SUPPORTING INFORMATION

Structure-Based Design and Synthesis of Potent and Selective Matrix Metalloproteinase 13 Inhibitors

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Table of Contents	**Page Number**

I. Supplemental Figures and Tables

Table S1. X-ray data collection and refinement statistics

	MMP-13:(*S*)-**10a**	MMP-13:(*S*)-**17a***	MMP-13:(*R*)-**17a**[§]	MMP-13:**10d**
PDB code	5UWK	5UWL	5UWM	5UWN
Data collection				
Space group	$P2_12_12_1$	$P6_422$	$R3$	$I4_122$
Cell dimensions				
a, *b*, *c* (Å)	45.8, 57.2, 120.6	130.0, 130.0, 142.1	140.1, 140.1, 46.9	131.7, 131.7, 418.7
α, β, γ (°)	90, 90, 90	90, 90, 120	90, 90, 120 (hexagonal setting, *H*3)	90, 90, 90
Wavelength (Å)	0.97918	0.97918	0.97918	0.97918
Resolution (Å)	120.56-1.60 (1.69-1.60)[†]	60.07-2.55 (2.69-2.55)	32.77-1.62 (1.71-1.62)	125.66-3.20 (3.37-3.20)
R_{sym}	0.062 (0.768)	0.124 (0.967)	0.069 (0.646)	0.244 (1.030)
R_{pim}	0.035 (0.430)	0.047 (0.369)	0.046 (0.430)	0.120 (0.506)
Mean $I/\sigma I$	15.3 (1.9)	12.1 (2.1)	13.2 (2.0)	7.6 (1.9)
Completeness (%)	99.7 (100)	99.0 (99.1)	99.7 (99.9)	98.2 (99.4)
Redundancy	3.9 (4.0)	7.3 (7.4)	3.1 (3.1)	5.0 (5.0)
Wilson value (Å^2)	18.2	50.1	16.1	60.8
Refinement				
Resolution (Å)	51.65-1.60	60.07-2.55	32.77-1.62	95.80-3.20
No. reflections	42,432	23,266	43,436	30,163
No. monomers per asymmetric unit	2	2	2	5
R_{work}/ R_{free}	0.148/0.199	0.199/0.240	0.129/0.178	0.195/0.234
No. atoms				
Protein	2,556	2,548	2,580	6,588
Ligand/ion	80 [4 Zn^{2+}, 6 Ca^{2+}, 2 (*S*)-10a]	76 [3 Zn^{2+}, 5 Ca^{2+}, 2 (*S*)-17a]	80 [4 Zn^{2+}, 6 Ca^{2+}, 2 (*R*)-17a]	245 [10 Zn^{2+}, 10 Ca^{2+}, 5 10d]
Water	364	62	354	-
B-factors (Å^2)				
Protein	22.5	47.8	22.6	53.4
Ligand	20.6	39.5	20.3	61.2
Water	33.3	44.2	34.1	-
R.m.s deviations				
Bond lengths (Å)	0.008	0.010	0.010	0.011
Bond angles (°)	1.101	1.158	0.987	1.347
Ramachandran statistics - favored, allowed, outliers (%)	96.2, 3.2, 0.6	94.3, 5.7, 0.0	96.5, 3.5, 0.0	94.9, 5.1, 0.0

*Chain B has a significant deviation in residues 170-184 which disrupts Zn-binding. However, (*S*)-17a binds in the chain B active site despite this apparent crystal-packing artifact.

[§] An MMP-13 fragment (^{211}VTPLN^{215}) was observed bound at the active site Zn as a product complex. The fragment was likely carried along with intact MMP-13 during the purification and remained bound following addition of (*R*)-**17a**. Residues 213-215 align with an r.m.s.d. of 0.73-0.75 Å for backbone atoms when superimposed on similarly positioned peptides (residues 37-39 [PDB 4FU4] and 43-45 [PDB 4FVL,4G0D]) bound in the active site of MMP-13 crystal structures.[1] The peptide was not observed in the other crystal structures listed above and a modeling exercise suggests it will clash with crystal packing contacts in those structures.

[†]Highest resolution shell is shown in parentheses.

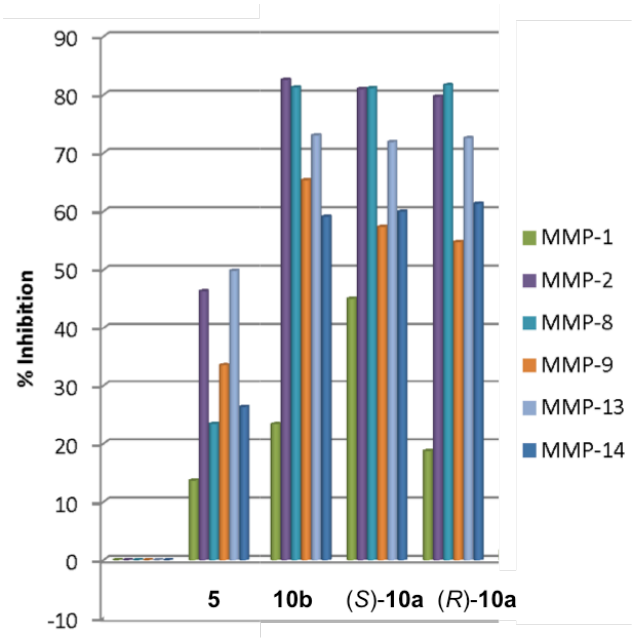


Figure S1. Results of compound selectivity against MMP isozyme panel. Compounds **5**, (*S*)-**10a**, (*R*)-**10a** and **10b** were tested at a single concentration of 20 μM. The inhibition of each isozyme was determined as a percent conversion of the substrate to product after 30 min of incubation.

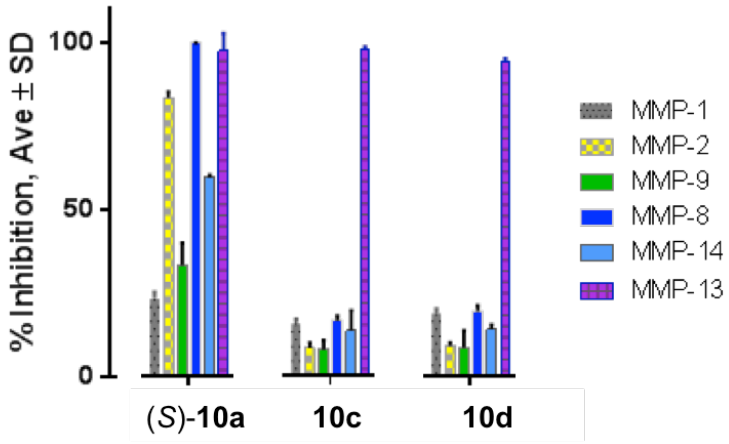


Figure S2. Selectivity of (*S*)-**10a**, **10c**, and **10d** against MMP isozyme panel. The compounds were tested at a single concentration of 200 nM.

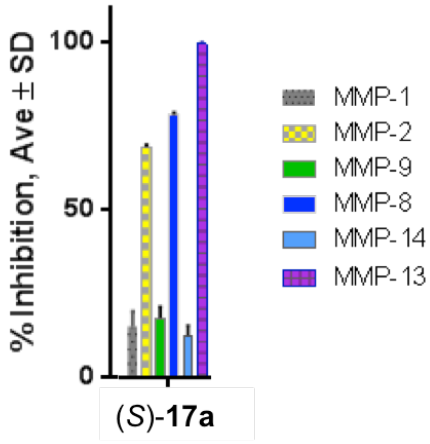


Figure S3. Promiscuity assay of (*S*)-**17a** against MMP isozymes. The inhibition of each isozyme was determined as a percent conversion of the substrate to product after 30 min of incubation. The compounds (*S*)-**17a** was tested against the MMP isozyme panel at a single concentration of 200 nM.

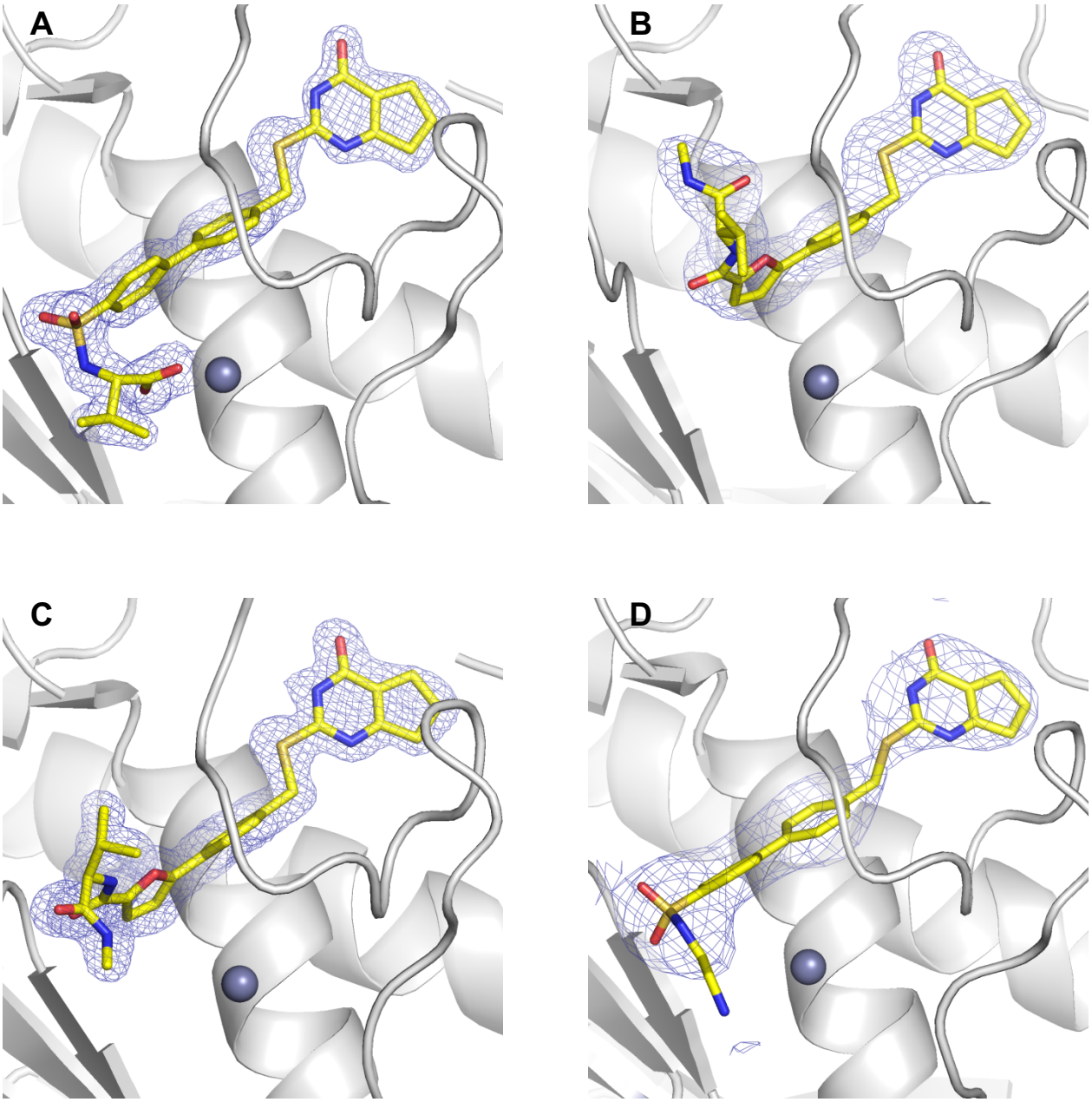


Figure S4. Representative electron density from crystal structures of MMP-13/inhibitor complexes of a) (*S*)-**10a**, b) (*S*)-**17a**, c) (*R*)-**17a** and d) **10d**. Composite omit maps with coefficients $2mF_o$-DF_c contoured at 1.0 **?** were calculated using PHENIX[5]. The density is contoured for the inhibitors only and the active site zinc position is shown as a blue sphere.

