max} (ATR): 2946, 2868, 1697 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{13}H_{24}NO_2$, 226.1807; found, 226.1801.

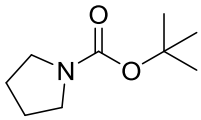
4-Cyclohexylbutyl pyrrolidine-1-carboxylate (29)



Prepared according to **general method B** using 1, 1'-carbonyldiimidazole (0.18 g, 1.1 mmol), anhydrous THF (1.0 mL), 4-cyclohexan-1-butanol (174 μL, 1.0 mmol) and pyrrolidine (84 μL, 1.0 mmol). The *carbamate* **29** was isolated as a colourless liquid (171 mg, 67%). TLC (EtOAc/petroleum ether (40-60), 4:1, v/v): R_f = 0.38; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.15 – 4.02 (m, 2H), 3.46 – 3.27 (m, 4H), 1.96 – 1.77 (m, 4H), 1.77 – 1.56 (m, 7H), 1.45 – 1.31 (m, 2H), 1.31 – 1.07 (m, 6H), 0.98 – 0.76 (m, 2H); ^{13}C NMR (125 MHz, MeOD) δ 155.4, 65.1, 46.1, 45.7, 37.5, 37.1, 33.4, 29.4, 26.7, 26.4, 25.8, 25.0, 23.2; ν_{max} (ATR):

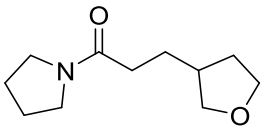
2919, 2849, 1698 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_{15}H_{28}NO_2$, 254.2120; found, 254.2113.

tert-Butyl pyrrolidine-1-carboxylate (30)



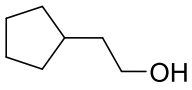
Pyrrolidine (100 µL, 1.2 mmol) and triethylamine (334 µL, 2.4 mmol) in anhydrous THF (3.0 mL) were cooled in an ice/ water bath. Di-*tert*-butyl dicarbonate (288 mg, 1.3 mmol) was added and the reaction mixture was stirred at 0 °C for 10 min, after which it was warmed up and stirred at room temperature for 18 h. The solvent was evaporated *in vacuo* and the crude residue was dissolved in EtOAc (30 mL) and washed with water (30 mL). The organic layer was concentrated *in vacuo*. Purification by automated flash chromatography afforded *tert*-butyl pyrrolidine-1-carboxylate **30** as a colourless liquid (105 mg, 51%). TLC (EtOAc/petroleum ether (40-60), 1:1, v/v): R_f = 0.56; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.37 – 3.27 (m, 4H), 1.89 – 1.80 (m, 4H), 1.47 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.7, 78.8, 45.8, 28.5, 25.4; ν_{max} (ATR): 2974, 2875, 1691 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_9H_{17}NO_2{}^{23}Na$, 194.1152; found, 194.1153.

1-(Pyrrolidin-1-yl)-3-(tetrahydrofuran-3-yl)propan-1-one (31)



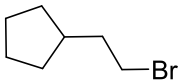
A mixture of (*E*)-3-(furan-3-yl)-1-(pyrrolidin-1-yl)prop-2-en-1-one **7** (20 mg, 0.1 mmol) and 30% wt Pd/C (3 mg) in ethyl acetate (2.0 mL) was thoroughly deoxygenated, put under hydrogen atmosphere and stirred vigorously at ambient temperature for 72 h. The catalyst was removed by filtration through celite. The filtrate was concentrated and dried *in vacuo* to give the desired *amide* **31** as a colourless oil (17 mg, 82%); TLC (DCM/MeOH, 9:1, v/v): R_f = 0.37; ^{1}H NMR (400 MHz, Acetone-d_6) δ 3.81 – 3.68 (m, 2H), 3.65 – 3.57 (m, 1H), 3.47 – 3.40 (m, 2H), 3.35 – 3.28 (m, 2H), 3.27 – 3.21 (m, 1H), 2.34 – 2.10 (m, 3H), 2.07 – 1.86 (m, 3H), 1.85 – 1.73 (m, 2H), 1.71 – 1.54 (m, 2H), 1.53 – 1.39 (m, 1H); ^{13}C NMR (125 MHz, acetone-d_6) δ 171.2, 74.0, 68.4, 47.2, 46.4, 40.2, 34.2, 33.4, 27.1, 25.4.

2-Cyclopentylethan-1-ol (32)[26]



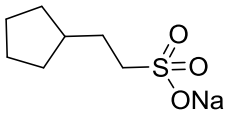
Lithium aluminium hydride in THF (1.0 M, 28 mL, 28 mmol) was added slowly to a solution of cyclopentylacetic acid (2.5 mL, 20 mmol) in anhydrous THF (2.0 mL) maintaining the internal temperature of the reaction mixture below 50 °C. After the addition was complete, the reaction mixture was stirred at ambient temperature overnight. 10% wt/wt aqueous NaOH (10 mL) was added and the quenched reaction mixture was stirred for approximately 30 min. The solids formed were removed by filtration and washed with THF (3 x 30 mL). The combined filtrate was concentrated *in vacuo* and re-dissolved in diethyl ether (50 mL). The bottom aqueous layer was separated and the organic layer was dried with anhydrous Na_2SO_4. The drying agent was removed by gravity filtration and the solvent was evaporated *in vacuo* to give the alcohol **32** as a colourless liquid (1.7 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.71-3.66 (m, 2H), 1.94-1.77 (m, 3H), 1.68-1.51 (m, 6H), 1.26 (br s, 1H), 1.18-1.09 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 62.5, 39.1, 36.6, 32.7, 25.0; IR, ν_{max} (ATR): 3292 (br, OH), 2945, 2867 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_7H_{15}O$, 115.1117; found, 115.1117.

(2-Bromoethyl)cyclopentane (33)[27]



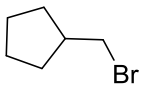
A solution of 2-cyclopentylethan-1-ol (1.0 g, 8.8 mmol) in anhydrous DCM (2.0 mL) was added to a solution of triphenylphosphine (2.3 g, 8.8 mmol) in anhydrous DCM (3.0 mL). The reaction mixture was stirred and cooled in ice/ water. *N*-Bromosuccinimide (1.56 g, 8.8 mmol) was added slowly and portion-wise (caution: effervescence). After the addition was complete, the ice/ water bath was removed and the reaction mixture was stirred at 22 °C overnight. The solvent was evaporated *in vacuo* and the desired bromide **33** was purified by flash chromatography as a pale yellow liquid (0.56 g, 36%).^{1}H NMR (400 MHz, $CDCl_3$) δ 3.45-3.41 (m, 2H), 2.03-1.78 (m, 5H), 1.68-1.52 (m, 4H), 1.17-1.08 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 39.3, 38.7, 33.1, 32.1, 25.0; IR, ν_{max} (ATR): 2947, 2865, 1451, 1259, 1214 cm^{-1}.

Sodium 2-cyclopentylethane-1-sulfonate (34)



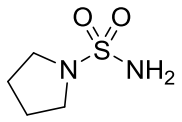
(2-Bromoethyl)cyclopentane (0.56 g, 3.2 mmol), sodium sulphite (0.48 g, 3.8 mmol) and distilled water (2.0 mL) were heated up to reflux with stirring for 36 h. The reaction mixture was then cooled to 22 °C and 1: 1 MeCN/ water (15 mL) was added to dissolve all solids. The solvents were removed *in vacuo* using a freeze dryer. The crude residue was dissolved in hot ethanol (15 mL) and the insoluble material was removed by gravity filtration through a hot funnel. The filtrate was slowly cooled to 22 °C and left in the fridge overnight. The crystals formed were filtered under suction, washed with cold ethanol (5 ml) and dried *in vacuo*. The desired *sulphonate* **34** was isolated as a white crystalline solid (130 mg, 21%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.40-2.36 (m, 2H), 1.81-1.65 (m, 3H), 1.60-1.53 (m, 4H), 1.51-1.42 (m, 2H), 1.08-0.99 (m, 2H); ^{13}C NMR (125 MHz, DMSO) δ 50.9, 38.9, 32.1, 31.4, 24.8; IR, ν_{max} (ATR): 3536, 3488, 2951, 2866, 1620, 1454 cm^{-1}; HRMS (*m/z*): $[M + Na]^+$ calcd for $C_7H_{13}O_3{}^{23}Na_2{}^{32}S$, 223.0375; found, 223.0365.

(Bromomethyl)cyclopentane (35)[28]



Cyclopentylmethanol (1.0 g, 10 mmol) and triphenylphosphine (2.8 g, 10.7 mmol) were dissolved in DMF (10 mL) and cooled in an ice-water bath. Bromine (0.51 mL, 10.7 mmol) was added over 10 minutes. After the addition was complete, the reaction mixture was allowed to warm up and was stirred at ambient temperature overnight. The crude reaction mixture was distilled at 55 °C, 1 Torr and the distillate was collected as a 31% wt/wt solution of the bromide in DMF. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.40-3.39 (m, 2H), 2.36-2.25 (m, 1H), 1.89-1.82 (m, 2H), 1.70-1.54 (m, 4H), 1.35-1.27 (m, 2H).

Pyrrolidine-1-sulfonamide (36)[29]

