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Article

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Remodeling oncogenic transcriptomes by small molecules targeting NONO

In the format provided by the authors and unedited

Supplementary information: Supplementary Tables 1-5, and Synthesis and characterization of compounds

Information regarding general reagents can be found in material and methods.

Table of Contents

Category	Parameter	Description
Assay	Type of assay	Quantigene
	Target	Androgen receptor full length and V7 splice variant mRNA
	Primary measurement	Fluorescence
	Key reagents	Quantigene probes for AR-FL and V7 (ThermoFisher)
	Assay protocol	Parental 22Rv1 cells or 22Rv1 cells with stable expression of shRNA targeting AR-FL were seeded at 15000 cells/well in 384 well plates and 24h later treated with compounds in 0.2% DMSO or with 0.2% DMSO only. After 6h treatment, cells were directly harvested by adding 0.5 volume of the QuantiGene Lysis Mixture (ThermoFisher) and processed immediately for hybridization or stored at -80°C. QuantiGene was performed according to the manufacturer's protocol. Briefly, 40 µl of the pre-warmed cell lysate was incubated with Blocking reagent, Protease K, Capture beads and the Probe set over night at 54°C. Next day the beads were washed, and the signal was amplified using Branch DNA technology by serial incubation with Preamplification, Amplification and Labeling reagent for 1h at 50°C each and finally visualized by incubation with SAPE for 30 min at RT as indicated by manufacturer's protocol. Beads were measured using the FLEXMAP 3D. Data interpretation followed a three-step normalization/transformation flow in which raw median fluorescent gene signals were transformed to fold change values with the use of housekeeping genes (SUCLA2 and TCEB2) and then to expression (% of DMSO) by normalizing to DMSO controls. This in-house developed method was previously published (Verbist et al., 2019) and the R based analysis tool, QGProfiler, has been made publicly available at: https://qgprofiler.openanalytics.eu/app/QGprofiler. QuantiGene probes and blocking reagents were designed and provided by Thermofisher using RefSeq ID below. FL-AR: NM_000044 Homo sapiens androgen receptor (AR), transcript variant 1 ARV7: HM055487 Homo sapiens androgen receptor isoform 8 (AR8) SUCLA2: NM_003850 Homo sapiens succinate-CoA ligase ADP-forming beta subunit (SUCLA2) TCEB2: NM_007108 Homo sapiens elongin B (ELOB), transcript variant 1
Library	Library size	536
	Library composition	Structurally diverse collection of cysteine-directed electrophilic compounds
	Source	Cravatt research group

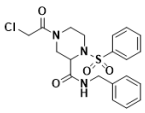
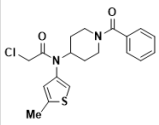
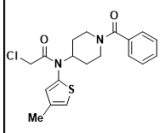
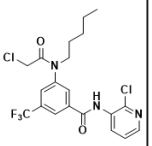
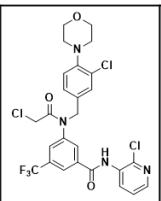
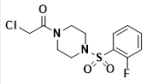
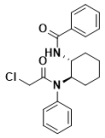
Screen	Format	384-well plate
	Concentration(s) tested	20 μM
	Plate controls	14 DMSO wells per 384-well plate
	Reagent/compounds dispensing system	Janus Liquid Handler workstation
	Detection instrument and software	FLEXMAP 3D, QGProfiler software
	Assay validation/QC	–
	Correction factors	–
	Normalization	Housekeeping genes: SUCLA2, TCEB2
Post-HTS analysis	Hit criteria	Reduction of AR-FL and V7 expression by ≥ 30% relative to DMSO
	Hit rate	1.10%
	Additional assay(s)	AR protein measurement
	Confirmation of hit purity and structure	Resynthesis, 1H NMR and LC/MS

Supplementary Table 1. Parameters for Quantigene AR mRNA screen

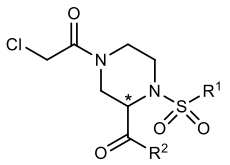
Category	Parameter	Description
Assay	Type of assay	High content imaging
	Target	Androgen receptor full length and V7 splice variant protein
	Primary measurement	Alexa488 fluorescence intensity
	Key reagents	AR antibody conjugated to Alexa488 (Cell Signaling #7395)
	Assay protocol	22Rv1 cells were seeded in 384 Cell Carrier Ultra plates (6057300, PerkinElmer) at 5000 cells per well in 30 µL medium and incubated overnight at 37°C. On the following day cells were treated with compounds (final DMSO = 0.2% (v/v)) and incubated for 24h. Cells were fixed by incubation with 4% formaldehyde for 20 min. The medium was completely removed, and the cells were permeabilized by 20 µL of ice-cold methanol for 10 min at 20°C followed by extensive washing using 1x PBS (11666789001, Roche) to remove the methanol. The cells were incubated with AR antibody conjugated with Alexa488 (Cell Signaling Technology) in 1X PBS supplemented with 5% Bovine Serum Albumin (Sigma, w/v) over night at 20°C in dark. The cells were washed 3x with 1X PBS and incubated with 0.625 µg/ml CellMask Deep Red (ThermoFisher) and 2 µg/ml Hoechst 33258 (ThermoFisher) in 1X PBS for 1h at 20°C in dark followed by 3X washing with 1X PBS. The cells were imaged on a Cell Voyager 7000 (Yokogawa, Tokyo, Japan) using a 40x dry objective lens (NA=0.95). PerkinElmer (Waltham, MA) Acapella/Columbus image analysis software was used to identify the boundaries of individual nuclei based on the Hoechst channel and the surrounding cytoplasm based on the CellMask Deep Red channel. Mean intensity of AR per cell median-aggregated over all cells per well and cell counts per well were then calculated. These measurements together with the original images and segmentation masks of the nuclei and cells were imported to Phaedra1 (www.phaedra.io) for visual and statistical quality control and plate-wise normalization as percentage of the median DMSO control value.
Library	Library size	536
	Library composition	Structurally diverse collection of cysteine-directed electrophilic compounds
	Source	Cravatt research group
Screen	Format	384-well plate
	Concentration(s) tested	10 µM
	Plate controls	14 DMSO wells per 384-well plate
	Reagent/compounds dispensing system	Janus Liquid Handler workstation
	Detection instrument and software	Cell Voyager 7000, PerkinElmer Acapella/Columbus software, Phaedra software
	Assay validation/QC	–
	Correction factors	–

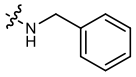
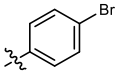
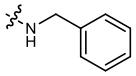
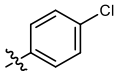
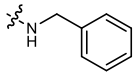
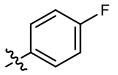
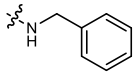
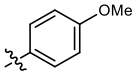
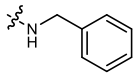
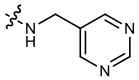
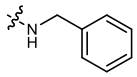
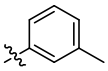
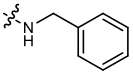
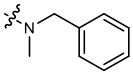
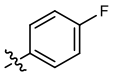
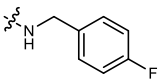
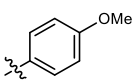
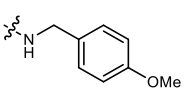
	Normalization	–
Post-HTS analysis	Hit criteria	Reduction of Total AR and AR-V7 protein expression by ≥ 50% relative to DMSO, ≤50% reduction in cell count relative to DMSO
	Hit rate	0.40%
	Additional assay(s)	AR mRNA measurement
	Confirmation of hit purity and structure	Resynthesis, 1H NMR and LC/MS

Supplementary Table 2. Parameters for high content imaging AR protein screen

Structure	 B21 (**1**)	 E124	 E126	 HS_175	 HS_189-2	 pipsulf	 E141tr
AR-FL mRNA	26	70	60	68	58	58	90
AR-V7 mRNA	69	70	61	67	53	70	98
Total AR protein	37	65	26	16	16	55	49
AR-V7 protein	33	39	64	61	50	48	34
Cell count	63	42	5	4	9	101	77

Supplementary Table 3. Structures of compounds that met hit criteria in either the AR mRNA (≥30% decrease of AR-FL and V7) or protein (≥50% decrease of total AR and AR-V7) expression screens. All values are percentages of DMSO control signal.



	R_1	R_2	IC_{50} (μM) AR-FL mRNA	AR-V7 mRNA	Cytotoxicity
B21			8.4 [7.2-10]	19 [14-24]	27 [21-45]
2			3.4 [2.8-4.4]	6.1 [4.0-8.3]	10 [9.3-12]
3			2.8 [2.4-3.2]	5.3 [4.3-5.9]	12 [11-55]
4			2.6 [2.4-2.8]	4.9 [4.7-5.1]	19 [15-25]
5			3.9 [3.4-4.5]	7.5 [6.4-10]	19 [15-29]
6			>50	>50	>50
7			>50	>50	22 [18-28]
8			>50	>50	17 [14-23]
9			>50	>50	18 [17-20]
(*R*)-**10**			2.7 [2.5-3.0]	4.9 [4.3-5.6]	35 [29-42]
(*S*)-**10**			>50	>50	31 [26-37]
(*R*)-SKBG-1			3.1 [2.7-3.4]	5.5 [4.9-6.2]	44 [34-62]
(*S*)-SKBG-1			>50	>50	37 [31-46]

Supplementary Table 4. Structure-activity relationship (SAR) analysis of analogues of hit compound B21.
Structures of B21 analogues, along with IC_{50} values for depleting AR-FL and AR-V7 mRNA (measured by Quantigene assays at 6 h post-compound treatment) and cytotoxicity estimates (measured by high-content imaging-based cell counting at 24 h post-compound treatment). 95% confidence intervals are shown in brackets. Data are mean values ± s.e.m. from two experiments each containing two independent replicates (four replicates total).

Mutagenesis primers	Mutated nucleotide in bold and underlined
NONO-C145S-fwd (T→A):	GTGTGCGCTTTGCC**A**GCCATAGTGCATCC
NONO-C145S-rev	GATGCACTATGGC**T**GGCAAAGCGCACAC
NONO-PAM-mutation-fwd (C→G)	ACCAGGAGAGAAGAC**G**TTCACCCAACGAAGC
NONO-PAM-mutation-rev	GCTTCGTTGGGTGAA**C**GTCTTCTCTCCTGGT
NONO-gateway-STOP-fwd (C→A)	CAAACAAACGTCGCCGATACTA**A**CCAACTTTCTTGTACAAAG
NONO-gateway-STOP-rev	CTTTGTACAAGAAAGTTGG**T**TAGTATCGGCGACGTTTGTTTG
CRISPR oligos (LentiCRISPR v2)	
Lenti-CRISPRv2:sgCRISPR-CTRL1-fwd	GCGAGGTATTCGGCTCCGCG
Lenti-CRISPRv2:sgCRISPR-CTRL1-rev	CGCGGAGCCGAATACCTCGC
Lenti-CRISPRv2:sgCRISPR-CTRL2-fwd	GCCTGCCCTAAACCCCGGAA
Lenti-CRISPRv2:sgCRISPR-CTRL2-rev	TTCCGGGGTTTAGGGCAGGC
Lenti-CRISPRv2:NONO-1-fwd	CTGGACAATATGCCACTCCG
Lenti-CRISPRv2:NONO-1-rev	CGGAGTGGCATATTGTCCAG
Lenti-CRISPRv2:NONO-2-fwd	AGTGGACCGCAACATCAAGG
Lenti-CRISPRv2:NONO-2-rev	CCTTGATGTTGCGGTCCACT
Lenti-CRISPRv2:NONO-3-fwd	GACCAGTTAGATGATGAAGA
Lenti-CRISPRv2:NONO-3-rev	TCTTCATCATCTAACTGGTC
Lenti-CRISPRv2:NONO-4-fwd	GCTCTGGACAGATGCAGTGA
Lenti-CRISPRv2:NONO-4-rev	TCACTGCATCTGTCCAGAGC
Lenti-CRISPRv2:NONO-5-fwd	AAGACGGCTTCGTTGGGTGA
Lenti-CRISPRv2:NONO-5-rev	TCACCCAACGAAGCCGTCTT
Alt-R CRISPR oligos	
Hs.Ca9.Control.1.AA	GCGAGGUAUUCGGCUCCGCG
Hs.Cas9.PSPC1.1.AA	CCGGTTGATGAAGACTTCGC
Hs.Cas9.PSPC1.1.AB	TCCAGAGGACCACCCGGACG
Hs.Cas9.SFPQ.1.AA	CCGGGTCCCTGAGCAACGAC
Hs.Cas9.SFPQ.1.AB	TGTATCATCCAGTTCGGCTT

Supplementary Table 5. Sequences of oligos used in this paper

Synthesis of compounds

General Analytical Information

All final compounds were characterized by ^{1}H and ^{13}C NMR spectroscopy and HRMS. Copies of the ^{1}H and ^{13}C spectra can be found at the end of the Supporting Information. All ^{1}H and ^{13}C NMR experiments are reported in δ units, parts per million (ppm), and were measured relative to the signals for residual DMSO-d_6 (2.50 ppm ^{1}H, 39.52 ^{13}C) or $CHCl_3$ (7.26 ppm ^{1}H, 77.16 ^{13}C) in the deuterated solvent. Reaction progress was monitored by LCMS unless otherwise noted. HRMS analyses were performed using an Agilent ESI-TOF instrument. The crystal structures of (*R*)-SKBG-1 and (*S*)-SKBG-1 were solved by the Emory University X-Ray Crystallography Center.

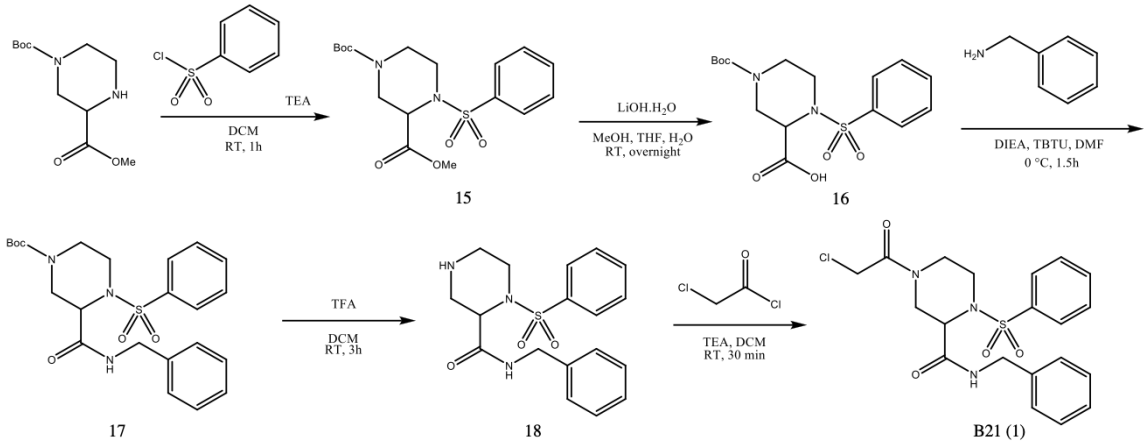
List of Abbreviations

Abbreviation	***Name***
HATU	*N*-[(Dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide
DIEA	*N,N*-Diisopropylethylamine
DCM	Dichloromethane
TFA	Trifluoroacetic acid
DMF	Dimethylformamide
THF	Tetrahydrofuran
PE	Petroleum ether
EtOAc	Ethyl acetate
EtOH	Ethanol
MeOH	Methanol
EtOAc	Ethyl acetate
TEA	Triethylamine
Boc_2O	di-*tert*-butyl dicarbonate
ACN	Acetonitrile
TBTU	2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate
HOBt	Hydroxybenzotriazole
TLC	Thin layer chromatography
LCMS	Liquid chromatography / mass spectrometry
SFC	Supercritical fluid chromatography

TBAC	Tetrabutylammonium chloride
DMAP	Dimethylaminopyride
DME	Dimethoxysilane
TsCl	4-toluenesulfonyl chloride
TBSCl	*Tert*-butyldimethylsilyl chloride

Synthetic procedures.

Synthesis of B21:



Synthesis of 15: Into a 250-mL 3-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed 1-tert-butyl 3-methyl piperazine-1,3-dicarboxylate (5 g, 20.467 mmol, 1 equiv.), DCM (100 mL), TEA (2.28 g, 22.514 mmol, 1.1 equiv.), benzenesulfonyl chloride (3.98 g, 22.514 mmol, 1.1 equiv.). The resulting solution was stirred for 1 h at 25ºC. The resulting mixture was concentrated. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (0:1-1:5). This resulted in 5 g (63.5%) of 1-tert-butyl 3-methyl 4-(benzenesulfonyl)piperazine-1,3-dicarboxylate (**15**) as a white solid.

Synthesis of 16: Into a 250-mL 3-necked round-bottom flask, was placed **15** (5 g, 13.006 mmol, 1 equiv.), oxolane (25 mL), methanol (25 mL), a solution of lithium hydroxide hydrate (2.73 g, 65.062 mmol, 5.00 equiv.) in H_2O (10 mL) . The resulting solution was stirred for 12 h at 25ºC. The resulting solution was diluted with 100 mL of H_2O. The pH value of the solution was adjusted to 3-4 with HCl (1 N). The resulting solution was extracted with 3x200 mL of dichloromethane. The resulting mixture was washed with 1x200 ml of brine. The mixture was dried over anhydrous sodium sulfate and concentrated under vacuum. This resulted in 4 g (83%) of 1-(benzenesulfonyl)-4-[(tert-butoxy)carbonyl]piperazine-2-carboxylic acid (**16**) as a white solid.

Synthesis of 17: Into a 250-mL 3-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed **16** (4 g, 10.799 mmol, 1 equiv.), DMF (40 mL). This was followed by the addition of DIEA (4.19 g, 32.396 mmol, 3 equiv.) and TBTU (4.51 g, 14.038 mmol, 1.3 equiv.) at 0ºC. The resulting solution was stirred for 30 min at 0ºC. To the mixture was added 1-phenylmethanamine (1.27 g, 11.878 mmol, 1.1 equiv.) at 0ºC. The resulting solution was stirred for 60 min at 0ºC. The resulting solution was diluted with 200 mL of H_2O. The resulting solution was extracted with 3x200 mL of ethyl acetate. The resulting mixture was washed with 1x200 ml of brine. The mixture was dried over anhydrous sodium sulfate and concentrated under vacuum. This resulted in 4 g (80.6%) of tert-butyl 4-(benzenesulfonyl)-3-(benzylcarbamoyl)piperazine-1-carboxylate (**17**) as a white solid.

Synthesis of 18: Into a 100-mL 3-necked round-bottom flask, was placed **17** (4 g, 8.704 mmol, 1 equiv.), DCM (40 mL), TFA (4.96 g, 43.500 mmol, 5.00 equiv.). The resulting

solution was stirred for 3 h at 25ºC. The resulting mixture was concentrated. The residue was dissolved in 20 mL of ACN. The pH value of the solution was adjusted to $NaHCO_3$ in water with 7-8 (0.5 N). The crude product was purified by Flash-Prep-HPLC with the following conditions (IntelFlash-1): Column, silica gel; mobile phase, H_2O (NH_4HCO_3, 0.1%)/ACN=3/7 increasing to H_2O/ACN=5/5 within 20 min. This resulted in 2.29 g (73.2%) of 1-(benzenesulfonyl)-N-benzylpiperazine-2-carboxamide (**18**) as a white solid.

Synthesis of B21: Into a 50-mL 3-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed **18** (300 mg, 0.835 mmol, 1 equiv.), DCM (6 mL), TEA (101.35 mg, 1.002 mmol, 1.2 equiv.), 2-chloroacetyl chloride (103.69 mg, 0.918 mmol, 1.1 equiv.). The resulting solution was stirred for 30 min at 25ºC. The resulting mixture was concentrated. The crude product was purified by Flash-Prep-HPLC with the following conditions (IntelFlash-1): Column, C18; mobile phase, H_2O (NH_4HCO_3, 0.1%)/ACN=20/80 increasing to H_2O/ACN=50/50 within 30 min. This resulted in 182 mg (49.02%) of 1-(benzenesulfonyl)-N-benzyl-4-(2-chloroacetyl)piperazine-2-carboxamide (**B21** (**1**)) as a white solid.

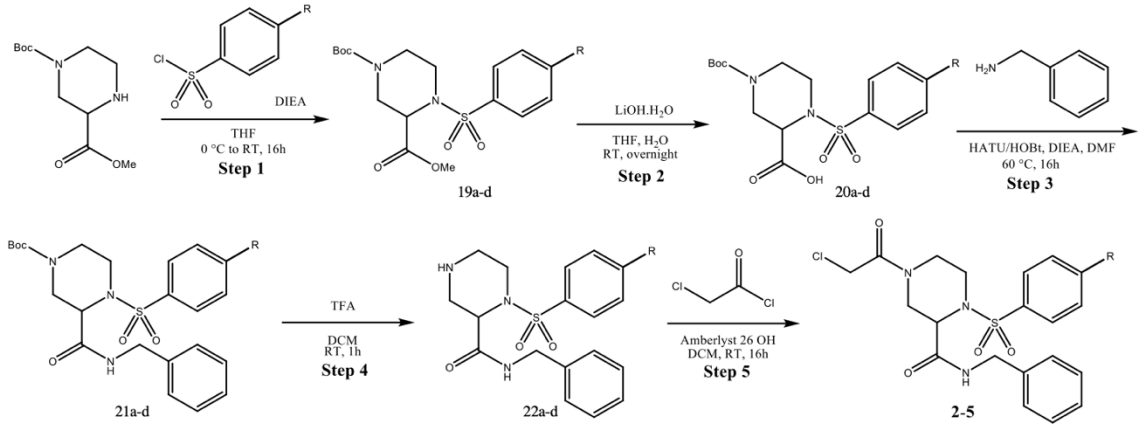
^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ 7.86-7.90 (m, 2H), 7.66-7.72 (m, 1H), 7.57-7.62 (m, 2H), 7.32-7.40 (m, 3H), 7.21-7.23 (m, 2H), 6.90-7.00 (m, 1H), 4.45-4.55 (m, 5H), 4.29-4.33 (m, 1H), 4.08-4.12 (m, 1H), 3.90-3.95 (m, 1H), 3.12-3.22 (m, 1H), 2.84-2.89 (m, 1H), 2.39-2.48 (m, 1H).

^{13}C NMR (101 MHz, $CDCl_3$, ppm) δ 167.44, 166.14, 139.50, 137.26, 134.03, 130.12, 129.07, 127.97, 127.60, 127.13, 77.48, 77.16, 76.84, 56.49, 44.20, 44.14, 43.19, 41.11, 40.20, 0.14.

HRMS ESI-TOF (*m/z*): $[M+H]^+$ for $C_{20}H_{22}N_3O_4SCl$: expected 436.1092, measured 436.10936

Synthesis of Compounds 2-5:

General Scheme:



	2	**3**	**4**	**5**
	a	b	c	d
R=	Br	Cl	F	OMe

Step 1:
Dipea (1.3 eq.) was added to a stirred solution of 1-tert-butyl 3-methyl piperazine-1,3-dicarboxylate in dry THF at 0 ºC. Then, the corresponding sulfonyl chloride (1 eq.) was added portionwise to the reaction mixture at 0 ºC. The reaction mixture was stirred at room temperature for 16 h. Solvent was concentrated in vacuo. The residue was diluted with EtOAc and washed with Brine, dried over $MgSO_4$ anhydrous, filtered and concentrated in vacuo.

Step 2:
Lithium hydroxide monohydrate (1.5 eq.) was added to a stirred solution of **19** in THF:H_2O (4:1). The reaction mixture was stirred at room temperature until complete conversion of the starting material. Solvent was concentrated in vacuo.

Step 3:
HATU (1.3 eq.) and HOBt (0.3 eq.) were sequentially added to a stirred solution of **20** and Dipea (3 eq.) in DMF at room temperature. The mixture was stirred at the same temperature for 15 min. The amine was added and the reaction mixture was stirred at 60 ºC for 16 h. The mixture was diluted with EtOAc and NH4Cl saturated solution saturated solution, the aqueous layer was extracted with EtOAc. The combined organic phases were washed with $NaHCO_3$ saturated solution and Brine, dried over MgSO4 anhydrous, filtered and solvent was concentrated in vacuo.

Step 4:
Trifluoroacetic acid (20 eq.) was added to a solution of **21** in Dichloromethane (6 mL/mmol) at room temperature. The crude was dissolved in DCM: MeOH (20:1) and Amberlyst 26A OH form (10 eq.) was added. The mixture was stirred at room temperature for 1h. The solution was filtered and the solvent was concentrated in vacuo.

Step 5:
Chloroacetyl chloride (2.2 eq.) was added to a stirred solution of **22** and Amberlyst 26A OH form (3 eq.) in Dichloromethane at room temperature. The reaction mixture was stirred until complete conversion of the starting material. The solid was removed by filtration and washed with DCM twice. The filtrate was concentrated under reduced pressure.

Synthesis of 19a: Following the procedure for Step 1, from 1-tert-butyl 3-methyl piperazine-1,3-dicarboxylate (440 mg, 1.8 mmol) and 4-Bromobenzenesulfonyl chloride (460 mg, 1.8 mmol). The crude was purified by flash column chromatography (Silica; EtOAc in Heptane from 0/100 to 30/70). The desired fractions were collected and concentrated in vacuo to yield 505 mg (59%) of **19a** as a colourless oil.

Synthesis of 19b: Following the procedure for Step 1, from 1-tert-butyl 3-methyl piperazine-1,3-dicarboxylate (400 mg, 1.6 mmol) and 4-Chlorobenzenesulfonyl chloride (345 mg, 1.6 mmol). The crude was purified by flash column chromatography (Silica; EtOAc in Heptane from 0/100 to 20/80). The desired fractions were collected and concentrated in vacuo to yield 336 mg (49%) of **19b** as a colourless oil.

Synthesis of 19c: Following the procedure for Step 1, from 1-tert-butyl 3-methyl piperazine-1,3-dicarboxylate (500 mg, 2 mmol) and 4-Fluorobenzenesulfonyl chloride (345 mg, 1.6 mmol). The crude was purified by flash column chromatography (Silica; EtOAc in Heptane

from 0/100 to 30/70). The desired fractions were collected and concentrated in vacuo to yield 550 mg (65%) of **19c** as a colourless oil.

Synthesis of 19d: Following the procedure for Step 1, from 1-tert-butyl 3-methyl piperazine-1,3-dicarboxylate (500 mg, 2 mmol) and 4-Methoxybenzene sulfonyl chloride (423 mg, 2 mmol). The crude was purified by flash column chromatography (silica; EtOAc in Heptane from 0/100 to 60/40). The desired fractions were collected and solvent was concentrated in vacuo to yield 790 mg (92%) of **19d** as a colourless oil.

Synthesis of 20a: Following the procedure for Step 2, from **19a** (505 mg, 1.06 mmol). The reaction mixture was stirred at room temperature for 16 h. Solvent was concentrated in vacuo and EtOAc was added and the mixture was filtered through a short pad of $MgSO_4$ anhydrous. The filtrate was concentrated in vacuo to yield 494 mg (99%) of **20a** as a white solid. The crude was used in the next reaction step without further purification.

Synthesis of 20b: Following the procedure for Step 2, from **19b** (428 mg, 1.02 mmol). The reaction mixture was stirred at room temperature for 16 h. Solvent was concentrated in vacuo to yield 435 mg (95%) of **20b** as a colourless oil. The crude was used in the next reaction step without further purification.

Synthesis of 20c: Following the procedure for Step 2, from **19c** (550 mg, 1.3 mmol). The reaction mixture was stirred at room temperature for 16 h. Solvent was concentrated in vacuo to yield 540 mg (99%) of **20c** as a colourless oil. The crude was used in the next reaction step without further purification.

Synthesis of 20d: Following the procedure for Step 2, from **19d** (790 mg, 1.9 mmol). The reaction mixture was stirred at room temperature for 16 h. Solvent was concentrated in vacuo to yield 770 mg (99%) of **20d** as a colourless oil. The crude was used in the next reaction step without further purification.

Synthesis of 21a: Following the procedure for Step 3, from **20a** (406 mg, 0.9 mmol) and Benzylamine (2 eq.). The reaction mixture was stirred at 60 ºC for 16 h. The mixture was diluted with EtOAc and NH4Cl saturated solution, the aqueous layer was extracted with EtOAc. The combined organic phases were washed with $NaHCO_3$ saturated solution and Brine, dried over $MgSO_4$ anhydrous, filtered and solvent was concentrated in vacuo. The crude was purified by flash column chromatography (silica, EtOAc in Heptane from 0/100 to 30/70). The desired fractions were collected and concentrated in vacuo to yield 176 mg (34%) of **21a** as a colourless oil.

Synthesis of 21b: Following the procedure for Step 3, from **20b** (205 mg, 0.5 mmol) and Benzylamine (1.5 eq.). The reaction mixture was stirred at 60 ºC for 16 h. The mixture was diluted with EtOAc and NH4Cl saturated solution, the aqueous layer was extracted with EtOAc. The combined organic phases were washed with $NaHCO_3$ saturated solution and Brine, dried over $MgSO_4$ anhydrous, filtered and solvent was concentrated in vacuo. The crude was purified by flash column chromatography (silica, EtOAc in Heptane from 0/100 to 30/70). The desired fractions were collected and concentrated in vacuo to yield 69 mg (27%) of **21b** as a colourless oil.

Synthesis of 21c: Following the procedure for Step 3, from **20c** (540 mg, 1.39 mmol) and Benzylamine (2 eq.). The reaction mixture was stirred at 60 ºC for 16 h. The mixture was diluted with EtOAc and NH_4Cl saturated solution, the aqueous layer was extracted with EtOAc. The combined organic phases were washed with $NaHCO_3$ saturated solution and Brine, dried over $MgSO_4$ anhydrous, filtered and solvent was concentrated in vacuo. The crude was purified by flash column chromatography (silica, EtOAc in Heptane from 0/100 to

30/70). The desired fractions were collected and concentrated in vacuo to yield 226 mg (33%) of **21c** as a colourless oil.