	$T_{1/2}$ in rat liver microsomes (min)*	Solubility (µM)
10d	163	24.2
(*S*)-17b	9.2	15.7

Table S2. In-vitro PK data for **10d** and (*S*)-**17b**. *As further animal studies are planned in rats the in-vitro half-life time was determined in rat liver microsomes.

II. Supplemental Methods

MMP-13 enzyme activation: Full-length recombinant human pro-MMP-13 (rhMMP-13) was purchased from R&D Systems (catalog no. 511-MM; Minneapolis, MN). MMP-13 was activated by incubating pro-MMP-13 diluted in 100 µL enzyme assay buffer (EAB; 50 mM Tris•HCl, pH 7.5, 100 mM NaCl, 10 mM $CaCl_2$, 0.05% Brij-35) with 1 mM (p-aminophenyl)mercuric acid (APMA) for 2 h at 37 °C.[2] The stock of active MMP-13 was diluted to 384.6 nM and stored at -80 °C.

Inhibitor kinetics: Inhibition experiments were conducted as described previously [3]. Briefly, fTHP-15, MMP-13, and inhibitor working solutions were prepared in EAB. All reactions were conducted in 384-well black polystyrene plates (Greiner, North Carolina, catalog no. 784076). To determine the IC_{50} of each inhibitor, the compounds were screened in 10- point 3-fold dilution dose-response curve format in triplicates.

The assay began by dispensing 5 µL of test compounds in assay buffer followed by 5 µL of MMP-13. The enzyme was allowed to incubate with the test compounds for 30 min at 25 °C. The assays were initiated by addition of 5 µL of fTHP-15 or Knight substrate and immediately placed in the microplate reader to record fluorescence.

To determine IC_{50} values of each compound, the relative fluorescence units (RFU) from wells containing MMP-13, fTHP-15, and inhibitors were plotted vs. no-enzyme and untreated controls. For each compound, RFUs from the linear part of the curve were fitted with a four parameter equation describing a sigmoidal dose-response curve with adjustable baseline using GraphPad Prism® version 11 suite of programs. The IC_{50} values of the compounds were determined as the concentrations that resulted in 50% enzyme activity when compared to the activity of the control samples (without a compound). These values were generated from fitted curves by solving for the X-intercept at the 50% inhibition level of Y-intercept using the built-in dose-response model algorithm of GraphPad Prism (LaJolla, CA). Hill slopes were also determined.

Determinations of inhibition constants and modalities were conducted by incubating a range of fTHP-15 substrate concentrations (2-25 µM) with 4 nM MMP-13 at room temperature in the presence of varying concentrations of inhibitors. Fluorescence was measured on a BioTek H1 microplate reader using $\lambda_{excitation}$ = 324 nm and $\lambda_{emission}$ = 393 nm. Rates of hydrolysis were obtained from plots of fluorescence versus time using data points from only the linear portion of the hydrolysis curve. All kinetic parameters were calculated using GraphPad Prism, version 5.01 (GraphPad Software, Inc., La Jolla, CA).

K_M values were determined by nonlinear regression analysis using the one-site hyperbolic binding model [4] and additionally evaluated by linear analysis. All K_i values were determined by nonlinear regression (hyperbolic equation) analysis using the mixed inhibition model, which allows for simultaneous determination of the mechanism of inhibition. The mechanism of inhibition was determined using the "α" parameter derived from a mixed-model inhibition by GraphPad Prism. The mechanism of inhibition was additionally confirmed by Lineweaver-Burke plots.

Selectivity assay: To determine the selectivity of each inhibitor, the compounds were tested against a selected protease panel consisting of MMP-1, MMP-2, MMP-8, MMP-9, and MMP-14. All enzymes were purchased from R&D Systems and activated according to manufacturer's instructions. Upon activation, each enzyme was diluted in EAB to 200 µM and stored at -80°C until further use. The compounds were screened as described above in 10- point 3-fold dilution dose-response curve format in triplicate utilizing fTHP-15 as substrate except for MMP-1, for which Knight substrate was used [2].

Type II collagen assay: To assess the potency of probes using a physiologically relevant substrate we tested compounds in an assay utilizing type II collagen (Sigma-Aldrich, St. Louis, MO, Cat# 234184). All experiments were performed in 384-well white microtiter plates. The assay was initiated by dispensing 9 μL of 333 nM type II collagen in EAB. 2 μL of test compounds in EAB were added. Reactions were initiated by addition of 9 μL of 4 nM MMP-13 in EAB. After 22 h of incubation at 37 °C, the samples were resolved by electrophoresis on a 8% SDS-PAGE gel. The gel was stained with Coomassie Blue and band intensities quantified vs. no-enzyme and untreated controls. For each compound, band intensity data were fitted with a four parameter equation describing a sigmoidal dose-response curve with adjustable baseline using GraphPad Prism® version 11 suite of programs. The IC_{50} values were generated from fitted curves by solving for X-intercept at the 50% inhibition level of Y-intercept.

Crystallization, structure determination and refinement. Protein was prepared as previously described.[3] Automated screening for crystallization was carried out using the sitting drop vapor-diffusion method with an Art Robbins Instruments Phoenix system in the *X-ray Crystallography Core Laboratory* at UTHSCSA. MMP-13 inhibitor complexes were prepared in a 1:5 molar ratio of protein to inhibitor prior to mixing 0.2 μL of protein complexes at 10 mg/mL with 0.2 μL of crystallization reagents from commercial screens. MMP13:(*S*)-**10a** crystals were obtained from Microlytic (Woburn, MA) MCSG-1 screen condition #17 (0.2 M magnesium chloride, 0.1 M Tris•HCl pH 8.5, 25% polyethylene glycol 3350) at 22 °C. MMP-13:(*S*)-**17a** crystals were obtained from Qiagen JCSG Core III screen condition #34 (0.2 M sodium chloride, 0.1 M Tris pH 7.0, 1.0 M sodium citrate) at 4 °C. MMP-13:(*R*)-**17a** crystals were obtained from Microlytic MCSG-4 screen condition #70 (0.2 M lithium sulfate 0.1 M Tris•HCl pH 8.5, 30% polyethylene glycol 4000) at 22 °C. MMP-13:**10d** crystals were obtained from Qiagen pHClear screen condition #58 (0.1 M HEPES, 1.6 M ammonium sulfate, pH 7.0) at 4 °C. All crystals were mounted in undersized nylon loops with excess mother liquor wicked off and flash-cooled in liquid nitrogen prior to data collection. The structures were determined by the molecular replacement method implemented in PHASER[5] using PDB entry 4L19 as the search model. Coordinates for all models were refined using PHENIX[6], including simulated annealing with torsion angle dynamics, and alternated with manual rebuilding using COOT[7]. Non-crystallographic symmetry restraints were used in the refinement of the MMP-13:**10d** complex. Data were collected at the Advanced Photon Source NE-CAT beamline 24-ID-E and integrated and scaled using XDS[8]. Data collection and refinement statistics are shown in Table 1. Figures were generated using PyMOL (http://www.pymol.org)[9].

ACCESSION CODES

Coordinates and structure factors have been deposited in the Protein Data Bank[10] under accession codes 5UWK, 5UWL, 5UWM and 5UWN.

Molecular modeling.

The X-ray crystallographic structures of MMP-13•**5** (PDB ID: 4L19), MMP-13•**6** (1ZTQ), and MMP13•**11** (1I7I) were refined with Protein Preparation Wizard implemented in Maestro 9.3. Briefly, the protein structure was imported into workspace and preprocessed to assign bond orders, add hydrogen atoms, create zero-order bonds to metals, create disulfide bonds, and to delete water molecules beyond 5 Å from hetero groups. In addition, missing atoms in residues were added using Prime to generate a complete protein structure, and only chain A was used for further refinement. The protein structure was refined via automated H-bond assignment and restrained minimization with OPLS 2005 force field by converging heavy atoms to 0.5 Å RMSD. The refined 3D structures of MMP-13 bound to each inhibitor were aligned and superimposed to analyze and compare binding poses of inhibitors.

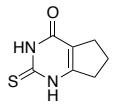
Receptor grids for Glide dock were generated from the refined structures. The grid was set to a 25 $Å^3$ box centered on the each bound ligand in MMP-13. Van der Waals radius was scaled by decreasing the default value of scaling factor to 0.8 to soften the potential for nonpolar parts of the receptor.

Ligand structures were sketched from structure **5** and prepared with LigPrep in Schrödinger program suite.

Ligand structures of designed inhibitors were docked into the active site (receptor grid) of MMP-13 by using Glide5.5 in standard precisions (SP) modes without any constraint. The binding poses of inhibitors in the MMP-13 active site were subsequently analyzed.

II. Procedures for the Synthesis of Inhibitors and Spectroscopic Analyses

General experimental details: All non-aqueous reactions were carried out under a positive pressure of argon using oven-dried (140 °C) or flame-dried glassware (under vacuum). Dichloromethane, diethyl ether, *N,N*-dimethylformamide, toluene and tetrahydrofuran were dried by being passed through a column of desiccant (activated A-1 alumina). Triethylamine was distilled from calcium hydride under an argon atmosphere prior to use. All other commercially available reagents were used without further purification. Reactions were either monitored by thin layer chromatography or analytical LC-MS. Thin layer chromatography was performed on Kieselgel 60 F254 glass plates pre-coated with a 0.25 mm thickness of silica gel. TLC plates were visualized with UV light and/or by staining with Hanessian solution [H_2SO_4 (conc., 22 mL), phosphormolybdic acid (20 g), $Ce(SO_4)_2$ (0.5 g), 378 mL H_2O)].
Column chromatography was performed on a Biotage Isolera automated flash system. Compound was loaded onto pre-filled cartridges filled with KP-Sil 50 µm irregular silica.
NMR spectra were recorded on a 400 MHz spectrometer and measured in $CDCl_3$, MeOD or DMSO ($CHCl_3$: ^1H, δ = 7.26, ^{13}C, δ = 77.16, MeOH: ^1H, δ = 3.31, ^{13}C, δ = 49.00, DMSO: ^1H, δ = 2.50, ^{13}C, δ = 39.50). All ^1H and ^{13}C shifts are given in ppm and coupling constants *J* are given in Hz.
High-resolution mass spectra were recorded on a spectrometer (ESI) at the University of Illinois Urbana-Champaign Mass Spectrometry Laboratory.



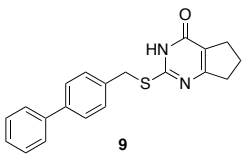
8

DBU (11.5 mL, 77.33 mmol, 1.1 eq) was added to a suspension of methyl-2-cyclopentanone-1-carboxylate (10 g, 70.3 mmol, 1.0 eq) and thiourea (8.03 g, 105.45 mmol, 1.5 eq) in 70 mL CH_3CN and the mixture was stirred at 80 °C for 16 h. The reaction mixture was cooled to 0 °C while a white solid precipitated. The solid product was filtered, washed with 2M HCl (2x 30 mL) and water (2x 30 mL) and was dried under vacuum to give **8** (7.54 g, 64%) as a white powder.

^1H NMR (400 MHz, DMSO) δ = 12.59 (s, 1H), 12.21 (s, 1H), 2.76 – 2.63 (m, 2H), 2.56 – 2.44 (m, 2H), 2.05 – 1.87 (m, 2H).

^{13}C NMR (101 MHz, DMSO) δ 175.54, 159.51, 156.45, 115.54, 30.98, 26.60, 20.78.

MS (ESI) for $C_7H_8N_2OS$ $[M+H]^+$ 169.10.



