ChemBioChem

Supporting Information

[4.3.1]Bicyclic FKBP Ligands Inhibit *Legionella Pneumophila* Infection by *Lp*Mip-Dependent and *Lp*Mip-Independent Mechanisms

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Table of content:

1. Supplementary figures

a.

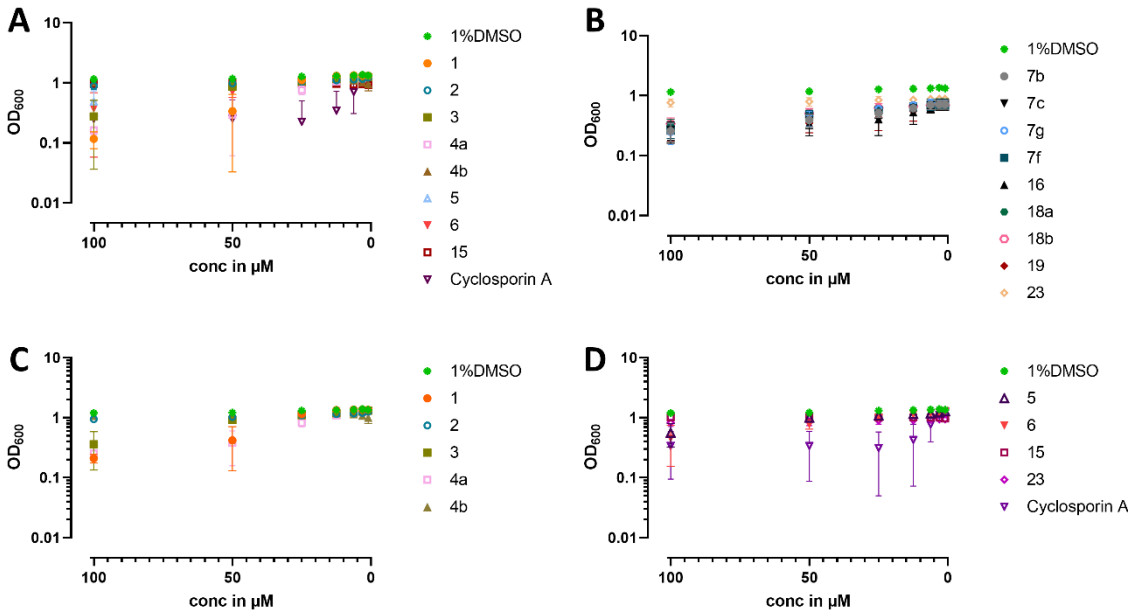


Figure S1: Impact of compounds on the growth of *L. pneumophila* wild type (A+B) and *Δmip* strain (C+D) after 24 hours. The bacterial growth was quantified by measuring the OD_{600nm}. 1% DMSO references (green stars) were included as negative controls for each set of compound concentrations. The MIC values were determined using GraphPad regression analysis. The 95% confidence interval for the calculated values was ± 5%.

b.

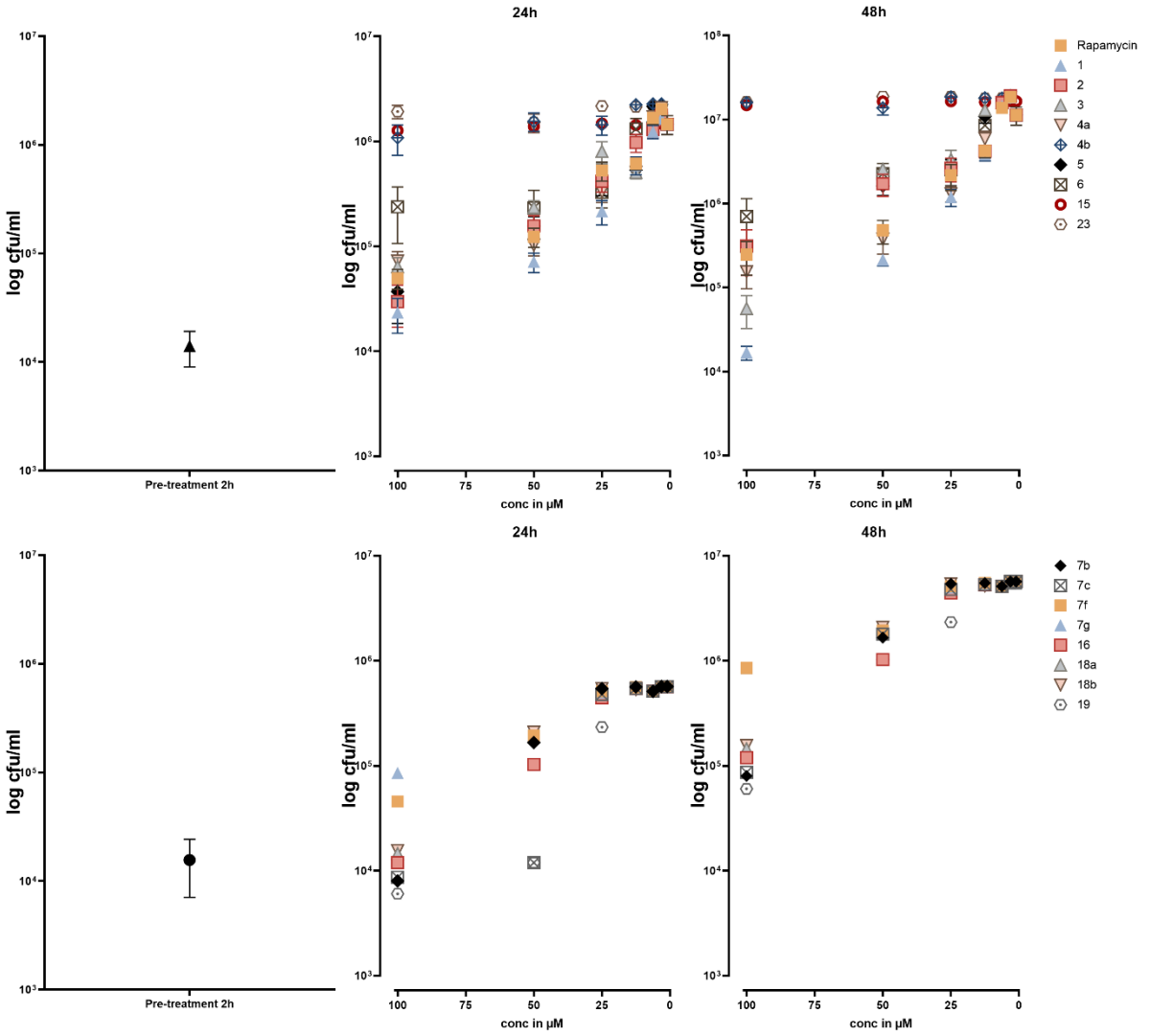


Figure S2: Intracellular replication of *L. pneumophila* wild type and in A549 lung epithelial cells was determined following 24 and 48 hours of exposure to different substance concentrations. The results obtained at 24 and 48 hours are presented and compared to the control group, which consisted of 1% DMSO. For compounds Rapamycin, **1**-**6**, **15** and **23** 12 replicates were done, for compounds **7b**, **7c**, **7f**, **7g**, **16**, **18a**, **18b** and **19** 4 replicates were done.

c.

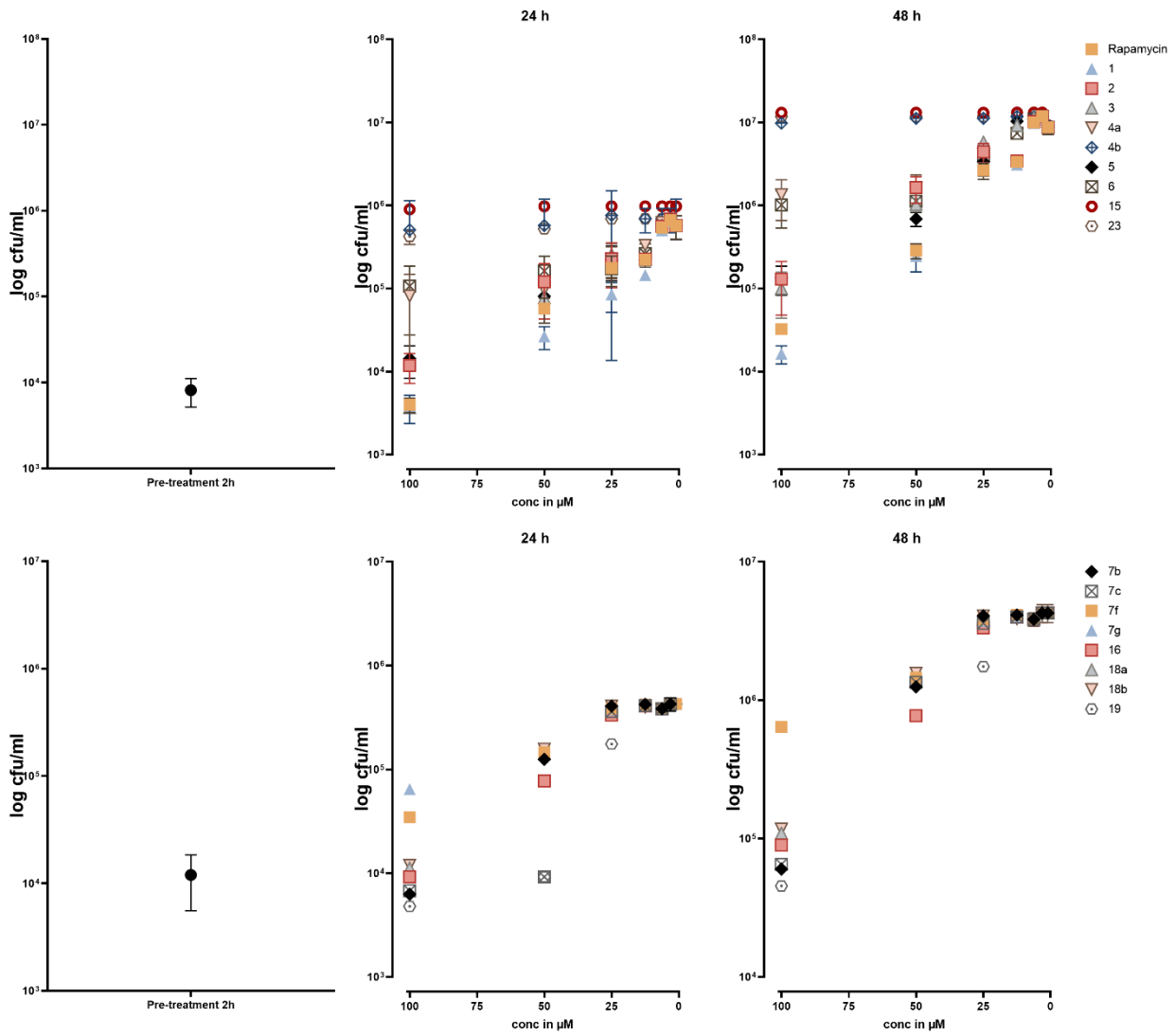


Figure S3: Intracellular replication of *L. pneumophila* wild type and in THP1 macrophages was determined following 24 and 48 hours of exposure to different substance concentrations. The results obtained at 24 and 48 hours are presented and compared to the control group, which consisted of 1% DMSO. For compounds Rapamycin, **1**-**6**, **15** and **23** 12 replicates were done, for compounds **7b**, **7c**, **7f**, **7g**, **16**, **18a**, **18b** and **19** 4 replicates were done.

d.

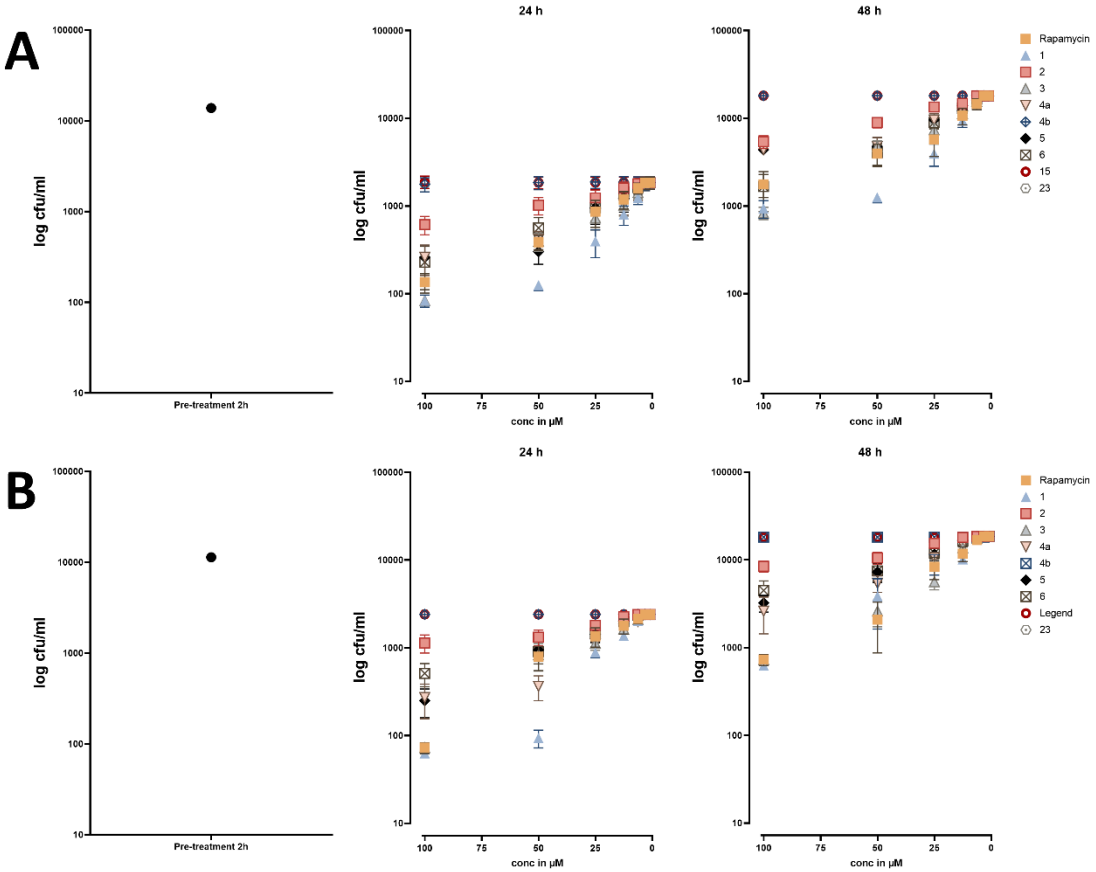


Figure S4: Intracellular replication of *L. pneumophila Δmip* strains in A549 lung epithelial cells (A) and THP1 macrophages (B) was determined following 24 and 48 hours of exposure to different substance concentrations. The results obtained at 24 and 48 hours are presented and compared to the control group, which consisted of 1% DMSO. A Dunnet's multiple comparison test was performed on 6 replicates for the cellular infection assays.

e.

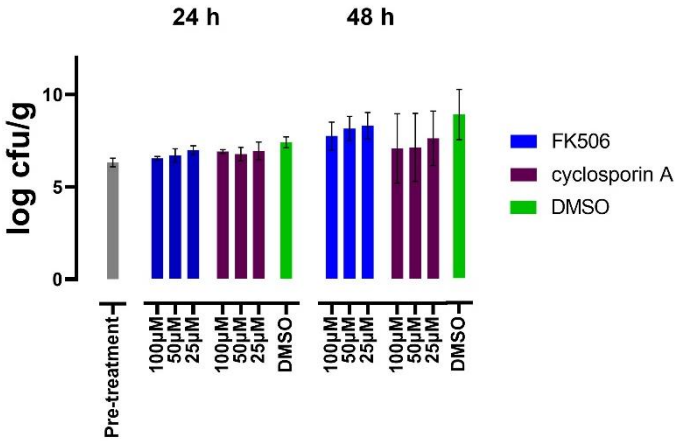


Figure S5: Intracellular replication of *L. pneumophila* wild type strains in human lung tissue explants (HLTEs) was analyzed after 24 and 48 hours of exposure to FK506 and Cyclosporin in various substance concentrations. The results obtained at 24 and 48 hours are presented and compared to the control group, which consisted of 1% DMSO. A Dunnet's multiple comparison test was performed on 6 replicates.

2. Competitive fluorescence polarisation assays for binding to FKBP target proteins

Fluorescence Polarization assay for the determination of the binding affinity

FKBP12 was recombinantly expressed in *E. coli* BL21DE3Gold and had a final purity of >95% as visually judged by Coomassie gel and SEC. The proteins were stored in HEPES buffer (20 mM HEPES, 20 mM NaCl (150 mM NaCl for FKBP12.6), +/- 5% (v/v) Glycerol, pH 8.0). The fluorescent ligand **SI1** was developed by Pomplun *et al.* [1] (Figure S6).

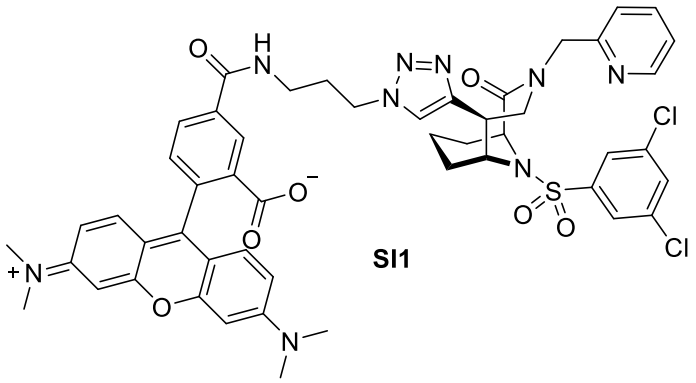


Figure S6: Chemical structure of the fluorescent ligand **SI1**.

Fluorescent ligand dissociation constants as well as final concentrations of the fluorescent ligand **SI1** and proteins FKBP12, full length LpMip(1-214) and shortened LpMip(77-214) are summarized in **Table S1**. The competition curves were analyzed using Prism 8.0 (GraphPad Software).

Table S1. Concentrations of proteins and fluorescent ligand FT and dissociation constants of FT.

Proteins	FKBP12	LpMip(1-214)	LpMip(77-214)
[Protein] / nM	1	50	90
[Tracer] / nM	0.5	1	1
K_i [Tracer] / nM	0.30 ± 0.06	31.82 ± 2.46	38.37 ± 1.61

For the analysis of K_i values, data were fitted to the following equation (provided in Kozany *et al.* [2]; Supporting Information, Appendix 3).

$$A = (A_{max} - A_{min}) / [L]_t \times (([L]_t \times ((2 \times ((K_{lig} + K_{comp} + [L]_t + [I] - [R]_t)^2 - 3(K_{comp} \times ([L]_t - [R]_t) + K_{lig} \times ([I]_t - [R]_t) + K_{lig} \times K_{comp}))^{0.5} \times \cos(\arccos((-2(K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t)^3 + 9(K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t) \times (K_{comp} \times ([L]_t - [R]_t) + K_{lig} \times ([I]_t - [R]_t) + K_{lig} \times K_{comp}) - 27(-1 K_{lig} \times K_{comp} \times [R]_t)) / (2 \times ((((K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t)^2 - 3 \times (K_{comp} \times ([L]_t - [R]_t) + K_{lig} \times ([I]_t - [R]_t) + K_{lig} \times K_{comp}))^3)^{0.5}))) / 3)) - (K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t))) / ((3 \times K_{lig}) + ((2((K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t)^2 - 3(K_{comp} \times ([L]_t - [R]_t) + K_{lig} \times ([I]_t - [R]_t) + K_{lig} \times K_{comp}))^{0.5} \times \cos(\arccos((-2(K_{lig} +$$

$K_{comp} + [L]_t + [I]_t - [R]_t)^3 + 9x(K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t) \times (K_{comp} \times ([L]_t - [R]_t) + K_{lig} \times ([I]_t - [R]_t) + K_{lig} \times K_{comp}) - 27x(-1x\ K_{lig} \times K_{comp} \times [R]_t)) / (2x(((K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t)^2\ 3 \times (K_{comp} \times ([L]_t - [R]_t) + K_{lig} \times ([I]_t - [R]_t) + K_{lig} \times K_{comp}))^3)^0.5)))/3)) - (K_{lig} + K_{comp} + [L]_t + [I]_t - [R]_t)))) + A_{min}$

In this equation K_{lig} and K_{comp} stand for the K_i values of the used tracer or competing ligand, $[L]_t$ and $[R]_t$ are referring to the total concentrations of the used tracer or the protein and $[I]_t$ is referring to the total concentration of the titrated ligand. A stands for the fluorescence anisotropy (A_{min} = minimal measured anisotropy, A = maximal measured anisotropy). The competition curves were analyzed using Prism 8.0 (GraphPad Software).

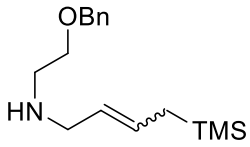
For most pipetting steps, a Beckman Coulter FX_P Laboratory Automation Workstation was used. The compound was diluted in a 1:2 serial dilutions in DMSO and then mixed in pseudo-duplicates with protein and tracer in buffer (20 mM HEPES, pH 8.0, 0.002 % v/v Triton X-100, 150 mM NaCl) in a black, non-binding 384-well plate, and then incubated with light protection for 30 min. Polarization was measured on a Tecan Spark at room temperature with an excitation wavelength of 535 nm and an emission wavelength of 595 nm.

3. Compound synthesis and characterization

If not indicated otherwise, reagents and solvents were purchased from commercial suppliers and used without further treatment. All reactions were followed by TLC analysis or LCMS. Flash silica gel column chromatography was performed with a Biotage® Isolera One system with Biotage® Sfär Silica HC D columns. Column chromatography was performed manually with silica gel 60 (0.04-0.063 mm) from Machery Nagel GmbH & Co. KG. Semi-Preparative HPLC was performed with an Interchim PuriFlash 5250 system with a Luna® 5 µm C18(2) 100 Å, 250x21.2 mm column from Phenomenex. Eluents were 0.1% TFA in water (Eluent A) and 0.1% TFA in acetonitrile (Eluent B), methods are given in percentage B. All key compounds were >95% purity by HPLC. Compound purity and low-resolution mass spectra were determined using an Agilent 1260 Infinity II system with a Poroshell 120 EC-C18 1.9 µm, 2.1 x 50 mm column from Agilent. Eluents were 0.1% formic acid in water (Eluent A) and 0.1% formic acid in acetonitrile (Eluent B), the used method was 5% B to 100% B in 2 min. MS was recorded with an Agilent InfinityLab G6125B LC/MSD. NMR spectroscopy was performed by the NMR department at TU Darmstadt. NMR spectra were recorded either on a 300 MHz Avance II NMR spectrometer from Bruker BioSpin GmbH (for 1H-NMR only), a 300 MHz Avance III NMR spectrometer from Bruker BioSpin GmbH (for ^{1}H-, ^{13}C-NMR), or a 500 MHz NMR spectrometer DRX 500 from Bruker BioSpin GmbH (for ^{1}H- and ^{13}C-NMR). NMR spectra were recorded at room temperature. Chemical shifts are given in parts per million, referenced to the respective solvent (^{1}H: $CDCl_3$ = 7.26 ppm, [D_6] DMSO = 2.50 ppm, ^{13}C: $CDCl_3$ = 77.16 ppm, [D_6] DMSO = 39.52 ppm). Coupling constants (*J*) are given in hertz (Hz), peak multiplicities are given as singlet (s), doublet (d), triplet (t), quartet (q) or multiplet (m). HRMS was performed by the mass spectrometry department at TU Darmstadt. Mass spectra were recorded on an Impact II, quadrupol-time-of-flight spectrometer from Bruker Daltonics. TLC was performed on TLC Silica gel 60 F254 Aluminum sheets from Merck Millipore. All final test compounds had a purity ≥95% as determined by HPLC and UV detection at 220nm.