Synthesis of 21d: Following the procedure for Step 3, from **20d** (760 mg, 1.89 mmol) and Benzylamine (2.2 eq.). The reaction mixture was stirred at 60 ºC for 16 h. The mixture was diluted with EtOAc and NH_4Cl saturated solution, the aqueous layer was extracted with EtOAc. The combined organic phases were washed with $NaHCO_3$ saturated solution and Brine, dried over $MgSO_4$ anhydrous, filtered and solvent was concentrated in vacuo. The crude was purified by flash column chromatography (silica, EtOAc in Heptane from 0/100 to 30/70). The desired fractions were collected and concentrated in vacuo to yield 730 mg (78%) of **21d** as a beige solid.

Synthesis of 22a: Following the procedure for Step 4, from **21a** (175 mg, 0.32 mmol). The reaction mixture was stirred for 2 h. Solvent was concentrated in vacuo. The crude was dissolved in DCM: MeOH (20:1) and Amberlyst 26A OH form (10 eq.) was added. The mixture was stirred at room temperature for 1h. The solution was filtered and the solvent was concentrated in vacuo to afford 149 mg (78%) of **22a** as colorless oil.

Synthesis of 22b: Following the procedure for Step 4, from **21b** (226 mg, 0.45 mmol). The reaction mixture was stirred for 2 h. Solvent was concentrated in vacuo. The crude was dissolved in DCM: MeOH (20:1) and Amberlyst 26A OH form (10 eq.) was added. The mixture was stirred at room temperature for 1h. The solution was filtered and the solvent was concentrated in vacuo to afford 189 mg (99%) of **22b** as colorless oil.

Synthesis of 22c: Following the procedure for Step 4, from **21c** (226 mg, 0.45 mmol). The reaction mixture was stirred for 2 h. Solvent was concentrated in vacuo. The crude was dissolved in DCM: MeOH (20:1) and Amberlyst 26A OH form (10 eq.) was added. The mixture was stirred at room temperature for 1h. The solution was filtered and the solvent was concentrated in vacuo to afford 178 mg (99%) of **22c** as colorless oil.

Synthesis of 22d: Following the procedure for Step 4, from **21d** (250 mg, 0.51 mmol). The reaction mixture was stirred for 2 h. Solvent was concentrated in vacuo. The crude was dissolved in DCM: MeOH (20:1) and Amberlyst 26A OH form (10 eq.) was added. The mixture was stirred at room temperature for 1h. The solution was filtered and the solvent was concentrated in vacuo to afford 203 mg (97%) of **22d** as colorless oil.

Synthesis of Compound 2: Following the procedure for Step 5, from **22a** (156 mg, 0.35 mmol). The reaction mixture was stirred for 16 h. The solid was removed by filtration and washed with DCM twice. The filtrate was concentrated under reduced pressure. The crude product was purified by flash chromatography (silica, EtOAc in Heptane from 0/100 to 70/30). The desired fractions were collected and concentrated under reduced pressure to afford 112 mg (60%) of **2** as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ 7.73 (s, 4H), 7.36 (s, 2H), 7.30 – 7.18 (m, 5H), 6.86 (s, 1H), 4.59 – 4.42 (m, 5H), 4.35 (d, *J* = 13.8 Hz, 1H), 4.13 (dd, *J* = 12.5, 7.2 Hz, 2H), 4.00 – 3.84 (m, 1H), 3.25 – 3.09 (m, 1H), 2.92 (dd, *J* = 14.4, 4.2 Hz, 1H), 2.56 – 2.40 (m, 1H).

^{13}C NMR (101 MHz, DMSO-d_6, ppm) δ 169.73, 167.43, 167.16, 164.37, 163.91, 138.24, 137.60, 137.36, 131.83, 131.61, 128.44, 128.36, 127.71, 127.65, 126.70, 126.56, 126.31, 126.18, 59.15, 54.21, 53.66, 45.40, 43.62, 43.01, 41.78, 41.62, 41.52, 41.41, 41.04, 40.84, 40.07, 39.55, 39.34, 39.13, 38.92, 38.71, 38.51, 38.30, 20.16, 13.48, -0.50.

HRMS ESI-TOF (*m/z*): $[M+H]^+$ for $C_{20}H_{21}N_3O_4SClBr$: expected 514.0198, measured 514.0197

Synthesis of Compound 3: Following the procedure for Step 5, from **22b** (180 mg, 0.45 mmol). The reaction mixture was stirred for 16 h. The solid was removed by filtration and washed with DCM twice. The filtrate was concentrated under reduced pressure. The crude product was purified by flash chromatography (silica, EtOAc in Heptane from 0/100 to 70/30). The desired fractions were collected and concentrated under reduced pressure to afford 159 mg (73%) of **3** as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ (d, *J* = 28.1 Hz, 1H), 7.80 (dd, *J* = 27.9, 8.2 Hz, 2H), 7.61 (dd, *J* = 13.3, 8.1 Hz, 2H), 7.42 – 7.09 (m, 5H), 4.57 (t, *J* = 17.2 Hz, 2H), 4.47 – 3.92 (m, 5H), 3.92 – 3.53 (m, 2H), 3.16 (s, 1H).

^{13}C NMR (101 MHz, DMSO-d_6, ppm) δ 170.81, 168.52, 168.25, 165.45, 164.99, 139.32, 138.61, 138.36, 138.28, 138.03, 129.97, 129.75, 129.46, 129.37, 128.79, 128.73, 127.77, 127.63, 127.40, 127.27, 60.23, 55.30, 54.74, 46.47, 44.69, 44.08, 42.86, 42.71, 42.60, 42.48, 42.12, 41.92, 41.15, 40.63, 40.42, 40.21, 40.00, 39.80, 39.59, 39.38, 21.24, 14.56, 0.58.

HRMS ESI-TOF (*m/z*): $[M+H]^+$ for $C_{20}H_{21}N_3O_4SCl_2$: expected 470.0703, measured 470.0699

Synthesis of Compound 4: Following the procedure for Step 5, from **22c** (178 mg, 0.47 mmol). The reaction mixture was stirred for 16 h. The solid was removed by filtration and washed with DCM twice. The filtrate was concentrated under reduced pressure. The crude product was purified by flash chromatography (silica, EtOAc in Heptane from 0/100 to 70/30). The desired fractions were collected and concentrated under reduced pressure to afford 105 mg (49%) of **4** as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 8.65 (dt, *J* = 28.6, 5.5 Hz, 1H), 7.87 (dt, *J* = 28.1, 6.8 Hz, 2H), 7.32 (ddt, *J* = 33.2, 24.9, 8.0 Hz, 7H), 4.54 (d, *J* = 20.0 Hz, 2H), 4.45 – 3.93 (m, 6H), 3.93 – 3.55 (m, 2H), 3.25 – 3.08 (m, 1H).

^{13}C NMR (126 MHz, DMSO-d_6, ppm) δ 167.50, 167.24, 165.01, 164.88, 164.36, 163.90, 163.01, 162.87, 138.26, 134.75, 134.52, 129.61, 129.53, 129.50, 129.42, 127.70, 127.64, 126.68, 126.55, 126.31, 126.18, 116.05, 115.86, 115.80, 115.62, 54.24, 53.64, 45.26, 43.58, 42.93, 41.78, 41.59, 41.47, 41.39, 41.05, 40.85, 40.02, 39.52, 39.42, 39.35, 39.26, 39.18, 39.09, 39.01, 38.92, 38.84, 38.76, 38.59, 38.42, -0.50.

HRMS ESI-TOF (*m/z*): $[M+H]^+$ for $C_{20}H_{21}N_3O_4SClF$: expected 454.0998, measured 454.0996

Synthesis of Compound 5: Following the procedure for Step 5, from **22d** (233 mg, 0.59 mmol). The reaction mixture was stirred for 16 h. The solid was removed by filtration and washed with DCM twice. The filtrate was concentrated under reduced pressure. The crude product was purified by flash chromatography (silica, EtOAc in Heptane from 0/100 to 70/30). The desired fractions were collected and concentrated under reduced pressure to afford 180 mg (64%) of **5** as a white solid.

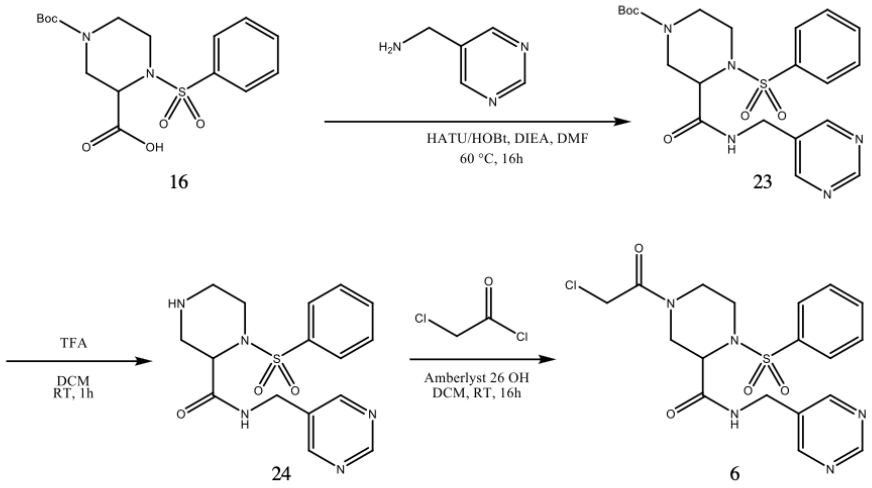
^{1}H NMR (300 MHz, DMSO-d_6, ppm) δ 8.80 – 8.42 (m, 1H), 7.71 (dd, *J* = 27.1, 7.9 Hz, 2H), 7.53 – 7.11 (m, 7H), 4.53 (d, *J* = 21.0 Hz, 2H), 4.43 – 3.92 (m, 5H), 3.69 (dt, *J* = 40.7, 14.2 Hz, 2H), 3.23 – 2.79 (m, 2H), 2.40 (d, *J* = 3.9 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d_6, ppm) δ 13C NMR (101 MHz, DMSO) δ 167.70, 167.41, 164.34, 163.87, 162.18, 161.99, 138.36, 130.03, 129.81, 128.75, 128.65, 127.68, 127.63, 126.61, 126.52, 126.26, 126.15, 113.98, 113.74, 64.31, 55.11, 55.08, 54.37, 53.71, 44.82,

43.41, 42.58, 41.83, 41.44, 41.07, 40.95, 39.85, 39.55, 39.34, 39.13, 38.92, 38.71, 38.50, 38.30, 14.57, -0.50.

HRMS ESI-TOF (*m/z*): $[M+H]^+$ for $C_{21}H_{24}N_3O_5SCl$: expected 466.1198, measured 466.1196

Synthesis of Compound 6:



Synthesis of 23: Following the procedure for Step 3 above, from **16** (508 mg, 1.3 mmol) and 5-Pyrimidinemethanamine (1.3 eq.). The reaction mixture was stirred at 60 ºC for 16 h. The mixture was diluted with EtOAc and NH_4Cl saturated solution, the aqueous layer was extracted with EtOAc. The combined organic phases were washed with $NaHCO_3$ saturated solution and Brine, dried over $MgSO_4$ anhydrous, filtered and solvent was concentrated in vacuo. The crude was purified by flash column chromatography (silica, EtOAc in Heptane from 0/100 to 30/70). The desired fractions were collected and concentrated in vacuo to yield 422 mg (66%) of **23** as a beige solid.

Synthesis of 24: Following the procedure for Step 4, from **23** (345 mg, 0.75 mmol). The reaction mixture was stirred for 2 h. Solvent was concentrated in vacuo. The crude was dissolved in DCM: MeOH (20:1) and Amberlyst 26A OH form (10 eq.) was added. The mixture was stirred at room temperature for 1h. The solution was filtered and the solvent was concentrated in vacuo to afford 206 mg (76%) of **24** as an orange solid.

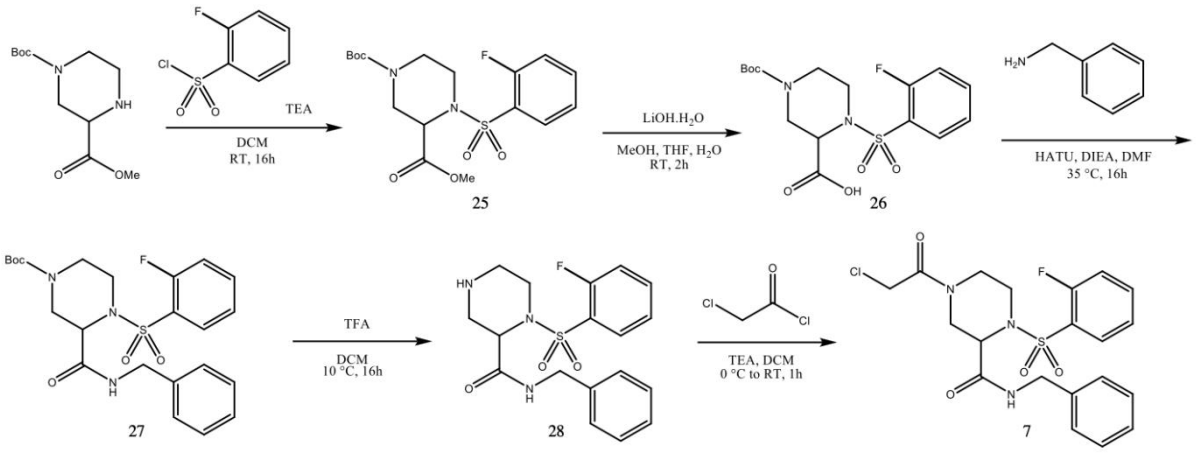
Synthesis of Compound 6: Following the procedure for Step 5, from **24** (236 mg, 0.65 mmol). The reaction mixture was stirred for 16 h. The solid was removed by filtration and washed with DCM twice. The filtrate was concentrated under reduced pressure. The crude product was purified by flash chromatography (silica, EtOAc in Heptane from 0/100 to 70/30). The desired fractions were collected and concentrated under reduced pressure to afford 156 mg (52%) of **6** as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ 7.94 (td, *J* = 7.6, 1.8 Hz, 1H), 7.66 (tdd, *J* = 7.3, 5.0, 1.8 Hz, 1H), 7.34 (dddd, *J* = 14.1, 8.9, 5.3, 3.6 Hz, 4H), 7.28 – 7.16 (m, 3H), 6.96 (s, 1H), 4.70 – 4.53 (m, 3H), 4.53 – 4.33 (m, 3H), 4.13 (d, *J* = 12.7 Hz, 1H), 3.96 – 3.77 (m, 1H), 3.26 – 2.99 (m, 2H), 2.58 (td, *J* = 13.0, 3.5 Hz, 1H).

^{13}C NMR (101 MHz, DMSO-d_6, ppm) δ 170.94, 169.32, 157.55, 156.53, 139.92, 133.48, 133.43, 132.83, 129.70, 127.40, 60.47, 55.54, 42.56, 41.16, 40.95, 40.74, 40.53, 40.32, 40.11, 39.91, 38.62, 0.46.

HRMS ESI-TOF (*m/z*): $[M+H]^+$ for $C_{18}H_{20}N_5O_4SCl$: expected 438.0997, measured 438.0994

Synthesis of Compound 7:



Synthesis of 25: To a solution of 1-tert-butyl 3-methyl piperazine-1,3-dicarboxylate (600 mg, 2.456 mmol) and TEA (0.41 mL, 2.95 mmol) in DCM (5 mL) was added 2-fluorobenzenesulfonyl chloride (525 mg, 2.702 mmol) at r.t.. The mixture was stirred at r.t. for 16 h. TLC (petroleum ether: ethyl acetate=4:1, UV) showed the completion of the reaction. The mixture was diluted with DCM (20 mL) and washed with H_2O (10 mL). The separated aqueous layer was extracted with DCM (10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under vacuum to give 1.2 g crude product which was purified by flash column chromatography over silica gel (eluent: ethyl acetate/ petroleum ether, from 0/100 to 30/70, gradient). The desired fractions were concentrated to dryness under vacuum to give **26** as a white solid. Yield: 462 mg (47%)

Synthesis of 26: Aq. $LiOH.H_2O$ (1.1 mL, 2M) was added to a solution of 85_A (462 mg, 1.1 mmol) in H_2O (2 mL) and MeOH (2mL) at r.t.. The solution was stirred at r.t. for 2 h. LCMS showed the desired mass ion was observed. The solution was concentrated to give **27** (453.8 mg, crude) as a white solid, which was used in the next step without further purification.

Synthesis of 27: To a mixture of 85_B (454 mg, 1.1 mmol), benzylamine (135 mg, 1.3 mmol) and DIEA (0.8 mL, 4.6 mmol) in DMF (5 mL) was added HATU (873 mg, 2.3 mmol) under N2 at r.t.. The reaction mixture was stirred at 35°C under N_2 overnight. LCMS showed the desired mass ion was observed. The mixture was concentrated in vacuo. The residue was diluted with DCM (10 mL) and washed with H_2O (5 mL), brine (5 mL), dried over MgSO4, filtered and concentrated under vacuum to give the crude product which was purified by flash column chromatography over silica gel (eluent: ethyl acetate/ petroleum ether, from

0/100 to 50/50, gradient). The desired fractions were concentrated to dryness under vacuum to give **27** (425 mg, 78% yield) as a white solid.

Synthesis of 28: TFA (0.37 mL, 5.1 mmol) was added to a solution of 85_C (200 mg, 0.42 mmol) in DCM (2 mL) at 10 °C (r.t.). The solution was stirred at 10 °C for 16 h. TLC (petroleum ether: ethyl acetate=3:1, UV) showed the completion of the reaction. The mixture was concentrated under vacuum to give **28** (205 mg, crude) as light yellow gum, which was used in the next step without further purification.

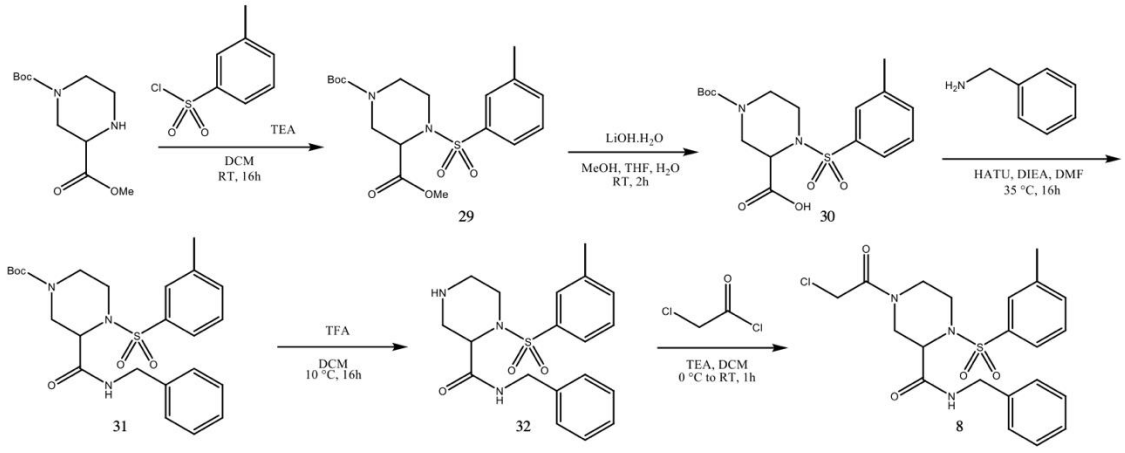
Synthesis of Compound 7: To a solution of **28** (205 mg, 0.4 mmol) in DCM (1.7 mL) was added TEA (0.2 mL, 1.3 mmol) in an ice-water bath. To the mixture was added a solution of 2-chloroacetyl chloride (36.7 µL) in DCM (0.3 mL) in an ice-water bath. The mixture was stirred at r.t. for 1 h. LCMS showed the desired mass ion was observed. The mixture was concentrated under vacuum to give a residue as a light yellow gum, which was purified by high-performance liquid chromatography. Column: Xtimate C18 150*25mm*5um Condition: A: water (water (0.2% FA)) B: CH_3CN at the beginning: A (55%) and B (45%) at the end: A: (25%) and B (75%) Gradient Time (min) 7; 100% B Hold Time (min) 0; Flow Rate (ml/min) 30. Pure fractions were collected and the organic solvent was evaporated under vacuum. The residue was lyophilized to give **7** (138.6 mg, yield=73%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.94 (t, *J*=7.13 Hz, 1 H) 7.61 - 7.73 (m, 1 H) 7.28 - 7.38 (m, 4 H) 7.19 - 7.26 (m, 3 H) 6.96 (br s, 1 H) 4.52 - 4.65 (m, 3 H) 4.38 - 4.52 (m, 3 H) 4.13 (d, *J*=12.55 Hz, 1 H) 3.90 (br d, *J*=14.05 Hz, 1 H) 3.05 - 3.22 (m, 2 H) 2.58 (td, *J*=12.92, 3.26 Hz, 1 H)

^{13}C NMR (101 MHz, DMSO-d_6, ppm) δ 167.45, 167.15, 164.40, 163.95, 159.05, 158.90, 156.52, 156.37, 138.12, 135.38, 135.29, 135.15, 135.07, 129.60, 129.40, 127.74, 127.61, 126.78, 126.50, 126.35, 126.15, 124.26, 124.09, 116.96, 116.82, 116.75, 116.61, 54.05, 53.63, 46.41, 44.10, 43.64, 41.93, 41.73, 41.58, 41.38, 41.07, 40.72, 40.58, 39.55, 39.34, 39.13, 38.92, 38.71, 38.50, 38.29, -0.50.

HRMS ESI-TOF (*m/z*): $[M+H]^+$ for $C_{20}H_{21}N_3O_4SClF$: expected 454.0998, measured 454.0997

Synthesis of Compound 8:



Synthesis of 29: To a solution of 1-tert-butyl 3-methyl piperazine-1,3-dicarboxylate (1 g, 4.1 mmol) and TEA (0.67 mL, 4.9 mmol) in DCM (8 mL) was added 3-methylbenzenesulfonyl chloride (0.65 mL, 4.5 mmol) at r.t.. The mixture was stirred at r.t. for 16 h. TLC (petroleum ether: ethyl acetate=4:1, UV) showed the completion of the reaction. The mixture was diluted with DCM (20 mL) and washed with H_2O (10 mL). The separated aqueous layer was extracted with DCM (10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under vacuum to give 1.6 g crude product which was purified by flash column chromatography over silica gel (eluent: ethyl acetate/ petroleum ether, from 0/100 to 20/80, gradient). The desired fractions were concentrated to dryness under vacuum to give **29** 1.2 g (Yield: 73.6%) as a white solid.

Synthesis of 30: Aq. $LiOH.H_2O$ (1.3 mL, 2M) was added to a solution of **29** (509 mg, 1.3 mmol) in H_2O (2.5 mL) and MeOH (2.5 mL) at r.t.. The solution was stirred at r.t. for 2 h. TLC (petroleum ether: ethyl acetate=4:1, UV) showed the completion of the reaction. The solution was concentrated to give **30** (500 mg, crude) as a white solid, which was used in the next step without further purification.

Synthesis of 31: To a mixture of **30** (500 mg, 1.3 mmol), benzylamine (151 mg, 1.4 mmol) and DIEA (0.9 mL, 5.1 mmol) in DMF (6 mL) was added HATU (971 mg, 2.6 mmol) under N2 at r.t.. The reaction mixture was stirred at 35°C under N2 overnight. LCMS showed the desired mass ion was observed. The mixture was concentrated in vacuo. The residue was diluted with DCM (10 mL) and washed with H2O (5 mL), brine (5 mL), dried over $MgSO_4$, filtered and concentrated under vacuum to give the crude product which was purified by flash column chromatography over silica gel (eluent: ethyl acetate/ petroleum ether, from 0/100 to 30/70, gradient). The desired fractions were concentrated to dryness under vacuum to give **31** (375 mg, 62% yield) as a white solid.

Synthesis of 32: TFA (0.7 mL, 9.5 mmol) was added to a solution of **31** (375 mg, 0.79 mmol) in DCM (6 mL) at 10 °C (r.t.). The solution was stirred at 10 °C for 16 h. TLC (petroleum ether: ethyl acetate=1:1, UV) showed the completion of the reaction. The mixture was concentrated under vacuum to give **32** (386 mg, crude) as light yellow gum, which was used in the next step without further purification.

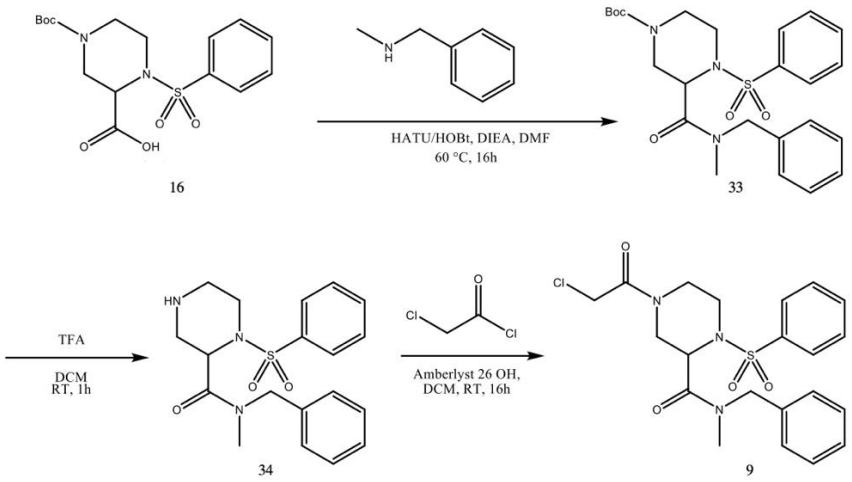
Synthesis of Compound 8: To a solution of **32** (386 mg, 0.8 mmol) in DCM (2.7 mL) was added TEA (0.3 mL, 2.4 mmol) in an ice-water bath. To the mixture was added a solution of 2-chloroacetyl chloride (69.5 µL, 0.87 mmol) in DCM (0.3 mL) in an ice-water bath. The mixture was stirred at r.t. for 1 h. LCMS showed the desired mass ion was observed. The mixture was concentrated under vacuum to give a residue as a light yellow gum, which was purified by high-performance liquid chromatography. Column: Xtimate C18 150*25mm*5um Condition: A: water (10mM NH_4HCO_3) B: CH_3CN at the beginning: A (56%) and B (44%) at the end: A: (26%) and B (74%) Gradient Time (min) 7; 100% B Hold Time (min) 2; Flow Rate (ml/min) 30. Pure fractions were collected and the organic solvent was evaporated under vacuum. The residue was lyophilized and Re-purified by high-performance liquid chromatography. Column: Xtimate C18 150*25mm*5um Condition: A: water (0.2%FA) B: CH_3CN at the beginning: A (56%) and B (44%) at the end: A: (26%) and B (74%) Gradient Time (min) 7; 100% B Hold Time (min) 2; Flow Rate (ml/min) 30. Pure fractions were collected and the organic solvent was evaporated under vacuum to give **8** (124.8 mg, yield 35%).

^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.99 (d, *J* = 7.9 Hz, 1H), 7.60 – 7.45 (m, 1H), 7.44 – 7.29 (m, 4H), 7.17 (d, *J* = 7.2 Hz, 2H), 6.89 (s, 1H), 4.59 (dd, *J* = 16.9, 13.3 Hz, 2H), 4.47 (d, *J* = 20.0 Hz, 2H), 4.39 (d, *J* = 5.6 Hz, 2H), 4.14 (d, *J* = 12.7 Hz, 1H), 3.77 (d, *J* = 13.9 Hz, 1H), 3.18 (dd, *J* = 13.8, 3.9 Hz, 1H), 3.14 – 3.01 (m, 1H), 2.61 (td, *J* = 12.8, 3.5 Hz, 1H), 2.54 (s, 3H).

^{13}C NMR (126 MHz, DMSO-d_6, ppm) δ 168.02, 167.63, 164.40, 163.95, 138.17, 138.13, 136.81, 136.66, 136.43, 136.41, 132.59, 132.43, 132.30, 132.18, 128.85, 128.65, 127.73, 127.61, 126.71, 126.44, 126.36, 126.14, 125.84, 125.70, 53.48, 53.05, 46.49, 44.21, 43.58, 41.70, 41.56, 41.36, 41.28, 41.10, 40.76, 40.69, 39.51, 39.42, 39.35, 39.25, 39.18, 39.09, 39.01, 38.92, 38.84, 38.75, 38.59, 38.42, 19.40, 19.28, -0.50.

HRMS ESI-TOF (*m/z*): $[M+H]^+$ for $C_{21}H_{24}N_3O_4SCl$: expected 450.1249, measured 450.1234

Synthesis of Compound 9:



Synthesis of 33: Following the procedure for Step 3 above, from **SI-2** (508 mg, 1.3 mmol) and N-Methylbenzylamine (2 eq.). The reaction mixture was stirred at 60 ºC for 16 h. The mixture was diluted with EtOAc and NH_4Cl saturated solution, the aqueous layer was extracted with EtOAc. The combined organic phases were washed with NaHCO3 saturated solution and Brine, dried over MgSO4 anhydrous, filtered and solvent was concentrated in vacuo. The crude was purified by flash column chromatography (silica, EtOAc in Heptane from 0/100 to 30/70). The desired fractions were collected and concentrated in vacuo to yield 359 mg (49%) of **33** as an orange sticky solid.

Synthesis of 34: Following the procedure for Step 4, from **33** (354 mg, 0.75 mmol). The reaction mixture was stirred for 2 h. Solvent was concentrated in vacuo. The crude was dissolved in DCM: MeOH (20:1) and Amberlyst 26A OH form (10 eq.) was added. The mixture was stirred at room temperature for 1h. The solution was filtered and the solvent was concentrated in vacuo to afford 290 mg (93%) of **34** as a yellow solid.