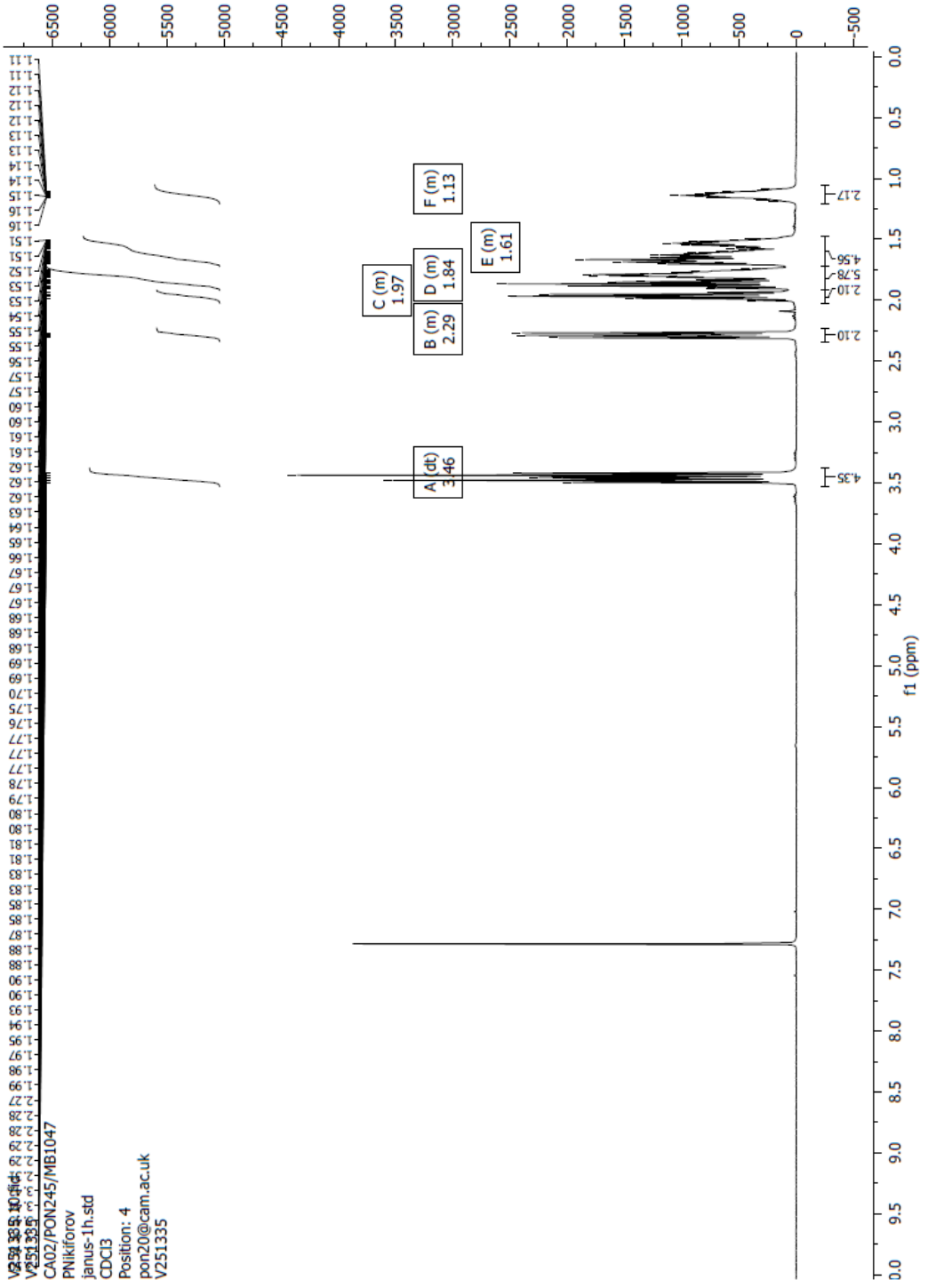


Sulfuric diamide (1.0 g, 10.4 mmol) was dissolved in DME (6.0 mL) and pyrrolidine (0.78 mL, 9.4 mmol) was added. The reaction mixture was heated under reflux for 6 h. After cooling to 22 °C, the solvents were removed *in vacuo* and the crude residue was purified by flash chromatography to afford the desired sulphonamide **36** as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.48 (br s, 2H), 3.35-3.31 (m, 4H), 1.99-1.93 (m, 4H); IR, ν_{max} (ATR): 3333, 3246, 2988, 2962, 2866, 1575 cm^{-1}; HRMS (*m/z*): $[M + H]^+$ calcd for $C_4H_{11}N_2O_2S$, 151.0536; found, 151.0535.

Selected Spectra

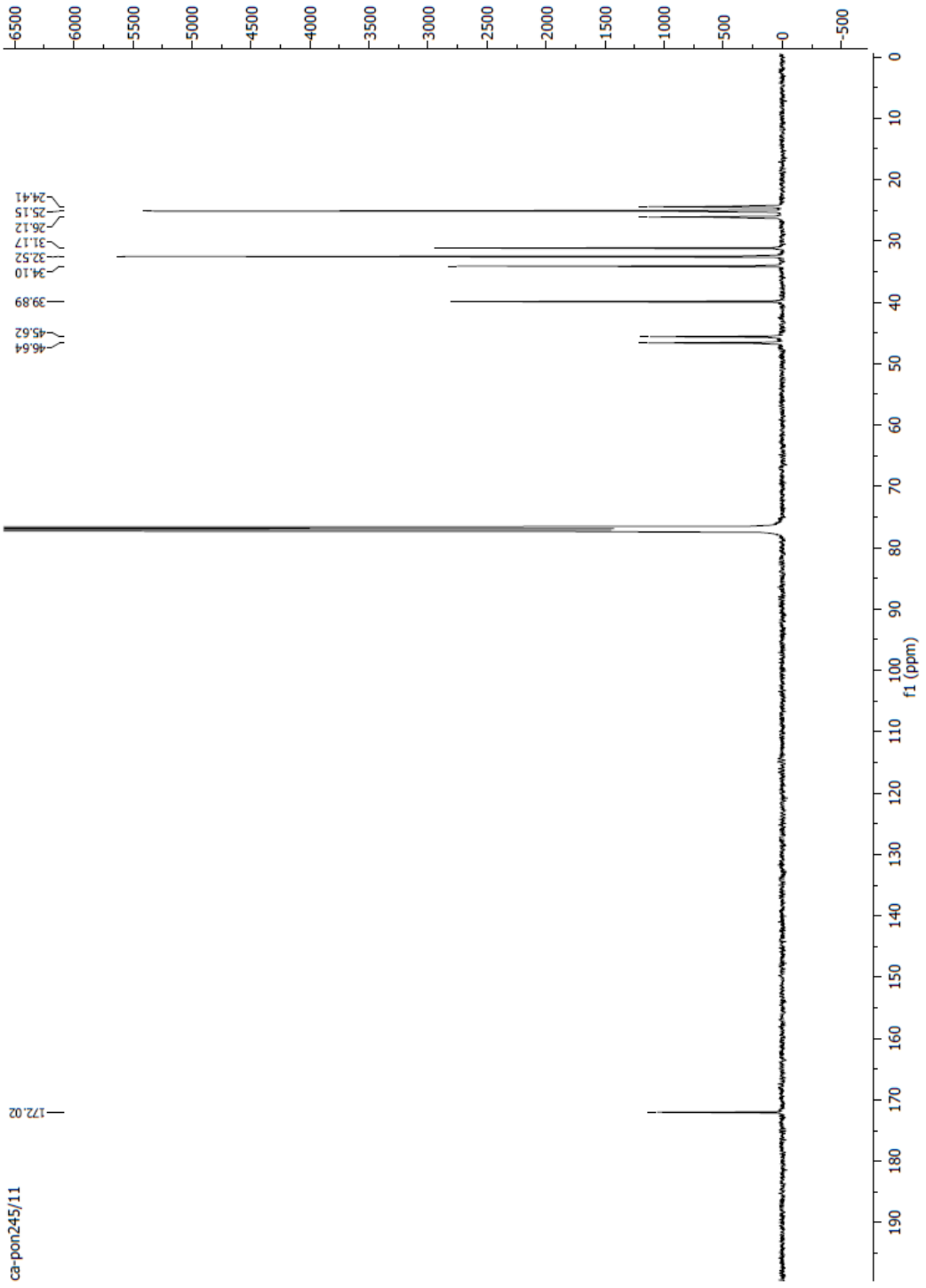
3-Cyclopentyl-1-(pyrrolidin-1-yl)propan-1-one (1)

^{1}H NMR



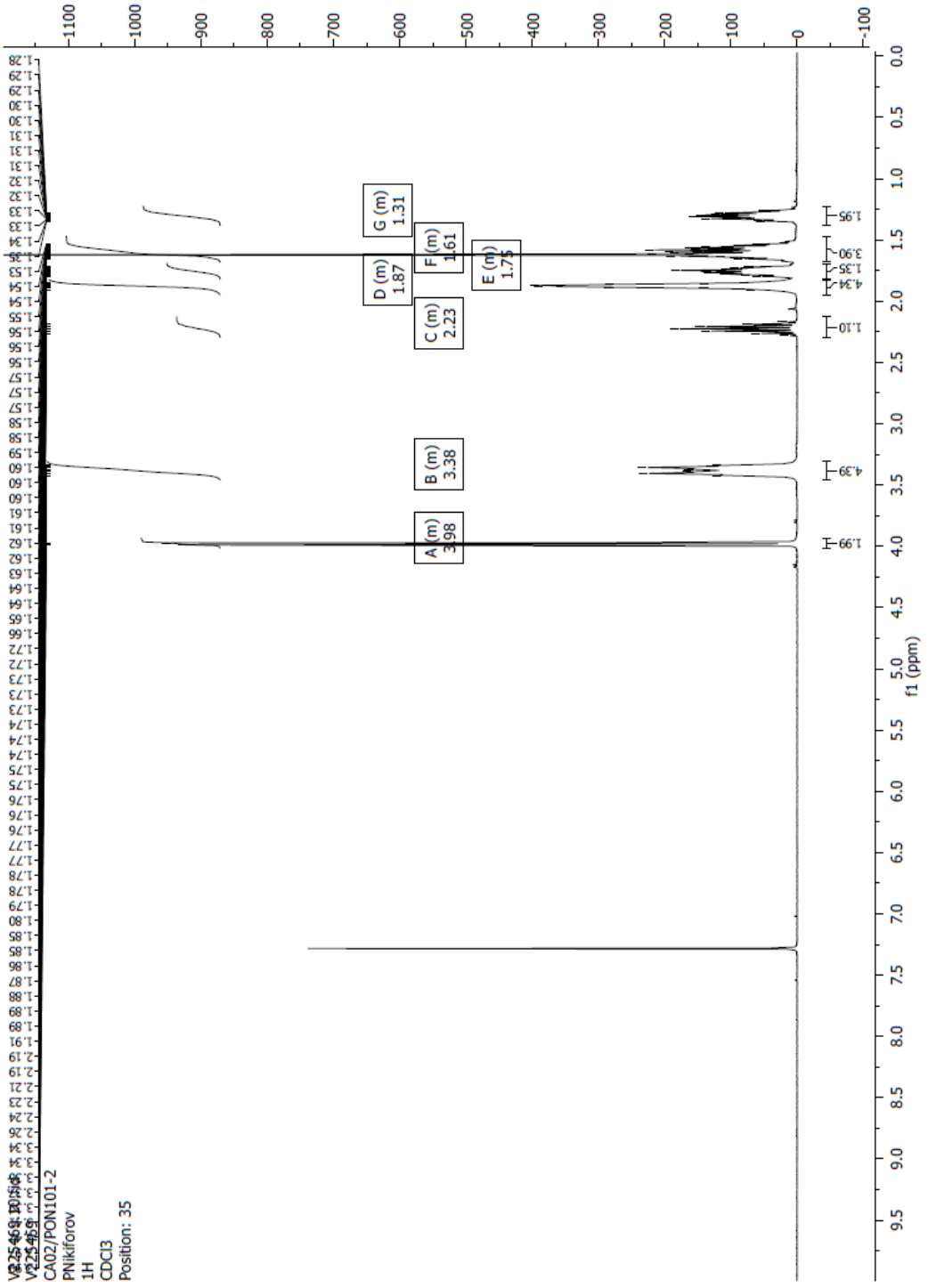
3-Cyclopentyl-1-(pyrrolidin-1-yl)propan-1-one (1)