Compound Synthesis

Amine 9



9

2-(4-(Trimethylsilyl)but-2-en-1-yl)isoindoline-1,3-dione (**8**) (1.0 g, 3.55 mmol, 1.0 eq.) was dissolved in methanol (37 mL). Hydrazine (35% in H_2O, 649 µL, 7.32 mmol, 2.0 eq.) was added to the reaction mixture, which was heated to reflux for 16 h: After the reaction mixture cooled to rt, DCM and aq. NaOH solution (1M) was added. The organic phase was extracted and washed again with 1 M aq. NaOH solution. The aqueous phases were combined and extracted with two times DCM. Organic phases were combined, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was dissolved in ethanol (18 mL) and 2-(benzyloxy)acetaldehyde (516 µL, 3.36 mmol, 1.0 eq.) was added to the reaction mixture. The reaction mixture was stirred for 2.5 h at rt under argon atmosphere, before $NaBH_4$ (206 mg, 5.44 mmol, 1.5 eq.) was added. Afterwards the reaction mixture was stirred for additional 1.5 h till no more gas emission was observed. 1 M aq. NaOH solution (5 mL) was added to the reaction mixture to quench the reaction. The organic phase was extracted with three times DCM and the organic phases were combined, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified using column chromatography (100 g SiO_2, EA + 3% TEA) to provide the product as a yellowish liquid (665 mg, 66% yield over two steps).

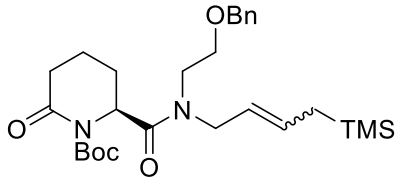
TLC R_f = 0.33 (EA + 3% TEA).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = -0.08 (d, *J* = 1.6 Hz, 9H), 1.37 (dd, *J* = 7.9, 1.1 Hz, 2H), 2.72 (dd, *J* = 5.6, 4.8 Hz, 2H), 3.11 (dq, *J* = 6.4, 0.9 Hz, 2H), 3.52 (dd, *J* = 5.6, 4.9 Hz, 2H), 4.45 (s, 2H), 5.28 (dtt, *J* = 15.2, 6.5, 1.2 Hz, 1H), 5.48 (dtt, *J* = 15.8, 7.8, 1.1 Hz, 1H), 7.09 – 7.35 (m, 5H) ppm.

^{13}C-NMR (75 MHz, $CDCl_3$): δ = -1.8, -1.7, 22.8, 48.7, 52.0, 69.8, 73.3, 73.4, 76.7, 77.2, 77.6, 126.9, 127.7, 127.8, 128.5, 129.3, 138.5 ppm.

MS (ESI): *m/z* calculated for $C_{16}H_{27}NOSi+H^+$: 278.2 [M+H$^+$]; found: 278.6.

Carboxylate 10



10

Amine **9** (645 mg, 2.32 mmol, 1.0 eq.), (*S*)-6-oxopiperidine-2-carboxylic acid (399 mg, 2.79 mmol, 1.2 eq.) and HATU (1.06 g, 2.79 mmol, 1.2 eq.) were dissolved under argon in DMF (15.5 mL, peptide grade). DIPEA (949 µL, 5.58 mmol, 2.4 eq.) was added and the reaction was stirred at rt for 2 h. Et_2O was added to the reaction mixture and the organic phase was washed four times with sat. aq. $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was used in the next reaction without further purification. The crude carboxamide (936 mg, 2.32 mmol, 1.0 eq.), di-*tert*-butyl dicarbonate (3.80 g, 17.4 mmol, 7.5 eq.), DMAP (633 mg, 5.23 mmol, 2.3 eq.) and DIPEA (119 µL, 6.97 mmol, 3.0 eq.) were dissolved in DCM (25 mL). The reaction mixture was stirred for 70 h at rt. Brine was added and the organic phase was extracted with DCM four times. Organic phases were combined, dried over $MgSO_4$ and concentrated under reduced pressure. The crude was purified twice by column chromatography (275 g, EA + 3%TEA; 200 g, Cy:EA = 2:1) to provide the product as a yellowish oil (896 mg, 74% yield over two steps).

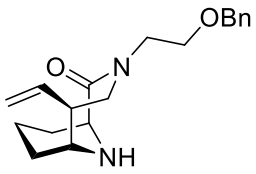
TLC R_f = 0.24 (Cy:EA = 2:1).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = -0.23 – 0.25 (m, 9H), 1.30 – 2.21 (m, 16H), 2.42 (ddd, *J* = 17.3, 9.8, 7.1 Hz, 1H), 2.57 (dt, *J* = 17.1, 5.1 Hz, 1H), 3.28 – 3.53 (m, 1H), 3.55 – 3.81 (m, 2H), 3.84 – 4.24 (m, 2H), 4.34 – 4.70 (m, 2H), 4.84 – 5.07 (m, 1H), 5.10 – 5.84 (m, 2H), 7.15 – 8.21 (m, 5H) ppm.

^{13}C-NMR (126 MHz, $CDCl_3$): δ = -1.8, -1.8, 14.3, 18.4, 18.4, 22.9, 23.0, 26.0, 26.0, 28.1, 34.5, 34.6, 45.6, 46.4, 48.6, 51.2, 55.7, 55.8, 67.9, 68.7, 73.2, 73.6, 83.0, 122.9, 127.7, 127.7, 127.9, 128.0, 128.5, 128.6, 131.2, 131.7, 137.9, 138.4, 153.4, 153.8, 170.9, 171.5, 171.6 ppm.

MS (ESI): *m/z* calculated for $C_{27}H_{42}N_2O_5Si+Na^+$: 525.2 [M+$Na^+$]; found: 525.6

Compound 11



11

Boc-protected amide 10 (4.00 g, 7.96 mmol, 1.0 eq.) was dissolved in dry THF (80 mL). The reaction mixture was cooled to -78°C and DIBAL-H (1 M solution in DCM, 9.75 mL, 9.75 mmol, 1.2 eq.) was added. The reaction was stirred for 3.5 h at -78°C, then Glauber's salt was added to quench the reaction. The reaction mixture was warmed to rt and Glauber's salt was added again. The reaction mixture was filtered over diatomaceous earth and concentrated under reduced pressure. The reduced crude product (7.93 mmol, 1.0 eq) was dissolved in DCM (400 mL) in a 1 L teflon flask. The solution was cooled to -78°C. HF·pyridine (20 mL, 1.10 mol, 139 eq.) was added and the reaction mixture was stirred for 10 minutes at -78°C. The reaction mixture was warmed to 0°C in an ice bath and stirred for 1 h. Sat. aq. $CaCO_3$ solution was added till no more gas evolution was observed. Aq. NaOH (10 M) was added till the pH value was 14. The mixture was filtrated, and the organic phase was extracted. The aqueous phase was extracted twice with DCM. Organic phases were combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was purified by column chromatography (150 g SiO_2, EA + 3% TEA) to provide the product as a yellowish oil (692 mg, 27% yield over two steps).

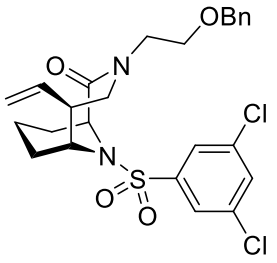
TLC R_f = 0.35 (EA + 3% TEA + 5% MeOH).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.54 (s, 1H), 1.57 – 1.74 (m, 4H), 2.16 – 2.34 (m, 1H), 2.65 – 2.78 (m, 1H), 2.83 (ddd, *J* = 6.9, 4.5, 1.8 Hz, 1H), 3.14 (dd, *J* = 13.8, 2.0 Hz, 1H), 3.49 – 3.87 (m, 5H), 4.06 (dd, *J* = 13.8, 10.7 Hz, 1H), 4.54 (s, 2H), 4.85 – 4.99 (m, 2H), 5.64 (ddd, *J* = 17.0, 10.3, 8.3 Hz, 1H), 7.15 – 7.45 (m, 5H) ppm.

^{13}C-NMR (126 MHz, $CDCl_3$): δ = 16.9, 28.3, 29.5, 49.6, 51.5, 52.7, 52.7, 57.8, 69.2, 73.3, 114.9, 127.7, 127.9 (2C), 128.4 (2C), 138.3, 139.3, 174.6 ppm.

MS (ESI): *m/z* calculated for $C_{19}H_{26}N_2O_2+H^+$: 315.2 [M+H^+]; found: 315.2.

Compound 12



12

Bicyclic compound 11 (0.670 g, 1.60 mmol, 1.0 eq.) and 3,5-dichlorobenzenesulfonyl chloride (1.05 g, 4.26 mmol, 2.0 eq.) were dissolved in MeCN (125 mL) under argon atmosphere. DIPEA (742 µl, 4.26 mmol, 2.0 eq.) was added to the solution, which was subsequently stirred for 3 d at rt. EA was added to the mixture. The organic phase was washed twice with H_2O, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was purified by column chromatography (145 g SiO_2, Cy:EA = 5:1) to provide the product as a colorless solid (619 mg, 74% yield over two steps).

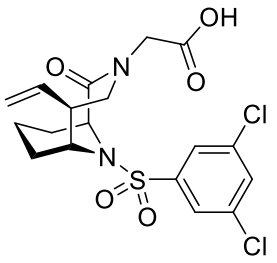
TLC R_f = 0.13 (Cy:EA = 5:1).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.23 (ddt, *J* = 12.9, 10.7, 5.7 Hz, 1H), 1.28 – 1.41 (m, 1H), 1.46 – 1.63 (m, 3H), 2.29 (dp, *J* = 15.0, 2.7 Hz, 1H), 2.72 (qd, *J* = 8.5, 1.9 Hz, 1H), 3.15 (dd, *J* = 14.2, 2.1 Hz, 1H), 3.45 (ddd, *J* = 13.9, 7.6, 3.8 Hz, 1H), 3.65 (ddd, *J* = 9.8, 5.5, 3.9 Hz, 1H), 3.75 (ddd, *J* = 10.1, 7.6, 3.7 Hz, 1H), 3.99 – 4.03 (m, 2H), 4.09 (dd, *J* = 14.2, 10.7 Hz, 1H), 4.46 – 4.66 (m, 2H), 4.71 (dt, *J* = 6.1, 2.0 Hz, 1H), 4.96 (dt, *J* = 17.0, 1.0 Hz, 1H), 5.06 (dd, *J* = 10.2, 1.2 Hz, 1H), 5.76 (ddd, *J* = 17.1, 10.2, 8.7 Hz, 1H), 7.19 – 7.49 (m, 5H), 7.59 (t, *J* = 1.9 Hz, 1H), 7.73 (d, *J* = 1.9 Hz, 2H) ppm.

^{13}C-NMR (126 MHz, $CDCl_3$): δ = 15.5, 26.7, 27.9, 49.2, 52.0, 53.3, 55.0, 57.0, 69.0, 73.5, 116.6, 125.0 (2C), 127.8, 128.0 (2C), 128.5 (2C), 132.7, 136.4 (2C), 137.5, 138.1, 144.3, 169.9 ppm.

MS (ESI): *m/z* calculated for $C_{25}H_{28}Cl_2N_2O_4S+H^+$: 523.2 [M+H$^+$]; found: 523.7.

Compound 13



13

Compound **12** (0.590 g, 1.13 mmol, 1.0 eq.) was dissolved under argon in DCM (11.3 mL) and boron trichloride methyl sulfide complex (0.590 g, 1.13 mmol, 1.0 eq.) was added. The reaction mixture was stirred for 2 h, before sat. aq. $NaHCO_3$ solution was added. The mixture was extracted three times with DCM. The organic phases were combined, dried over $MgSO_4$, filtered through a filter pad, and concentrated under reduced pressure. The resulting crude product was purified by column chromatography (27 g SiO_2, Cy:EA = 1:4) and dissolved under argon in acetone (50 mL). The solution was cooled to 0°C using an ice bath, Jones reagent (2 M CrO_3 in H_2SO_4, 969 µL, 1.94 mmol, 2.0 eq.) was added and the solution was stirred for 2 h at 0°C. Isopropanol (20 mL) was added to quench the reaction. The mixture was stirred for another hour before H_2O and DCM were added. The organic phase was extracted, and the aqueous phase was again extracted with H_2O. The organic phases were combined, dried over $MgSO_4$, filtered through a filter pad, and concentrated under reduced pressure. The resulting crude product was purified twice by column chromatography (42 g SiO_2, Cy:EA = 2:1 + 1% HCOOH; 80 g SiO_2, DCM:acetone = 20:1 + 1% HCOOH) to provide the product as a colorless solid (340 mg, 68% yield over two steps).

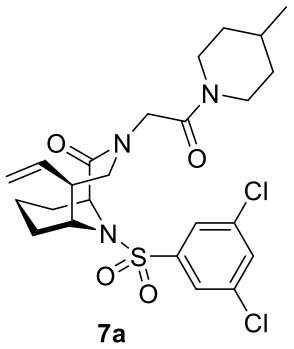
TLC R_f = 0.19 (DCM:acetone = 20:1 + 1% HCOOH).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.13 – 1.39 (m, 2H), 1.42 – 1.61 (m, 2H), 1.61 – 1.82 (m, 1H), 2.25 (dd, *J* = 13.6, 3.2 Hz, 1H), 2.91 (dd, *J* = 14.1, 2.0 Hz, 1H), 2.98 – 3.14 (m, 1H), 3.78 (d, *J* = 17.7 Hz, 1H), 3.98 (t, *J* = 5.7 Hz, 1H), 4.02 – 4.27 (m, 1H), 4.66 (d, *J* = 17.6 Hz, 1H), 4.75 (dt, *J* = 6.2, 1.9 Hz, 1H), 5.06 – 5.20 (m, 2H), 5.76 (ddd, *J* = 17.0, 10.1, 8.8 Hz, 1H), 7.56 (t, *J* = 1.9 Hz, 1H), 7.70 (d, *J* = 1.8 Hz, 2H), 10.16 (s, 1H) ppm.

^{13}C-NMR (126 MHz, $CDCl_3$): δ = 15.5, 26.4, 27.6, 48.6, 53.4, 53.8, 55.1, 56.8, 117.4, 125.0 (2C), 132.9, 136.5 (2C), 137.2, 144.1, 171.7, 173.7 ppm.

MS (ESI): *m/z* calculated for $C_{18}H_{20}Cl_2N_2O_5S+H^+$: 447.0 [$M+H^+$]; found: 447.4.

Inhibitor 7a:



7a

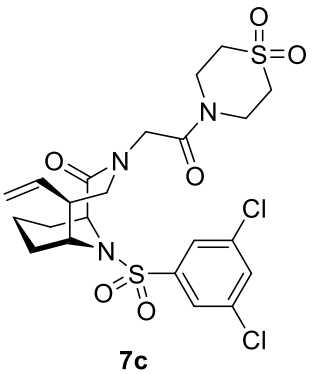
Compound **13** (70.5 mg, 0.16 mmol, 1.0 eq.), 4-methylpiperidine (32.7 μL, 0.32 mmol, 2.0 eq.) and HATU (149.8 mg, 0.39 mmol, 2.5 eq.) were dissolved in DMF (2.0 mL), DIPEA (137 μL, 0.79 mmol, 5.0 eq.) was added and the solution was stirred under argon atmosphere at room temperature for 17 h. The reaction mixture was then diluted by brine and the organic phase was extracted with DCM. The organic phases were combined, dried over $MgSO_4$, filtered, and the solvent was removed to afford the crude. The crude was purified by FLASH chromatography (10 g column, 0-15% MeOH/DCM) and reverse phase chromatography (30-100B) to provide the product (63,3 mg, 76% yield, >99% purity) as a colorless, amorphous solid.

^{1}H-NMR (500 MHz, CDCl3): δ = 7.68 (d, *J* = 1.8 Hz, 2H), 7.54 (t, *J* = 1.8 Hz, 1H), 5.77 (ddt, *J* = 17.0, 10.2, 8.4 Hz, 1H), 5.18 (dd, *J* = 17.1, 6.2 Hz, 1H), 5.08 (ddd, *J* = 10.3, 6.8, 1.4 Hz, 1H), 4.97 – 4.76 (m, 1H), 4.68 (dt, *J* = 6.3, 2.0 Hz, 1H), 4.53 – 4.37 (m, 1H), 4.06 (td, *J* = 14.0, 10.5 Hz, 1H), 4.00 – 3.92 (m, 1H), 3.66 (dt, *J* = 13.4, 2.3 Hz, 1H), 3.61 (d, *J* = 16.0 Hz, 1H), 3.32 (qd, *J* = 8.7, 2.0 Hz, 1H), 3.00 (dtd, *J* = 22.0, 13.0, 2.8 Hz, 1H), 2.87 (ddd, *J* = 16.5, 13.9, 2.1 Hz, 1H), 2.58 (dddd, *J* = 13.1, 10.2, 7.5, 3.6 Hz, 1H), 2.30 – 2.20 (m, 1H), 1.84 (qt, *J* = 13.8, 3.7 Hz, 1H), 1.73 – 1.52 (m, 3H), 1.48 (dq, *J* = 14.3, 3.3 Hz, 1H), 1.32 – 1.00 (m, 4H), 0.93 (dd, *J* = 6.5, 4.9 Hz, 3H) ppm.

^{13}C-NMR (126 MHz, CDCl3): δ = 170.82, 170.79, 165.95, 144.31, 137.67, 136.40, 132.72, 125.02, 116.92, 57.02, 56.98, 55.22, 53.58, 53.42, 53.31, 53.10, 47.99, 47.94, 45.26, 45.21, 42.74, 42.68, 34.53, 34.39, 33.76, 33.61, 31.11, 31.00, 29.78, 27.79, 26.64, 21.72, 15.65.ppm.

MS (ESI): *m/z* calcd for $C_{24}H_{31}Cl_2N_3O_4S+H^+$: 528.1 [M+H$^+$]; found: 528.2. HRMS (ESI): *m/z* calculated for $C_{24}H_{31}Cl_2N_3O_4S+H^+$: 528.1485 [M+H$^+$]; found: 528.1495.

Inhibitor 7b



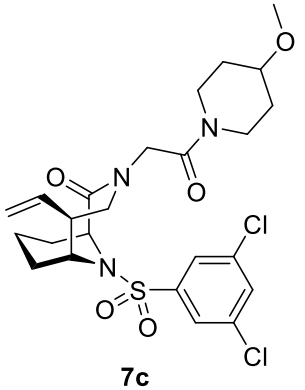
7c

Compound **13** (10.0 mg, 0.022 mmol, 1.0 eq.), thiomorpholine 1,1-dioxide (9.1 mg, 0.0066 mmol, 3.0 eq.), HBTU (21.20 mg, 0.056 mmol, 2.5 eq.) and HOBt (7.55 mg, 0.056 mol, 2.5 eq.) were dissolved in DMF (1.0 mL), DIPEA (19,5 μL, 0,112 mmol, 5.0 eq.) was added and the solution was stirred under argon atmosphere at room temperature for 20 h. The reaction mixture was then diluted by brine and the organic phase was extracted with DCM. The organic phases were combined, dried over $MgSO_4$, filtered and the solvent was removed to afford the crude. The crude product was purified by column chromatography (12 g SiO_2, EA/Cy = 1:1, then EA/acetone = 1:1) to provide the product (11,5 mg, 91% yield, 95% yield) as a colorless, amorphous solid.

^{1}H-NMR (500 MHz, [D_6] DMSO): δ = 7.99 (d, *J* = 1.9 Hz, 2H), 7.95 (t, *J* = 1.8 Hz, 1H), 5.75 – 5.66 (m, 1H), 5.14 – 5.09 (m, 1H), 5.06 (dd, *J* = 10.3, 1.4 Hz, 1H), 4.70 (dt, *J* = 6.1, 1.9 Hz, 1H), 4.60 (d, *J* = 16.4 Hz, 1H), 4.07 (d, *J* = 16.4 Hz, 1H), 4.03 – 3.88 (m, 2H), 3.89 – 3.82 (m, 3H), 3.78 (dd, *J* = 14.1, 10.5 Hz, 1H), 3.74 – 3.62 (m, 2H), 3.21 – 3.13 (m, 1H), 3.13 – 2.99 (m, 5H), 2.46 (p, *J* = 1.9 Hz, 1H), 1.99 – 1.91 (m, 1H), 1.56 (qt, *J* = 13.6, 3.7 Hz, 1H), 1.43 – 1.31 (m, 3H), 1.20 (d, *J* = 4.6 Hz, 1H), 1.16 – 1.00 (m, 2H) ppm.

MS (ESI): *m/z* calcd for $C_{22}H_{27}Cl_2N_3O_6S_2+Na^+$: 586.1 [M+Na$^+$]; found: 586.0. HRMS (ESI): *m/z* calculated for $C_{22}H_{27}Cl_2N_3O_6S_2+H^{+.}$ 564.07911 [M+H$^+$]; found: 564.07875.

Compound 7c



7c

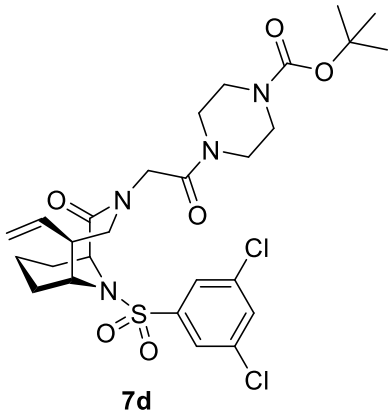
Compound **13** (10.0 mg, 0.022 mmol, 1.0 eq.), 4-methoxypiperidine hydrochloride (10.2 mg, 0.0067 mmol, 3.0 eq.), HBTU (21.20 mg, 0.056 mmol, 2.5 eq.) and HOBt (7.55 mg, 0.056 mol, 2.5 eq.) were dissolved in DMF (1.0 mL), DIPEA (137 μL, 0,79 mmol, 5.0 eq.) was added and the solution was stirred under argon atmosphere at room temperature for 20 h. The reaction mixture was then diluted by brine and the organic phase was extracted with DCM. The organic phases were merged, dried over $MgSO_4$, filtered and the solvent was removed to afford the crude. The crude was purified by column chromatography (20 g SiO_2, EA/CH = 1:1, then EA/acetone = 1:1) to provide the product (10,9 mg, 90% yield, >99% purity) as a colorless, amorphous solid.

^{1}H-NMR (500 MHz, [D_6] DMSO): δ = 8.02 (dd, *J* = 14.4, 1.9 Hz, 3H), 5.75 (ddd, *J* = 17.8, 10.3, 8.0 Hz, 1H), 5.14 (d, *J* = 17.2 Hz, 1H), 5.08 (d, *J* = 10.2 Hz, 1H), 4.75 – 4.70 (m, 1H), 4.59 (d, *J* = 16.3 Hz, 1H), 4.06 – 3.97 (m, 2H), 3.82 (dt, *J* = 13.0, 9.6 Hz, 2H), 3.54 (d, *J* = 26.2 Hz, 4H), 3.39 (d, *J* = 10.2 Hz, 1H), 3.26 (s, 3H), 3.20 – 3.05 (m, 3H), 3.01 – 2.91 (m, 1H), 1.99 (d, *J* = 13.4 Hz, 1H), 1.93 – 1.73 (m, 2H), 1.64 (q, *J* = 13.6 Hz, 1H), 1.54 – 1.21 (m, 3H), 1.15 (ddt, *J* = 19.2, 14.5, 5.1 Hz, 1H) ppm.