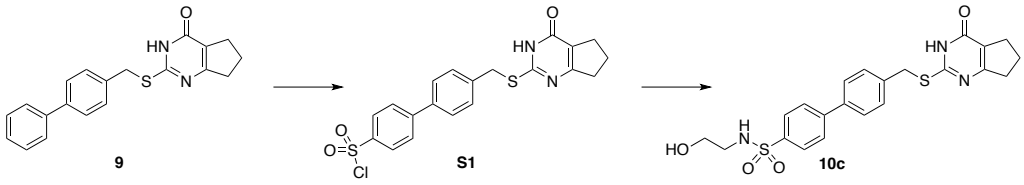
9

A suspension of **8** (1.22 g, 7.28 mmol, 1.2 eq) and triethylamine (1.0 mL, 7.28 mmol, 1.2 eq) in 12 mL DMF was stirred for 20 min at room temperature before 4-bromomethylbiphenyl (1.5 g, 6.07 mmol, 1.0 eq) was added and the reaction mixture was stirred for 16 h at room temperature. The solids were filtered, washed with small amounts of water, methanol and diethyl ether and the product was dried under vacuum to give **9** (1.9 g, 94%) as a white solid.

^{1}H NMR (400 MHz, DMSO) δ = 12.56 (bs, 1H), 7.71 – 7.56 (m, 4H), 7.54 – 7.41 (m, 4H), 7.40 – 7.31 (m, 1H), 4.43 (s, 2H), 2.85 – 2.73 (m, 2H), 2.63 – 2.56 (m, 2H), 2.03 – 1.90 (m, 2H).

^{13}C NMR (101 MHz, DMSO) δ 139.71, 139.14, 136.46, 129.65, 128.90, 127.43, 126.73, 126.58, 34.28, 33.23, 26.71, 20.56.

MS (ESI) for $C_{20}H_{18}N_2OS$ $[M+H]^+$ 335.11.



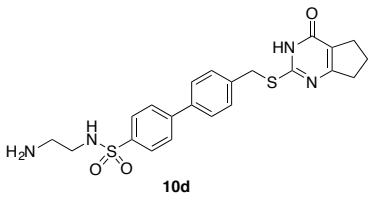
Chlorosulfonic acid (3M in DCM, 4 mL, 11.96 mmol, 20.0 eq) was added to **9** (200 mg, 0.598 mmol, 1.0 eq) at 0 °C, the cooling bath was removed and the deep blue solution was stirred for 16 hours at room temperature. The reaction was quenched by pouring it onto a mixture of ice-water (ca. 20 mL) and ethyl acetate (ca. 10 mL), further 10 mL of THF were added to dissolve the white solids formed during the quench. The phases were separated and the organic layer was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure to provide the crude sulfonyl chloride (**S1**, 200 mg, 77%) as a white solid.
The sulfonyl chloride (**S1**, 106 mg, 0.245 mmol, 1.0 eq) was dissolved in 2.2 mL THF and 2-ethanolamine (22 μL, 0.368 mmol, 1.5 eq) followed by tritehylamine (68 μL, 0.49 mmol, 2.0 eq) were added at room temperature. The reaction mixture was stirred for 16 h, the solvent was removed under reduced pressure and the product was purified by flash chromatography (0-10% methanol linear gradient in DCM) to provide **10c** (30 mg, 27%) as a white solid.

^{1}H NMR (400 MHz, DMSO) δ = 12.51 (bs, 1H), 7.87 (s, 4H), 7.74 – 7.63 (m, 3H), 7.54 (d, *J*=8.3 Hz, 2H), 4.80 – 4.64 (m, 1H), 4.45 (s, 2H), 3.45 – 3.30 (m, 4H), 2.81 – 2.74 (m, 2H), 2.59 (t, *J*=7.4 Hz, 2H), 2.02 – 1.91 (m, 2H).

^{13}C NMR (101 MHz, DMSO) δ 168.74, 160.69, 159.53, 143.36, 139.33, 137.75, 137.48, 129.82, 127.23, 127.15, 127.11, 119.41, 59.92, 45.11, 40.13, 39.92, 39.71, 39.50, 39.29, 39.08, 38.88, 34.28, 33.17, 26.72, 20.57.

IR (thin film) ν 3374, 2923, 1655, 1316, 1158, 1050 cm^{-1}.

HRMS (ESI) calcd. for $C_{22}H_{23}N_3O_4S_2$ $[M+H]^+$ 458.1130; found 458.1215.



Chlorosulfonic acid (3M in DCM, 4 mL, 11.96 mmol, 20.0 eq) was added to **9** (200 mg, 0.598 mmol, 1.0 eq) at 0 °C, the cooling bath was removed and the deep blue solution was stirred for 16 h at room temperature. The reaction was quenched by pouring it onto a mixture of ice-water (ca.

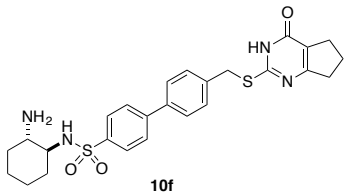
20 mL) and ethyl acetate (ca. 10 mL), further 10 mL of THF were added to completely dissolve all the solids. The phases were separated and the organic layer was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure to provide the crude sulfonyl chloride (**S1**, 200 mg, 77%) as a white solid.
The sulfonyl chloride (**S1**, 106 mg, 0.245 mmol, 1.0 eq) was dissolved in 2.2 mL THF and mono-Boc-protected ethylenediamine (59 mg, 0.368 mmol, 1.5 eq) followed by tritehylamine (68 μL, 0.49 mmol, 2.0 eq) were added at room temperature. The reaction mixture was stirred for 16 h, the solvent was removed under reduced pressure and the product was purified by flash chromatography (0-10% methanol linear gradient in DCM) to provide Boc-protected **10d** (41 mg, 30%) as a white solid. ^{1}H NMR (400 MHz, DMSO) δ = 12.58 (s, 1H), 7.93 – 7.81 (m, 4H), 7.76 – 7.67 (m, 3H), 7.58 – 7.50 (m, 2H), 6.81 (t, *J*=5.8, 1H), 4.45 (s, 2H), 3.03 – 2.93 (m, 2H), 2.83 – 2.75 (m, 4H), 2.64 – 2.57 (m, 2H), 2.03 – 1.92 (m, 2H), 1.34 (s, 9H).
Boc-protected **10d** (41 mg, 0.074 mmol) was suspended in 1 mL HCl in dioxane (4M). The reaction mixture was stirred for 90 min at room temperature and concentrated under reduced pressure. The crude product was triturated with diethyl ether (1x1.5 mL) followed by diethyl ether/methanol=20/1 (2x1.5 mL) and dried under vacuum to give **10d** (29 mg, 85%) as a slightly yellow solid.

^{1}H NMR (400 MHz, DMSO) δ = 8.34 – 8.12 (m, 4H), 7.89 (s, 4H), 7.69 (d, *J*=8.3, 2H), 7.54 (d, *J*=8.3, 2H), 4.45 (s, 2H), 3.08 – 2.99 (m, 2H), 2.91 – 2.82 (m, 2H), 2.81 – 2.74 (m, 2H), 2.58 (t, *J*=7.4, 2H), 2.01 – 1.89 (m, 2H).

^{13}C NMR (101 MHz, DMSO) δ 168.50, 161.15, 160.21, 143.76, 138.48, 137.88, 137.44, 129.92, 127.44, 127.40, 127.21, 119.41, 40.08, 38.57, 34.13, 33.25, 26.75, 20.67.

IR (thin film) ν 3376, 1645, 1566, 1046, 990 cm^{-1}.

HRMS (ESI) calcd. for $C_{22}H_{24}N_4O_3S_2$ $[M+H]^+$ 457.1290; found 457.1363.



10f

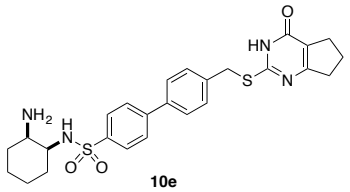
The sulfonyl chloride (**S1**) was synthesized as described above for **10c**.
The sulfonyl chloride (131 mg, 0.303 mmol, 1.0 eq) was dissolved in 2.75 mL THF and (±)-1,2-trans-cyclohexanediamine (52 mg, 0.455 mmol, 1.5 eq) followed by triethylamine (84 μL, 0.606 mmol, 2.0 eq) were added at room temperature. The reaction mixture was stirred for 16 h, the precipitate was filtered and washed with small amounts of THF and diethyl ether. The crude product was further purified by preparative HPLC (linear gradient 10-100% acetonitrile/MeOH = 1:1, 0.1% TFA, 10 min). Lyophilization gave 26 mg (28%) of **10f** as a slightly yellow powder.

^{1}H NMR (400 MHz, DMSO) δ = 12.57 (bs, 1H), 8.03 (d, *J*=8.8 Hz, 1H), 7.97 – 7.86 (m, 6H), 7.75 – 7.70 (m, 2H), 7.58 – 7.52 (m, 2H), 4.45 (s, 2H), 3.06 – 2.94 (m, 1H), 2.85 – 2.71 (m, 3H), 2.67 – 2.55 (m, 2H), 2.03 – 1.92 (m, 3H), 1.63 – 1.43 (m, 2H), 1.41 – 1.26 (m, 1H), 1.25 – 1.05 (m, 3H), 1.05 – 0.86 (m, 1H).

^{13}C NMR (101 MHz, DMSO) δ 158.26, 157.95, 143.58, 140.31, 137.99, 137.22, 129.89, 127.34, 127.14, 118.71, 54.81, 53.43, 34.27, 33.18, 30.46, 29.09, 26.75, 23.87, 22.97, 20.60.

IR (thin film) ν 2933, 2861, 1651, 1531, 1427, 1315, 1152, 1056 cm^{-1}.

HRMS (ESI) calcd. for $C_{26}H_{30}N_4O_3S_2$ $[M+H]^+$ 511.1759; found 511.1818.

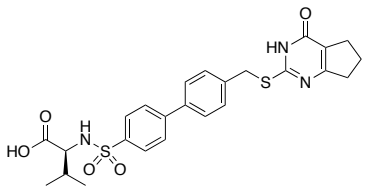


10e

Compound **10e** was prepared as described for **10c** using (±)-1,2-cis-cyclohexanediamine as the coupling partner for the sulfonyl chloride **S1**. **10e** (23 mg) was isolated in 24% yield.

^{1}H NMR (400 MHz, DMSO) δ = 12.57 (bs, 1H), 7.97 – 7.89 (m, 5H), 7.84 – 7.77 (m, 2H), 7.72 (d, *J*=8.4 Hz, 2H), 7.55 (d, *J*=8.4 Hz, 2H), 4.45 (s, 2H), 3.45 – 3.39 (m, 1H), 3.21 – 3.10 (m, 1H), 2.82 – 2.73 (m, 2H), 2.59 (t, *J*=7.3 Hz, 2H), 1.96 (p, *J*=7.3 Hz, 2H), 1.68 – 1.47 (m, 4H), 1.33 – 1.08 (m, 4H).

HRMS (ESI) calcd. for $C_{26}H_{30}N_4O_3S_2$ $[M+H]^+$ 511.1759; found 511.1823.



(S)-**10a**

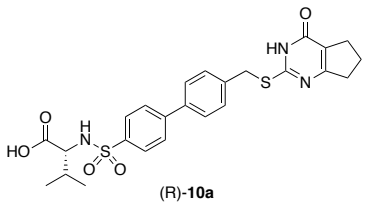
The sulfonyl chloride (**S1**) was synthesized as described above for **10c**.
A solution of L-valine methyl ester•HCl (150 mg, 0.897 mmol, 1.5 eq) in 0.9 mL THF and 0.9 mL water was added to the sulfonyl chloride (**S1**, 259 mg, 0.598 mmol, 1.0 eq) and triethylamine (249 μL, 1.794 mmol, 3.0 eq) was added at room temperature. The reaction mixture was stirred for 24 h before it was diluted with ethyl acetate (10 mL) and water (10 mL). The phases were separated and the aqueous phase was extracted with ethyl acetate (3x 10 mL). The combined organic extracts were dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude methyl ester was purified by flash chromatography (0-10% methanol linear gradient in DCM). The product was further purified by trituration with diethyl ether (1x 1.5 mL) followed by diethyl ether/methanol=20/1 (2x 1.5 mL) and dried under vacuum to give the corresponding valine methyl ester (67 mg, 21%) as a slightly yellow solid. ^{1}H NMR (400 MHz, DMSO) δ = 12.40 (s, 1H), 8.31 (d, *J*=9.4 Hz, 1H), 7.89 – 7.82 (m, 2H), 7.82 – 7.76 (m, 2H), 7.72 – 7.64 (m, 2H), 7.57 – 7.50 (m, 2H), 4.44 (s, 2H), 3.56 (dd, *J*=9.3, 7.1 Hz, 1H), 3.32 (s, 3H), 2.83 – 2.72 (m, 2H), 2.59 (t, *J*=7.4 Hz, 2H), 1.96 (p, *J*=7.4 Hz, 2H), 0.83 (d, *J*=6.7 Hz, 3H), 0.79 (d, *J*=6.7 Hz, 3H). MS (ESI) for $C_{26}H_{29}N_3O_5S_2$ $[M+H]^+$ 528.20.
Lithium hydroxide (7 mg, 0.29 mmol, 3.0 eq) was added to a solution of the methyl ester in 2 mL of a 1:1 mixture of THF and water. The reaction mixture was heated to 60 °C and stirred for 16 h before it was acidified with 1M HCl (pH~3). Filtration of the precipitated product gave (S)-**10a** (29 mg) in 58% yield.