^{13}C NMR



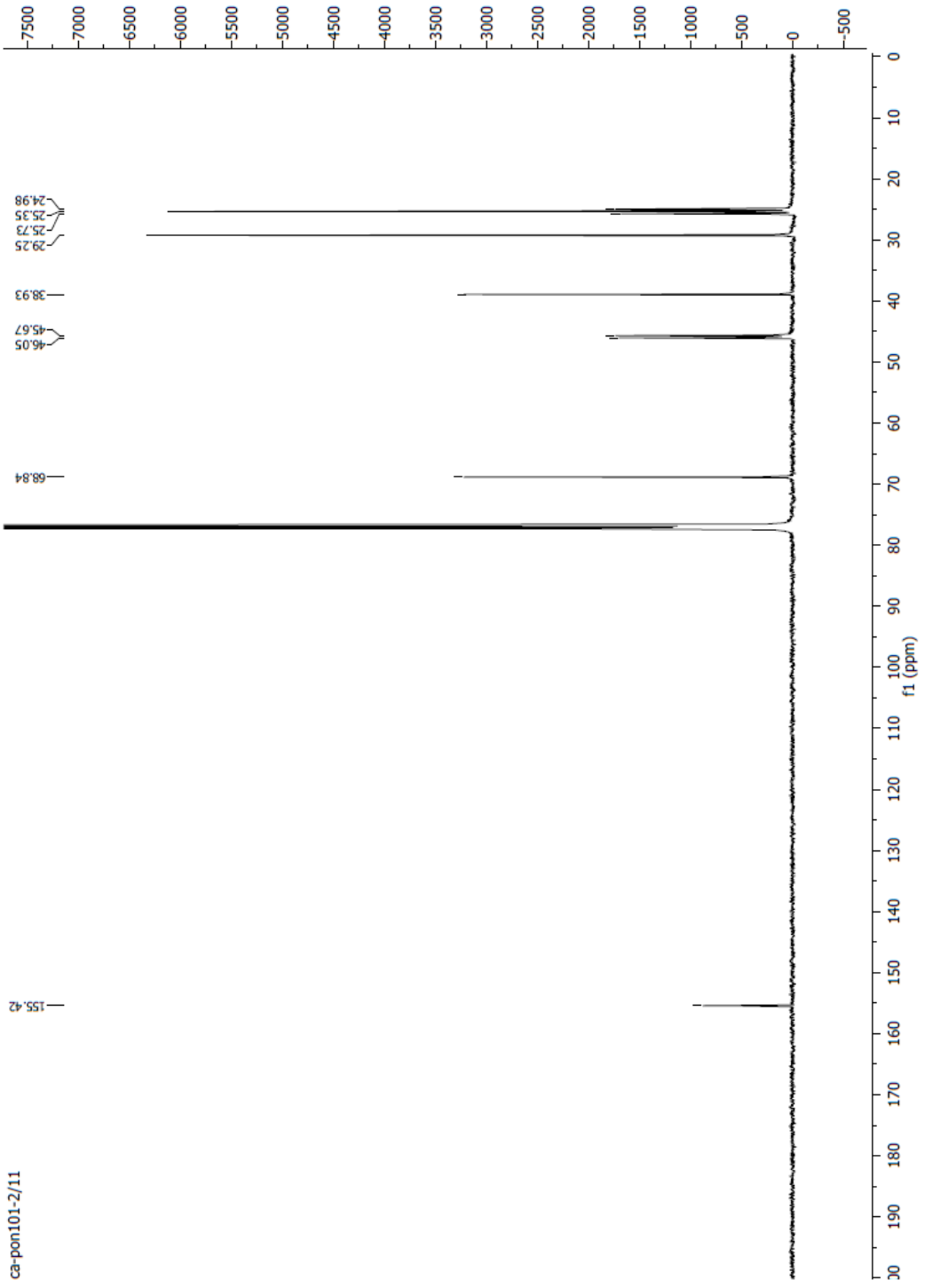
Cyclopentylmethyl pyrrolidine-1-carboxylate (2)

^{1}H NMR



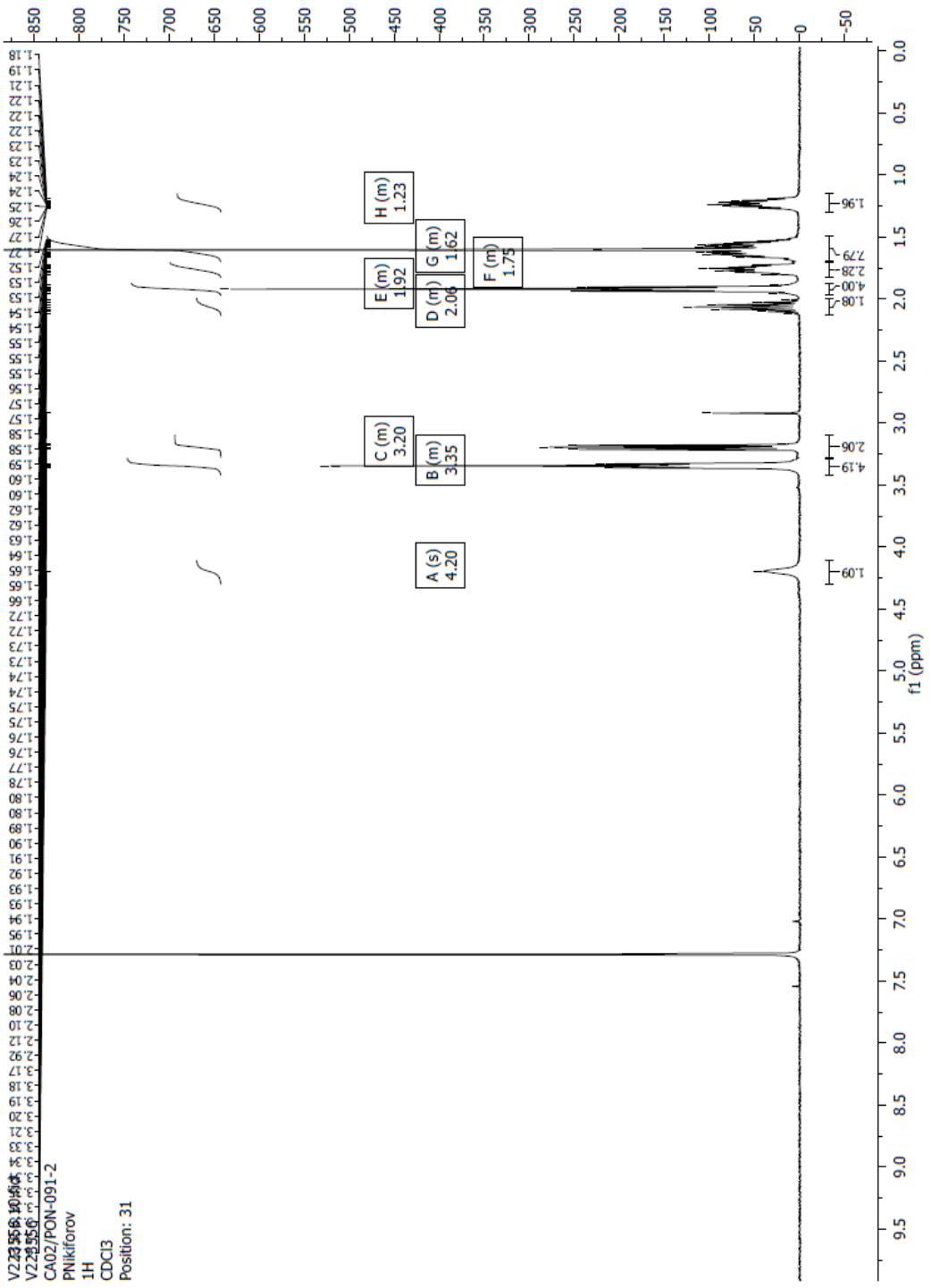
Cyclopentylmethyl pyrrolidine-1-carboxylate (2)

^{13}C NMR



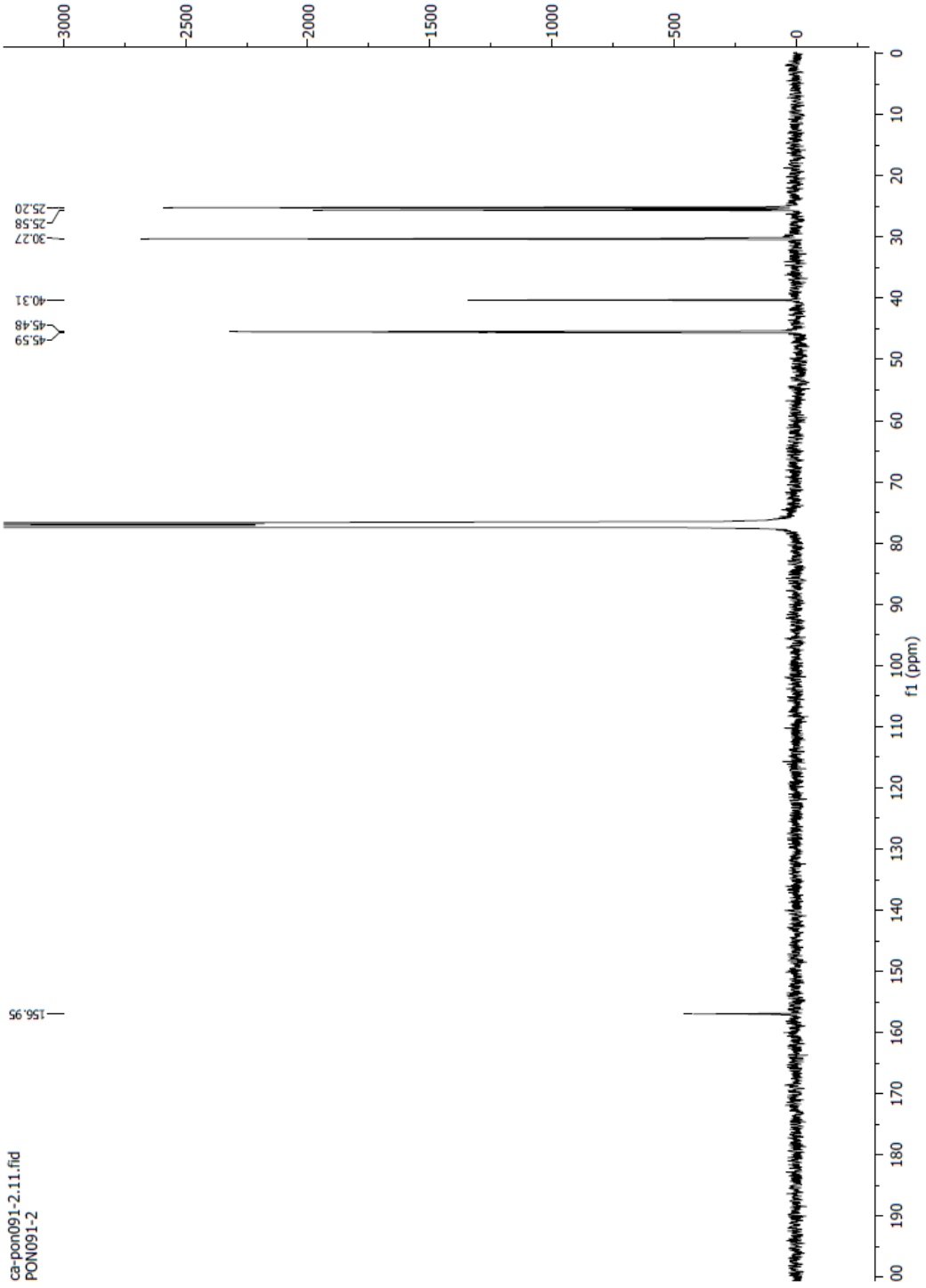
N-(Cyclopentylmethyl)pyrrolidine-1-carboxamide (3)