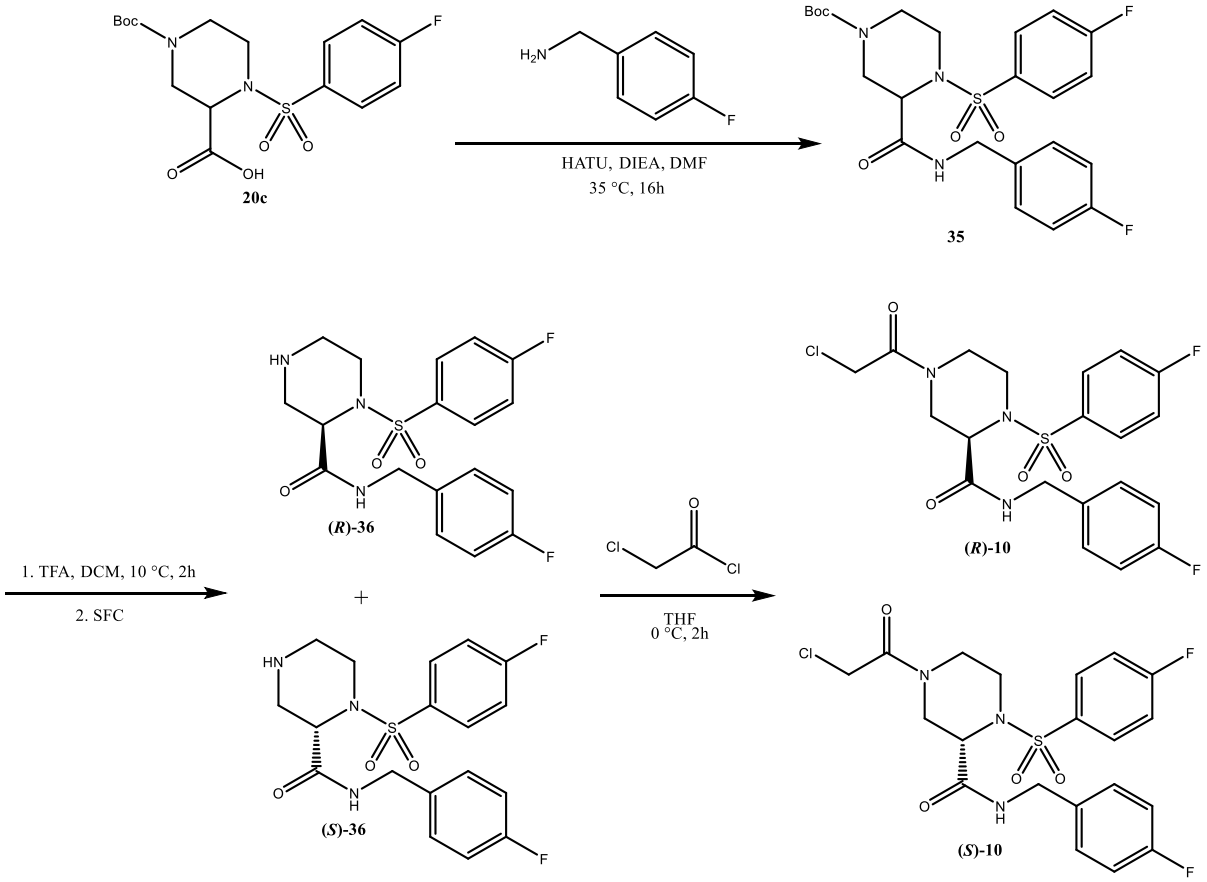
Synthesis of Compound 9: Following the procedure for Step 5, from **34** (290 mg, 0.77 mmol). The reaction mixture was stirred for 16 h. The solid was removed by filtration and washed with DCM twice. The filtrate was concentrated under reduced pressure. The crude product was purified by flash chromatography (silica, EtOAc in Heptane from 0/100 to 70/30). The desired fractions were collected and concentrated under reduced pressure to afford 137 mg (39%) of **9** as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ 7.78 (dt, *J* = 17.1, 8.3 Hz, 2H), 7.54 (dq, *J* = 25.2, 7.3 Hz, 4H), 7.40 – 7.26 (m, 3H), 7.26 – 7.03 (m, 2H), 5.09 – 4.91 (m, 1H), 4.77 (td, *J* = 28.9, 10.4 Hz, 1H), 4.60 – 4.22 (m, 2H), 4.22 – 3.88 (m, 4H), 3.88 – 3.58 (m, 2H), 3.45 (dq, *J* = 17.8, 4.8 Hz, 1H), 3.19 – 2.84 (m, 3H).

^{13}C NMR (126 MHz, DMSO-d_6, ppm) δ 167.92, 167.87, 167.78, 167.72, 164.28, 164.19, 138.25, 136.46, 136.40, 132.53, 132.33, 128.69, 128.63, 128.53, 128.50, 128.14, 128.06, 127.87, 127.78, 127.15, 126.99, 126.86, 126.69, 126.62, 126.33, 54.31, 51.67, 51.47, 50.18, 50.09, 49.88, 49.79, 49.75, 49.32, 45.79, 45.28, 43.85, 43.78, 42.98, 42.22, 41.42, 41.32, 41.05, 40.98, 40.90, 40.70, 40.42, 40.24, 39.51, 39.42, 39.35, 39.26, 39.18, 39.09, 39.01, 38.92, 38.84, 38.76, 38.59, 38.42, 34.34, 33.80, 32.90, 32.53, -0.50.

HRMS ESI-TOF (*m/z*): $[M+H]^+$ for $C_{21}H_{24}N_3O_4SCl$: expected 450.1249, measured 450.1251

Synthesis of Compounds (R)-10 and (S)-10:



Synthesis of 35: To a mixture of **20c** (500 mg, 1.287 mmol), 4-fluorobenzylamine (177 mg, 1.416 mmol) and DIEA (0.9 mL, 5.15 mmol) in DMF (6 mL) was added HATU (979 mg, 2.575 mmol) under N_2 at r.t.. The reaction mixture was stirred at 35°C under N_2 overnight. LCMS showed the desired mass was observed. The mixture was concentrated in vacuum. The residue was diluted with DCM (10 mL) and washed with H_2O (5 mL), brine (5 mL), dried over $MgSO_4$, filtered and concentrated under vacuum to give the crude product, which was purified by flash column chromatography over silica gel (eluent: ethyl acetate/ petroleum ether, from 0/100 to 100/0, gradient). The desired fractions were concentrated to dryness under vacuum to give **35** (250 mg, 84.36% purity, 33.06% yield) as a white solid.

Synthesis of (*R*)-36: TFA (0.844 mL, 11.394 mmol) was added to a solution of **35** (250 mg, 0.426 mmol) in DCM (4 mL) at 10 °C. The solution was stirred at 10 °C for 2 h. LCMS showed the completion of the reaction. The mixture was concentrated under vacuum to give a residue as a white solid, which was purified by high-performance liquid chromatography. Column: Boston Uni C18 40*150*5um: A: water (0.05%NH_3H_2O) B: CH_3CN at the beginning: A (67%) and B (33%) at the end: A: (37%) and B (63%) Gradient Time (min) 7.7; 100% B Hold Time (min) 1.8; Flow Rate (ml/min) 60. Pure fractions were collected and the organic solvent was evaporated under vacuum to give **36** as white solid. The residue was separated by Supercritical Fluid Chromatography. Column: DAICEL CHIRALPAK AD (250mm*30mm,10um); Mobile phase: A: Supercritical CO_2, B: 0.1%NH_3H_2O ETOH,A:B =70:30 at 70YmL/min; The pure fractions were collected and the volatiles were removed under vacuum. It was lyophilized to dryness to give (*R*)-**36** (90 mg, 53.479% yield) as a white solid.

Synthesis of Compound (*R*)-10: To a solution of (*R*)-**36** (90 mg, 0.228 mmol) in THF (2 mL) in an ice water bath was added chlororacetyl chloride (0.02 mL, 0.25 mmol) dropwise. The mixture was stirred at 0°C for 2 h. LCMS showed the desired mass was observed. The mixture was concentrated under vacuum to give a residue as a yellow oil, which was purified by high-performance liquid chromatography. Column: Boston Prime C18 150*30mm*5umCondition: A: water (0.05%NH_3H_2O+10mM NH_4HCO_3) B: CH_3CN at the beginning: A (50%) and B (50%) at the end: A: (20%) and B (80%) Gradient Time (min) 8; 100% B Hold Time (min) 2; Flow Rate (ml/min) 25. Pure fractions were collected and the organic solvent was evaporated under vacuum to give (*R*)-**10** (67.6 mg, yield 62.938%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.93 – 7.72 (m, 2H), 7.23 – 7.16 (m, 3H), 7.16 – 7.07 (m, 2H), 6.97 (t, *J* = 8.6 Hz, 2H), 6.87 (s, 1H), 4.43 (dd, *J* = 13.2, 3.0 Hz, 3H), 4.37 – 4.28 (m, 2H), 4.28 – 4.18 (m, 1H), 4.02 (d, *J* = 12.8 Hz, 1H), 3.80 (d, *J* = 14.4 Hz, 1H), 3.08 (ddd, *J* = 14.9, 12.0, 3.5 Hz, 1H), 2.80 (dd, *J* = 14.3, 4.3 Hz, 1H), 2.36 (td, *J* = 12.8, 3.5 Hz, 1H).

^{13}C NMR (101 MHz, DMSO-d_6, ppm) δ 168.60, 168.34, 166.35, 166.22, 165.46, 164.96, 163.85, 163.72, 162.86, 160.45, 135.84, 135.57, 130.69, 130.59, 130.49, 129.86, 129.78, 129.61, 129.53, 117.16, 116.93, 116.69, 115.60, 115.49, 115.39, 115.28, 55.34, 54.75, 46.23, 44.66, 43.99, 42.67, 42.55, 42.18, 42.10, 41.95, 41.77, 41.09, 40.62, 40.42, 40.21, 40.00, 39.79, 39.58, 39.37, 0.57.

HRMS ESI-TOF (*m/z*): $[M+H]^+$ for $C_{20}H_{20}N_3O_4SClF_2$: expected 472.0904, measured 472.0900

Synthesis of (*S*)-36: To a mixture of **35** (280 mg, 0.547 mmol, 96.746% purity) in DCM (4 mL) was added TFA (2 mL) at 15°C. The reaction mixture was stirred at 15°C for 16h. LCMS showed the desired mass was detected. The reaction mixture was concentrated in vacuum. The residue was purified by preparative high-performance liquid chromatography. Column: Phenomenex Gemini NX-C18 (75*30mm*3um) Condition: A: water (0.05%NH_3H_2O+10mM NH_4HCO_3) B: MeCN at the beginning: A (70%) and B (30%) at the end: A: (40%) and B (60%) Gradient Time (min) 6; 100%B Hold Time (min) 18; Flow Rate (ml/min) 25. The pure fractions were collected and the aqueous layer was lyophilized to dryness to give the crude product, which was separated by SFC. SFC condition Column: DAICEL CHIRALPAK AS (250mm*30mm,10um) Mobile phase: 0.1%NH_3H_2O ETOH Gradient: 25% B, Flow Rate(ml/min) 60. The pure fractions were collected and the solvent was evaporated under vacuum to give the desired product (*S*)-**36** (110 mg, 50.888% yield) as a white solid.

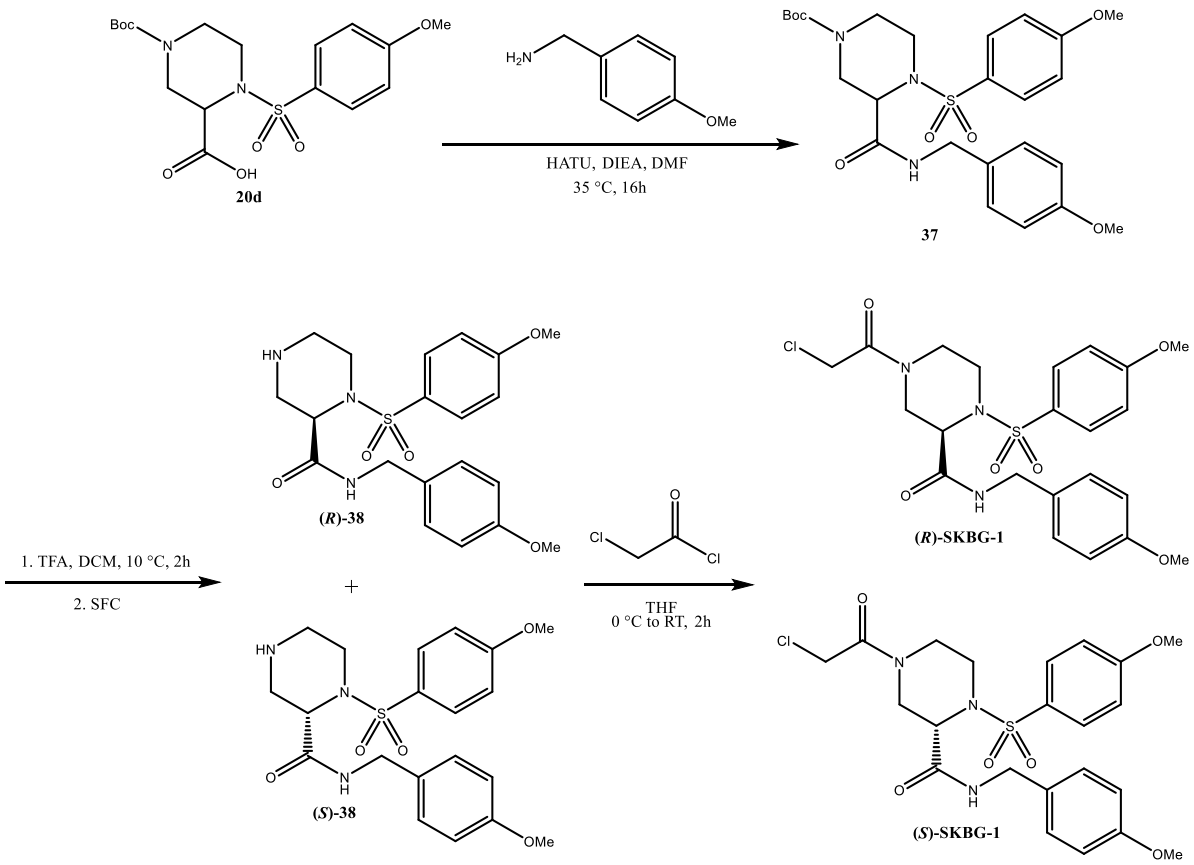
Synthesis of Compound (*S*)-10: To a solution of (*S*)-**36** (110 mg, 0.278 mmol) in THF (2 mL) in an ice water bath was added chlororacetyl chloride (0.024 mL, 0.306 mmol) dropwise.

The mixture was stirred at 0°C for 2 h. LCMS showed the desired mass was observed. The reaction mixture was concentrated in vacuum. The residue was purified by preparative high-performance liquid chromatography. Column: Phenomenex Gemini-NX 80*30mm*3um Condition: A: water (10mM NH_4HCO_3) B: MeCN at the beginning: A (65%) and B (35%) at the end: A: (35%) and B (65%) Gradient Time(min) 8; 100%B Hold Time(min) 2; Flow Rate(ml/min) 25. The pure fractions were collected and the solvent was evaporated under vacuum. The aqueous layer was lyophilized to dryness to give (*S*)-**10** (94.6 mg, 99.865% purity, 71.965% yield) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.88 – 7.73 (m, 2H), 7.20 (d, *J* = 14.8 Hz, 3H), 7.14 – 7.08 (m, 2H), 6.97 (t, *J* = 8.6 Hz, 2H), 6.85 (s, 1H), 4.48 – 4.40 (m, 2H), 4.34 (dd, *J* = 11.9, 5.9 Hz, 2H), 4.28 – 4.18 (m, 1H), 4.02 (d, *J* = 12.7 Hz, 1H), 3.80 (d, *J* = 14.6 Hz, 1H), 3.08 (ddd, *J* = 14.8, 12.0, 3.5 Hz, 1H), 2.80 (dd, *J* = 14.3, 4.4 Hz, 1H), 2.46 – 2.31 (m, 1H).
^{13}C NMR (101 MHz, DMSO-d_6, ppm) δ 167.52, 167.27, 165.14, 164.38, 163.88, 162.77, 162.64, 161.79, 159.38, 134.76, 134.50, 129.61, 129.51, 129.41, 128.78, 128.71, 128.54, 128.45, 116.09, 115.85, 115.62, 114.52, 114.42, 114.31, 114.21, 54.26, 53.67, 45.15, 43.58, 42.91, 41.59, 41.47, 41.09, 41.02, 40.86, 40.69, 40.01, 39.55, 39.34, 39.13, 38.92, 38.71, 38.50, 38.29, -0.50.

HRMS ESI-TOF (*m/z*): $[M+H]^+$ for $C_{20}H_{20}N_3O_4SClF_2$: expected 472.0904, measured 472.0903

Synthesis of Compounds (R)-SKBG-1 and (S)-SKBG-1:



Synthesis of 37: To a mixture of **20d** (800 mg, 1.827 mmol, 91.456% purity), 4-methoxybenzylamine (275.7 mg, 2.01 mmol) and DIEA (1.276 mL, 7.308 mmol) in DMF (8 mL) was added HATU (1389 mg, 3.654 mmol) under N2 at r.t. The reaction mixture was stirred at 35°C under N_2 overnight. LCMS showed the desired mass ion was observed. The

mixture was concentrated in vacuum. The residue was diluted with DCM (10 mL*3) and washed with H_2O (5 mL), brine (5 mL), dried over MgSO4, filtered and concentrated under vacuum to give the crude product which was purified by flash column chromatography over silica gel (eluent: ethyl acetate/ petroleum ether, from 0/100 to 100/0, gradient). The desired fractions were concentrated to dryness under vacuum to give **37** (790 mg, 90.733% purity, 75.501% yield) as a white solid.

Synthesis of (*R*)-38: TFA (1.086 mL, 14.668 mmol) was added to a solution of **37** (300 mg, 0.524 mmol, 90.732% purity) in DCM (4 mL) at 10 °C. The solution was stirred at 10 °C for 2h. LCMS showed the desired mass ion was observed. The mixture was concentrated under vacuum. The residue was purified by high-performance liquid chromatography. Column: Boston Uni C18 40*150*5um,Condition: A: water(0.05%NH_3H_2O) B: CH_3CN,at the beginning: A (67%) and B (33%),at the end: A: (37%) and B (63%),Gradient Time(min) 7.7; 100% B Hold Time(min) 1.8 Flow Rate(ml/min) 60 Pure fractions were collected and the organic solvent was evaporated under vacuum to give the crude product. The crude product was separated by Supercritical Fluid Chromatography. Column: DAICEL CHIRALCELOD-H(250mm*30mm,5um); Mobile phase: A: Supercritical CO_2, B: 0.1%NH_3H_2O ETOH,A:B =70:30 at 60mL/min; The pure fractions were collected and the volatiles were removed under vacuum. It was lyophilized to dryness to give (*R*)-**38** (110 mg, 50.057% yield) as a white solid.

Synthesis of (*R*)-SKBG-1: To a solution of **38** (*R*) (221 mg, 0.527 mmol) in THF (4 mL) in an ice water bath was added chlororacetyl chloride (0.023 mL, 0.288 mmol) dropwise. The mixture was stirred at r.t. for 2 h. LCMS showed the desired mass ion was observed. The mixture was concentrated under vacuum to give a residue as a light yellow gum, which was purified by high-performance liquid chromatography. Column: Boston Prime C18 150*30mm*5um,Condition: A: water (0.05%NH_3H_2O+10mM NH_4HCO_3) ,B: MeCN, at the beginning: A (55%) and B (45%),at the end: A: (25%) and B (75%),Gradient Time(min) 8; 100%B Hold Time(min) 2; Flow Rate(ml/min) 25.,The pure fractions were collected and the solvent was evaporated under vacuum. The aqueous layer was lyophilized to dryness to give the desired product (*R*)-SKBG-1 ((*R*)-**11**) (99.7 mg, 100% purity, 76.66% yield) as a white solid. Absolute stereochemistry of (*R*)-SKBG-1 was confirmed with crystallography. See crystallography section below.

^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.78 – 7.63 (m, 2H), 7.14 – 7.01 (m, 2H), 7.01 – 6.91 (m, 2H), 6.91 – 6.76 (m, 3H), 4.50 – 4.37 (m, 3H), 4.29 (d, *J* = 5.8 Hz, 2H), 4.20 (d, *J* = 13.8 Hz, 1H), 4.03 (d, *J* = 12.7 Hz, 1H), 3.83 (s, 4H), 3.74 (d, *J* = 0.9 Hz, 3H), 3.04 (ddd, *J* = 14.8, 12.0, 3.5 Hz, 1H), 2.79 (dd, *J* = 14.0, 4.3 Hz, 1H), 2.36 (td, *J* = 12.8, 3.4 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$, ppm) δ 167.32, 166.00, 163.77, 159.20, 130.60, 129.27, 129.24, 128.86, 115.07, 114.29, 77.29, 77.04, 76.78, 56.32, 55.77, 55.32, 43.96, 43.54, 42.91, 41.04, 40.08.

HRMS ESI-TOF (*m/z*): $[M+H]^+$ for $C_{22}H_{26}N_3O_6SCl$: expected 496.1304, measured 496.1303

Synthesis of (*S*)-38: (1.324 mL, 17.883 mmol) was added to a solution of **37** (517 mg, 0.637 mmol, 64.024% purity) in DCM (5 mL) at r.t. The solution was stirred at r.t. for 2 h. LCMS showed the desired mass ion was observed. The mixture was concentrated under vacuum to give a residue as a light white gum, which was purified by high-performance liquid chromatography. Column: DAICEL CHIRALCEL OD-H (250mm*30mm,5um) Condition: A: water (10mM NH_4HCO_3) B: CH_3CN at the beginning: A (70%) and B (30%) at the end: A: (70%) and B (30%) Gradient Time (min) 7; 100% B Hold Time (min) 2; Flow Rate (ml/min) 60. Pure fractions were collected and the organic solvent was evaporated under vacuum.

The residue was lyophilized to give (*S*)-**38** (221 mg, 100% purity, yield 82.7%) as a white solid.

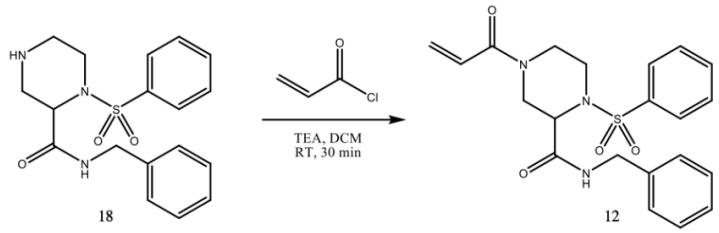
Synthesis of (*S*)-SKBG-1: To a solution of (S)-**38** (221 mg, 0.527 mmol) in THF (4 mL) in an ice water bath was added TEA (0.220 mL, 1.580 mmol). To the mixture was added a solution of chloroacetyl chloride (0.046 mL, 0.58 mmol) in DCM (2 mL) in an ice-water bath. The mixture was stirred at r.t. for 2 h. LCMS showed the desired mass was observed. The mixture was concentrated under vacuum to give a residue as a light yellow gum, which was purified by high-performance liquid chromatography. Column: XtiPhenomenex：Gemini-NX ：80*30mm*3um Condition: A: water (10mM NH_4HCO_3) B: CH_3CN at the beginning: A (66%) and B (34%) at the end: A: (36%) and B (64%) Gradient Time (min) 9; 100% B Hold Time (min) 1.5; Flow Rate (ml/min) 30. Pure fractions were collected and the organic solvent was evaporated under vacuum. The residue was lyophilized to give (*S*)-SKBG-1 ((*S*)-**11**) (66.7 mg, 100% purity, 25.527% yield) as a white solid. Absolute stereochemistry of (S)-SKBG-1 was confirmed with crystallography. See crystallography section below.

^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 7.70 (d, *J* = 8.7 Hz, 2H), 7.06 (d, *J* = 8.2 Hz, 2H), 6.94 (d, *J* = 8.6 Hz, 2H), 6.80 (d, *J* = 8.2 Hz, 3H), 4.55 – 4.35 (m, 3H), 4.29 (d, *J* = 5.8 Hz, 2H), 4.20 (d, *J* = 13.6 Hz, 1H), 4.03 (d, *J* = 12.7 Hz, 1H), 3.83 (s, 3H), 3.74 (s, 3H), 3.03 (t, *J* = 12.4 Hz, 1H), 2.80 (dd, *J* = 14.1, 4.4 Hz, 1H), 2.36 (t, *J* = 12.3 Hz, 1H).

^{13}C NMR (126 MHz, $CDCl_3$, ppm) δ 167.32, 166.00, 163.77, 159.20, 130.60, 129.27, 129.24, 128.86, 115.07, 114.29, 77.29, 77.04, 76.79, 56.32, 55.77, 55.32, 43.96, 43.54, 42.91, 41.04, 40.08.

HRMS ESI-TOF (*m/z*): $[M+H]^+$ for $C_{22}H_{26}N_3O_6SCl$: expected 496.1304, measured 496.1301

Synthesis of Compound 12:



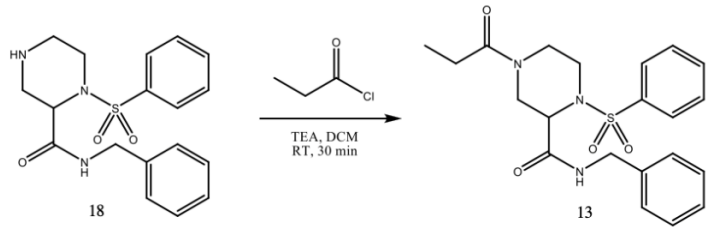
Into a 50-mL 3-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed **18** (300 mg, 0.835 mmol, 1 equiv.), DCM (6 ml) , TEA (101.35 mg, 1.002 mmol, 1.2 equiv.), prop-2-enoyl chloride (83.10 mg, 0.918 mmol, 1.10 equiv.). The resulting solution was stirred for 30 min at 25°C. The resulting mixture was concentrated. The residue was dissolved in 5 mL of ACN. The crude product was purified by Flash-Prep-HPLC with the following conditions (IntelFlash-1): Column, C18; mobile phase, H_2O (NH_4HCO_3, 0.1%)/ACN=20/80 increasing to H_2O/ACN=50/50 within 30 min. This resulted in 176 mg (49.98%) of 1-(benzenesulfonyl)-N-benzyl-4-(prop-2-enoyl)piperazine-2-carboxamide (**12**) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ 7.87-7.89 (m, 2H), 7.65-7.69 (m, 1H), 7.56-7.60 (m, 2H), 7.31-7.38 (m, 3H), 7.21-7.23 (m, 2H), 6.85-6.95 (m, 1H), 6.74-6.81 (m, 1H), 6.25-6.29 (m, 1H),5.70-5.73 (m, 1H), 4.65-4.69 (m, 1H), 4.50-4.54 (m, 1H), 4.41-4.47 (m, 2H), 4.33-4.36 (m, 1H), 3.90-3.93 (m, 1H), 3.22-3.25 (m, 1H), 2.80-2.83 (m, 1H), 2.41-2.53 (m, 1H).

^{13}C NMR (126 MHz, $CDCl_3$, ppm) δ 167.31, 166.08, 139.39, 139.38, 137.36, 133.74, 129.86, 128.84, 128.66, 127.68, 127.49, 127.30, 127.07, 77.32, 77.06, 76.81, 56.57, 43.94, 43.71, 43.21, 39.82.

HRMS ESI-TOF (*m/z*): $[M+H]^+$ for $C_{21}H_{23}N_3O_4S$: expected 414.1482, measured 414.1480

Synthesis of Compound 13:



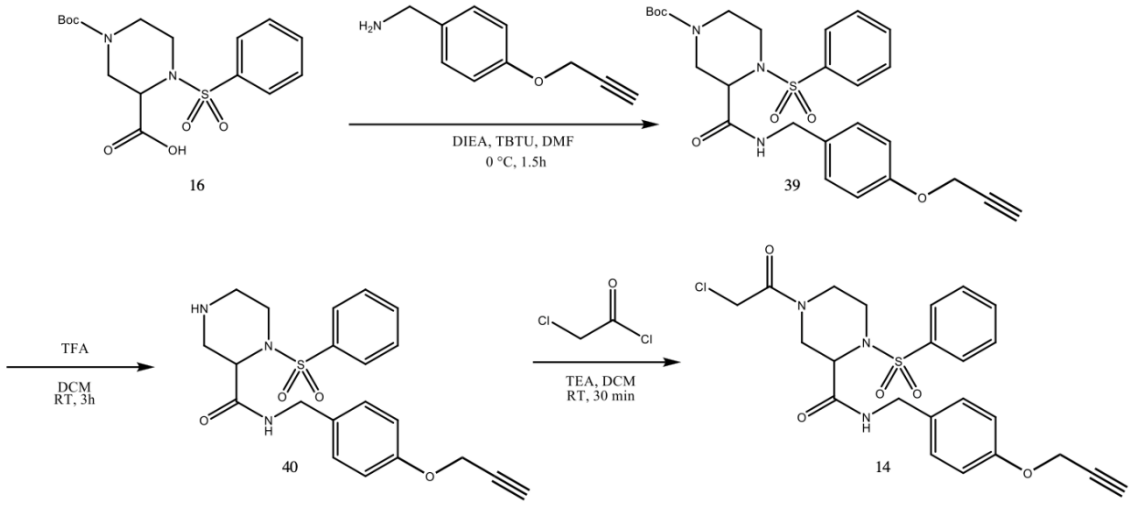
Into a 50-mL 3-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed **18** (300 mg, 0.835 mmol, 1 equiv.), DCM (6 mL), TEA (101.35 mg, 1.002 mmol, 1.20 equiv.), propanoyl chloride (84.94 mg, 0.918 mmol, 1.1 equiv.). The resulting solution was stirred for 30 min at 25ºC. The resulting mixture was concentrated. The residue was dissolved in 5 mL of ACN. The crude product was purified by Flash-Prep-HPLC with the following conditions (IntelFlash-1): Column, C18; mobile phase, H_2O (NH_4HCO_3, 0.1%)/ACN=20/80 increasing to H_2O (NH_4CO_3, 0.1%)/ACN =50/50 within 30 min. This resulted in 229 mg (64.71%) of 1-(benzenesulfonyl)-N-benzyl-4-propanoylpiperazine-2-carboxamide (**13**) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ 7.86-7.89 (m, 2H), 7.65-7.69 (m, 1H), 7.55-7.60 (m, 2H), 7.31-7.39 (m, 3H), 7.21-7.23 (m, 2H), 6.85-6.95 (m, 1H), 4.30-4.59 (m, 5H), 3.84-3.89 (m, 1H), 3.14-3.24 (m, 1H), 2.75-2.81 (m, 1H), 2.56-2.69 (m, 1H), 2.24-2.45 (m, 2H), 1.09 (d, *J* = 7.5 Hz,1H).