^{1}H NMR (400 MHz, DMSO) δ = 12.57 (s, 2H), 8.07 (d, *J*=9.3 Hz, 1H), 7.83 (s, 4H), 7.69 (d, *J*=8.2 Hz, 2H), 7.53 (d, *J*=8.2 Hz, 2H), 4.44 (s, 2H), 3.55 (dd, *J*=9.3, 5.9 Hz, 1H), 2.78 (t, *J*=7.7 Hz, 2H), 2.59 (t, *J*=7.7 Hz, 2H), 2.06-1.86 (m, 3H), 0.83 (d, *J*=6.7 Hz, 3H), 0.80 (d, *J*=6.7 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 172.18, 168.77, 160.77, 159.92, 143.10, 139.96, 137.73, 137.38, 129.82, 127.19, 127.04, 126.84, 119.52, 61.26, 40.13, 34.28, 33.15, 30.40, 26.71, 20.57, 19.02, 17.86.

IR (thin film) ν 2965, 1647, 1548, 1192, 1166, 1096 cm^{-1}.

HRMS (ESI) calcd. for $C_{25}H_{27}N_3O_5S_2$ $[M+H]^+$ 514.1392; found 514.1476.



(R)-**10a**

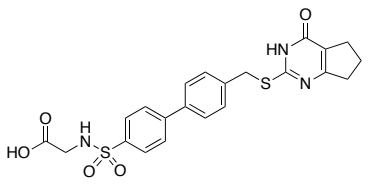
Compound (R)-**10a** was prepared as described for (S)-**10a** using D-valine methyl ester•HCl as the coupling partner for the sulfonyl chloride (**S1**). (R)-**10a** (44 mg) was isolated in 62% yield.

^{1}H NMR (400 MHz, DMSO) δ = 12.57 (s, 2H), 8.07 (d, *J*=9.3 Hz, 1H), 7.83 (s, 4H), 7.69 (d, *J*=8.2 Hz, 2H), 7.53 (d, *J*=8.2 Hz, 2H), 4.44 (s, 2H), 3.55 (dd, *J*=9.3, 5.9 Hz, 1H), 2.78 (t, *J*=7.7 Hz, 2H), 2.59 (t, *J*=7.7 Hz, 2H), 2.06-1.86 (m, 3H), 0.83 (d, *J*=6.7 Hz, 3H), 0.80 (d, *J*=6.7 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 172.18, 168.77, 160.77, 159.98, 143.10, 139.96, 137.73, 137.38, 129.82, 127.19, 127.04, 126.84, 119.52, 61.26, 40.13, 34.28, 33.15, 30.40, 26.71, 20.57, 19.02, 17.86.

IR (thin film) ν 2965, 1647, 1548, 1192, 1166, 1096 cm^{-1}.

HRMS (ESI) calcd. for $C_{25}H_{27}N_3O_5S_2$ $[M+H]^+$ 514.1392; found 514.1465.



10b

Compound **10b** was prepared as described for (S)-**10a** using glycine-tertbutyl-ester (150 mg, 0.897 mmol) as the coupling partner for the sulfonyl chloride (**S1**; yield (tert-butyl ester): 70 mg, 45%).
Ester hydrolysis: The tert-butyl ester (65 mg, 0.123 mmol, 1.0 eq) was dissolved in 2 mL of a 1:1 mixture of CH_2Cl_2 and trifluoroacetic acid. The reaction mixture was stirred for 2 h, concentrated under reduced pressure and the crude product was purified by trituration with diethyl ether (1x

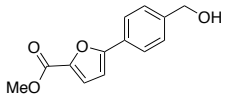
1.5 mL) followed by diethyl ether/methanol=20/1 (2x1.5 mL) and dried under vacuum to give **10b** (26 mg, 45%) as a slightly yellow solid.

^{1}H NMR (400 MHz, DMSO) δ = 12.61 (s, 2H), 8.09 (t, *J*=6.1 Hz, 1H), 7.85 (s, 4H), 7.69 (d, *J*=8.3 Hz, 1H), 7.54 (d, *J*=8.3 Hz, 1H), 4.44 (s, 2H), 3.62 (d, *J*=5.4 Hz, 2H), 2.78 (t, *J*=7.7 Hz, 2H), 2.59 (t, *J*=7.4 Hz, 2H), 2.04-1.88 (2H).

^{13}C NMR (101 MHz, DMSO) δ 170.24, 143.38, 139.46, 137.75, 137.44, 129.81, 127.14, 127.11, 127.09, 43.80, 34.27, 33.15, 26.71, 20.56.

IR (thin film) ν 2939, 1650, 1548, 1192, 1159, 1096 cm^{-1}.

HRMS (ESI) calcd. for $C_{22}H_{21}N_3O_5S_2$ $[M+H]^+$ 472.0923; found 472.1000.



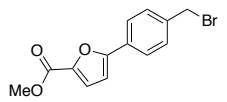
S2

To a solution of G (1.0 g, 4.88 mmol, 1.0 eq) in 150 mL dioxane was added $Pd(PPh_3)_4$ (276 mg, 0.239 mmol, 0.05 eq) and the resulting yellow solution was stirred for 15 min at room temperature. 4-Hydroxymethylbenzeneboronic acid (741 mg, 4.88 mmol, 1.0 eq) dissolved in 45 mL water followed by K_2CO_3 (810 mg, 5.86 mmol, 1.2 eq) were added and the reaction mixture was stirred at 60 °C for 16 h. After cooling to room temperature most of the dioxane was removed under reduced pressure. The residue was diluted with water (ca. 50 mL) and the product was extracted with ethyl acetate (3x 50 mL). The combined organic extracts were dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (6-37% ethyl acetate gradient in hexanes) providing **S2** (987 mg, 87%) as a slightly yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.75 (d, *J*=8.1 Hz, 2H), 7.39 (d, *J*=8.1 Hz, 2H), 7.23 (d, *J*=3.6 Hz, 1H), 6.72 (d, *J*=3.6 Hz, 1H), 4.71 (s, 2H), 3.90 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 159.40, 157.51, 143.56, 141.86, 128.82, 127.39, 125.10, 120.25, 106.97, 64.94, 52.03.

MS (ESI) for $C_{13}H_{12}O_4$ $[M+H]^+$ 233.09.



15a

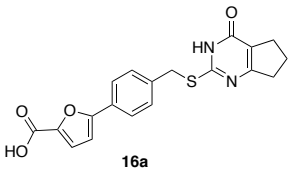
To a stirring solution of benzylic alcohol **S2** (980 mg, 4.22 mmol, 1.0 eq) and CBr_4 (1.82 g, 5.49 mmol, 1.3 eq) in 14 mL methylene chloride was added PPh_3 (1.44 g, 5.49 mmol, 1.3 eq) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C, quenched with water and the product was

extracted with methylene chloride (3x 15 mL). The combined organic extracts were dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (4-34% ethyl acetate gradient in hexanes) giving **15a** (1.14 g, 91%) as a slightly yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.73 (d, *J*=8.3 Hz, 1H), 7.42 (d, *J*=8.3 Hz, 2H), 7.23 (d, *J*=3.6 Hz, 1H), 6.74 (d, *J*=3.4 Hz, 1H), 4.49 (s, 2H), 3.91 (s, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 159.20, 156.92, 143.87, 138.51, 129.65, 129.54, 125.26, 120.11, 107.49, 52.01, 33.08.

MS (ESI) for $C_{13}H_{11}BrO_3$ $[M+H]^+$ 295.05.



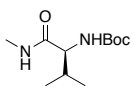
16a

A suspension of **8** (476 mg, 2.83 mmol, 1.0 eq) and triethylamine (470 μL, 3.39 mmol, 1.2 eq) in 7 mL DMF was stirred for 20 min at room temperature before **15a** (1 g, 3.39 mmol, 1.2 eq) was added and the reaction mixture was stirred for 16 h at room temperature. The solids were filtered, washed with small amounts of water, methanol and diethyl ether and the product was dried under vacuum to give the corresponding methyl ester (1.16 g, 89%) as a white solid.^{1}H NMR (400 MHz, DMSO) δ = 12.55 (bs, 1H), 7.76 (d, *J*=8.4, 2H), 7.52 (d, *J*=8.4, 2H), 7.41 (d, *J*=3.7, 1H), 7.15 (d, *J*=3.7, 1H), 4.42 (s, 2H), 3.83 (s, 3H), 2.84 – 2.73 (m, 2H), 2.64 – 2.54 (m, 2H), 2.07 – 1.87 (m, 2H). MS (ESI) for $C_{20}H_{18}N_2O_4S$ $[M+H]^+$, 383.10.
An aqueous solution of NaOH (1M, 4.96 mL, 4.96 mmol, 3.2 eq) was added to a suspension of the methyl ester from above (594 mg, 1.55 mmol, 1.0 eq) in 16 mL of a 2:1 mixture of THF and methanol and the reaction mixture was heated to 60 °C for 2 h. After cooling to room temperature the mixture was diluted with water (ca. 2 mL) and acidified with 1 M HCl (pH~2, ca. 5 mL). The precipitated product was filtered and washed with water providing **16a** (554 mg, 97%) as a white solid.

^{1}H NMR (400 MHz, DMSO) δ = 13.07 (bs, 1H), 12.59 (bs, 1H), 7.75 (d, *J*=8.4 Hz, 2H), 7.52 (d, *J*=8.4 Hz, 2H), 7.31 (d, *J*=3.6 Hz, 1H), 7.12 (d, *J*=3.6 Hz, 1H), 4.42 (s, 2H), 2.84 – 2.73 (m, 2H), 2.63 – 2.55 (m, 2H), 2.03 – 1.89 (m, 2H).

^{13}C NMR (101 MHz, DMSO) δ 168.76, 160.72, 159.89, 159.27, 156.05, 144.13, 138.30, 129.84, 128.14, 124.46, 119.90, 119.44, 107.96, 34.28, 33.30, 26.74, 20.58.

MS (ESI) for $C_{19}H_{16}N_2O_4S$ $[M+H]^+$ 368.17.



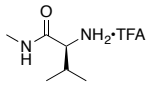
S3

To a solution of Boc-potected L-valine (3.0 g, 13.8 mmol, 1.0 eq), EDCI-HCl (3.17 g, 16.56 mmol, 1.2 eq), and DMAP (100 mg, 0.82 mmol, 0.05 eq) in 138 mL methylene chloride was

added methylamine (2 M in THF, 8.25 mL, 16.56 mmol, 1.2 eq). The reaction mixture was stirred for 18 h at room temperature before it was transferred in a separatory funnel and washed with 1 M HCl (2x 100 mL), aqueous saturated solution of $NaHCO_3$ (2x 100 mL) and brine (1x 100 mL). The organic extract was dried over Na_2SO_4 and concentrated under reduced pressure to provide **S3** (2.8 g, 88%) as a yellow oil, which was used for the next step without any further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.05 (s, 1H), 5.06 (bd, *J*=9.0 Hz, 1H), 3.87 (dd, *J*=9.0 Hz, 6.3, 1H), 2.82 (d, *J*=4.9 Hz, 3H), 2.18 – 2.04 (m, 1H), 1.44 (s, 9H), 0.94 (d, *J*=7.0 Hz, 3H), 0.91 (d, *J*=7.0 Hz, 3H).

