Supporting Information

Discovery of TAK-981, a first-in-class inhibitor of Sumo Activating Enzyme for the treatment of cancer

Steven P. Langston*[1], Stephen Grossman*[1], Dylan England[1], Roushan Afroze[1], Neil Bence[1], Douglas Bowman[1], Nancy Bump[1], Ryan Chau[1], Bei-Ching Chuang[1], Christopher Claiborne,[1] Larry Cohen, Kelly Connolly[1], Matthew Duffey, Nitya Durvasula, Scott Freeze, Melissa Gallery, Katherine Galvin[1], Jeffrey Gaulin[1], Rachel Gershman[1], Paul Greenspan[1], Jessica Grieves[1], Jianping Guo[1], Nanda Gulavita[1], Shumet Hailu[1], Xingyue He[1], Kara Hoar[1], Yongbo Hu[1], Zhigen Hu[1], Mitsuhiro Ito[2], Mi-Sook Kim[1], Scott Weston Lane[1], David Lok[1], Anya Lublinsky[1], William Mallender[1], Charles McIntyre[1], James Minissale[1], Hirotake Mizutani[1], Miho Mizutani[1], Nina Molchinova[1], Koji Ono[2], Ashok Patil[1], Mark Qian[1], Jessica Riceberg[1], Vaishali Shindi[1], Michael D. Sintchak[1], Keli Song[1], Teresa Soucy[1], Yana Wang[1], He Xu[1], Xiaofeng Yang[1], Agatha Zawadzka[1], Ji Zhang[1], and Sai M Pulukuri[1].

[1]Millennium Pharmaceuticals, a wholly owned subsidiary of Takeda Pharmaceuticals Company Ltd., Cambridge, Massachusetts 02139, USA.

[2]Takeda Pharmaceuticals, Fujisawa, Kanagawa 251-0012, Japan.

*Email steve.langston@takeda.com

*Email Stephen.Grossman@Takeda.com

Enzyme Assays

The E1-E2 HTRF transthiolation (HTRF) assay was run as previously described to determine the IC_{50} value of compounds against recombinantly purified SAE, UAE, NAE. Each E1 assay was run at concentrations of ATP respective to the Km of each enzyme. SUMO1 was used as the substrate for the SAE HTRF assay.

Soucy, T. A. *et al.* An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. *Nature* **458**, 732 (2009).

ATP-PPi exchange assays were conducted as previously described. In brief, **ML-792** was serially diluted into a 96-well assay plate in 50 mM HEPES (pH 7.5), 25 mM NaCl, 5 mM $MgCl_2$, 0.05% BSA, 0.01% Tween-20, and 1 mM TCEP, and a mixture containing 2 nM wild-type SAE, 1 mM ATP, and 0.1 mM PPi (containing 50 cpm/pmol [^{32}P] PPi) was added. Reactions were initiated by adding 1 μM SUMO1 or SUMO2 and were incubated for 60 min at 37 °C before stopping with 5% (w/v) trichloroacetic acid (TCA) containing 10 mM PP_i. The stopped reactions were transferred to a dot-blot apparatus fitted with activated charcoal filter paper presoaked in 2% TCA and 10 mM PPi and washed in the same solution. Filters were dried and exposed to an imaging plate for 1 h and quantified using a phosphoimager. After correcting for background (measured in the absence of SUMO), raw counts were converted to pmol/min of ATP using the slope of an [α-^{32}P]-ATP standard curve. ATP-PPi exchange assays for UAE and NAE were conducted under similar assay conditions except the concentrations of UAE and Ub were 1 nM and 500 nM and the concentrations of NAE and NEDD8 were 1 nM and 160 nM in their respective assays.

Bruzzese, F. J. *et al.* Development of a charcoal paper adenosine triphosphate:pyrophosphate exchange assay: kinetic characterization of NEDD8 activating enzyme. *Analytical biochemistry* **394**, 24-29, (2009).

SUMO immunofluorescent Assay

In the SUMO IF assay, 1×10^4 cells were plated in 100 μL of McCoy's 5A media supplemented with 10% FBS in each well of 96-well plate. All cells were maintained at approximately 37°C in a humidified cell culture chamber containing 5% CO_2. Seeding time is 16 to 20 hours at 37°C in a humidified cell culture incubator containing 5% CO_2 before compound treatment. The compound was prepared in a series of 3-fold serial dilutions in a 96 well polypropylene U-bottom plate. The compound was diluted in McCoy's 5A media to 5-fold of final concentration of cells. 25 μL compounds in media were added to 100 μL cells prepared previously to reach final desired concentration. Positive control was 10 μM **12** and negative control was 0.25% DMSO. Treated cells were then incubated at approximately 37°C in the tissue culture incubator for 4 hours before immunofluorescent staining. The experiment was conducted with triplicate plates.

While in the SUMO washout IF assay, 2.5×10^3 cells were plated in 100 μL of McCoy's 5A media supplemented with 10% FBS in each well of the 96-well plate and incubated for 36 to 40 hours before compound treatment. Compound treatment was similar as described above. Treated

cells were then incubated at approximately 37°C in the tissue culture incubator for 2 hours before washout. The experiment was conducted with triplicate plates. Three washes of 100 μL McCoy's 5A medium supplemented with 10% FBS was conducted to remove extra compound in the well. Then 100 μL McCoy's 5A medium supplemented with 10% FBS was added to the cells. The plates were incubated for 8 hours at approximately 37°C in the tissue culture incubator before immunofluorescence staining.

Immunofluorescent Staining

In both assays, compound treated cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 15 minutes, permeated with 0.5% Triton X−100 in PBS for 15 minutes, and washed once in PBS. Fixed cells were treated with blocking reagent for 1 hour at room temperature and stained for 60 minutes at room temperature with anti-SUMO antibody (1:1250) in blocking reagent. The cells were washed two times with PBS containing 0.05% Tween-20 and then stained for 60 minutes at room temperature with Alexa 488-conjugated goat anti-mouse IgG (1:500) in blocking reagent. The cells were washed three times with PBS, then stained with a solution of DRAQ5 (a nuclei staining dye, 1:5000) in PBS and maintained in this solution for imaging.

Image Visualization

Immunofluorescent cells in the SUMO IF/Washout IF studies were visualized using an Image Xpress Ultra High Content Imaging System (Molecular Devices, Inc [Sunnyvale, CA, USA]). Images from 9 to 16 pre-set locations per well were captured with a 20× objective lens. Inhibition of target was determined by measuring the redistribution of SUMO from nucleus to cytosol within HCT116 cells using MetaXpress software, Version 5.0 (Molecular Devices, Inc). The module of translocation-enhanced was applied and the parameter of "cell: inner to outer mean intensity" was used as raw readout.

Statistical Analysis

The IC_{50} value and the 95% confidence interval were calculated from a dose-response curve generated using Genedata Condoseo v11 (Genedata AG [Lexington, MA, USA]). The curves were generated using SUMO translocation percentage calculated from normalization of raw data to DMSO controls (0% inhibition) and positive control (10 μM **ML-93** 100% inhibition). The IC_{50} values were determined from the curves, representing the concentration that produces the 50 percent inhibition.

Western Blot

HCT116 cells were obtained from American Type Culture Collection and were cultured in McCoy's 5A medium supplemented with 10% fetal bovine serum. Samples were fractionated by nonreducing SDS–PAGE and Western blotted with antibodies against Sumo2/3 (Epitomics 1:1000), Nedd8 (monoclonal rabbit antibody generated by Takeda). Secondary Alexa-680-labelled antibody to rabbit/mouse IgG (Invitrogen, 1:4000 dilution) was used and the blots were imaged using the LI-COR Odyssey Infrared Imaging System.

CellTiter-Glo viability assay

Cells were plated in 96-well optical bottom plates and treated with DMSO or a dilution series of compounds for 72 h. Cell viability was determined using CellTiter-Glo® Luminescent Cell Proliferation Assay (Promega Corp). Luminescence was measured using a PHERAstar multi-label counter (BMG Labtech).

Human carbonic anhydrase enzyme assay

To determine that ability of adenosine sulfamate compounds to inhibit human carbonic anhydrase I (hCA-I) and II, (hCA-II), a fluorometric esterase assay for the known promiscuous esterase activity of the enzyme was utilized (*references below*). For this assay, the activated fluorescent ester Fluorescein Diacetate (FL-DA) was utilized to permit maximal sensitivity for monitoring inhibition by potent compounds. Chemical reagents and enzymes were purchased from Sigma Aldrich. Adenosine sulfamate compounds were added by multichannel pipette into 384-well black assay plate in 1μL DMSO. The buffer used for this assay was 25 mM MOPS (pH 7.5) and 0.02% Triton-X-100. A 2X stock solution for enzyme was made in assay buffer containing either 50 nM hCA-I or 5 nM hCA-II. A 2X stock solution of 50 μM FL-DA was made in assay buffer using a freshly prepared 20 mM DMSO stock solution. Reactions were initiated by a 25 μL addition of 2X substrate stock solution and a 25 μL addition of 2X enzyme stock solution. Final assay (1X) conditions were 25 nM hCA-I or 2.5 nM hCA-II with 25 μM FL-DA in assay buffer with 2% DMSO final and a serial dilution of adenosine sulfamate inhibitor starting at 10 μM or 100 μM depending on compound potency. For 0% inhibition control wells, a 1 μL DMSO spot was utilized. Known hCA inhibitors Acetazolamide and Chlorothiazide were used as controls for the assay. For 100% inhibition control wells, a 1 μL DMSO addition of 10 mM Acetazolamide, final concentration 200 μM, was used for hCA-I reactions and a 1 μL DMSO addition of 10 mM Chlorothiazide, final concentration 200 μM, was used for hCA-II. Reactions were run in duplicate at room temperature on a BMG POLARstar or BMG PHERAstar multi-label counter (BMG LabTech) and read in kinetics mode. Data was analyzed using Microsoft Excel and XLFit and Genedata Condoseo software.

Reference publications:

Pocker, Y. and Stone J.T. (1967) The catalytic versatility of erythrocyte carbonic anhydrase. 3. Kinetic studies of the enzyme-catalyzed hydrolysis of p-nitrophenyl acetate. Biochemistry **6 (3)**, 668-678.

Elleby, B, Sjöblom, S., and Lindskog, S. (1999) Changing the efficiency and specificity of the esterase activity of human carbonic anhydrase II by site-specific mutagenesis. Eur. J. Biochem. **262**, 516-521.

Gould, S.M. and Tawfik, D.S. (2005) Directed evolution of the promiscuous esterase activity of carbonic anhydrase II. Biochemistry **44 (14)**, 5444-5452.

Mouse Models

All animal studies were conducted under the approval of the Takeda Oncology Institutional Animal Care and Use Committee and complied with all relevant ethical regulations. Mice were maintained in a specific pathogen-free facility in accordance with American Association for Laboratory Animal Science guidelines. To establish the HCT-116 model, six to twelve-week-old female NCr nude mice (Taconic Farms, Inc.) were inoculated subcutaneously along the right flank with 1.0 x 10^6 HCT-116 cells (ATCC CCL-247) cultured according to vendor recommendations and re-suspended for injection in 50% McCoy's 5a Medium/50% Matrigel™ [BD Biosciences (Bedford, MA)]. To establish the OCI-Ly10 model, six to twelve-week-old

female CB17 SCID mice (Taconic Farms, Inc.) were inoculated subcutaneously along the right flank with 4.0 x 10^6 OCI-Ly10 cells (from National Cancer Institute) cultured in IMDM media supplemented with 20% fetal bovine serum and re-suspended for injection in 50% Iscove's Modified Dulbecco's Medium/50% Matrigel™ [BD Biosciences (Bedford, MA)]. Tumors measurements were calculated with Vernier calipers across 2 dimensions and volumes were calculated using the formula (V) = W^2 x L/2, where W and L and the width and length of the tumor, respectively.

Sample Collection and Handling

For plasma collection, approximately 500 µL of whole blood was obtained via cardiac puncture, placed into tubes coated with dipotassium ethylenediaminetetraacetic acid (K2EDTA) to prevent clotting and centrifuged at 10,000 rpm for 5 minutes. Approximately 200 µL of plasma was then transferred into 1.4-mL sterile Micronic non-coded push cap U-bottom tubes and capped with a pierceable thermo plastic elastomer cap (MP32022; MP53007 [Nova Biostorage + (Canonsburg, PA, USA)]), snap frozen on dry ice and stored frozen at approximately −80°C. Tumor samples were excised from the mouse and cut into 2 pieces. One piece was placed into 2 mL FastPrep tubes (Cat #5076-400 [MP Biomedicals (Santa Ana, CA, USA)]), snap frozen on dry ice and stored frozen at approximately −80°C for PK analysis. One piece was thinly sliced, placed into a bar-coded histology cassette and submerged in 10% neutral buffered formalin for a minimum of 24 hours. Tumors were embedded in paraffin cut into 5-micron sections and used for IHC staining for pharmacodynamic analysis. Samples of plasma and tumor were transferred to the Bioanalysis group at Takeda for PK analysis.

Plasma and Tumor Processing for Liquid Chromatography With Tandem Mass Spectrometry

A protein precipitation extraction method was used for preparation of plasma samples. 20 µL of plasma was diluted with 380 µL of acetonitrile (ACN) with 0.1% formic acid (FA). The sample was mixed, and then centrifuged at 3000 g for 10 minutes, and the supernatant analyzed by LC/MS/MS. Frozen tumor tissues were homogenized with water using the MP FastPrep®-24 bead homogenizing system (MP Biomedicals). The final diluted tumor homogenate was processed and analyzed as described for plasma samples.

Quantitation of Compound Using a Liquid Chromatography With Tandem Mass Spectrometry Method

The method was established on a API-5500 mass spectrometer (SCIEX [Concord, Ontario, Canada]) equipped with a Shimadzu UFLC (ultra-fast liquid chromatography) system and autosampler. Concentrations of the SUMO inhibitor in mouse plasma were determined by a liquid chromatography UFLC system with a tandem mass spectrometry based method, operated in the positive ionization mode using a turbo ion spray. Multiple-reaction monitoring was used for detection of the compound.

The calibration curve standards were prepared from a dimethyl sulfoxide stock (1 mg/mL) and serially diluted with ACN with 0.1% FA. The dynamic range for the curve was 0.050-5.0 µM (or 25.0 µM if the curve was still linear). In brief, an aliquot of 20 µL of plasma samples or standards was transferred into a True Taper™ 2 mL Square 96-well plate with 100 µL tapered reservoir (Analytical Sales & Service, Inc. [Pompton Plains, NJ, USA]). 380 µL of ACN with

0.1% FA, (which contained carbutamide at a concentration of approximately 100 ng/mL), was added to the samples to either precipitate the proteins from the plasma or dilute the calibration curve standards. The carbutamide in the samples was used to ensure the reproducibility of the autosampler injections. The sample wells were mixed (aspirated and dispensed) several times with a multi-channel pipette and then centrifuged at 3000 rpm for 10 minutes. The resulting supernatant was then injected onto an LC/MS/MS system and the compound concentrations were determined by a LC/MS/MS based
assay.

Measuring SUMO2/3 Conjugates by IHC

Sumo2/3 expression was measured via IHC on formalin fixed paraffin embedded tumors using an anti-SUMO2/3 rabbit monoclonal antibody [EPR300(2)] (Abcam [Cambridge, MA, USA] [Catalog No 109196, Lot GR96828-7]) at a 1:1,000 dilution. Leica BOND™ Polymer Refine Detection kit (Leica Biosystems [Buffalo Grove, IL, USA] [Catalog No DS9800, Lot# 43319]) was used for SUMO 2/3 detection. Staining was performed on the Leica BOND™ RX automated
machine (Leica Biosystems [Buffalo Grove, IL, USA]). SUMO 2/3 expression was visualized by 3, 3'-diaminobenzidine (DAB) staining. After staining, whole slide images were captured using an Aperio Scanscope® XT (Aperio ePathology Solutions [Vista, CA, USA]). Quantitative analysis was done using a Definiens Tissue Studio® (Definiens Tissue Studio image analysis software [Cambridge, MA,
USA]) designed to measure DAB staining. Data are represented as the percentage of SUMO2/3 positive nuclei relative to the total number of nuclei in the viable tumor region. The mean result of the 3 tumors per group was compared to the mean of the vehicle group, to determine the mean percent inhibition of SUMO2/3 conjugates at each timepoint.

Measuring SUMO-Compound Adduct by IHC

For detection of SUMO-**ML-792** adduct, the antibody MIL-79 (Epitomics [Cambridge, MA, USA] [subclone MIL-79-84-6]) was used at a 1:8000 dilution. IHC assays for MIL-79 were carried using a Ventana XT Staining Module (Ventana Medical Systems). Data are shown as a percentage of MIL-79 positive area within the viable tumor area.

For detection of SUMO-TAK-981 adduct, the antibody MIL-113 (Epitomics [Cambridge, MA, USA] [subclone MIL-113-67-2]) was used at a 1:30,000 dilution. Leica BOND™ Polymer Refine Detection kit (Leica Biosystems [Buffalo Grove, IL, USA] [Catalog No DS9800, Lot# 43319]) was used for MIL113 detection. IHC assays for MIL-113 were carried out using a Leica BOND™ RX automated stainer (Leica Biosystems [Buffalo Grove, IL, USA]). MIL-113 was visualized by 3, 3'-diaminobenzidine (DAB) staining. After staining, whole slide images were captured using an Aperio Scanscope® XT (Aperio ePathology Solutions [Vista, CA, USA]). Data analysis was performed by using a Definiens Tissue Studio® (Definiens [Cambridge, MA, USA]) designed to measure DAB staining. Data are shown as a percentage of MIL-113 positive area within the viable tumor area.

Measuring SUMO-Compound Adduct by Mass Spectrometry

To quantify the amounts of SAE and Sumo–compound **ML-792** adducts in biological samples subjected to treatment with **ML-792**, an internal standard sample for mass spectrometric quantification was prepared using the purified heavy SILAC-labeled SAE (SAE*) and ^{13}C- and ^{15}N-labeled Sumo1 and Sumo2 (Sumo1* and Sumo2*). In detail, 2 μM SAE*, 1 μM Sumo1* or 1 μM Sumo2*, and 2 μM **ML-792** were mixed with 150 μM ATP and 10 mM $MgCl_2$ in 20 mM Tris buffer pH 8.0 at room temperature for 1 h. The reaction was quenched by EDTA at final concentration of 30 mM. The conversion of free Sumo1* or Sumo2* to Sumo*–**ML-792** adducts was confirmed to be complete by intact protein mass spectrometric analysis as described previously (Nature Chemical Biology, 2017 November 13: 1164-71). The internal standard solution was stored in aliquots at −80 °C until use.
To quantify the absolute amounts of SAE and Sumo–**ML-792** adducts, xenograft tissue lysates from **ML-792** treated animals were mixed with known amounts of the internal standard (SAE* and Sumo*–**ML-792**) and NuPage 4 × LDS sample buffer (Invitrogen) containing reducing agent (Invitrogen) and were resolved by SDS-PAGE on a NuPage 4–12 % gel (Invitrogen). As described previously (Cell Biochemistry and Biophysics. 2013 Sep 67(1): 139-47.), SAE subunits gel fractions were excised and an in-gel digestion by trypsin (Roche) was performed. While Sumo fraction was excised, and in-gel digested by the combination of chymotrypsin (Roche) and Glu-C (Roche). After digestion, 20 μl solution containing 1 % formic acid (Sigma-Aldrich) and 5 % acetonitrile (J.T. Baker) (v/v) was added to each digest and vortexed at room temperature for 5 min. After centrifugation, the supernatant was subjected to NanoLC–MS/MS analysis for quantification of SAE and SUMO-adducts. The SAE amount in each sample was calculated from the ratio of the chromatogram peak areas of the light and SILAC heavy labeled peptides of SAE as described previously (Cell Biochemistry and Biophysics. 2013 Sep 67(1): 139-47). The Sumo1-**ML-792** adduct amount in each sample was calculated from the ratio of the peak areas of QTGG-**ML-792** to Q*T*G*G*-**ML-792** in the chromatogram. The Sumo2/3-**ML-792** adduct amount was calculated from the ratio of the peak areas of QQQTGG-**ML-792** to Q*Q*Q*T*G*G*-**ML-792** in the chromatogram. Finally, the Sumo-adducts to SAE molar ratios were calculated to represent the SAE pathway inhibition extent.
The procedures to quantify the amounts of SAE and Sumo-adducts in biological samples were reproduced for compounds **ML-93** and TAK-981.

Reference publications:
Probing the roles of SUMOylation in cancer cell biology by using a selective SAE inhibitor. Nature Chemical Biology, 2017 November 13: 1164-71
Absolute Quantification of E1, Ubiquitin-like Proteins and Nedd8-MLN4924 Adduct by Mass Spectrometry. Cell Biochemistry and Biophysics. 2013 Sep 67(1): 139-47.

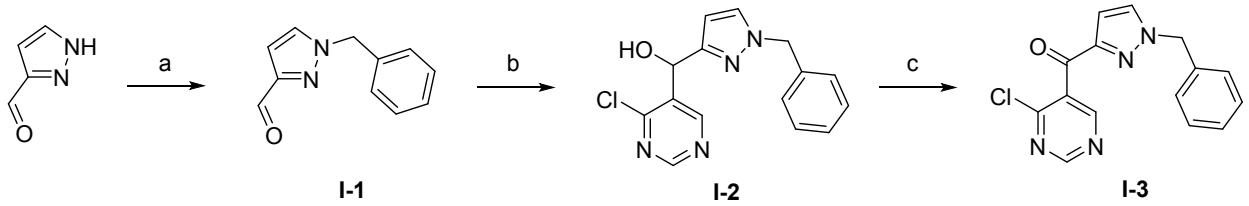
Antitumor activity against the HCT-116 (colon carcinoma) model
To determine the antitumor activities of compounds **ML-792**, **ML-93**, and TAK-981 in HCT-116 tumor-bearing mice, 6-10 week-old female NCr nude mice were inoculated with HCT-116 cells to establish the model as previously described. When the mean tumor volume reached approximately 200 mm^3, animals were randomized into treatment groups (n=8/group) and dosed SC (**ML-792**, **ML-93**) or IV (TAK-981) at the doses and schedules indicated. Compounds were prepared fresh weekly and stored at 4 degrees. Dosing volume for all compounds was 200 μL. Tumor size and body weight were measured twice weekly. Animals were monitored daily for signs of toxicity.

Antitumor activity against OCI-Ly10 (DLBCL) model

To determine the antitumor activities of **ML-792**, **ML-93** and TAK-981 in OCI-Ly10 tumor-bearing mice, 7-10 week-old female CB17 SCID mice were inoculated with OCI-Ly10 cells to establish the model as previously described. When the mean tumor volume reached approximately 200 mm^3, animals were randomized into treatment groups (n=8/group) and dosed SC (**ML-792**, **ML-93**) or IV (TAK-981) at the doses and schedules indicated. Compounds were prepared fresh weekly and stored at 4 degrees. Dosing volume for all compounds was 200 µL. Tumor size and body weight were measured twice weekly. Animals were monitored daily for signs of toxicity.

Procedures for the syntheses of compounds in Table 1

Synthesis of intermediate I-3



Reagents: (a) Benzylbromide, Cs_2CO_3, DMF, rt, 1 h, 77%; (b) 4-chloro-5-iodopyrimidine, *n*-BuLi, THF, -78 °C, 0.5 h, 85%; (c) MnO_2, DCM, rt, 15 h, 91%.