^{13}C NMR (126 MHz, $CDCl_3$, ppm) δ 173.22, 167.52, 139.36, 137.30, 133.72, 129.85, 128.88, 127.72, 127.45, 127.08, 77.30, 77.04, 76.79, 56.69, 43.99, 43.24, 43.04, 39.30, 25.80, 8.99.

HRMS ESI-TOF (*m/z*): $[M+H]^+$ for $C_{21}H_{25}N_3O_4S$: expected 416.1639, measured 416.1636

Synthesis of alkyne probe 14:



Synthesis of 39: Into a 10-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed **SI-2** (250.00 mg, 0.675 mmol, 1.00 equiv.), TBTU (281.71 mg, 0.877 mmol, 1.30 equiv.), DIEA (261.68 mg, 2.025 mmol, 3.00 equiv.), DMF (3.00 mL), 1-[4-(prop-2-yn-1-yloxy)phenyl]methanamine hydrochloride (146.74 mg, 0.742 mmol, 1.10 equiv.). The resulting solution was stirred for 1.5 h at 0°C. The crude product was purified by Flash-Prep-HPLC with the following conditions (IntelFlash-1): Column, C18; mobile phase, ACN: 0.05%$NH_3.H_2O$-water=20:80 increasing to ACN: 0.05%$NH_3.H_2O$-water=100:0 within 30 min; Detector, UV, 254 nm. This resulted in 220 mg (63.47%) of tert-butyl 4-(benzenesulfonyl)-3-([[4-(prop-2-yn-1-yloxy)phenyl]methyl]carbamoyl)piperazine-1-carboxylate (**39**) as a white solid.

Synthesis of 40: Into a 10-mL round-bottom flask, was placed **39** (220.00 mg, 0.428 mmol, 1.00 equiv.), trifluoroacetic acid (0.2 mL), DCM (2 mL). The resulting solution was stirred overnight at 25°C. The resulting mixture was concentrated under vacuum. The residue was dissolved in of ACN. The pH value of the solution was adjusted to 7-8 with aqueous $NaHCO_3$ solution. The crude product was purified by Flash-Prep-HPLC with the following conditions (IntelFlash-1): Column, C18; mobile phase, ACN: 0.05%$NH_3.H_2O$-water=20:80 increasing to ACN: 0.05%$NH_3.H_2O$-water=80:20 within 30 min; Detector, UV, 254 nm. This resulted in 147 mg (83.00%) of 1-(benzenesulfonyl)-N-[[4-(prop-2-yn-1-yloxy)phenyl]methyl]piperazine-2-carboxamide (**40**) as a white solid.

Synthesis of alkyne probe 14: Into a 10-mL round-bottom flask, was placed **40** (147.00 mg, 0.356 mmol, 1.00 equiv.), DCM (3.00 mL), TEA (43.17 mg, 0.427 mmol, 1.2 equiv.), chloroacetyl chloride (44.17 mg, 0.391 mmol, 1.1 equiv.). The resulting solution was stirred for 30 min at 25°C. The resulting mixture was concentrated under vacuum. The crude product was purified by Flash-Prep-HPLC with the following conditions (IntelFlash-1): Column, C18; mobile phase, ACN: 0.05% $NH_3.H_2O$-water=20:80 increasing to ACN: 0.05% $NH_3.H_2O$-water=100:0 within 30min.Freeze-drying; Detector, UV 254 nm. This resulted in 65 mg (37.32%) of 1-(benzenesulfonyl)-4-(2-chloroacetyl)-N-[[4-(prop-2-yn-1-yloxy)phenyl]methyl]piperazine-2-carboxamide (**14**) as a white solid.

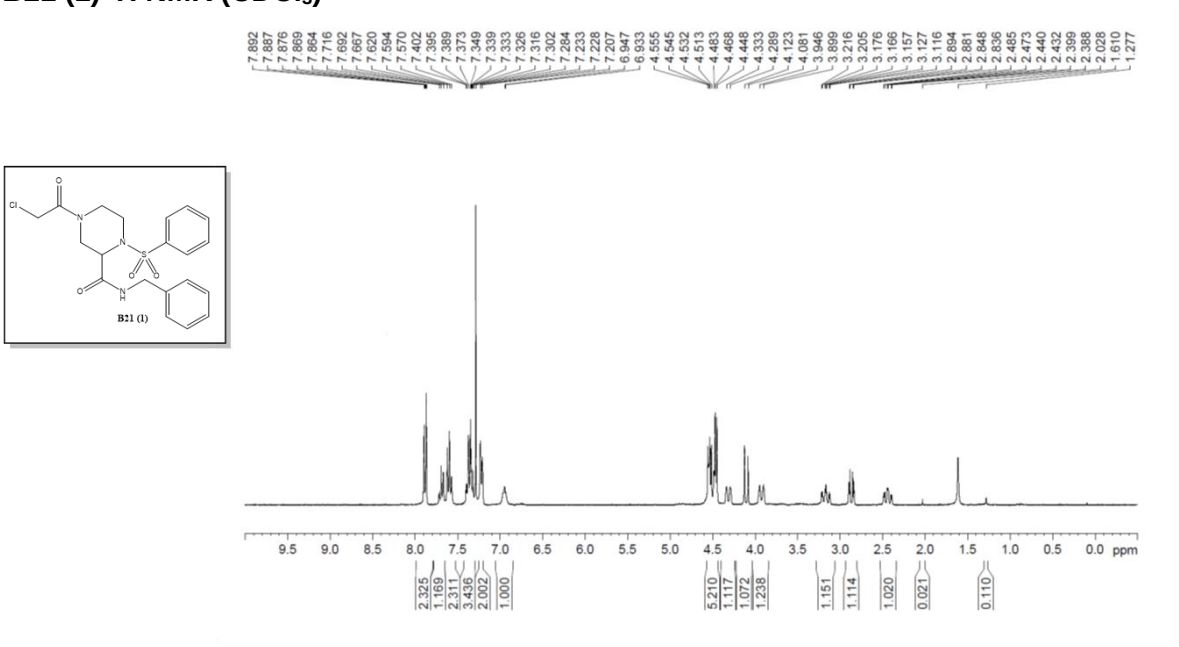
^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ 7.89-7.87 (2H, m), 7.71-7.68 (1H, m), 7.62-7.54 (2H, m), 7.18-7.16 (2H, m), 7.02-6.97 (2H, m), 6.87-6.86 (1H, m), 4.73-4.72 (2H, m), 4.55-4.49 (3H, m), 4.43-4.39 (2H, m), 4.36-4.29 (1H, m), 4.13-4.09 (1H, m), 3.94-3.90 (1H, m), 3.19-3.11 (1H, m), 2.89-2.84 (1H, m), 2.56-2.55 (1H, m), 2.47-2.40 (1H, m).

^{13}C NMR (126 MHz, $CDCl_3$, ppm) δ 167.20, 166.00, 157.16, 139.37, 133.89, 130.22, 129.99, 128.86, 126.98, 115.32, 78.46, 77.29, 77.04, 76.78, 75.67, 56.34, 55.87, 44.00, 43.51, 43.04, 41.00, 40.07.

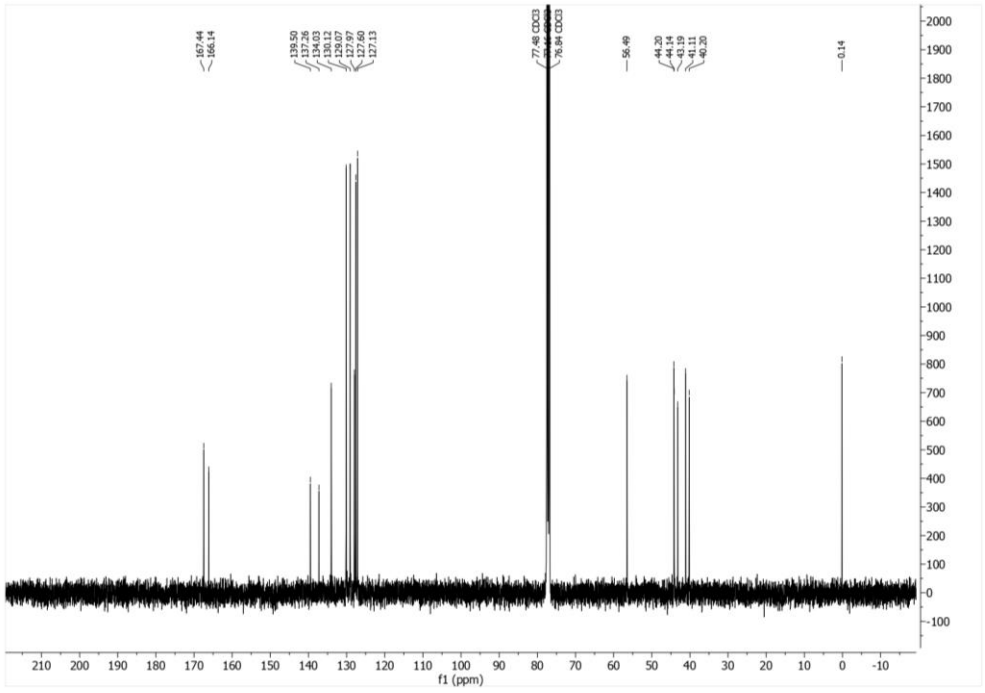
HRMS ESI-TOF (*m/z*): $[M+H]^+$ for $C_{23}H_{24}N_3O_5SCl$: expected 490.1198, measured 490.1197

NMR Spectra

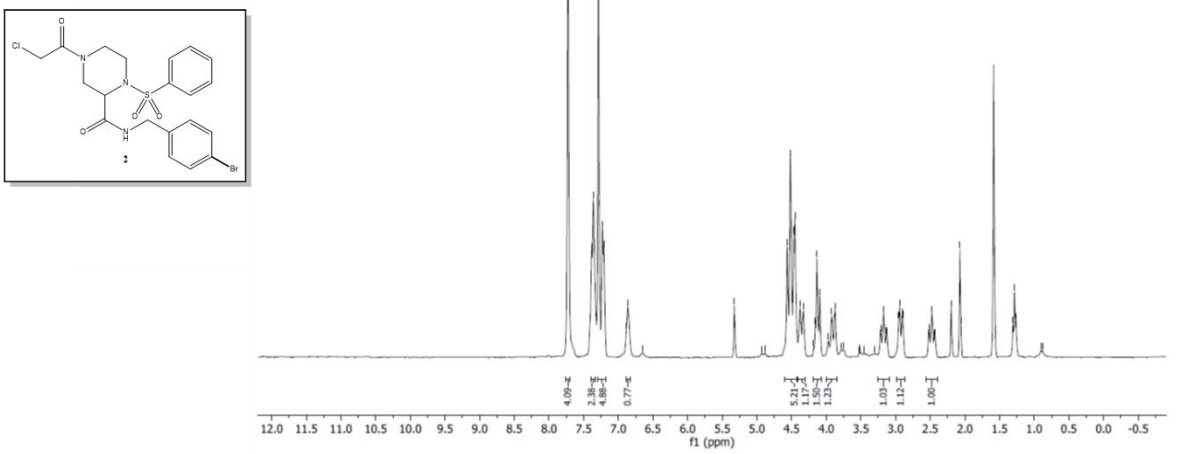
B21 (1) ^{1}H NMR ($CDCl_3$)



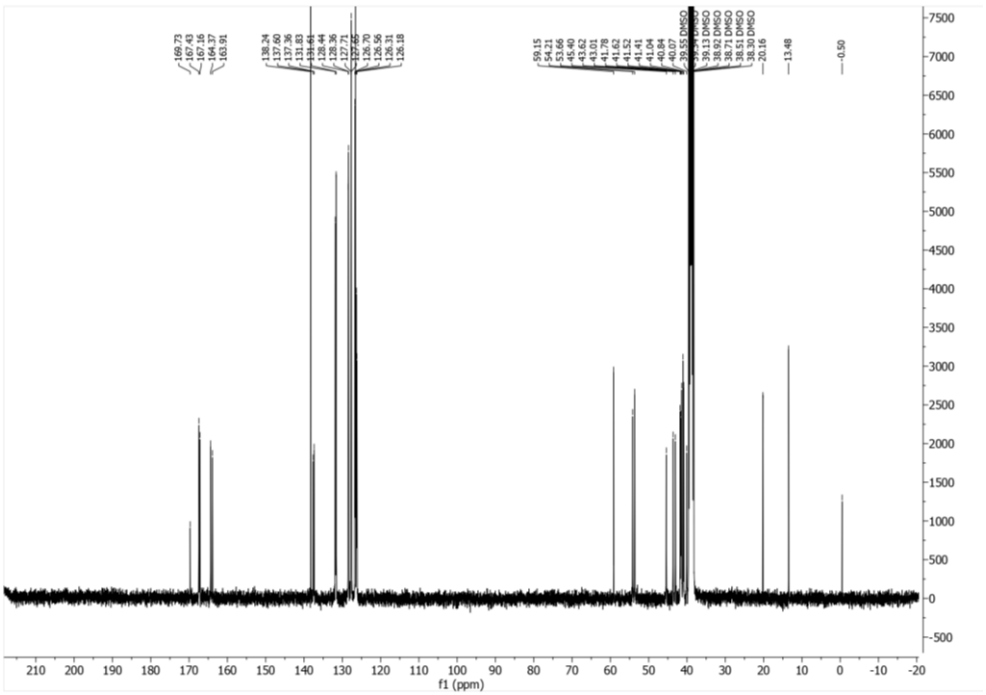
B21 (1) ^{13}C NMR ($CDCl_3$)



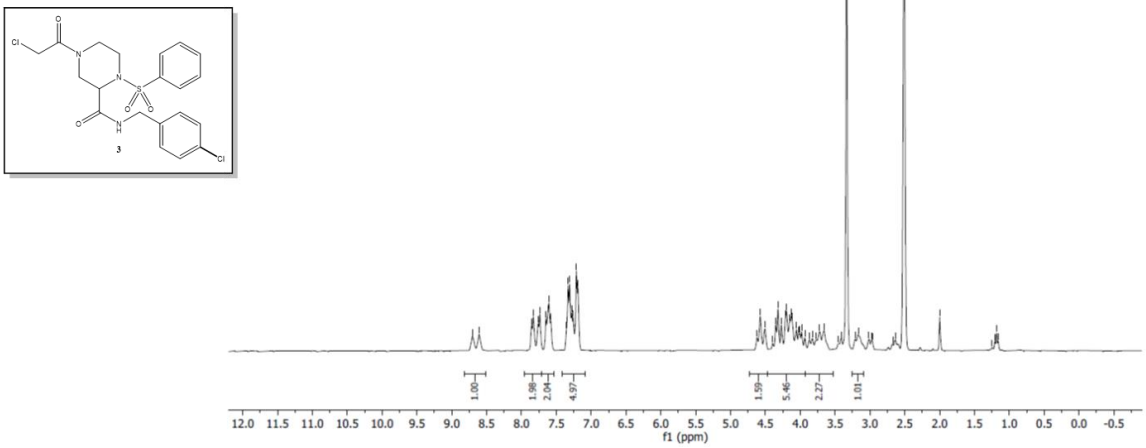
Compound 2 ^{1}H NMR ($CDCl_3$)



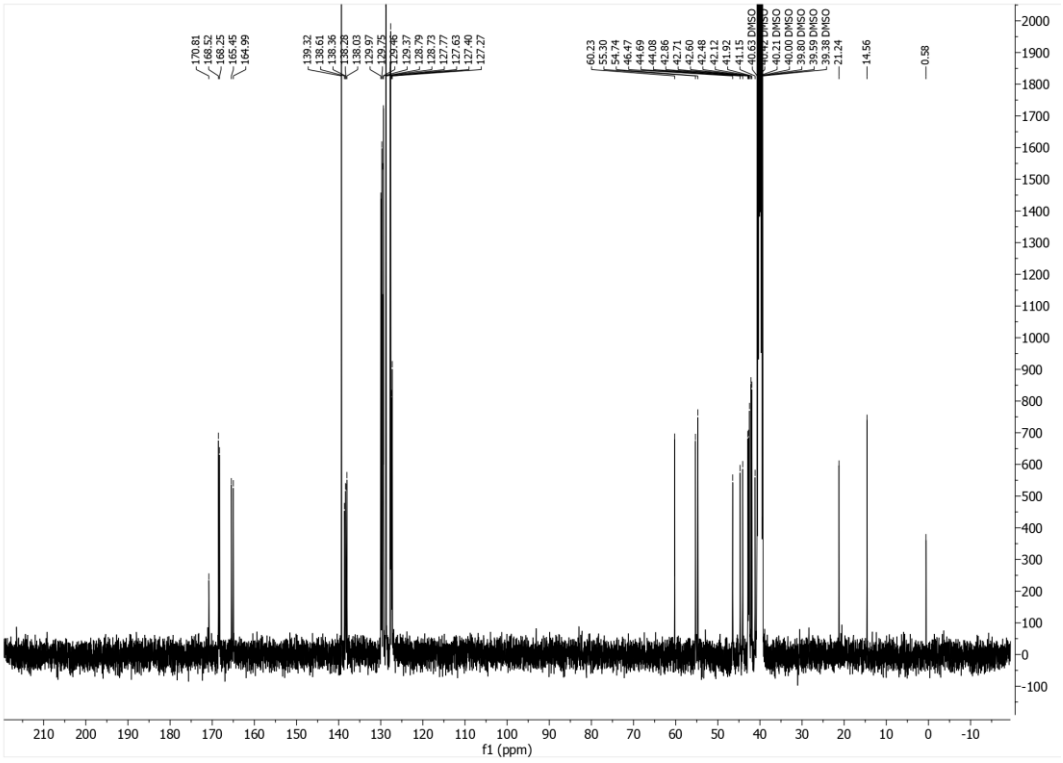
Compound 2 ^{13}C NMR ($DMSO\text{-}d_6$)



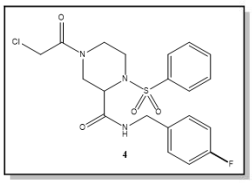
Compound 3 ^{1}H NMR (DMSO-d$_6$)

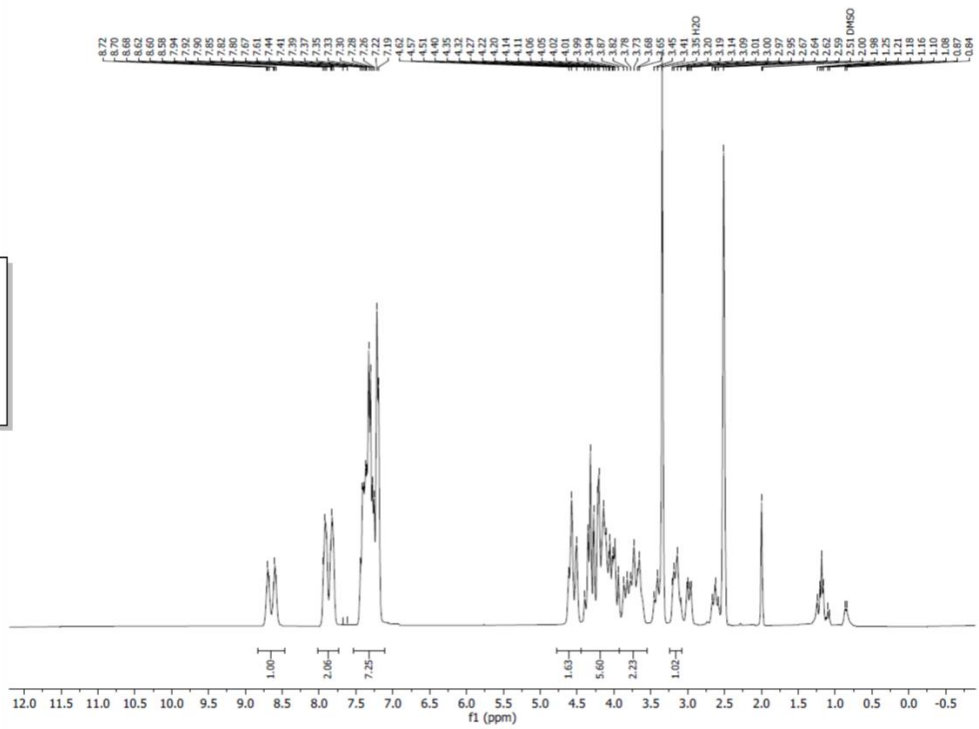


Compound 3 ^{13}C NMR (DMSO-d$_6$)

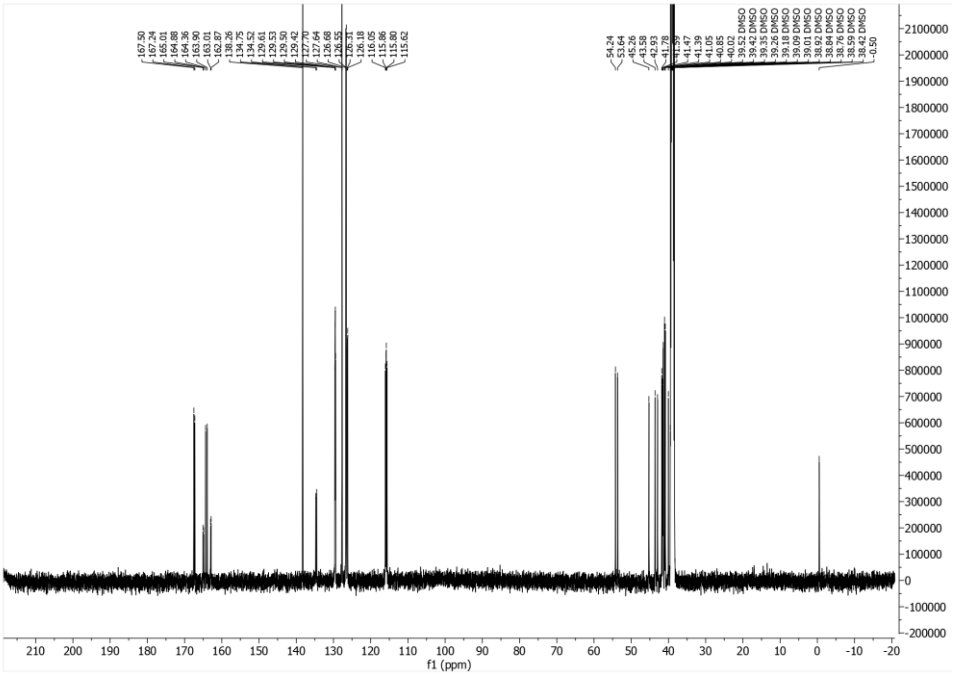


Compound 4 ^{1}H NMR (DMSO-d_6)

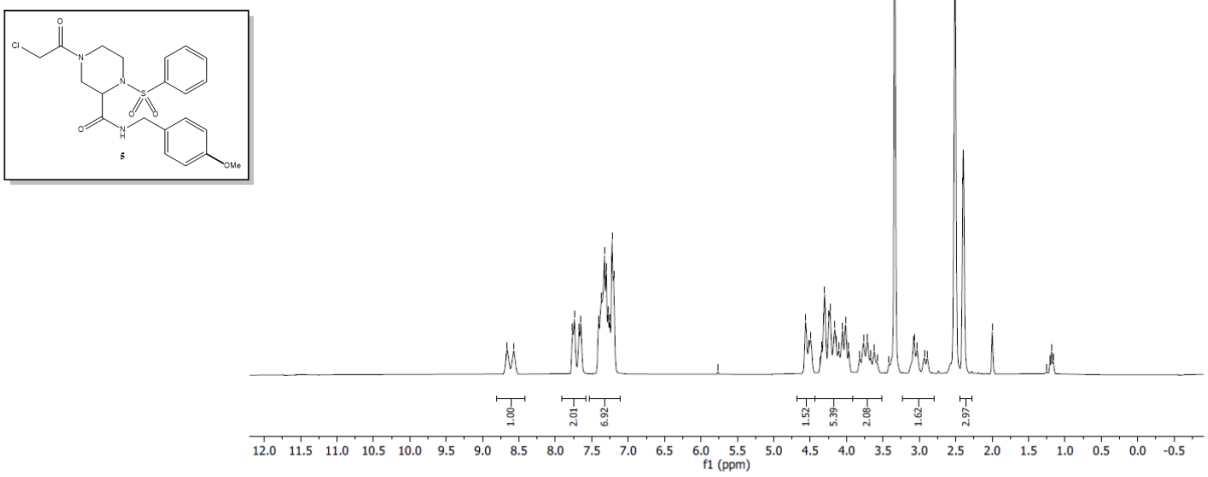




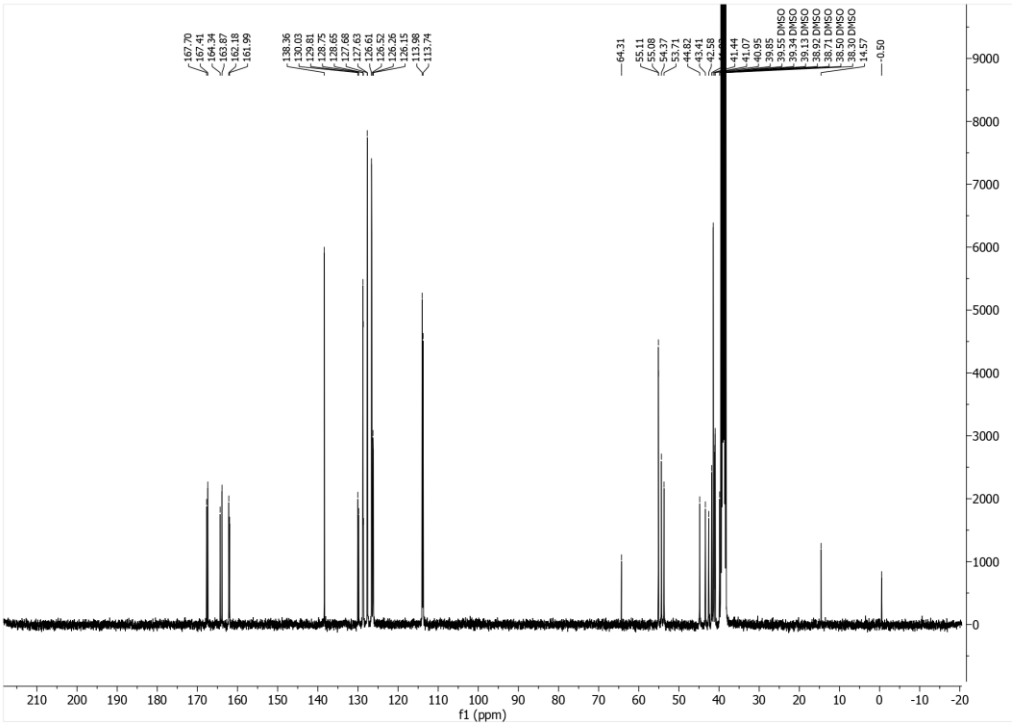
Compound 4 ^{13}C NMR (DMSO-d_6)



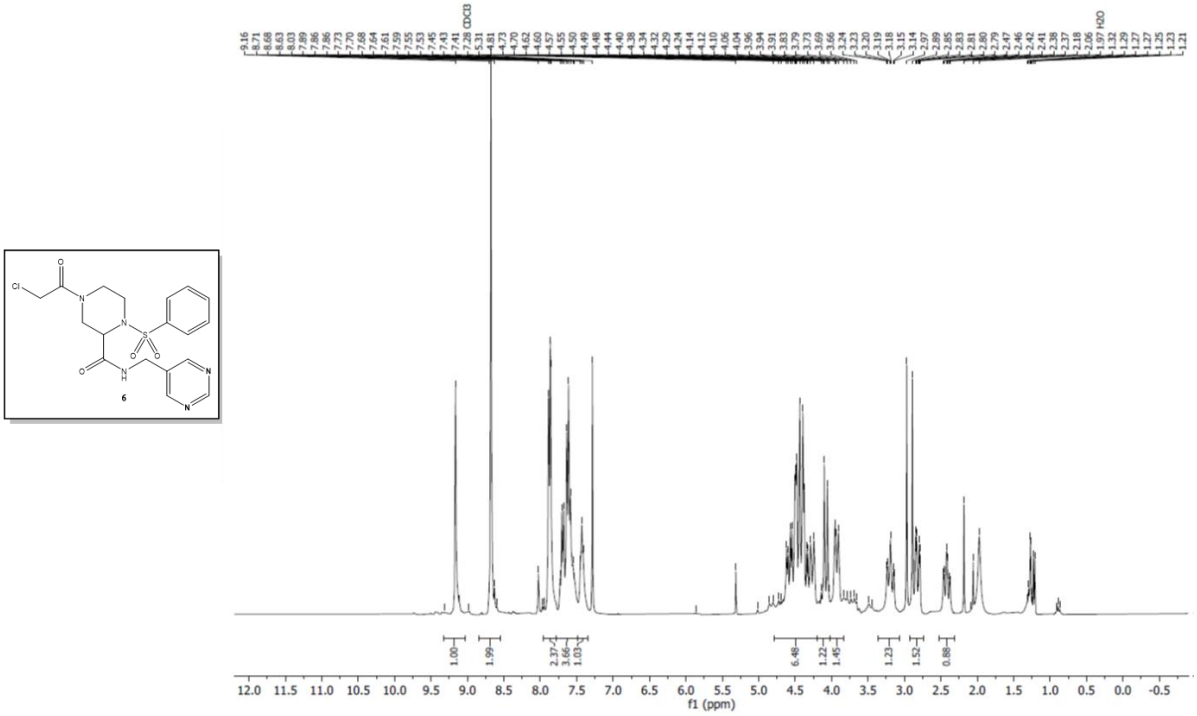
Compound 5 ^{1}H NMR (DMSO-d_6)