These spectral characteristics are identical to those previously reported.[11]

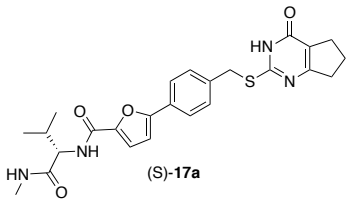


S4

Compound **S3** (1.0 g, 4.34 mmol, 1.0 eq) was dissolved in a 1:1 mixture of CH_2Cl_2 and trifluoroacetic acid (44 mL) and stirred for 90 min. The reaction mixture was concentrated under reduced pressure, the remaining yellow oil was re-dissolved in chloroform (ca. 10 mL) and the solvent was removed again in *vacuo*. This last step was repeated three times to eliminate all traces of trifluoroacetic acid and **S4** (TFA-salt, 1.02 g) was isolated in 99% yield. The NMR of the crude material showed clean product, which was used without any further purification for the next step.

^{1}H NMR (400 MHz, MeOD) δ = 3.60 (d, *J*=6.1 Hz, 1H), 2.82 (s, 3H), 2.23 – 2.10 (m, 1H), 1.05 (d, *J*=6.9 Hz, 6H).

These spectral characteristics were identical to those previously reported.[11]

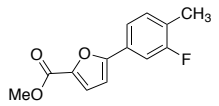


(S)-**17a**

To a solution of **16a** (460 mg, 1.25 mmol, 1.0 eq), **S4** (366 mg, 1.50 mmol, 1.2 eq) and HOBt (186 mg, 1.375 mmol, 1.1 eq) was added NEt_3 (487 µL, 2.75 mmol, 2.2 eq). After stirring for 5 min at room temperature EDCI-HCl (264 mg, 1.38 mmol, 1.1 eq) was added and the clear solution was stirred for 6 h. The reaction mixture was diluted with ethyl acetate and washed with 0.1 M HCl (2x 20 mL), sat. $NaHCO_3$ (1x 20 mL) and brine (1x 20 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (0-10% methanol linear gradient in DCM) providing (S)-**17a** (467 mg, 79%) as a white solid.

^{1}H NMR (400 MHz, DMSO) δ = 12.55 (s, 1H), 8.27 (d, *J*=8.9 Hz, 1H), 8.12 – 8.05 (m, 1H), 7.86 (d, *J*=8.4 Hz, 2H), 7.51 (d, *J*=8.4 Hz, 2H), 7.28 (d, *J*=3.6 Hz, 1H), 7.07 (d, *J*=3.6 Hz, 1H), 4.42 (s, 2H), 4.21 (t, *J*=8.7 Hz, 1H), 2.85 – 2.72 (m, 2H), 2.64 – 2.54 (m, 5H), 2.19 – 2.05 (m, 1H), 2.03 – 1.87 (m, 2H), 0.89 (t, *J*=6.9 Hz, 6H).

HRMS (ESI) calcd. for $C_{25}H_{28}N_4O_4S$ $[M+H]^+$ 481.1831; found 481.1902.



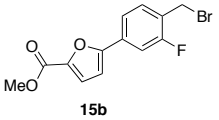
S5

To a solution of methyl-2-bromo-5-furanocarboxylate (1.11 g, 5.42 mmol, 1.0 eq) in 22 mL toluene were added 3-fluoro-4-methylbenzeneboronic acid (1.0 g, 6.50 mmol, 1.2 eq) in 1.9 mL methanol followed by $Pd(PPh_3)_4$ (220 mg, 0.19 mmol, 0.035 eq) and K_2CO_3 (2 M in water, 3.34 mL, 6.67 mmol, 1.23 eq) at room temperature. The reaction mixture was heated to 80 °C for 16 h before it was diluted with water and the product was extracted with ethyl acetate (3x 15 mL). The combined organic extracts were dried over Na_2SO_4, the solvent was removed under reduced pressure and the product was purified by flash chromatography (4-34% EtOAc linear gradient in hexanes) to give **S5** (1.06 g, 83%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.48 – 7.38 (m, 2H), 7.25 – 7.18 (m, 2H), 6.69 (d, *J*=3.6 Hz, 1H), 3.91 (s, 3H), 2.29 (d, *J*=2.0 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 161.58 (d, *J*=244.9 Hz), 159.23, 156.61 (d, *J*=3.0 Hz), 143.71, 132.01 (d, *J*=5.5 Hz), 129.07 (d, *J*=8.5 Hz), 125.96 (d, *J*=17.5 Hz), 120.37 (d, *J*=3.3 Hz), 120.12, 111.52 (d, *J*=24.7 Hz), 107.15, 52.02, 14.66 (d, *J*=3.5 Hz).

MS (ESI) for $C_{13}H_{11}FO_3$ $[M+H]^+$ 235.09.



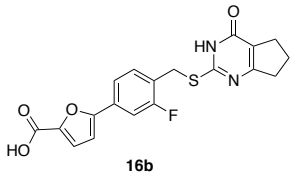
15b

Azobisisobutyronitrile (35 mg, 0.213 mmol, 0.1 eq) and N-bromosuccinimide (416 mg, 2.34 mmol, 1.1 eq) were added to a solution of **S5** (500 mg, 2.13 mmol, 1.0 eq) in 23 mL CCl_4 and the reaction mixture was stirred for 12 h at 96 °C. The yellow suspension was filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (4-34% EtOAc linear gradient in hexanes) providing **15b** (425 mg, 64%) as a slightly yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.52 (dd, *J*=8.0, 1.7 Hz, 1H), 7.46 (dd, *J*=10.6, 1.7 Hz, 1H), 7.42 (t, *J*=8.0 Hz, 1H), 7.23 (d, *J*=3.6 Hz, 1H), 6.76 (d, *J*=3.6 Hz, 1H), 4.51 (d, *J*=1.1 Hz, 2H), 3.91 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ = 160.94 (d, *J*=250.4 Hz), 159.07, 155.60 (d, *J*=2.9 Hz), 144.31, 131.89 (d, *J*=8.1 Hz) 131.87 (d, *J*=3.7 Hz), 125.73 (d, *J*=14.9 Hz), 120.87 (d, *J*=3.5 Hz), 120.01, 112.13 (d, *J*=24.0 Hz), 108.37, 52.12, 25.38 (d, *J*=4.3 Hz).

MS (ESI) for $C_{13}H_{10}BrFO_3$ $[M+H]^+$ 312.93.



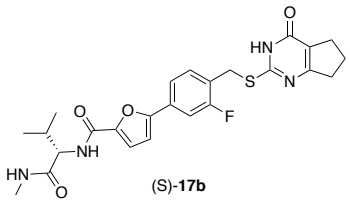
16b

A suspension of **8** (1.33 g, 7.91 mmol, 1.2 eq) and triethylamine (1.32 mL, 9.49 mmol, 1.2 eq) in 15 mL DMF was stirred for 15 min at room temperature before **15b** (2.96 g, 9.49 mmol, 1.0 eq) was added and the reaction mixture was stirred for 16 h at room temperature. The solids were filtered, washed with small amounts of water, methanol and diethyl ether, and the product was dried under vacuum to give the corresponding methyl ester (2.98 g, 94%) as a white solid. ^{1}H NMR (400 MHz, DMSO) δ = 12.56 (bs, 1H), 7.69 – 7.56 (m, 3H), 7.42 (d, *J*=3.7, 1H), 7.25 (d, *J*=3.7, 1H), 4.42 (s, 2H), 3.83 (s, 3H), 2.81-2.71 (m, 2H), 2.62-2.54 (m, 2H), 2.01-1.89 (m, 2H). MS (ESI) for $C_{20}H_{17}FN_2O_4S$ $[M+H]^+$ 401.05.
An aqueous 1 M solution of sodium hydroxide (4.12 mL, 4.12 mmol, 3.2 eq) was added to the methyl ester (515 mg, 1.29 mmol, 1.0 eq) in 13 mL of a 2:1 mixture of THF and methanol and the reaction mixture was heated to 60 °C for 2 h. After cooling down to room temperature the mixture was diluted with 3 mL water and acidified with 1 M HCl (pH~2, ca. 4.5 mL). The precipitated product was filtered and washed with water providing **16b** (490 mg, 98%) as a white solid.

^{1}H NMR (400 MHz, DMSO) δ = 12.83 (s, 2H), 7.67 – 7.55 (m, 3H), 7.32 (d, *J*=3.6 Hz, 1H), 7.21 (d, *J*=3.6 Hz, 1H), 4.42 (s, 2H), 2.83 – 2.71 (m, 2H), 2.61 – 2.53 (m, 2H), 2.03 – 1.87 (m, 2H).

^{13}C NMR (101 MHz, DMSO) δ = 168.70, 160.84, 160.72 (d, *J*=246.4 Hz), 159.16, 154.63 (d, *J*=2.9 Hz), 144.55, 132.31 (d, *J*=4.3 Hz), 130.45 (d, *J*=8.9 Hz), 124.86 (d, *J*=14.8 Hz), 120.20 (d, *J*=3.3 Hz), 119.83, 119.41, 111.15 (d, *J*=24.0 Hz), 109.18, 34.18, 27.13, 26.70, 20.56.

MS (ESI) for $C_{19}H_{15}FN_2O_4S$ $[M+H]^+$ 387.15.



(S)-**17b**

To a solution of **16b** (60 mg, 0.155 mmol, 1.0 eq), EDCI•HCl (45 mg, 0.233 mmol, 1.5 eq), HOBt (31 mg, 0.233 mmol, 1.5 eq) and DIPEA (40 µL, 0.233 mmol, 1.5 eq) in 1 mL DMF was added **S4** (76 mg, 0.31 mmol, 2.0 eq). The reaction mixture was stirred for 4 h at room temperature before it was diluted with ethyl acetate (ca. 5 mL) and washed with 0.1M HCl (2x10 mL). The aqueous phase was extracted with ethyl acetate (3x 10 mL) and the combined organic extracts were washed with an aqueous saturated solution of $NaHCO_3$ (1x 10 mL) and brine (1x 10 mL). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced

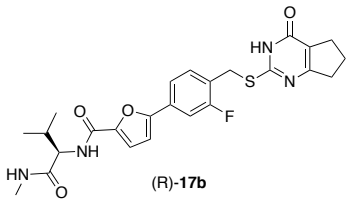
pressure. The crude product was purified by flash chromatography (0-10% methanol linear gradient in DCM) and preparative HPLC (linear gradient 10-100% acetonitrile/MeOH = 1:1, 0.1% TFA, 10 min) providing (S)-**17b** (61 mg, 79%) as a white solid.

^{1}H NMR (400 MHz, DMSO) δ = 12.59 (bs, 1H), 8.40 (d, *J*=8.9 Hz, 1H), 8.06 (q, *J*=4.5 Hz, 1H), 7.86 (dd, *J*=11.2, 1.7 Hz, 1H), 7.71 (dd, *J*=8.0 Hz, 1.7, 1H), 7.60 (t, *J*=8.0 Hz, 1H), 7.26 (d, *J*=3.6 Hz, 1H), 7.18 (d, *J*=3.6 Hz, 1H), 4.43 (s, 2H), 4.20 (t, *J*=8.8 Hz, 1H), 2.84 – 2.71 (m, 2H), 2.67 – 2.54 (m, 5H), 2.21 – 2.07 (m, 1H), 2.03 – 1.89 (m, 2H), 0.90 (d, *J*=6.7 Hz, 3H), 0.88 (d, *J*=6.7 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ = 171.24, 168.54, 160.80 (d, *J*=245.9 Hz), 160.62, 157.31, 153.09 (d, *J*=2.9 Hz), 147.12, 132.05, 130.78 (d, *J*=9.0 Hz), 124.31 (d, *J*=14.6 Hz), 120.30, 116.23, 111.18 (d, *J*=24.0 Hz), 108.92, 58.45, 34.24, 29.90, 27.16, 26.70, 25.42, 20.57, 19.35, 19.04.