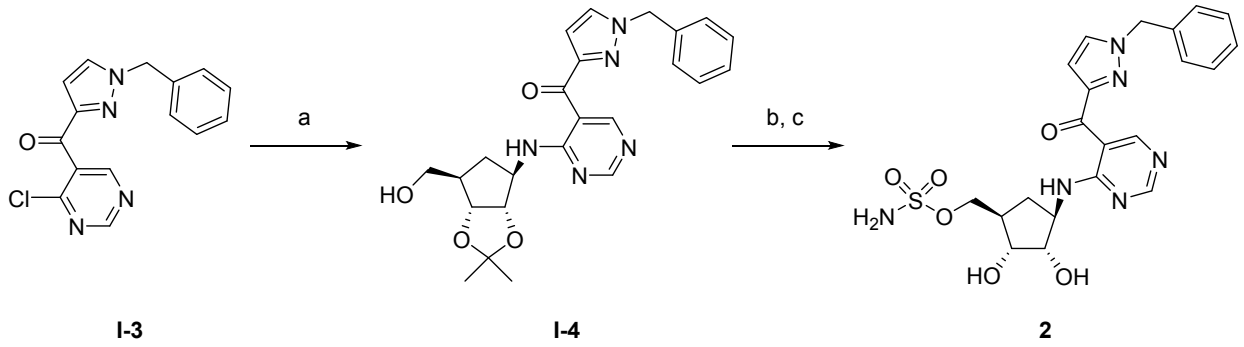
Step 1: 1-Benzyl-1*H*-pyrazole-3-carbaldehyde (I-1). A 100 mL round bottom flask was charged with 1-pyrazole-3-carbaldehyde (250 mg, 2.60 mmol), Cs_2CO_3 (2.12 g, 6.50 mmol), and DMF (10 mL). To the suspension was added benzyl bromide (0.33 mL, 2.70 mmol), and the reaction was stirred for 1 h. The reaction mixture was poured into water (50 mL) and the mixture was extracted with EtOAc (50 mL) three times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to provide **I-1** (372 mg, 77%). ^{1}H NMR ($CDCl_3$) δ 10.01–9.98 (m, 1H), 7.42 (d, 1H, J = 2.3 Hz), 7.41–7.32 (m, 3H), 7.28–7.23 (m, 2H), 6.82 (d, 1H, J = 2.4 Hz), 5.40 (s, 2H).

Step 2: (1-Benzyl-1*H*-pyrazol-3-yl)(4-chloropyrimidin-5-yl)methanol (I-2). 4-Chloro-5-iodopyrimidine (300 mg, 1.25 mmol) was weighed into a 100 mL 2 necked round bottom flask and the flask was purged with argon. This starting material was dissolved in THF (10 mL) and the solution was cooled to -78 °C. To the solution was added *n*-BuLi (2.50 M in hexane; 1.0 mL, 2.5 mmol) at -78 °C and then the mixture was stirred for 30 min. To this mixture was added dropwise a solution of 1-benzyl-1*H*-pyrazole-3-carbaldehyde (211 mg, 1.1 mmol) in THF (4 mL), and the resulting mixture was stirred for 30 min. The reaction was quenched by addition of saturated NH_4Cl (50 mL) and extracted with EtOAc (50 mL) four times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The

residue was purified on silica gel to provide **I-2** (304 mg, 85%) as a light-yellow oil. LCMS (FA): *m/z* = 301.4 (M+H).

Step 3: (1-Benzyl-1*H*-pyrazol-3-yl)(4-chloropyrimidin-5-yl)methanone (I-3). To a solution of (1-benzyl-1*H*-pyrazol-3-yl)(4-chloropyrimidin-5-yl)methanol (285 mg, 0.95 mmol) in DCM (10 mL) was added MnO_2 (0.82 g, 9.5 mmol), and the mixture was stirred for 15 h at rt. The reaction was filtered through a pad of celite and the residual solid was washed with DCM several times. The filtrate was concentrated in vacuo and the residue was purified on silica gel to give **I-3** (257 mg, 91%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 9.09 (s, 1H) 8.90 (s, 1H) 7.45 (d, 1H, *J* = 2.5 Hz) 7.32–7.41 (m, 3H) 7.22 (m, 2H) 7.02 (d, 1H, *J* = 2.5 Hz) 5.33 (s, 2H); LCMS (FA): *m/z* = 299.4 (M+H).

Synthesis of 2



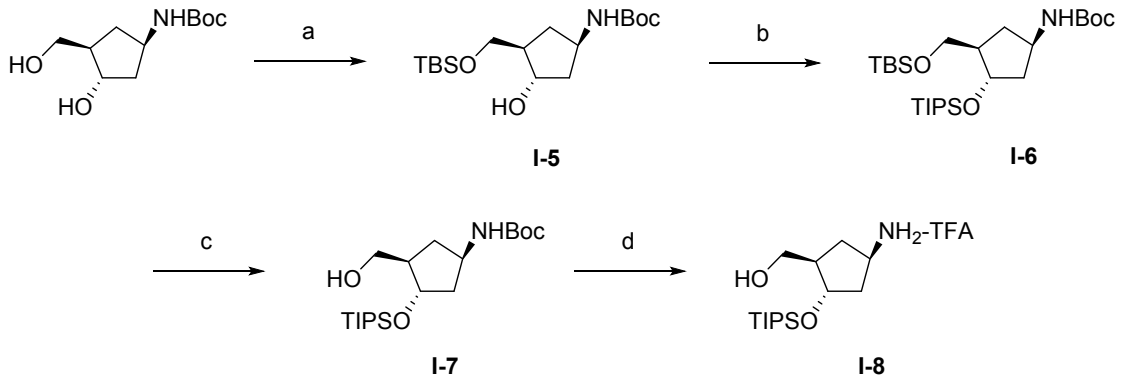
Reagents: (a) (1*R*,2*S*,3*R*,4*R*)-1-amino-2,3-(isoproplydenyl)dihydroxy-4-hydroxymethyl cyclopentane, DIPEA, 1-BuOH, 105 °C, 2 h, 92%, (b) chlorosulfonamide, DMF, 10 min, 81%; (c) conc. HCl, THF, H_2O, rt, 89%.

Step 1: (1-Benzyl-1*H*-pyrazol-3-yl)(4-{[(3a*S*,4*R*,6*R*,6a*R*)-6-(hydroxymethyl)-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-yl]amino}pyrimidin-5-yl)methanone (I-4). (1-Benzyl-1*H*-pyrazol-3-yl)(4-chloropyrimidin-5-yl)methanone (**I-3**, 120 mg, 0.40 mmol) and (1*R*,2*S*,3*R*,4*R*)-1-amino-2,3-(isoproplydenyl)dihydroxy-4-hydroxymethyl cyclopentane (90 mg, 0.48 mmol) (for synthesis of this starting material see: Claiborne, C. F. et al. PCT Application Publication WO2008/019124) were weighed into a reaction vessel. To this mixture was added 1-BuOH (2.4 mL) and *N,N*-diisopropylethylamine (0.14 mL, 0.80 mmol) and the tube was sealed under an atmosphere of argon. The resulting mixture was stirred for 2 h at 105 °C. After cooling to rt, the reaction was concentrated in vacuo. To this residue was added water (30 mL) and the resulting mixture was extracted with EtOAc (50 mL) four times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to give **I-4** (175 mg, 92%) as a colorless solid. LCMS (FA): *m/z* = 450.5 (M+H).

Step 2: [(1*R*,2*R*,3*S*,4*R*)-4-({5-[(1-Benzyl-1*H*-pyrazol-3-yl)carbonyl]pyrimidin-4-yl}amino)-2,3-dihydroxycyclopentyl]methyl sulfamate (2).
To a solution of (1-benzyl-1*H*-pyrazol-3-yl)(4-{[(3a*S*,4*R*,6*R*,6a*R*)-6-(hydroxymethyl)-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-yl]amino}pyrimidin-5-yl)methanone (162

mg, 0.36 mmol) in DMF (2.0 mL) was added chlorosulfonamide (83 mg, 0.72 mmol) at rt, and the mixture was stirred for 10 min. The reaction was quenched by addition of saturated $NaHCO_3$ (30 mL) and extracted with EtOAc (50 mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to give the sulfamate intermediate [(162 mg, 81%); LCMS (FA): *m/z* = 529.5 (M+H)] which was then dissolved in THF (2.0 mL). To this solution was added water (2 mL) and conc. HCl (0.35 mL, 4.2 mmol) at rt, and the mixture was stirred for 2 h. The reaction was quenched by addition of saturated $NaHCO_3$ (30 mL) and extracted with EtOAc (50 mL) four times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The residue was purified on silica gel to give **2** (128 mg, 89%). ^{1}H NMR (DMSO-d_6) δ 9.47 (s, 1H), 9.07 (d, 1H, *J* = 7.4 Hz), 8.61 (s, 1H), 8.07 (d, 1H, *J* = 2.4 Hz), 7.45 (s, 2H), 7.41–7.26 (m, 5H), 6.92 (d, 1H, *J* = 2.4 Hz), 5.51 (s, 2H), 4.92 (d, 1H, *J* = 6.0 Hz), 4.74 (d, 1H, *J* = 4.8 Hz), 4.50–4.41 (m, 1H), 4.07 (dd, 1H, *J* = 9.7, 6.1 Hz), 3.98 (dd, 1H, *J* = 9.7, 6.6 Hz), 3.79 (dd, 1H, *J* = 13.0, 5.8 Hz), 3.71 (dd, 1H, *J* = 9.5, 4.8 Hz), 2.38–2.28 (m, 1H), 2.25–2.15 (m, 1H), 1.15 (dt, 1H, *J* = 12.9, 8.7 Hz); LCMS (FA): *m/z* = 489.5 (M+H); HRMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{25}N_6O_6S$ 489.1556, obsd 489.1558.

Synthesis of cyclopentane intermediate I-8



Reagents: (a) TBSCl, imidazole, DMF, DCM, rt, 12 h, 87%; (b) TIPSCl, imidazole, DMF, rt, 61 h, 93%; (c) 1% HCl in EtOH, EtOH, 4 °C for 13 h and rt for 4 h, 93%; (d) TFA, 5 min, rt, 100%.

Step 1: *tert*-Butyl [(1*R*,3*R*,4*S*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-hydroxycyclopentyl]carbamate (I-5). A solution of *tert*-butyl [(1*R*,3*S*,4*R*)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]carbamate (4.0 g, 17 mmol) (for synthesis of starting material see: Ober, M. et al. *J. Am. Chem. Soc*. **2005**, *127*, 18143-18149) and imidazole (1.4 g, 21 mmol) in DMF (40 mL) was diluted with DCM (200 mL) and cooled in an ice/water bath. TBSCl (2.9 g, 19 mmol) was added as a solution in DCM (40 mL). The reaction was allowed to warm to ambient temperature and stirred overnight. The reaction was quenched by addition of water (150 mL) and the mixture was transferred to separatory funnel. The organic layer was collected and the residual water layer was extracted with DCM (150 mL) twice. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The residue was purified on silica gel to provide **I-5** (5.21 g, 87%). ^{1}H NMR ($CDCl_3$) δ 4.73 (s, 1H), 4.20–4.05 (m, 2H), 3.81 (dd, 1H, *J* =

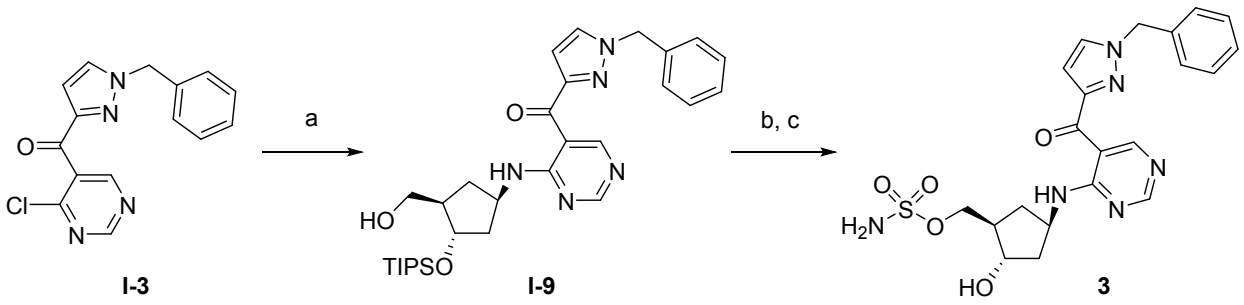
9.8, 4.2 Hz), 3.54 (dd, 1H, *J* = 9.7, 7.1 Hz), 2.33–2.10 (m, 2H), 2.05–1.79 (m, 3H), 1.43 (s, 9H), 1.20–1.08 (m, 1H), 0.90 (s, 9H), 0.08 (s, 6H). LCMS (FA): *m/z* = 346.6 (M+H).

Step 2: *tert*-Butyl {(1*R*,3*R*,4*S*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}carbamate (I-6). To a solution of *tert*-butyl [(1*R*,3*R*,4*S*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-hydroxycyclopentyl]carbamate (3.8 g, 11 mmol) in DMF (57 mL) under an atmosphere of argon was added imidazole (2.25 g, 33 mmol) followed by TIPSCl (4.7 mL, 22 mmol) at rt, and the mixture was stirred for 61 h. The reaction was quenched by addition of saturated NH_4Cl (150 mL) and extracted with EtOAc (200 mL) five times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to afford **I-6** (5.13 g, 93%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 4.90 (s, 1H), 4.35–4.20 (m, 1H), 4.19–3.99 (m, 1H), 3.75–3.44 (m, 2H), 2.37–2.17 (m, 1H), 2.03 (s, 1H), 1.96–1.69 (m, 2H), 1.43 (s, 9H), 1.31–1.13 (m, 1H), 1.04 (s, 21H), 0.90 (s, 9H), 0.06 (s, 6H). LCMS (FA): *m/z* = 402.6 (M+H) - Boc.

Step 3: *tert*-Butyl {(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}carbamate (I-7). To a solution of *tert*-butyl {(1*R*,3*R*,4*S*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}carbamate (**I-6**, 535 mg, 0.91 mmol) in EtOH (9.7 mL) was added 1% HCl in EtOH (9.7 mL, 1.2 mmol) at rt, and the mixture was allowed to stand at 4 °C for 13 h. The reaction was then stirred for 4 h at rt. The reaction was quenched by addition of saturated $NaHCO_3$ (50 mL) and extracted with DCM (50 mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to give **I-7** (327 mg, 93%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 4.65 (s, 1H), 4.22 (dd, 1H, *J* = 10.9, 5.1 Hz), 4.19–4.05 (m, 1H), 3.72–3.58 (m, 2H), 2.41–2.27 (m, 1H), 2.13–2.04 (m, 1H), 2.00 (m, 1H), 1.80–1.63 (m, 2H), 1.44 (s, 9H), 1.23–1.09 (m, 1H), 1.06 (s, 21H).

Step 4: {(1*R*,2*S*,4*R*)-4-Amino-2-[(triisopropylsilyl)oxy]cyclopentyl}methanol-TFA (I-8). A 250 mL round bottom flask was charged with *tert*-butyl {(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}carbamate (1.0 g, 2.6 mmol). To the reaction vessel was added TFA (6.5 mL, 84 mmol) at rt, and the mixture was stirred for 5 min. To the mixture was added toluene (50 mL) and the mixture was concentrated in vacuo. This was repeated twice more to remove TFA and the resulting residue was dried under high vacuum to give {(1*R*,2*S*,4*R*)-4-amino-2-[(triisopropylsilyl)oxy]cyclopentyl}methanol-TFA (**I-8**, 1.31 g, 100%) as a colorless oil. LCMS (FA): *m/z* = 288.6 (M+H).

Synthesis of 3



Reagents: (a) **I-8**, K_2CO_3, DMF, rt, 13 h, 75%; (b) chlorosulfonamide, DMF, rt, 20 min, 80%; (c) 4N HCl, THF, rt, 1 h, 95%.

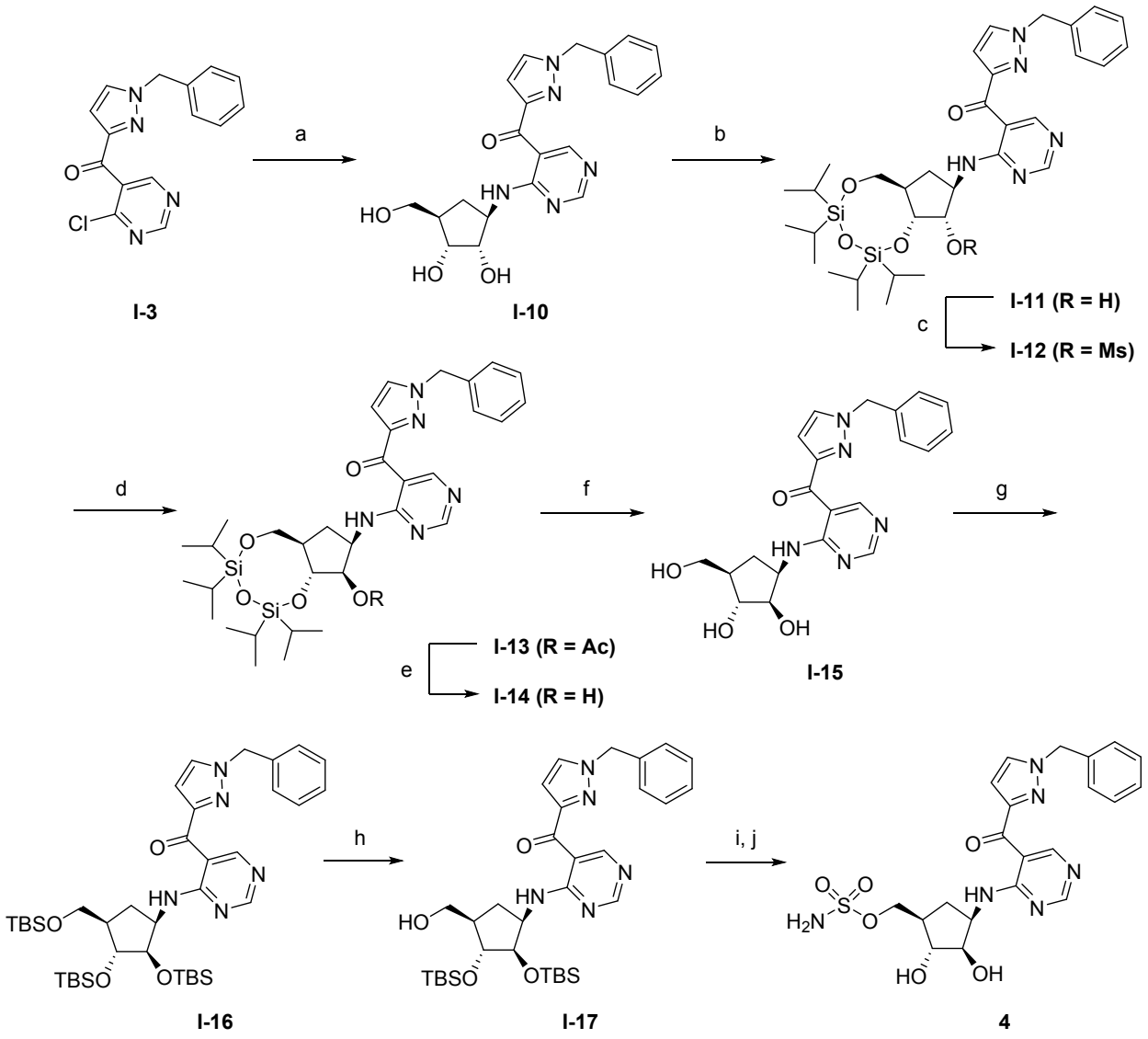
Step 1: (1-Benzyl-1*H*-pyrazol-3-yl)[4-({(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]methanone (I-9). To a solution of {(1*R*,2*S*,4*R*)-4-amino-2-[(triisopropylsilyl)oxy]cyclopentyl}methanol-TFA (**I-8**, 1.04 g, 2.58 mmol) in DMF (20 mL) was added K_2CO_3 (1.1 g, 7.7 mmol) followed by (1-benzyl-1*H*-pyrazol-3-yl)(4-chloropyrimidin-5-yl)methanone (**I-3**, 0.85 g, 2.8 mmol) at rt, and the mixture was stirred for 13 h. The reaction was then concentrated in vacuo. To the residue was added water (100 mL) and the mixture was extracted with EtOAc (100 mL) three times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to give **I-9** (1.08 g, 75%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 9.66 (s, 1H), 9.25 (br d, 1H, *J* = 7.3 Hz), 8.64 (s, 1H), 7.42 (d, 1H, *J* = 2.5 Hz), 7.40–7.31 (m, 3H), 7.28–7.23 (m, 2H), 6.89 (d, 1H, *J* = 2.3 Hz), 5.39 (s, 2H), 4.87–4.76 (m, 1H), 4.32 (q, 1H, *J* = 4.7 Hz), 3.70 (t, 2H, *J* = 5.4 Hz), 2.50 (td, 1H, *J* = 13.1, 8.0 Hz), 2.24–2.14 (m, 2H), 1.92–1.81 (m, 2H), 1.32 (td, 1H, *J* = 13.1, 8.1 Hz), 1.11–1.03 (m, 21H). LCMS (FA): *m/z* = 550.7 (M+H).

Step 2: [(1*R*,2*S*,4*R*)-4-({5-[(1-Benzyl-1*H*-pyrazol-3-yl)carbonyl]pyrimidin-4-yl}amino)-2-hydroxycyclopentyl]methyl sulfamate (3).
To a solution of (1-benzyl-1*H*-pyrazol-3-yl)[4-({(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]methanone (1.0 g, 1.8 mmol) in DMF (30 mL) was added chlorosulfonamide (650 mg, 5.6 mmol) at rt, and the mixture was stirred for 20 min. The reaction was quenched by addition of saturated $NaHCO_3$ (100 mL) and water (50 mL). The mixture was extracted with EtOAc (150 mL) three times, and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to give the sulfamate intermediate [(0.91 g, 80%); LCMS (FA): *m/z* = 629.7 (M+H)] which was then dissolved in THF (20 mL) and HCl (4 M in water; 20 mL, 80 mmol) was added at rt. This mixture was stirred for 1 h and then quenched by addition of saturated $NaHCO_3$ (150 mL) and extracted with EtOAc (200 mL) four times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. To the residue was added DCM and the resulting suspension was filtered through a glass frit funnel and the residual solid was washed with DCM twice. The filtrate was concentrated in vacuo and the residue was purified on silica gel. The purification product and solid from filtration were combined to give **3** (652 mg, 95%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 9.46 (s, 1H), 9.00 (d, 1H, *J* = 7.5 Hz), 8.62 (s, 1H), 8.07 (d, 1H, *J* = 2.4 Hz), 7.45 (s, 2H), 7.41–7.25 (m, 5H), 6.90 (d, 1H, *J* = 2.4 Hz), 5.50 (s, 2H), 4.92 (d, 1H, *J* = 4.6 Hz), 4.76–4.64 (m, 1H), 4.10 (dd, 1H, *J* = 9.7, 5.9 Hz), 4.01–3.92 (m, 2H), 2.41–2.30 (m, 1H), 2.19–2.07 (m, 1H), 2.00 (ddd, 1H, *J* = 11.7, 7.7, 3.8 Hz), 1.83–1.72 (m, 1H), 1.28

(dt, 1H, J = 12.9, 9.2 Hz). LCMS (FA): m/z = 473.5 (M+H). HRMS m/z $[M+H]^+$ calcd for $C_{21}H_{25}N_6O_5S$ 473.1607, obsd 473.1608.

Procedures for the syntheses of compounds in Table 2

Synthesis of 4



Reagents: (a) (1*R*,2*S*,3*R*,5*R*)-3-amino-5-(hydroxymethyl)cyclopentane-1,2-diol hydrochloride, DIPEA, 1-PrOH, 50 °C, 18 h, 100%; (b) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, imidazole, DMF, rt, 16 h, 92%; (c) MsCl, DMAP, TEA, DCM, rt, 15 h, 100%; (d) CsOAc, 1,4,7,10,13,16-hexaoxacyclooctadecane, benzene, 80 °C, 7 h, 24%; (e) K_2CO_3, MeOH, rt, 20 h, 99%; (f) TBAF, THF, rt, 5 h, 84%; (g) TBSCl, DMAP, imidazole, DMF, rt, 77 h, 26%; (h) 1% HCl in EtOH, EtOH, 4 °C for 2 h and -20 °C for 60 h, 79%; (i) chlorosulfonamide, TEA, DMF, rt, 2 h; (j) 3N HCl, THF, 60 °C, 7 h, 61% for 2 steps.