^{1}H NMR



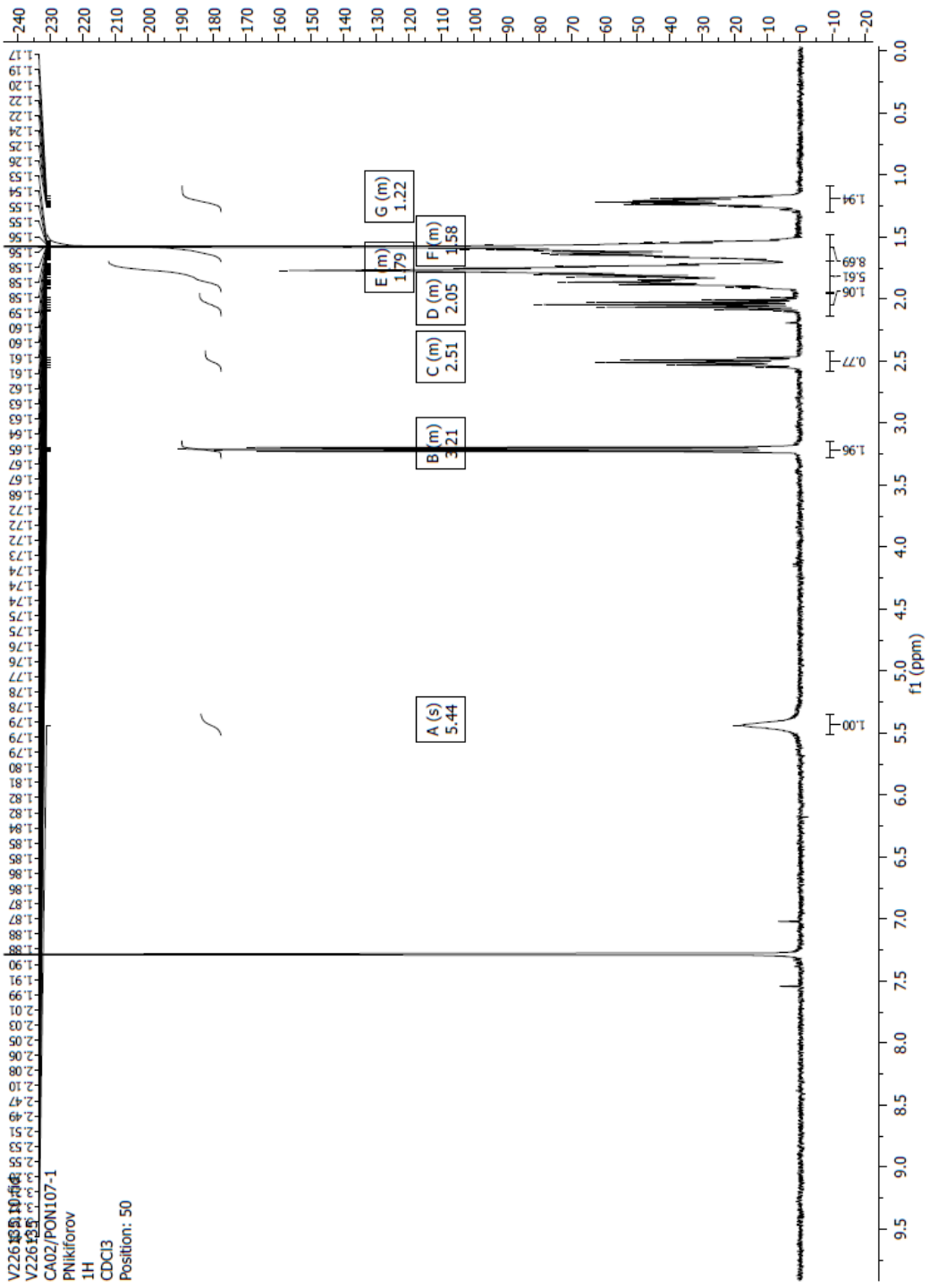
N-(Cyclopentylmethyl)pyrrolidine-1-carboxamide (3)

^{13}C NMR



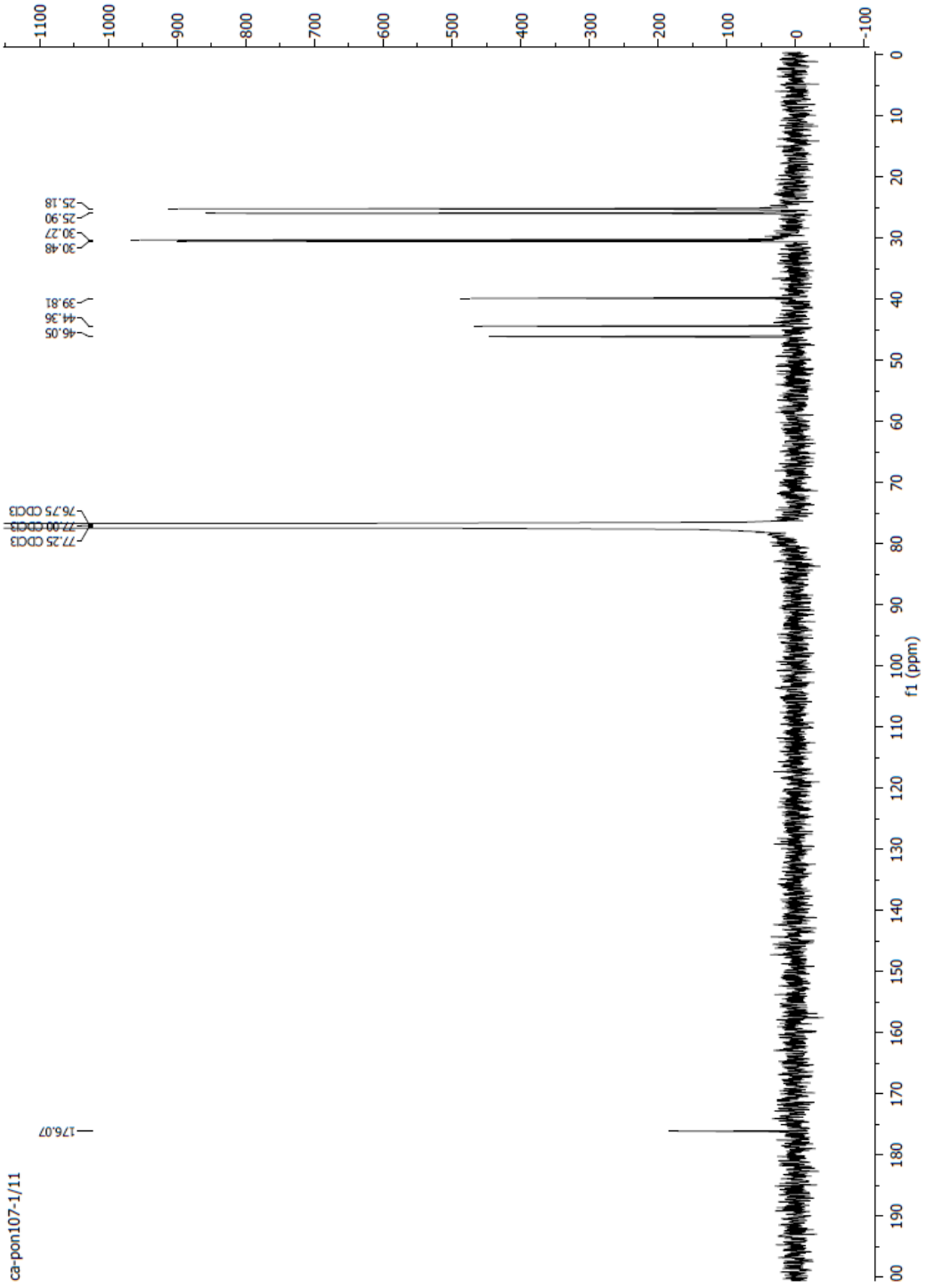
N-(Cyclopentylmethyl)cyclopentanecarboxamide (4)

^{1}H NMR



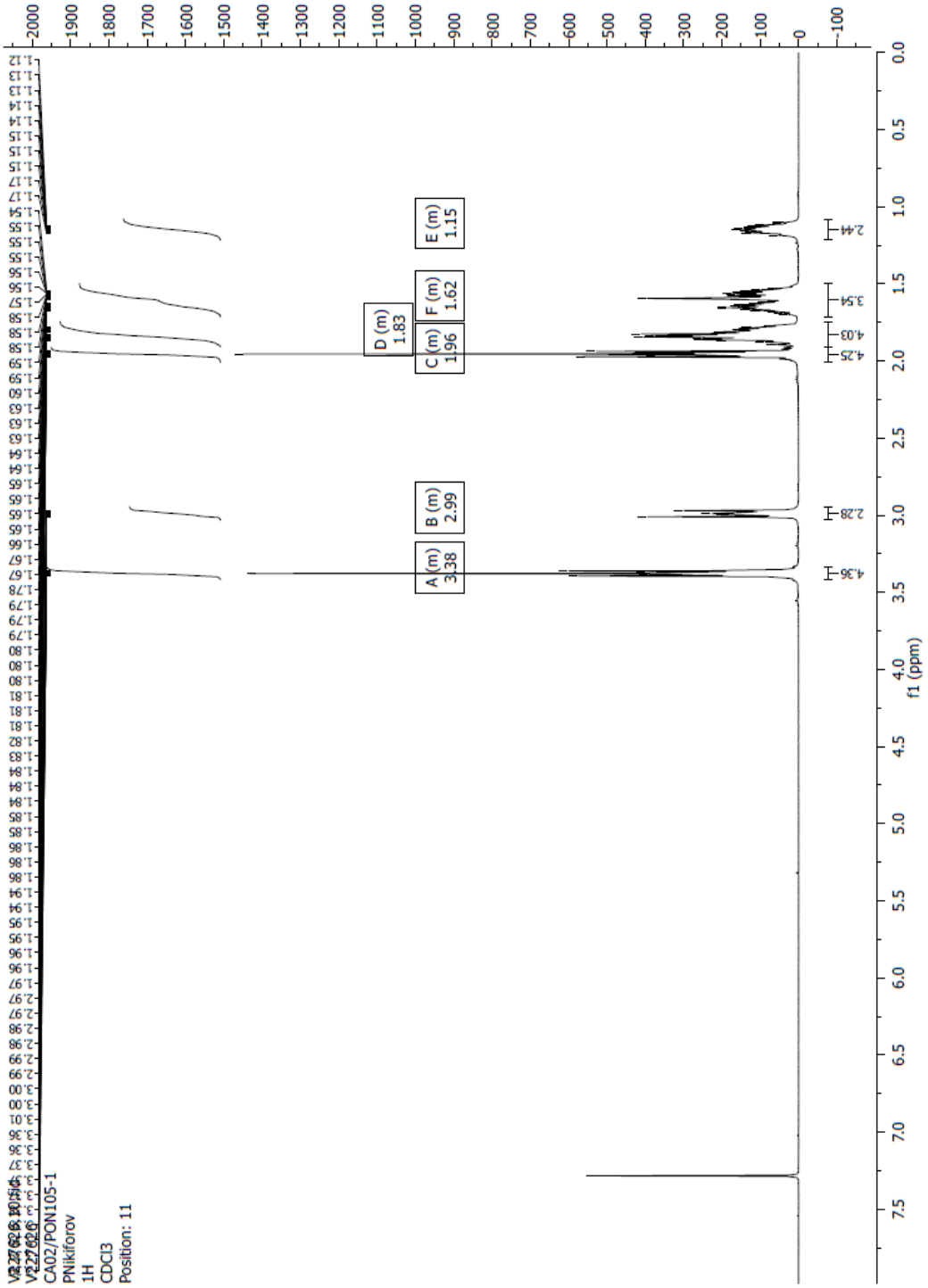
***N*-(Cyclopentylmethyl)cyclopentanecarboxamide (4)**