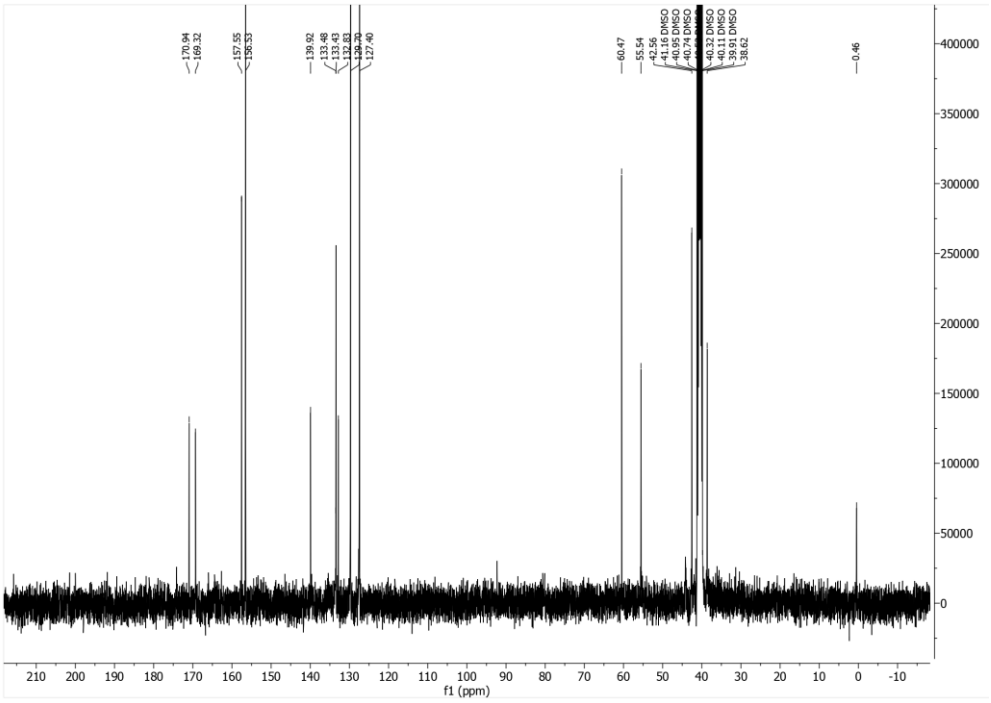
Compound 5 ^{13}C NMR (DMSO-d_6)



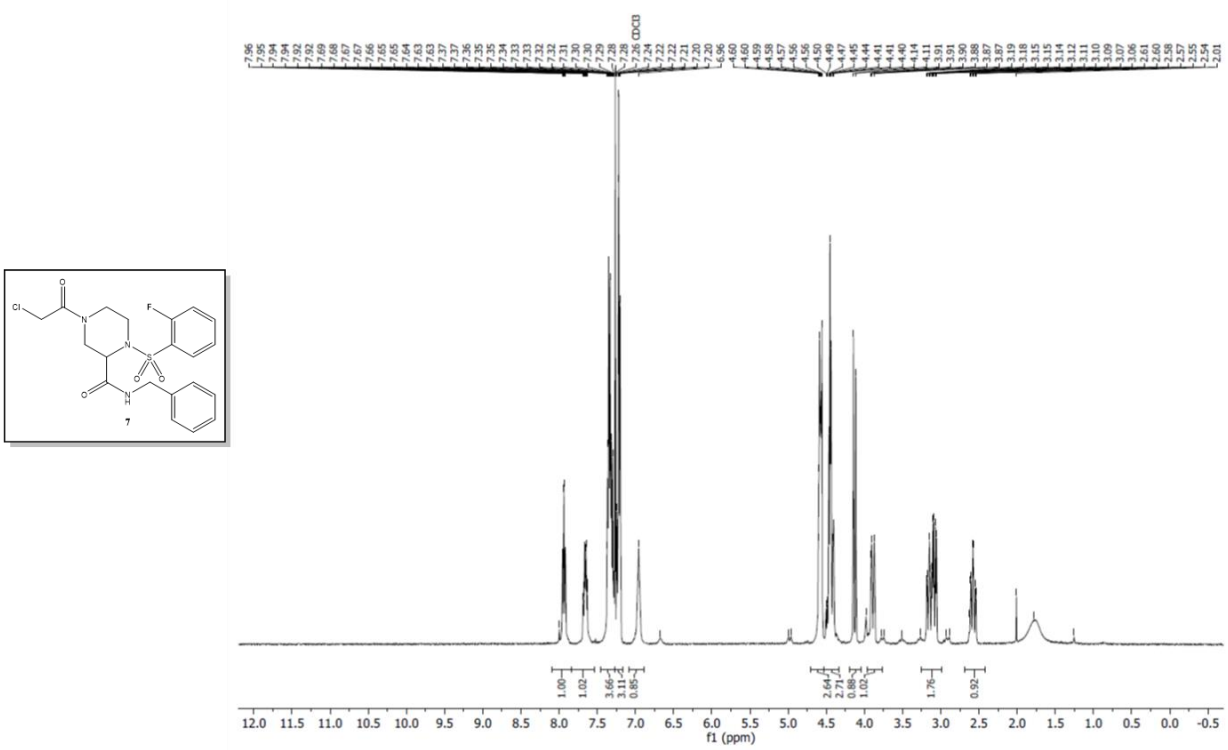
Compound 6 ^{1}H NMR ($CDCl_3$)



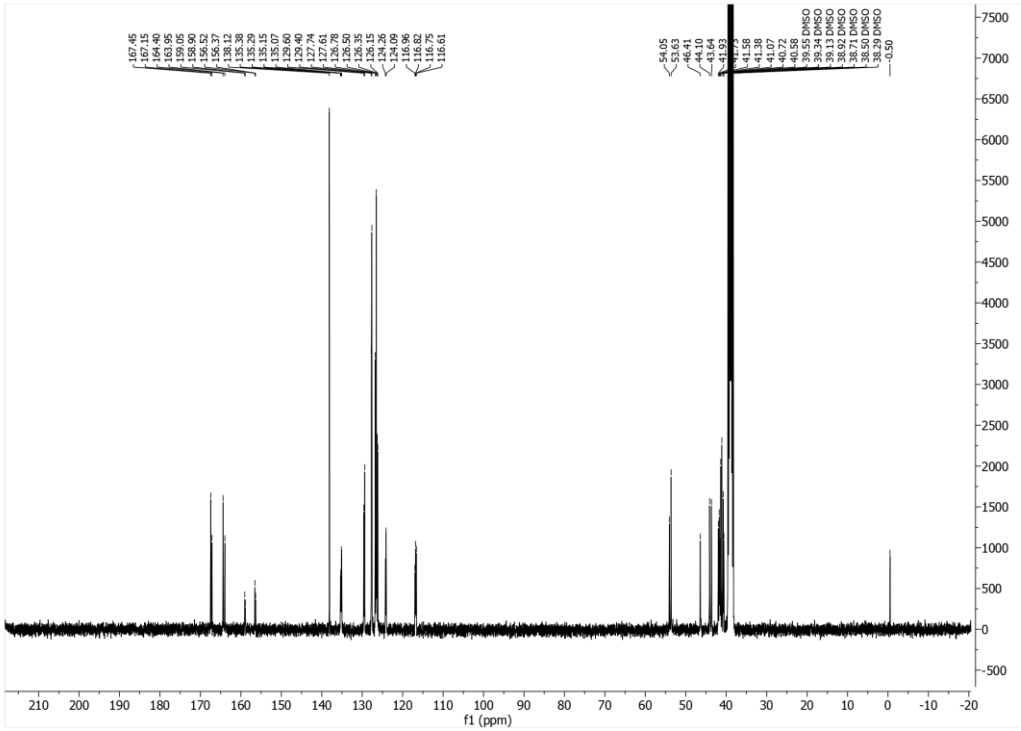
Compound 6 ^{13}C NMR (DMSO-d_6)



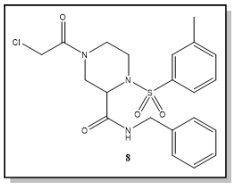
Compound 7 ^{1}H NMR ($CDCl_3$)

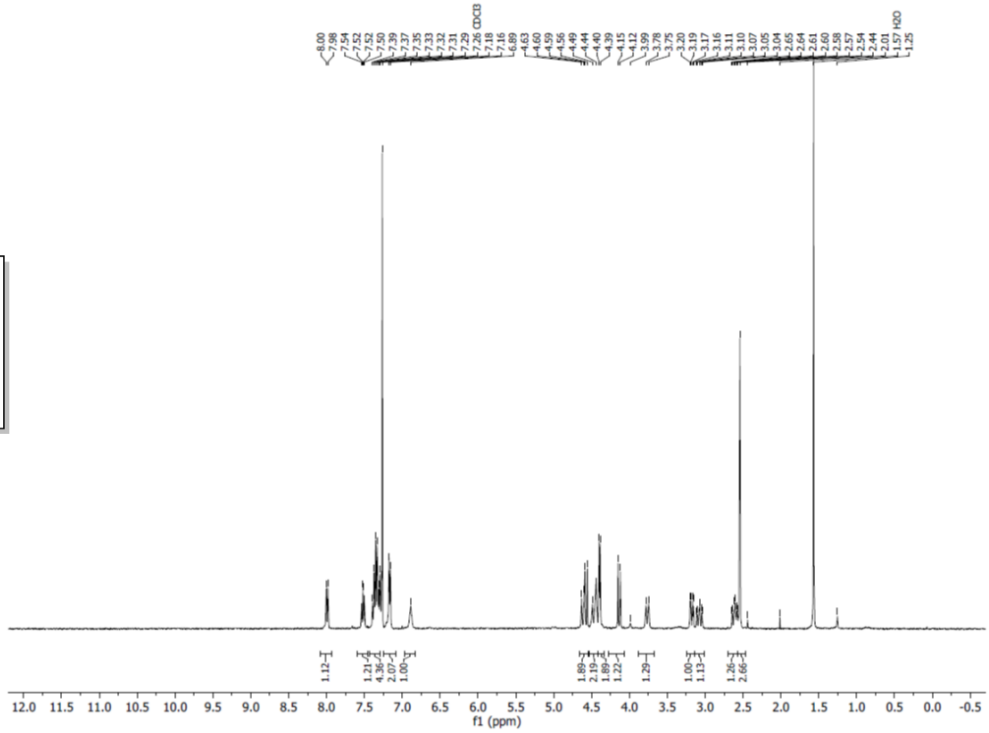


Compound 7 ^{13}C NMR (DMSO-d_6)

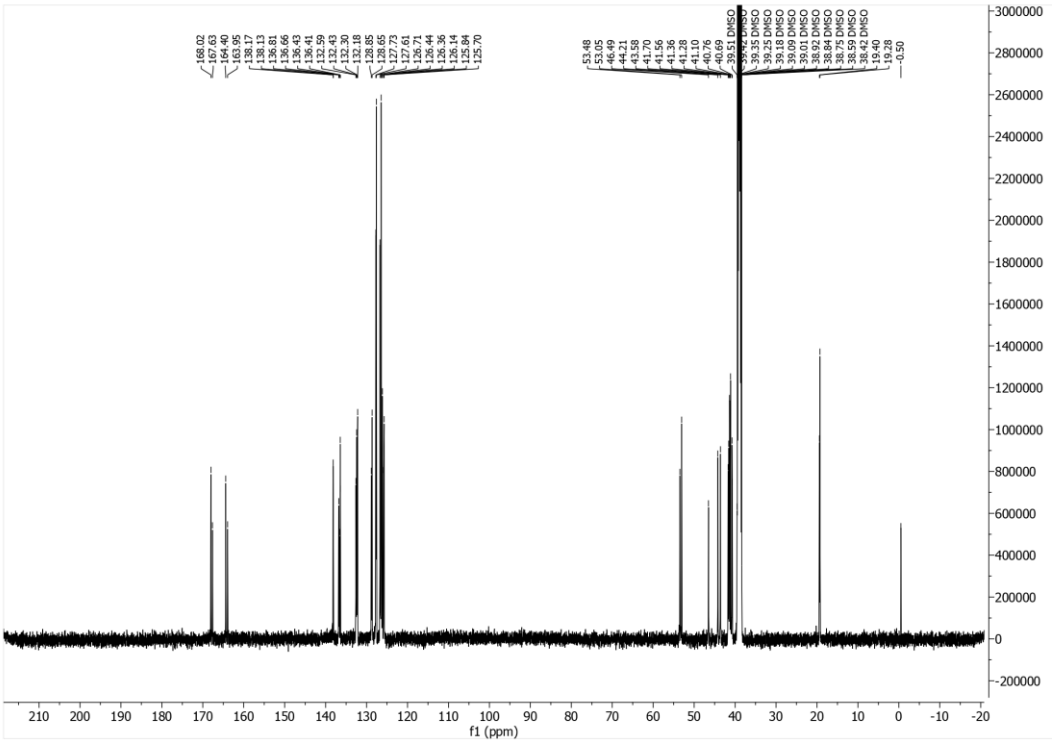


Compound 8 ^{1}H NMR ($CDCl_3$)

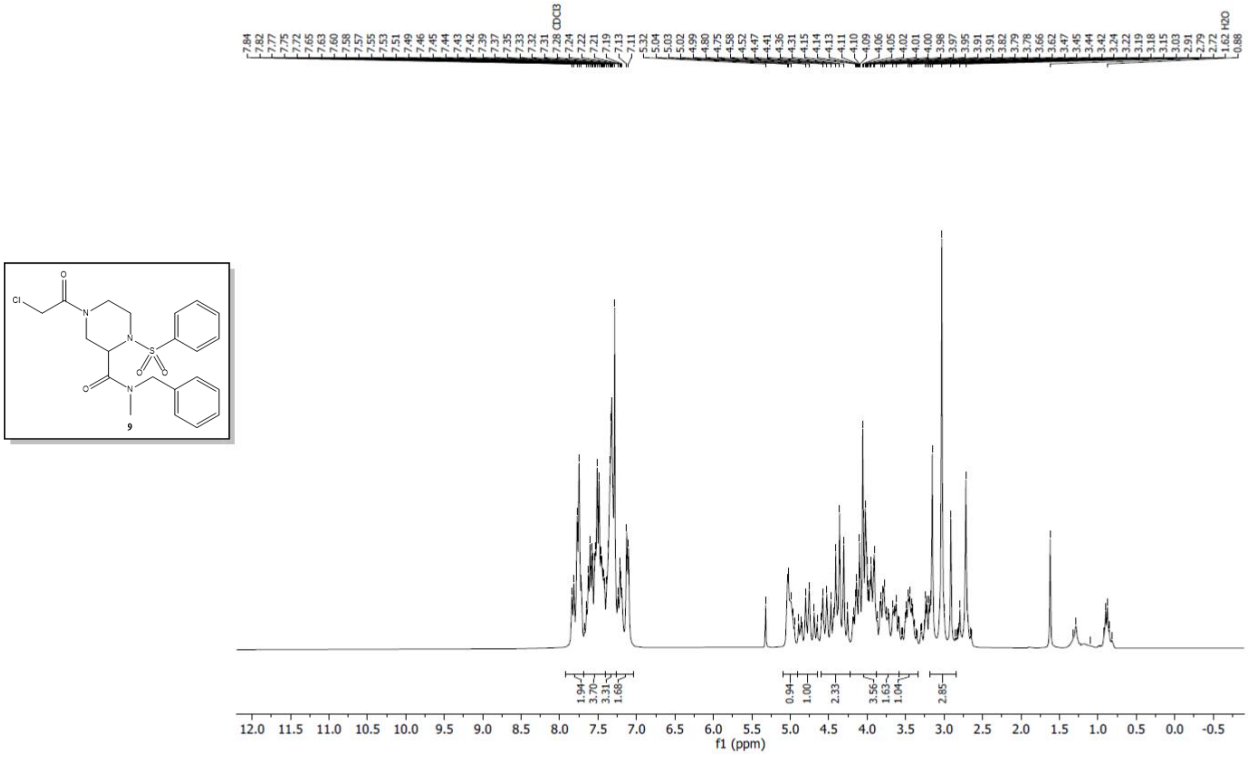




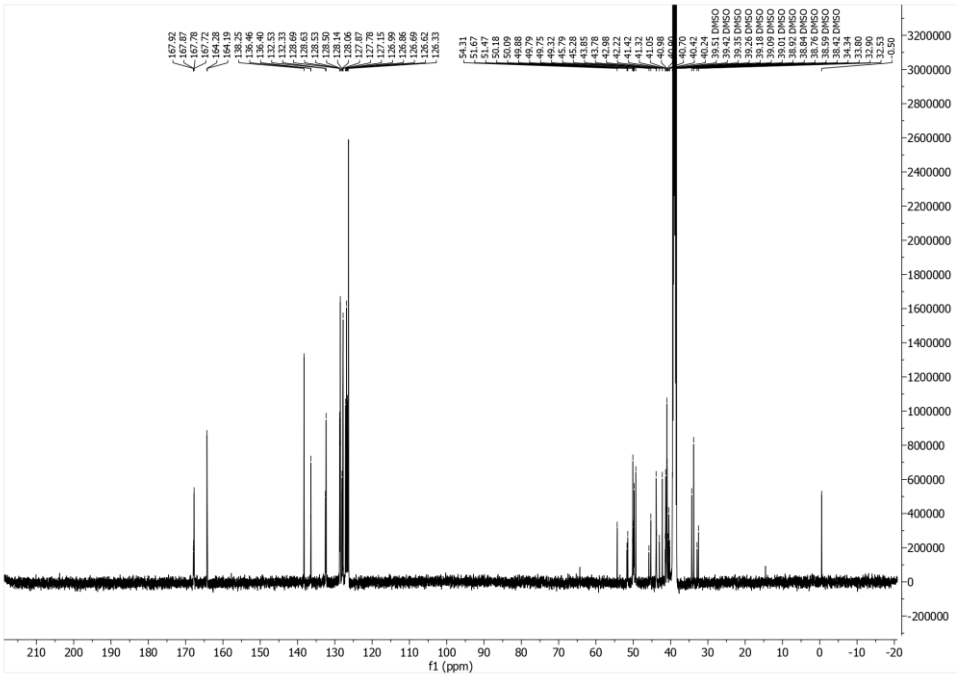
Compound 8 ^{13}C NMR ($DMSO-d_6$)



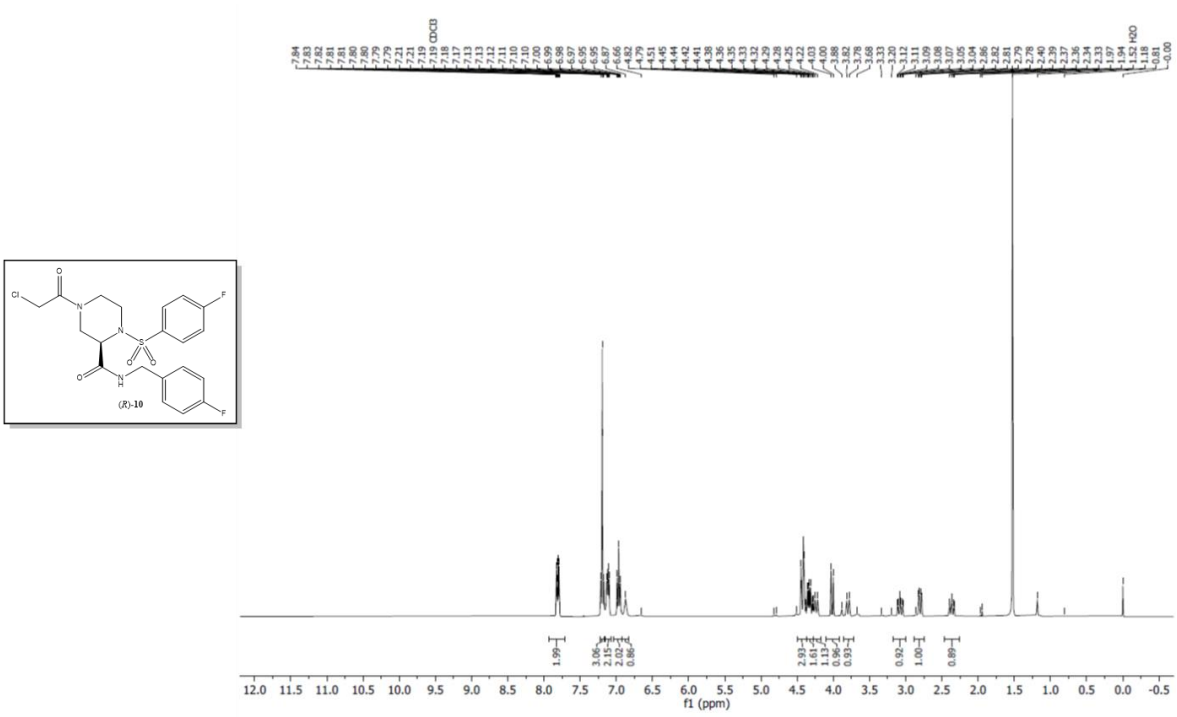
Compound 9 ^{1}H NMR ($CDCl_3$)



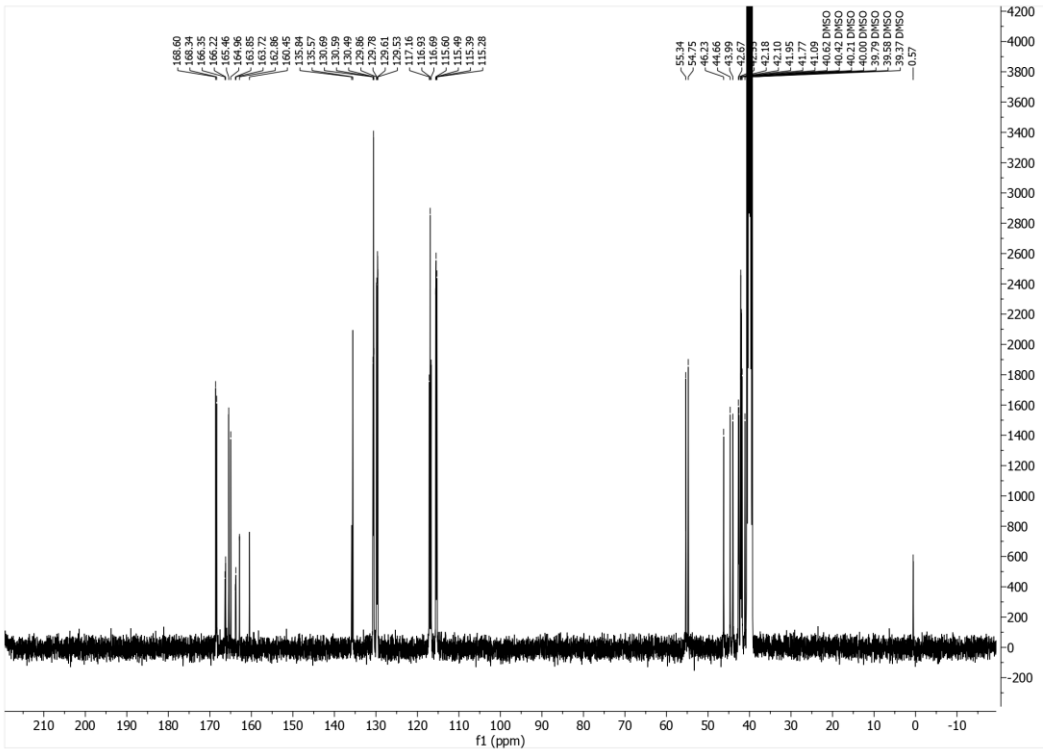
Compound 9 ^{13}C NMR (DMSO-d_6)



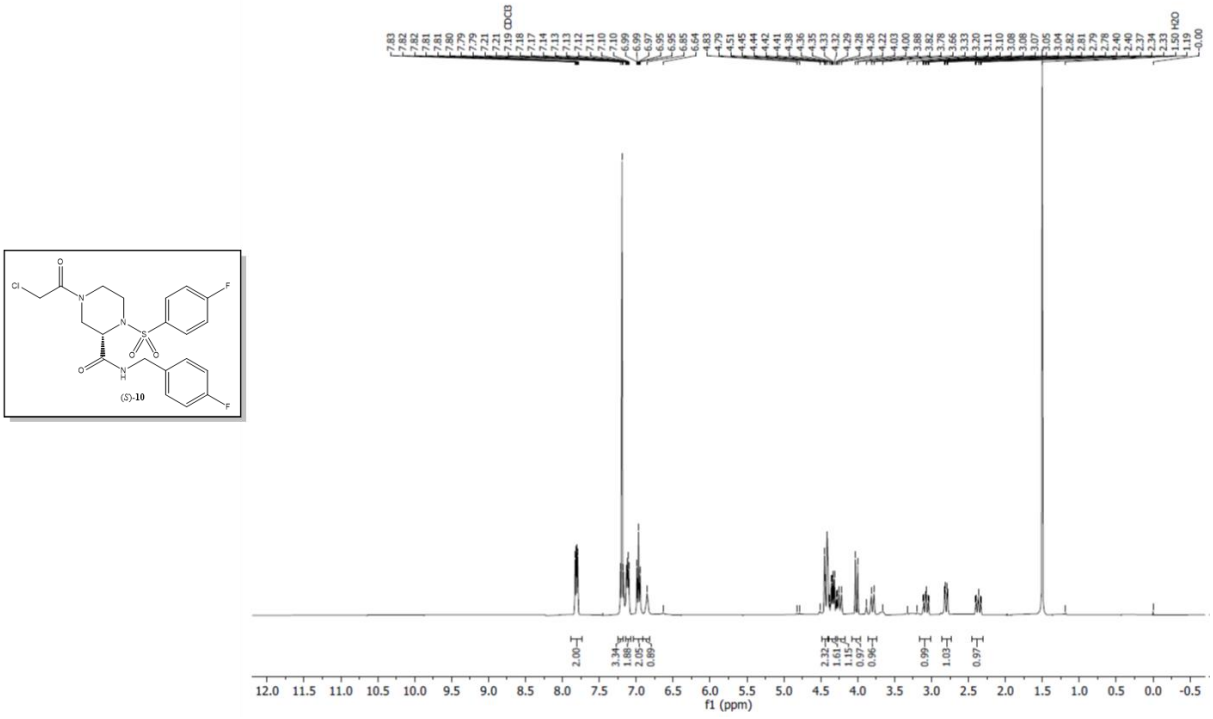
Compound (*R*)-10 ^{1}H NMR ($CDCl_3$)



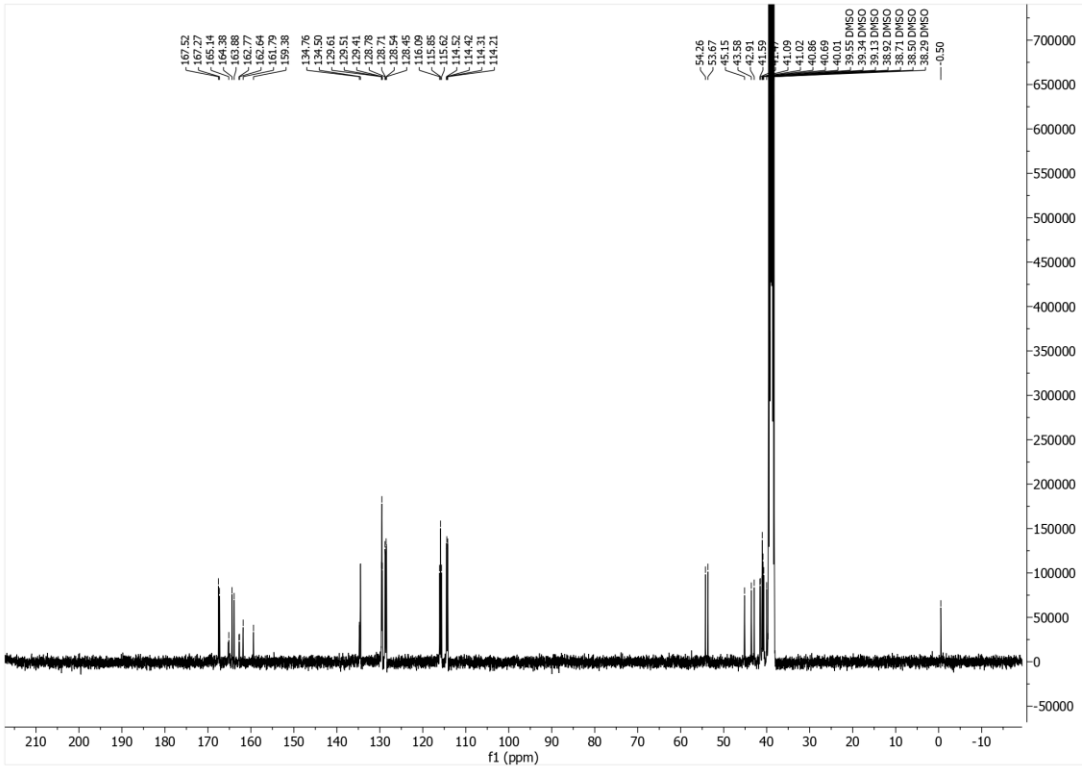
Compound (*R*)-10 ^{13}C NMR (DMSO-d_6)



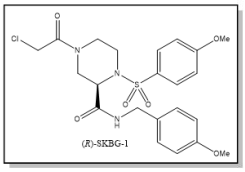
Compound (*S*)-10 ^{1}H NMR ($CDCl_3$)