Step 1: (1-Benzyl-1*H*-pyrazol-3-yl)(4-{[(1*R*,2*S*,3*R*,4*R*)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]amino}pyrimidin-5-yl)methanone (I-10). (1-Benzyl-1*H*-pyrazol-3-yl)(4-chloropyrimidin-5-yl)methanone (**I-3**, 1.00 g, 3.35 mmol) and (1*R*,2*S*,3*R*,5*R*)-3-amino-5-(hydroxymethyl)cyclopentane-1,2-diol hydrochloride (for synthesis of this starting material see: Claiborne, C.F. et al. PCT Application Publication WO2008/019124) (0.68 g, 3.68 mmol) were weighed into a 250 mL round bottom flask fitted with a reflux condenser. To this mixture was added 1-PrOH (48 mL) and *N,N*-diisopropylethylamine (1.75 mL, 10.0 mmol). The resulting mixture was stirred at 50 °C for 18 h. The reaction was allowed to cool to rt and the solution was concentrated. The residue was purified on silica gel to yield **I-10** (1.3 g, 100%). ^{1}H NMR (DMSO-d_6) δ 9.45 (s, 1H), 9.35–9.00 (m, 3H), 8.60 (s, 1H), 8.07 (d, 1H, J = 2.3 Hz), 7.40–7.26 (m, 5H), 6.90 (d, 1H, J = 2.5 Hz), 5.51 (s, 2H), 4.77 (d, 1H, J = 5.3 Hz), 4.66 (t, 1H, J = 5.1 Hz), 4.51–4.39 (m, 2H), 3.76–3.69 (m, 2H), 2.26 (ddd, 1H, J = 13.3, 9.3, 8.0 Hz), 1.99–1.90 (m, 1H), 1.18–1.08 (m, 1H).

Step 2: (1-Benzyl-1*H*-pyrazol-3-yl)(4-{[(6a*R*,8*R*,9*S*,9a*R*)-9-hydroxy-2,2,4,4-tetraisopropylhexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-8-yl]amino}pyrimidin-5-yl)methanone (I-11). A 100 mL round bottom flask under nitrogen was charged with (1-benzyl-1*H*-pyrazol-3-yl)(4-{[(1*R*,2*S*,3*R*,4*R*)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]amino}pyrimidin-5-yl)methanone (1.3 g, 3.2 mmol), DMF (13 mL), and imidazole (0.87 g, 12.7 mmol). A solution of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (1.1 mL, 3.5 mmol) in DMF (10 mL) was added dropwise over 1 h. The resulting mixture was allowed to stir at rt overnight. After 16 h reaction was poured into a saturated $NaHCO_3$ solution and the mixture was extracted with EtOAc three times. The combined organic portions were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified on silica gel to give **I-11** (1.9 g, 92%). ^{1}H NMR ($CDCl_3$) δ 9.77 (s, 1H), 9.45–9.35 (br d, 1H, J = 4.3 Hz), 8.71 (s, 1H), 7.44 (d, 1H, J = 2.3 Hz), 7.42–7.33 (m, 3H), 7.30–7.27 (m, 2H), 6.92 (d, 1H, J = 2.3 Hz), 5.41 (s, 2H), 4.50–4.41 (m, 1H), 4.25 (dd, 1H, J = 7.3, 6.3 Hz), 4.02–3.93 (m, 2H), 3.77 (dd, 1H, J = 11.8, 5.3 Hz), 2.99–2.96 (m, 1H), 2.35–2.25 (m, 2H), 1.39–1.32 (m, 1H), 1.13–0.98 (m, 28H). LCMS (FA): *m/z* = 653.0 (M+H).

Step 3: (6a*R*,8*R*,9*S*,9a*R*)-8-({5-[(1-Benzyl-1*H*-pyrazol-3-yl)carbonyl]pyrimidin-4-yl}amino)-2,2,4,4-tetraisopropylhexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-yl methanesulfonate (I-12). A 500 mL round bottom flask under nitrogen was charged with (1-benzyl-1*H*-pyrazol-3-yl)(4-{[(6a*R*,8*R*,9*S*,9a*R*)-9-hydroxy-2,2,4,4-tetraisopropylhexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-8-yl]amino}pyrimidin-5-yl)methanone (1.8 g, 2.8 mmol), DCM (8 mL), triethylamine (0.42 mL, 3.0 mmol) and DMAP (0.37 g, 3.0 mmol). The reaction was cooled in an ice bath and a solution of methanesulfonyl chloride (0.24 mL, 3.0 mmol) in DCM (6 mL) was added dropwise. The reaction was then allowed to slowly warm to rt and stir overnight. After stirring 15 h, the reaction was quenched by the addition of water. The mixture was extracted with EtOAc three times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to give **I-12** (2 g, 100%). ^{1}H NMR ($CDCl_3$) δ 9.78 (s, 1H), 9.17 (d, 1H, J = 6.3 Hz), 8.68 (s, 1H), 7.44 (d, 1H, J=2.5 Hz), 7.41–7.34 (m, 3H), 7.29–7.26 (m, 2H), 6.92 (d, 1H, J = 2.5 Hz), 5.40 (s, 2H), 4.98 (d, 1H, J = 4.8 Hz), 4.58–4.50 (m, 1H), 4.24 (dd, 1H, J = 10.8, 4.8 Hz), 4.01 (dd, 1H, J = 11.8, 3.0 Hz), 3.83 (dd, 1H,

J = 10.5, 1.3 Hz), 3.24 (s, 3H), 2.33 (ddd, 1H, *J* = 13.1, 9.0, 7.5 Hz), 2.27–2.17 (m, 1H), 1.69 (ddd, 1H, *J* = 13.1, 11.5, 7.3 Hz), 1.13–1.03 (m, 28H). LCMS (FA): *m/z* = 731.0 (M+H).

Step 4: (6a*R*,8*R*,9*R*,9a*R*)-8-({5-[(1-Benzyl-1*H*-pyrazol-3-yl)carbonyl]pyrimidin-4-yl}amino)-2,2,4,4-tetraisopropylhexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-yl acetate (I-13). A 500 mL round bottom flask under nitrogen was charged with (6a*R*,8*R*,9*S*,9a*R*)-8-({5-[(1-benzyl-1*H*-pyrazol-3-yl)carbonyl]pyrimidin-4-yl}amino)-2,2,4,4-tetraisopropylhexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-yl methanesulfonate (1.1 g, 1.5 mmol), benzene (20 mL), 1,4,7,10,13,16-hexaoxacyclooctadecane (0.21 g, 0.78 mmol), and CsOAc (0.91 g, 4.7 mmol). The reaction was then heated at 80 °C while stirring. After 7 h, additional CsOAc (0.91 g, 4.7 mmol) was added and the reaction continued to stir overnight at 80 °C. The reaction was subsequently quenched by the addition of water then extracted with EtOAc three times. The combined extracts were concentrated and the crude material purified on silica gel to provide **I-13** (0.25 g, 24%). LCMS (FA): *m/z* = 695.0 (M+H).

Step 5: (1-Benzyl-1*H*-pyrazol-3-yl)(4-{[(6a*R*,8*R*,9*R*,9a*R*)-9-hydroxy-2,2,4,4-tetraisopropylhexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-8-yl]amino}pyrimidin-5-yl)methanone (I-14). (6a*R*,8*R*,9*R*,9a*R*)-8-({5-[(1-Benzyl-1*H*-pyrazol-3-yl)carbonyl]pyrimidin-4-yl}amino)-2,2,4,4-tetraisopropylhexahydrocyclopenta[*f*][1,3,5,2,4]trioxadisilocin-9-yl acetate (0.15 g, 0.22 mmol), MeOH (7 mL), and K_2CO_3 (90 mg, 0.6 mmol) were stirred in a 20 mL scintillation vial at rt for 20 h. The reaction mixture was then concentrated and a solution of saturated $NaHCO_3$ (aq.) (20 mL) was added. The resulting mixture was extracted with EtOAc three times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to yield **I-14** (0.14 g, 99%). ^{1}H NMR ($CDCl_3$) δ 9.71 (br s, 1H), 9.48 (d, 1H, *J* = 6.4 Hz), 8.63 (s, 1H), 7.45–7.30 (m, 5H), 7.26–7.23 (m, 1H), 6.92 (d, 1H, *J* = 2.3 Hz), 5.39 (s, 2H), 4.65–4.55 (m, 1H), 4.26–4.20 (m, 1H), 4.13–4.08 (m, 1H), 3.99 (dd, 1H, *J* = 11.6, 3.5 Hz), 3.72 (dd, 1H, *J* = 11.6, 5.8 Hz), 2.76–2.57 (br s, 1H), 2.34–2.25 (m, 1H), 2.03–1.93 (m, 1H), 1.62–1.52 (m, 1H), 1.12–1.02 (m, 28H). LCMS (FA): *m/z* = 653.0 (M+H).

Step 6: (1-Benzyl-1*H*-pyrazol-3-yl)(4-{[(1*R*,2*R*,3*R*,4*R*)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]amino}pyrimidin-5-yl)methanone (1-15). (1-Benzyl-1*H*-pyrazol-3-yl)(4-{[(6a*R*,8*R*,9*R*,9a*R*)-9-hydroxy-2,2,4,4-tetraisopropylhexahydrocyclopenta[f][1,3,5,2,4]trioxadisilocin-8-yl]amino}pyrimidin-5-yl)methanone (0.36 g, 0.55 mmol) was dissolved in THF (5 mL). TBAF (1.0 M solution in THF; 0.58 mL, 0.58 mmol) was added and the solution was allowed to stir at rt. After stirring for 1 h, additional TBAF (1.0 M solution in THF; 0.2 mL, 0.2 mmol) was added and the reaction stirred an additional 4 h. The reaction mixture was concentrated and purified on silica gel to afford **I-15** (0.19 g, 84%). ^{1}H NMR (DMSO-d_6) δ 9.48 (s, 1H), 9.37 (d, 1H, *J* = 7.7 Hz), 8.60 (s, 1H), 8.05 (d, 1H, *J* = 2.4 Hz), 7.40–7.27 (m, 5H), 6.90 (d, 1H, *J* = 2.4 Hz), 5.50 (s, 2H), 5.34 (d, 1H, *J* = 4.8 Hz), 4.81 (d, 1H, *J* = 4.6 Hz), 4.61–4.52 (m, 2H), 3.80–3.75 (m, 1H), 3.65–3.61 (m, 1H), 3.52–3.45 (m, 1H), 3.39–3.31 (m, 1H), 2.22 (td, 1H, *J* = 12.5, 7.7 Hz), 1.89–1.79 (m, 1H), 1.34–1.24 (m, 1H). LCMS (FA): *m/z* = 410.4 (M+H).

Step 7: (1-Benzyl-1*H*-pyrazol-3-yl)(4-{[(1*R*,2*R*,3*R*,4*R*)-2,3-bis{[*tert*-butyl(dimethyl)silyl]oxy}-4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)cyclopentyl]amino}pyrimidin-5-yl)methanone (I-16). To a

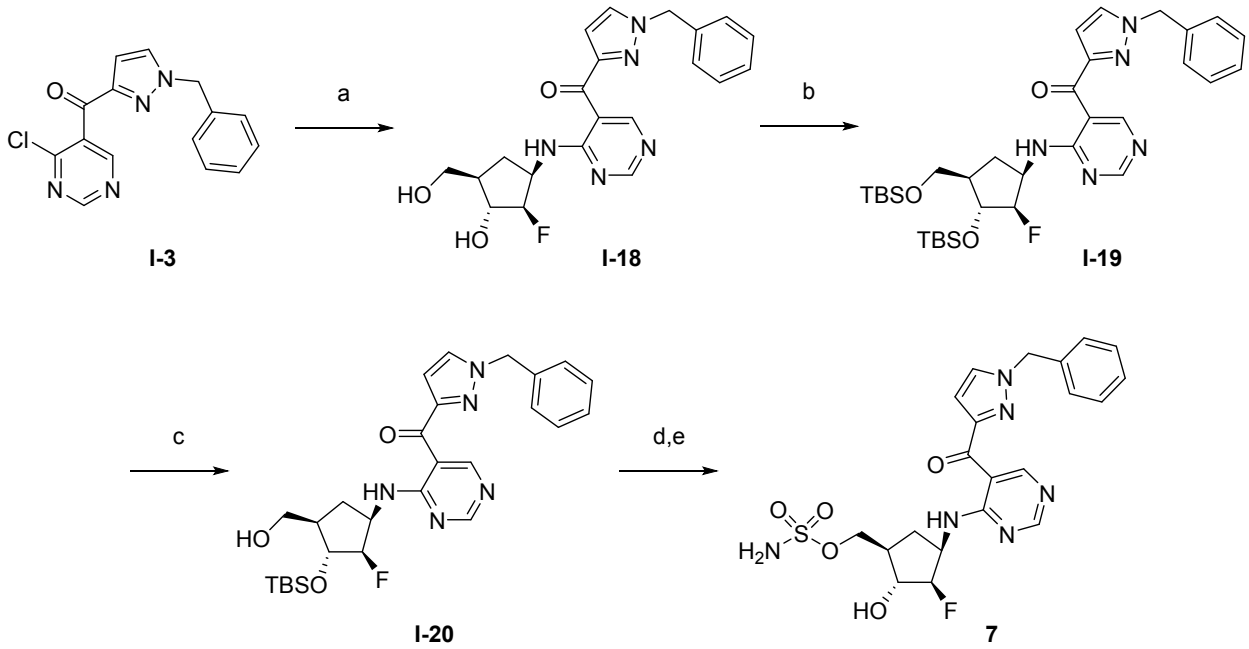
solution of (1-benzyl-1*H*-pyrazol-3-yl)(4-{[(1*R*,2*R*,3*R*,4*R*)-2,3-dihydroxy-4-(hydroxymethyl)cyclopentyl]amino}pyrimidin-5-yl)methanone (0.19 g, 0.45 mmol) in DMF (8.0 mL) was added imidazole (0.22 g, 3.2 mmol) and DMAP (5.5 mg, 0.05 mmol) followed by TBSCl (0.34 g, 2.3 mmol) at rt, and the mixture was stirred for 3 days. Additional imidazole (0.09 g, 1.4 mmol) and TBSCl (0.14 g, 0.90 mmol) were added and the reaction was allowed to continue to stir at rt. After 5 h, TLC and LCMS showed no starting material, but a mixture of the bis-silylated and tris-silylated desired products. The reaction was quenched by addition of water and extracted with EtOAc three times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified on silica gel to give **I-16** (0.09g, 26%). ^{1}H NMR ($CDCl_3$) δ 9.68 (s, 1H), 9.26 (d, 1H, *J* = 8.3 Hz), 8.65 (s, 1H), 7.43–7.39 (m, 1H), 7.39–7.33 (m, 3H), 7.29–7.25 (m, 2H), 6.90 (d, 1H, *J* = 2.5 Hz), 5.39 (s, 2H), 4.92–4.83 (m, 1H), 3.98 (d, 1H, *J* = 3.5 Hz), 3.94–3.90 (m, 1H), 3.67–3.62 (m, 1H), 3.57 (dd, 1H, *J* = 9.6, 7.0 Hz), 2.32 (td, 1H, *J* = 12.4, 8.4 Hz), 2.15–2.06 (m, 1H), 1.39–1.29 (m, 1H), 0.92 (s, 9H), 0.91 (s, 9H), 0.90 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H), 0.06 (s, 3H), 0.05 (s, 6H), -0.02 (s, 3H). LCMS (FA): *m/z* = 753.2 (M+H).

Step 8: (1-Benzyl-1*H*-pyrazol-3-yl)(4-{[(1*R*,2*R*,3*R*,4*R*)-2,3-bis{[*tert*-butyl(dimethyl)silyl]oxy}-4-(hydroxymethyl)cyclopentyl]amino}pyrimidin-5-yl)methanone (I-17). To a solution of 1-benzyl-1*H*-pyrazol-3-yl)(4-{[(1*R*,2*R*,3*R*,4*R*)-2,3-bis{[*tert*-butyl(dimethyl)silyl]oxy}-4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)cyclopentyl]amino}pyrimidin-5-yl)methanone (90.0 mg, 0.12 mmol) in EtOH (3.0 mL) was added a solution of 1% HCl in EtOH (3.0 mL, 0.36 mmol) at rt. The reaction mixture was sealed and placed in a refrigerator (4 °C) for 2 h then moved to a -20 °C freezer for 60 h. The reaction was then quenched by the addition of aq. saturated $NaHCO_3$ (10 mL). To the residue was added water (20mL) and it was extracted with EtOAc four times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was purified on silica gel to yield **I-17** (0.06 g, 79%). ^{1}H NMR ($CDCl_3$) δ 9.74 (s, 1H), 9.44–9.22 (br s, 1H), 8.66 (s, 1H), 7.44–7.33 (m, 4H), 7.30–7.26 (m, 2H), 6.92 (d, 1H, *J* = 2.2 Hz), 5.40 (s, 2H), 4.99–4.91 (m, 1H), 4.03–3.99 (m, 1H), 3.94–3.90 (m, 1H), 3.77–3.63 (m, 2H), 2.41–2.31 (m, 1H), 2.22–2.14 (m, 1H), 2.04–1.96 (br s, 1H), 1.66–1.59 (m, 1H), 0.93 (s, 9H), 0.92 (s, 9H), 0.13 (s, 3H), 0.12 (s, 3H), 0.07 (s, 3H), - 0.01 (s, 3H).

Step 9: [(1*R*,2*R*,3*R*,4*R*)-4-({5-[(1-Benzyl-1*H*-pyrazol-3-yl)carbonyl]pyrimidin-4-yl}amino)-2,3-dihydroxycyclopentyl]methyl sulfamate (4).
To a solution of (1-benzyl-1*H*-pyrazol-3-yl)(4-{[(1*R*,2*R*,3*R*,4*R*)-2,3-bis{[*tert*-butyl(dimethyl)silyl]oxy}-4-(hydroxymethyl)cyclopentyl]amino}pyrimidin-5-yl)methanone (0.06 g, 0.09 mmol) in DMF (1.5 mL) and triethylamine (0.08 mL, 0.56 mmol) was added chlorosulfonamide (0.05 g, 0.42 mmol) at rt, and the mixture was allowed to stir for 1 h. Additional chlorosulfonamide (25.0 mg, 0.21 mmol) was then added and the reaction stirred at rt an additional 1 h. The reaction was quenched with sat $NaHCO_3$, and then extracted with EtOAc four times. The combined organic layers were dried over $MgSO_4$, filtered and concentrated to give the crude sulfamate intermediate which was immediately dissolved in THF (4 mL). HCl (3.0 M solution in water; 1 mL, 3 mmol) was added to the solution. The reaction flask was fitted with a reflux condenser and the reaction stirred at 60 °C for 7 h. The reaction was then quenched with saturated $NaHCO_3$, and then extracted with EtOAc four times. The combined organic layers

were dried over $MgSO_4$, filtered and concentrated. The crude product was triturated with DCM and collected as a white solid to provide **4** (40 mg, 61%). ^{1}H NMR (DMSO-d_6) δ 9.50 (s, 1H), 9.39 (d, 1H, J = 7.5 Hz), 8.62 (s, 1H), 8.07 (d, 1H, J = 2.4 Hz), 7.43 (s, 2H), 7.41–7.27 (m, 5H), 6.91 (d, 1H, J = 2.4 Hz), 5.59 (d, 1H, J = 4.4 Hz), 5.51 (s, 2H), 5.12 (d, 1H, J = 4.7 Hz), 4.67–4.53 (m, 1H), 4.13 (dd, 1H, J = 9.4, 6.5 Hz), 4.01–3.92 (m, 1H), 3.87–3.79 (m, 1H), 3.66 (dd, 1H, J = 6.7, 4.4 Hz), 2.38–2.28 (m, 1H), 2.14–2.03 (m, 1H), 1.40–1.25 (m, 1H). LCMS (FA): m/z = 489.5 (M+H). HRMS m/z $[M+H]^+$ calcd for $C_{21}H_{25}N_6O_6S$ 489.1556, obsd 489.1556.

Synthesis of 7



Reagents: (a) (1*R*,2*R*,3*R*,5*R*)-3-amino-2-fluoro-5-(hydroxymethyl)cyclopentanol hydrochloride, DIPEA, 1-PrOH, 50 °C, 2 h, 94%; (b) TBSCl, imidazole, DMF, rt, 72 h, 92%; (c) 1% HCl in EtOH, EtOH, 4 °C, 22 h, 71%; (d) chlorosulfonamide, DMF, rt, 1 h; (e) 3N HCl, THF, rt, 3 h, 44% for 2 steps.

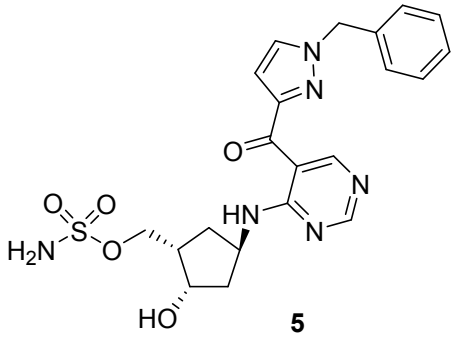
Step 1: (1-Benzyl-1*H*-pyrazol-3-yl)(4-{[(1*R*,2*R*,3*R*,4*R*)-2-fluoro-3-hydroxy-4-(hydroxymethyl)cyclopentyl]amino}pyrimidin-5-yl)methanone (I-18). (1-Benzyl-1*H*-pyrazol-3-yl)(4-chloropyrimidin-5-yl)methanone (**I-3**, 0.30 g, 1.0 mmol) and (1*R*,2*R*,3*R*,5*R*)-3-amino-2-fluoro-5-(hydroxymethyl)cyclopentanol hydrochloride (0.21 g, 1.11 mmol) (for synthesis see: Biggadike, K. et al. *J. Chem. Soc. Perkin Trans.* **1988**, *3*, 549-554; Borthwick, A.D. et al. *J. Med. Chem.* **1990**, *33*, 179-186) were weighed into a 20 mL reaction vessel. To this mixture was added 1-PrOH (14 mL) and *N,N*-diisopropylethylamine (0.52 mL, 3.0 mmol). The resulting mixture was sealed and the vessel allowed to stir while heating at 50 °C for 2 h. The reaction was then cooled to rt and the reaction was concentrated. The crude product was purified on silica gel to afford **I-18** (0.38 g, 94%). ^{1}H NMR (DMSO-d_6) δ 9.55 (s, 1H), 9.23 (d, 1H, J = 7.8 Hz), 8.67 (s, 1H), 8.07 (d, 1H, J = 2.3 Hz), 7.40–7.35 (m, 2H), 7.34–7.27 (m, 3H), 6.93 (d, 1H, J = 2.5 Hz), 5.51 (s, 2H), 5.29 (d, 1H, J = 5.0 Hz), 4.87–4.71 (m, 2H), 4.69 (t, 1H, J = 5.3 Hz), 3.95–3.85 (m, 1H), 3.51–3.45 (m, 1H), 3.43–3.37 (m, 1H), 2.29–2.21 (m, 1H), 1.96–1.86 (m, 1H), 1.45–1.35 (m, 1H). LCMS (FA): m/z = 412.4 (M+H).

Step 2: (1-Benzyl-1*H*-pyrazol-3-yl)(4-{[(1*R*,2*R*,3*R*,4*R*)-3-{[*tert*-butyl(dimethyl)silyl]oxy}-4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-2-fluorocyclopentyl]amino}pyrimidin-5-yl)methanone (I-19). To a solution of (1-benzyl-1*H*-pyrazol-3-yl)(4-{[(1*R*,2*R*,3*R*,4*R*)-2-fluoro-3-hydroxy-4-(hydroxymethyl)cyclopentyl]amino}pyrimidin-5-yl)methanone (0.37 g, 0.90 mmol) in DMF (14.8 mL) was added imidazole (0.24 g, 3.6 mmol) followed by TBSCl (0.34 g, 2.2 mmol) at rt, and the mixture was stirred for 3 d. The reaction was then quenched by addition of water and extracted with EtOAc three times. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified on silica gel to give **I-19** (0.53 g, 92%).