MS (ESI): *m/z* calcd for $C_{24}H_{31}Cl_2N_3O_5S+H^+$: 544.1[M+$H^+$]; found: 544.2.

HRMS (ESI): *m/z* calcd for $C_{24}H_{31}Cl_2N_3O_5S+H^+$: 544.14348 [M+$H^+$]; found: 544.14348.

Compound 7d



7d

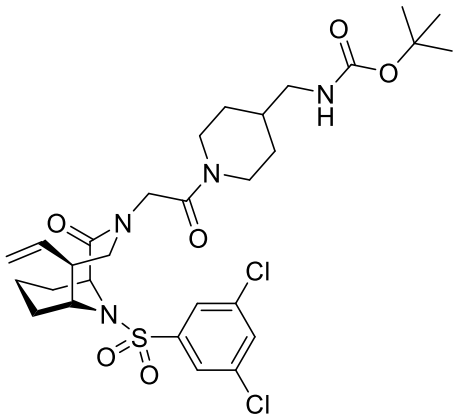
Compound **13** (70.5 mg, 0.16 mmol, 1.0 eq.), tert-butyl piperazine-1-carboxylate (27.1 mg, 0.145 mmol, 1.3 eq.) were dissolved in DMF (0.32 mL)and DIPEA (58 μL, 0,79 mmol, 5.0 eq.) was added. After 5 minutes HATU (60.0 mg, 0.156 mmol, 1.4 eq.) was added and the solution was stirred under argon atmosphere at room temperature for 1 h. The reaction mixture was then diluted by sat. aq. $NaHCO_3$-solution and the organic phase was washed with brine. The organic phase was dried over $MgSO_4$, filtered and the solvent was removed to afford the crude. The crude was purified by FLASH chromatography (10 g column, 0-100% EA/CH) to provide the product (63 mg, 92% yield) as an orange oil.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.67 (d, J = 1.8 Hz, 2H), 7.54 (t, J = 1.8 Hz, 1H), 5.76 (ddd, J = 17.1, 10.1, 8.7 Hz, 1H), 5.17 (d, J = 17.1 Hz, 1H), 5.08 (dd, J = 10.2, 1.3 Hz, 1H), 4.86 (d, J = 15.9 Hz, 1H), 4.73 –4.64 (m, 1H), 4.08 (dd, J = 14.0, 10.6 Hz, 1H), 3.96 (dt, J = 6.6, 3.6 Hz, 1H), 3.64 (d, J = 16.0 Hz, 1H), 3.54 –3.32 (m, 7H), 3.28 (q, J = 8.8, 8.0 Hz, 1H), 2.93 (s, 2H), 2.85 (s, 2H), 2.77 (s, 5H), 2.23 (dd, J = 13.6, 2.9 Hz, 1H), 1.80 (qt, J = 13.8, 3.7 Hz, 1H), 1.59 –1.46 (m, 2H), 1.44 (s, 9H), 1.33 –1.13 (m, 3H) ppm.

^{13}C-NMR (126 MHz, $CDCl_3$): δ = 170.95, 166.57, 162.58, 154.54, 144.18, 137.47, 136.40, 132.77, 124.97, 117.05, 80.47, 56.92, 55.16, 53.59, 53.18, 48.05, 44.75, 41.93, 38.68, 36.55, 28.44, 27.72, 26.54, 15.61 ppm.

MS (ESI): *m/z* calcd for $C_{27}H_{35}Cl_2N_4NaO_6S+H^+$: 637.2 [M+H$^+$]; found: 637.0.

Compound 7e



7e

Compound **13** (30.0 mg, 0.007 mmol, 1.0 eq.), *tert*-butyl-4-piperidinylmethylcarbamate (57,5 mg, 0.27 mmol, 4.0 eq.) and HATU (63.8 mg, 0.17 mmol, 2.5 eq.) were dissolved in DMF (2.0 mL), DIPEA (46.7 μL, 0.27 mmol, 4.0 eq.) was added and the solution was stirred under argon atmosphere at room temperature for 18 h. The reaction mixture was then diluted by brine and the organic phase was extracted with DCM. The organic phases were merged, dried over $MgSO_4$, filtered and the solvent was removed to afford the crude. The crude was purified by FLASH chromatography (10 g column, 0-100% EA/CH) to provide the product (30,4 mg, 70% yield) as a colorless, amorphous solid.

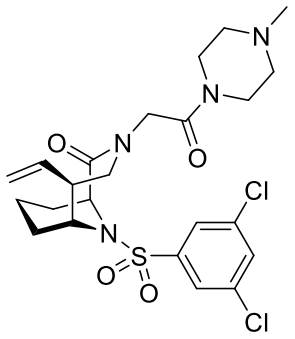
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.70 (d, *J* = 1.8 Hz, 2H), 7.56 (t, *J* = 1.8 Hz, 1H), 5.78 (dq, *J* = 8.5, 17.0 Hz, 1H), 5.19 (d, *J* = 17.0 Hz, 1H), 5.10 (dd, *J* = 6.4, 10.2 Hz, 1H), 4.87 (dd, *J* = 16.0, 43.5 Hz, 1H), 4.69 (d, *J* = 5.7 Hz, 1H), 4.54 (t, *J* = 13.5 Hz, 1H), 4.08 (td, *J* = 8.3, 13.3 Hz, 1H), 3.98 (t, *J* = 5.9 Hz, 1H), 3.73 (d, *J* = 13.6 Hz, 1H), 3.65 (dd, *J* = 16.0, 26.7 Hz, 1H), 3.31 (t, *J* = 8.8 Hz, 1H), 3.01 (td, *J* = 7.1, 15.0, 17.1 Hz, 3H), 2.88 (dd, *J* = 13.9, 21.7 Hz, 1H), 2.59 (q, *J* = 12.5 Hz, 1H), 2.25 (d, *J* = 13.6 Hz, 1H), 1.90 – 1.79 (m, 1H), 1.79 – 1.65 (m, 3H), 1.60 – 1.54 (m, 1H), 1.50 (dt, *J* = 3.3, 14.7 Hz, 1H), 1.43 (s, 9H), 1.31 – 1.08 (m, 4H) ppm.

^{13}C-NMR (126 MHz, $CDCl_3$): δ = 170.90, 166.07, 156.21, 144.28, 137.60, 136.45, 132.80, 125.05, 117.05, 79.57, 57.01, 55.24, 53.55, 53.35, 53.20, 48.04, 44.93, 42.35, 42.29, 36.86, 30.21, 30.10, 29.42, 29.28, 28.53, 27.80, 26.62, 15.68 ppm.

MS (ESI): *m/z* calcd for $C_{29}H_{40}Cl_2N_4O_6S+Na^+$: 665.2 [M+$Na^+$]; found: 665.2.

HRMS (ESI): *m/z* calcd for $C_{29}H_{40}Cl_2N_4O_6S+H^+$: 643.21184 [M+H$^+$]; found 643.21156.

Compound 7f



7f

Compound **13** (9.5 mg, 0.021 mmol, 1.0 eq.), 4-methylpiperazine (11.8 μL, 0.106 mmol, 5.0 eq.) and HATU (20.19 mg, 0.053 mmol, 2.5 eq.) were dissolved in DMF (1.0 mL), DIPEA (18.5 μL, 0.106 mmol, 5.0 eq.) was added and the solution was stirred under argon atmosphere at room temperature for 10 d. The reaction mixture was then diluted by brine (30 mL) and the organic phase was extracted with DCM (2 times 50 ml). The organic phases were merged, dried over $MgSO_4$, filtered and the solvent was removed to afford the crude. The crude was purified by FLASH chromatography (10 g column, 0-25% MeOH/DCM) and reverse phase chromatography (5-100B) to provide the product (8.2 mg, 73% yield, >99% purity) as a colorless, amorphous solid.

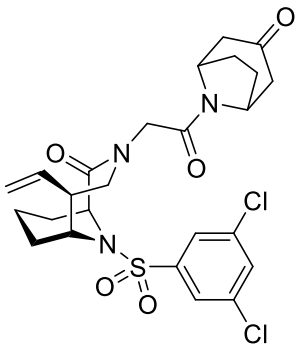
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.69 (d, *J* = 1.7 Hz, 2H), 7.58 (d, *J* = 1.8 Hz, 1H), 5.77 (dt, *J* = 17.0, 9.5 Hz, 1H), 5.18 (d, *J* = 17.0 Hz, 1H), 5.14 (d, *J* = 10.2 Hz, 1H), 4.96 (s, 1H), 4.68 (d, *J* = 6.0 Hz, 1H), 4.14 (s, 1H), 3.99 (t, *J* = 6.0 Hz, 1H), 3.88 (s, 2H), 3.53 (s, 3H), 3.29 (s, 2H), 3.09 – 2.86 (m, 2H), 2.84 (s, 3H), 2.20 (d, *J* = 13.6 Hz, 1H), 1.76 (s, 1H), 1.56 (dd, *J* = 27.3, 13.7 Hz, 2H), 1.33 – 1.13 (m, 2H) ppm.

^{13}C-NMR (126 MHz, $CDCl_3$): δ = 166.80, 162.20, 161.90, 143.92, 136.91, 136.44, 132.86, 124.86, 117.41, 117.07, 114.77, 56.75, 55.03, 53.22, 43.59, 42.28, 39.24, 27.49, 26.25, 15.45 ppm.

MS (ESI): *m/z* calcd for $C_{23}H_{30}Cl_2N_4O_4S+H^+$: 529.1 [M+H$^+$]; found: 529.2.

HRMS (ESI): *m/z* calcd for $C_{23}H_{30}Cl_2N_4O_4S+H^+$: 529.14376 [M+H$^+$]; found: 529.14413.

Compound 7g



7g

Compound **13** (14.0 mg, 0.031 mmol, 1.0 eq.), nortropinone hydrochloride (15.2 mg, 0.094 mmol, 5.0 eq.), HBTU (26.1 mg, 0.069 mmol, 2.2 eq.) and HOBt (9.3 mg, 0.069 mol, 2.2 eq.)were dissolved in DMF (1.0 mL), DIPEA (27.3 μL, 0.156 mmol, 5.0 eq.) was added and the solution was stirred under argon atmosphere at room temperature for 18 h. The reaction mixture was then diluted by brine (30 mL) and the organic phase was extracted with DCM (2 times 50 ml). The organic phases were merged, dried over $MgSO_4$, filtered and the solvent was removed to afford the crude. The crude was purified by FLASH chromatography (10 g column, 0-100% CH/EA) to provide the product (12.7 mg, 73% yield, >99% purity) as a colorless, amorphous solid.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 8.03 (d, *J* = 2.1 Hz, 2H), 8.00 (t, *J* = 1.6 Hz, 1H), 5.77 (dddd, *J* = 17.8, 10.6, 8.0, 2.5 Hz, 1H), 5.16 (d, *J* = 17.2 Hz, 1H), 5.09 (d, *J* = 10.3 Hz, 1H), 4.74 (d, *J* = 5.7 Hz, 1H), 4.71 (d, *J* = 16.8 Hz, 1H), 4.68 (d, *J* = 5.1 Hz, 1H), 4.56 (d, *J* = 10.2 Hz, 0H), 4.53 (d, *J* = 5.8 Hz, 1H), 4.21 (d, *J* = 16.4 Hz, 1H), 4.08 – 3.98 (m, 2H), 3.87 (ddd, *J* = 16.8, 14.1, 10.4 Hz, 1H), 3.23 – 3.13 (m, 1H), 3.12 – 3.03 (m, 1H), 2.82 (ddd, *J* = 21.6, 16.3, 4.0 Hz, 1H), 2.54 (dd, *J* = 15.9, 4.6 Hz, 1H), 2.28 (t, *J* = 16.1 Hz, 2H), 2.16 – 2.03 (m, 1H), 2.03 – 1.96 (m, 1H), 1.93 (dt, *J* = 12.5, 5.5 Hz, 1H), 1.70 (ddd, *J* = 13.0, 9.5, 4.2 Hz, 1H), 1.66 – 1.59 (m, 1H), 1.56 (dq, *J* = 9.4, 4.4 Hz, 1H), 1.45 (d, *J* = 13.7 Hz, 1H), 1.42 – 1.35 (m, 1H), 1.32 – 0.99 (m, 2H) ppm.

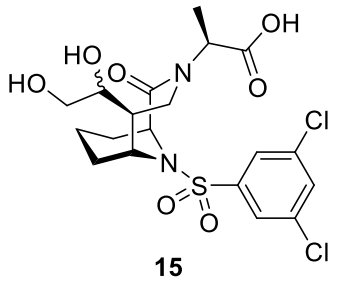
^{13}C-NMR (126 MHz, $CDCl_3$): δ = 207.52, 170.06, 169.96, 165.10, 164.75, 143.90, 137.94, 137.86, 135.44, 132.66, 125.09, 116.08, 116.01, 56.08, 54.07, 53.01, 52.90, 52.62, 52.47,

52.36, 52.04, 51.19, 51.03, 48.87, 48.16, 47.14, 47.03, 29.76, 29.53, 27.27, 27.14, 26.10, 26.04, 14.81 ppm.

MS (ESI): *m/z* calcd for $C_{25}H_{29}Cl_2N_2O_5S+H^+$: 554.1 [M+H$^+$]; found: 554.0.

HRMS (ESI): *m/z* calcd for $C_{25}H_{29}Cl_2N_2O_5S+H^+$: 554.12777 [M+H$^+$]; found: 554.12740.

Inhibitor 15



15

Compound **14** (52 mg, 113 µmol, 1.0 eq.) was dissolved in acetone/water (9:1, 2 mL), then 2,6-lutidine (38 µL, 327 µmol, 2.9 eq.), NMO (23 mg, 196 µmol, 1.7 eq.) and OsO_4 (2.5 wt.-% in ^{t}BuOH, 70 µL, 5.6 µmol, 5 mol-%) were added and the reaction was stirred at room temperature. After 6 h, additional NMO (18 mg, 154 µmol, 1.4 eq.) and OsO_4 (2.5 wt-% in *t*BuOH, 70 µL, 5.6 µmol, 5 mol-%) were added and it was stirred for another 19 h. The reaction was quenched with sat. aq. $Na_2S_2O_3$ solution and stirred for 30 min, then the solution was acidified with 1 M HCl and extracted with EA. The organic phase was extracted with 1 M NaOH, then the aqueous phase was again acidified with 1 M HCl and extracted with EA. The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by semi-preparative HPLC (30-40% MeCN in H_2O) to provide the product as colorless solid (33 mg, 59% yield, 98% purity).

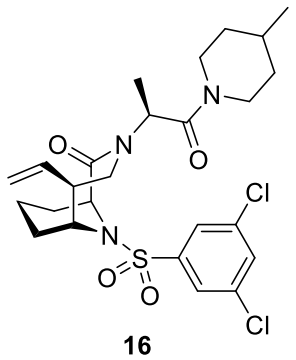
TLC R_f = 0.23 (EA + 1 % HCOOH).

^{1}H-NMR (500 MHz, [D_6] DMSO): δ = 1.11-1.25 (m, 2H), 1.25-1.33 (m, 3H), 1.34-1.44 (m, 2H), 1.44- 1.54 (m, 1H), 1.95-2.13 (m, 2H), 3.02-3.24 (m, 1H), 3.30-3.57 (m, 4H), 4.08-4.32 (m, 1H), 4.70-4.79 (m, 1H), 4.91-5.07 (m, 1H), 7.86-8.04 (m, 3H) ppm.

^{13}C-NMR (125 MHz, [D_6] DMSO): δ = 14.3, 14.8, 14.9, 26.9, 27.8, 28.0, 44.4, 45.5, 46.2, 47.3, 51.0, 51.9, 55.2, 55.3, 56.3, 56.4, 63.1, 71.7, 72.4, 125.1, 132.6, 135.4, 143.8, 168.9, 169.0,

172.4, 172.4 ppm. HR-MS (ESI): *m/z* calculated for $C_{19}H_{23}Cl_2N_2O_7S+H^+$: 495.07540 [M+H$^+$]; found: 495.07534.

Compound 16:



16

Compound **14** (50.0 mg, 0.108 mmol, 1.0 eq.), 4-methylpiperidine (64.0 μL, 0.542 mmol, 5.0 eq.) and HATU (103.0 mg, 0.271 mmol, 2.5 eq.) were dissolved in anhydrous DMF (2.0 mL), DIPEA (94.4 μL, 0.542 mmol, 5.0 eq.) was added and the solution was stirred under argon atmosphere at room temperature for 3 d. The reaction mixture was then diluted by brine (50 mL) and the organic phase was extracted with DCM (2 times 30 ml). The organic phases were merged, dried over $MgSO_4$, filtered and the solvent was removed to afford the crude. The crude was purified by FLASH chromatography (10 g column, 0-10% MeOH in DCM) to provide the product (24.7 mg, 42% yield, >99% purity) as a colorless, amorphous solid.

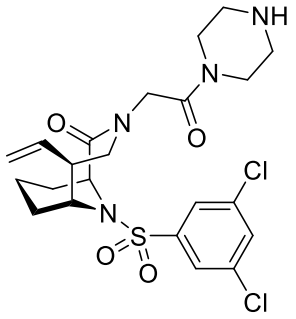
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.68 (dd, *J* = 4.6, 1.8 Hz, 2H), 7.57 (t, *J* = 1.8 Hz, 1H), 5.82 – 5.69 (m, 1H), 5.55 (dq, *J* = 58.8, 6.6 Hz, 1H), 5.15 (dd, *J* = 5.9, 4.1 Hz, 1H), 5.13 – 5.10 (m, 1H), 4.87 – 4.64 (m, 1H), 4.51 (dp, *J* = 13.3, 2.3 Hz, 1H), 3.97 (dt, *J* = 32.6, 6.0 Hz, 1H), 3.83 – 3.68 (m, 1H), 3.68 – 3.48 (m, 1H), 3.16 (dd, *J* = 14.6, 1.8 Hz, 0.5H), 3.01 (td, *J* = 13.6, 13.1, 2.4 Hz, 1H), 2.93 (td, *J* = 13.1, 2.6 Hz, 0.5H), 2.65 (dtd, *J* = 25.7, 12.9, 2.9 Hz, 1H), 2.54 – 2.42 (m, 1H), 2.30 – 2.22 (m, 1H), 1.75 – 1.65 (m, 2H), 1.61 (ddq, *J* = 10.7, 7.0, 3.2 Hz, 0H), 1.58 – 1.45 (m, 3H), 1.32 (q, *J* = 6.6, 4.6 Hz, 0H), 1.28 (dd, *J* = 6.8, 1.8 Hz, 3H), 1.20 (tt, *J* = 12.4, 7.5 Hz, 1H), 1.09 (ddt, *J* = 18.6, 12.6, 7.4 Hz, 2H), 0.95 (dd, *J* = 21.1, 6.5 Hz, 3H) ppm.

^{13}C-NMR (126 MHz, $CDCl_3$): δ = 170.04, 169.68, 168.62, 168.47, 144.13, 144.05, 136.59, 136.56, 136.49, 132.98, 132.95, 125.03, 124.92, 117.61, 117.54, 57.04, 56.81, 55.10, 55.05, 51.99, 51.38, 50.48, 50.01, 46.12, 46.08, 45.94, 45.27, 43.59, 43.17, 34.81, 34.43, 34.32, 33.87, 31.29, 30.86, 27.63, 27.45, 26.04, 25.80, 21.74, 21.55, 15.61, 15.58, 14.83, 14.70 ppm.

MS (ESI): *m/z* calcd for $C_{25}H_{33}Cl_2N_3O_4S+H^+$: 542.2 [M+H$^+$]; found: 542.0.

HRMS (ESI): *m/z* calcd for $C_{25}H_{33}Cl_2N_3O_4S+H^+$: 542.16416 [M+H$^+$]; found: 542.16413.

Compound 17a



17a

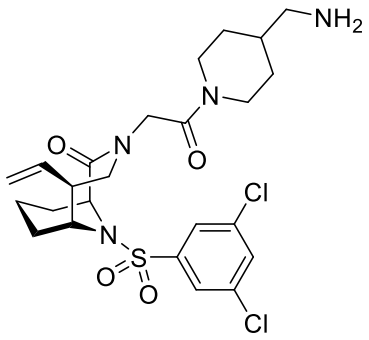
Compound **7d** (70.0 mg, 0.114 mmol, 1.0 eq.), dissolved in DCM (3.5 mL), was treated with TFA (870 μL, 11.37 mmol, 100 eq.). The solution was stirred for 30 min at rt. After completion of the reaction, aq. sat. solution of $NaHCO_3$ (2 ml) was slowly added and the mixture was diluted with DCM (10 mL). The aqueous phase was extracted with $CHCl_3$ iPrOH (3:1, 3x30 mL) and dried over $MgSO_4$. Solvents were removed under reduced pressure. The crude product was purified by FLASH chromatography (10g column, 0-40% MeOH in DCM) quantitatively (58.6 mg) yielding a dark orange oil.

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.62 (d, J = 1.8 Hz, 2H), 7.50 (t, J = 1.9 Hz, 1H), 5.69 (ddd, J = 17.1, 10.1, 8.6 Hz, 1H), 5.11 (d, J = 17.1 Hz, 1H), 5.04 (dd, J = 10.0, 1.2 Hz, 1H), 4.71 (d, J = 16.2 Hz, 1H), 4.66 –4.58 (m, 1H), 4.02 (dd, J = 14.1, 10.6 Hz, 1H), 3.90 (t, J = 5.9 Hz, 1H), 3.88 –3.80 (m, 1H), 3.80 –3.66 (m, 4H), 3.26 –3.11 (m, 5H), 3.06 (q, J = 7.3 Hz, 19H), 2.84 (dd, J = 14.2, 2.1 Hz, 1H), 2.14 (dd, J = 13.9, 3.2 Hz, 1H), 1.71 (qt, J = 14.1, 3.5 Hz, 1H), 1.56 –1.41 (m, 2H) ppm.

^{13}C-NMR (126 MHz, $CDCl_3$): δ = 171.04, 166.60, 162.51, 162.23, 161.96, 161.68, 144.01, 137.11, 136.34, 132.74, 124.84, 120.25, 117.92, 117.04, 115.59, 113.26, 56.79, 55.03, 53.57, 52.90, 47.97, 45.82, 43.03, 41.79, 38.78, 27.59, 26.38, 15.41 ppm.

MS (ESI): *m/z* calcd for $C_{22}H_{28}Cl_2N_4O_4S+H^+$: 515.2 [M+H$^+$]; found: 515.2.

Compound 17b



17b

Compound **7e** (25.0 mg, 0.039 mmol, 1.0 eq.), dissolved in DCM (5 mL), was treated with TFA (599 μL, 7.77 mmol, 200 eq.). The solution was stirred for 28 h at rt. After completion of the reaction, brine (40 ml) was added. The aqueous phase was extracted with DCM (2x25 mL) and dried over $MgSO_4$. Solvents were removed under reduced pressure. The crude product was purified twice by FLASH chromatography (10g column, 0-20% MeOH in DCM; 10g column, 0-20% MeOH + 3% TEA in EA+3%TEA) and twice by preparative HPLC (5-100% B) yielding a colorless amorphous solid (11,4 mg, 54% yield).