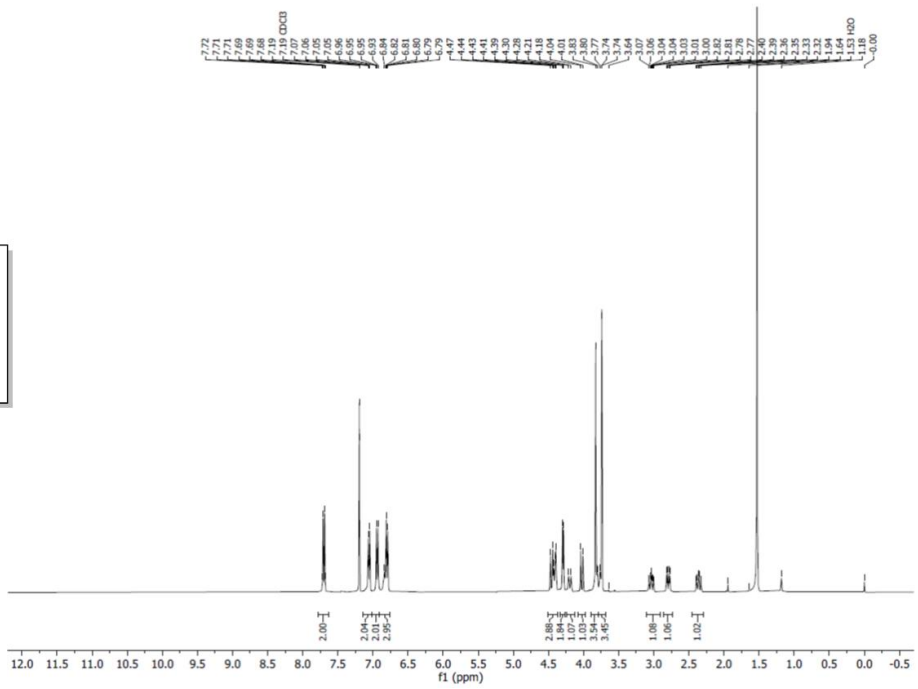


Compound (*S*)-10 ^{13}C NMR (DMSO-d_6)

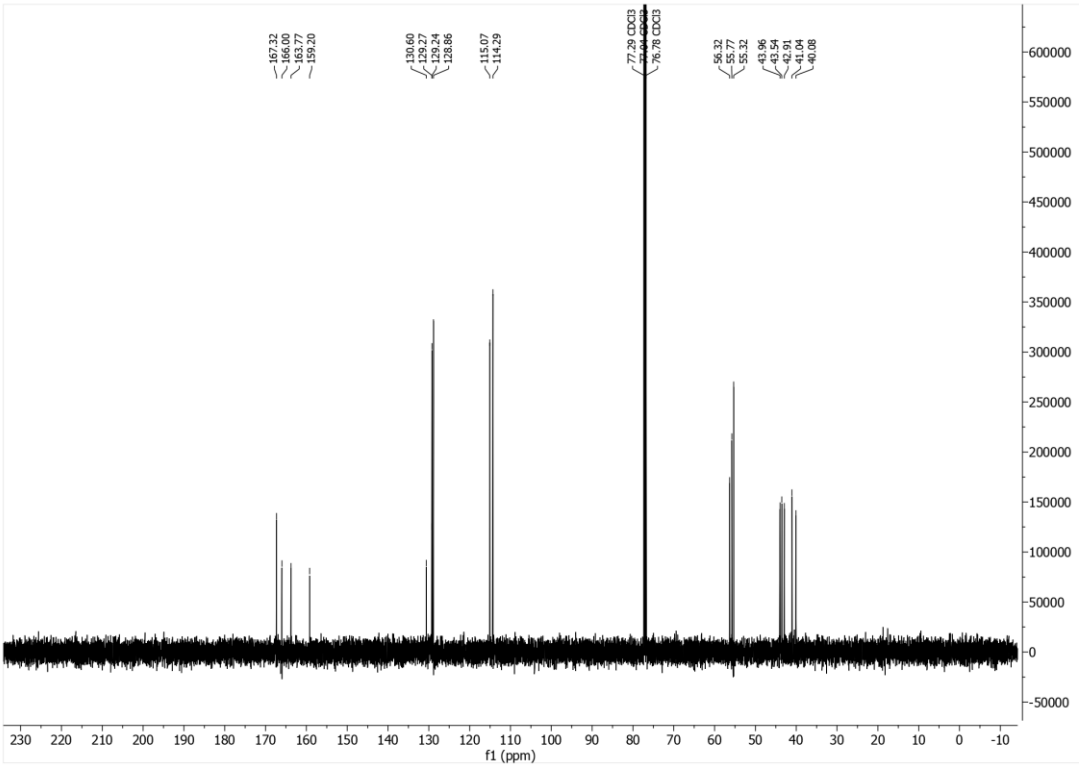


(*R*)-SKBG-1 ^{1}H NMR ($CDCl_3$)

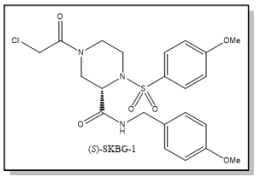


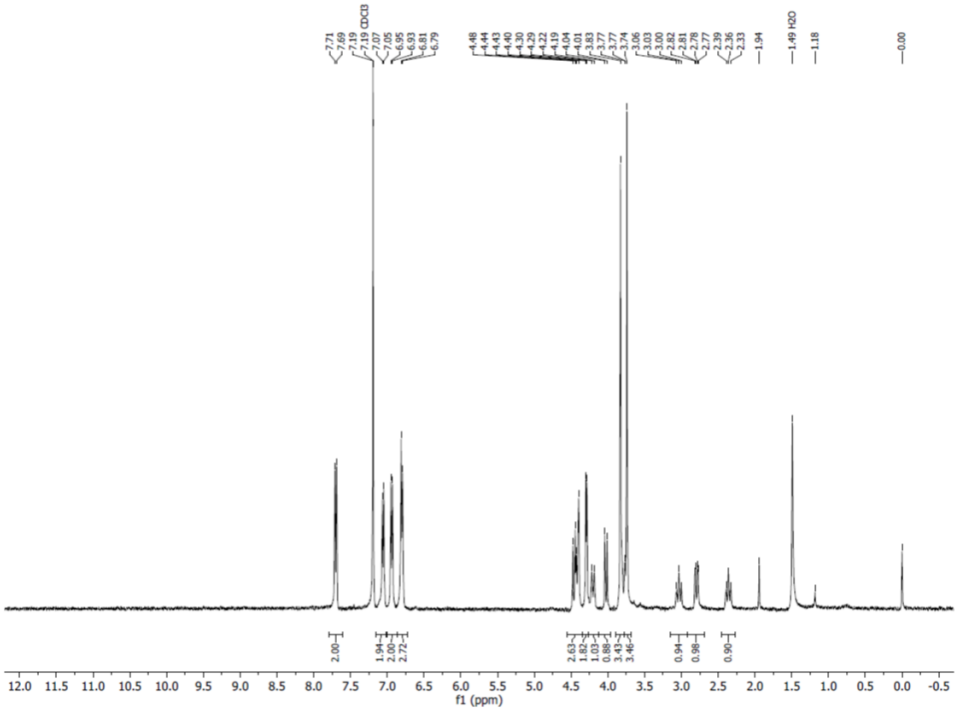


(*R*)-SKBG-1 ^{13}C NMR ($CDCl_3$)

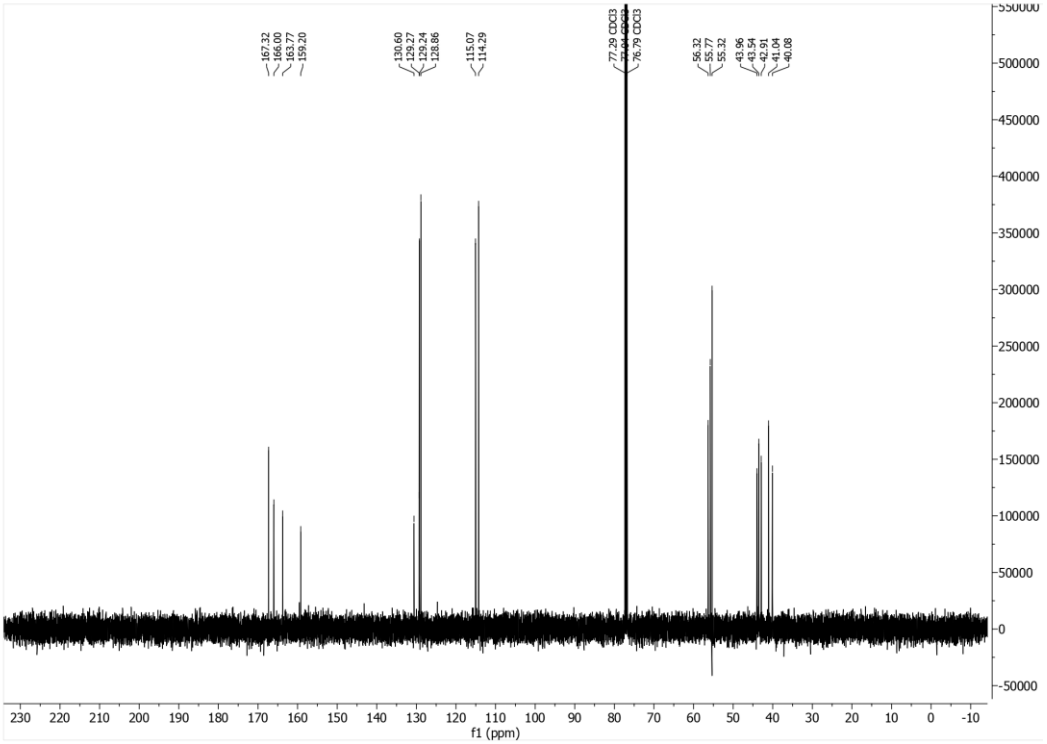


(*S*)-SKBG-1 ^{1}H NMR ($CDCl_3$)

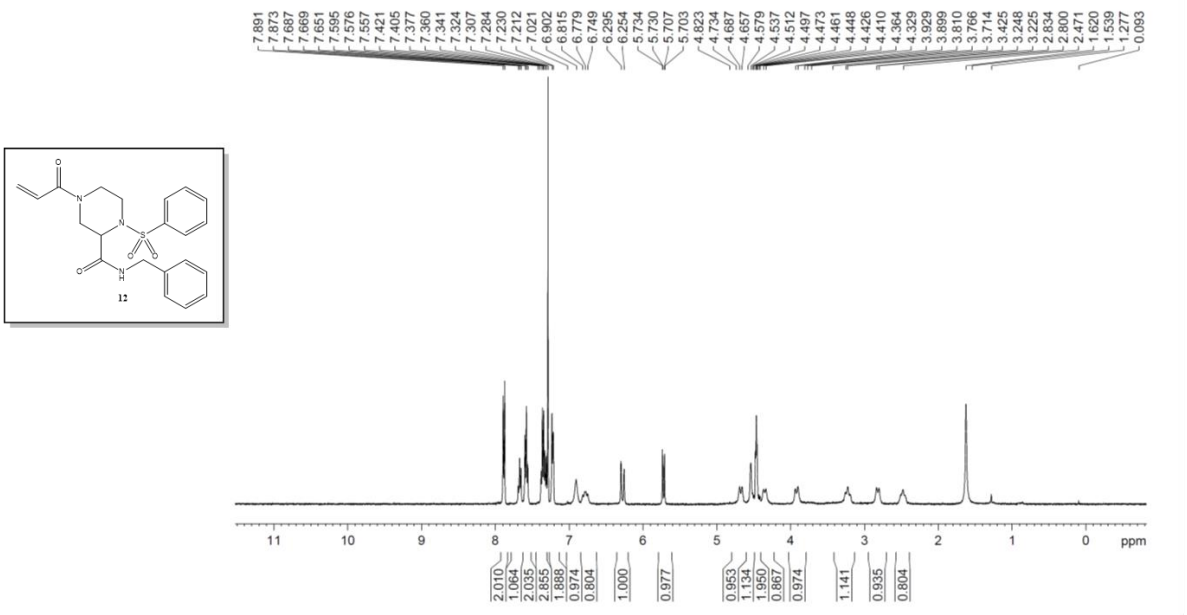




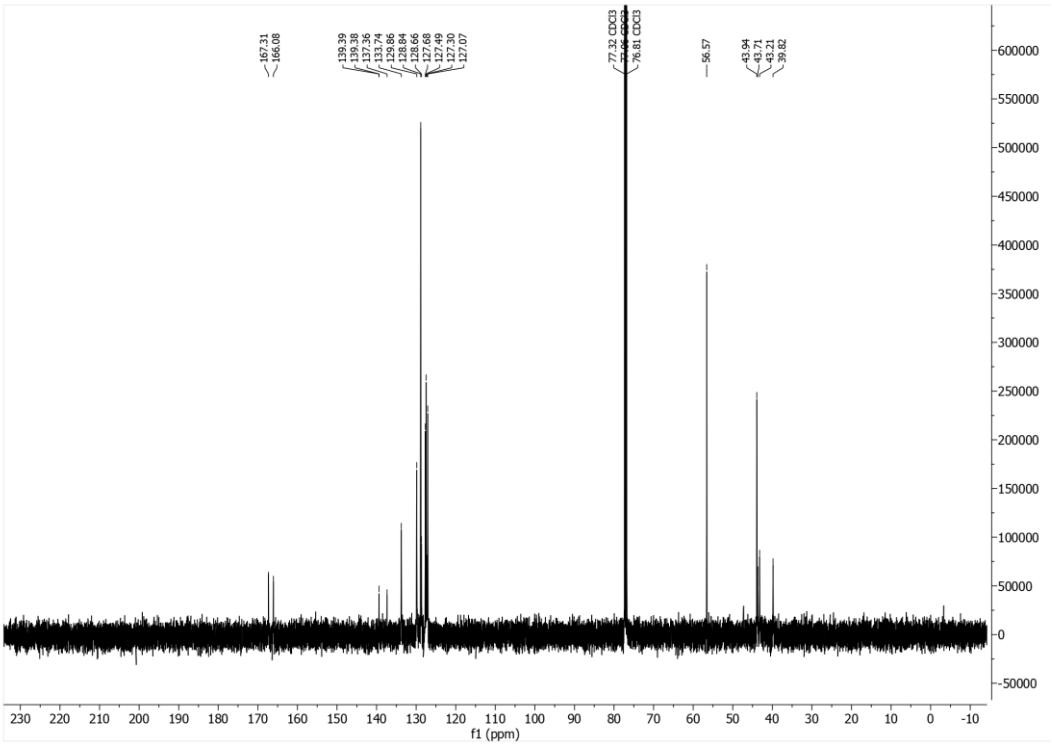
(*S*)-SKBG-1 ^{13}C NMR ($CDCl_3$)



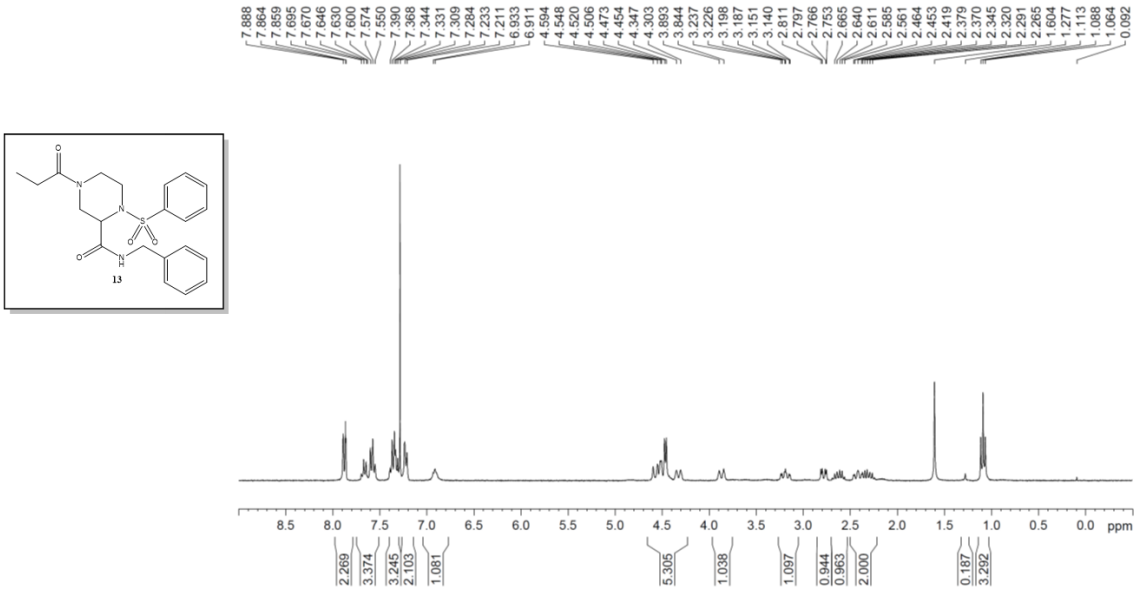
Compound 12 ^{1}H NMR ($CDCl_3$)



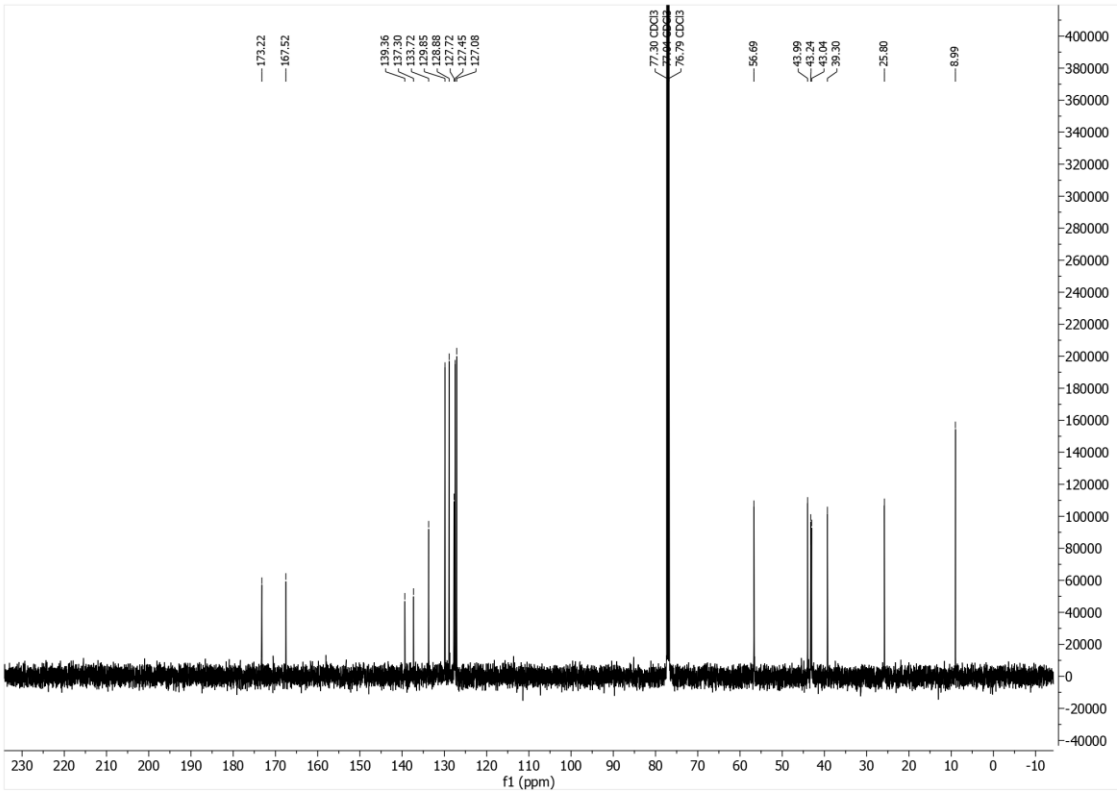
Compound 12 ^{13}C NMR ($CDCl_3$)



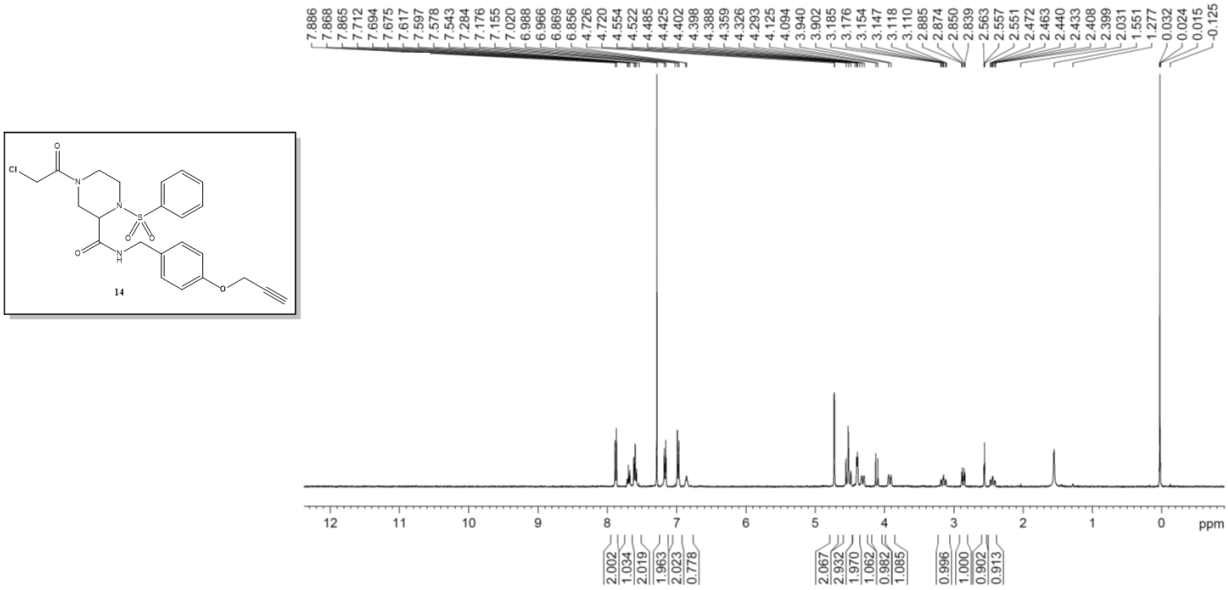
Compound 13 ^{1}H NMR ($CDCl_3$)



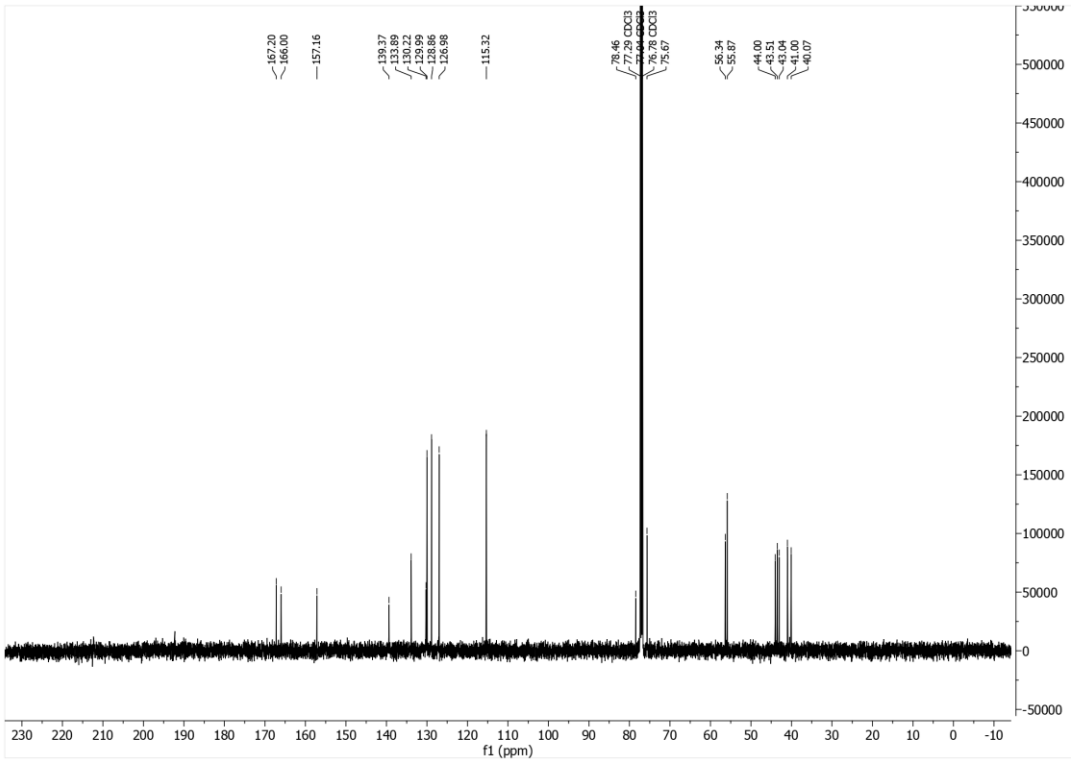
Compound 13 ^{13}C NMR ($CDCl_3$)



Alkyne probe 14 ^{1}H NMR ($CDCl_3$)

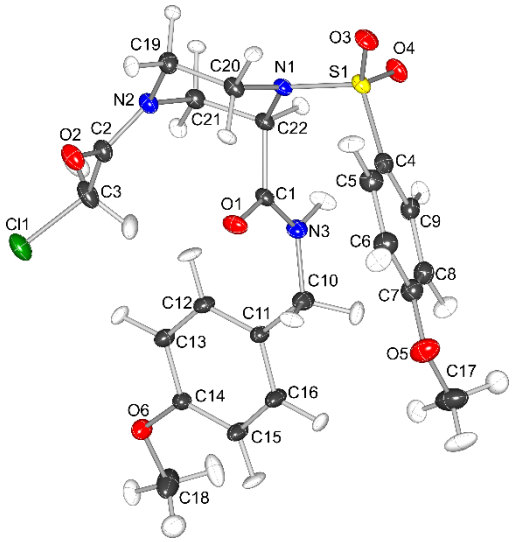


Alkyne probe 14 ^{13}C NMR ($CDCl_3$)



Crystallography of (*R*)-SKBG-1

Crystal Data and Experimental



Experimental. Single colorless prism-shaped crystals of (*R*)-SKBG-1 were crystallized from a mixture of ethanol and chloroform by slow evaporation. A suitable crystal with dimensions 0.33 × 0.12 × 0.10 mm^3 was selected and mounted on a loop with paratone on a Rigaku Synergy-S diffractometer. The crystal was kept at a steady T = 100.0(1) K during data collection. The structure was solved with the **ShelXT** 2018/2 (Sheldrick, 2018) solution program using dual methods and by using **Olex2** 1.3-alpha (Dolomanov et al., 2009) as the graphical interface. The model was refined with **olex2.refine** 1.3-alpha (Bourhis et al., 2015) using full matrix least squares minimisation on $\boldsymbol{F^2}$.

Crystal Data. $C_{22}H_{26}ClN_3O_6S$, M_r = 495.987, orthorhombic, $P2_12_12_1$ (No. 19), a = 7.7661(1) Å, b = 15.3418(2) Å, c = 19.6444(2) Å, $a = b = g = 90^\circ$, V = 2340.55(5) Å^3, T = 100.02(10) K, Z = 4, Z' = 1, m(Cu K$_a$) = 2.657, 13148 reflections measured, 4475 unique (R_{int} = 0.0431) which were used in all calculations. The final wR_2 was 0.0465 (all data) and R_1 was 0.0212 (I≥2 s(I)).

Compound	(*R*)-SKBG-1
Formula	$C_{22}H_{26}ClN_3O_6S$
$D_{calc.}$/ g cm^{-3}	1.408
m/mm^{-1}	2.657
Formula Weight	495.987
Color	colorless
Shape	prism-shaped
Size/mm^3	0.33×0.12×0.10

T/K	100.02(10)
Crystal System	orthorhombic
Flack Parameter	0.013(11)
Hooft Parameter	0.013(7)
Space Group	$P2_12_12_1$
a/Å	7.7661(1)
b/Å	15.3418(2)
c/Å	19.6444(2)
a/°	90
b/°	90
g/°	90
V/Å^3	2340.55(5)
Z	4
Z'	1
Wavelength/Å	1.54184
Radiation type	Cu K$_a$
Q_{min}/°	3.66
Q_{max}/°	73.01
Measured Refl's.	13148
Indep't Refl's	4475
Refl's I≥2 s(I)	4361
R_{int}	0.0431
Parameters	533
Restraints	252
Largest Peak	0.2379
Deepest Hole	-0.1357
GooF	1.0514
wR_2 (all data)	0.0465
wR_2	0.0461
R_1 (all data)	0.0218
R_1	0.0212

Structure Quality Indicators

Reflections: d min (Cu\a) 2θ=146.0° 0.81 | I/σ(I) 24.1 | Rint 4.31% | CAP 130.0° 97% to 146.0° 100

Refinement: Shift 0.000 | Max Peak 0.2 | Min Peak -0.1 | GooF 1.051 | Flack .013(11)

A colourless prism-shaped-shaped crystal with dimensions 0.33 × 0.12 × 0.10 mm^3 was mounted on a loop with paratone. Data were collected using a Rigaku Synergy-S diffractometer equipped with an Oxford Cryosystems low-temperature device operating at T = 100.02(10) K.

Data were measured using *w* scans using Cu K_a radiation. The diffraction pattern was indexed and the total number of runs and images was based on the strategy calculation from the program CrysAlisPro 1.171.41.108a (Rigaku OD, 2021). The maximum resolution that was achieved was Q = 73.01° (0.81 Å).

The unit cell was refined using CrysAlisPro 1.171.41.108a (Rigaku OD, 2021) on 11351 reflections, 86% of the observed reflections.