Step 3: (1-Benzyl-1*H*-pyrazol-3-yl)(4-{[(1*R*,2*R*,3*R*,4*R*)-3-{[*tert*-butyl(dimethyl)silyl]oxy}-2-fluoro-4-(hydroxymethyl)cyclopentyl]amino}pyrimidin-5-yl)methanone (I-20). To a solution of (1-benzyl-1*H*-pyrazol-3-yl)(4-{[(1*R*,2*R*,3*R*,4*R*)-3-{[*tert*-butyl(dimethyl)silyl]oxy}-4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-2-fluorocyclopentyl]amino}pyrimidin-5-yl)methanone (0.50 g, 0.78 mmol) in EtOH (50 mL) was added 1% HCl in EtOH (25.9 mL, 3.12 mmol) at rt. The reaction vessel was sealed and placed in a refrigerator (4 °C) for 22 h. The reaction was quenched by addition of a saturated aq. solution of $NaHCO_3$. To the residue was added water which was extracted with EtOAc four times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was purified on silica gel to give **I-20** (0.29 g, 71%). ^{1}H NMR ($CDCl_3$) δ 9.76 (s, 1H), 9.48–9.42 (br d, 1H, *J* = 7.8 Hz), 8.68 (s, 1H), 7.43 (d, 1H, *J* = 2.5 Hz), 7.41–7.34 (m, 3H), 7.29–7.25 (m, 2H), 6.94 (d, 1H, *J* = 2.3 Hz), 5.40 (s, 2H), 4.99–4.72 (m, 2H), 4.15 (ddd, 1H, *J* = 21.1, 5.0, 1.5 Hz), 3.77–3.67 (m, 2H), 2.48–2.39 (m, 1H), 2.20–2.09 (m, 1H), 1.58–1.46 (m, 2H), 0.92 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H). LCMS (FA): m/z = 526.7 (M+H).

Step 4: [(1*R*,2*R*,3*R*,4*R*)-4-({5-[(1-Benzyl-1*H*-pyrazol-3-yl)carbonyl]pyrimidin-4-yl}amino)-3-fluoro-2-hydroxycyclopentyl]methyl sulfamate (7).
To a solution of (1-benzyl-1*H*-pyrazol-3-yl)(4-{[(1*R*,2*R*,3*R*,4*R*)-3-{[*tert*-butyl(dimethyl)silyl]oxy}-2-fluoro-4-(hydroxymethyl)cyclopentyl]amino}pyrimidin-5-yl)methanone (0.28 g, 0.53 mmol) in DMF (4.0 mL) was added chlorosulfonamide (123 mg, 1.1 mmol) at rt, and the mixture was stirred for 1 h. The reaction was quenched by addition of a saturated solution of $NaHCO_3$ and diluted with water. The mixture was extracted with EtOAc four times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude sulfamate intermediate was dissolved in THF (11 mL) and HCl (3.0 M in water; 5.50 mL, 16.5 mmol) was added to the solution. The reaction was stirred for 3 h at rt. The reaction was quenched by the addition of a saturated solution of $NaHCO_3$ (30mL) and extracted with EtOAc four times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by prep HPLC to afford **7** (115 mg, 44%). ^{1}H NMR (DMSO-d_6) δ 9.56 (d, 1H, *J* = 1.1 Hz), 9.23 (d, 1H, *J* = 7.6 Hz), 8.68 (s, 1H), 8.07 (dd, 1H, *J* = 2.3, 1.2 Hz), 7.50 (s, 2H), 7.43–7.22 (m, 5H), 6.93 (d, 1H, *J* = 2.4 Hz), 5.57 (s, 1H), 5.51 (s, 2H), 4.93–4.70 (m, 2H), 4.12 (dd, 1H, *J* = 9.8, 6.0 Hz), 4.03 (dd, 1H, *J* = 9.8, 6.9 Hz), 3.94 (dd, 1H, *J* = 21.9, 4.7 Hz), 2.41–2.28 (m, 1H), 2.25–2.12 (m, 1H), 1.47 (dd, 1H, *J* = 22.4, 11.4 Hz). LCMS (FA): m/z = 491.5 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{24}FN_6O_5S$ 491.1513, obsd 491.1515.

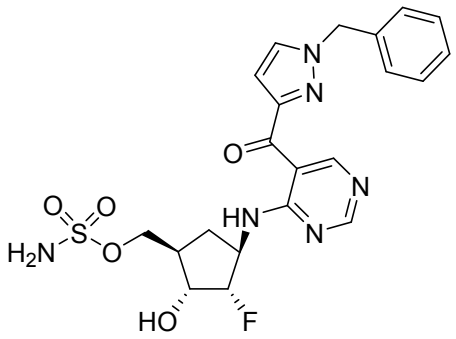
Synthesis of 5



5

[(1*S*,2*S*,4*R*)-4-({5-[(1-benzyl-1*H*-pyrazol-3-yl)carbonyl]pyrimidin-4-yl}amino)-2-hydroxycyclopentyl]methyl sulfamate (5).
Prepared by the method described for the preparation of **7** using intermediate **I-3** and (1*S*,2*S*,4*R*)-4-amino-2-(hydroxymethyl)cyclopentanol hydrobromide (for synthesis see: Armitage, I. et al. US Patent Application Publication 2009/0036678) as the starting material in Step 1. ^{1}H NMR (MeOD) δ 9.61 (s, 1H), 8.54 (s, 1H), 7.79 (d, 1H, *J* = 2.3 Hz), 7.38–7.29 (m, 5H), 6.93 (d, 1H, *J* = 2.5 Hz), 5.45 (s, 2H), 4.92–4.84 (m, 1H), 4.41–4.31 (m, 2H), 4.18–4.14 (m, 1H), 2.61–2.51 (m, 1H), 2.36–2.31 (m, 1H), 2.19–2.10 (m, 1H), 1.91–1.76 (m, 2H). LCMS (FA): m/z = 471.0 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{25}N_6O_5S$ 473.1607, obsd 473.1595.

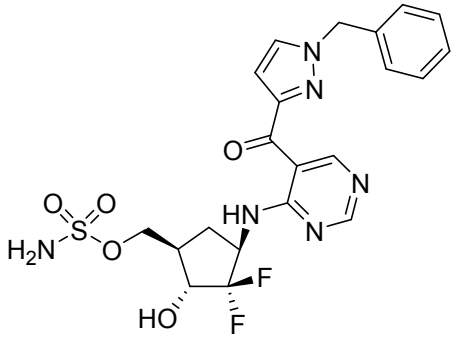
Synthesis of 6



6

[(1*R*,2*R*,3*S*,4*R*)-4-({5-[(1-Benzyl-1*H*-pyrazol-3-yl)carbonyl]pyrimidin-4-yl}amino)-3-fluoro-2-hydroxycyclopentyl]methyl sulfamate (6).
Prepared by the method described for the preparation of **7** using intermediate **I-3** and (1*R*,2*S*,3*R*,5*R*)-3-amino-2-fluoro-5-(hydroxymethyl)cyclopentanol hydrochloride (for synthesis see: Biggadike, K. et al. *J. Chem. Soc. Perkin Trans.* **1988**, *3*, 549-554; Borthwick, A.D. et al. *J. Med. Chem.* **1990**, *33*, 179-186) as the starting material in Step 1. ^{1}H NMR (DMSO-d_6) δ 9.44 (s, 1H), 8.97 (d, 1H, *J* = 7.4 Hz), 8.65 (s, 1H), 8.07 (d, 1H, *J* = 1.5 Hz), 7.47 (s, 2H), 7.39–7.28 (m, 5H), 6.92 (d, 1H, *J* = 1.4 Hz), 5.51 (s, 2H), 5.26–5.25 (m, 1H), 4.86–4.72 (m, 2H), 4.19–4.15 (m, 1H), 4.06–4.01 (m, 1H), 3.92–3.84 (m, 1H), 2.39–2.22 (m, 2H), 1.38–1.31 (m, 1H). LCMS (FA): m/z = 491.0 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{24}FN_6O_5S$ 491.1513, obsd 491.1511.

Synthesis of 8

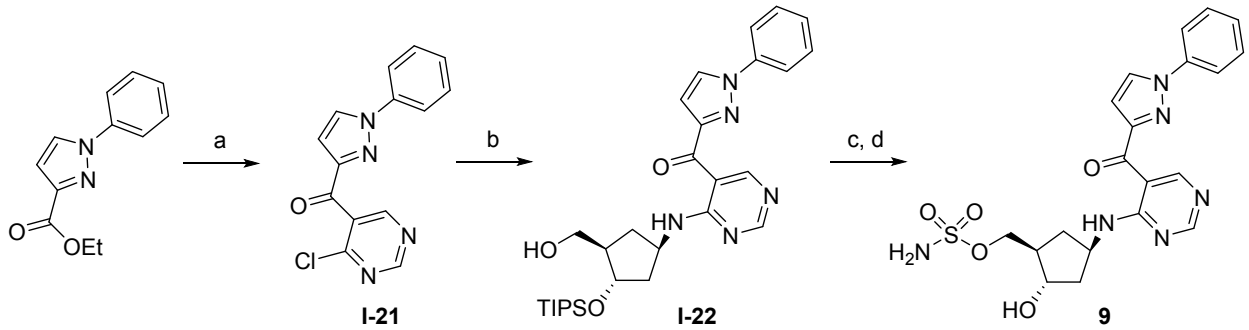


8

[(1*R*,2*R*,4*R*)-4-({5-[(1-Benzyl-1*H*-pyrazol-3-yl)carbonyl]pyrimidin-4-yl}amino)-3,3-difluoro-2-hydroxycyclopentyl]methyl sulfamate (8).
Prepared by the method described for the preparation of **7** using intermediate **I-3** and (1*R*,3*R*,5*R*)-3-amino-2,2-difluoro-5-(hydroxymethyl)cyclopentanol hydrochloride (for synthesis see: Biggadike, K. et al. *J. Chem. Soc. Perkin Trans.* **1988**, *3*, 549-554; Borthwick, A.D. et al. *J. Med. Chem.* **1990**, *33*, 179-186) as the starting material in Step 1. ^{1}H NMR (DMSO-d_6) δ 9.57 (s, 1H), 9.18 (d, 1H, *J* = 8.4 Hz), 8.69 (s, 1H), 8.08 (d, 1H, *J* = 2.2 Hz), 7.75–7.03 (m, 7H), 6.95 (d, 1H, *J* = 2.2 Hz), 5.52 (s, 2H), 5.10–4.92 (m, 1H), 4.19–3.97 (m, 2H), 3.84 (dt, 1H, *J* = 15.8, 8.0 Hz), 2.40–2.28 (m, 1H), 2.27–2.13 (m, 1H), 1.44 (dd, 1H, *J* = 22.8, 11.1 Hz). LCMS (FA): m/z = 509.4 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{23}F_2N_6O_5S$ 509.1419, obsd 509.1427.

Procedures for the syntheses of compounds in Table 3

Synthesis of 9



Reagents: (a) 4-chloro-5-iodopyrimidine, *n*-BuLi, THF, -78 °C, 0.5 h, 54%; (b) **I-8**, K_2CO_3, DMF, rt, 2 h, 83%; (c) chlorosulfonamide, DMF, 0.5 h, rt; (e) 4N HCl, THF, rt, 5 h, 88% for 2 steps.

Step 1: (4-Chloropyrimidin-5-yl)(1-phenyl-1*H*-pyrazol-3-yl)methanone (I-21). A round bottom flask was charged with 4-chloro-5-iodopyrimidine (360 mg, 1.5 mmol) and the reaction vessel was purged with argon. The content was dissolved in THF (12 mL) and the solution was

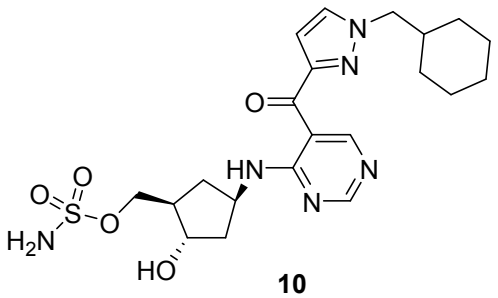
cooled to -78 °C. To the solution was added dropwise *n*-BuLi (2.50 M solution in hexane; 1.2 mL, 3.0 mmol), and the mixture was stirred for 30 min at -78 °C. To this mixture was added dropwise a solution of ethyl 1-phenyl-1*H*-pyrazole-3-carboxylate (300 mg, 1.4 mmol) in THF (6 mL), and the resulting mixture was stirred for 30 min. The reaction was quenched by addition of saturated NH_4Cl (50 mL) and extracted with EtOAc (50 mL) four times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to give **I-21** (210 mg, 54%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 9.14 (s, 1H), 9.01 (s, 1H), 8.06 (d, 1H, *J* = 2.8 Hz), 7.72–7.67 (m, 2H), 7.53–7.47 (m, 2H), 7.43–7.38 (m, 1H), 7.25 (d, 1H, *J* = 2.5 Hz). LCMS (FA): *m/z* = 285.1 (M+H).

Step 2: [4-({(1*R*,3*R*,4*S*)-3-(Hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl](1-phenyl-1*H*-pyrazol-3-yl)methanone (I-22). A reaction vessel was charged with {(1*R*,2*S*,4*R*)-4-amino-2-[(triisopropylsilyl)oxy]cyclopentyl}methanol-TFA (**I-8**, 293 mg, 0.73 mmol) and K_2CO_3 (277 mg, 2.00 mmol). To the mixture was added a solution of (4-chloropyrimidin-5-yl)(1-phenyl-1*H*-pyrazol-3-yl)methanone (190 mg, 0.67 mmol) in DMF (6 mL), and the resulting mixture was stirred for 2 h at rt. The reaction was concentrated in vacuo. To the residue was added water (40 mL) and the mixture was extracted with EtOAc (50 mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to give **I-22** (311 mg, 83%) as a yellow sticky oil. ^{1}H NMR ($CDCl_3$) δ 9.85 (s, 1H), 9.33 (br d, 1H, *J* = 6.9 Hz), 8.69 (s, 1H), 8.02 (d, 1H, *J* = 2.0 Hz), 7.78 (br d, 1H, *J* = 7.9 Hz), 7.52 (br t, 2H, *J* = 7.7 Hz), 7.40 (br t, 1H, *J* = 7.4 Hz), 7.11 (d, 1H, *J* = 2.0 Hz), 4.93–4.81 (m, 1H), 4.37 (q, 1H, *J* = 4.7 Hz), 3.74 (br t, 2H, *J* = 4.8 Hz), 2.60–2.49 (m, 1H), 2.29–2.18 (m, 2H), 1.96–1.86 (m, 1H), 1.85–1.78 (m, 1H), 1.42–1.23 (m, 2H), 1.10 (s, 21H). LCMS (FA): *m/z* = 536.4 (M+H).

Step 3: [(1*R*,2*S*,4*R*)-2-Hydroxy-4-({5-[(1-phenyl-1*H*-pyrazol-3-yl)carbonyl]pyrimidin-4-yl}amino)cyclopentyl]methyl sulfamate (9).
To a solution of [4-({(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl](1-phenyl-1*H*-pyrazol-3-yl)methanone (300 mg, 0.56 mmol) in DMF (2.0 mL) was added chlorosulfonamide (129 mg, 1.12 mmol) at rt, and the mixture was stirred for 30 min. The reaction was quenched by addition of saturated $NaHCO_3$ (50 mL) and extracted with EtOAc (60 mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to give the sulfamate intermediate [(267 mg, 78%); LCMS (FA): *m/z* = 615.2 (M+H)] which was dissolved in THF (2 mL). To this solution was added HCl (4.0 M solution in water; 2.0 mL, 8.0 mmol) at rt and the mixture was stirred for 5 h at rt. The reaction was quenched by addition of saturated $NaHCO_3$ and solid NaCl. The mixture was extracted with a 9:1 EtOAc:MeOH solution (60 mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. To the residue was added a small amount of DCM and the resulting suspension was filtered through a glass frit funnel. The residual solid was rinsed with DCM twice and dried under high vacuum overnight to give **9** (168 mg, 88%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 9.61 (s, 1H), 9.05 (d, 1H, *J* = 7.3 Hz), 8.69 (m, 2H), 7.98–7.89 (m, 2H), 7.63–7.54 (m, 2H), 7.47–7.39 (m, 3H), 7.14 (s, 1H), 4.91 (d, 1H, *J* = 4.5 Hz), 4.81–4.69 (m, 1H), 4.12 (dd, 1H, *J* = 9.5, 6.0 Hz), 4.03–3.95 (m, 2H), 2.44–2.34 (m, 1H), 2.21–2.10 (m, 1H), 2.08–

1.99 (m, 1H), 1.86–1.76 (m, 1H), 1.32 (dd, 1H, J = 21.8, 9.3 Hz). LCMS (FA): m/z = 459.2 (M+H); HRMS m/z $[M+H]^+$ calcd for $C_{20}H_{23}N_6O_5S$ 459.1451, obsd 459.1448.

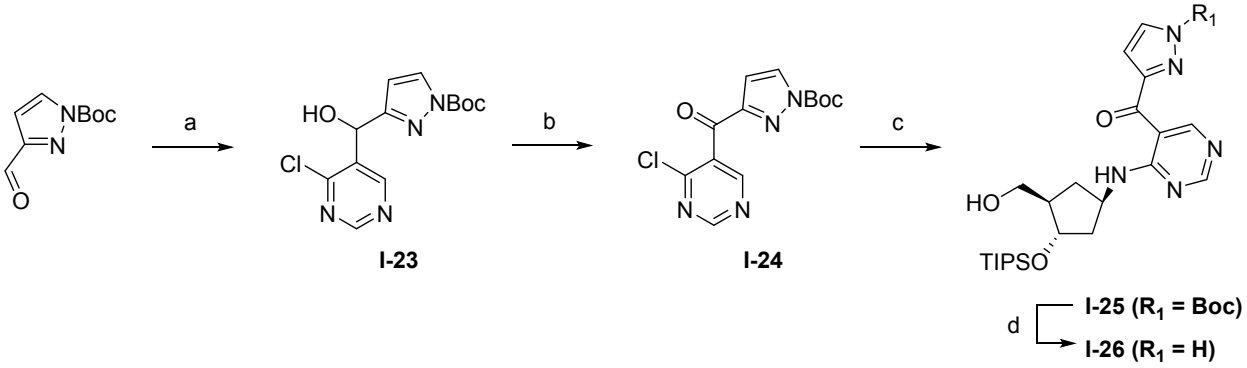
Synthesis of 10



10

{(1*R*,2*S*,4*R*)-4-[(5-{[1-(Cyclohexylmethyl)-1*H*-pyrazol-3-yl]carbonyl}pyrimidin-4-yl)amino]-2-hydroxycyclopentyl}methyl sulfamate (10).
Prepared by the method described for the preparation of **3** using (bromomethyl)cyclohexane as the starting material instead of benzyl bromide in Step 1 of the synthesis of **I-3**. ^{1}H NMR (DMSO-d_6) δ 9.50 (s, 1H), 9.03 (d, 1H, J = 7.4 Hz), 8.65 (s, 1H), 7.94 (s, 1H), 7.47 (s, 2H), 6.88 (d, 1H, J = 2.3 Hz), 4.98–4.88 (m, 1H), 4.78–4.65 (m, 1H), 4.17–4.07 (m, 3H), 4.04–3.93 (m, 2H), 2.44–2.32 (m, 1H), 2.20–2.08 (m, 1H), 2.06–1.95 (m, 1H), 1.91–1.74 (m, 2H), 1.73–1.58 (m, 3H), 1.58–1.48 (m, 2H), 1.34–1.23 (m, 1H), 1.23–1.10 (m, 3H), 1.06–0.92 (m, 2H). LCMS (FA): m/z = 479.6 (M+H). HRMS m/z $[M+H]^+$ calcd for $C_{21}H_{31}N_6O_5S$ 497.2077, obsd 479.2081.

Synthesis of intermediate I-26



Reagents: (a) 4-chloro-5-iodopyrimidine, *n*-BuLi, THF, -95 °C to -78 °C, 0.5 h, 64%; (b) Dess-Martin periodinane, DCM, 40 °C, 2 h, 90%; (c) **I-8**, DIPEA, *i*-PrOH, rt, 16 h, 63%; (d) K_3PO_4, EtOH, rt, 2 h, 79%.

Step 1: *tert*-Butyl 3-[(4-chloropyrimidin-5-yl)(hydroxy)methyl]-1*H*-pyrazole-1-carboxylate (I-23). Into a flame dried round bottom flask with stir bar was added 4-chloro-5-iodopyrimidine (12.3 g, 51 mmol) dissolved in THF (200 mL). The flask was purged with argon and cooled to -95 °C. To this solution was added dropwise *n*-BuLi (2.5 M in hexane; 43 mL, 107 mmol) at -95 °C and the mixture was stirred for 10 min. To this mixture was added *tert*-butyl 3-formyl-1*H*-pyrazole-1-carboxylate (9.1 g, 46 mmol) dissolved in THF (30 mL) dropwise at -95 °C. The reaction was stirred at -78 °C for 30 min. The reaction was quenched with a solution of acetic

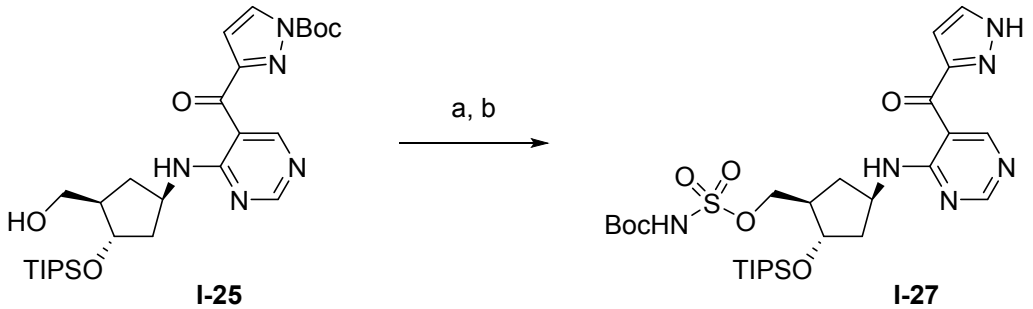
acid (7.9 mL) in THF (15 mL) and then allowed to warm to rt. Water (80 mL) was added and the mixture extracted with EtOAc three times. The combined organic layers were then washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified on silica gel to give **I-23** (10.2 g, 64%). ^{1}H NMR (DMSO-d_6) δ 9.01 (d, 2H, J = 4.8 Hz), 8.21 (d, 1H, J = 2.8 Hz), 6.61 (d, 1H, J = 5.0 Hz), 6.55 (d, 1H, J = 3.0 Hz), 5.98 (d, 1H, J = 5.0 Hz), 1.55 (s, 9H). LCMS (AA): *m/z* = 311.7 (M+H).

Step 2: *tert*-Butyl 3-[(4-chloropyrimidin-5-yl)carbonyl]-1*H*-pyrazole-1-carboxylate (I-24). *tert*-Butyl 3-[(4-chloropyrimidin-5-yl)(hydroxy)methyl]-1*H*-pyrazole-1-carboxylate (1.0 g, 3.3 mmol) was dissolved in DCM (30 mL) and Dess-Martin periodinane (2.1 g, 4.9 mmol) was added to this solution. The mixture was then stirred at 40 °C for 2 h. TLC showed the reaction was done. The reaction was concentrated and purified on silica gel to provide **I-24** (0.92 g, 90%). ^{1}H NMR ($CDCl_3$) δ 9.12 (s, 1H), 9.00 (s, 1H), 8.20 (d, 1H, J = 2.9 Hz), 7.09 (d, 1H, J = 2.8 Hz), 1.67 (s, 9H). LCMS (AA): *m/z* = 309.7 (M+H).