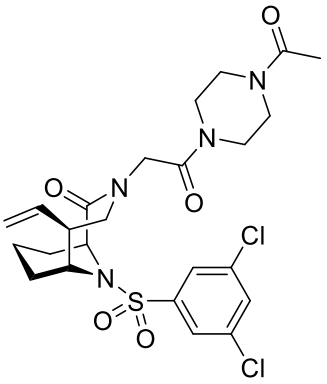
^{1}H-NMR (500 MHz, [D_6] DMSO): δ = 8.04 – 7.96 (m, 5H), 5.74 (dtd, *J* = 17.5, 9.1, 4.8 Hz, 1H), 5.13 (dd, *J* = 17.3, 6.8 Hz, 1H), 5.07 (dd, *J* = 10.4, 3.5 Hz, 1H), 4.72 (d, *J* = 5.7 Hz, 1H), 4.58 (dd, *J* = 38.5, 16.4 Hz, 1H), 4.31 (d, *J* = 13.2 Hz, 1H), 4.08 – 3.95 (m, 2H), 3.82 (ddd, *J* = 31.4, 15.6, 10.9 Hz, 2H), 3.17 (h, *J* = 7.8, 7.1 Hz, 1H), 3.02 – 2.85 (m, 2H), 2.71 (h, *J* = 6.5 Hz, 2H), 2.61 – 2.52 (m, 1H), 1.98 (d, *J* = 13.5 Hz, 1H), 1.88 – 1.67 (m, 2H), 1.63 (d, *J* = 16.5 Hz, 1H), 1.41 (dd, *J* = 27.8, 13.6 Hz, 2H), 1.20 – 0.95 (m,4H). ppm.

^{13}C-NMR (126 MHz, [D_6] DMSO): δ = 169.82, 166.01, 143.93, 137.96, 135.46, 132.70, 125.11, 116.01, 56.12, 54.04, 52.65, 52.40, 52.33, 47.00, 43.53, 43.40, 40.98, 40.91, 33.79, 33.76, 29.30, 29.24, 28.63, 27.16, 26.09, 14.84. ppm.

MS (ESI): *m/z* calcd for $C_{24}H_{32}Cl_2N_4O_4S+H^+$: 543.2 [$M+H^+$]; found: 543.4.

HRMS (ESI): *m/z* [$M+H^+$] calcd for $C_{24}H_{32}Cl_2N_4O_4S+H^+$: 543.15941; found: 543.15955.

Compound 18a



17a

Compound **17a** (16.0 mg, 0.031 mmol, 1.0 eq.), dissolved in anhydrous DCM (4 mL), was treated with acetyl chloride (3.3 μL, 0.047 mmol, 1.5 eq.) and anhydrous DIPEA (10.8 μL, 0.062 mmol, 2.0 eq.). The solution was stirred for 1 h at rt. After completion of the reaction, aq. sat. solution of $NaHCO_3$ (40 ml) was added. The aqueous phase was extracted with DCM (2x25 mL) and dried over $MgSO_4$. Solvents were removed under reduced pressure. The crude product was purified by column chromatography (Biotage, 10g column, 0-20% MeOH in DCM) and preparative HPLC (5-100% B) yielding a colorless amorphous solid (5,6 mg, 31% yield, 98% purity).

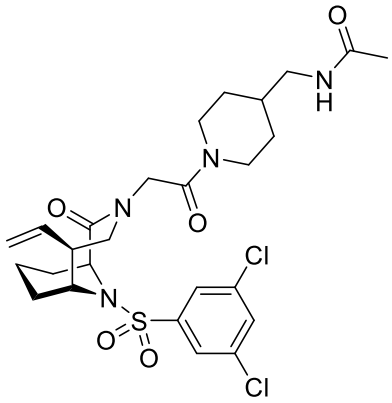
^{1}H-NMR (500 MHz, [D_6] DMSO): δ = 8.03 (d, *J* = 1.7 Hz, 2H), 8.00 (d, *J* = 2.0 Hz, 1H), 5.75 (ddd, *J* = 17.8, 10.1, 8.1 Hz, 1H), 5.13 (d, *J* = 17.2 Hz, 1H), 5.08 (d, *J* = 10.2 Hz, 1H), 4.73 (d, *J* = 5.8 Hz, 1H), 4.62 (dd, *J* = 16.4, 9.2 Hz, 1H), 4.05 (d, *J* = 16.3 Hz, 1H), 4.01 (s, 1H), 3.84 (dd, *J* = 14.1, 10.6 Hz, 1H), 3.46 (d, *J* = 13.8 Hz, 4H), 3.41 (dp, *J* = 11.3, 5.7, 5.0 Hz, 4H), 3.17 (q, *J* = 8.7 Hz, 1H), 2.96 (d, *J* = 14.0 Hz, 1H), 2.02 (d, *J* = 3.4 Hz, 3H), 1.99 (d, *J* = 14.4 Hz, 1H), 1.62 (dddd, *J* = 17.4, 13.8, 8.9, 3.8 Hz, 1H), 1.48 – 1.34 (m, 2H), 1.12 (dtt, *J* = 23.4, 13.0, 4.4 Hz, 2H) ppm.

^{13}C-NMR (126 MHz, [D_6] DMSO $_3$): δ = 170.37, 168.94, 167.05, 144.40, 138.39, 135.94, 133.17, 125.60, 116.53, 56.57, 54.53, 53.14, 52.80, 47.51, 45.95, 45.93, 45.75, 44.57, 44.54, 41.96, 41.95, 41.59, 41.57, 41.20, 41.18, 27.65, 26.56, 21.70, 15.30 ppm.

MS (ESI): *m/z* calcd for $C_{24}H_{30}Cl_2N_4O_5S+H^+$: 557.1 [M+H$^+$]; found 557.2.

HRMS (ESI): *m/z* calcd for $C_{24}H_{30}Cl_2N_4O_5S+H^+$: 557.13867 [M+H$^+$]; found 557.13881.

Compound 18b



18b

Compound **17b** (9.0 mg, 0.017 mmol, 1.0 eq.), dissolved in anhydrous DCM (1 mL), was treated with acetyl chloride (3.2 μL, 0.050 mmol, 3.0 eq.) and anhydrous DIPEA (5.8 μL, 0.033 mmol, 2.0 eq.). The solution was stirred for 2 h at rt. After completion of the reaction, the solvent was removed by pressurized air and crude product was purified by preparative HPLC (5-100% B) yielding a colorless amorphous solid (8,7 mg, 90% yield, >99% purity).

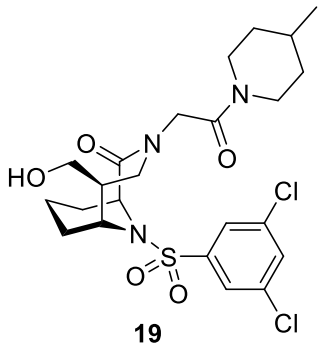
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.70 (s, 2H), 7.57 (d, *J* = 2.5 Hz, 1H), 6.02 (dt, *J* = 13.3, 6.2 Hz, 1H), 5.77 (dq, *J* = 17.0, 9.4 Hz, 1H), 5.19 (d, *J* = 17.1 Hz, 1H), 5.12 (t, *J* = 9.5 Hz, 1H), 4.91 (d, *J* = 16.0 Hz, 0.5H), 4.76 (dd, *J* = 9.8, 6.1 Hz, 1H), 4.60 (d, *J* = 15.7 Hz, 0.5H), 4.53 (t, *J* = 13.7 Hz, 1H), 4.11 (td, *J* = 14.1, 10.2 Hz, 1H), 4.04 – 3.93 (m, 1.5H), 3.80 – 3.72 (m, 1H), 3.67 (d, *J* = 16.0 Hz, 0.5H), 3.35 – 3.10 (m, 3H), 3.10 – 2.84 (m, 2H), 2.62 (dtd, *J* = 16.1, 13.1, 2.9 Hz, 1H), 2.22 (d, *J* = 13.8 Hz, 1H), 2.07 (s, 3H), 1.90 – 1.66 (m, 4H), 1.62 – 1.46 (m, 2H), 1.36 – 1.10 (m, 4H) ppm.

^{13}C-NMR (126 MHz, $CDCl_3$): δ = 172.50, 166.47, 166.27, 144.12, 137.25, 137.15, 136.60, 136.55, 133.02, 132.94, 125.04, 117.39, 117.36, 56.94, 56.90, 55.20, 53.91, 53.53, 53.31, 48.11, 48.01, 45.24, 45.14, 45.14, 44.98, 42.64, 42.52, 36.09, 35.90, 30.16, 29.93, 29.36, 29.21, 27.63, 27.61, 27.06, 26.44, 26.31, 22.91, 15.59, 15.57 ppm.

MS (ESI): *m/z* calcd for $C_{26}H_{34}Cl_2N_4O_5S+H^+$: 585.2 [M+H$^+$]; found: 585.2.

HRMS (ESI): *m/z* [M+H$^+$] calcd for $C_{26}H_{34}Cl_2N_4O_5S+H^+$: 585.16997; found: 585.17053.

Inhibitor 19



19

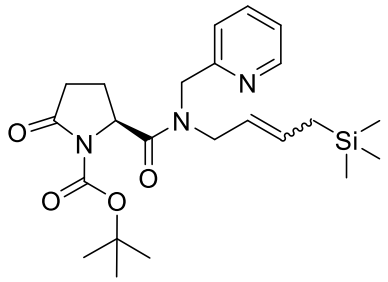
Compound **7a** (63.3 mg, 0.120 mmol, 1.0 eq.) was dissolved in a 3:1 mixture of dioxane and H_2O (6 mL) and cooled to 0°C with an ice bath. 1,6-lutidine (17.9 μL, 0.240 mmol, 2.0 eq.), osmium tetroxide (2.5 wt-% solution in *t*BuOH, 71.1 μL, 0.006 mmol, 0.05 eq.) and sodium periodate (102.5 mg, 0.479 mmol, 4.0 eq.) were added consecutively. The mixture was stirred for 22 h while slowly warming to rt. After completion of the reaction, aq. sat. solution of $Na_2S_2O_3$ (10 mL) was added. The organic phase was extracted with DCM and dried over $MgSO_4$. Solvents were removed under reduced pressure. The crude product was dissolved in ethanol (15 mL), the solution cooled to 0°C and $NaBH_4$ (6.8 mg, 0.180 mmol, 1.5 eq.) was added. The solution was stirred for 1 h at 0°C. Then, the organic phase was removed, 50 mL half concentrated brine was added, the organic phase was extracted with DCM (3x20 mL), dried over $MgSO_4$ and the solvent was removed. The crude product was purified by FLASH chromatography (10g column, 0-10% MeOH in DCM) to yield a colorless amorphous solid (48.2 mg, 76% yield, >99% yield).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.70 (d, *J* = 1.8 Hz, 2H), 7.56 (d, *J* = 1.9 Hz, 1H), 7.26 (s, 0H), 4.98 (t, *J* = 17.6 Hz, 1H), 4.71 (d, *J* = 5.6 Hz, 1H), 4.52 – 4.39 (m, 1H), 3.92 (d, *J* = 12.3 Hz, 2H), 3.69 (d, *J* = 13.8 Hz, 1H), 3.64 (d, *J* = 16.1 Hz, 1H), 3.60 (s, 0H), 3.52 (q, *J* = 8.9, 7.4 Hz, 1H), 3.15 (t, *J* = 14.2 Hz, 1H), 3.02 (dt, *J* = 22.8, 12.5 Hz, 1H), 2.89 (dq, *J* = 13.3, 7.5 Hz, 1H), 2.59 (q, *J* = 12.2, 9.8 Hz, 1H), 2.27 (d, *J* = 13.7 Hz, 1H), 2.19 (s, 3H), 1.90 – 1.77 (m, 1H), 1.76 – 1.45 (m, 3H), 1.35 (tt, *J* = 13.2, 4.4 Hz, 1H), 1.28 – 1.01 (m, 3H), 0.99 – 0.91 (m, 3H) ppm.

^{13}C-NMR (126 MHz, $CDCl_3$): δ = 170.88, 166.37, 144.24, 136.45, 132.81, 125.07, 63.68, 57.19, 52.89, 52.74, 52.60, 50.83, 50.66, 46.27, 45.35, 42.79, 34.50, 34.36, 33.76, 33.62, 31.05, 27.99, 27.93, 21.75, 15.77 ppm.

MS (ESI): *m/z* calcd for $C_{23}H_{31}Cl_2N_3O_5S+H^+$: 532.1 [M+H$^+$]; found: 532.0. HRMS (ESI): *m/z* calculated for $C_{23}H_{31}Cl_2N_3O_5S+H^+$: 532.14342 [M+H$^+$]; found: 532.14347.

Carboxylate 21



21

Compound **20** (2065 mg, 8.809 mmol, 1.0 eq.), L-pyroglutamic acid (1145 mg, 8.868 mmol, 1.0 eq.), EDC-HCl (1970 mg, 10.276 mmol, 1.2 eq.) and HOBt-H_2O (1585 mg, 10.350 mmol, 1.2 eq.) were dissolved in DMF (100 mL) at 0°C under argon athmosphere. The reaction was allowed to warm to room temperature and stirred for 17 h. Brine was added to the reaction mixture and it was extracted with Et_2O. The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude intermediate was dissolved in DCM (60 mL) and Boc_2O (11 mL, 47.881 mmol, 5.4 eq.) and DIPEA (10 mL, 57.408 mmol, 6.5 eq.) were added. Then DMAP was added in portions until gas formation was visible. The reaction was stirred at room temperature for 15 h, then brine was added and it was extracted with DCM. The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified twice by silica gel column chromatography (EA; Cy/EA 1:1) to afford as a mixture of *E*/*Z*-isomers (2248 mg, 57% yield over 2 steps).

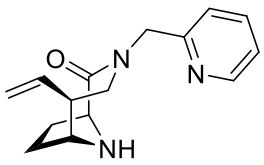
TLC R_f = 0.36 (Cy/EA 1:1).

^{1}H-NMR (300 MHz, $CDCl_3$): δ = -0.06-0.04 (m, 9H), 1.41-1.54 (m, 11H), 1.79-2.00 (m, 1H), 2.07-2.57 (m, 2H), 2.57-2.87 (m, 1H), 3.81-4.04 (m, 1H), 4.05-4.24 (m, 1H), 4.46-4.63 (m, 1H),

4.67-4.90 (m, 1H), 4.90-4.54 (m, 1H), 5.20-5.39 (m, 1H), 5.55-5.77 (m, 1H), 7.12-7.28 (m, 1H), 7.27-7.39 (m, 1H), 7.58-7.78 (m, 1H), 8.46-8.63 (m, 1H) ppm.

MS (ESI): *m/z* calculated for $C_{23}H_{35}N_3O_4+H^+$: 446.3 [$M+H^+$]; found: 446.2.

Compound 22



22

Compound **21** (2187 mg, 4.908 mmol, 1.0 eq.) was dissolved in dry THF (60 mL) under argon atmosphere and cooled to -98°C. DIBAL-H (1 M in THF, 8.4 mL, 8.400 mmol, 1.7 eq.) was added dropwise. After stirring for 5 min at -98°C, Glauber's salt was added and it was allowed to warm to room temperature. The mixture was diluted with Et_2O, filtered over celite and concentrated *in vacuo*. The residue was taken up in DCM (200 mL) and cooled to -84°C. HF-pyridine (70 wt.-%, 14 mL, 530 mmol, 110 eq.) was added slowly and the reaction was allowed to warm to 0°C. After 3 h the reaction was carefully quenched by addition of aqueous $CaCO_3$ slurry (200 mL) and NaOH (10 M, 200 mL). The mixture was filtered and extracted with DCM. The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified twice by silica gel column chromatography (EA + 5% MeOH + 3% TEA; Cy/EA 3:1 + 3% TEA → EA + 5% MeOH + 3% TEA) to provide the product as orange oil (506 mg, 40% yield over 2 steps).

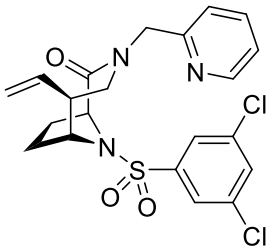
TLC R_f = 0.27 (EA + 5% MeOH + 3% TEA).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.67-1.75 (m, 1H), 1.92-2.04 (m, 2H), 2.12-2.19 (m, 1H), 2.22-2.31 (m, 1H), 3.00-3.25 (s, 1H), 3.32 (dd, 1H, *J* = 14.9/4.5 Hz), 3.51-3.56 (m, 1H), 3.59 (d, 1H, *J* = 14.9 Hz), 4.16 (d, 1H, *J* = 9.8 Hz), 4.38 (d, 1H, *J* = 14.8 Hz), 4.88 (d, 1H, *J* = 14.8 Hz), 5.00-5.09 (m, 2H), 5.69-5.79 (m, 1H), 7.10-7.16 (m, 1H), 7.23 (d, 1H, *J* = 7.9 Hz), 7.56-7.63 (m, 1H), 8.42-8.48 (m, 1H) ppm.

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 27.1, 30.2, 46.5, 49.8, 55.5, 60.8, 63.6, 116.8, 122.4, 122.8, 136.8, 137.6, 149.0, 157.6, 177.9 ppm.

MS (ESI): *m/z* calculated for $C_{15}H_{18}N_3O+H^+$: 258.2 [M+H$^+$]; found: 258.3.

Inhibitor 23



23

Compound **22** (31 mg, 0.121 mmol, 1.0 eq.) and 3,5-dichlorobenzenesulfonyl chloride (55 mg, 0.222 mmol, 1.8 eq.) were dissolved in dry MeCN (15 mL) under argon atmosphere. DIPEA (50 µL, 0.287 mmol, 2.4 eq.) was added and the reaction was stirred at room temperature for 18 h. The solvent was evaporated *in vacuo* and the crude product was purified by silica gel column chromatography (Cy/EA 1:1) to provide the product as colorless solid (45 mg, 80% yield, 99% purity).

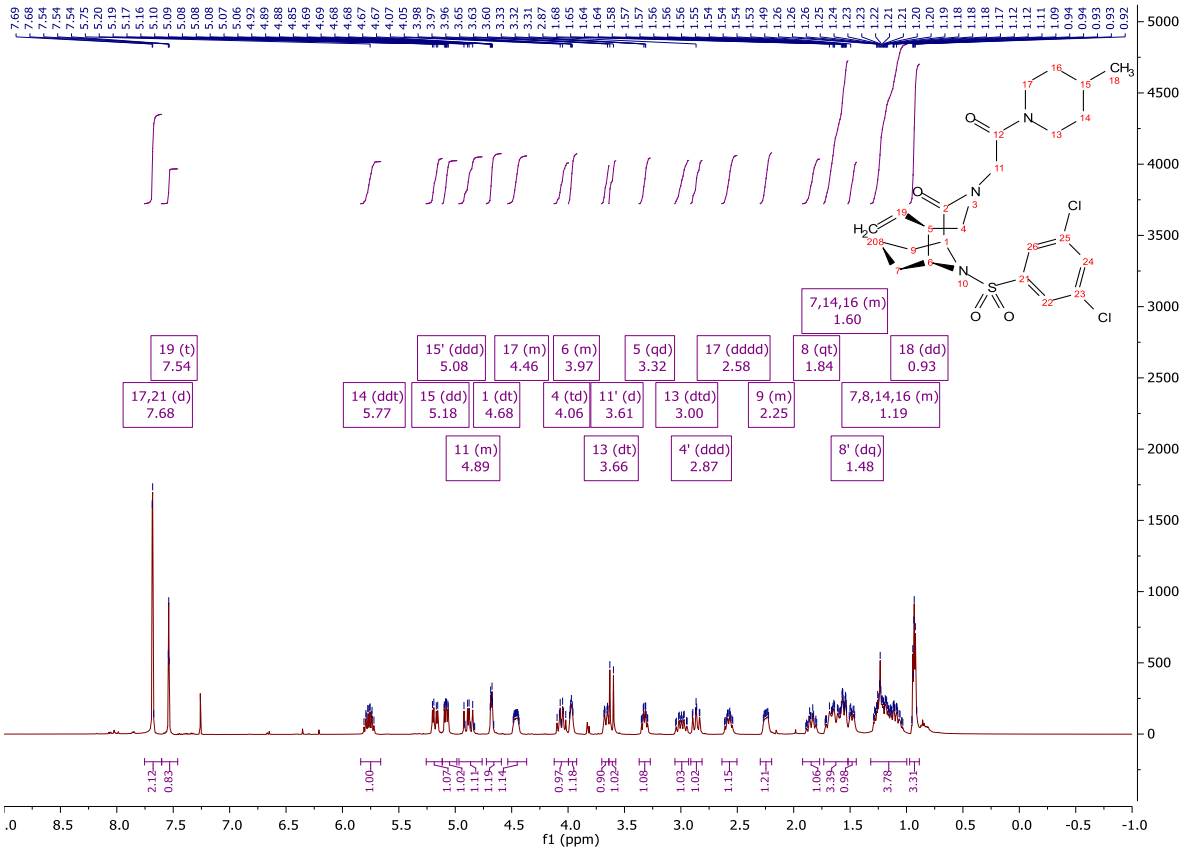
TLC R_f = 0.15 (Cy/EA 1:1).