^{13}C NMR



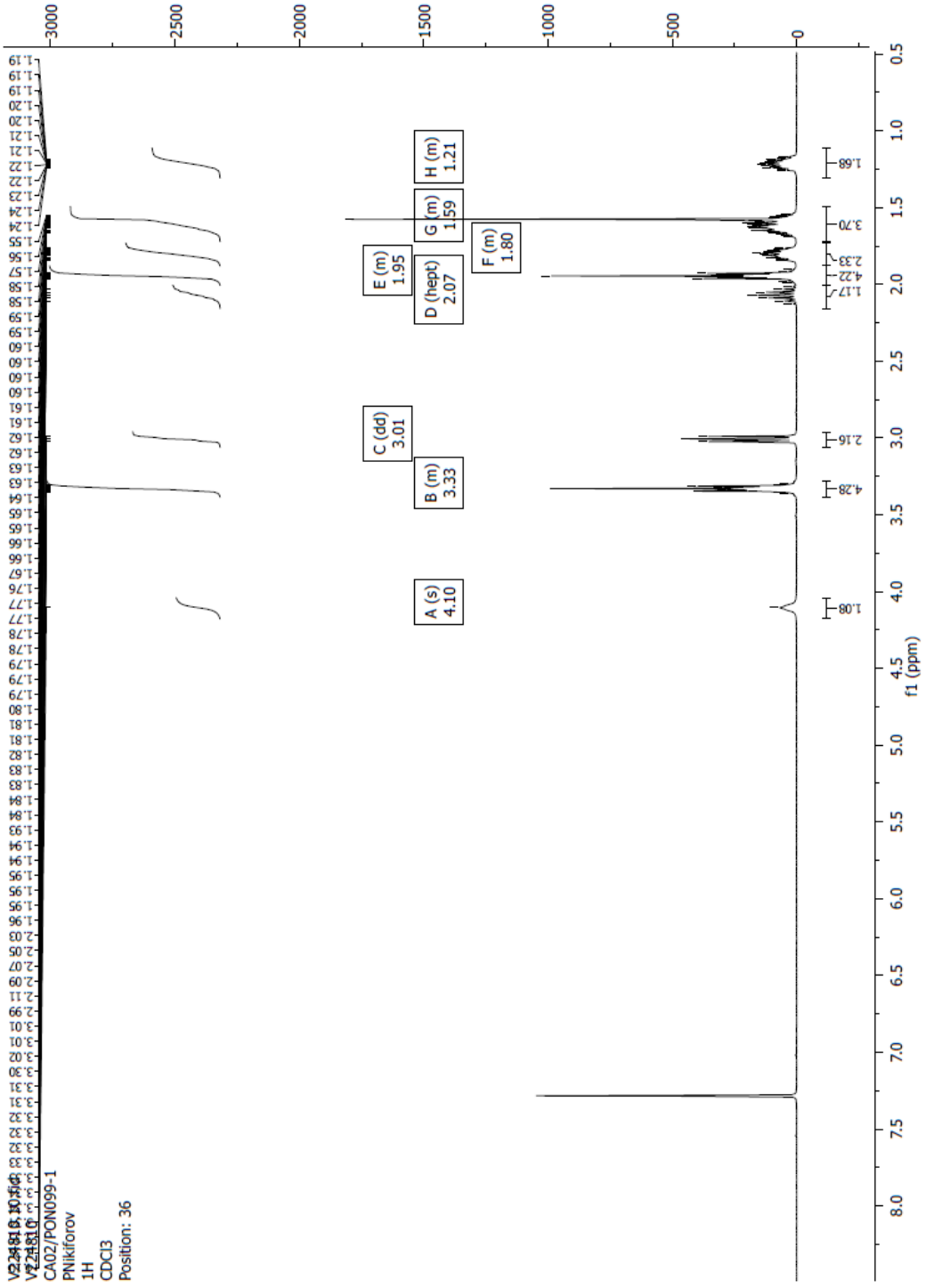
1-((2-Cyclopentylethyl)sulfonyl)pyrrolidine (5)

^{1}H NMR



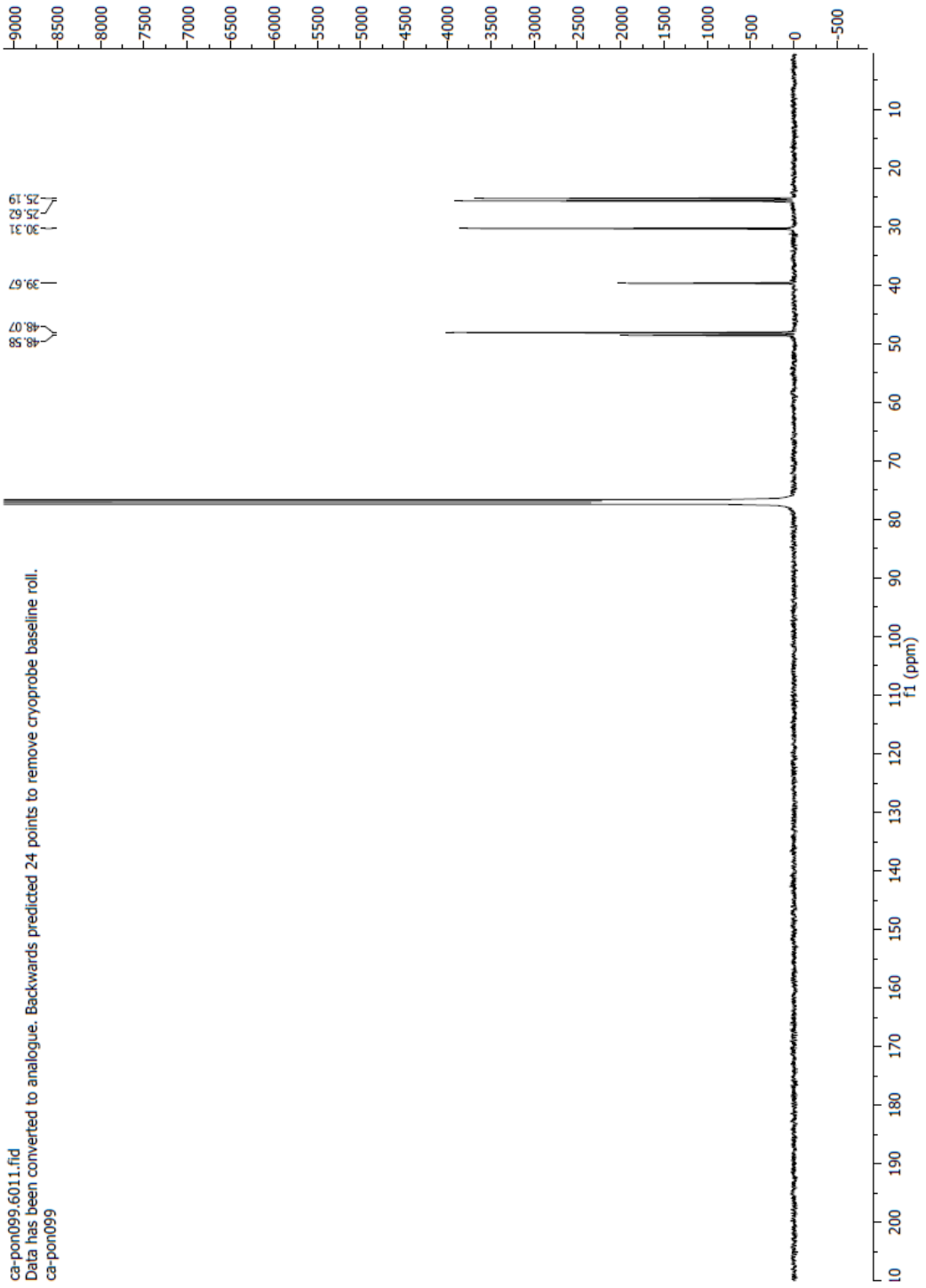
N-(Cyclopentylmethyl)pyrrolidine-1-sulfonamide (6)

^{1}H NMR



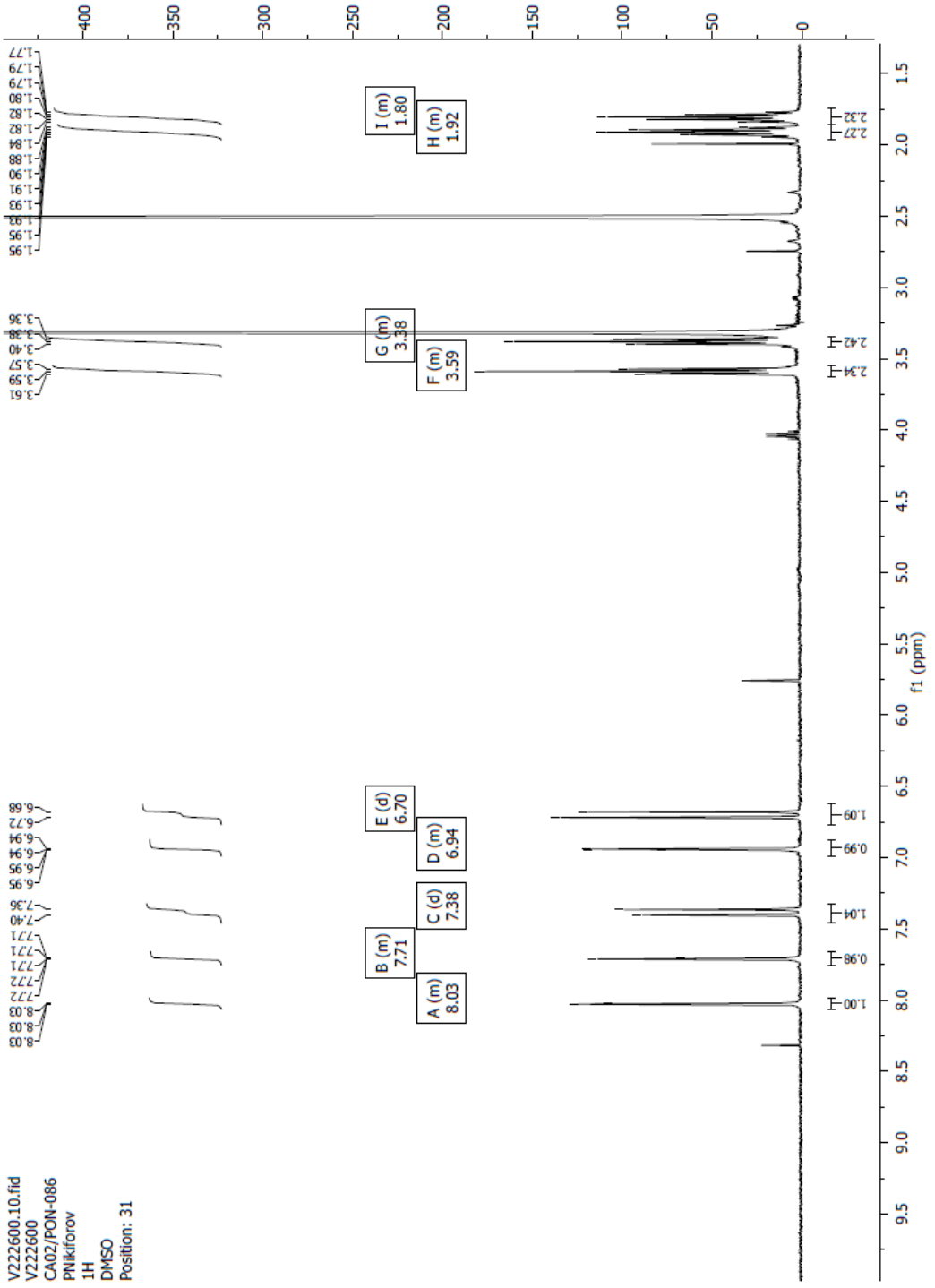
N-(Cyclopentylmethyl)pyrrolidine-1-sulfonamide (6)