Step 3: *tert*-Butyl 3-{[4-({(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]carbonyl}-1*H*-pyrazole-1-carboxylate (I-25). *tert*-Butyl 3-[(4-chloropyrimidin-5-yl)carbonyl]-1*H*-pyrazole-1-carboxylate (2.2 g, 7.1 mmol) was dissolved in *i*-PrOH (80 mL). *N,N*-Diisopropylethylamine (3.7 mL, 21 mmol) and {(1*R*,2*S*,4*R*)-4-amino-2-[(triisopropylsilyl)oxy]cyclopentyl}methanol-TFA (**I-8**, 2.9 g, 7.1 mmol) dissolved in *i*-PrOH (20 mL) was added to the solution and it was stirred at rt for 16 h. The reaction mixture was concentrated and the crude product was purified on silica gel to provide **I-25** (2.5 g, 63%). ^{1}H NMR (DMSO-d_6) δ 9.44 (s, 1H), 9.06 (d, 1H, J = 7.3 Hz), 8.66 (s, 1H), 8.45 (d, 1H, J = 3.0 Hz), 7.01 (d, 1H, J = 3.0 Hz), 4.84–4.74 (m, 1H), 4.71 (t, 1H, J = 5.0 Hz), 4.31–4.25 (m, 1H), 3.47–3.37 (m, 2H), 2.40–2.31 (m, 1H), 2.08–1.98 (m, 2H), 1.85–1.77 (m, 1H), 1.62 (s, 9H), 1.34–1.25 (m, 1H), 1.07–1.02 (m, 21H). LCMS (AA): *m/z* = 560.8 (M+H).

Step 4: [4-({(1*R*,3*R*,4*S*)-3-(Hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl](1*H*-pyrazol-3-yl)methanone (I-26). To a solution of *tert*-butyl 3-{[4-({(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]carbonyl}-1*H*-pyrazole-1-carboxylate (1.7 g, 3.1 mmol) in EtOH (30 mL) was added a solution of K_3PO_4 (1.4 g, 6.5 mmol) in water (4.5 mL). This mixture was stirred at rt for 2 h. Water was added to the mixture and it was extracted with DCM (50mL) twice. The combined extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified on silica gel to give **I-26 (**1.13 g, 79%). ^{1}H NMR ($CDCl_3$) δ 13.93 (br s, 1H) 10.15 (br s, 1H) 9.47 (br s, 1H) 8.72 (s, 1H) 7.72 (d, 1H, J = 2.5 Hz) 7.00 (d, 1H, J = 2.2 Hz) 4.94–4.81 (m, 1H) 4.40–4.32 (m, 1H) 3.78–3.69 (m, 2H) 2.60–2.49 (m, 1H) 2.30–2.18 (m, 2H) 1.97–1.83 (m, 2H) 1.43–1.32 (m, 1H) 1.10 (s, 21H). LCMS (FA): *m/z* = 460.4 (M+H).

Synthesis of intermediate I-27

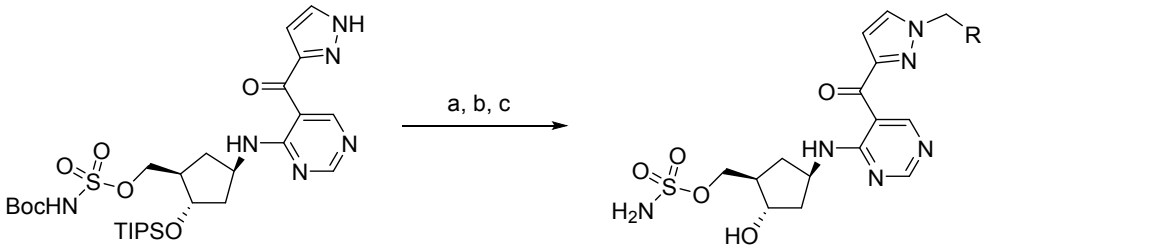


I-25 **I-27**

Reagents: (a) *tert*-butyl (chlorosulfonyl)carbamate, DIPEA, THF, 0 °C, 4 h; (b) K_3PO_4, EtOH, rt, 2 h, 56% for 2 steps.

Step 1: *tert*-Butyl [({(1*R*,2*S*,4*R*)-4-{[5-(1*H*-pyrazol-3-ylcarbonyl)pyrimidin-4-yl]amino}-2-[(triisopropylsilyl)oxy]cyclopentyl}methoxy)sulfonyl]carbamate (I-27). To a solution of *tert*-butyl 3-{[4-({(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]carbonyl}-1*H*-pyrazole-1-carboxylate (**I-25**, 2.5 g, 4.4 mmol) and *N,N*-diisopropylethylamine (2.3 g, 18 mmol) in THF (40 mL) was added *tert*-butyl (chlorosulfonyl)carbamate (1.9 g, 8.8 mmol) at 0 °C. It was then stirred at 0 °C for 4 h. The reaction solvent was evaporated and the residue was dissolved in EtOH (60 mL). A solution of K_3PO_4 (0.47 g, 2.2 mmol) in water (4 mL) was then added and the resulting mixture stirred at rt for 2 h. The reaction solvent was evaporated and the crude product was purified on silica gel to provide **I-27** (1.6 g, 56%). LCMS (AA): *m/z* = 639.0 (M+H).

General method for pyrazole alkylation of intermediate I-27 in library fashion for the preparation of compounds in Table 3



I-27

11: R = 2-chloro-1-phenyl
13: R = 4-chloro-1-phenyl
14: R = 3-fluoro-1-phenyl
17: R = 3-trifluoromethyl-1-phenyl
18: R = 3-trifluoromethoxy-1-phenyl

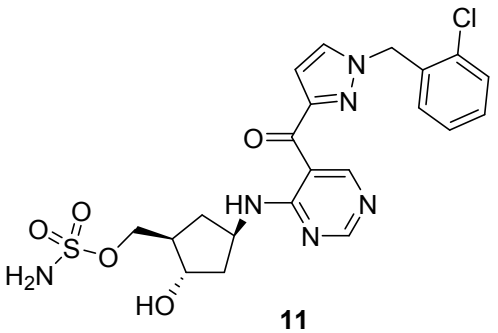
Reagents: (a) $ArCH_2Br$, Cs_2CO_3, THF, rt, 4 h; (b) TFA, H_2O, rt, 16 h; (c) MP PL-CO_3, MeOH

Step 1

To a 3-dram vial was added the substituted benzyl bromide (0.10 mmol), *tert*-butyl [({(1*R*,2*S*,4*R*)-4-{[5-(1*H*-pyrazol-3-ylcarbonyl)pyrimidin-4-yl]amino}-2-[(triisopropylsilyl) oxy] cyclopentyl}methoxy)sulfonyl]carbamate (**I-27**, 32.0 mg, 0.05 mmol) in THF (1.0 mL) followed by Cs_2CO_3 (0.16 g, 0.50 mmol). The mixture was shaken at rt for 4 h then the mixture was

filtered to remove solids. To the clear THF solution was added TFA (3.0 mL), water (0.3 mL). This mixture was then shaken at rt for 16 h. The solvent was then evaporated and to the resulting residue was added MeOH (3.0 mL) and MP PL-CO_3 resin. After shaking at rt for 30 min, the resin was filtered off and rinsed with MeOH (10 mL). The solvent was then evaporated and purified by prep-HPLC to give the desired products.

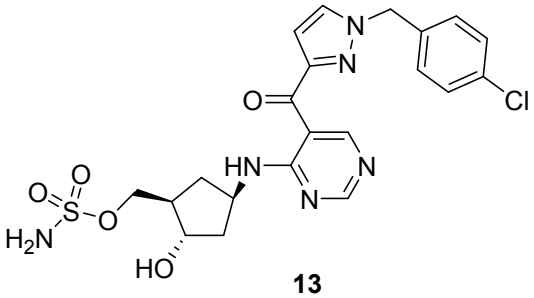
Synthesis of 11



11

{(1*R*,2*S*,4*R*)-4-[(5-{[1-(2-Chlorobenzyl)-1*H*-pyrazol-3-yl]carbonyl}pyrimidin-4-yl)amino]-2-hydroxycyclopentyl}methyl sulfamate (11).
Prepared by the general method described above using intermediate **I-27** and 1-(bromomethyl)-2-chlorobenzene as the starting material. LCMS (FA): *m/z* = 507.6 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{24}ClN_6O_5S$ 507.1217, obsd 507.1213.

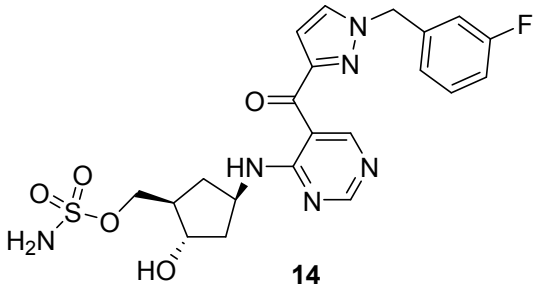
Synthesis of 13



13

{(1*R*,2*S*,4*R*)-4-[(5-{[1-(4-Chlorobenzyl)-1*H*-pyrazol-3-yl]carbonyl}pyrimidin-4-yl)amino]-2-hydroxycyclopentyl}methyl sulfamate (13).
Prepared by the general method described above using intermediate **I-27** and 1-(bromomethyl)-4-chlorobenzene as the starting material. LCMS (FA): *m/z* = 507.6 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{24}ClN_6O_5S$ 507.1217, obsd 507.1216.

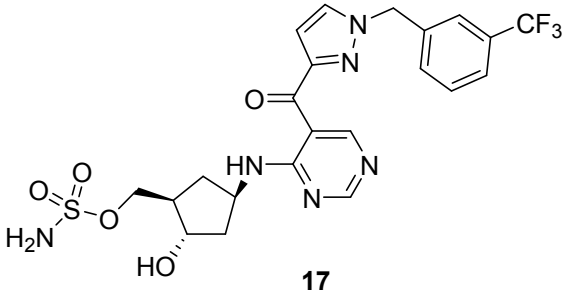
Synthesis of 14



14

{(1*R*,2*S*,4*R*)-4-[(5-{[1-(3-Fluorobenzyl)-1*H*-pyrazol-3-yl]carbonyl}pyrimidin-4-yl)amino]-2-hydroxycyclopentyl}methyl sulfamate (14).
Prepared by the general method described above using intermediate **I-27** and 1-(bromomethyl)-3-fluorobenzene as the starting material. LCMS (FA): *m/z* = 491.5 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{24}FN_6O_5S$ 491.1513, obsd 491.1512.

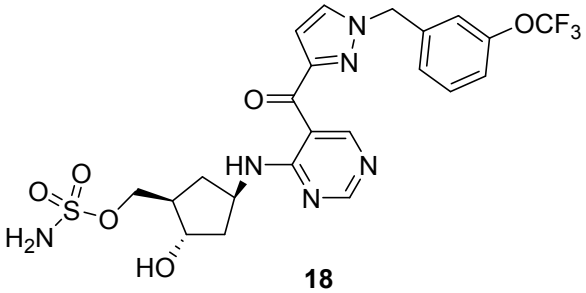
Synthesis of 17



17

[(1*R*,2*S*,4*R*)-2-Hydroxy-4-{[5-({1-[3-(trifluoromethyl)benzyl]-1*H*-pyrazol-3-yl}carbonyl) pyrimidin-4-yl]amino}cyclopentyl]methyl sulfamate (17).
Prepared by the general method described above using intermediate **I-27** and 3-(trifluoromethyl)benzyl bromide as the starting material. LCMS (FA): *m/z* = 541.0 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{22}H_{24}F_3N_6O_5S$ 541.1481, obsd 541.1480.

Synthesis of 18

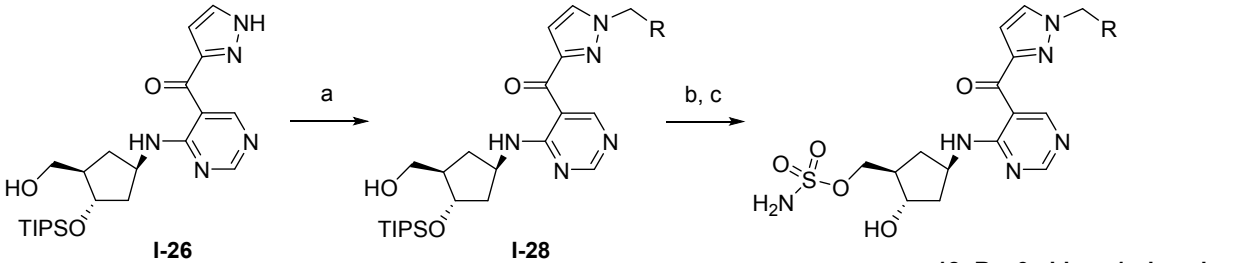


18

[(1*R*,2*S*,4*R*)-2-Hydroxy-4-{[5-({1-[3-(trifluoromethoxy)benzyl]-1*H*-pyrazol-3-yl}carbonyl) pyrimidin-4-yl]amino}cyclopentyl]methyl sulfamate (18).

Prepared by the general method described above using intermediate **I-27** and 1-(bromomethyl)-3-(trifluoromethoxy)benzene as the starting material. LCMS (FA): *m/z* = 557.0 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{22}H_{24}F_3N_6O_6S$ 557.1430, obsd 557.1432.

General method for pyrazole alkylation of intermediate I-26 for the preparation of compounds in Table 3



I-26 **I-28**

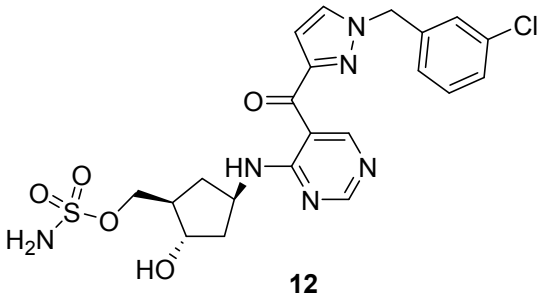
12: R = 3-chloro-1-phenyl
15: R = 3-methyl-1-phenyl
16: R = 3-propargyl-1-phenyl
19: R = 5-chloro-2-furyl
20: R = 5-chloro-2-thienyl
21: R = 6-chloro-2-pyridyl
22: R = 6-bromo-2-pyridyl

Reagents: (a) $ArCH_2X$, Cs_2CO_3, THF, rt, 20 h; (b) chlorosulfonamide, TEA, DMF, rt, 2 h; (c) 4N HCl, THF, 5 h, 40 °C.

Step 1
[4-({(1*R*,3*R*,4*S*)-3-(Hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl](1*H*-pyrazol-3-yl)methanone (**I-26**, 0.64 g, 1.4 mmol) was dissolved in THF (25 mL). To this solution was added Cs_2CO_3 (1.6 g, 4.9 mmol) and the substituted benzyl halide (2.1 mmol) and the reaction was stirred at rt for 20 h. The reaction was filtered and the filtrate concentrated. The residue was purified on silica gel to give the desired product, **I-28**.

Step 2
To a solution of the above alcohol (**I-28**, 1.16 mmol) and triethylamine (0.5 mL, 2.4 mmol) in DMF (10 mL) was added chlorosulfonamide (400 mg, 3.3 mmol) at rt, and the mixture was stirred for 2 h. The reaction was quenched by addition of a saturated solution of $NaHCO_3$ and diluted with water. The mixture was extracted with EtOAc four times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was purified on silica gel to give the sulfamate intermediate which was dissolved in THF (7 mL) and HCl (4.0 M in water; 7 mL, 28 mmol) was added to the solution. The reaction was stirred for 5 h at 40 °C. The reaction was cooled to rt and quenched by the addition of a saturated solution of $NaHCO_3$ and extracted with EtOAc four times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was triturated with the addition of DCM to give the desired product.

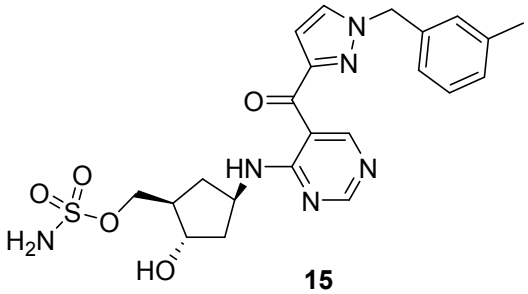
Synthesis of 12



12

((1*R*,2*S*,4*R*)-4-((5-(1-(3-Chlorobenzyl)-1*H*-pyrazole-3-carbonyl)pyrimidin-4-yl)amino)-2-hydroxycyclopentyl)methyl sulfamate (12).
Prepared by the general method described above using intermediate **I-26** and 1-(bromomethyl)-3-chlorobenzene as the starting material. ^{1}H NMR (MeOD) δ 9.84 (s, 1H), 9.69 (s, 1H), 9.23 (s, 1H), 8.84–8.69 (m, 1H), 7.90 (d, 1H, *J* = 2.4 Hz), 7.38–7.28 (m, 3H), 7.27–7.19 (m, 1H), 7.04 (d, 1H), 5.48 (s, 2H), 5.01–4.92 (m, 1H), 4.26–4.10 (m, 3H), 2.57–2.49 (m, 1H), 2.36–2.24 (m, 1H), 2.24–2.13 (m, 1H), 2.08–1.95 (m, 1H), 1.61–1.50 (m, 1H). LCMS (FA): *m/z* = 507.1 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{24}ClN_6O_5S$ 507.1217, obsd 507.1215.

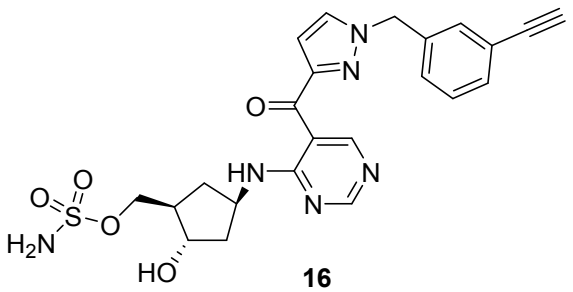
Synthesis of 15



15

{(1*R*,2*S*,4*R*)-2-Hydroxy-4-[(5-{[1-(3-methylbenzyl)-1*H*-pyrazol-3-yl]carbonyl}pyrimidin-4-yl)amino]cyclopentyl}methyl sulfamate (15).
Prepared by the general method described above using intermediate **I-26** and 1-(chloromethyl)-3-methylbenzene as the starting material. ^{1}H NMR (MeOD) δ 9.63 (s, 1H), 8.56 (s, 1H), 7.78 (d, 1H, *J* = 2.4 Hz), 7.27–7.22 (m, 1H), 7.16–7.08 (m, 3H), 6.93 (d, 1H, *J* = 2.4 Hz), 5.42 (s, 2H), 4.86–4.77 (m, 1H), 4.25–4.13 (m, 3H), 2.58–2.49 (m, 1H), 2.33 (s, 3H), 2.31–2.23 (m, 1H), 2.22–2.14 (m, 1H), 1.97–1.88 (m, 1H), 1.44 (dt, 1H, *J* = 13.0, 9.0 Hz). LCMS (FA): *m/z* = 487.4 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{22}H_{27}N_6O_5S$ 487.1764, obsd 487.1763.

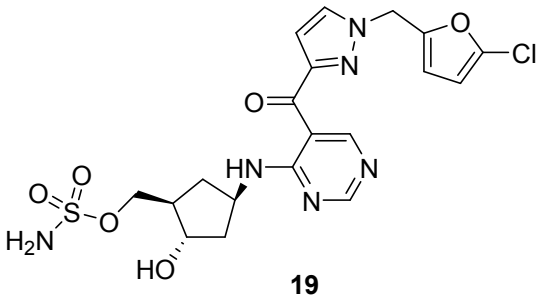
Synthesis of 16



16

{(1*R*,2*S*,4*R*)-4-[(5-{[1-(3-Ethynylbenzyl)-1*H*-pyrazol-3-yl]carbonyl}pyrimidin-4-yl)amino]-2-hydroxycyclopentyl}methyl sulfamate (16).
Prepared by the general method described above using intermediate **I-26** and 3-ethynylbenzyl methanesulfonate **I-30** as the starting material. ^{1}H NMR (MeOD) δ 9.62 (s, 1H), 8.56 (s, 1H), 7.84 (d, 1H, *J* = 2.4 Hz), 7.44–7.30 (m, 4H), 6.96 (d, 1H, *J* = 2.4 Hz), 5.47 (s, 2H), 4.86–4.77 (m, 1H), 4.19 (m, 3H), 3.51 (s, 1H), 2.58–2.48 (m, 1H), 2.33–2.22 (m, 1H), 2.22–2.14 (m, 1H), 1.98–1.87 (m, 1H), 1.44 (dt, 1H, *J* = 13.0, 9.1 Hz). LCMS (FA): *m/z* = 497.5 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{23}H_{25}N_6O_5S$ 497.1607, obsd 497.1606.

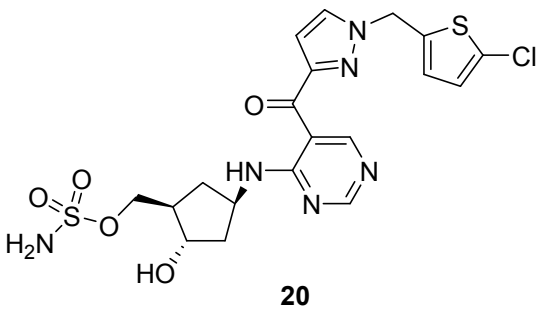
Synthesis of 19



19

[(1*R*,2*S*,4*R*)-4-{[5-({1-[(5-Chloro-2-furyl)methyl]-1*H*-pyrazol-3-yl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (19).
Prepared by the general method described above using intermediate **I-26** and 2-(bromomethyl)-5-chlorofuran as the starting material. LCMS (FA): *m/z* = 497.3 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{19}H_{22}ClN_6O_6S$ 497.1010, obsd 497.1012.

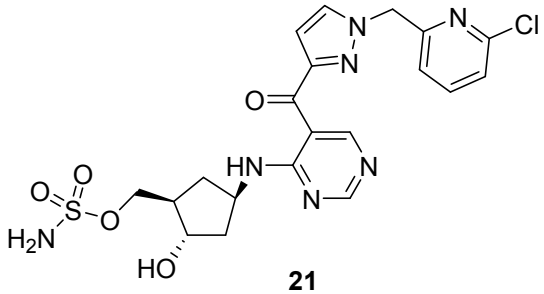
Synthesis of 20



20

[(1*R*,2*S*,4*R*)-4-{[5-({1-[(5-Chloro-2-thienyl)methyl]-1*H*-pyrazol-3-yl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (20).
Prepared by the general method described above using intermediate **I-26** and 2-(bromomethyl)-5-chlorothiophene as the starting material. The following alternative conditions were employed in the reaction scheme: Step 2: desilylating agent/solvent used was TFA/water. LCMS (FA): *m/z* = 513.0 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{19}H_{22}ClN_6O_5S_2$ 513.0782, obsd 513.0779.

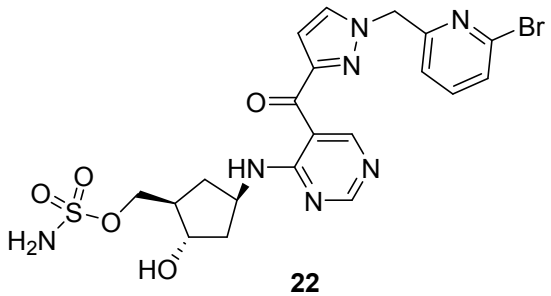
Synthesis of 21



21

[(1*R*,2*S*,4*R*)-4-{[5-({1-[(6-Chloropyridin-2-yl)methyl]-1*H*-pyrazol-3-yl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (21).
Prepared by the general method described above using intermediate **I-26** and 2-(bromomethyl)-6-chloropyridine as the starting material. ^{1}H NMR (DMSO-d_6) δ 9.41 (s, 1H), 8.99 (d, 1H, *J* = 7.2 Hz), 8.62 (s, 1H), 8.10 (d, 1H, *J* = 2.4 Hz), 7.88 (t, 1H, *J* = 7.8 Hz), 7.48 (d, 1H, *J* = 7.9 Hz), 7.43 (s, 2H), 7.18 (d, 1H, *J* = 7.6 Hz), 6.94 (d, 1H, *J* = 2.4 Hz), 5.63 (s, 2H), 4.91 (d, 1H, *J* = 4.5 Hz), 4.71 (dd, 1H, *J* = 16.0, 7.8 Hz), 4.10 (dd, 1H, *J* = 9.7, 5.9 Hz), 4.01–3.94 (m, 2H), 2.41–2.31 (m, 1H), 2.18–2.08 (m, 1H), 2.04–1.96 (m, 1H), 1.83–1.73 (m, 1H), 1.28 (dt, 1H, *J* = 12.9, 9.2 Hz). LCMS (FA): *m/z* = 508.0 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{20}H_{23}ClN_7O_5S$ 508.1170, obsd 508.1167.