Data reduction, scaling and absorption corrections were performed using CrysAlisPro 1.171.41.108a (Rigaku OD, 2021). The final completeness is 99.84 % out to 73.01° in Q. A numerical absorption correction based on gaussian integration over a multifaceted crystal model was performed using CrysAlisPro 1.171.41.108a (Rigaku Oxford Diffraction, 2021). An empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm was also applied. The absorption coefficient m of this material is 2.657 mm^{-1} at this wavelength (l = 1.54184Å) and the minimum and maximum transmissions are 0.338 and 1.000.

The structure was solved and the space group $P2_12_12_1$ (# 19) determined by the ShelXT 2018/2 (Sheldrick, 2018) structure solution program using dual methods and refined by full matrix least squares minimisation on $\boldsymbol{F^2}$ using version of **olex2.refine** 1.3-alpha (Bourhis et al., 2015). All atoms, even hydrogen atoms, were refined anisotropically. Hydrogen atom positions were located from the electron densities and freely refined using Hirshfeld scattering factors. Refinement was by using NoSpherA2, an implementation of non-spherical atom-form-factors (F. Kleemiss, H. Puschmann, O. Dolomanov, S.Grabowsky - https://doi.org/10.1039/D0SC05526C – 2020). NoSpherA2 implementation of HAR makes use of tailor-made aspherical atomic form factors calculated from a Hirshfeld-partitioned electron density (ED) not from spherical-atom form factors. The ED was calculated from a Gaussian basis set single determinant SCF wavefunction from DFT using selected functionals for a fragment of this crystal. The following options were used: SOFTWARE: ORCA PARTITIONING: NoSpherA2 INT ACCURACY: High METHOD: B3LYP BASIS SET: def2-TZVP CHARGE: 0 MULTIPLICITY: 1 DATE: 2021-07-04_17-01-48

The Flack parameter was refined to 0.013(11). Determination of absolute structure using Bayesian statistics on Bijvoet differences using the Olex2 results in 0.013(7). Note: The Flack parameter is used to determine chirality of the crystal studied, the value should be near 0, a value of 1 means that the stereochemistry is wrong and the model should be inverted. A value of 0.5 means that the crystal consists of a racemic mixture of the two enantiomers.

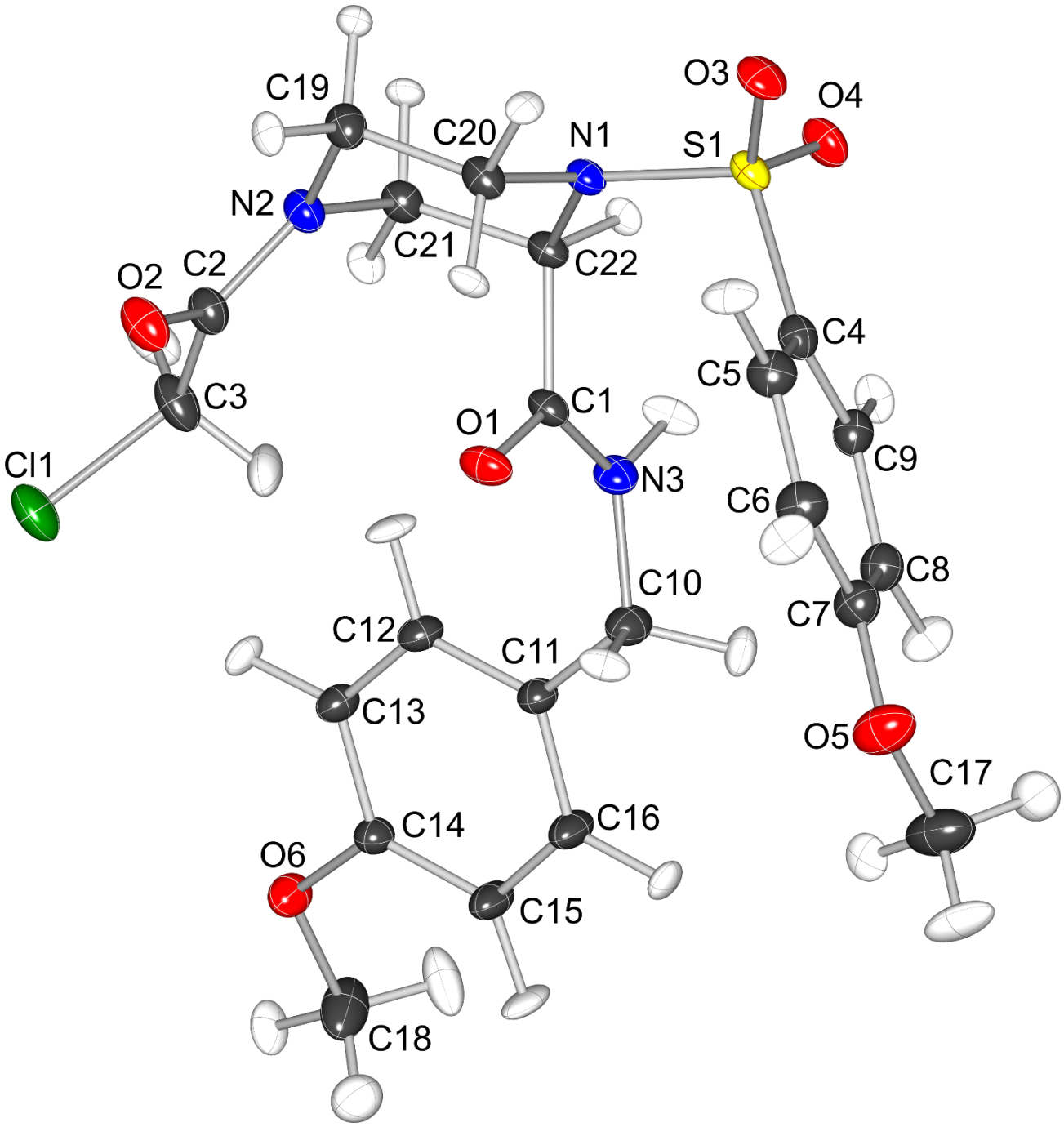
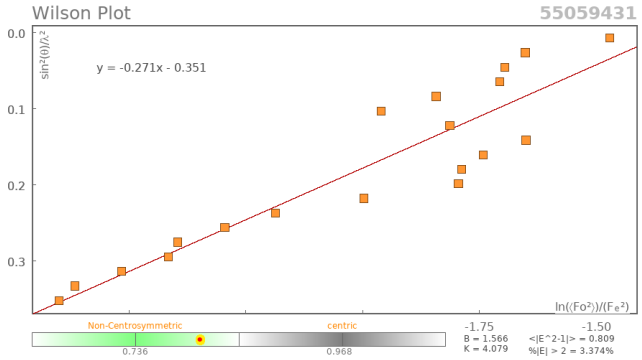
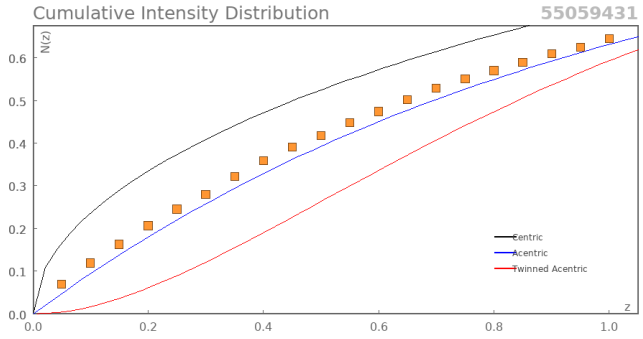
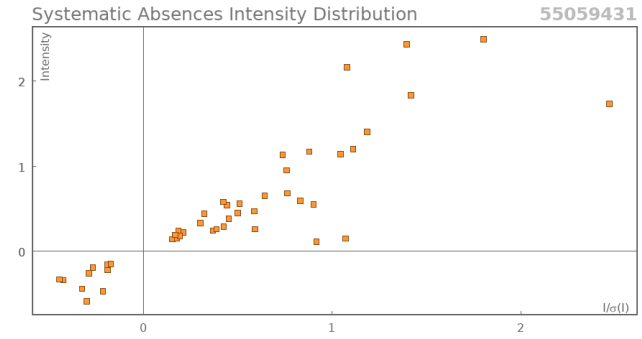


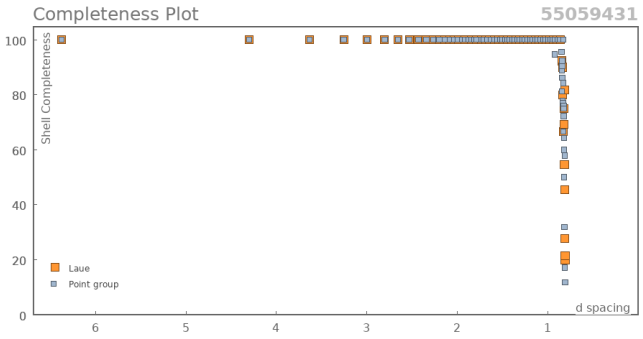
Figure: A thermal ellipsoid representation of molecular structure showing the orientation of the substituents. There is a single molecule in the asymmetric unit, which is represented by the reported sum formula. In other words: Z is 4 and Z' is 1. The chiral center has R configuration.

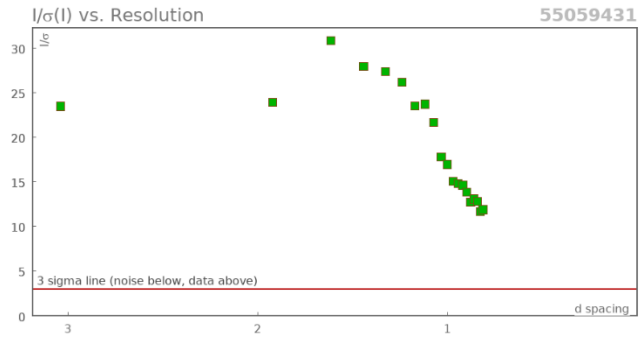
Data Plots: Diffraction Data



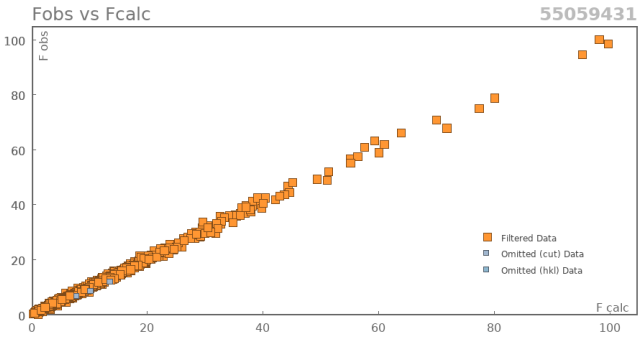


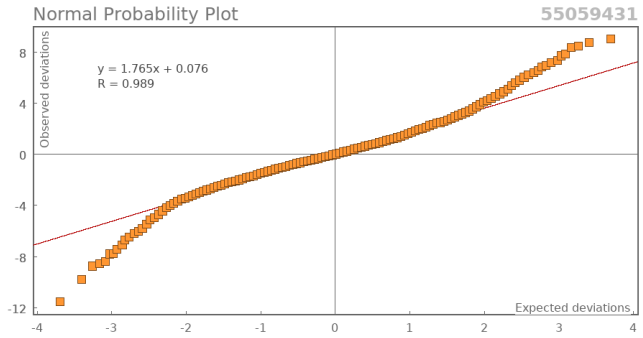






Data Plots: Refinement and Data





Images of the Crystal on the Diffractometer

Table: Fractional Atomic Coordinates (×10^4) and Equivalent Isotropic Displacement Parameters (Å^2×10^3) for (*R*)-SKBG-1. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij}.

Atom	x	y	z	U_{eq}
Cl1	-473.5(4)	2814.91(17)	3330.01(16)	26.01(8)
S1	5813.2(4)	6802.03(16)	3164.98(13)	16.24(7)
O1	3483.1(12)	4811.7(5)	3948.7(4)	21.31(19)
O2	-693.5(12)	4657.9(5)	3087.6(4)	24.08(19)
O3	5153.6(12)	7657.1(5)	3020.7(4)	21.85(19)
O4	7429.6(12)	6538.4(5)	2880.7(4)	22.14(19)
O5	5898.4(14)	6377.0(6)	6138.2(4)	31.5(2)
O6	5462.3(12)	144.6(5)	4289.3(4)	20.81(18)
N1	4370.6(13)	6107.2(6)	2891.1(4)	14.68(19)
N2	1819.5(13)	4896.4(6)	2547.4(5)	17.7(2)
N3	6158.2(15)	4263.8(6)	3749.4(5)	17.7(2)
C1	4769.2(16)	4742.9(7)	3586.5(5)	15.4(2)
C2	703.9(17)	4381.7(7)	2886.3(5)	18.1(2)
C3	1218.4(18)	3431.9(8)	2978.7(7)	24.4(3)
C4	5935.5(16)	6688.9(7)	4053.8(6)	17.3(2)
C5	4740.3(17)	7130.7(7)	4461.4(6)	21.3(3)
C6	4763.9(18)	7011.8(8)	5158.5(6)	23.7(3)
C7	5969.1(17)	6441.4(8)	5453.3(6)	21.5(2)
C8	7150.1(17)	5996.1(8)	5044.3(6)	20.3(3)
C9	7138.0(16)	6129.0(7)	4341.8(6)	18.2(2)
C10	6317.9(18)	3821.8(7)	4403.3(6)	19.9(3)
C11	6078.1(16)	2844.2(7)	4366.2(5)	15.8(2)
C12	5226.0(17)	2434.8(7)	3831.1(5)	17.5(2)
C13	5003.3(16)	1534.3(7)	3824.8(5)	17.2(2)
C14	5683.0(16)	1029.4(7)	4348.3(5)	16.0(2)
C15	6545.4(17)	1425.5(8)	4888.9(6)	20.7(3)
C16	6719.3(18)	2331.4(7)	4892.5(6)	21.0(3)
C17	7094(2)	5810.8(11)	6463.9(8)	36.9(4)
C18	6551(2)	-397.3(9)	4687.6(9)	35.0(4)
C19	1454.9(17)	5828.2(7)	2486.2(6)	19.2(2)
C20	2531.8(16)	6328.6(7)	3000.0(6)	17.3(2)
C21	3614.0(16)	4688.9(7)	2397.5(6)	17.0(2)
C22	4843.0(16)	5177.2(7)	2884.9(6)	14.8(2)

Table: Anisotropic Displacement Parameters (×10^4) for (*R*)-SKBG-1. The anisotropic displacement factor exponent takes the form: $-2p^2[h^2a^{*2} \times U_{11} + ... + 2hka^* \times b^* \times U_{12}]$

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
Cl1	24.65(17)	16.30(13)	37.07(16)	-4.74(11)	8.14(13)	-0.87(11)
S1	15.85(14)	12.28(12)	20.60(13)	-1.0(1)	2.72(11)	2.43(10)
O1	19.3(5)	23.1(4)	21.5(4)	3.5(3)	6.8(4)	6.8(3)
O2	16.6(5)	18.7(4)	36.9(4)	1.7(3)	7.4(4)	2.7(3)
O3	25.3(5)	12.1(4)	28.1(4)	0.2(3)	2.1(4)	4.9(3)
O4	15.9(4)	22.2(4)	28.3(4)	-1.1(3)	7.4(4)	2.1(3)
O5	29.2(6)	42.9(5)	22.5(4)	8.6(4)	1.9(4)	1.9(4)
O6	25.4(5)	15.2(4)	21.8(4)	-1.2(3)	-5.0(4)	0.5(3)
N1	14.8(5)	12.3(4)	17.0(4)	0.4(4)	1.9(4)	2.7(3)
N2	15.3(5)	15.9(4)	21.9(5)	0.1(4)	2.1(4)	1.6(4)
N3	18.2(6)	14.4(4)	20.7(5)	2.2(4)	-0.0(4)	3.7(4)

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C1	14.5(6)	12.9(5)	18.8(5)	0.2(4)	3.1(5)	3.2(4)
C2	14.7(6)	16.5(5)	23.2(5)	-0.1(5)	2.6(5)	-0.9(4)
C3	19.7(7)	14.6(5)	38.9(7)	-1.7(5)	6.4(6)	-0.8(5)
C4	16.0(6)	13.5(5)	22.4(5)	0.1(4)	0.5(5)	-0.4(4)
C5	21.4(6)	18.4(5)	24.2(6)	4.9(5)	1.5(5)	-1.0(4)
C6	22.5(7)	23.8(6)	24.7(6)	5.4(5)	2.4(5)	-1.2(4)
C7	18.7(6)	23.0(5)	22.7(5)	2.3(5)	0.4(5)	-0.4(4)
C8	18.0(7)	20.1(6)	22.8(6)	1.2(5)	-0.6(5)	-1.6(4)
C9	16.9(6)	15.3(5)	22.6(6)	0.9(5)	-1.6(5)	-1.5(4)
C10	25.1(7)	15.0(5)	19.6(6)	1.0(5)	-3.5(5)	1.8(4)
C11	17.2(6)	15.6(5)	14.7(5)	0.7(5)	-2.2(4)	2.1(4)
C12	20.9(6)	17.4(5)	14.3(5)	3.3(4)	-2.9(5)	2.0(4)
C13	20.3(6)	16.7(5)	14.6(5)	2.0(4)	-3.1(5)	0.2(4)
C14	17.7(6)	14.7(5)	15.7(5)	0.1(5)	-1.8(5)	1.8(4)
C15	27.4(7)	16.2(6)	18.5(5)	-0.5(5)	-8.7(5)	2.7(4)
C16	27.8(7)	16.2(6)	19.0(6)	-0.7(5)	-9.4(5)	2.9(4)
C17	35.3(9)	50.4(9)	25.1(7)	8.9(7)	2.6(7)	10.4(7)
C18	35.0(9)	16.5(6)	53.4(10)	-1.6(6)	-19.1(8)	4.6(6)
C19	16.0(6)	16.8(5)	24.9(6)	1.3(4)	-1.0(5)	1.4(4)
C20	15.5(6)	13.6(5)	22.8(6)	2.0(4)	3.7(5)	1.3(4)
C21	17.0(6)	15.7(5)	18.3(5)	0.6(4)	2.8(4)	-1.0(4)
C22	14.8(6)	12.9(5)	16.7(5)	1.0(4)	3.3(4)	1.9(4)
H3	65(16)	24(11)	41(11)	10(11)	27(12)	2(9)
H3a	82(15)	38(9)	52(5)	-11(9)	28(5)	-14(3)
H3b	64(9)	45(12)	105(12)	-25(8)	-47(5)	29(9)
H5	43(10)	55(11)	41(9)	29(5)	6(5)	16(6)
H6	45(12)	69(13)	42(9)	26(6)	9(4)	-15(6)
H8	45(11)	50(11)	57(11)	22(5)	-14(6)	6(6)
H9	34(9)	36(10)	48(9)	4(5)	14(4)	-13(5)
H10a	50(9)	28(9)	35(7)	12(5)	16(4)	7(5)
H10b	36(4)	38(10)	70(12)	-4(4)	-24(3)	1(8)
H12	67(14)	37(9)	29(6)	10(6)	-19(5)	12(3)
H13	56(13)	33(9)	32(7)	-4(6)	-23(4)	-5(4)
H15	89(17)	33(10)	47(9)	-6(7)	-44(6)	17(4)
H16	47(12)	42(10)	28(7)	-9(6)	-17(4)	-5(4)
H17a	21(10)	53(3)	58(10)	9(3)	10(8)	0(2)
H17b	74(16)	127(18)	24(2)	35(12)	6.5(18)	11(2)
H17c	39(4)	97(15)	54(11)	-3(3)	11(3)	8(10)
H18a	38(3)	39(12)	150(20)	-7(3)	-6(3)	11(11)
H18b	77(16)	58(13)	55(3)	-4(11)	-10(3)	1(3)
H18c	53(13)	21(4)	79(12)	-8(4)	-12(10)	-7(3)
H19a	30(10)	34(9)	26(3)	-3(7)	1(2)	5(2)
H19b	18(3)	36(9)	50(9)	4(2)	3(2)	-2(7)
H20a	48(12)	30(9)	25(3)	-9(7)	11(3)	1(3)
H20b	42(11)	14(2)	34(8)	2.7(17)	-8(8)	2.9(15)
H21a	37(11)	37(9)	20(3)	-6(7)	4(3)	4(2)
H21b	35(11)	16(2)	51(9)	2.8(15)	0(8)	0.4(18)
H22	19(3)	36(9)	36(8)	2(3)	12(2)	0(7)

Table: Bond Lengths in Å for (*R*)-SKBG-1.

Atom	Atom	Length/Å
Cl1	C3	1.7604(13)
S1	O3	1.4365(8)
S1	O4	1.4322(9)
S1	N1	1.6374(10)
S1	C4	1.7572(11)
O1	C1	1.2309(14)
O2	C2	1.2303(15)
O5	C7	1.3504(15)
O5	C17	1.4235(18)
O6	C14	1.3732(13)
O6	C18	1.4204(16)
N1	C20	1.4834(16)
N1	C22	1.4733(13)
N2	C2	1.3481(15)
N2	C19	1.4622(14)
N2	C21	1.4595(16)
N3	C1	1.3440(16)
N3	C10	1.4579(15)
C1	C22	1.5319(15)
C2	C3	1.5218(16)
C4	C5	1.4008(17)
C4	C9	1.3892(16)
C5	C6	1.3816(18)
C6	C7	1.4062(18)
C7	C8	1.3976(17)
C8	C9	1.3950(17)
C10	C11	1.5131(15)
C11	C12	1.3920(16)
C11	C16	1.3914(16)
C12	C13	1.3924(16)
C13	C14	1.3914(16)
C14	C15	1.3948(16)
C15	C16	1.3963(16)
C19	C20	1.5191(17)
C21	C22	1.5456(16)

Table: Bond Angles in ° for (*R*)-SKBG-1.

Atom	Atom	Atom	Angle/°
O4	S1	O3	119.57(5)
N1	S1	O3	106.60(5)
N1	S1	O4	106.72(5)
C4	S1	O3	107.79(5)
C4	S1	O4	108.19(6)
C4	S1	N1	107.41(5)
C17	O5	C7	117.77(11)
C18	O6	C14	117.25(10)
C20	N1	S1	117.53(7)
C22	N1	S1	117.57(8)
C22	N1	C20	117.56(9)
C19	N2	C2	119.26(10)
C21	N2	C2	125.84(10)
C21	N2	C19	112.43(9)
C10	N3	C1	122.18(11)
N3	C1	O1	124.09(10)
C22	C1	O1	120.88(10)
C22	C1	N3	114.96(10)
N2	C2	O2	121.60(10)
C3	C2	O2	121.53(11)
C3	C2	N2	116.81(11)
C2	C3	Cl1	111.45(9)
C5	C4	S1	118.97(9)
C9	C4	S1	120.14(9)
C9	C4	C5	120.79(10)
C6	C5	C4	119.60(12)
C7	C6	C5	119.94(12)
C6	C7	O5	115.39(11)
C8	C7	O5	124.32(11)
C8	C7	C6	120.29(11)
C9	C8	C7	119.52(11)
C8	C9	C4	119.85(11)
C11	C10	N3	114.09(9)
C12	C11	C10	122.83(10)
C16	C11	C10	118.73(10)
C16	C11	C12	118.44(10)
C13	C12	C11	120.89(10)
C14	C13	C12	119.91(11)
C13	C14	O6	116.14(10)
C15	C14	O6	123.70(10)
C15	C14	C13	120.16(10)
C16	C15	C14	118.95(11)
C15	C16	C11	121.62(11)
C20	C19	N2	109.44(10)
C19	C20	N1	108.57(9)
C22	C21	N2	111.04(9)
C1	C22	N1	113.85(9)
C21	C22	N1	108.70(9)
C21	C22	C1	108.87(9)

Table: Torsion Angles in ° for (*R*)-SKBG-1.

Atom	Atom	Atom	Atom	Angle/°
Cl1	C3	C2	O2	4.81(12)

Atom	Atom	Atom	Atom	Angle/°
Cl1	C3	C2	N2	-172.38(8)
S1	N1	C20	C19	157.55(8)
S1	N1	C22	C1	77.61(8)
S1	N1	C22	C21	-160.86(8)
S1	C4	C5	C6	176.62(10)
S1	C4	C9	C8	-175.57(9)
O1	C1	N3	C10	-4.36(14)
O1	C1	C22	N1	50.67(12)
O1	C1	C22	C21	-70.77(11)
O2	C2	N2	C19	7.26(14)
O2	C2	N2	C21	168.03(10)
O5	C7	C6	C5	179.37(12)
O5	C7	C8	C9	-178.29(12)
O6	C14	C13	C12	-177.58(11)
O6	C14	C15	C16	178.99(13)
N1	C20	C19	N2	55.65(10)
N1	C22	C1	N3	-132.19(10)
N1	C22	C21	N2	-50.20(9)
N2	C21	C22	C1	74.33(10)
N3	C1	C22	C21	106.36(10)
N3	C10	C11	C12	-20.75(14)
N3	C10	C11	C16	159.95(11)
C4	C5	C6	C7	-0.81(14)
C4	C9	C8	C7	-1.17(13)
C5	C6	C7	C8	0.28(15)
C6	C7	C8	C9	0.72(15)
C10	C11	C12	C13	-178.65(12)
C10	C11	C16	C15	-179.83(12)
C11	C12	C13	C14	-1.96(14)
C11	C16	C15	C14	-1.01(15)
C12	C13	C14	C15	1.78(15)
C13	C14	C15	C16	-0.32(14)

Table: Hydrogen Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for (*R*)-SKBG-1. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij}.

Atom	x	y	z	U_{eq}
H3	7180(30)	4298(11)	3424(9)	43(6)
H3a	1560(30)	3146(11)	2501(9)	57(6)
H3b	2270(30)	3387(12)	3338(12)	71(7)
H5	3810(30)	7575(12)	4225(8)	46(5)
H6	3890(30)	7355(13)	5505(9)	52(5)
H8	8110(30)	5574(12)	5261(9)	51(5)
H9	8070(20)	5809(11)	4005(8)	39(5)
H10a	5350(30)	4106(10)	4756(8)	38(4)
H10b	7640(30)	3968(12)	4615(10)	48(5)
H12	4730(30)	2807(10)	3404(8)	44(5)
H13	4320(30)	1200(10)	3407(8)	40(5)
H15	7120(30)	1071(11)	5306(9)	56(6)
H16	7430(30)	2642(11)	5317(7)	39(5)
H17a	6850(20)	5157(13)	6313(9)	44(4)
H17b	6910(30)	5847(16)	6985(9)	75(7)

Atom	x	y	z	U_{eq}
H17c	8420(30)	5975(15)	6329(9)	63(6)
H18a	7850(30)	-219(12)	4609(14)	76(7)
H18b	6240(30)	-338(13)	5225(10)	64(6)
H18c	6310(30)	-1064(10)	4497(10)	51(5)
H19a	1790(20)	6015(10)	1972(7)	30(4)
H19b	100(20)	5950(11)	2585(9)	35(4)
H20a	2080(20)	6168(10)	3514(8)	34(4)
H20b	2410(20)	7039(9)	2921(7)	30(4)
H21a	3830(20)	4901(10)	1876(7)	31(4)
H21b	3830(20)	3985(9)	2430(8)	34(4)
H22	6170(20)	5123(10)	2669(8)	30(4)

Table: Hydrogen Bond information for (*R*)-SKBG-1.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/deg
N3	H3	O2[1]	1.02(2)	1.86(2)	2.8344(15)	158.1(17)

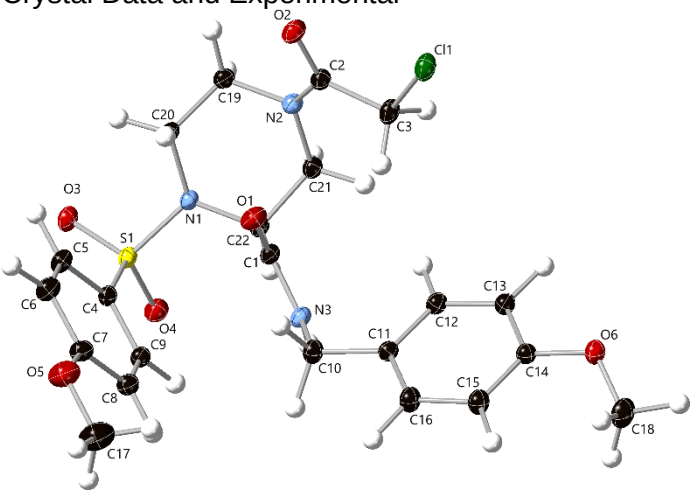
[1]1+x,+y,+z

Table: Selected Bond Lengths in Å for (*R*)-SKBG-1.

Atom	Atom	Length/Å
N3	H3	1.02(2)
C3	H3a	1.068(17)
C3	H3b	1.08(2)
C5	H5	1.095(17)
C6	H6	1.095(17)
C8	H8	1.073(17)
C9	H9	1.099(16)
C10	H10a	1.111(17)
C10	H10b	1.127(19)
C12	H12	1.085(14)
C13	H13	1.105(16)
C15	H15	1.078(16)
C16	H16	1.110(15)
C17	H17a	1.06(2)
C17	H17b	1.036(17)
C17	H17c	1.09(2)
C18	H18a	1.06(2)
C18	H18b	1.09(2)
C18	H18c	1.105(16)
C19	H19a	1.081(15)
C19	H19b	1.087(17)
C20	H20a	1.096(15)
C20	H20b	1.105(14)
C21	H21a	1.088(14)
C21	H21b	1.094(14)
C22	H22	1.120(17)

Crystallography of (*S*)-SKBG-1

Crystal Data and Experimental



Experimental. Single colorless needle-shaped crystals of (*S*)-SKBG-1 were crystallized from chloroform by slow evaporation. A suitable crystal with dimensions 0.33 × 0.12 × 0.10 mm^3 was selected and mounted on a loop paratone on a XtaLAB Synergy-S diffractometer. The crystal was kept at a steady T = 100.0(1) K during data collection. The structure was solved with the **ShelXT** 2018/2 (Sheldrick, 2018) solution program using dual methods and by using **Olex2** 1.3-alpha (Dolomanov et al., 2009) as the graphical interface. The model was refined with **ShelXL** 2018/3 (Sheldrick, 2015) using full matrix least squares minimisation on $\boldsymbol{F^2}$.