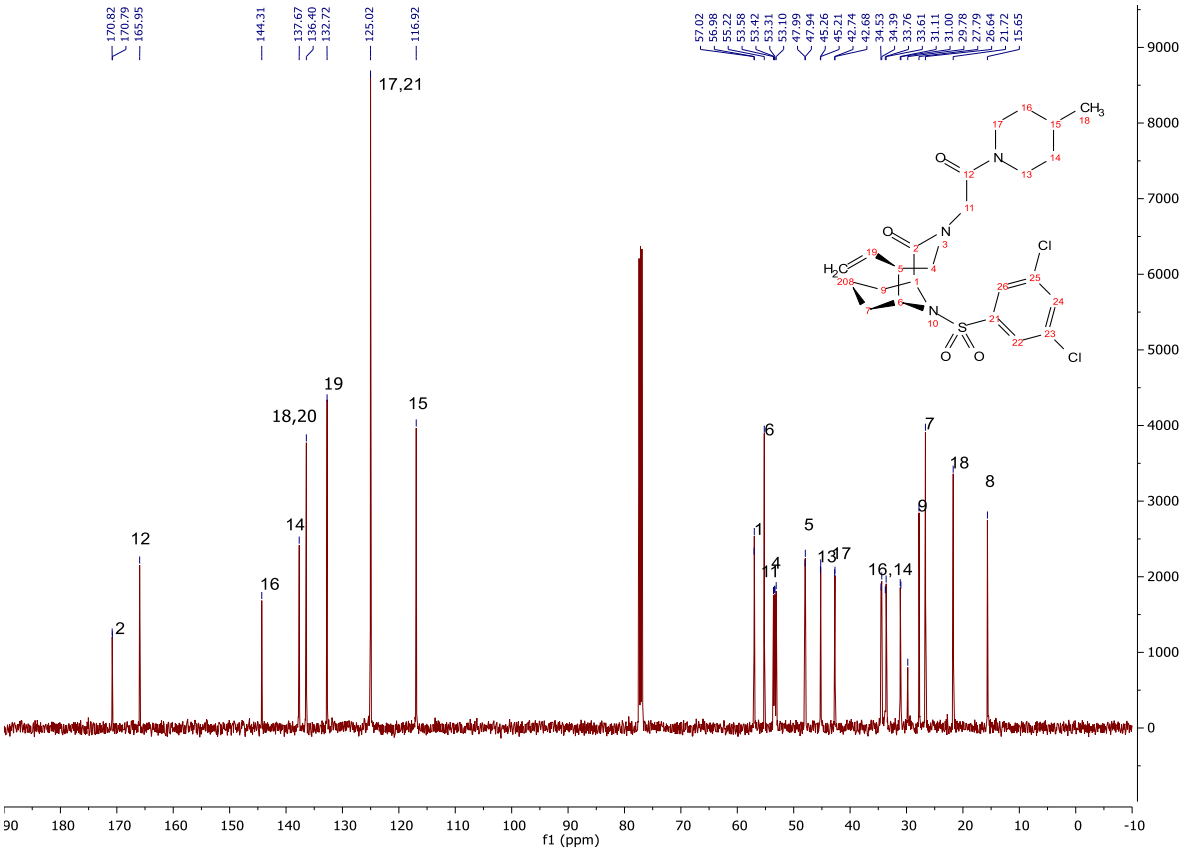
^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.65-1.79 (m, 2H), 2.06-2.22 (m, 2H), 2.29-2.37 (m, 1H), 3.50-2.61 (m, 2H), 4.62 (d, 1H, *J* = 15.0 Hz), 4.75 (d, 1H, *J* = 15.0 Hz), 4.82 (dd, 1H, *J* = 10.0/2.7 Hz), 4.98 (d, 1H, *J* = 10.4 Hz), 5.07 (d, 1H, *J* = 17.2 Hz), 5.60-5.71 (m, 1H), 7.23 (dd, 1H, *J* = 7.1/5.3 Hz), 7.30 (d, 1H, *J* = 7.9 Hz), 7.55-7.58 (m, 1H), 7.68-7.72 (m, 1H), 7.73 (d, 2H, *J* = 1.9 Hz), 8.49 (d, 1H, *J* = 4.8 Hz) ppm.

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 28.3, 29.8, 30.3, 48.8, 50.7, 55.2, 63.7, 117.4, 122.9, 123.3, 125.6, 133.1, 136.2, 136.4, 137.8, 142.8, 148.4, 156.7, 172.3 ppm.

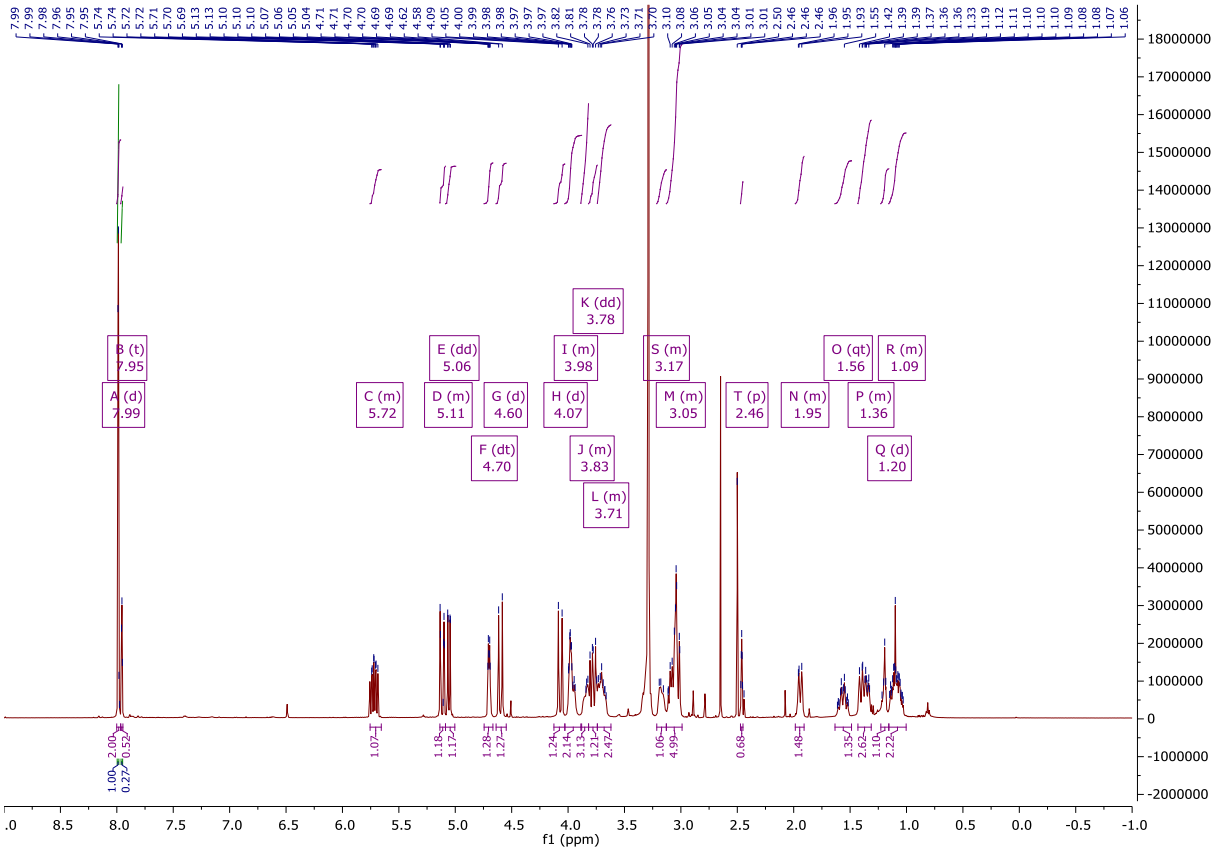
HRMS (ESI): *m/z* calculated for $C_{21}H_{20}Cl_2N_3O_3S+H^+$: 466.07534 [M+H$^+$]; found: 466.07580.

Compound 7a

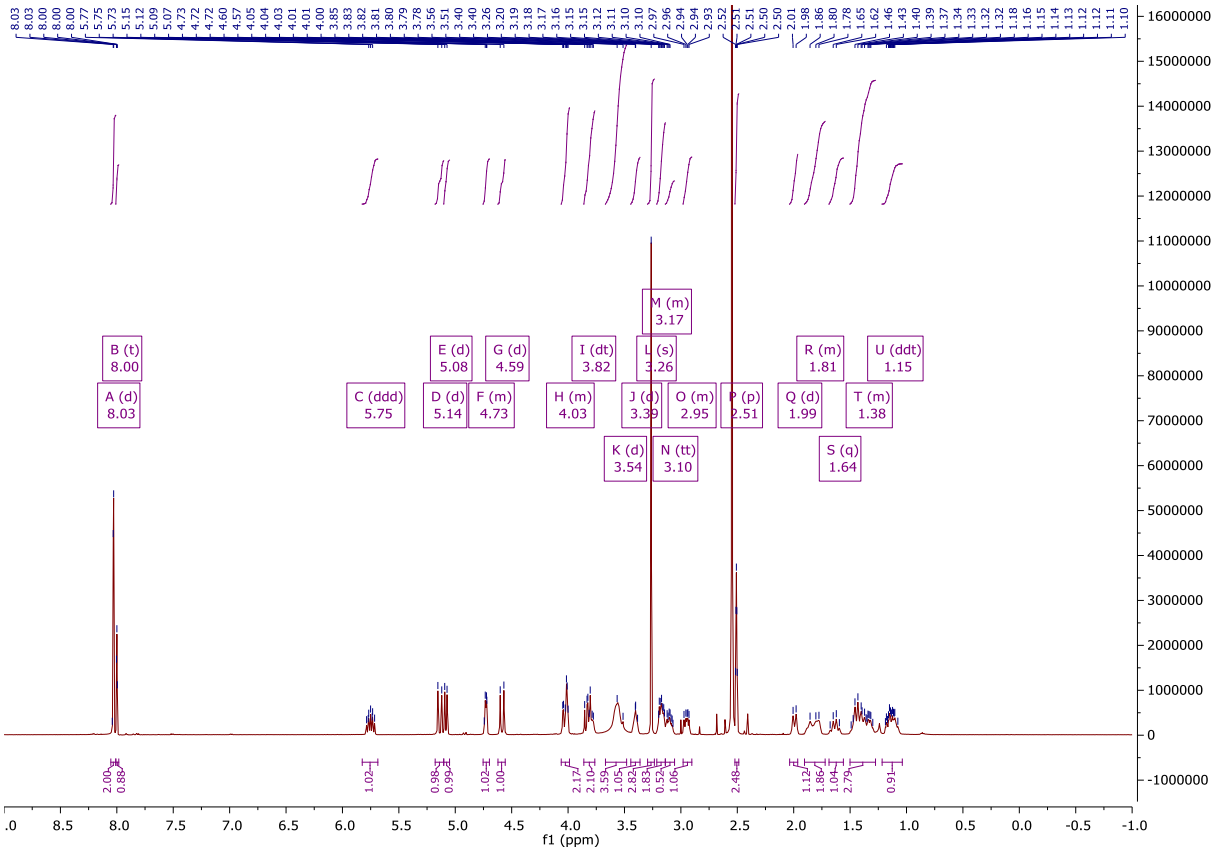

19 (t) 7.54
17,21 (d) 7.68
14 (ddt) 5.77
15' (ddd) 5.08
15 (dd) 5.18
1 (dt) 4.68
11 (m) 4.89
17 (m) 4.46
6 (m) 3.97
4 (td) 4.06
13 (dt) 3.66
11' (d) 3.61
5 (qd) 3.32
13 (dtd) 3.00
4' (ddd) 2.87
17 (dddd) 2.58
9 (m) 2.25
8 (qt) 1.84
7,14,16 (m) 1.60
8' (dq) 1.48
18 (dd) 0.93
7,8,14,16 (m) 1.19
f1 (ppm)


170.82
170.79
165.95
144.31
137.67
136.40
132.72
125.02
116.92
17,21
19
18,20
15
12
14
16
2
6
1
4
11
5
13
17
16,14
7
18
8
9
f1 (ppm)

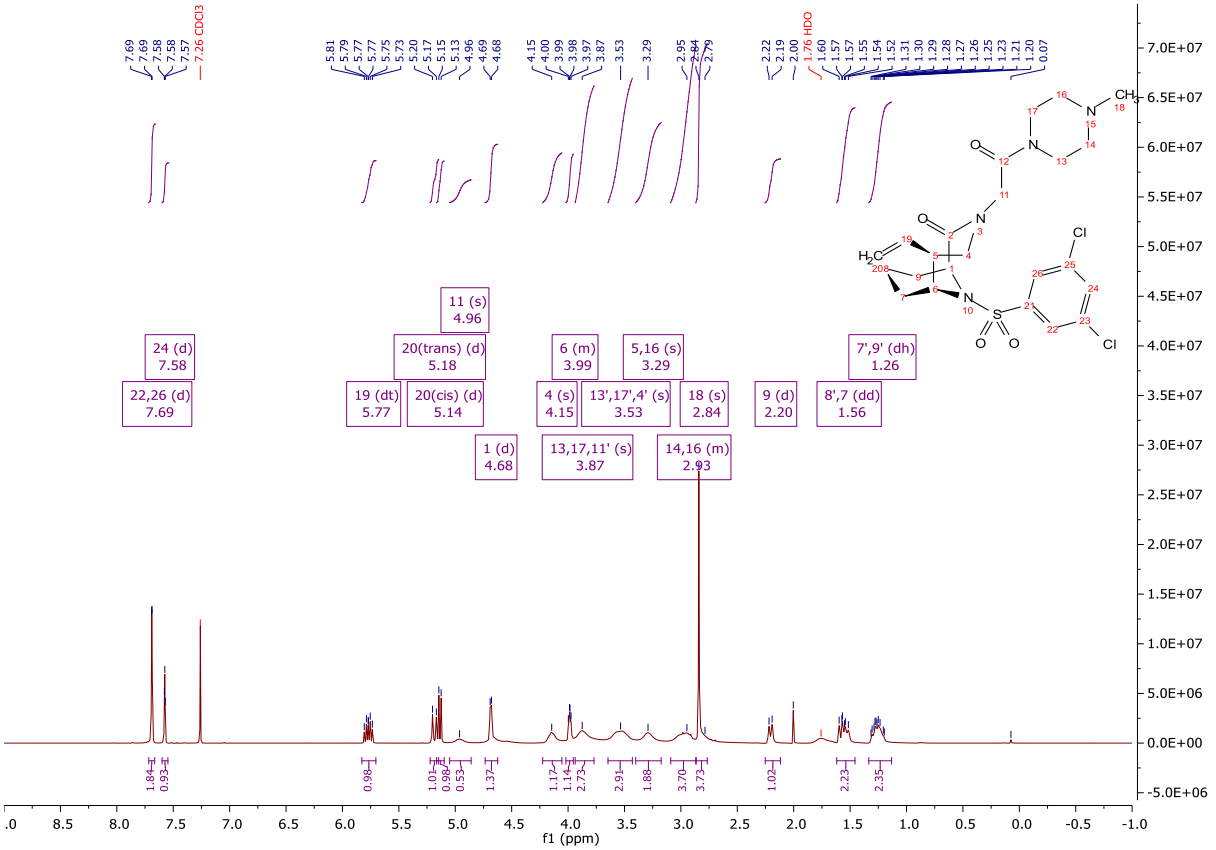
Compound 7b

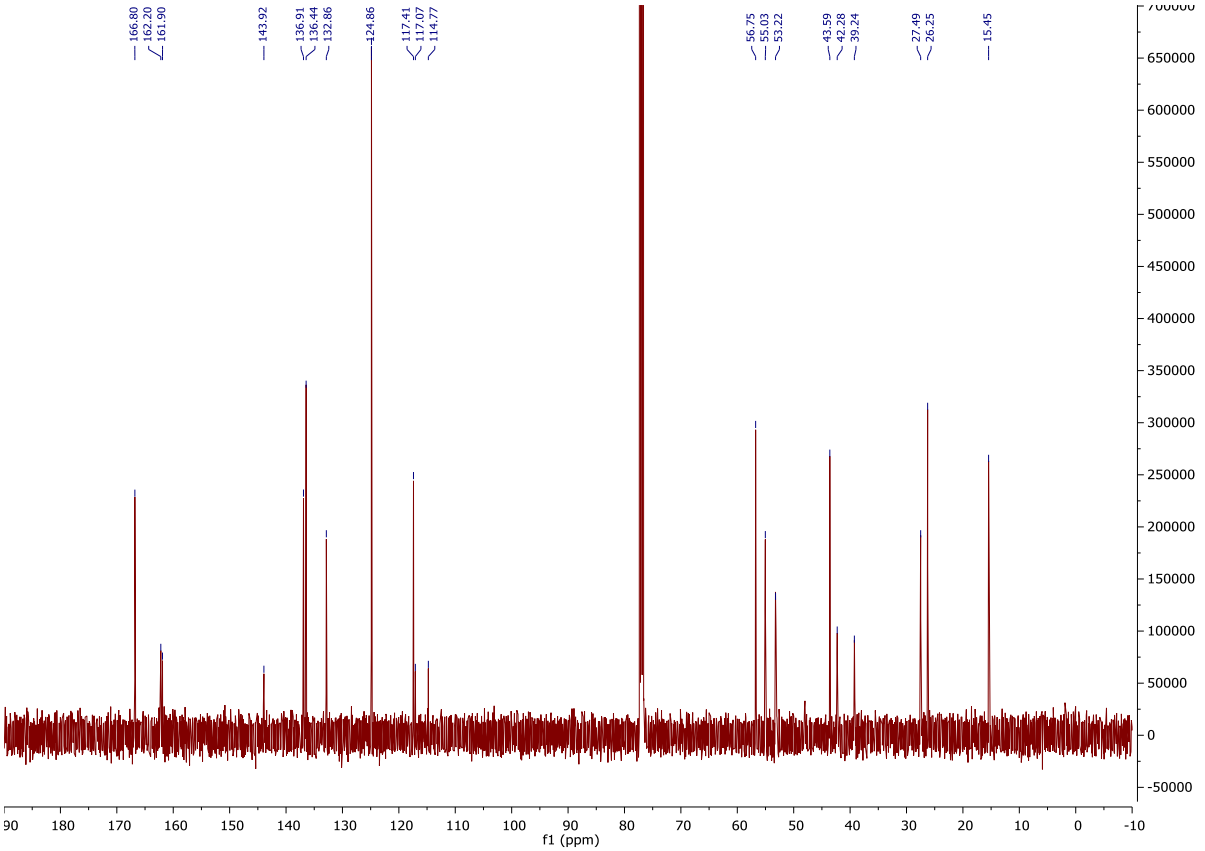

B (t)
7.95
A (d)
7.99
E (dd)
5.06
C (m)
5.72
D (m)
5.11
G (d)
4.60
F (dt)
4.70
K (dd)
3.78
I (m)
3.98
H (d)
4.07
J (m)
3.83
L (m)
3.71
S (m)
3.17
M (m)
3.05
T (p)
2.46
N (m)
1.95
O (qt)
1.56
R (m)
1.09
P (m)
1.36
Q (d)
1.20
f1 (ppm)