Synthesis of 22

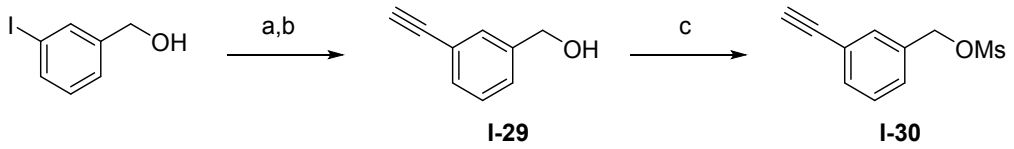


22

[(1*R*,2*S*,4*R*)-4-{[5-({1-[(6-Bromopyridin-2-yl)methyl]-1*H*-pyrazol-3-yl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (22).
Prepared by the general method described above using intermediate **I-26** and 2-bromo-6-bromomethyl-pyridine as the starting material. ^{1}H NMR (DMSO-d_6) δ 9.43 (s, 1H), 9.00 (d, 1H,

J = 7.4 Hz), 8.63 (s, 1H), 8.11 (d, 1H, *J* = 2.4 Hz), 7.78 (t, 1H, *J* = 7.8 Hz), 7.62 (d, 1H, *J* = 7.9 Hz), 7.45 (s, 2H), 7.21 (d, 1H, *J* = 7.5 Hz), 6.95 (d, 1H, *J* = 2.4 Hz), 5.64 (s, 2H), 4.92 (d, 1H, *J* = 4.3 Hz), 4.78–4.66 (m, 1H), 4.12 (dd, 1H, *J* = 9.7, 5.9 Hz), 4.03–3.94 (m, 2H), 2.42–2.31 (m, 1H), 2.20–2.08 (m, 1H), 2.06–1.96 (m, 1H), 1.84–1.74 (m, 1H), 1.29 (dt, 1H, *J* = 12.8, 9.2 Hz). LCMS (FA): *m/z* = 554.4 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{20}H_{23}BrN_7O_5S$ 552.0665, obsd 552.0659.

Synthesis of intermediate I-30

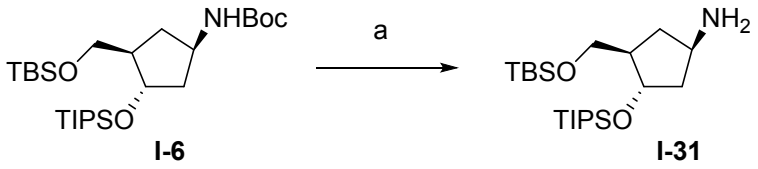


Reagents: (a) (trimethylsilyl)acetylene, $Pd(PPh_3)_2Cl_2$, CuI, TEA, 100 °C, 2 h, 52%; (b) K_2CO_3, MeOH, rt, 1 h, 97%; (c) MsCl, TEA, DCM, 0 °C, 1 h, 82%.

Step 1: (3-Ethynylphenyl)methanol (I-29). A reaction vial was charged with 3-iodobenzyl alcohol (1.0 g, 4.27 mmol), $Pd(PPh_3)_2Cl_2$ (60 mg, 0.08 mmol), CuI (33 mg, 0.17 mmol), and triethylamine (5.0 mL, 36 mmol). The vial was purged with argon and then sealed with a cap. After sonication, (trimethylsilyl)acetylene (0.90 mL, 6.4 mmol) was added to the mixture, and the resulting mixture was heated at 100 °C for 2 h. The reaction was diluted with EtOAc (100mL) and the EtOAc layer was washed with 1 M HCl (60 mL) twice, water (60 mL) and brine (60 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified on silica gel to give the TMS protected alkyne (855 mg, 98%) as light yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.50 (s, 1H), 7.42–7.38 (m, 1H), 7.35–7.28 (m, 2H), 4.68 (s, 2H), 0.26 (s, 9H). To a solution of alkyne (1.30 g, 6.4 mmol) in MeOH (15 mL) was added K_2CO_3 (4.40 g, 31.8 mmol) at rt, and the mixture was stirred for 1 h. The reaction was concentrated in vacuo. To the residue was added water (100 mL) and extracted with Et_2O (100 mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography to give **I-29** (813 mg, 97%) as a light yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.52 (s, 1H), 7.43 (br d, 1H, *J* = 7.3 Hz), 7.40–7.30 (m, 2H), 4.70 (s, 2H), 3.08 (s, 1H), 1.69 (br s, 1H).

Step 2: 3-Ethynylbenzyl methanesulfonate (I-30). To a solution of (3-ethynylphenyl)methanol (106 mg, 0.80 mmol) in DCM (7 mL) was added triethylamine (0.17 mL, 1.2 mmol) followed by MsCl (65 uL, 0.84 mmol) at 0 °C under an atmosphere of argon. The mixture was stirred for 1 h. The reaction was quenched by addition of water (50 mL) and DCM (50 mL). After separation, the DCM layer was washed with water (50 mL) followed by brine (50 mL) and then dried over Na_2SO_4. The mixture was filtered and concentrated in vacuo. The residue was purified on silica gel to give **I-30** (138 mg, 82%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.50–7.60 (m, 2H), 7.35–7.45 (m, 2H), 5.23 (s, 2H), 3.15 (s, 1H), 2.95 (s, 3H).

Synthesis of cyclopentane intermediate I-31

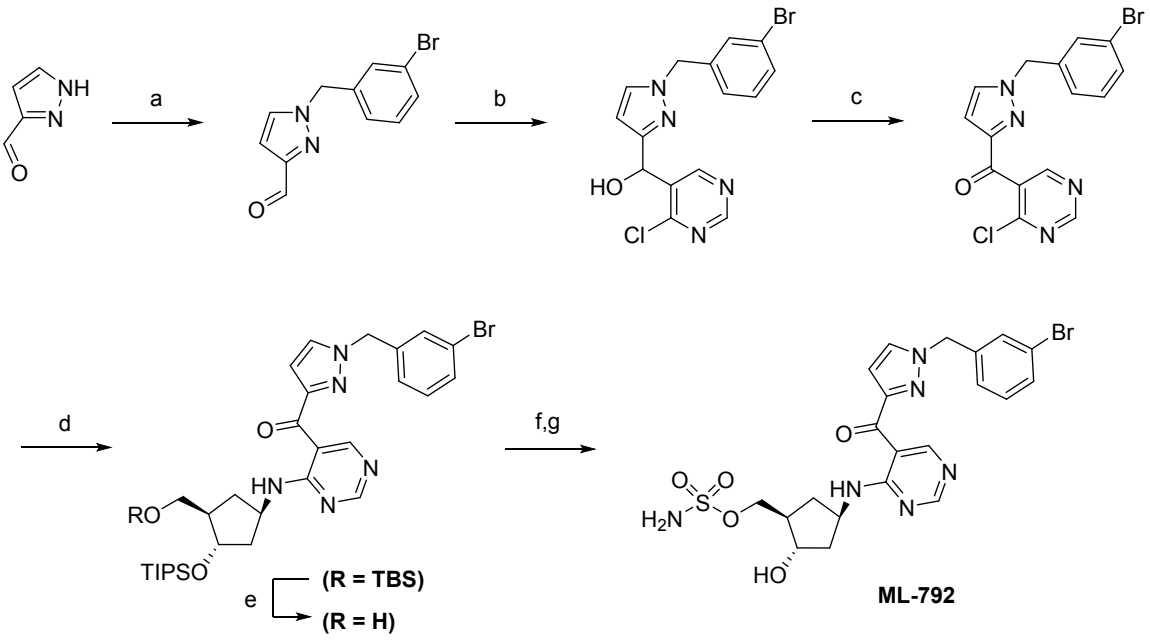


I-6 **I-31**

Reagents: (a) $ZnBr_2$, EtOH, DCM, rt, 37 h, 91%.

Step 1: (1*R*,3*R*,4*S*)-3-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentanamine (I-31). To solution of *tert*-butyl {(1*R*,3*R*,4*S*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}carbamate (**I-6**, 1.5 g, 3.0 mmol) in DCM (100 mL) was added EtOH (0.38 mL, 6.6 mmol) followed by zinc bromide (5.4 g, 24 mmol) at rt, and the mixture was stirred for 37 h. The reaction was quenched by addition of 1N NaOH (100 mL) and extracted with DCM (100 mL) five times. The combined organic layers were washed with brine and then dried over Na_2SO_4. After filtration, the filtrate was concentrated in vacuo. The residue was purified on silica gel to give **I-31** (1.09 g, 91%) as a colorless oil. ^{1}H NMR (MeOD) δ 4.33–4.4.29 (m, 1H), 3.63 (dd, 1H, *J* = 10.0, 5.5 Hz), 3.58 (dd, 1H, J = 10.0, 6.0 Hz), 3.53–3.44 (m, 1H), 2.22–2.13 (m, 1H), 2.11–2.02 (m, 1H), 1.92 (ddd, 1H, *J* = 12.7, 6.8, 3.1 Hz), 1.57 (ddd, 1H, *J* = 12.7, 9.0, 6.0 Hz), 1.09–0.96 (m, 22H), 0.82 (s, 9H), 0.07 (s, 6H). LCMS (FA): *m/z* = 402.6 (M+H).

Synthesis of ML-792



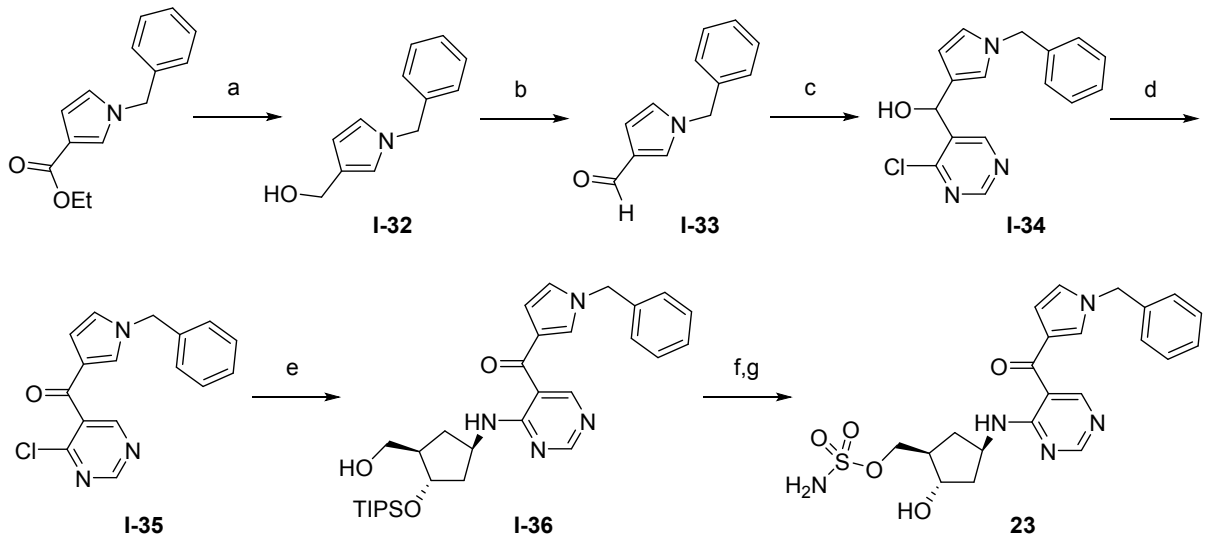
Reagents: (a) 3-bromobenzyl bromide, K_2CO_3, DMF, rt, 14 h, 76%; (b) 4-chloro-5-iodopyrimidine, *n*-BuLi, THF, -78 °C, 0.5 h, 71%; (c) MnO_2, DCM, rt, 12 h, 82%; (d) **I-31**, K_2CO_3, DMF, rt, 12 h, 94%; (e) 1% HCl in EtOH, EtOH, 4 °C for 14 h followed by rt for 9 h, 90%; (f) chlorosulfonamide, DMF, 0 °C, 20 min, 86%; (g) 4N HCl, THF, rt, 14 h, 90%.

{(1*R*,2*S*,4*R*)-4-[(5-{[1-(3-Bromobenzyl)-1*H*-pyrazol-3-yl]carbonyl}pyrimidin-4-yl)amino]-2-hydroxycyclopentyl}methyl sulfamate (ML-792).

For a detailed synthesis see: He, X. et al. *Nature Chemical Biology* **2017**, 13(11), 1164-1171. MP: 137-138 °C; ^{1}H NMR (DMSO-d_6) δ 9.44 (s, 1H), 9.00 (d, 1H, J = 7.4 Hz), 8.63 (s, 1H), 8.09 (d, 1H, J = 2.4 Hz), 7.56–7.50 (m, 2H), 7.43 (s, 2H), 7.37–7.31 (m, 1H), 7.28 (d, 1H, J = 7.7 Hz), 6.91 (d, 1H, J = 2.4 Hz), 5.52 (s, 2H), 4.91 (d, 1H, J = 4.3 Hz), 4.77–4.65 (m, 1H), 4.10 (dd, 1H, J = 9.7, 5.9 Hz), 4.01–3.93 (m, 2H), 2.41–2.31 (m, 1H), 2.18–2.08 (m, 1H), 2.05–1.95 (m, 1H), 1.78 (ddd, 1H, J = 13.2, 8.4, 6.9 Hz), 1.28 (dt, 1H, J = 12.8, 9.2 Hz). ^{13}C NMR (DMSO-d_6) δ 186.4, 161.2, 160.4, 159.8, 150.4, 139.3, 132.5, 130.9, 130.8, 130.4, 126.7, 121.8, 110.9, 109.1, 71.4, 70.4, 54.7, 48.7, 45.9, 40.9, 34.2. LCMS (FA): *m/z* = 553.3 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{24}BrN_6O_5S$ 551.0707, obsd 551.0703; analysis (%calcd, %found for $C_{21}H_{23}BrN_6O_5S$: C (45.74, 45.76), H (4.20, 4.30), Br (14.49, 14.74), N (15.24, 15.51), S (5.81, 5.94).

Procedures for the syntheses of compounds in Table 4

Synthesis of 23



Reagents: (a) LAH, THF, 50 °C, 4 h, 88%; (b) MnO_2, DCM, rt, 19 h, 83%; (c) 4-chloro-5-iodopyrimidine, *n*-BuLi, -78 °C, 2 h, 41%; (d) MnO_2, DCM, rt, 19 h, 83%; (e) **I-8**, DIPEA, 2-PrOH, rt, 20 h, 42%; (f) chlorosulfonamide, DMF, rt, 1 h; (g) 4N HCl, THF, rt, 3 h, 36% for 2 steps.

Step 1: (1-Benzyl-1*H*-pyrrol-3-yl)methanol (I-32). A solution of lithium aluminum hydride (0.66 g, 17 mmol) in THF (100 mL) was stirred at 0 °C. A solution of 1-benzylpyrrole-3-carboxylic acid ethyl ester (2.0 g, 8.7 mmol) in THF (10 mL) was added dropwise. The resulting reaction mixture was heated at 50 °C for 3 h. Additional lithium aluminum hydride (0.75 g, 20 mmol) was added and the reaction continued to stir at 50 °C for 1 h. The reaction mixture was then cooled to 0 °C, and sodium sulfate decahydrate (8 g) was added and the mixture was stirred for 16 h. EtOAc was added and the mixture was filtered over a pad of celite, then washed with additional EtOAc. The filtrate was concentrated and the crude residue purified on silica gel to give **I-32** (1.4 g, 88%). ^{1}H NMR (DMSO-d_6) δ 7.35–7.30 (m, 2H), 7.28–7.23 (m, 1H), 7.21–7.17

(m, 2H), 6.73 (dd, 1H, *J* = 2.5, 2.3 Hz), 6.70–6.68 (m, 1H), 5.98–5.95 (m, 1H), 5.02 (s, 2H), 4.56 (t, 1H, *J* = 5.5 Hz), 4.27 (d, 2H, *J* = 5.5 Hz). LCMS (FA): *m/z* = 188.4 (M+H).

Step 2: 1-Benzyl-1*H*-pyrrole-3-carbaldehyde (I-33). To a solution of (1-benzyl-1*H*-pyrrol-3-yl)methanol (1.4 g, 7.7 mmol) in DCM (39 mL) was added MnO_2 (6.7 g, 77 mmol). The suspension was stirred for 19 h at rt. The reaction was then filtered through a celite pad and the residual solid was washed with DCM several times. The filtrate was then concentrated in vacuo and the residue was purified on silica gel to afford **I-33** (1.2 g, 83%). ^{1}H NMR (DMSO-d_6) δ 9.64 (s, 1H), 7.73 (t, 1H, *J* = 1.9 Hz), 7.39–7.34 (m, 2H), 7.33–7.26 (m, 3H), 6.99 (t, 1H, *J* = 2.1 Hz), 6.47 (dd, 1H, *J* = 2.9, 1.6 Hz), 5.20 (s, 2H). LCMS (AA): *m/z* = 186.1 (M+H).

Step 3: (1-Benzyl-1*H*-pyrrol-3-yl)(4-chloropyrimidin-5-yl)methanol (I-34). Into a flame dried round bottom flask with stir bar was added 4-chloro-5-iodopyrimidine (1.4 g, 5.8 mmol) dissolved in THF (40 mL). The flask was purged with argon and cooled to -78 °C. To this solution was added dropwise *n*-BuLi (2.5 M in hexane; 4.6 mL, 11.5 mmol) at -78 °C and the mixture was stirred for 30 min. To this mixture was added 1-benzyl-1*H*-pyrrole-3-carbaldehyde (1.2 g, 6.3 mmol) dissolved in THF (10 mL) dropwise. The reaction was stirred at -78 °C for 2 h. The reaction was quenched with a saturated solution of NH_4Cl and then extracted with EtOAc three times. The combined organic layers were then washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified on silica gel to give **I-34** (0.70 g, 41%). LCMS (AA): *m/z* = 300.2 (M+H).

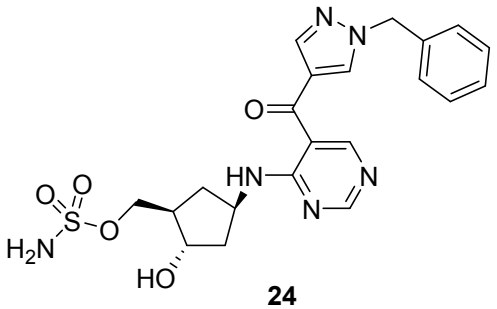
Step 4: (1-Benzyl-1*H*-pyrrol-3-yl)(4-chloropyrimidin-5-yl)methanone (I-35). To a solution of (1-benzyl-1*H*-pyrrol-3-yl)(4-chloropyrimidin-5-yl)methanol (0.70 g, 2.4 mmol) in DCM (23 mL) was added MnO_2 (2.0 g, 23 mmol). The suspension was stirred for 19 h at rt. The reaction was then filtered through a celite pad and the residual solid was washed with DCM several times. The filtrate was then concentrated in vacuo and the residue was purified on silica gel to afford **I-35** (0.59 g, 83%). ^{1}H NMR ($CDCl_3$) δ 9.07 (s, 1H), 8.72 (s, 1H), 7.41–7.32 (m, 3H), 7.19–7.15 (m, 3H), 6.75–6.73 (m, 1H), 6.63 (dd, 1H, *J* = 2.8, 1.8 Hz), 5.09 (s, 2H). LCMS (FA): *m/z* = 298.4 (M+H).

Step 5: (1-Benzyl-1*H*-pyrrol-3-yl)[4-({(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]methanone (I-36). (1-Benzyl-1*H*-pyrrol-3-yl)(4-chloropyrimidin-5-yl)methanone (147 mg, 0.49 mmol) and {(1*R*,2*S*,4*R*)-4-amino-2-[(triisopropylsilyl)oxy]cyclopentyl}methanol-TFA (**I-8**, 180 mg, 0.45 mmol) were weighed into a reaction vessel with stir bar. To this mixture was added 2-PrOH (5 mL) and *N,N*-diisopropylethylamine (0.24 mL , 1.35 mmol). The resulting mixture was allowed to stir at rt for 20 h. The reaction was then concentrated and the crude product was purified on silica gel to afford **I-36** (104 mg, 42%). LCMS (FA): *m/z* = 549.7 (M+H).

Step 6: [(1*R*,2*S*,4*R*)-4-({5-[(1-Benzyl-1*H*-pyrrol-3-yl)carbonyl]pyrimidin-4-yl}amino)-2-hydroxycyclopentyl]methyl sulfamate (23).
To a solution of (1-benzyl-1*H*-pyrrol-3-yl)[4-({(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]methanone (0.12 g, 0.21 mmol) in DMF (4 mL) was added chlorosulfonamide (74 mg, 0.64 mmol) at rt, and the mixture was stirred for 1 h. The reaction was quenched by addition of a saturated solution of $NaHCO_3$ and diluted

with water. The mixture was extracted with EtOAc four times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude sulfamate intermediate was dissolved in THF (2 mL) and HCl (4.0 M in water; 2.1 mL, 8.4 mmol) was added to the solution. The reaction was stirred for 3 h at rt. The reaction was quenched by the addition of a saturated solution of $NaHCO_3$ and extracted with EtOAc four times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by prep HPLC to afford **23** (26 mg, 36%). ^{1}H NMR (DMSO-d_6) δ 8.66 (s, 1H), 8.60 (s, 1H), 8.44 (d, 1H, J = 7.4 Hz), 7.67 (s, 1H), 7.50–7.33 (m, 3H), 7.30 (t, 3H, J = 6.3 Hz), 7.00 (s, 1H), 6.54 (s, 1H), 5.21 (s, 2H), 5.05–4.75 (m, 1H), 4.74–4.57 (m, 1H), 4.15–4.03 (m, 1H), 4.03–3.88 (m, 2H), 2.96–2.52 (m, 1H), 2.40–2.25 (m, 1H), 2.20–2.05 (m, 1H), 2.03–1.90 (m, 1H), 1.79–1.66 (m, 1H), 1.31–1.18 (m, 1H). LCMS (FA): *m/z* = 472.5 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{22}H_{26}N_5O_5S$ 472.1655, obsd 472.1666.