IR (thin film) ν 3295, 2958, 1737, 1668, 1519, 1315, 1184 cm^{-1}.

HRMS (ESI) calcd. for $C_{25}H_{27}FN_4O_4S$ $[M+H]^+$ 499.1737; found 499.1805.



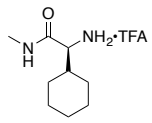
(R)-**17b**

Compound (R)-**17b** was synthesized following the same procedure as described for (S)-**17b** using (R)-**S4** as the coupling partner for **16b**. Yield: 79%

^{1}H NMR (400 MHz, DMSO) δ = 12.59 (bs, 1H), 8.40 (d, *J*=8.9 Hz, 1H), 8.06 (q, *J*=4.5 Hz, 1H), 7.86 (dd, *J*=11.2, 1.7 Hz, 1H), 7.71 (dd, *J*=8.0 Hz, 1.7, 1H), 7.60 (t, *J*=8.0 Hz, 1H), 7.26 (d, *J*=3.6 Hz, 1H), 7.18 (d, *J*=3.6 Hz, 1H), 4.43 (s, 2H), 4.20 (t, *J*=8.8 Hz, 1H), 2.84 – 2.71 (m, 2H), 2.67 – 2.54 (m, 5H), 2.21 – 2.07 (m, 1H), 2.03 – 1.89 (m, 2H), 0.90 (d, *J*=6.7 Hz, 3H), 0.88 (d, *J*=6.7 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ = 171.24, 168.54, 160.80 (d, *J*=245.9 Hz), 160.62, 157.31, 153.09 (d, *J*=2.9 Hz), 147.12, 132.05, 130.78 (d, *J*=9.0 Hz), 124.31 (d, *J*=14.6 Hz), 120.30, 116.23, 111.18 (d, *J*=24.0 Hz), 108.92, 58.45, 34.24, 29.90, 27.16, 26.70, 25.42, 20.57, 19.35, 19.04.

HRMS (ESI) calcd. for $C_{25}H_{27}FN_4O_4S$ $[M+H]^+$ 499.1737; found 499.1809.



S6

To a suspension of L-cyclohexylglycine (1 g, 6.36 mmol, 1.0 eq) in 10.5 mL water and 5 mL THF were added di-tert-butyl dicarbonate (2.08 g, 9.54 mmol, 1.5 eq) and Na_2CO_3 (1.35 g, 12.72

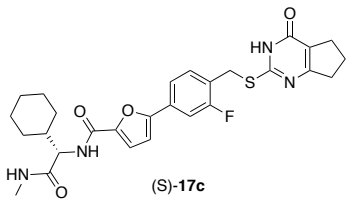
mmol, 2.0 eq) at room temperature. Further 420 mg (0.3 eq) of di-tert-butyl dicarbonate was added after 12 h as the reaction was not complete (TLC: n-BuOH/conc. AcOH/water = 4/1/1, R_f (product) = 0.35, ninhydrin staining). The reaction mixture was stirred for another 12 h at room temperature before it was quenched by the addition of 2 M HCl (pH~2). After stirring for another 30 min to hydrolyze unreacted di-tert-butyl dicarbonate the product was extracted with ethyl acetate (3x 20 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure providing Boc-potected L-cyclohexylglycine (1.6 g, 98%) as a yellow oil. The crude product was used for the next step without any further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.99 (d, *J*=9.0 Hz, 1H), 4.23 (dd, *J*=9.0, 5.0 Hz, 1H), 1.88 – 1.57 (m, 6H), 1.45 (s, 9H), 1.23 – 1.01 (m, 4H).

To a solution of Boc-potected L-cyclohexylglycine (1.6 g, 6.22 mmol, 1.0 eq), EDCI•HCl (1.43 g, 7.46 mmol, 1.2 eq), and DMAP (100 mg, 0.82 mmol, 0.13 eq) in 63 mL methylene chloride was added methylamine (2M in THF, 3.73 mL, 7.46 mmol, 1.2 eq). The reaction mixture was stirred for 18 h at room temperature before it was transferred in a separatory funnel and washed with 1 M HCl (2x 30 mL), aqueous saturated solution of $NaHCO_3$ (2x 30 mL), and brine (1x 30 mL). The organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to provide *tert*-butyl-(*S*)-(1-cyclohexyl-2-(methylamino)-2-oxoethyl)carbamate (1.32 g, 77%) as a yellow oil, which was used for the next step without any further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.02 (bs, 1H), 5.06 (d, *J*=8.6 Hz, 1H), 3.85 (dd, *J*=8.6, 6.6 Hz, 1H), 2.81 (d, *J*=4.9 Hz, 3H), 1.79 – 1.61 (m, 6H), 1.30 – 0.89 (m, 4H).

Compound *tert*-butyl-(*S*)-(1-cyclohexyl-2-(methylamino)-2-oxoethyl)carbamate (500 mg, 1.85 mmol, 1.0 eq) was dissolved in a 3:1 mixture of methylene chloride and trifluoroacetic acid (20 mL) and stirred for 45 min. The reaction mixture was concentrated under reduced pressure, the remaining yellow oil was re-dissolved in chloroform (ca. 10 mL), and the solvent was again removed in *vacuo*. This last step was repeated three times to eliminate all traces of trifluoroacetic acid and **S6** (TFA salt, 498 mg) was isolated in 99% yield. The NMR of the crude material showed clean product, which was used without any further purification for the next step.

^{1}H NMR (400 MHz, MeOD) δ = 3.55 (d, *J*=6.4, 1H), 2.86 – 2.80 (m, 4H), 1.90 – 1.66 (m, 6H), 1.36 – 1.06 (m, 4H).

MS (ESI) for $C_9H_{18}N_2O$ $[M+H]^+$ 171.14.



(S)-**17c**

16b (70 mg, 0.18 mmol, 1.0 eq) was suspended in 2.2 mL THF and pentafluorophenyl trifluoroacetate (34 µL, 0.198 mmol, 1.1 eq) followed by triethylamine (75 µL, 0.54 mmol, 3.0 eq) were added ad room temperature. After stirring for 2 h **S6** (66 mg, 0.234 mmol, 1.3 eq) was added and the reaction mixture was stirred for 18 h at room temperature. Dilution with ethyl acetate (5 mL) and THF (5 mL) was followed by the addition of water (10 mL). The phases were separated and the product was extracted with ethyl acetate (3x 15 mL). The combined organic extracts were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude

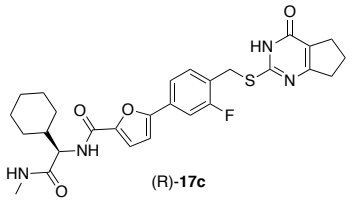
product was purified by preparative HPLC (linear gradient 10-100% acetonitrile/MeOH = 1:1, 0.1% TFA, 10 min). Lyophilization gave 60 mg (63%) of (S)-**17c** as a white powder.

^{1}H NMR (400 MHz, DMSO) δ = 12.58 (bs, 1H), 8.38 (d, *J*=8.9 Hz, 1H), 8.08 (q, *J*=4.5 Hz, 1H), 7.87 (dd, *J*=11.2, 1.7 Hz, 1H), 7.71 (dd, *J*=8.0, 1.7 Hz, 1H), 7.60 (t, *J*=8.0 Hz, 1H), 7.25 (d, *J*=3.6 Hz, 1H), 7.17 (d, *J*=3.6 Hz, 1H), 4.43 (s, 2H), 4.25 (t, *J*=8.9 Hz, 1H), 2.84 – 2.74 (m, 2H), 2.66 – 2.55 (m, 5H), 2.03 – 1.91 (m, 2H), 1.86 – 1.50 (m, 6H), 1.27 – 0.88 (m, 5H).

^{13}C NMR (176 MHz, DMSO) δ = 171.10, 168.50, 160.84, 160.80 (d, *J*=245.8 Hz), 157.26, 153.09 (d, *J*=2.7 Hz), 147.13, 132.03 (d, *J*=4.2 Hz), 130.79 (d, *J*=8.9 Hz), 124.30 (d, *J*=14.8 Hz), 120.31 (d, *J*=3.2 Hz), 119.58, 116.20, 111.19 (d, *J*=24.1 Hz), 108.90, 57.50, 38.98, 34.20, 29.36, 28.95, 27.18, 26.70, 25.84, 25.46, 25.39, 20.58.

IR (thin film) ν 3289, 2921, 1736, 1668, 1517, 1185, 1167 cm^{-1}.

HRMS (ESI) calcd. for $C_{28}H_{31}FN_4O_4S$ $[M+H]^+$ 539.2040; found 539.2109.



(R)-**17c**

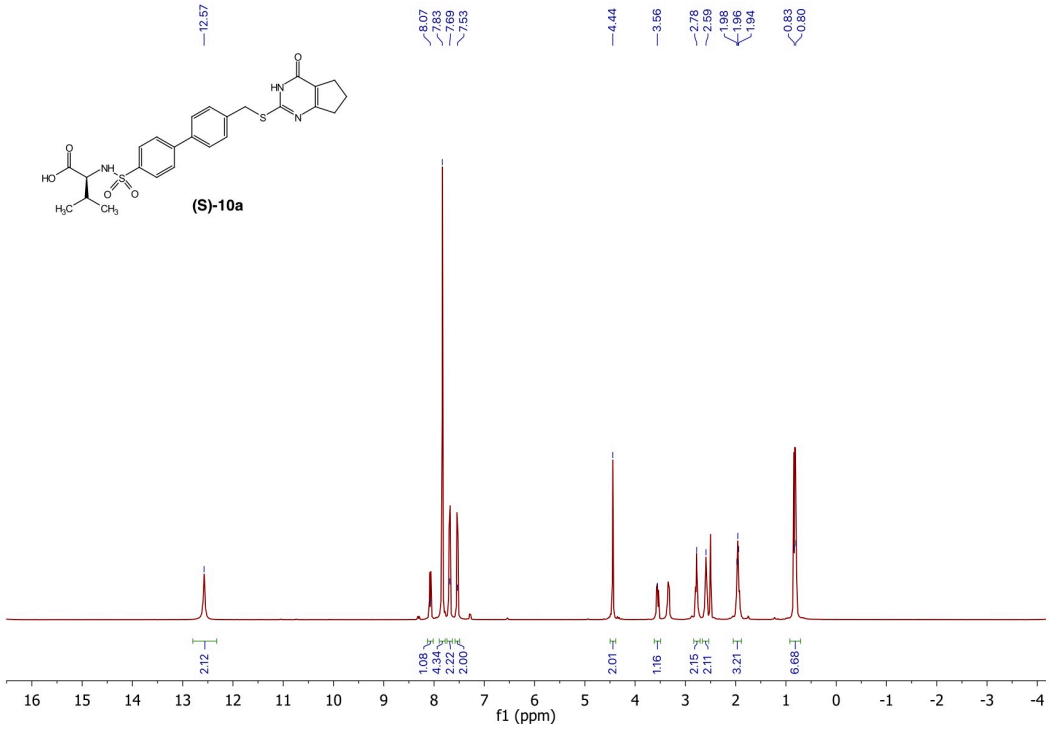
Compound (R)-**17c** was synthesized following the same procedure as described for (S)-**17c** using (R)-**S6** as the coupling partner for **16b**. Yield: 79%

^{1}H NMR (400 MHz, DMSO) δ = 12.58 (bs, 1H), 8.38 (d, *J*=8.9 Hz, 1H), 8.08 (q, *J*=4.5 Hz, 1H), 7.87 (dd, *J*=11.2, 1.7 Hz, 1H), 7.71 (dd, *J*=8.0, 1.7 Hz, 1H), 7.60 (t, *J*=8.0 Hz, 1H), 7.25 (d, *J*=3.6 Hz, 1H), 7.17 (d, *J*=3.6 Hz, 1H), 4.43 (s, 2H), 4.25 (t, *J*=8.9 Hz, 1H), 2.84 – 2.74 (m, 2H), 2.66 – 2.55 (m, 5H), 2.03 – 1.91 (m, 2H), 1.86 – 1.50 (m, 6H), 1.27 – 0.88 (m, 5H).