Compound 7c

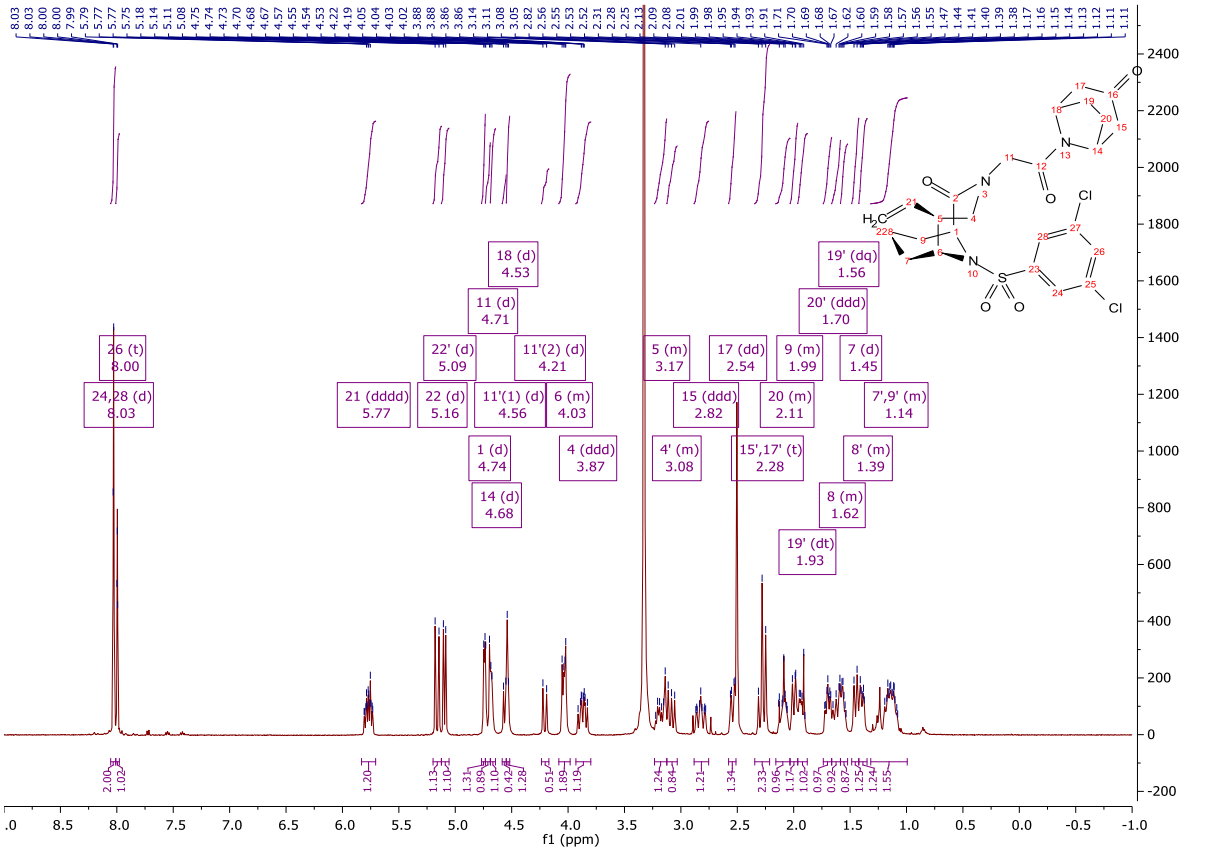


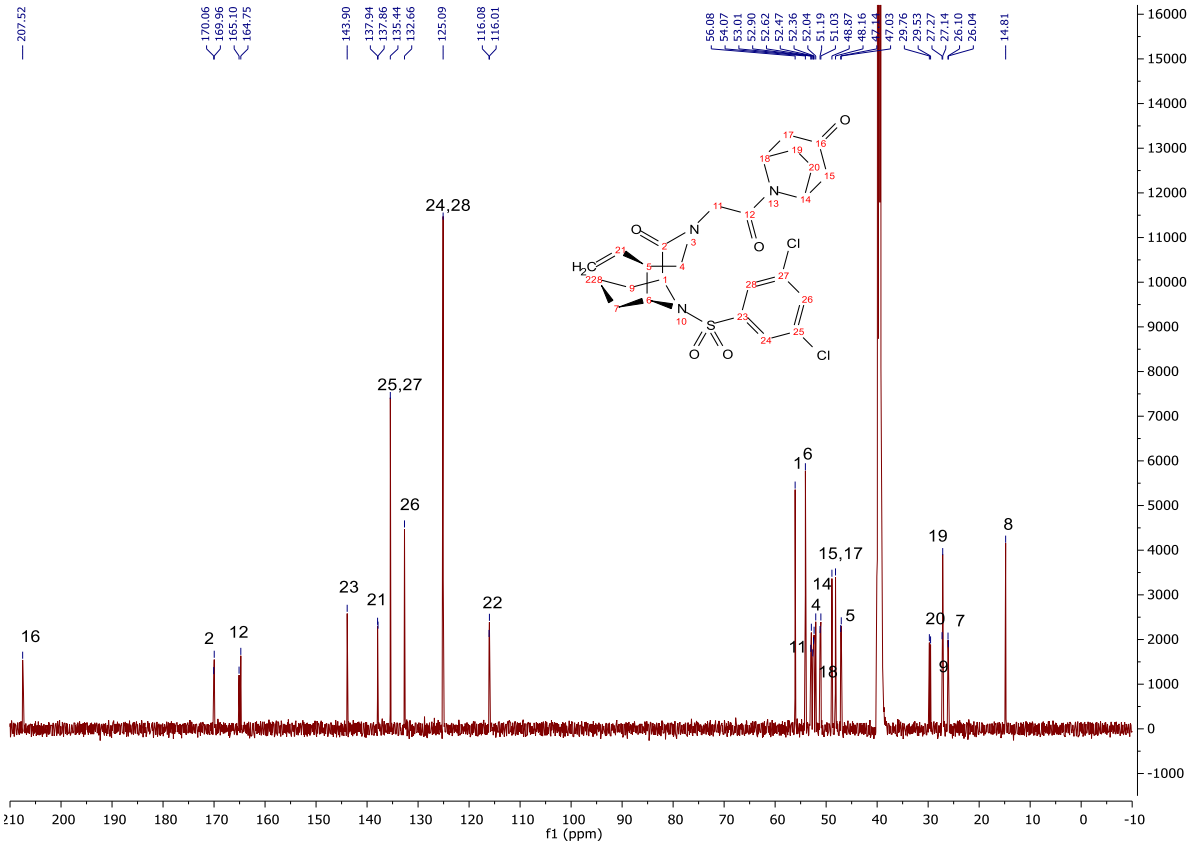
Compound 7f



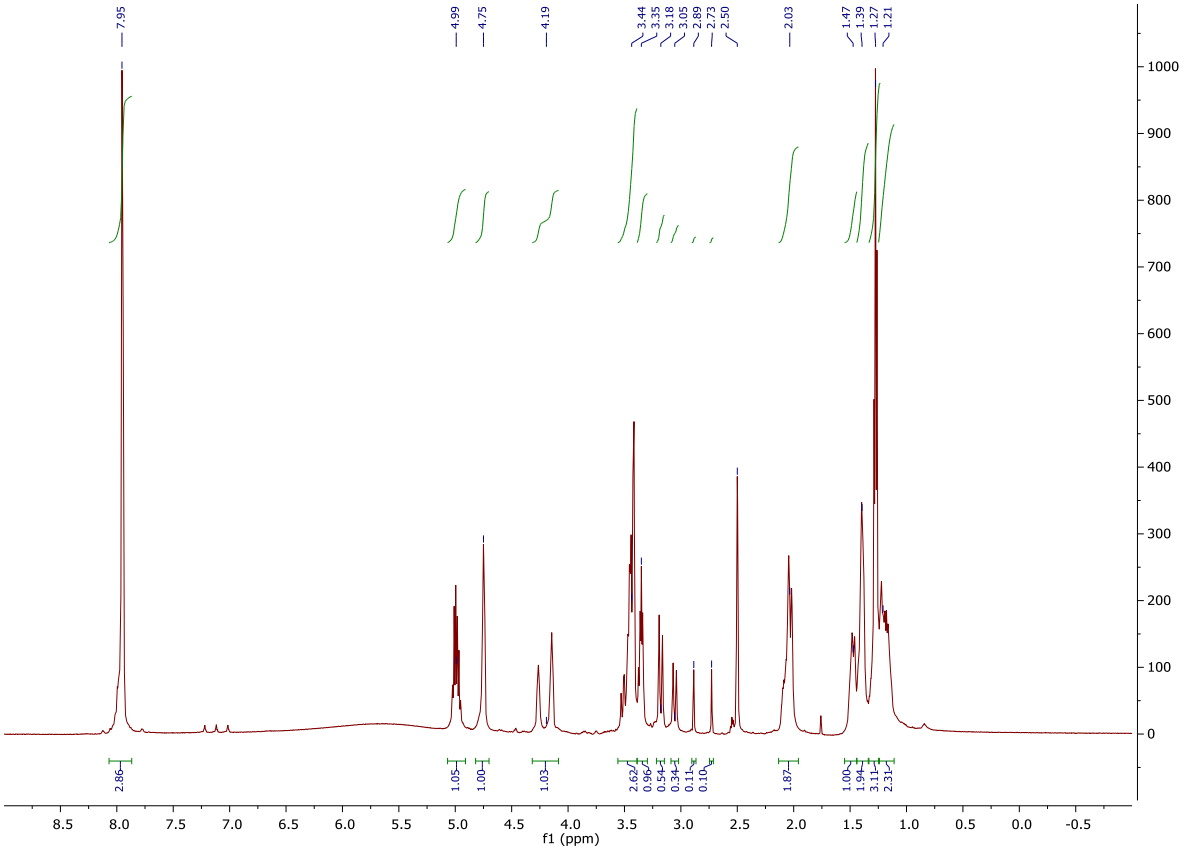

166.80
162.20
161.90
143.92
136.91
136.44
132.86
124.86
117.41
117.07
114.77
56.75
55.03
53.22
43.59
42.28
39.24
27.49
26.25
15.45
f1 (ppm)

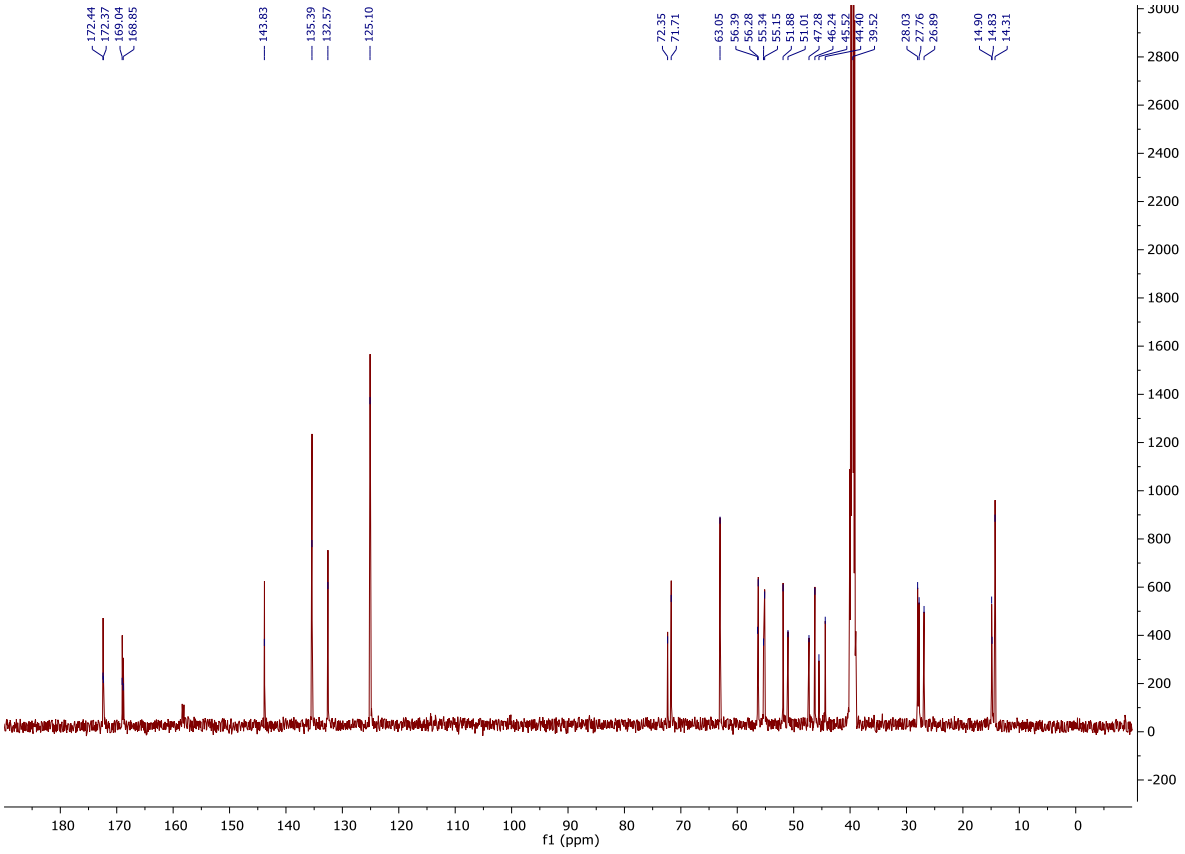
Compound 7g


26 (t) 8.00
24,28 (d) 8.03
21 (dddd) 5.77
22 (d) 5.16
22' (d) 5.09
11'(1) (d) 4.56
11'(2) (d) 4.21
6 (m) 4.03
18 (d) 4.53
11 (d) 4.71
1 (d) 4.74
14 (d) 4.68
4 (ddd) 3.87
5 (m) 3.17
4' (m) 3.08
15 (ddd) 2.82
17 (dd) 2.54
15',17' (t) 2.28
9 (m) 1.99
20 (m) 2.11
19' (dq) 1.56
20' (ddd) 1.70
7 (d) 1.45
7',9' (m) 1.14
8' (m) 1.39
8 (m) 1.62
19' (dt) 1.93
f1 (ppm)

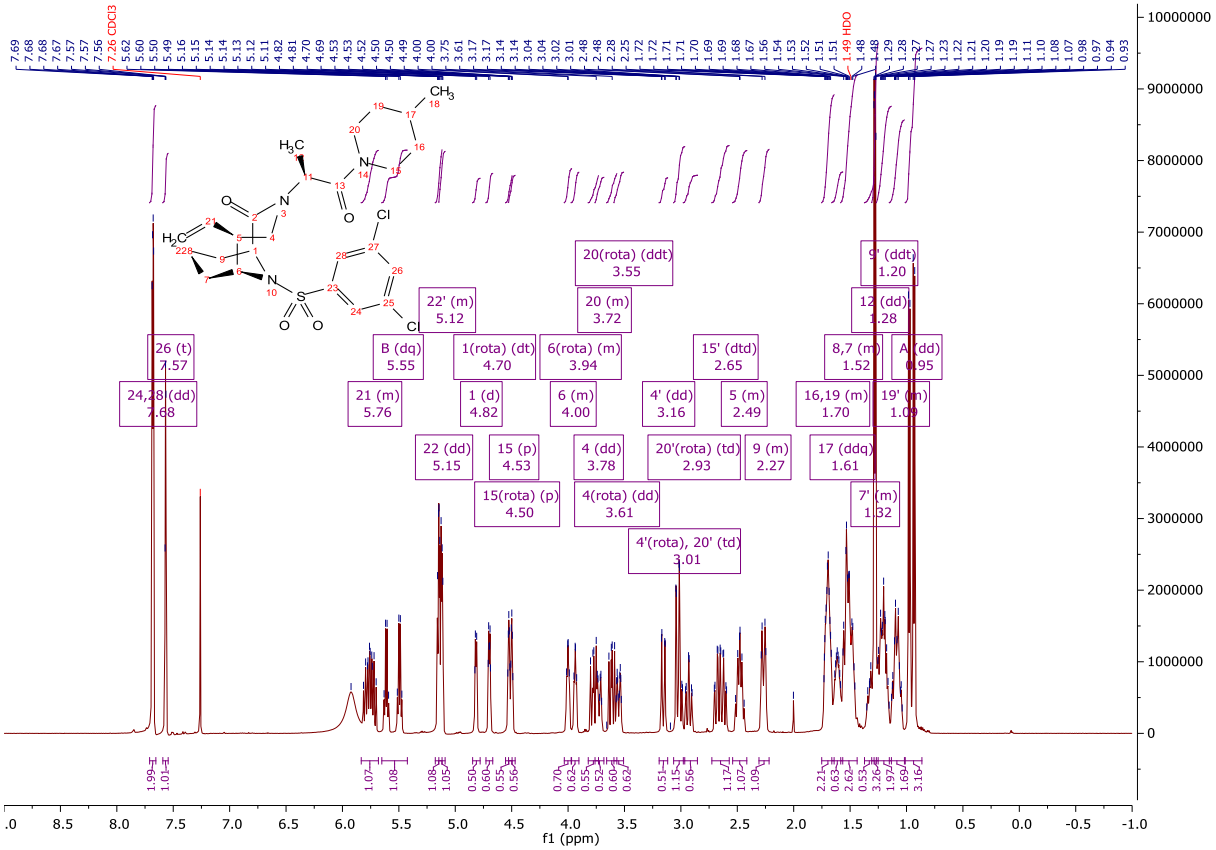

207.52
170.06
169.96
165.10
164.75
143.90
137.94
137.86
135.44
132.66
125.09
116.08
116.01
56.08
54.07
53.01
52.90
52.62
52.47
52.36
52.04
51.19
51.03
48.87
48.16
47.14
47.03
29.76
29.53
27.27
27.14
26.10
26.04
14.81
24,28
25,27
26
23
21
22
16
2
12
1
6
15,17
14
4
5
11
18
19
20
7
9
8
f1 (ppm)

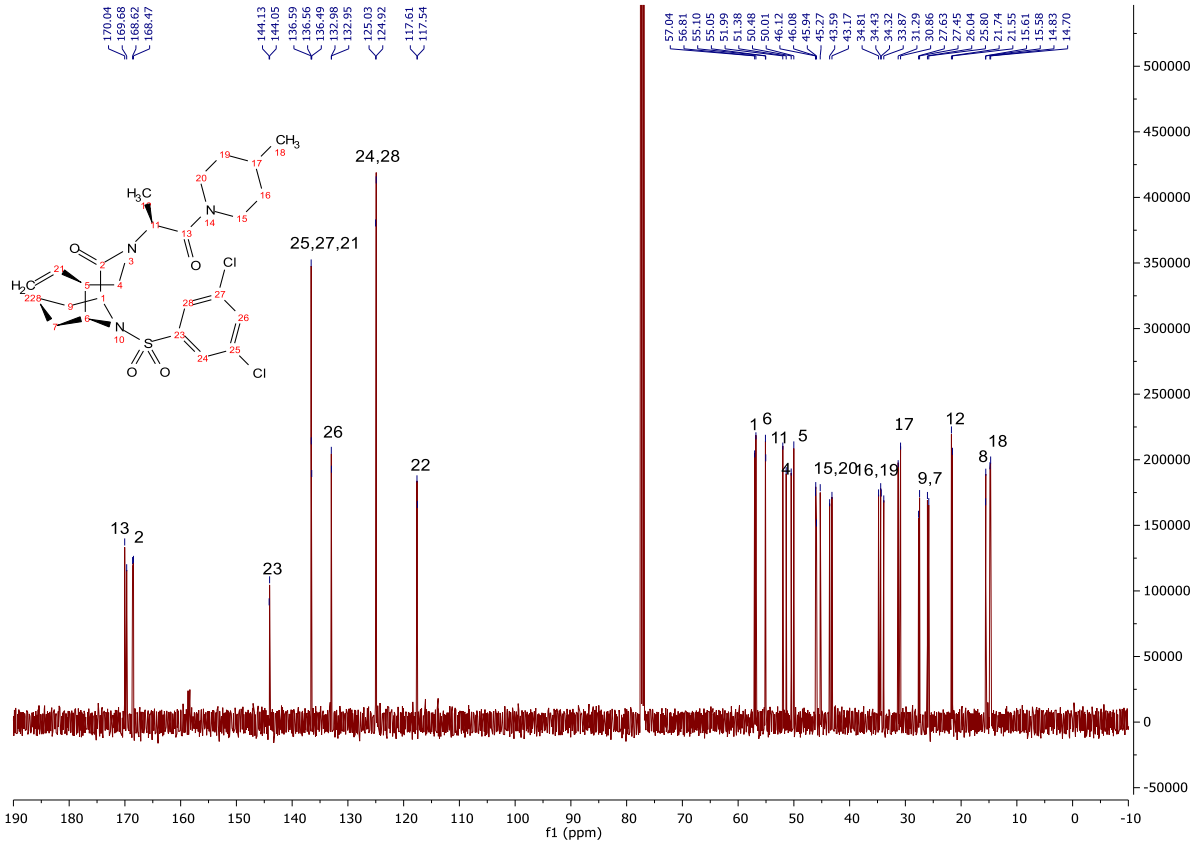
Compound 15


7.95
4.99
4.75
4.19
3.44
3.35
3.18
3.05
2.89
2.73
2.50
2.03
1.47
1.39
1.27
1.21
2.86
1.05
1.00
1.03
2.62
0.96
0.54
0.34
0.11
0.10
1.87
1.00
1.94
3.11
2.31
f1 (ppm)

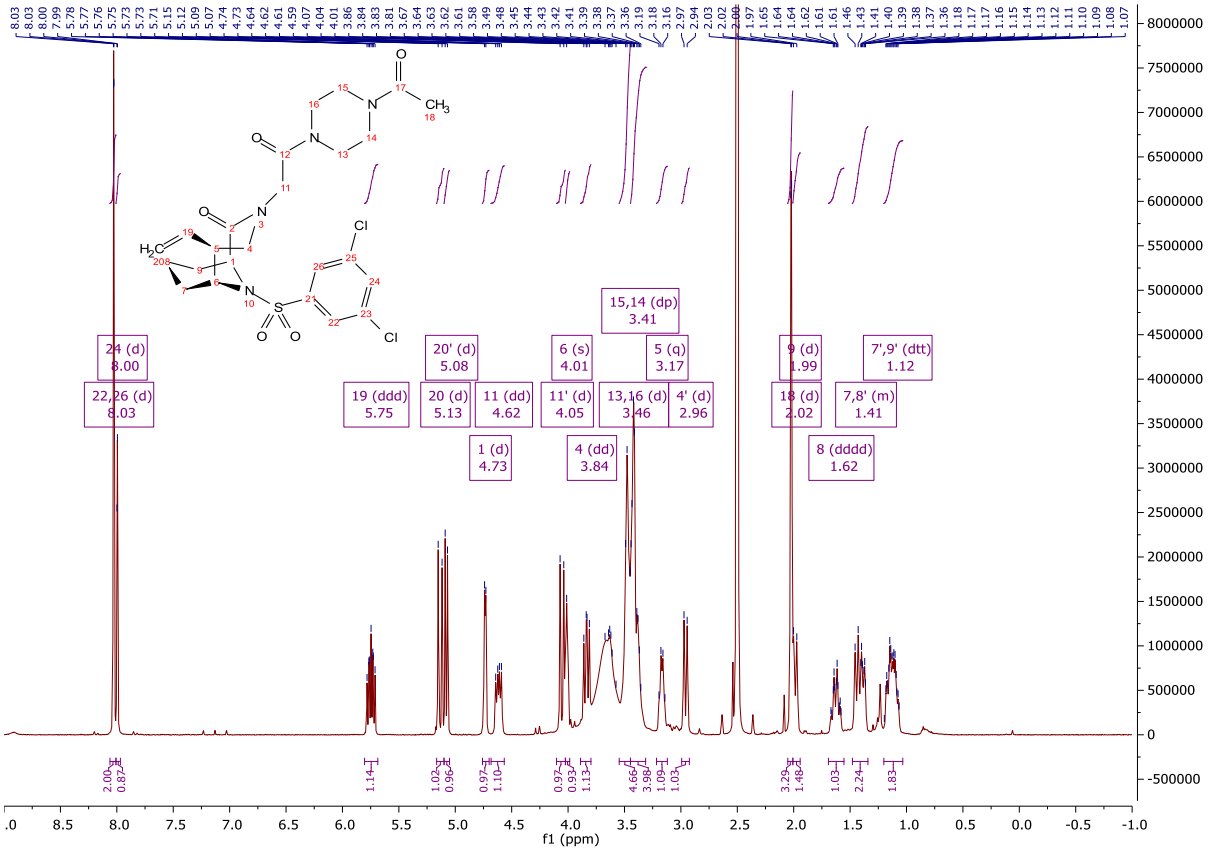

172.44
172.37
169.04
168.85
143.83
135.39
132.57
125.10
72.35
71.71
63.05
56.39
56.28
55.34
55.15
51.88
51.01
47.28
46.24
45.52
44.40
39.52
28.03
27.76
26.69
14.90
14.83
14.31
f1 (ppm)

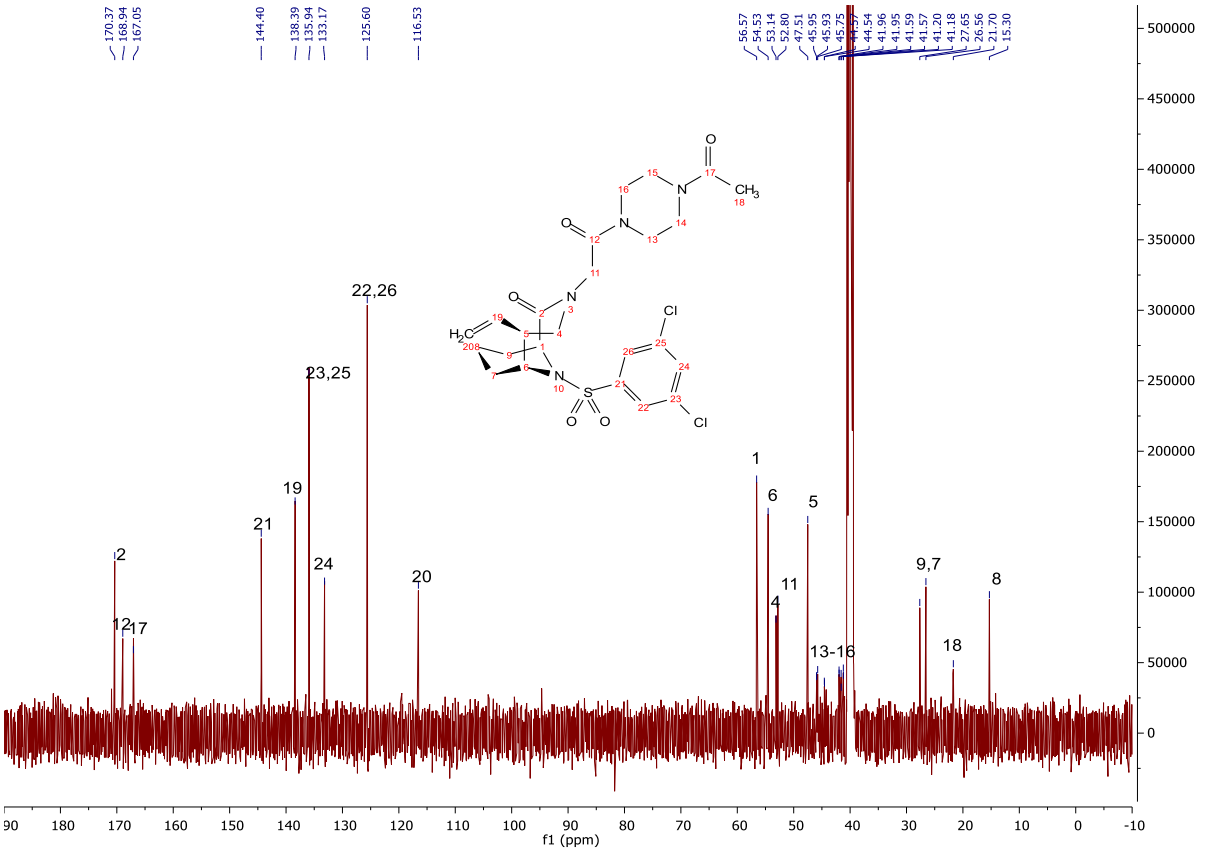
Compound 16


7.26 CDCl3
1.49 HDO
26 (t) 7.57
24,28 (dd) 7.68
B (dq) 5.55
21 (m) 5.76
22' (m) 5.12
1(rota) (dt) 4.70
1 (d) 4.82
22 (dd) 5.15
15 (p) 4.53
15(rota) (p) 4.50
6(rota) (m) 3.94
6 (m) 4.00
4 (dd) 3.78
4(rota) (dd) 3.61
20(rota) (ddt) 3.55
20 (m) 3.72
4'(rota), 20' (td) 3.01
4' (dd) 3.16
20'(rota) (td) 2.93
15' (dtd) 2.65
5 (m) 2.49
9 (m) 2.27
9' (ddt) 1.20
12 (dd) 1.28
8,7 (m) 1.52
A (dd) 0.95
16,19 (m) 1.70
19' (m) 1.09
17 (ddq) 1.61
7' (m) 1.32
f1 (ppm)