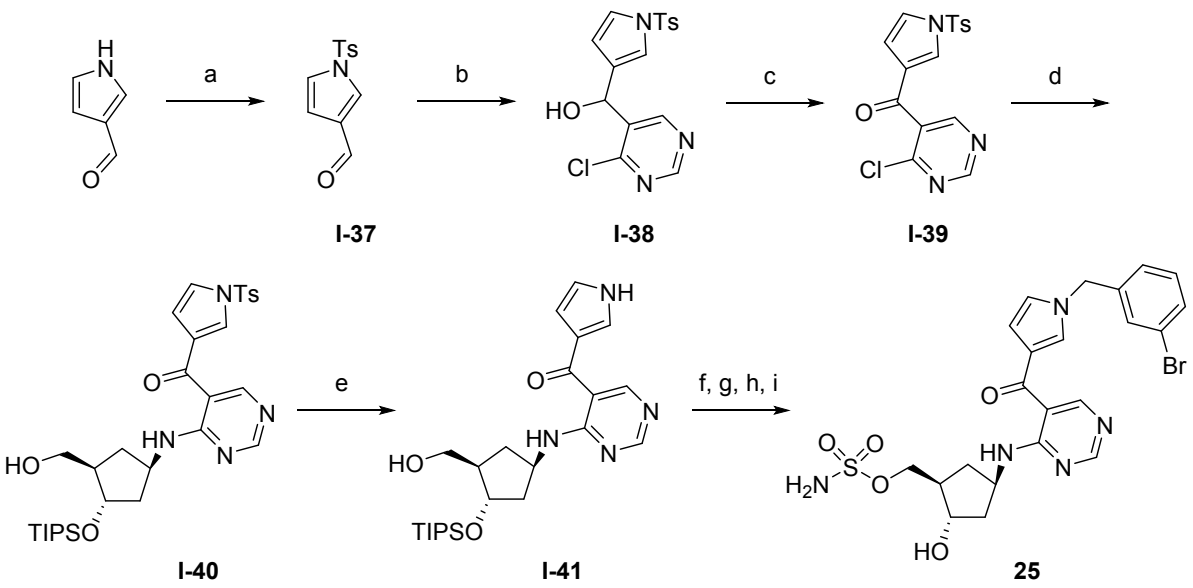
Synthesis of 24



24

[(1*R*,2*S*,4*R*)-4-({5-[(1-Benzyl-1*H*-pyrazol-4-yl)carbonyl]pyrimidin-4-yl}amino)-2-hydroxycyclopentyl]methyl sulfamate (24).
Prepared by the method described for the preparation of **23** using ethyl 1-benzyl-1*H*-pyrazole-4-carboxylate as the starting material in Step 1. ^{1}H NMR (MeOD) δ 8.73 (s, 1H), 8.57 (s, 1H), 8.35 (s, 1H), 7.98 (s, 1H), 7.38–7.30 (m, 5H), 5.42 (s, 2H), 4.83–4.75 (m, 1H), 4.21–4.13 (m, 3H), 2.55–2.47 (m, 1H), 2.30–2.12 (m, 2H), 1.95–1.87 (m, 1H), 1.47–1.37 (m, 1H). LCMS (FA): *m/z* = 473.5 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{25}N_6O_5S$ 473.1607, obsd 473.1607.

Synthesis of 25



Reagents: (a) TsCl, NaOH, DCM, rt, 19 h, 88%; (b) 4-chloro-5-iodopyrimidine, *n*-BuLi, THF, -78 °C, 3 h, 69%; (c) MnO_2, DCM, rt, 19 h, 97%; (d) **I-8**, K_2CO_3, DMF, rt, 24 h, 81%; (e) K_2CO_3, MeOH, rt, 3 h, 64%; (f), 3-bromobenzyl bromide, Cs_2CO_3, DMF, rt, 2 h; (g) chlorosulfonamide, DMF, rt, 1 h; (h) TFA, H_2O, rt, 2 h; (i) MP PL-CO_3 resin, MeOH, rt, 0.5 h, 53% for 4 steps.

Step 1: 1-[(4-Methylphenyl)sulfonyl]-1*H*-pyrrole-3-carbaldehyde (I-37). 1*H*-Pyrrole-3-carbaldehyde (4.5 g, 47 mmol) was dissolved in DCM (400 mL) and the solution was cooled to 0 °C in an ice bath. NaOH (2.3 g , 57 mmol) was added and the mixture stirred for 10 min at 0 °C. The reaction mixture was kept cool and *p*-toluenesulfonyl chloride (18.0 g, 95 mmol) was added in 2 portions (10 min apart). The reaction mixture was allowed to stir at 0 °C and then slowly warm to rt and stir for 19 h. The reaction was then diluted with DCM (100 mL) and the mixture washed with water twice. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified on silica gel to yield **I-37** (10.4 g, 88%). ^{1}H NMR ($CDCl_3$) δ 9.82 (s, 1H), 7.84–7.80 (m, 2H), 7.77 (t, 1H, *J* = 1.8 Hz), 7.36 (d, 2H, *J* = 8.0 Hz), 7.19–7.17 (m, 1H), 6.71 (dd, 1H, *J* = 3.3, 1.6 Hz), 2.44 (s, 3H). LCMS (FA): *m/z* = 250.1 (M+H).

Step 2: (4-Chloropyrimidin-5-yl){1-[(4-methylphenyl)sulfonyl]-1*H*-pyrrol-3-yl}methanol (I-38). Into a flame dried round bottom flask with stir bar was added 4-chloro-5-iodopyrimidine (2.3 g, 9.5 mmol) dissolved in THF (20 mL). The flask was purged with argon and cooled to -78 °C. To this solution was added dropwise *n*-BuLi (2.5 M in hexane; 7.9 mL, 19.8 mmol) at -78 °C and the mixture was stirred for 30 min. To this mixture was added 1-[(4-methylphenyl)sulfonyl]-1*H*-pyrrole-3-carbaldehyde (2.15 g, 8.6 mmol) dissolved in THF (10 mL) dropwise. The reaction was stirred at -78 °C for 3 h. The reaction was quenched with a saturated solution of NH_4Cl and then extracted with EtOAc three times. The combined organic layers were then washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified on silica gel to give **I-38** (2.2 g, 69%). ^{1}H NMR ($CDCl_3$) δ 8.93 (d, 2H, *J* = 1.8 Hz), 7.76–7.72 (m, 2H), 7.33–7.29 (m, 2H), 7.16–7.12 (m, 2H), 6.24 (dd, 1H, *J* = 3.0, 1.8 Hz), 6.02 (s, 1H), 2.43 (s, 3H), 1.90–1.60 (m, 1H). LCMS (FA): *m/z* = 364.0 (M+H).

Step 3: (4-Chloropyrimidin-5-yl){1-[(4-methylphenyl)sulfonyl]-1*H*-pyrrol-3-yl}methanone (I-39). To a solution of (4-chloropyrimidin-5-yl){1-[(4-methylphenyl)sulfonyl]-1*H*-pyrrol-3-yl}methanol (2.2 g, 6.0 mmol) in DCM (60 mL) was added MnO_2 (5.2 g, 60 mmol). The suspension was stirred for 19 h at rt. The reaction was then filtered through a celite pad and the residual solid was washed with DCM several times. The filtrate was then concentrated in vacuo and the residue was purified on silica gel to afford **I-39** (2.1 g, 97%). ^{1}H NMR ($CDCl_3$) δ 9.13 (s, 1H), 8.71 (s, 1H), 7.81 (d, 2H, *J* = 8.3 Hz), 7.53 (s, 1H), 7.37 (d, 2H, *J* = 8.2 Hz), 7.24–7.21 (m, 1H), 6.76–6.74 (m, 1H), 2.46 (s, 3H). LCMS (FA): *m/z* = 362.0 (M+H).

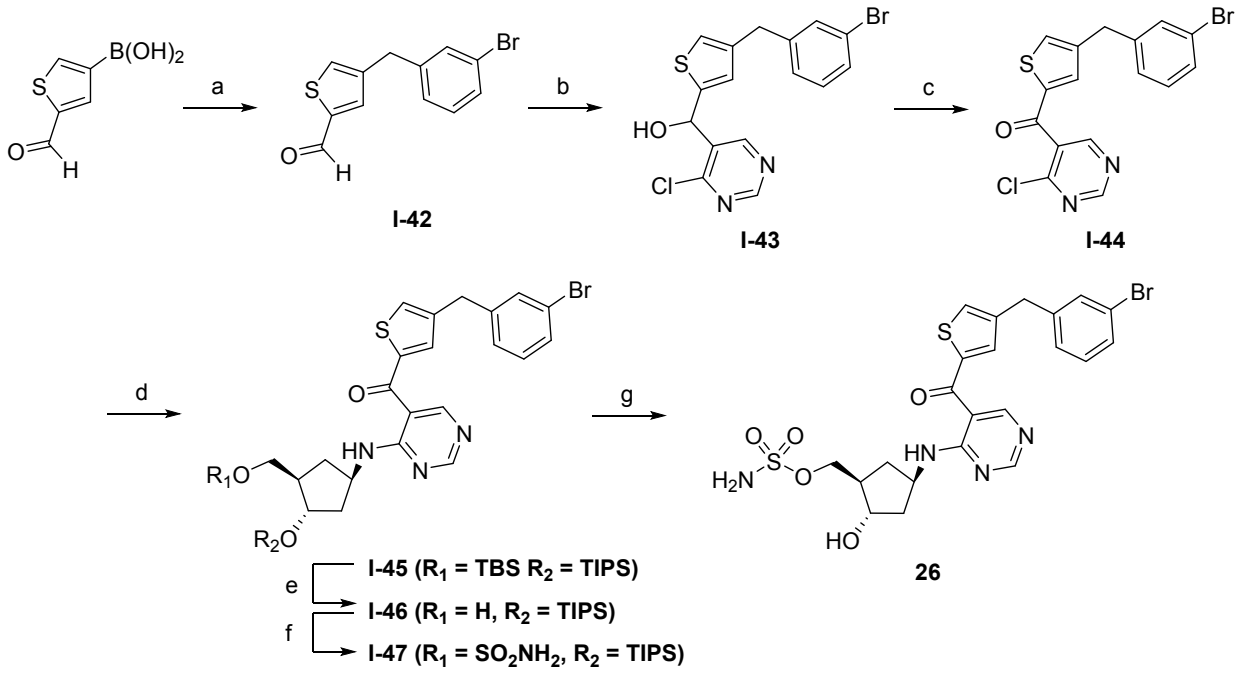
Step 4: [4-({(1*R*,3*R*,4*S*)-3-(Hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]{1-[(4-methylphenyl)sulfonyl]-1*H*-pyrrol-3-yl}methanone (I-40). Into a round bottom flask was added {(1*R*,2*S*,4*R*)-4-amino-2-[(triisopropylsilyl)oxy]cyclopentyl}methanol-TFA (**I-8**, 0.75 g, 1.9 mmol) dissolved in DMF (3 mL), and added to a solution of (4-chloropyrimidin-5-yl){1-[(4-methylphenyl)sulfonyl]-1*H*-pyrrol-3-yl}methanone (0.59 g, 1.6 mmol) in DMF (8 mL). K_2CO_3 (0.67 g, 4.9 mmol) was added to the reaction vessel and the resulting mixture was stirred at rt for 24 h. The reaction was then quenched by addition of water and the mixture extracted with EtOAc three times. The combined organic layers were then washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified on silica gel to provide **I-40** (0.80 g, 81%). ^{1}H NMR ($CDCl_3$) δ 8.96–8.88 (m, 1H), 8.71 (s, 1H), 8.65 (s, 1H), 7.82 (d, 2H, *J* = 8.4 Hz), 7.62 (t, 1H, *J* = 1.9 Hz), 7.36 (d, 2H, *J* = 8.2 Hz), 7.23 (dd, 1H, *J* = 3.1, 2.2 Hz), 6.69 (dd, 1H, *J* = 3.3, 1.6 Hz), 4.86–4.76 (m, 1H), 4.35–4.30 (m, 1H), 3.73–3.66 (m, 2H), 2.50 (ddd, 1H, *J* = 13.3, 8.3, 7.9 Hz), 2.44 (s, 3H), 2.24–2.15 (m, 2H), 1.89–1.80 (m, 1H), 1.77–1.72 (m, 1H), 1.32 (dt, 1H, *J* = 13.3, 7.8 Hz), 1.09–1.05 (m, 21H). LCMS (FA): *m/z* = 613.2 (M+H).

Step 5: [4-({(1*R*,3*R*,4*S*)-3-(Hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl](1*H*-pyrrol-3-yl)methanone (I-41). Into a round bottom flask with stir bar was added [4-({(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]{1-[(4-methylphenyl)sulfonyl]-1*H*-pyrrol-3-yl}methanone (0.80 g, 1.3 mmol), MeOH (20 mL) and K_2CO_3 (0.54 g, 3.9 mmol) and the mixture was stirred at rt for 3 h. The reaction was then quenched by addition of water and the mixture extracted with EtOAc three times. The combined organic layers were then washed with water and brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified on silica gel to provide **I-41** (0.38 g, 64%). ^{1}H NMR ($CDCl_3$) δ 9.06–8.90 (m, 2H), 8.79 (s, 1H), 8.64 (s, 1H), 7.36–7.33 (m, 1H), 6.90–6.87 (m, 1H), 6.72–6.69 (m, 1H), 4.86–4.76 (m, 1H), 4.36–4.31 (m, 1H), 3.71 (d, 2H, *J* = 5.8 Hz), 2.51 (dt, 1H, *J* = 13.0, 8.0 Hz), 2.25–2.16 (m, 2H), 2.10–1.90 (br s, 1H), 1.88 (ddd, 1H, *J* = 13.6, 7.2, 6.4 Hz), 1.34 (dt, 1H, *J* = 13.2, 7.9 Hz), 1.10–1.06 (m, 21H). LCMS (FA): *m/z* = 459.2 (M+H).

Step 6: {(1*R*,2*S*,4*R*)-4-[(5-{[1-(3-Bromobenzyl)-1*H*-pyrrol-3-yl]carbonyl}pyrimidin-4-yl)amino]-2-hydroxycyclopentyl}methyl sulfamate (25).
To a 3-dram vial with 3-bromobenzyl bromide (0.02 g, 0.08 mmol) was added [4-({(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy] cyclopentyl}amino)pyrimidin-5-yl](1*H*-pyrrol-3-yl)methanone (0.03 g, 0.06 mmol) in DMF (1.0 mL) followed by Cs_2CO_3 (0.08 g, 0.25 mmol). The mixture was shaken at rt for 2 h. The solid Cs_2CO_3 was filtered and rinsed with DMF (1 mL). To the combined clear DMF solution was added chlorosulfonamide (0.03g, 0.25 mmol).

The solution was shaken at rt for 1 h, then saturated $NaHCO_3$ (2 mL) and EtOAc (5 mL) were added. After separation, the aqueous layer was extracted with EtOAc (5 mL). The combined organic phases were then concentrated. To the resulting solid in a 20 mL vial was added TFA (2.0 mL) and water (0.2 mL) and then shaken at rt for 2 h. The solvent was then evaporated and to the resulting residue was added MeOH (3.0 mL) and MP PL-CO_3 resin. After shaking at rt for 30 min, the resin was filtered off and rinsed with MeOH (10 mL). The filtrate was concentrated and the residue purified by prep-HPLC to provide **25** (18 mg, 53%). ^{1}H NMR (DMSO-d_6) δ 8.68 (s, 1H), 8.53 (s, 1H), 7.49–7.42 (m, 3H), 7.30–7.21 (m, 2H), 6.90 (m, 1H), 6.64–6.61 (m, 1H), 5.20 (s, 2H), 4.80–4.72 (m, 1H), 4.23–4.13 (m, 3H), 2.54–2.47 (m, 1H), 2.30–2.11 (m, 2H), 1.93–1.86 (m, 1H), 1.45–1.37 (m, 1H); LCMS (FA): *m/z* = 550.0 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{22}H_{25}BrN_5O_5S$ 550.0760, obsd 550.0764.

Synthesis of 26



Reagents: (a) 3-bromobenzyl bromide, $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane, 80 °C, 24 h, 70%; (b) 4-chloro-5-iodopyrimidine, *n*-BuLi, THF, -78 °C, 0.5 h, 86%; (c) Dess-Martin periodinane, DCM, rt, 15 min, 96%; (d) **I-31**, K_2CO_3, DMF, rt, 13 h, 94%; (e) 1% HCl in EtOH, EtOH, rt, 8 h, 89%; (f) chlorosulfonamide, DMF, rt, 15 min, 78%; (g) 4N HCl, THF, rt, 4 h, 87%.

Step 1: 4-(3-Bromobenzyl)thiophene-2-carbaldehyde (I-42). To a degassed solution of 2-formyl-4-thiopheneboronic acid (500 mg, 3.21 mmol), 3-bromobenzyl bromide (0.88 g, 3.53 mmol) and K_2CO_3 (1.33 g, 9.6 mmol) in 1,4-dioxane (15 mL) was added $Pd(PPh_3)_4$ (0.185 g, 0.16 mmol). The reaction mixture was stirred at 80 °C for 1 d then quenched with water and extracted with EtOAc. The combined organic layers were dried over Mg_2SO_4, filtered and concentrated in vacuo. The crude material was purified on silica gel to provide **I-42** (631 mg, 70%). ^{1}H NMR ($CDCl_3$) δ 9.86 (d, 1H, *J* = 1.2 Hz), 7.55 (d, 1H, *J* = 1.2 Hz), 7.42–7.32 (m, 3H), 7.20 (t, 1H, *J* = 7.7 Hz), 7.13 (d, 1H, *J* = 7.7 Hz), 3.97 (s, 2H). LCMS (FA): *m/z* = 282.9 (M+H).

Step 2: rac-[4-(3-Bromobenzyl)-2-thienyl](4-chloropyrimidin-5-yl)methanol (I-43). A solution of 4-chloro-5-iodopyrimidine (216 mg, 0.90 mmol) in THF (5.0 mL) was cooled to -78 °C with dry-ice bath. To the solution was added dropwise 2.50 M of *n*-BuLi in hexane (0.36 mL, 0.90 mmol) at -78 °C, and the mixture was stirred for 20 min. To the mixture was added a solution of 4-(3-bromobenzyl)thiophene-2-carbaldehyde (210 mg, 0.75 mmol) in THF (2.0 mL) at -78 °C, and the resulting mixture was stirred for 30 min. The reaction was quenched by addition of saturated NH_4Cl (50mL) and extracted with EtOAc (50mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified on silica gel to provide **I-43** as a colorless oil (260 mg, 86%). ^{1}H NMR ($CDCl_3$) δ 9.02 (s, 1H), 8.94 (s, 1H), 7.35 (d, 1H, *J* = 7.9 Hz), 7.32 (s, 1H), 7.17 (t, 1H, *J* = 7.7 Hz), 7.09 (d, 1H, *J* = 7.7 Hz), 6.91 (s, 1H), 6.82 (s, 1H), 6.27 (s, 1H), 3.86 (s, 2H), 2.86–2.60 (br s, 1H). LCMS (FA): *m/z* = 396.9 (M+H).

Step 3: [4-(3-Bromobenzyl)-2-thienyl](4-chloropyrimidin-5-yl)methanone (I-44). To a solution of rac-[4-(3-bromobenzyl)-2-thienyl](4-chloropyrimidin-5-yl)methanol (255 mg, 0.64 mmol) in DCM (10.0 mL) was added Dess-Martin periodinane (410 mg, 0.97 mmol) at rt, and the mixture was stirred for 15 min. The reaction was quenched by addition of saturated $NaHCO_3$ (50mL) and extracted with DCM (50mL) three times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to afford **I-44** as a colorless oil (247 mg, 96%). ^{1}H NMR ($CDCl_3$) δ 9.12 (s, 1H), 8.75 (s, 1H), 7.49–7.45 (m, 1H), 7.38 (d, 1H, *J* = 8.0 Hz), 7.31 (s, 1H), 7.28 (d, 1H, *J* = 1.4 Hz), 7.18 (t, 1H, *J* = 7.8 Hz), 7.09 (d, 1H, *J* = 7.8 Hz), 3.94 (s, 2H). LCMS (FA): *m/z* = 394.9 (M+H).

Step 4: [4-(3-Bromobenzyl)-2-thienyl][4-({(1*R*,3*R*,4*S*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]methanone (I-45). To a solution of [4-(3-bromobenzyl)-2-thienyl](4-chloropyrimidin-5-yl)methanone (245 mg, 0.62 mmol) in DMF (10 mL) was added (1*R*,3*R*,4*S*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentanamine (**I-31**, 375 mg, 0.93 mmol) followed by K_2CO_3 (215 mg, 1.56 mmol), and the reaction was stirred for 13 h at rt. The reaction was concentrated in vacuo. To the residue was added water (50mL) and the mixture was extracted with EtOAc (50mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to give **I-45** as a light yellow oil (445 mg, 94%). ^{1}H NMR ($CDCl_3$) δ 8.78 (s, 1H), 8.69–8.56 (m, 2H), 7.42–7.30 (m, 4H), 7.18 (t, 1H, *J* = 7.7 Hz), 7.12 (d, 1H, *J* = 7.7 Hz), 4.86–4.72 (m, 1H), 4.33–4.25 (m, 1H), 3.96 (s, 2H), 3.61 (dd, 1H, *J* = 10.1, 5.4 Hz), 3.55 (dd, 1H, *J* = 10.1, 5.8 Hz), 2.49–2.36 (m, 1H), 2.24–2.08 (m, 2H), 1.77–1.66 (m, 1H), 1.34–1.17 (m, 1H), 1.06 (s, 21H), 0.88 (s, 9H), 0.03 (s, 6H).

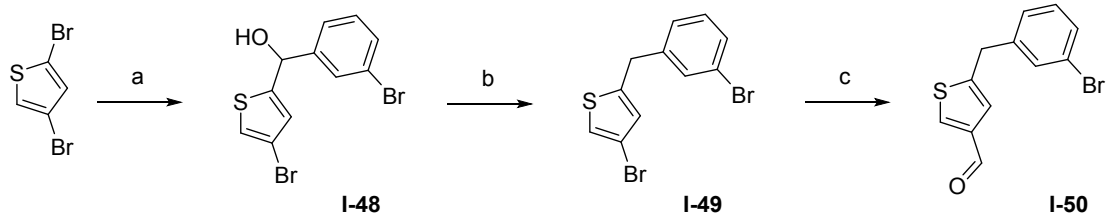
Step 5: [4-(3-Bromobenzyl)-2-thienyl][4-({(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]methanone (I-46). To a solution of [4-(3-bromobenzyl)-2-thienyl][4-({(1*R*,3*R*,4*S*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]methanone (150 mg, 0.20 mmol) in EtOH (8.0 mL) was added 1% HCl in EtOH solution (2.0 mL, 0.24 mmol), and the mixture was stirred for 8 h at rt. The reaction was quenched by addition of saturated $NaHCO_3$ (50mL) and extracted with EtOAc (60mL) three times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to

provide **I-46** as a light yellow oil (113 mg, 89%). ^{1}H NMR ($CDCl_3$) δ 8.79 (s, 1H), 8.71 (d, 1H, *J* = 7.3 Hz), 8.66 (s, 1H), 7.40–7.32 (m, 4H), 7.19 (dd, 1H, *J* = 7.8, 7.6 Hz), 7.15–7.11 (m, 1H), 4.86–4.76 (m, 1H), 4.35–4.30 (m, 1H), 3.97 (s, 2H), 3.74–3.66 (m, 2H), 2.54–2.45 (m, 1H), 2.24–2.15 (m, 2H), 1.89–1. 81 (m, 1H), 1.75 (t, 1H, *J* = 4.8 Hz), 1.32 (dt, 1H, *J* = 13.2, 7.8 Hz), 1.10–1.04 (m, 21H). LCMS (FA): *m/z* = 646.1 (M+H).

Step 6: {(1*R*,2*S*,4*R*)-4-[(5-{[4-(3-Bromobenzyl)-2-thienyl]carbonyl}pyrimidin-4-yl)amino]-2-[(triisopropylsilyl)oxy]cyclopentyl}methyl sulfamate (I-47). To a solution of [4-(3-bromobenzyl)-2-thienyl][4-({(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]methanone (110 mg, 0.17 mmol) in DMF (2.0 mL) was added chlorosulfonamide (39.4 mg, 0.34 mmol) at rt, and the mixture was stirred for 15 min. The reaction was cooled to 0 °C and quenched by addition of saturated $NaHCO_3$ (50 mL). The mixture was extracted with EtOAc (50 mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to give **I-47** as a light yellow sticky oil (101 mg, 78%). ^{1}H NMR ($CDCl_3$) δ 8.79 (s, 1H), 8.72–8.59 (m, 2H), 7.45–7.30 (m, 4H), 7.19 (t, 1H, *J* = 7.7 Hz), 7.12 (d, 1H, *J* = 7.5 Hz), 5.15 (s, 2H), 4.88–4.75 (m, 1H), 4.34 (q, 1H, *J* = 5.2 Hz), 4.27 (d, 2H, *J* = 4.7 Hz), 3.96 (s, 2H), 2.67–2.53 (m, 1H), 2.43–2.30 (m, 1H), 2.22–2.11 (m, 1H), 1.90 (dt, 1H, *J* = 13.1, 6.4 Hz), 1.46 (dt, 1H, *J* = 13.2, 6.6 Hz), 1.05 (s, 21H). LCMS (FA): *m/z* = 725.1 (M+H).