^{13}C NMR



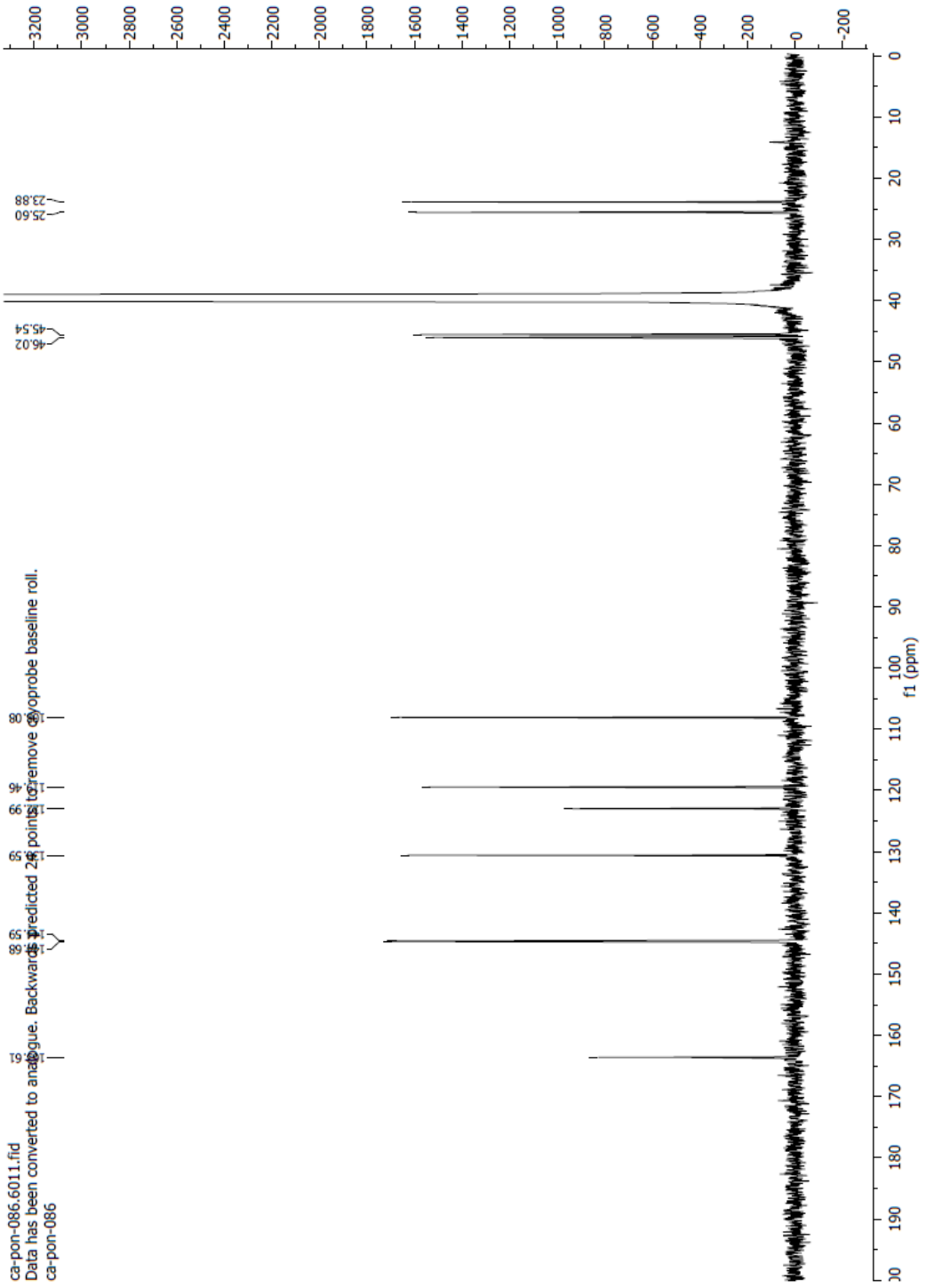
(*E*)-3-(Furan-3-yl)-1-(pyrrolidin-1-yl)prop-2-en-1-one (7)

^{1}H NMR



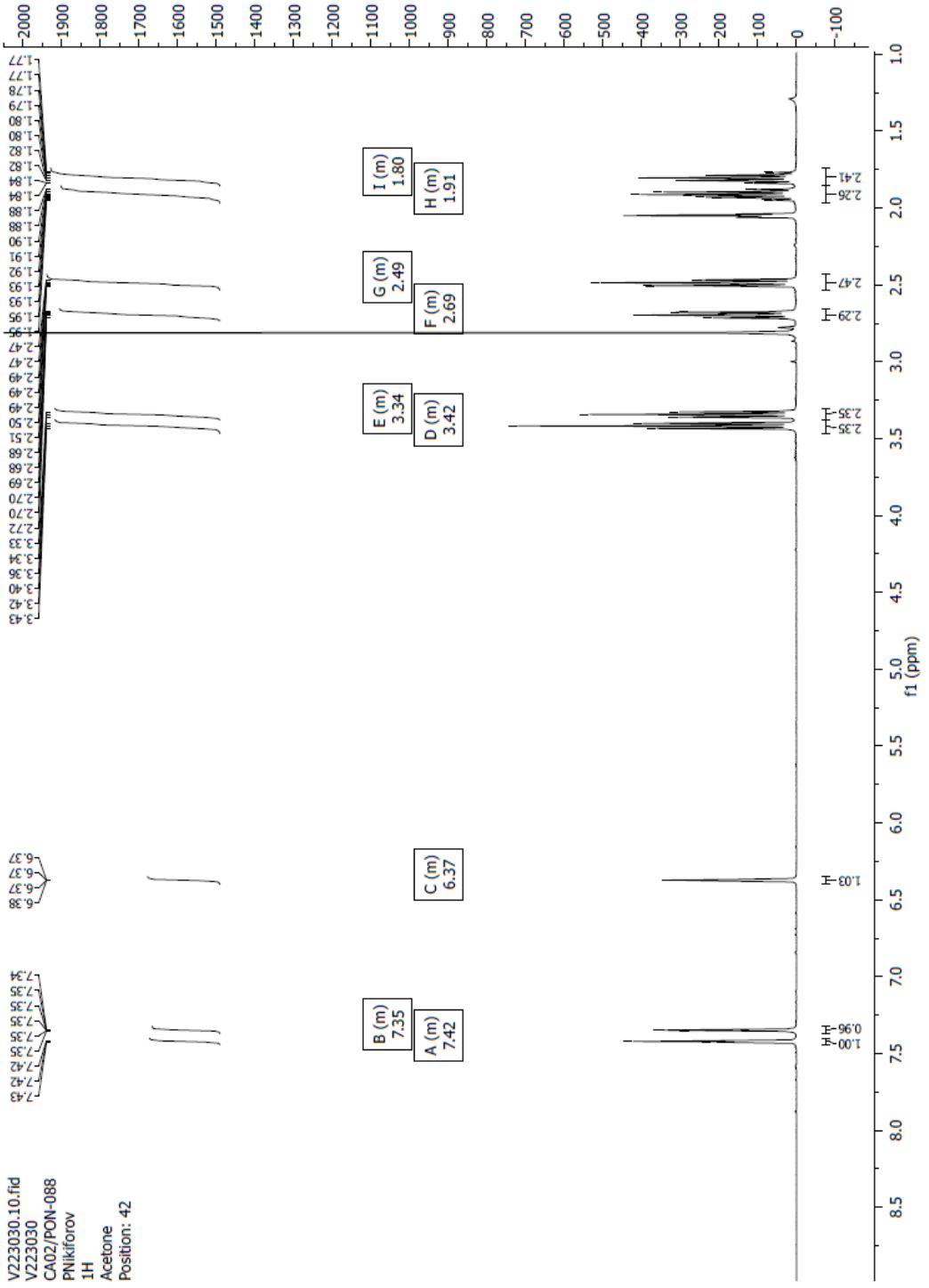
(*E*)-3-(Furan-3-yl)-1-(pyrrolidin-1-yl)prop-2-en-1-one (7)