^{13}C NMR (176 MHz, DMSO) δ = 171.10, 168.50, 160.84, 160.80 (d, *J*=245.8 Hz), 157.26, 153.09 (d, *J*=2.7 Hz), 147.13, 132.03 (d, *J*=4.2 Hz), 130.79 (d, *J*=8.9 Hz), 124.30 (d, *J*=14.8 Hz), 120.31 (d, *J*=3.2 Hz), 119.58, 116.20, 111.19 (d, *J*=24.1 Hz), 108.90, 57.50, 38.98, 34.20, 29.36, 28.95, 27.18, 26.70, 25.84, 25.46, 25.39, 20.58.

IR (thin film) ν 3289, 2921, 1736, 1668, 1517, 1185, 1167 cm^{-1}.

HRMS (ESI) calcd. for $C_{28}H_{31}FN_4O_4S$ $[M+H]^+$ 539.2040; found 539.2124.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0.80
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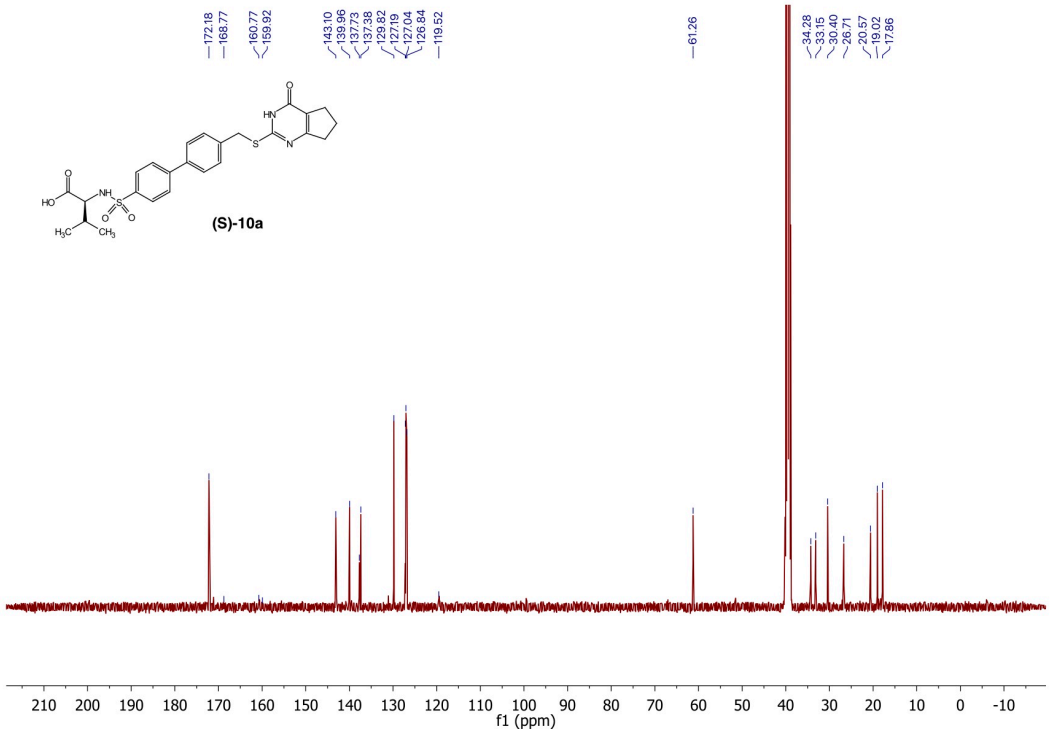
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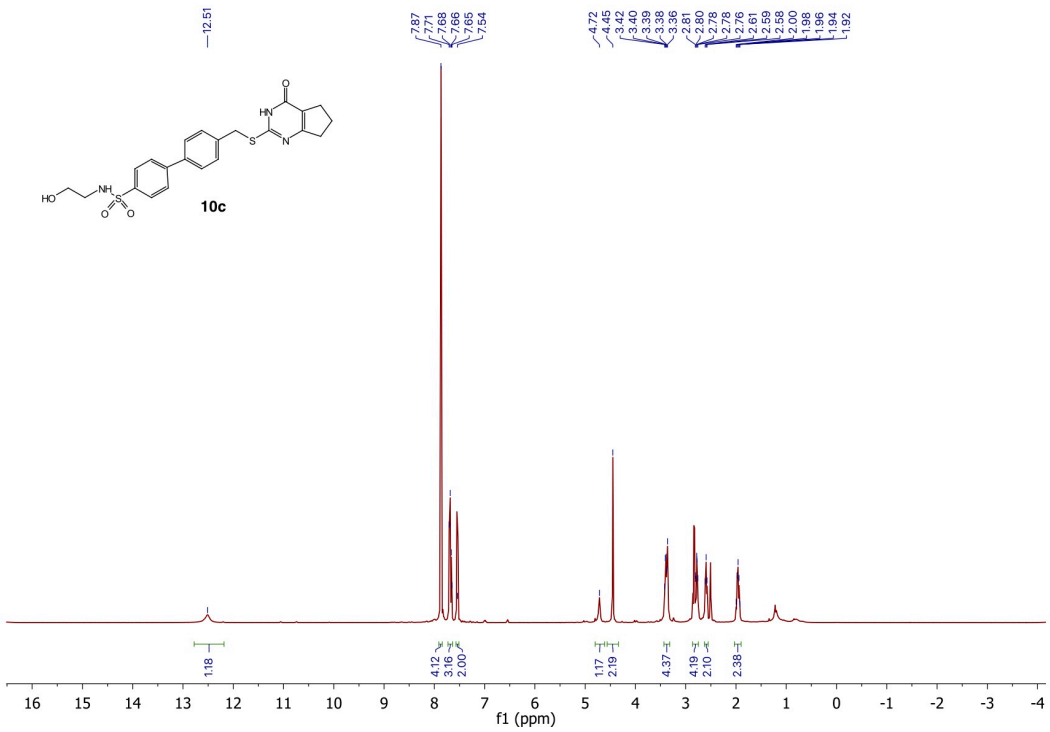