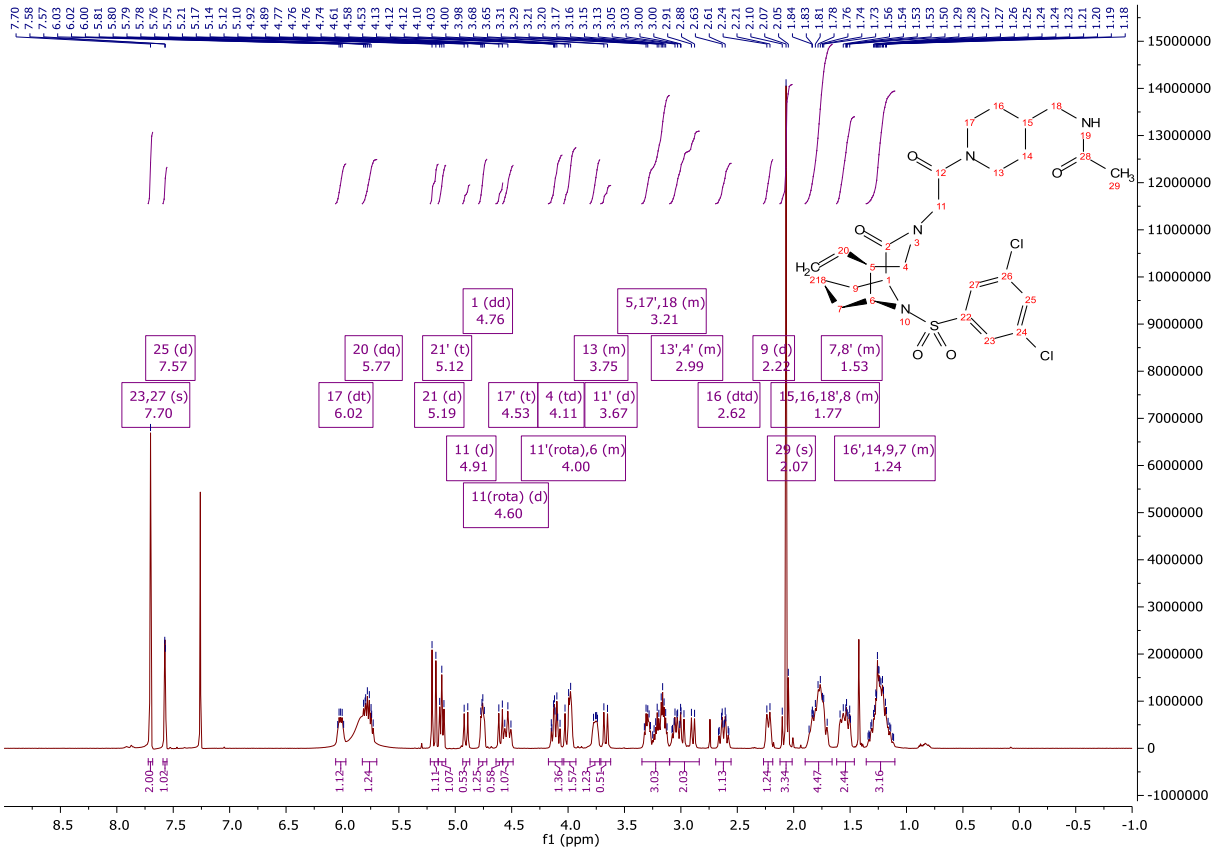


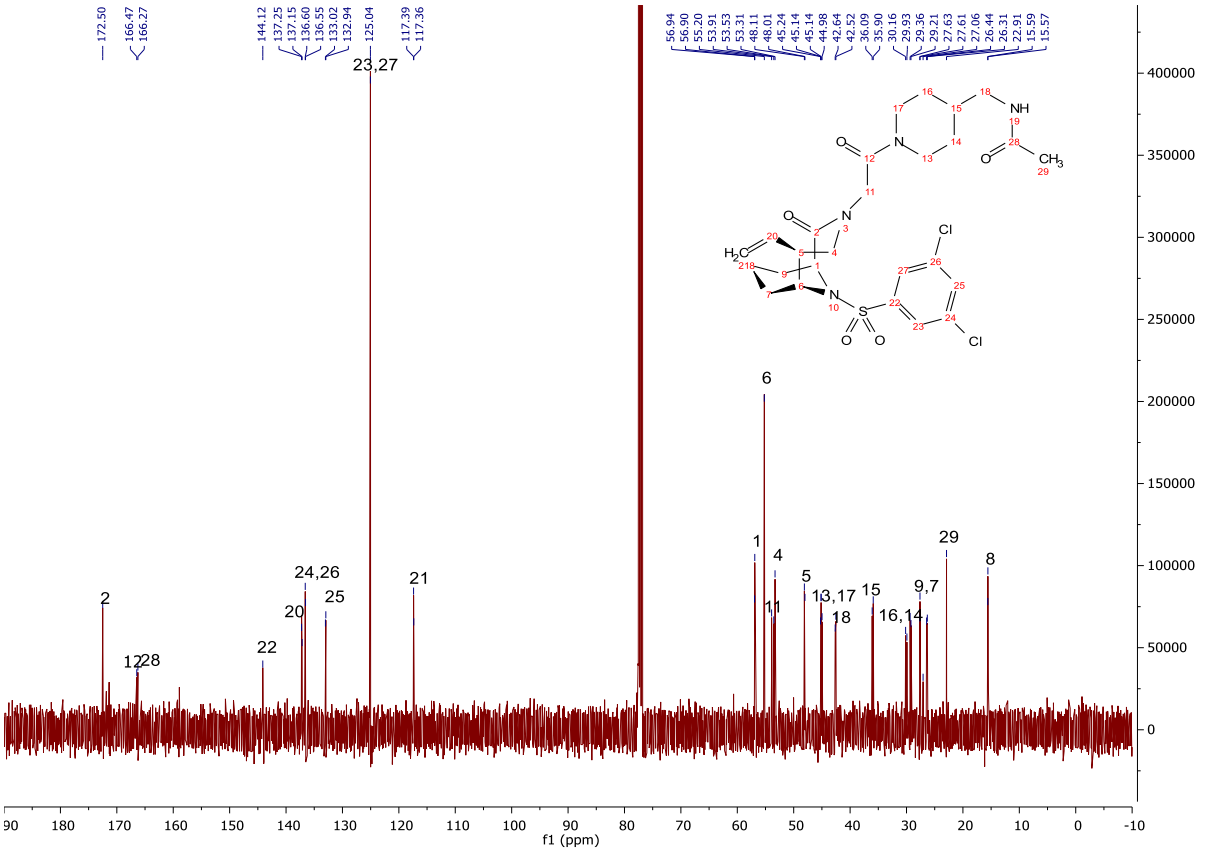
Compound 18a



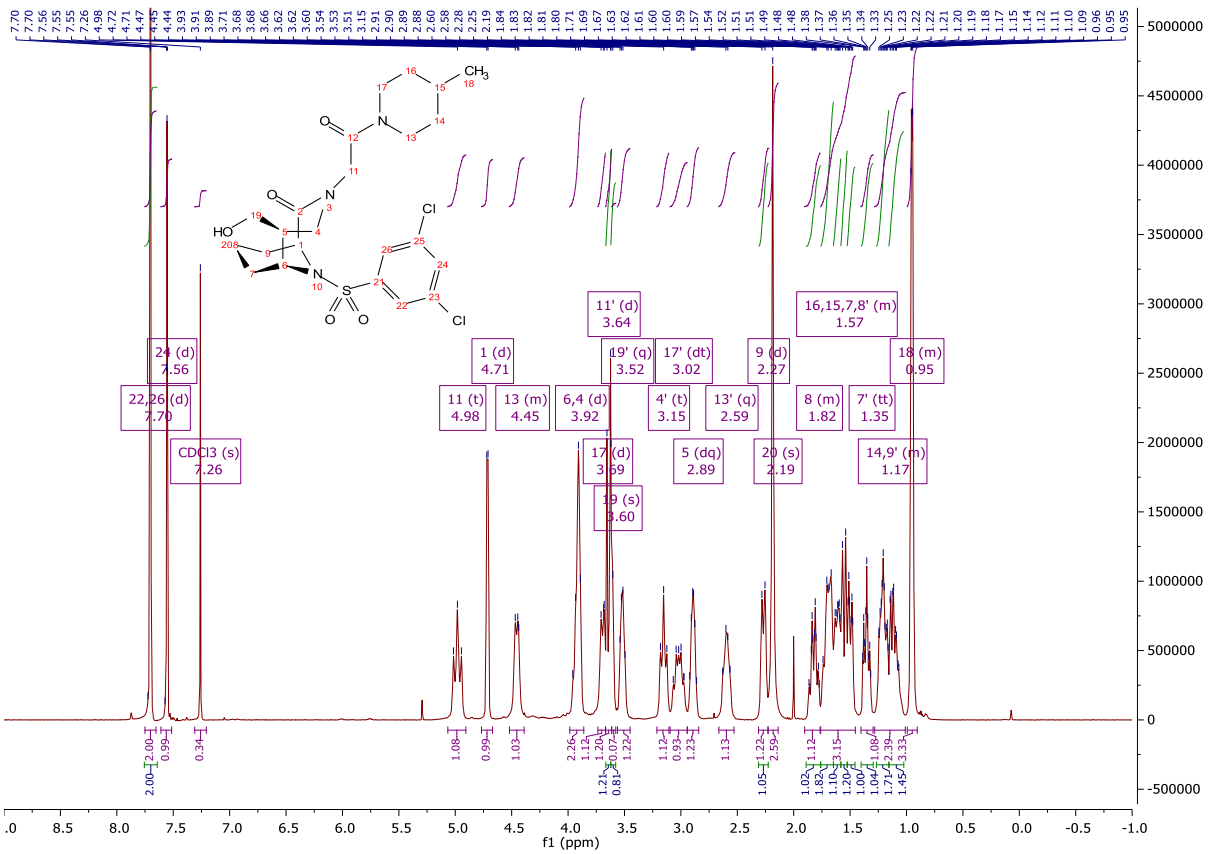


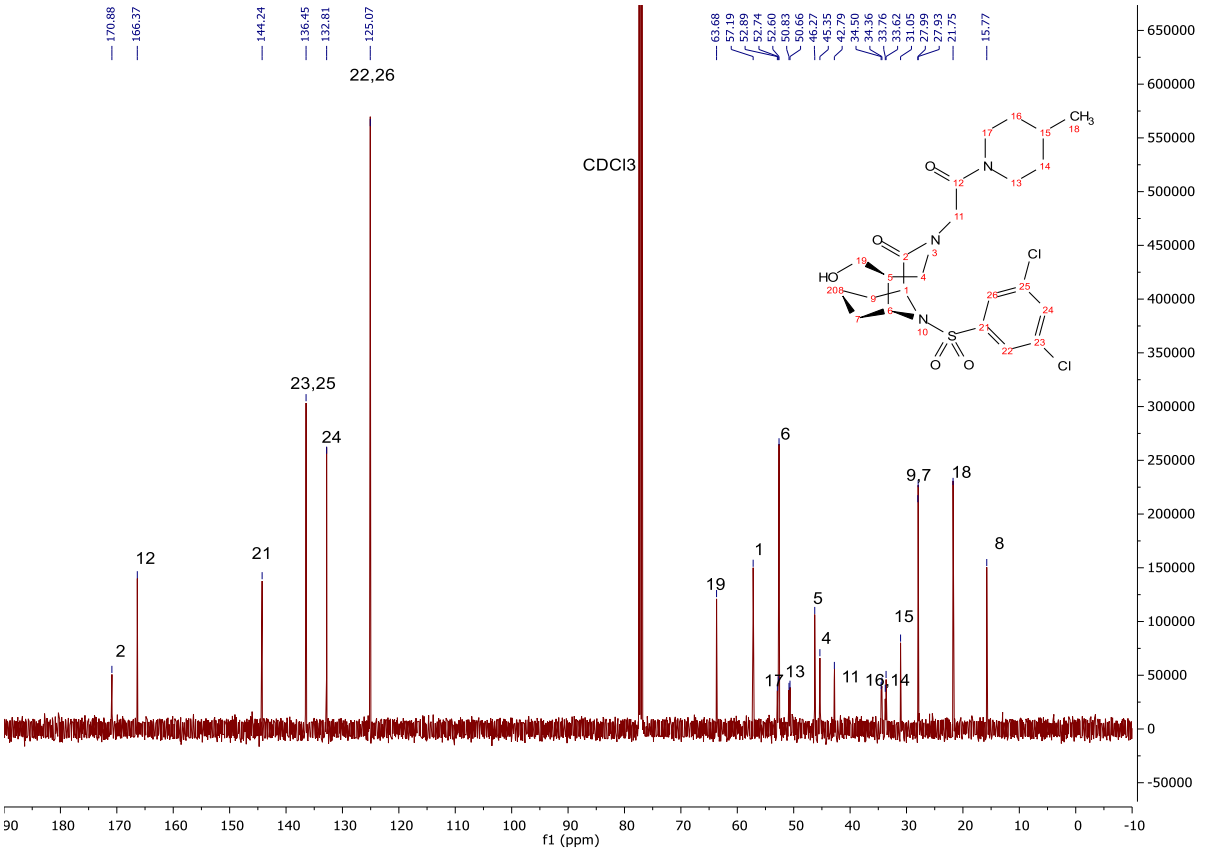
Compound 18b



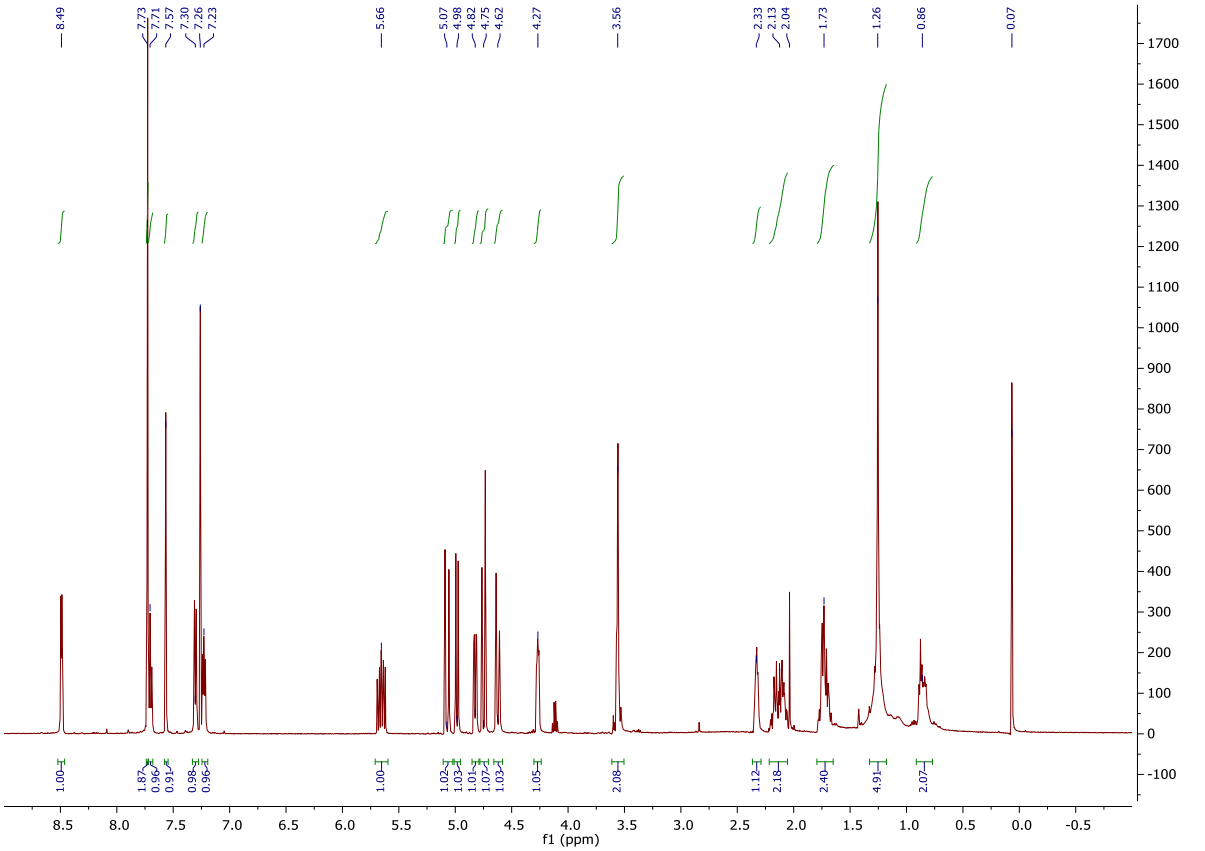

f1 (ppm)

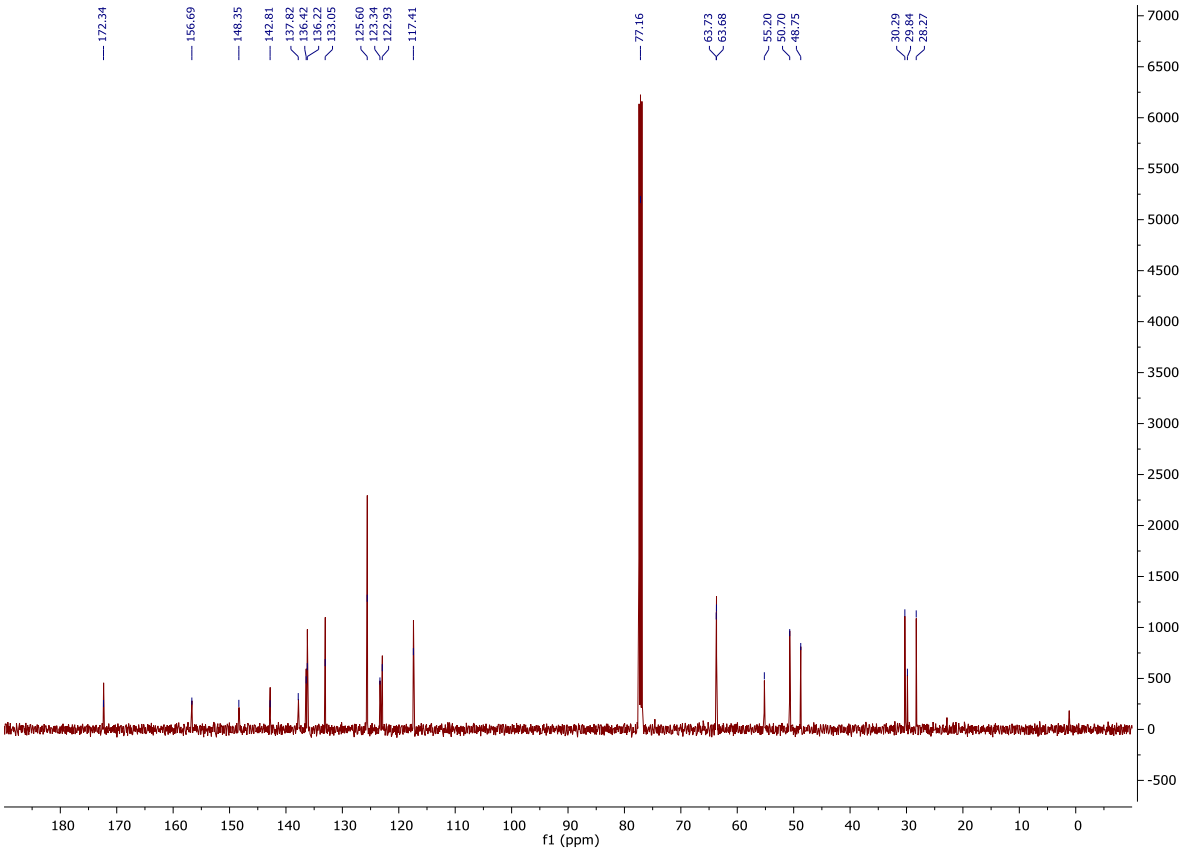
Compound 19


24 (d) 7.56
22,26 (d) 7.70
CDCl3 (s) 7.26
1 (d) 4.71
11 (t) 4.98
13 (m) 4.45
6,4 (d) 3.92
11' (d) 3.64
19' (q) 3.52
17' (dt) 3.02
17 (d) 3.59
19 (s) 3.60
4' (t) 3.15
13' (q) 2.59
5 (dq) 2.89
9 (d) 2.27
20 (s) 2.19
16,15,7,8' (m) 1.57
8 (m) 1.82
7' (tt) 1.35
18 (m) 0.95
14,9' (m) 1.17
f1 (ppm)

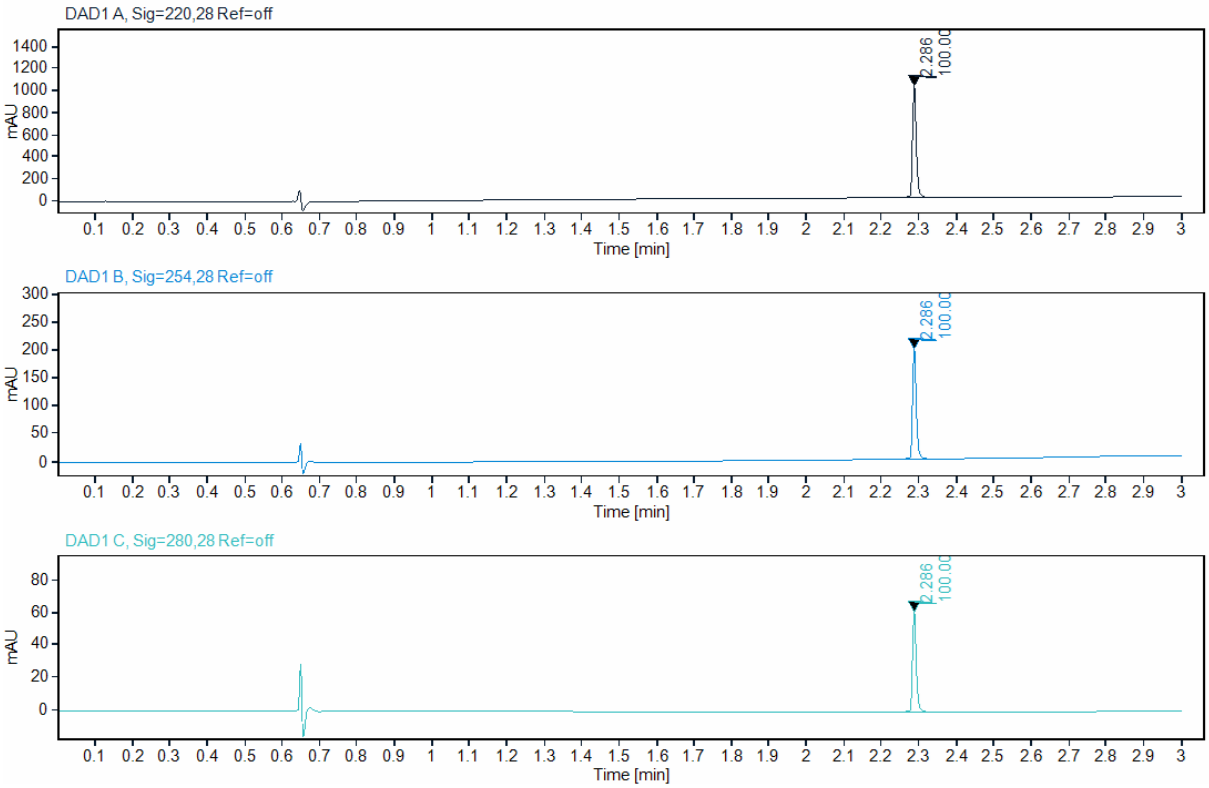

170.88
166.37
144.24
136.45
132.81
125.07
63.68
57.19
52.89
52.74
52.60
50.83
50.66
46.27
45.35
42.79
34.50
34.36
33.76
33.62
31.05
27.99
27.93
21.75
15.77
22,26
CDCl3
23,25
24
12
21
2
6
1
19
5
4
9,7
18
8
15
17
13
11
16
14
f1 (ppm)