^{13}C NMR



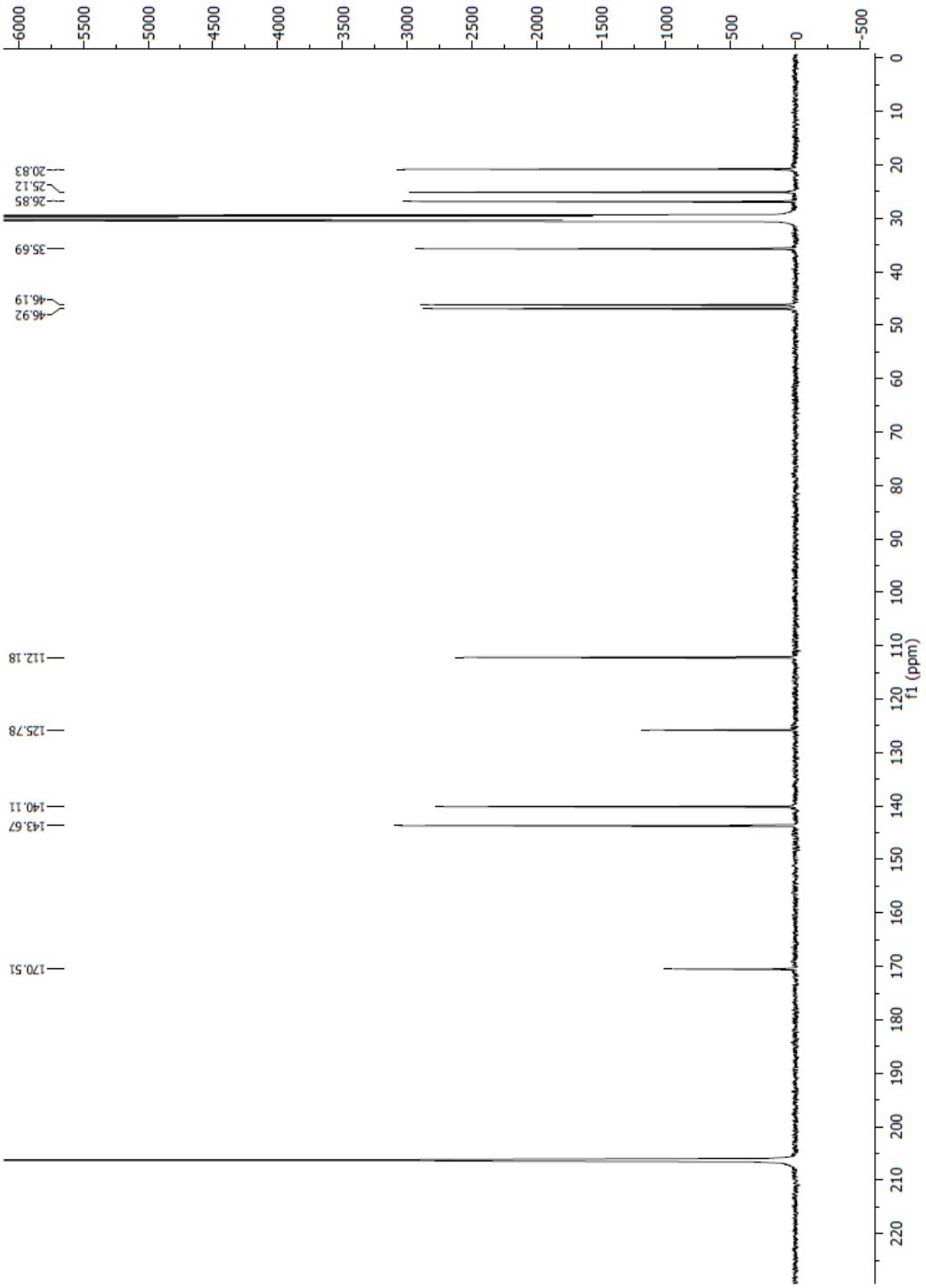
3-(Furan-3-yl)-1-(pyrrolidin-1-yl)propan-1-one (8)

^{1}H NMR



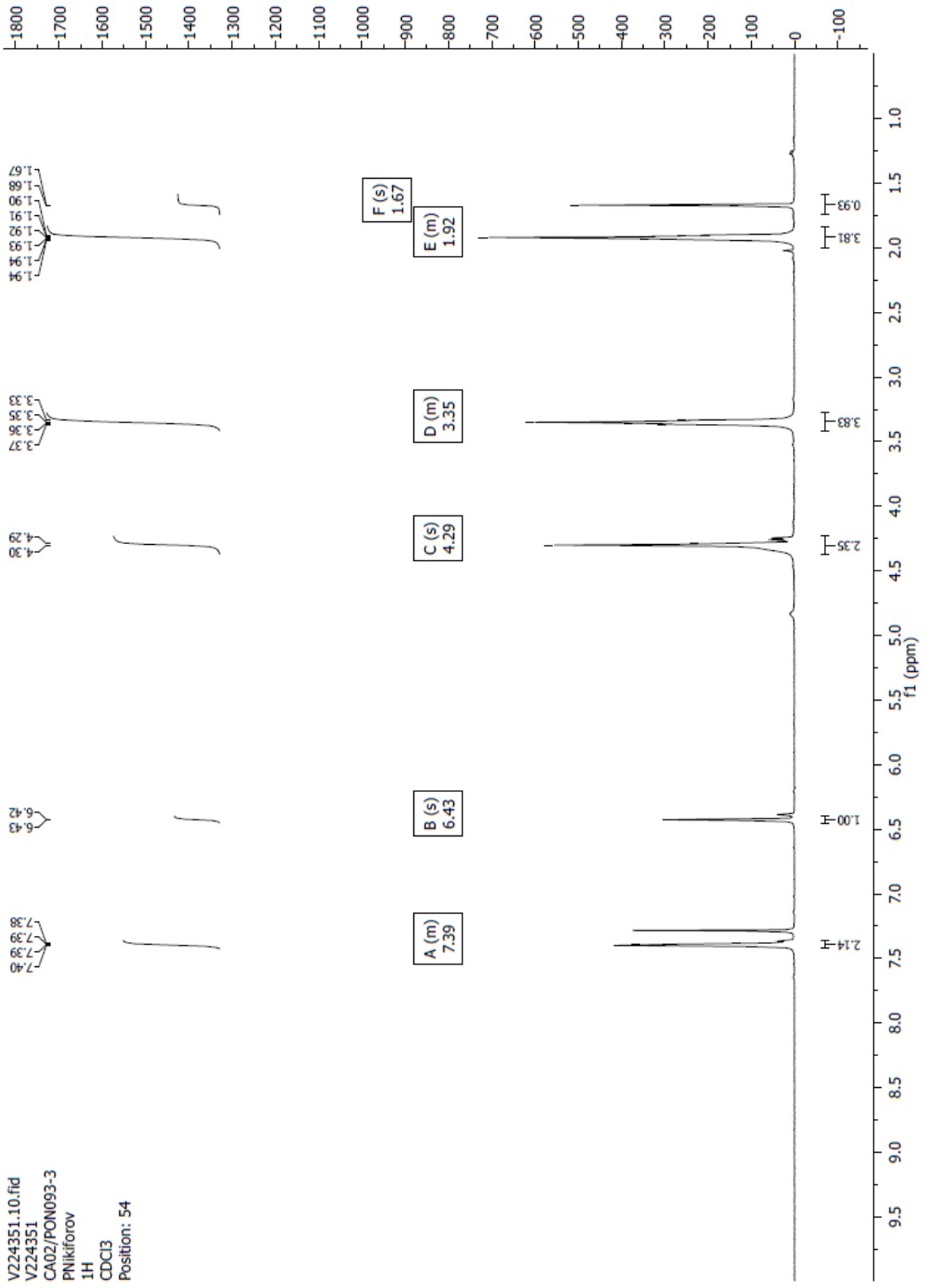
3-(Furan-3-yl)-1-(pyrrolidin-1-yl)propan-1-one (8)

^{13}C NMR



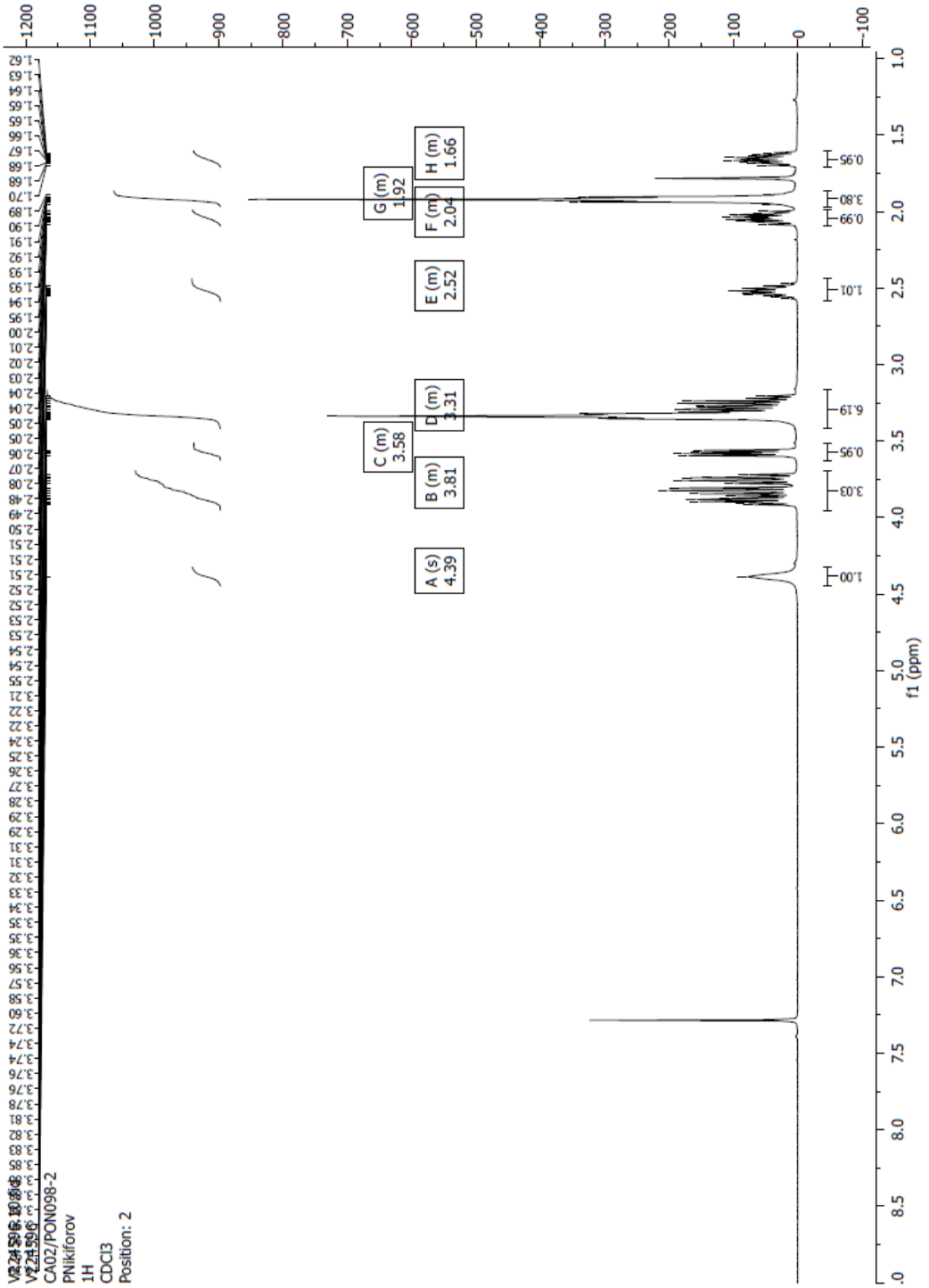
N-(Furan-3-ylmethyl)pyrrolidine-1-carboxamide (9)