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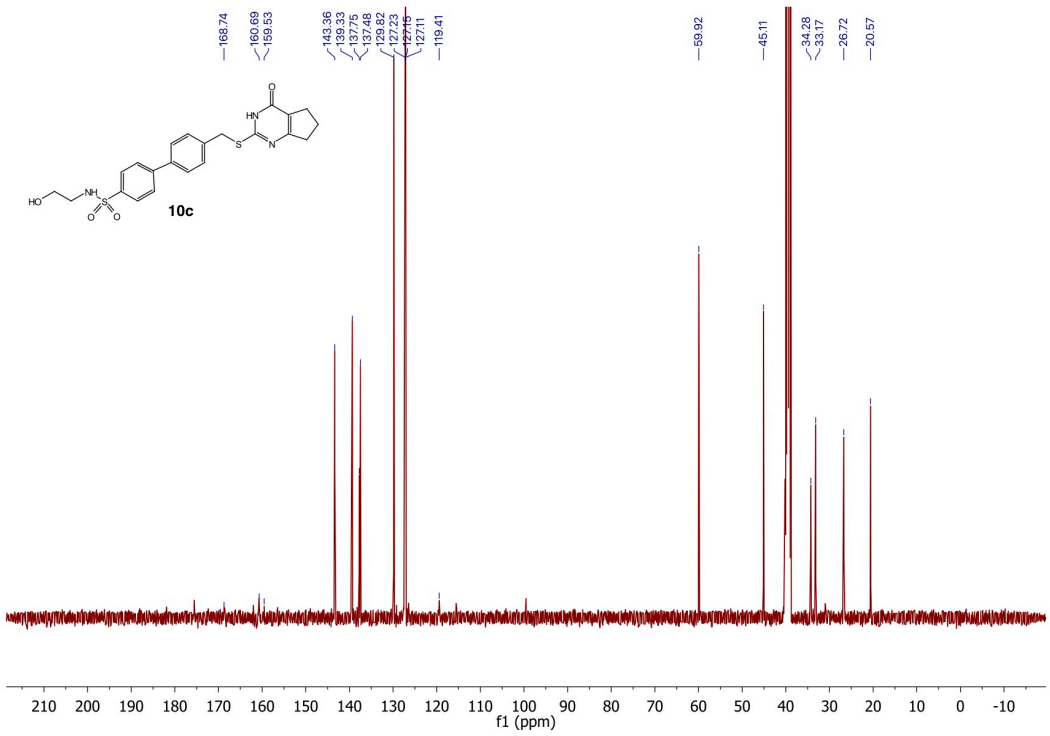
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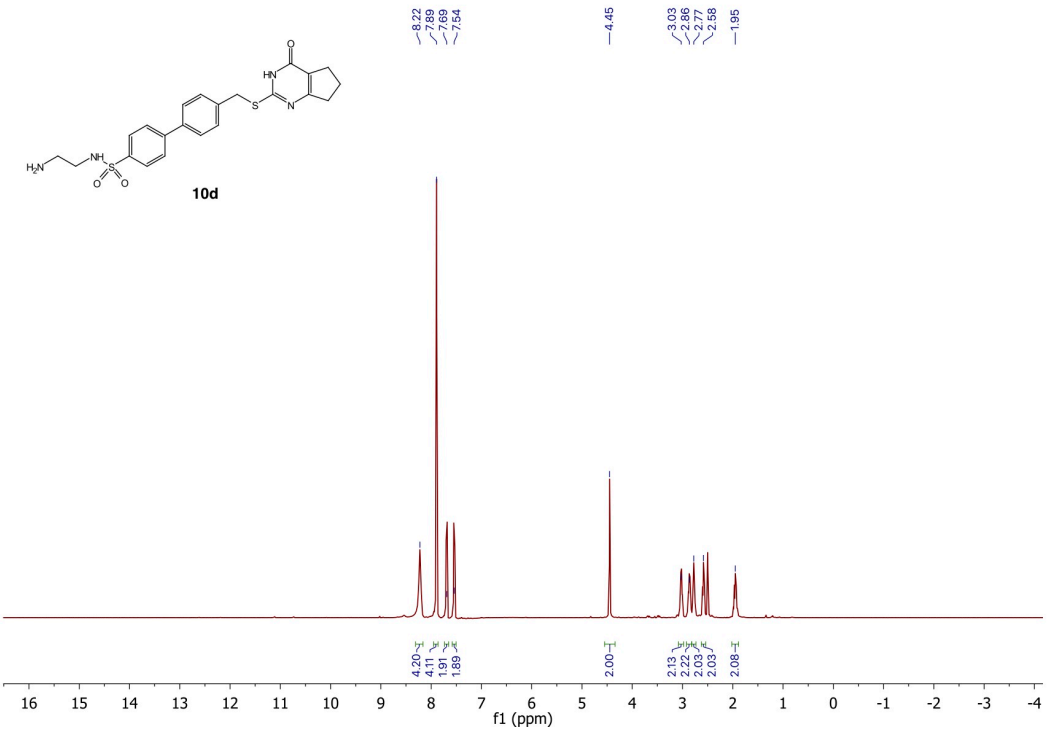
f1 (ppm)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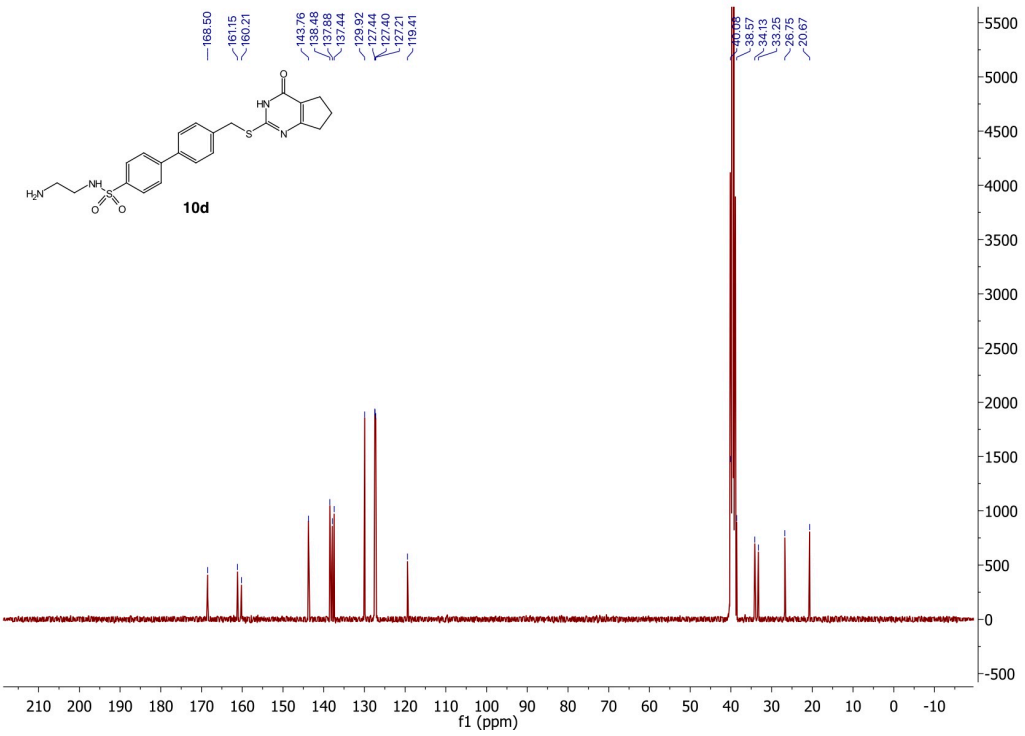
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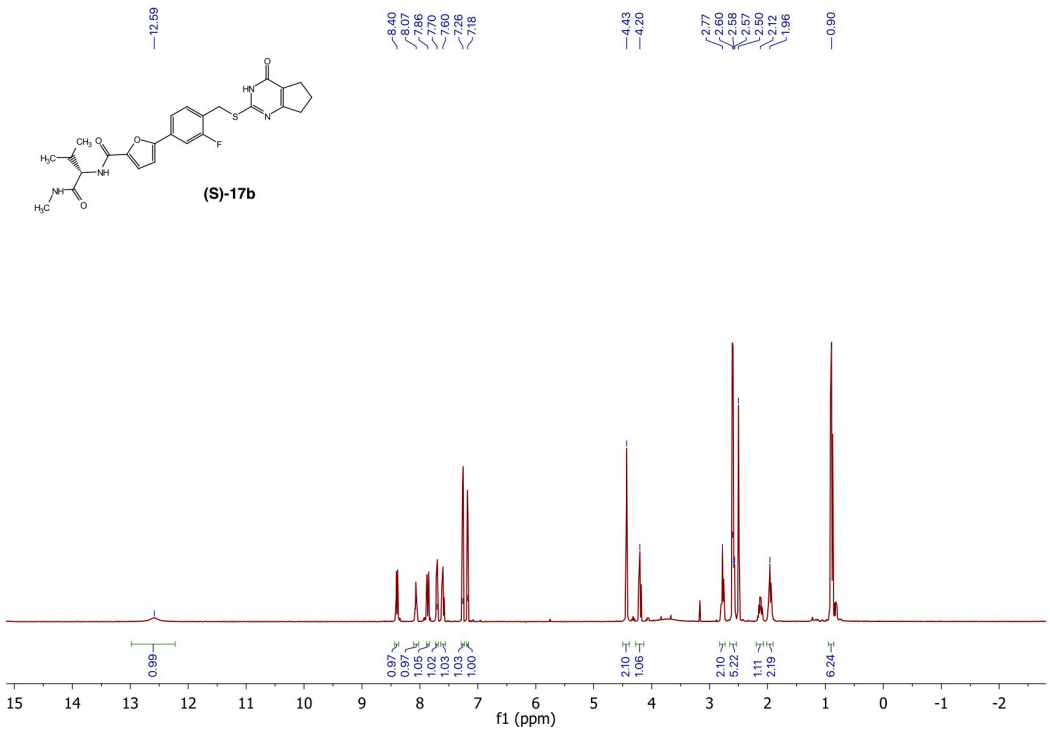
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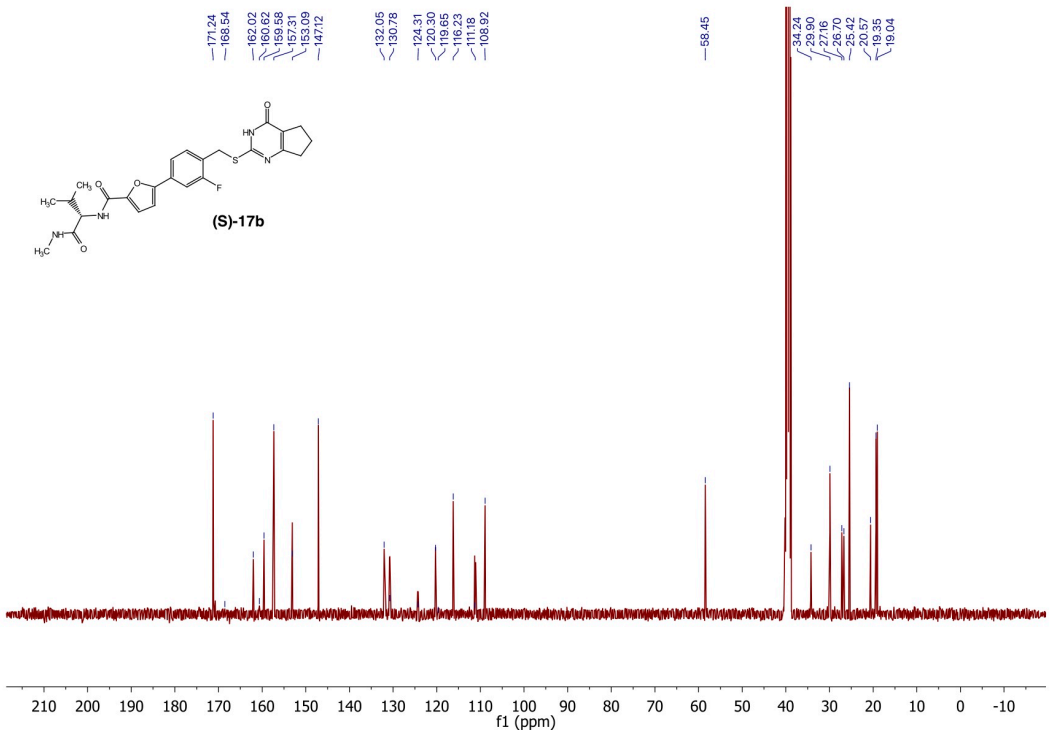
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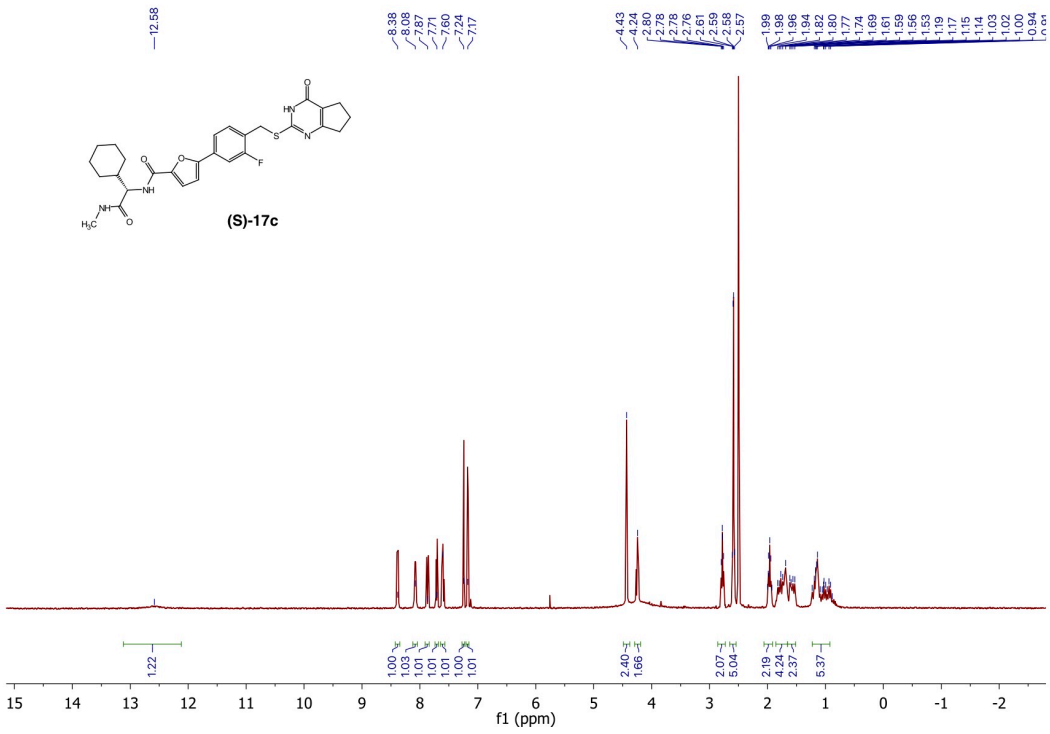
(S)-17b
f1 (ppm)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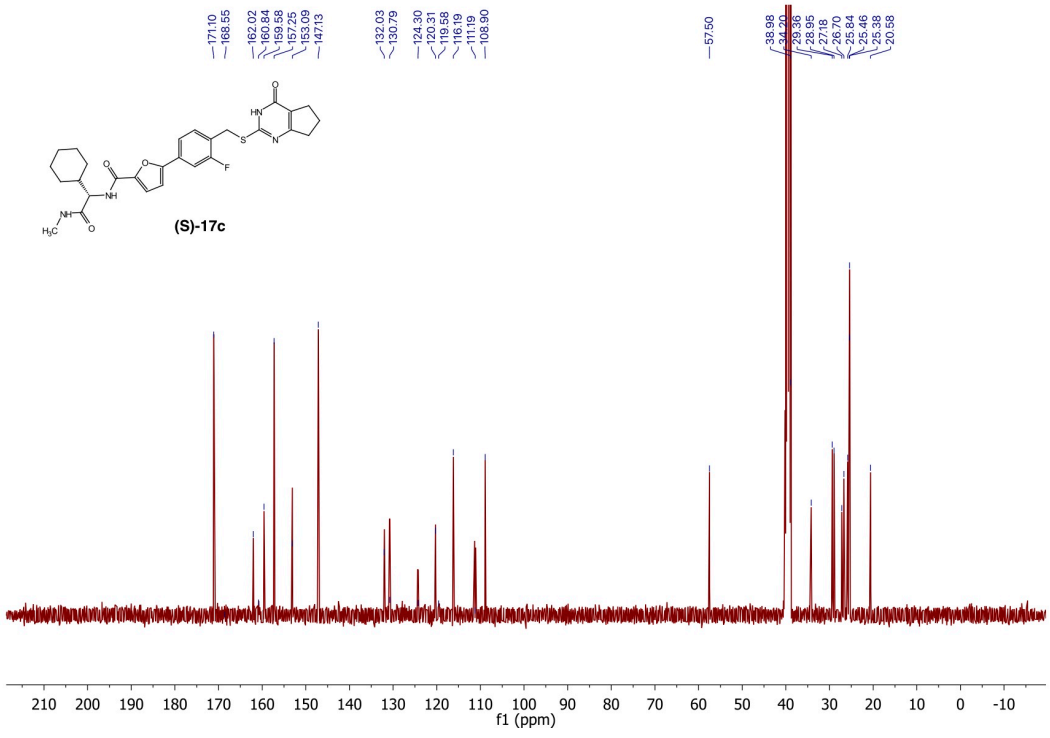
f1 (ppm)
(S)-17c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f1 (ppm)
(S)-17c

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