Crystal Data. $C_{22}H_{26}ClN_3O_6S$, M_r = 495.97, orthorhombic, $P2_12_12_1$ (No. 19), a = 7.7967(9) Å, b = 15.3379(14) Å, c = 19.594(3) Å, $a = b = g = 90^\circ$, V = 2343.1(5) Å^3, T = 100.01(10) K, Z = 4, Z' = 1, m(Cu K$_a$) = 2.654, 3132 reflections measured, 1744 unique (R_{int} = 0.0619) which were used in all calculations. The final wR_2 was 0.2326 (all data) and R_1 was 0.0861 (I≥2 s(I)).

Compound	(*S*)-SKBG-1
Formula	$C_{22}H_{26}ClN_3O_6S$
$D_{calc.}$/ g cm^{-3}	1.406
m/mm^{-1}	2.654
Formula Weight	495.97
Color	colorless
Shape	needle-shaped
Size/mm^3	0.33×0.12×0.10
T/K	100.01(10)
Crystal System	orthorhombic
Flack Parameter	0.03(4)
Hooft Parameter	-0.02(4)
Space Group	$P2_12_12_1$
a/Å	7.7967(9)
b/Å	15.3379(14)
c/Å	19.594(3)
a/°	90
b/°	90
g/°	90
V/Å^3	2343.1(5)
Z	4
Z'	1
Wavelength/Å	1.54184
Radiation type	Cu K$_a$
Q_{min}/°	3.660
Q_{max}/°	44.269
Measured Refl's.	3132
Indep't Refl's	1744
Refl's I≥2 s(I)	1283
R_{int}	0.0619
Parameters	1
Restraints	66
Largest Peak	0.957
Deepest Hole	-0.563
GooF	1.048
wR_2 (all data)	0.2326
wR_2	0.2102
R_1 (all data)	0.1199
R_1	0.0861

Structure Quality Indicators

Reflections: d min (Cu\a) 2θ=88.5° 1.10 | I/σ(I) 8.6 | Rint 6.19% | CAP 88.5° 99.0

Refinement: Shift 0.000 | Max Peak 1.0 | Min Peak -0.6 | GooF 1.048 | Flack .03(4)

A colourless needle-shaped-shaped crystal with dimensions 0.33 × 0.12 × 0.10 mm^3 was mounted on a loop with paratone. Data were collected using a XtaLAB Synergy, Dualflex, HyPix diffractometer equipped with an Oxford Cryosystems low-temperature device operating at T = 100.01(10) K.

Data were measured using *w* scans using Cu K_a radiation. The diffraction pattern was indexed and the total number of runs and images was based on the strategy calculation from the program CrysAlisPro 1.171.41.98a (Rigaku OD, 2021). The maximum resolution that was achieved was Q = 44.269° (1.10 Å).

The unit cell was refined using CrysAlisPro 1.171.41.98a (Rigaku OD, 2021) on 847 reflections, 27% of the observed reflections.

Data reduction, scaling and absorption corrections were performed using CrysAlisPro 1.171.41.98a (Rigaku OD, 2021). The final completeness is 98.90 % out to 44.269° in Q. A numerical absorption correction based on gaussian integration over a multifaceted crystal model was performed using CrysAlisPro 1.171.41.108a (Rigaku Oxford Diffraction, 2021). An empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm was also applied. The absorption coefficient m of this material is 2.654 mm^{-1} at this wavelength (l = 1.54184Å) and the minimum and maximum transmissions are 0.867 and 0.976.

The structure was solved and the space group $P2_12_12_1$ (# 19) the **ShelXT** 2018/2 (Sheldrick, 2018) solution program using dual methods and by using **Olex2** 1.3-alpha (Dolomanov et al., 2009) as the graphical interface and refined by full matrix least squares minimisation on $\boldsymbol{F^2}$ using version 2018/3 of **ShelXL** 2018/3 (Sheldrick, 2015). All non-hydrogen atoms were refined anisotropically. Hydrogen atom positions were calculated geometrically and refined using the riding model.

There is a single molecule in the asymmetric unit, which is represented by the reported sum formula. In other words: Z is 4 and Z' is 1.

The Flack parameter was refined to 0.03(4). The configuration of the chiral center is S. Determination of absolute structure using Bayesian statistics on Bijvoet differences using the Olex2 results in -0.02(4).

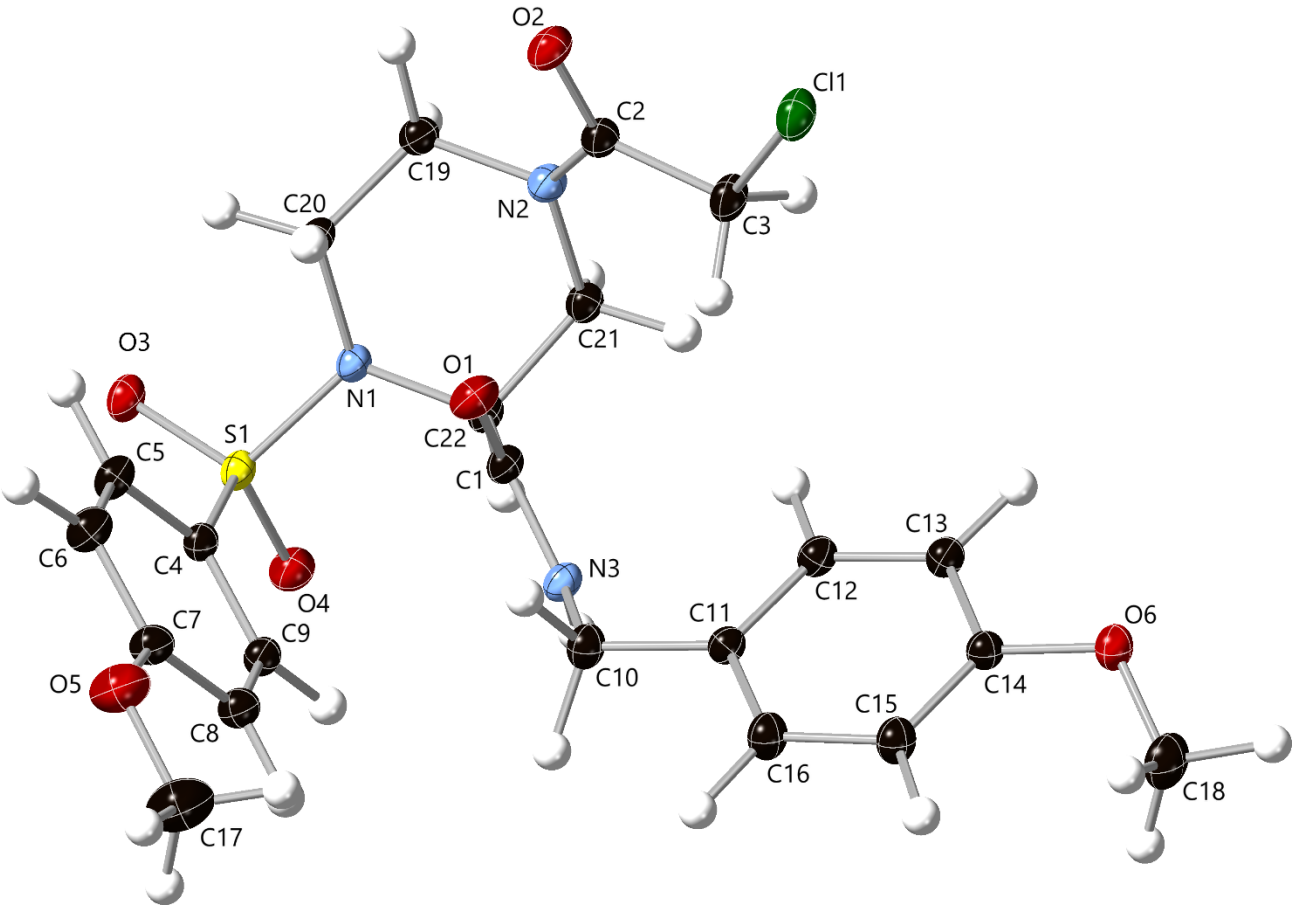


Figure: The independent molecule and the asymmetric unit. There is one chiral center C22 which has S-configuration.

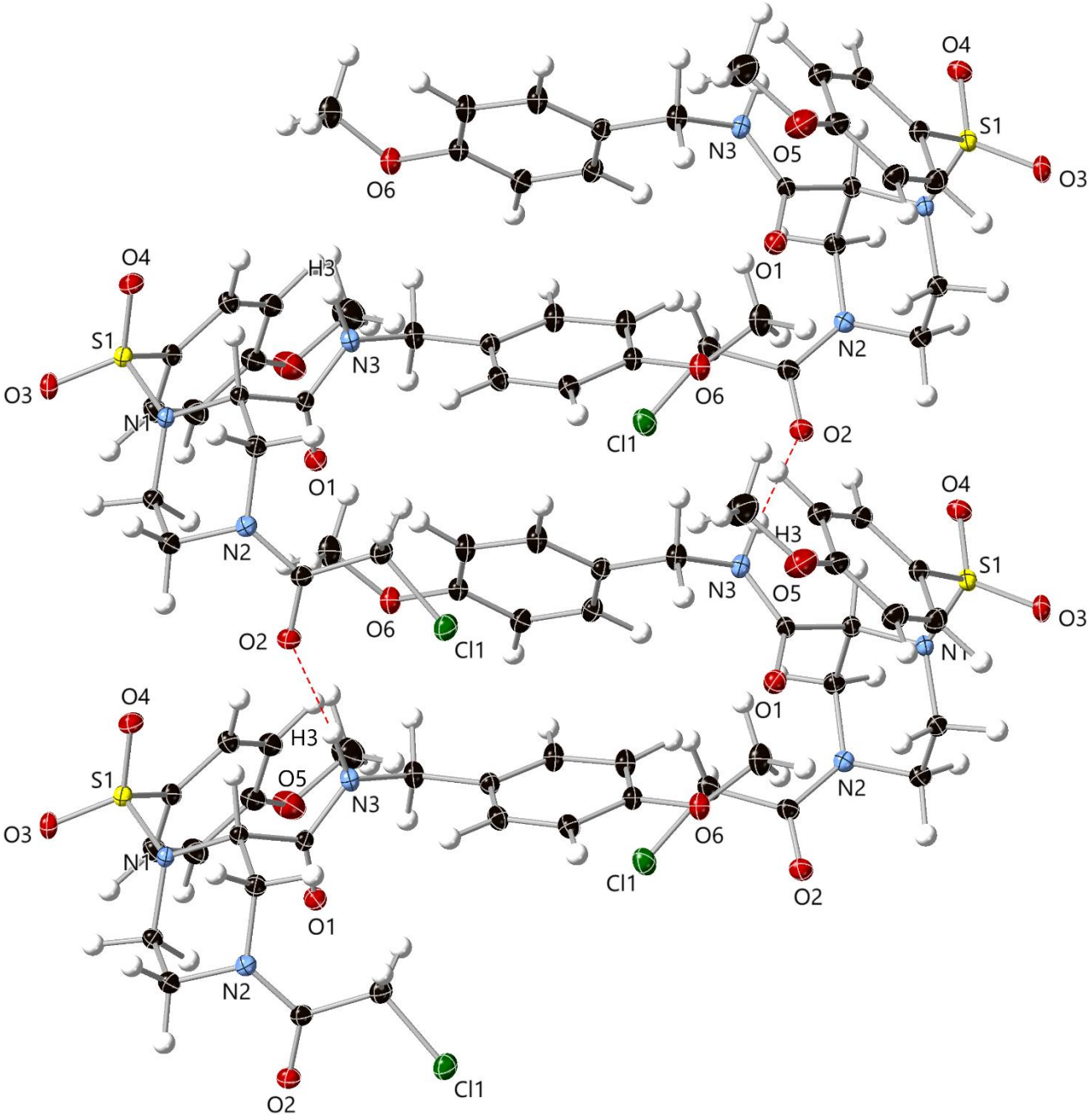
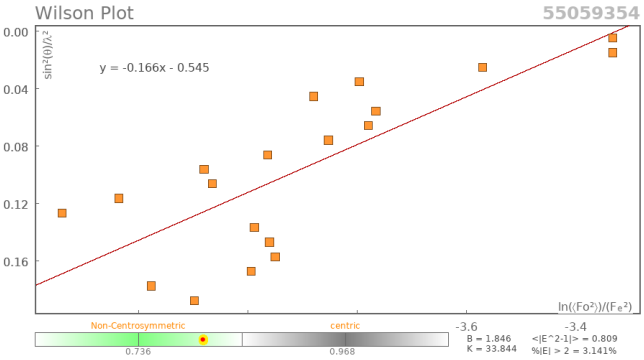
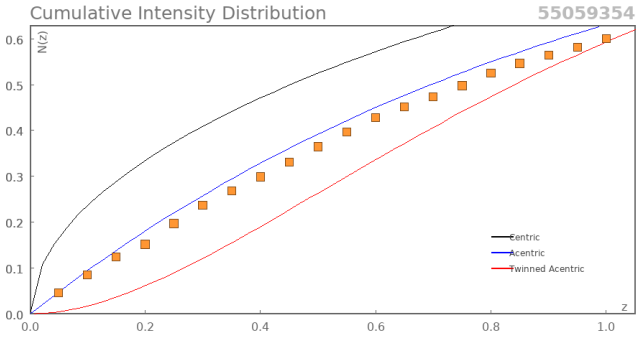
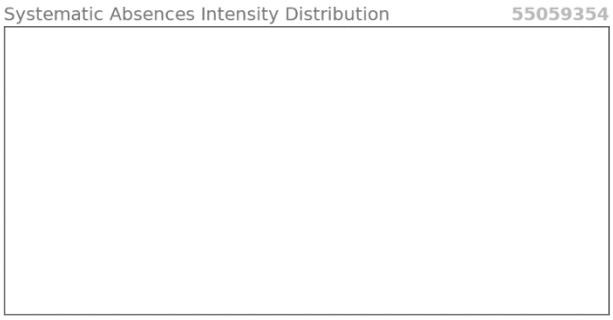


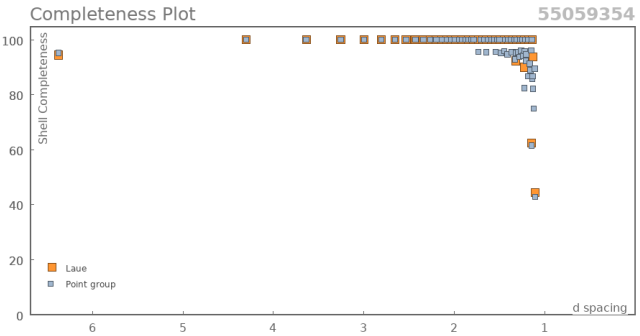
Figure: The structure consists of infinite one-dimensional hydrogen bonded chains that follow the a-axis.

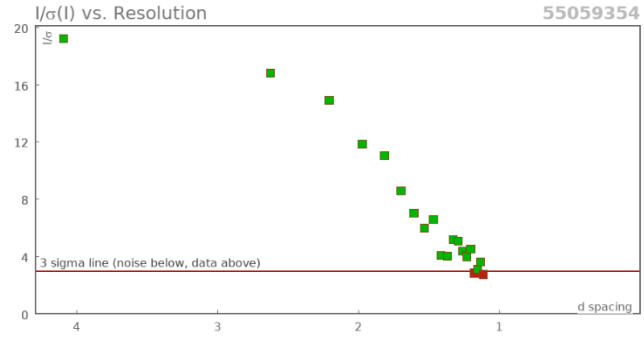
Data Plots: Diffraction Data



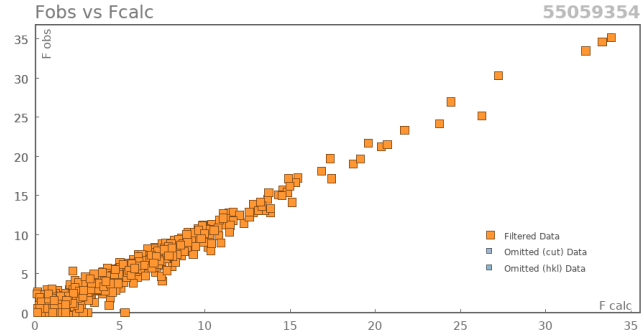








Data Plots: Refinement and Data



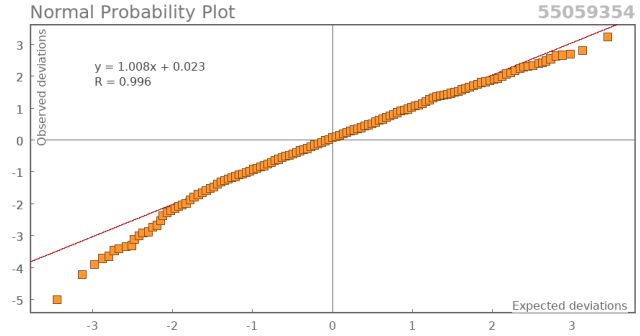


Table: Fractional Atomic Coordinates (×10^4) and Equivalent Isotropic Displacement Parameters (Å^2×10^3) for **10** *S*. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij}.

Atom	x	y	z	U_{eq}
Cl1	473.4	-2814.9	-3329.99	26
S1	-5813.2	-6802	-3165	16

Atom	x	y	z	U_{eq}
O1	-3483.19	-4811.7	-3948.7	21
O2	693.3	-4657.91	-3087.59	24
O3	-5153.51	-7657.2	-3020.7	22
O4	-7429.8	-6538.31	-2880.7	22
O5	-5898.2	-6376.99	-6138.31	31
O6	-5462.2	-144.61	-4289.4	21
N1	-4370.99	-6107.2	-2891.2	15
N2	-1819.59	-4896.5	-2547.4	18
N3	-6157.6	-4263.8	-3749.39	18
C1	-4768.9	-4742.9	-3586.39	15
C2	-703.4	-4381.79	-2886.3	18
C3	-1218.19	-3432	-2978.71	24
C4	-5935.41	-6688.9	-4053.8	17
C5	-4740.5	-7130.71	-4461.39	21
C6	-4764.1	-7011.9	-5158.61	24
C7	-5969.4	-6441.3	-5453.31	21
C8	-7149.6	-5996.1	-5044.31	20
C9	-7138.11	-6129	-4341.79	18
C10	-6317.89	-3821.7	-4403.31	20
C11	-6078.4	-2844.1	-4366.19	16
C12	-5225.81	-2434.91	-3831.1	17
C13	-5003	-1534.2	-3824.8	17
C14	-5683	-1029.4	-4348.39	16
C15	-6545.6	-1425.5	-4888.79	21
C16	-6719.99	-2331.4	-4892.5	21
C17	-7094.4	-5810.79	-6463.9	37
C18	-6550.3	397.4	-4687.8	35
C19	-1454.71	-5828.3	-2486.19	19
C20	-2531.51	-6328.59	-3000	17
C21	-3614.3	-4688.9	-2397.5	17
C22	-4843.1	-5177.2	-2884.9	15

Table: Anisotropic Displacement Parameters (×10^4) for (*S*)-SKBG-1. The anisotropic displacement factor exponent takes the form: $-2p^2[h^2a^{*2} \times U_{11} + ... + 2hka^* \times b^* \times U_{12}]$

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
Cl1	25	16	37	0	8	0
S1	16	12	21	2	3	0
O1	19	23	21	7	7	4
O2	16	19	37	3	7	2
O3	25	12	28	5	2	0
O4	16	22	28	2	7	0
O5	29	43	22	2	2	9
O6	25	15	22	1	0	0
N1	15	12	17	3	2	0
N2	15	16	22	2	2	0
N3	18	14	21	4	0	2
C1	15	13	19	3	3	0
C2	15	16	23	0	3	0
C3	20	14	39	0	6	0
C4	16	13	22	0	0	0
C5	22	18	24	0	2	5

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C6	23	24	25	0	2	5
C7	18	23	23	0	0	2
C8	18	20	23	0	0	1
C9	17	15	23	0	0	1
C10	25	15	20	2	0	1
C11	17	15	15	2	0	1
C12	21	17	14	2	0	3
C13	20	17	15	0	0	2
C14	18	15	16	2	0	0
C15	27	16	18	3	0	0
C16	28	16	19	3	0	0
C17	35	50	25	10	3	9
C18	35	16	53	5	0	0
C19	16	17	25	1	0	1
C20	15	14	23	1	4	2
C21	17	16	18	0	3	1
C22	15	13	17	2	3	1
H3	64	24	42	2	26	11
H3A	81	37	52	0	28	0
H3B	65	45	107	30	0	0
H5	42	56	41	17	6	29
H6	45	68	42	0	9	25
H8	46	51	57	6	0	23
H9	31	35	48	0	13	2
H10A	51	28	36	7	16	12
H10B	36	37	68	1	0	0
H12	67	37	29	12	0	10
H13	54	33	32	0	0	0
H15	91	33	47	17	0	0
H16	46	41	28	0	0	0
H17A	21	52	57	1	10	9
H17B	73	128	24	11	6	37
H17C	39	96	53	8	10	0
H18A	38	40	149	10	0	0
H18B	76	58	55	1	0	0
H18C	50	21	81	0	0	0
H19A	31	34	26	5	1	0
H19B	17	35	50	0	3	3
H20A	47	30	25	1	11	0
H20B	44	14	33	3	0	3
H21A	40	36	20	4	5	0
H21B	35	16	51	1	1	3
H22	19	36	36	0	12	2

Table: Bond Lengths in Å for (*S*)-SKBG-1.

Atom	Atom	Length/Å
Cl1	C3	1.76325(13)
S1	O3	1.43703(10)
S1	O4	1.43615(14)
S1	N1	1.63946(12)
S1	C4	1.7527(2)

Atom	Atom	Length/Å
O1	C1	1.23286(11)
O2	C2	1.23319(12)
O5	C7	1.34693(18)
O5	C17	1.42512(9)
O6	C14	1.37283(12)

Atom	Atom	Length/Å
O6	C18	1.42132(10)
N1	C20	1.48918(16)
N1	C22	1.47319(13)
N2	C2	1.34964(9)
N2	C19	1.46216(13)
N2	C21	1.46479(16)
N3	C1	1.34695(11)
N3	C10	1.45502(16)
C1	C22	1.52848(18)
C2	C3	1.52187(13)
C4	C5	1.40177(10)
C4	C9	1.39111(10)
C5	C6	1.3783(2)
C6	C7	1.40800(9)
C7	C8	1.39828(10)
C8	C9	1.39153(18)
C10	C11	1.51275(14)
C11	C12	1.39104(12)
C11	C16	1.39000(11)
C12	C13	1.39243(13)
C13	C14	1.39036(12)
C14	C15	1.39377(11)
C15	C16	1.39611(13)
C19	C20	1.51895(11)
C21	C22	1.54623(11)

Table: Bond Angles in ° for (*S*)-SKBG-1.

Atom	Atom	Atom	Angle/°
O3	S1	N1	106.467(7)
O3	S1	C4	107.8
O4	S1	O3	119.661(2)
O4	S1	N1	106.975(8)
O4	S1	C4	108.048(4)
N1	S1	C4	107.343(3)
C7	O5	C17	117.628(4)
C14	O6	C18	117.199(5)
C20	N1	S1	117.740(7)
C22	N1	S1	117.435(6)
C22	N1	C20	117.6
C2	N2	C19	119.120(5)
C2	N2	C21	125.978(6)
C19	N2	C21	112.451(1)
C1	N3	C10	122.147(1)
O1	C1	N3	124.315(7)
O1	C1	C22	120.749(5)
N3	C1	C22	114.870(4)
O2	C2	N2	121.727(6)
O2	C2	C3	121.572(1)
N2	C2	C3	116.641(6)
C2	C3	Cl1	111.285(6)
C5	C4	S1	118.823(5)
C9	C4	S1	120.052(3)
C9	C4	C5	121.019(8)
C6	C5	C4	119.465(5)
C5	C6	C7	119.839(3)
O5	C7	C6	115.256(4)
O5	C7	C8	124.226(4)
C8	C7	C6	120.511(7)
C9	C8	C7	119.417(5)
C4	C9	C8	119.737(4)
N3	C10	C11	114.142(4)
C12	C11	C10	122.849(4)
C16	C11	C10	118.729(4)
C16	C11	C12	118.418(7)
C11	C12	C13	120.927(4)
C14	C13	C12	119.894(4)
O6	C14	C13	116.139(5)
O6	C14	C15	123.737(2)
C13	C14	C15	120.121(7)
C14	C15	C16	118.998(4)
C11	C16	C15	121.610(4)
N2	C19	C20	109.383(5)
N1	C20	C19	108.798(6)
N2	C21	C22	111.270(6)
N1	C22	C1	113.890(4)
N1	C22	C21	108.624(5)
C1	C22	C21	108.715(7)

Table: Torsion Angles in ° for (*S*)-SKBG-1.

Atom	Atom	Atom	Atom	Angle/°
S1	N1	C20	C19	-157.550(1)
S1	N1	C22	C1	-77.791(5)
S1	N1	C22	C21	160.905(2)
S1	C4	C5	C6	-176.611(1)
S1	C4	C9	C8	175.544(1)
O1	C1	C22	N1	-50.681(1)

Atom	Atom	Atom	Atom	Angle/°
O1	C1	C22	C21	70.573(7)
O2	C2	C3	Cl1	-4.8
O3	S1	N1	C20	39.320(5)
O3	S1	N1	C22	-171.144(1)
O3	S1	C4	C5	-31.119(2)
O3	S1	C4	C9	152.584(2)
O4	S1	N1	C20	168.353(1)
O4	S1	N1	C22	-42.112(5)
O4	S1	C4	C5	-161.739(1)
O4	S1	C4	C9	21.965(2)
O5	C7	C8	C9	178.3
O6	C14	C15	C16	-178.977(0)
N1	S1	C4	C5	83.209(8)
N1	S1	C4	C9	-93.087(9)
N2	C2	C3	Cl1	172.418(1)
N2	C19	C20	N1	-55.526(4)
N2	C21	C22	N1	50.081(4)
N2	C21	C22	C1	-74.348(6)
N3	C1	C22	N1	132.158(1)
N3	C1	C22	C21	-106.588(7)
N3	C10	C11	C12	20.869(4)
N3	C10	C11	C16	-159.863(2)
C1	N3	C10	C11	-105.964(4)
C2	N2	C19	C20	-101.499(10)
C2	N2	C21	C22	102.706(9)
C4	S1	N1	C20	-75.882(4)
C4	S1	N1	C22	73.653(3)
C4	C5	C6	C7	0.814(1)
C5	C4	C9	C8	-0.670(1)
C5	C6	C7	O5	-179.4
C5	C6	C7	C8	-0.272(1)
C6	C7	C8	C9	-0.7
C7	C8	C9	C4	1.204(1)
C9	C4	C5	C6	-0.4
C10	N3	C1	O1	4.4
C10	N3	C1	C22	-178.592(0)
C10	C11	C12	C13	178.6
C10	C11	C16	C15	179.9
C11	C12	C13	C14	1.951(1)
C12	C11	C16	C15	-0.8
C12	C13	C14	O6	177.574(1)
C12	C13	C14	C15	-1.773(1)
C13	C14	C15	C16	0.319(1)
C14	C15	C16	C11	1.006(1)
C16	C11	C12	C13	-0.644(1)
C17	O5	C7	C6	179.8
C17	O5	C7	C8	0.680(1)
C18	O6	C14	C13	-161.502(0)
C18	O6	C14	C15	17.820(1)
C19	N2	C2	O2	-7.212(2)
C19	N2	C2	C3	175.560(1)
C19	N2	C21	C22	-59.218(6)
C20	N1	C22	C1	71.801(1)

Atom	Atom	Atom	Atom	Angle/°
C20	N1	C22	C21	-49.503(6)
C21	N2	C2	O2	-168.049(2)
C21	N2	C2	C3	14.724(2)
C21	N2	C19	C20	61.797(8)
C22	N1	C20	C19	52.952(5)

Table: Hydrogen Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for (*S*)-SKBG-1. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij}.

Atom	x	y	z	U_{eq}
H3	-7184.51	-4298.4	-3425.7	43
H3A	-1559.71	-3146.1	-2500.8	57
H3B	-2266.2	-3386.18	-3337.3	72
H5	-3811.99	-7573.31	-4224.41	46
H6	-3891.7	-7355.51	-5505.21	52
H8	-8105	-5574.61	-5260.6	51
H9	-8076.19	-5808.91	-4005.31	38
H10A	-5351.6	-4107.2	-4756.19	38
H10B	-7639.01	-3969.39	-4613.5	47
H12	-4732.3	-2806.5	-3403.7	44
H13	-4316.69	-1199.5	-3406.7	40
H15	-7118.1	-1071.6	-5305	57
H16	-7438	-2642.51	-5317.59	38
H17A	-6847.19	-5156.71	-6314.3	43
H17B	-6904.31	-5844.8	-6985.4	75
H17C	-8414.9	-5973.11	-6329.9	63
H18A	-7855.91	218.81	-4609.8	76
H18B	-6235.81	338.2	-5224.91	63
H18C	-6310.19	1064.6	-4497.1	51
H19A	-1789.99	-6014.6	-1971.8	31
H19B	-100.6	-5951	-2584.4	34
H20A	-2083.4	-6167.39	-3515	34
H20B	-2409.8	-7039.51	-2921	31
H21A	-3835.91	-4901	-1875.51	32
H21B	-3826	-3984.7	-2429.6	34
H22	-6180.9	-5122.31	-2668.5	31

Table: Hydrogen Bond information for (*S*)-SKBG-1.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/deg
N3	H3	O2[1]	1.0228(1)	1.8657(2)	2.8417(3)	158.401(2)

[1]-1+x,+y,+z

Citations

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