Compound 23

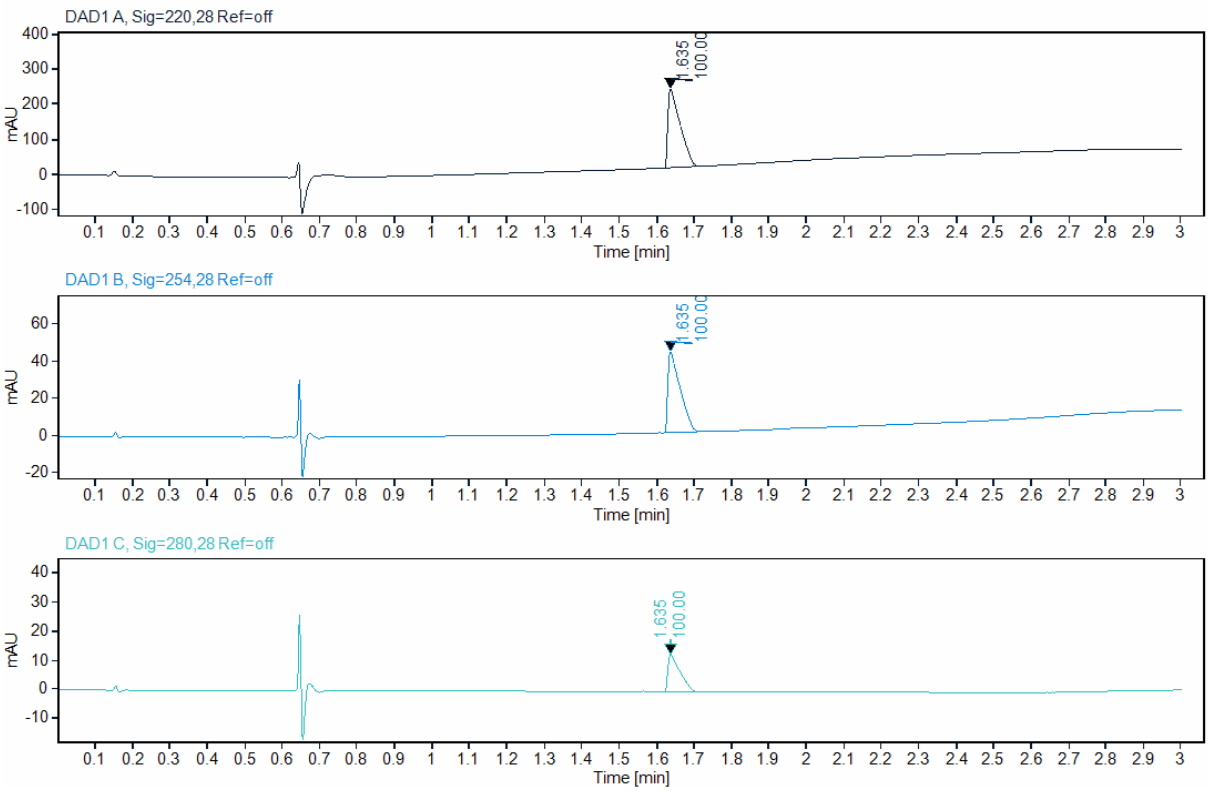

8.49
7.73
7.71
7.57
7.30
7.26
7.23
5.66
5.07
4.98
4.82
4.75
4.62
4.27
3.56
2.33
2.13
2.04
1.73
1.26
0.86
0.07
f1 (ppm)



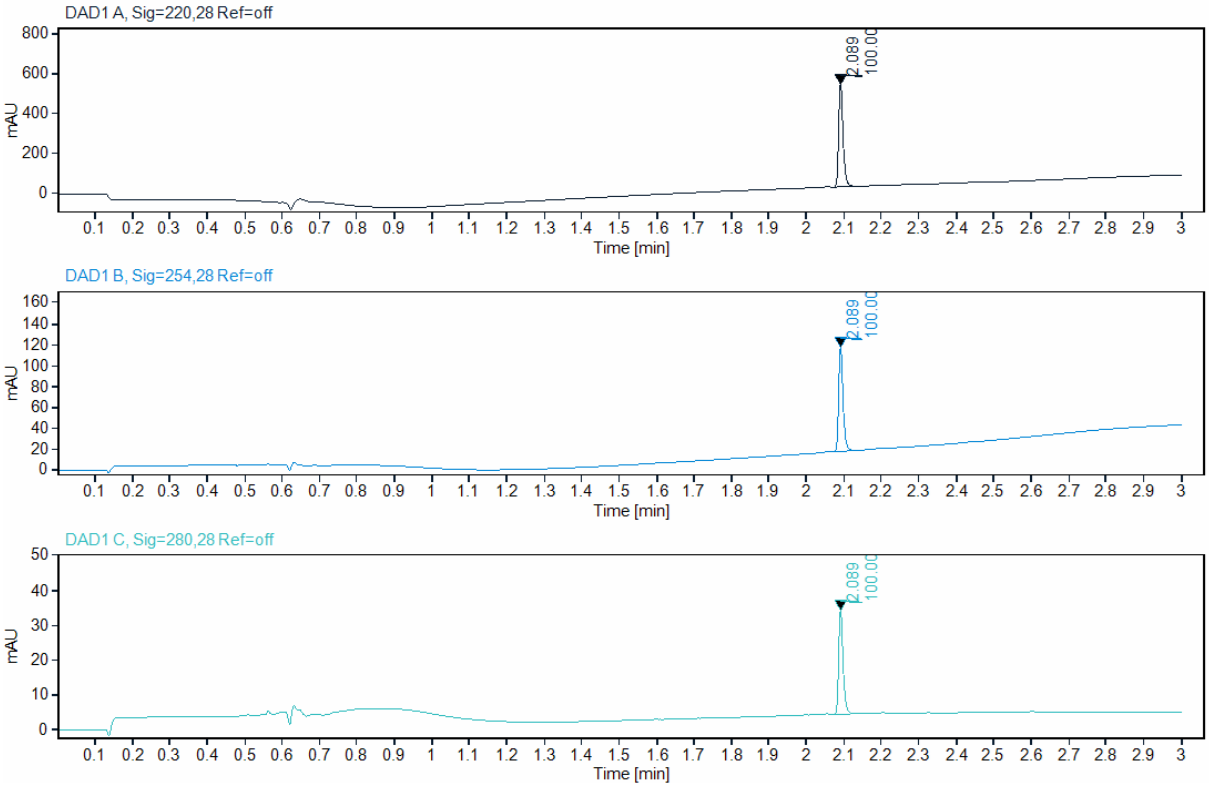
Compound **7a**



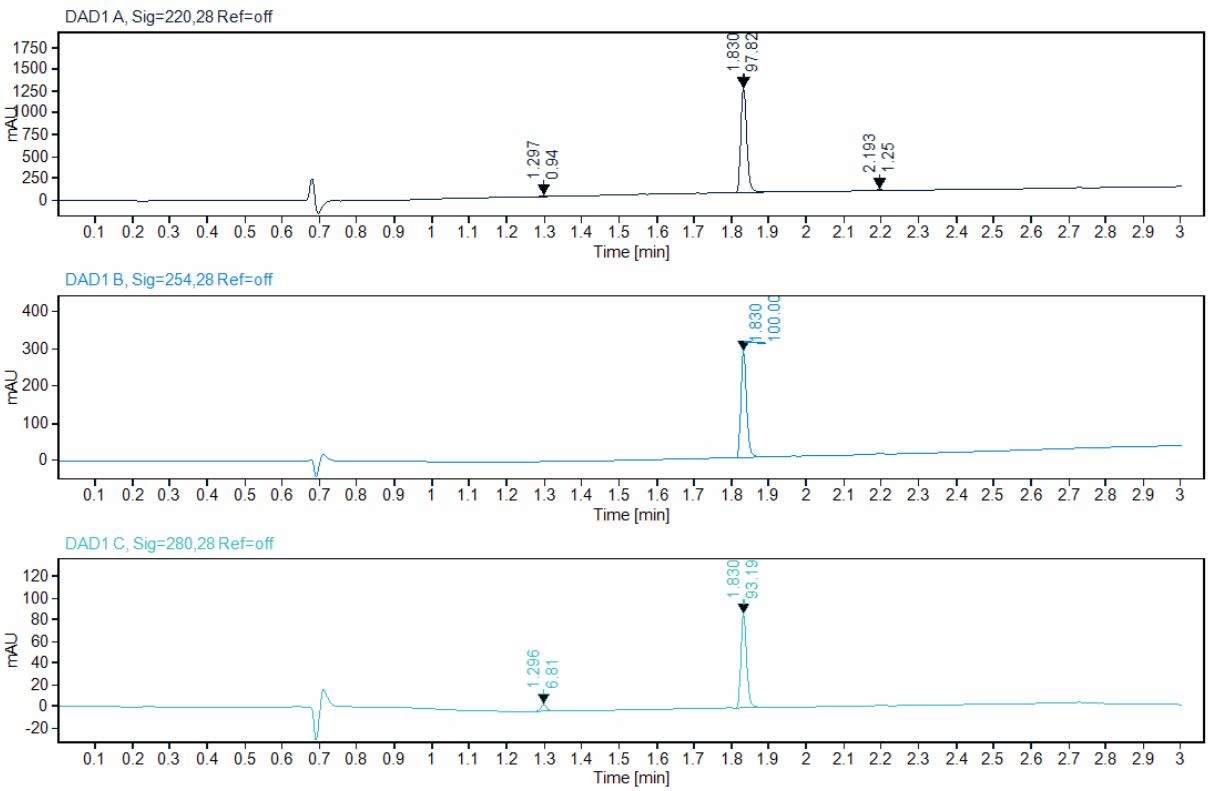
Compound **7f**



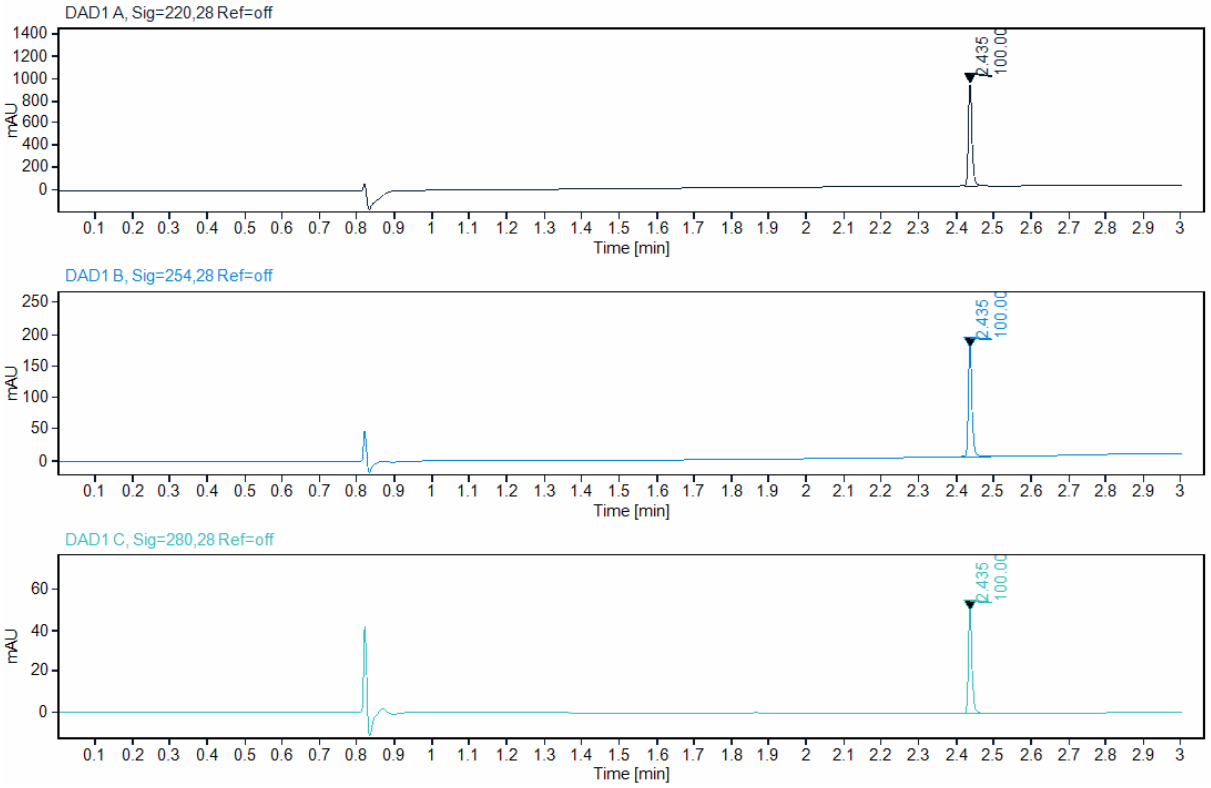
Compound **7g**



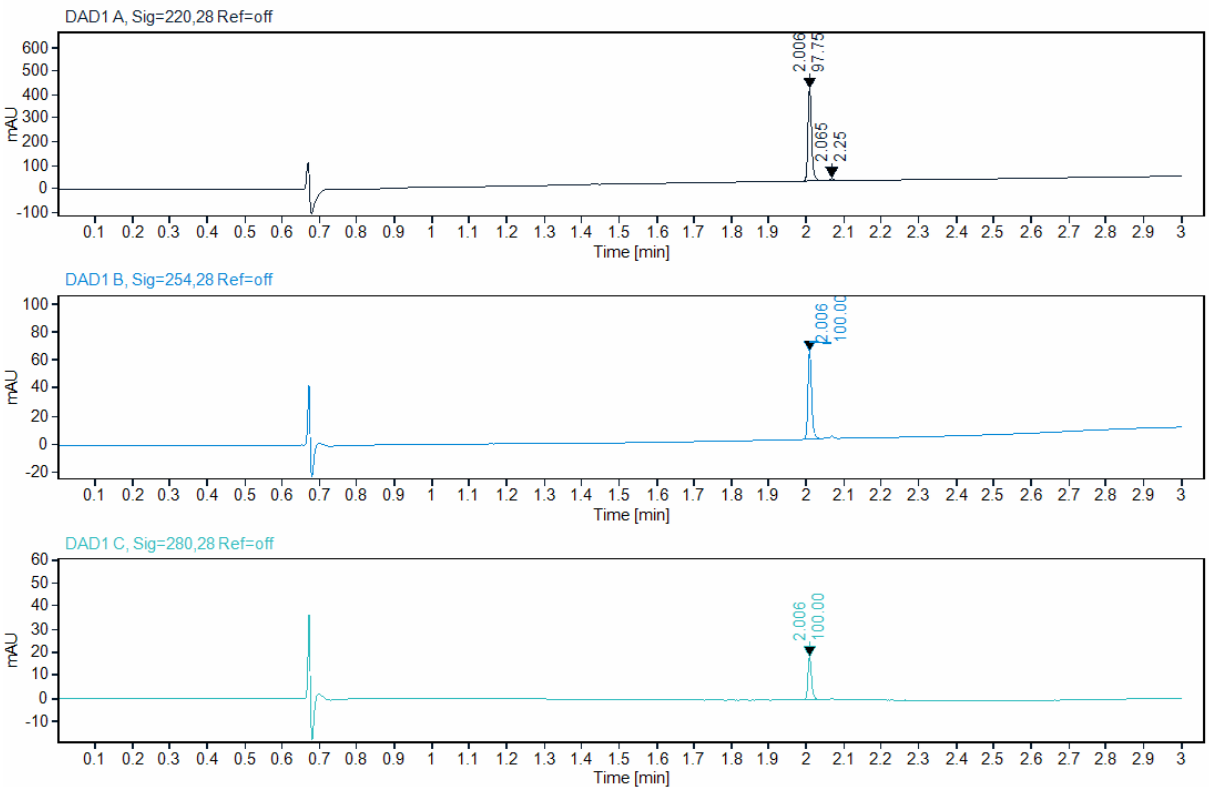
Compound **15**



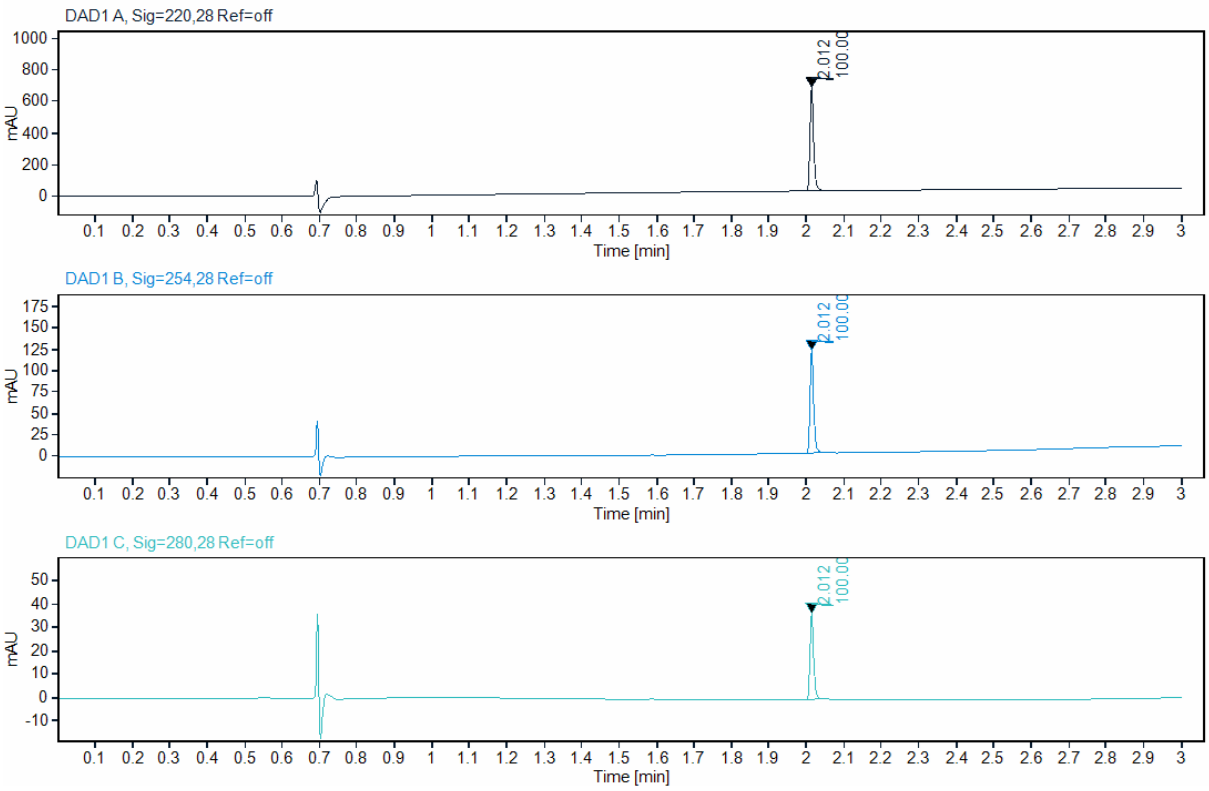
Compound **16**



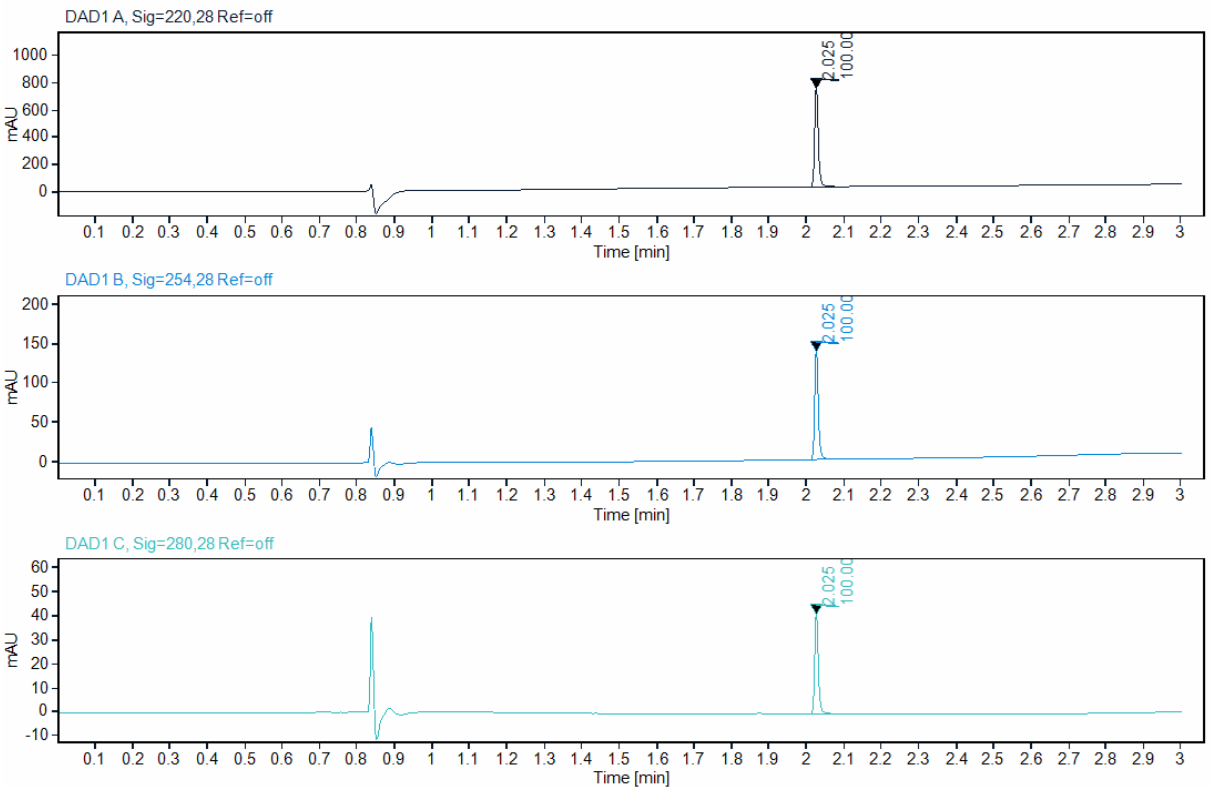
Compound **18a**



Compound **18b**



Compound **19**



4. Literature

References

(1) Pomplun, S.; Sippel, C.; Hähle, A.; Tay, D.; Shima, K.; Klages, A.; Ünal, C. M.; Rieß, B.; Toh, H. T.; Hansen, G.; Yoon, H. S.; Bracher, A.; Preiser, P.; Rupp, J.; Steinert, M.; Hausch, F. Chemogenomic Profiling of Human and Microbial FK506-Binding Proteins. *J. Med. Chem.* **2018,** *61,* 3660–3673.

(2) Kozany, C.; März, A.; Kress, C.; Hausch, F. Fluorescent probes to characterise FK506-binding proteins. *Chembiochem* **2009,** *10,* 1402–1410.