^{1}H NMR



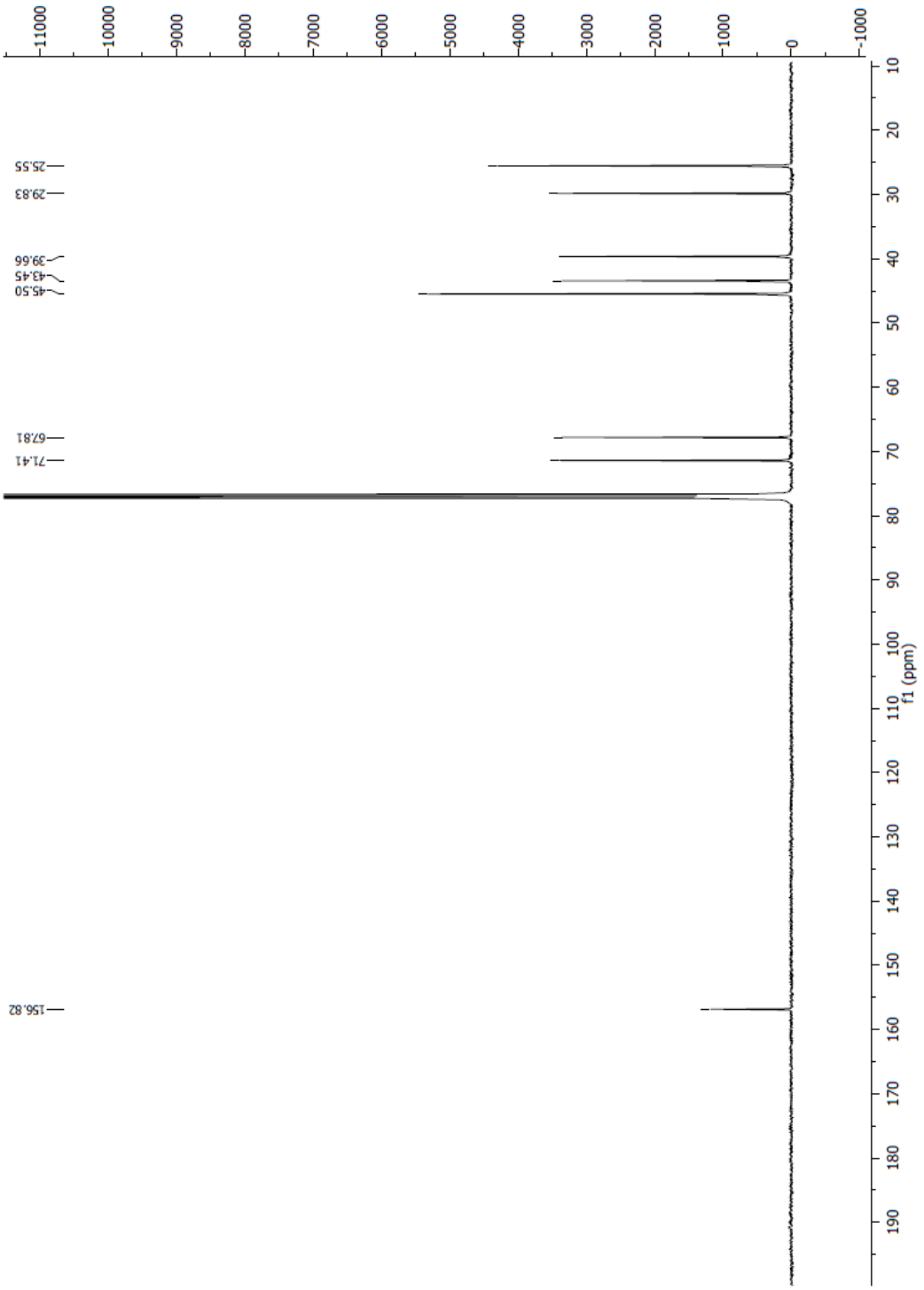
N-((Tetrahydrofuran-3-yl)methyl)pyrrolidine-1-carboxamide (10)

^{1}H NMR



_N_-((Tetrahydrofuran-3-yl)methyl)pyrrolidine-1-carboxamide (10)

^{13}C NMR



References

(1) Peränen, J., Rikkonen, M., Hyvönen, M., and Kääriäinen, L. (1996) T7 vectors with modified T7lac promoter for expression of proteins in *Escherichia coli*. *Analytical Biochemistry 236*, 371-373.

(2) Surade, S., Ty, N., Hengrung, N., Lechartier, B., Cole, S. T., Abell, C., and Blundell, T. L. (2014) A structure-guided fragment-based approach for the discovery of allosteric inhibitors targeting the lipophilic binding site of transcription factor EthR. *Biochem. J. 458*, 387-394.

(3) Vonrhein, C., Flensburg, C., Keller, P., Sharff, A., Smart, O., Paciorek, W., Womack, T., and Bricogne, G. (2011) Data processing and analysis with the autoPROC toolbox. *Acta Crystallographica D67*, 293-302.

(4) Kabsch, W. (2010) Software XDS for image rotation, recognition and crystal symmetry assignment. *Acta Crystallographica D66*, 125-132.

(5) Leslie, A. G. W., and Powell, H. R. (2007) Processing diffraction data with Mosfilm. In Evolving Methods for Macromolecular Crystallography (Read, R. J. and Sussman, J. L., editors), Springer, Berlin, 41-51.

(6) Evans, P. R. (2011) An introduction to data reduction: Space-group determination, scaling and intensity statistics. *Acta Crystallographica D67*, 282-292.

(7) McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. and Read, R. J. (2007) Phaser crystallographic software, *Journal of Applied Crystallography 40*, 658-674.

(8) Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallographica D53*, 240-255.

(9) Winn, M. D., Ballard, C. C., Cowtan, K. D., Dodson, E. J., Emsley, P., Evans, P. R., Keegan, R. M., Krissinel, E. B., Leslie, A. G. W., and McCoy, A. (2011) Overview of the CCP4 suite and current developments. *Acta Crystallographica D67*, 235–242.

(10) Adams, P. D., Afonine, P. V., Bunkóczi, G., Chen, V. B., Echols, N., Headd, J. J., Hung, L.-W., Jain, S., Kapral, G. J., Grosse Kunstleve, R. W., McCoy, A. J., Moriarty, N. W., Oeffner, R. D., Read, R. J., Richardson, D. C., Richardson, J. S., Terwilliger, T. C., and Zwart, P. H. (2011) The Phenix software for automated

determination of macromolecular structures. *Methods* (Amsterdam, Netherlands) *55*, 94-106.

(11) Bricogne, G., Blanc, E., Brandl, M., Flensburg, C., Keller, P., Paciorek, W., Roversi, P., Sharff, A., Smart, O. S., Vonrhein, C., and Womack, T. O. (2011) BUSTER version X.Y.Z. Cambridge, United Kingdom: Global Phasing Ltd.

(12) Schuttelkopf, A. W., and van Aalten, D. M. F. (2004) PRODRG: a tool for high-throughput crystallography of protein-ligand complexes. *Acta Crystallographica D60*, 1355-1363.

(13) Vagin, A. A., Murshudov, G. N., and Strokopytov, B. V. (1998) BLANC: the program suite for protein crystallography. *Journal of Applied Crystallography 31*, 98-102.

(14) Emsley, P., Lohkamp, B., Scott, W. G., and Cowtan, K. (2010) Features and development of Coot. *Acta Crystallographica D66*, 486-501.

(15) Engohang-Ndong, J., Baillat, D., Aumercier, M., Bellefontaine, F., Besra, G. S., Locht, C., and Baulard, A. R. (2004) EthR, a repressor of the TetR/CamR family implicated in ethionamide resistance in mycobacteria, octamerizes cooperatively on its operator. *Mol. Microbiol. 51*, 175-188.

(16) Willand, N., Dirié, B., Carette, X., Bifani, P., Singhal, A., Desroses, M., Leroux, F., Willery, E., Mathys, V., Déprez-Poulain, R., Delcroix, G., Frénois, F., Aumercier, M., Locht, C., Villeret, V., Déprez, B., and Baulard, A. R. (2009) Synthetic EthR inhibitors boost antituberculous activity of ethionamide. *Nat. Med. 15*, 537-544.

(17) Christophe, T., Jackson, M., Jeon, H. K., Fenistein, D., Contreras-Dominguez, M., Kim, J., Genovesio, A., Carralot, J.-P., Ewann, F., Kim, E. H., Lee, S. Y., Kang, S., Seo, M. J., Park, E. J., Škovierová, H., Pham, H., Riccardi, G., Nam, J. Y., Marsollier, L., Kempf, M., Joly-Guillou, M.-L., Oh, T., Shin, W. K., No, Z., Nehrbass, U., Brosch, R., Cole, S. T., and Brodin, P. (2009) High content screening identifies decaprenyl-phosphoribose 2' epimerase as a target for intracellular antimycobacterial inhibitors. *PLoS Pathog. 5*, e1000645.

(18) Christophe, T., Ewann, F., Jeon, H. K., Cechetto, J., and Brodin, P. (2010) High-content imaging of *Mycobacterium tuberculosis*-infected macrophages: an in vitro model for tuberculosis drug discovery. *Future Med. Chem. 2*, 1283-1293.

(19) Brodin, P., and Christophe, T. (2011) High-content screening in infectious diseases. *Curr. Opin. Chem. Biol. 15*, 534-539.

(20) El-Faham, A., Funosas, R. S., Prohens, R., and Albericio, F. (2009) COMU: a safer and more effective replacement for benzotriazole-based uronium coupling reagents. *Chem. Eur. J. 15*, 9404-9416.

(21) Negi, S., Sasho, M., Yamanaka, M., Sugiyama, I., Komatsu, Y., Tsuruoka, A., Kamada, A., Tsukada, I., Hiruma, R., Katsu, K., and Machida, Y. (1994) Studies on orally active cephalosporins II. Synthesis and structure-activity relations of new [(*E*) or (*Z*) 3-substituted carbamoyloxy]-1-propenyl cephalosporins. *The Journal of Antibiotics 47*, 1526-1540.

(22) Peng, B., Geerdink, D., Farès, C., and Maulide, N. (2014) Chemoselective intermolecular α-arylation of amides. *Angew. Chem. Int. Ed. 53*, 5462-5466.

(23) Bai, J., Zambroń, B. K., and Vogel, P. (2014) Amides in one-pot from carboxylic acids and amines via sulfinylamides. *Org. Lett. 16*, 604-607.

(24) Szostak, M., Spain, M., and Procter, D. J. (2013) Uncovering the importance of proton donors in TmI_2-promoted electron transfer: facile C-N bond cleavage in unactivated amides. *Angew. Chem. Int. Ed. 52*, 7237-7241.

(25) Kasemura, K., Fujita, S., Okada, Y., Fujihara, Y., and Nomura, M. (2003) Miticidal activity of monoterpenyl carboxypyrrolidinamides and piperidinamides. *J. Oleo Sci. 52*, 41-46.

(26) Cane, D. E., and Thomas, P. J. (1984) Synthesis of *dl*-pentalenolactones E and F. *J. Am. Chem. Soc. 106*, 5295-5303.

(27) Lednicer, D., Von Voigtlander, P. F., and Emmert, D. E. (1981) 4-Amino-4-arylcyclohexanones and their derivatives: a novel class of analgesics. 2. Modification of the carbonyl function. *J. Med. Chem. 24*, 404-408.

(28) Samsel, E. G., and Kochi, J. K. (1986) Oxidative alkylation of cobalt complexes with hydrazines. *Inorg. Chem. 25*, 2450-2457.

(29) PCT *Intl. Appl.* 2011/096462 A1, 11 Aug 2011, p. 51.