Step 7: {(1*R*,2*S*,4*R*)-4-[(5-{[4-(3-Bromobenzyl)-2-thienyl]carbonyl}pyrimidin-4-yl)amino]-2-hydroxycyclopentyl}methyl sulfamate (26).
To a solution of {(1*R*,2*S*,4*R*)-4-[(5-{[4-(3-bromobenzyl)-2-thienyl]carbonyl}pyrimidin-4-yl)amino]-2-[(triisopropylsilyl)oxy]cyclopentyl}methyl sulfamate (95.0 mg, 0.13 mmol) in THF (2.0 mL) was added 4.0 M of HCl (2.00 mL, 8.00 mmol) at rt, and the mixture was stirred for 4 h. The reaction was quenched by addition of saturated $NaHCO_3$ (60 mL) and extracted with EtOAc (60 mL) three times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to provide **26** as an off-white solid (66 mg, 87%). ^{1}H NMR (DMSO-d_6) δ 8.68 (s, 1H), 8.64 (s, 1H), 8.26 (d, 1H, *J* = 7.4 Hz), 7.82 (s, 1H), 7.70 (s, 1H), 7.51 (s, 1H), 7.48–7.35 (m, 3H), 7.35–7.20 (m, 2H), 4.88 (d, 1H, *J* = 4.5 Hz), 4.77–4.62 (m, 1H), 4.09 (dd, 1H, *J* = 9.6, 6.1 Hz), 4.04–3.87 (m, 4H), 2.31 (dt, 1H, *J* = 13.6, 7.5 Hz), 2.17–2.05 (m, 1H), 2.01–1.90 (m, 1H), 1.82–1.70 (m, 1H), 1.33–1.20 (m, 1H). LCMS (FA): *m/z* = 569.1 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{22}H_{24}BrN_4O_5S_2$ 567.0371, obsd 567.0370.

Synthesis of Intermediate I-50



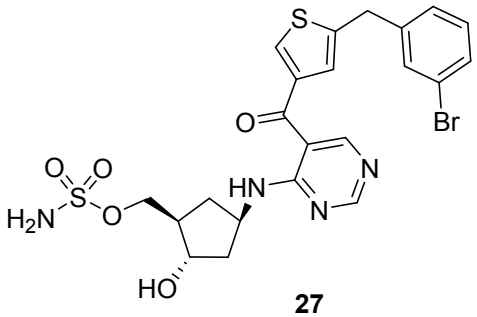
Reagents: (a) 3-bromobenzaldehyde, *n*-BuLi, THF, -45 °C, 15 min, 54%; (b) Et_3SiH, BF_3-Me_2O, DCM, rt, 2 h, 45%; (c) *n*-BuLi, DMF, THF, -65 °C, 30 min, 38%.

Step 1: rac-(3-Bromophenyl)(4-bromo-2-thienyl)methanol (I-48). To a solution of 2.50 M of *n*-BuLi in hexane (3.71 mL, 9.29 mmol) in THF (20 mL) at -78 °C was added 2,4-dibromothiophene (1.0 mL, 8.85 mmol) and the mixture was stirred for 5 min. To the mixture was added 3-bromobenzaldehyde (1.08 mL, 9.29 mmol) quickly and the resulting mixture was stirred for 15 min at -45 °C. The reaction was quenched by addition of saturated NH_4Cl (50mL) and extracted with EtOAc (50mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified on silica gel to provide **I-48** (1.65 g, 54%). ^{1}H NMR ($CDCl_3$) δ 7.60 (s, 1H), 7.49–7.45 (m, 1H), 7.37–7.34 (m, 1H), 7.28–7.24 (m, 1H), 7.20 (d, 1H, *J* = 1.4 Hz), 6.81 (s, 1H), 5.97 (d, 1H, *J* = 3.8 Hz), 2.45 (d, 1H, *J* = 4.0 Hz). LCMS (FA): *m/z* = 331.0 (M+H).

Step 2: 4-Bromo-2-(3-bromobenzyl)thiophene (I-49). A solution of rac-(3-bromophenyl)(4-bromo-2-thienyl)methanol (1.65 g, 4.74 mmol) and triethylsilane (1.13 mL, 7.08 mmol) in DCM (9.0 mL) was cooled to 0 °C. BF_3-Me_2O (0.71 mL, 7.14 mmol) was added dropwise slowly and the reaction was warmed to rt and stirred for 2 h. The reaction was quenched by addition of saturated $NaHCO_3$ (50mL) and extracted with DCM (50mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified on silica gel to provide **I-49** (0.71 g, 45%). ^{1}H NMR ($CDCl_3$) δ 7.41–7.39 (m, 2H), 7.23–7.15 (m, 2H), 7.08 (s, 1H), 6.73 (s, 1H), 4.08 (s, 2H).

Step 3: 5-(3-Bromobenzyl)thiophene-3-carbaldehyde (I-50). A solution of 4-bromo-2-(3-bromobenzyl)thiophene (0.71 g, 2.13 mmol) in THF (10.0 mL) was cooled to -78 °C with dry-ice bath. To the solution was added dropwise 2.50 M of *n*-BuLi in hexane (0.90 mL, 2.24 mmol) at -78 °C, and the mixture was stirred for 5 min. To the mixture was added DMF (0.20 mL, 2.56 mmol) and the resulting mixture was stirred at -65 °C for 30 min. The reaction was quenched by addition of saturated NH_4Cl (50mL) and extracted with EtOAc (50mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified on silica gel to provide **I-50** as an orange oil (0.23 g, 38%). ^{1}H NMR ($CDCl_3$) δ 9.82 (s, 1H), 7.97 (s, 1H), 7.42–7.39 (m, 2H), 7.25–7.23 (m, 1H), 7.21–7.16 (m, 2H), 4.12 (s, 2H). LCMS (FA): *m/z* = 283.0 (M+H).

Synthesis of 27

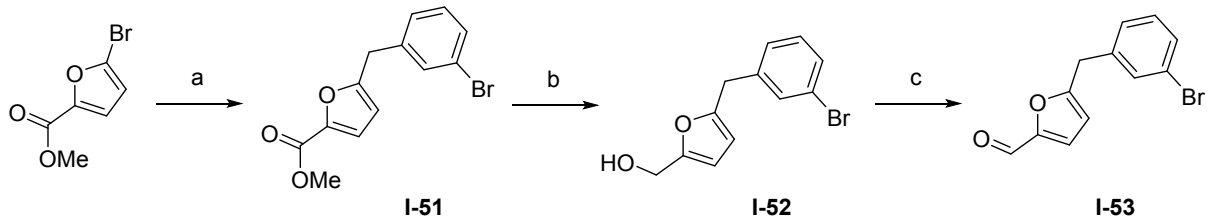


27

[(1*R*,2*S*,4*R*)-4-({5-[5-(3-Bromobenzyl)-2-furoyl]pyrimidin-4-yl}amino)-2-hydroxycyclopentyl]methyl sulfamate (27).
Prepared by the method described for the preparation of **26** using 4-chloro-5-iodopyrimidine and intermediate **I-50** in Step 2. The following alternative conditions were employed in the reaction

scheme: Step 7: desilylating agent/solvent used was H_3PO_4/CH_3CN. ^{1}H NMR (DMSO-d_6) δ 8.66 (s, 1H), 8.57 (s, 1H), 7.89 (s, 1H), 7.47 (s, 1H), 7.42–7.39 (m, 1H), 7.30–7.22 (m, 3H), 4.84–4.77 (m, 1H), 4.24–4.14 (m, 3H), 4.20 (s, 2H), 2.55–2.2.48 (m, 1H), 2.31–2.24 (m, 1H), 2.20–2.13 (m, 1H), 1.95–1.88 (m, 1H), 1.51–1.39 (m, 1H). LCMS (FA): *m/z* = 569.1 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{22}H_{24}BrN_4O_5S_2$ 567.0371, obsd 567.0367.

Synthesis of Intermediate I-53



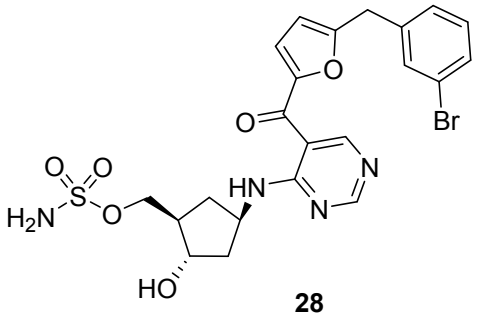
Reagents: (a) 3-bromobenzylzinc bromide, $Pd(PPh_3)_4$, THF, 70 °C, 24 h, 54%; (b) LAH, THF, 0 °C, 2 h; (c) Dess-Martin periodinane, DCM, rt, 1 h, 28% for 2 steps.

Step 1: Methyl 5-(3-bromobenzyl)-2-furoate (I-51). A microwave reaction tube was charged with methyl 5-bromofuran-2-carboxylate (775 mg, 3.78 mmol) and $Pd(PPh_3)_4$ (218 mg, 0.19 mmol). The flask was sealed and purged with argon for 5 min, and then THF (10.0 mL) was added to the reaction vessel. 0.5 M of 3-bromobenzylzinc bromide in THF (8.32 mL, 4.16 mmol) was then added to the solution and the reaction was heated at 70 °C for 1 d. The reaction was quenched with saturated NH_4Cl and extracted with EtOAc three times. The combined organic layers were then washed with water, brine, dried using Na_2SO_4, filtered and concentrated. The residue was purified on silica gel to give **I-51** (0.60 g, 54%). ^{1}H NMR ($CDCl_3$) δ 7.40–7.36 (m, 2H), 7.20–7.16 (m, 2H), 7.10 (d, 1H, *J* = 3.4 Hz), 6.11 (d, 1H, *J* = 3.4 Hz), 4.01 (s, 2H), 3.88 (s, 3H).

Step 2: [5-(3-Bromobenzyl)-2-furyl]methanol (I-52). To a round bottom flask was added methyl 5-(3-bromobenzyl)-2-furoate (733 mg, 2.48 mmol) in THF (8.0 mL) and cooled at 0 °C. 1.0 M of lithium aluminum hydride in Et_2O (3.23 mL, 3.23 mmol) was then added slowly and the resulting mixture was stirred at 0 °C for 2 h. Added 1mL of water slowly to quench reaction mixture, then added solid Na_2SO_4. The mixture was stirred at rt for 1 hour and then filtered through a pad of celite. The filtrate was concentrated to dryness and no further purification was done to give crude **I-52** (610 mg). ^{1}H NMR ($CDCl_3$) δ 7.39–7.37 (m, 1H), 7.32–7.28 (m, 1H), 7.25–7.21 (m, 2H), 7.18–7.15 (m, 1H), 6.22–6.17 (m, 1H), 5.98–5.91 (m, 1H), 4.56 (s, 2H), 3.94 (d, 2H, *J* = 13.7 Hz).

Step 3: 5-(3-Bromobenzyl)-2-furaldehyde (I-53). Into a round bottom flask was added crude [5-(3-bromobenzyl)-2-furyl]methanol (609 mg, 2.28 mmol) dissolved in DCM (10 mL). Dess-Martin periodinane (1.16 g, 2.74 mmol) was added and the resulting reaction mixture was stirred at rt for 1 h. The reaction was then quenched by the addition of saturated $Na_2S_2O_3$ and extracted with DCM three times. The combined organic layers were then washed with water, brine, dried using Na_2SO_4, filtered and concentrated. The residue was purified on silica gel to provide **I-53** (167 mg, 28% for 2 steps). ^{1}H NMR ($CDCl_3$) δ 9.56 (s, 1H), 7.43–7.37 (m, 2H), 7.23–7.14 (m, 3H), 6.22 (d, 1H, *J* = 3.5 Hz), 4.03 (s, 2H).

Synthesis of 28

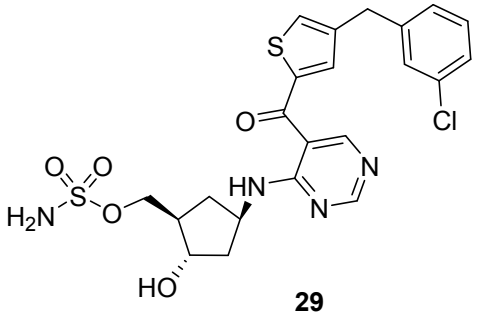


28

[(1*R*,2*S*,4*R*)-4-({5-[5-(3-Bromobenzyl)-2-furoyl]pyrimidin-4-yl}amino)-2-hydroxycyclopentyl]methyl sulfamate (28).
Prepared by the method described for the preparation of **26** using 4-chloro-5-iodopyrimidine and intermediate **I-53** in Step 2. ^{1}H NMR (DMSO-d_6) δ 8.80 (s, 1H), 8.62 (s, 1H), 8.43 (d, 1H, J = 7.4 Hz), 7.54 (s, 1H), 7.51–7.45 (m, 1H), 7.44 (s, 2H), 7.37 (d, 1H, J = 3.6 Hz), 7.34–7.29 (m, 2H), 6.52 (d, 1H, J = 3.5 Hz), 4.90 (d, 1H, J = 4.5 Hz), 4.74–4.62 (m, 1H), 4.17 (s, 2H), 4.08 (dd, 1H, J = 9.7, 6.0 Hz), 3.99–3.90 (m, 2H), 2.37–2.26 (m, 1H), 2.16–2.07 (m, 1H), 2.00–1.91 (m, 1H), 1.80–1.69 (m, 1H), 1.25 (dt, 1H, J = 12.5, 9.1 Hz). LCMS (FA): *m/z* = 552.9 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{22}H_{24}BrN_4O_6S$ 551.0600, obsd 551.0589.

Procedures for the syntheses of compounds in Table 5

Synthesis of 29

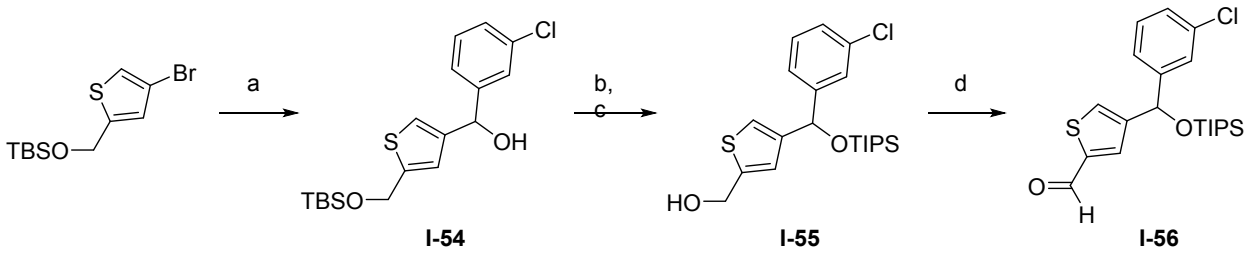


29

{(1*R*,2*S*,4*R*)-4-[(5-{[4-(3-Chlorobenzyl)-2-thienyl]carbonyl}pyrimidin-4-yl)amino]-2-hydroxycyclopentyl}methyl sulfamate (29).
Prepared by the method described for the preparation of **26** using 2-formyl-4-thiopheneboronicacid and 1-(bromomethyl)-3-chlorobenzene in Step 1. ^{1}H NMR ($CDCl_3$) δ 8.82 (s, 1H), 8.67 (s, 1H), 8.62 (d, 1H, J = 7.5 Hz), 7.42 (d, 1H, J = 1.4 Hz), 7.39 (s, 1H), 7.26–7.23 (m, 1H), 7.18 (s, 1H), 7.09 (d, 1H, J = 7.1 Hz), 5.41 (br, 2H), 4.87–4.68 (m, 1H), 4.43–4.31 (m, 2H), 4.30–4.21 (m, 1H), 3.99 (s, 2H), 2.63–2.49 (m, 1H), 2.42–2.27 (m, 1H), 2.21–2.09 (m, 1H),

2.08–1.97 (m, 1H), 1.54–1.37 (m, 1H). LCMS (FA): *m/z* = 523.2 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{22}H_{24}ClN_4O_5S_2$ 523.0877, obsd 523.0878.

Synthesis of Intermediate I-56



Reagents: (a) 3-chlorobenzaldehyde, *n*-BuLi, THF, -78 °C, 15 min, 60%; (b) TIPSCl, NaH, DCM, rt, 12 h; (c) 1% HCl in EtOH, rt, 15 min, 86% for 2 steps, (d) MnO_2, DCM, rt, 12 h, 93%.

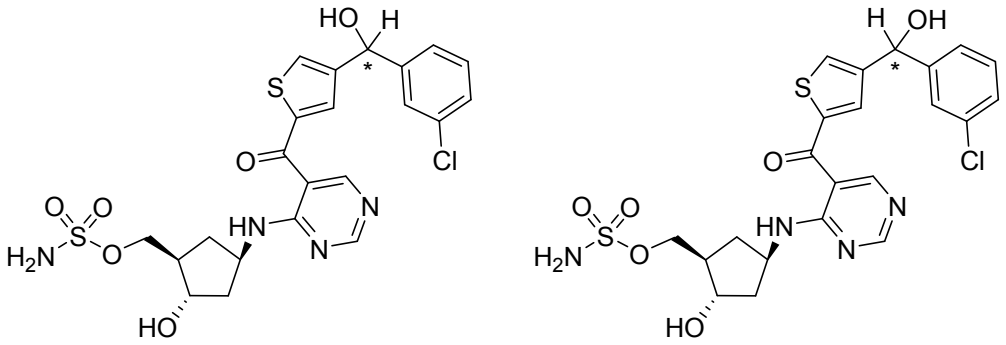
Step 1: rac-[5-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-3-thienyl](3-chlorophenyl)methanol (I-54). 2.50 M of *n*-BuLi in hexane (4.14 mL, 10.4 mmol) was dissolved into THF (100 mL) at -78 °C, then ((4-bromothiophen-2-yl)methoxy)(*tert*-butyl)dimethylsilane (2.45 g, 7.97 mmol) was added to this solution at -78 °C and the mixture was stirred for 2 min. 3-chlorobenzaldehyde (1.12 g, 7.99 mmol) was added to the solution at -78 °C and the reaction was stirred at -78 °C for 15 min. The solution was poured into 100 mL of 5 g acetic acid in water solution and the resulting mixture was extracted with DCM (70 mL) twice. The combined organic layers were concentrated in vacuo. The residue was purified on silica gel to provide **I-54** (1.76 g, 60%). ^{1}H NMR ($CDCl_3$) δ 7.40 (s, 1H), 7.28–7.26 (m, 3H), 7.09–7.08 (m, 1H), 6.79–6.78 (m, 1H), 5.77 (s, 1H), 4.81 (s, 2H), 2.40 (br s, 1H), 0.93 (s, 9H), 0.10 (s, 6H).

Step 2: rac-(4-{(3-Chlorophenyl)[(triisopropylsilyl)oxy]methyl}-2-thienyl)methanol (I-55). rac-[5-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-3-thienyl](3-chlorophenyl)methanol (0.40 g, 1.08 mmol) was dissolved in THF (20 mL), then 60% NaH in mineral oil (78 mg, 3.25 mmol) was added to this solution. The solution was stirred at 50 °C for 30 min. TIPSCl (0.35 mL, 1.63 mmol) was added and the reaction mixture stirred at rt overnight. The solution was poured into 30 mL saturated NH_4Cl solution. The solution was extracted with EtOAc (20 mL) twice. The combined organic layers were dried over Na_2SO_4, filtered, concentrated in vacuo to provide the disiylated intermediate. The residue was dissolved in 15 mL of 1% HCl in EtOH solution and the reaction mixture was stirred for 15 min. at rt. The reaction was quenched by addition of saturated NaHCO3 solution (30 mL). The solution was extracted with DCM (30 mL) twice. The combined organic layers were washed with brine, dried over Na2SO4, filtered, and concentrated in vacuo. The residue was purified on silica gel to provide **I-55** (382 mg, 86%). LCMS (FA): *m/z* = 412.1 (M+H).

Step 3: rac-4-{(3-Chlorophenyl)[(triisopropylsilyl)oxy]methyl}thiophene-2-carbaldehyde (I-56). rac-(4-{(3-Chlorophenyl)[(triisopropylsilyl)oxy]methyl}-2-thienyl)methanol (0.40 g, 0.98 mmol) was dissolved into DCM (50 mL), then MnO_2 (1.28 g, 14.7 mmol) was added to this solution. The reaction was stirred at rt overnight. The mixture was filtered through a celite pad and washed with DCM. The filtrate was concentrated in vacuo and the residue was purified on

silica gel to provide **I-56** as a white solid (372 mg, 93%). ^{1}H NMR ($CDCl_3$) δ 9.82 (d, 1H, *J* = 1.2 Hz), 7.65 (q, 1H, *J* = 1.3 Hz), 7.60 (d, 1H, *J* = 1.4 Hz), 7.40 (s, 1H), 7.30–7.26 (m, 2H), 7.23 (ddt, 1H, *J* = 6.6, 4.5, 2.6 Hz), 5.86 (s, 1H), 1.16–1.05 (m, 3H), 1.04–0.96 (m, 18H).

Synthesis of 30 and 31



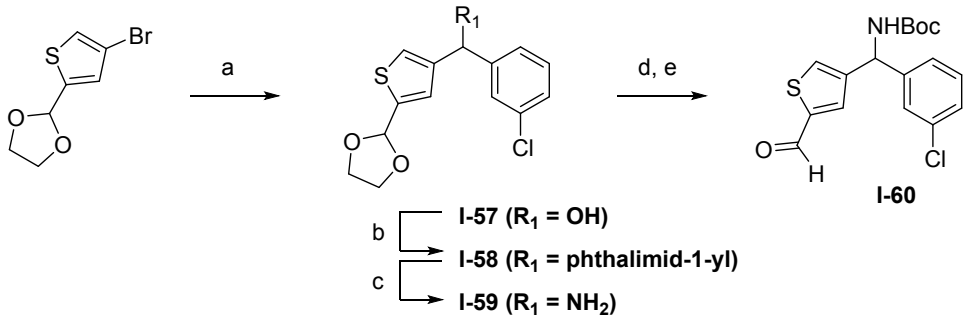
30 and 31

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(*R*)-(3-Chlorophenyl)(hydroxy)methyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate and [(1*R*,2*S*,4*R*)-4-{[5-({4-[(*S*)-(3-Chlorophenyl)(hydroxy)methyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (30 and 31). Prepared by the method described for the preparation of **26** using 4-chloro-5-iodopyrimidine and intermediate **I-56** in Step 2. The following alternative conditions were employed in the reaction scheme: Step 3: oxidant used was MnO_2; Step 4: Base/solvent used was *N,N*-diisopropylethylamine/*i*-PrOH; Step 7: desilylating agent/solvent used was TFA/water. The individual diastereomers were obtained using preparative scale chiral SCF.

30: ^{1}H NMR (MeOD) δ 8.67 (s, 1H), 8.56 (s, 1H), 7.72 (s, 1H), 7.54 (s, 1H), 7.43 (s, 1H), 7.34–7.29 (m, 2H), 7.28–7.20 (m, 1H), 5.84 (s, 1H), 4.82–4.70 (m, 1H), 4.22–4.11 (m, 3H), 2.53–2.40 (m, 2H), 2.28–2.18 (m, 1H), 2.17–2.08 (m, 1H), 1.44–1.36 (m, 1H). LCMS (FA): *m/z* = 539.1 (M+H).

31: ^{1}H NMR (MeOD) δ 8.67 (s, 1H), 8.56 (s, 1H), 7.72 (s, 1H), 7.54 (s, 1H), 7.43 (s, 1H), 7.34–7.29 (m, 2H), 7.28–7.20 (m, 1H), 5.84 (s, 1H), 4.82–4.70 (m, 1H), 4.22–4.11 (m, 3H), 2.53–2.40 (m, 2H), 2.28–2.18 (m, 1H), 2.17–2.08 (m, 1H), 1.42–1.34 (m, 1H). LCMS (FA): *m/z* = 539.1 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{22}H_{24}ClN_4O_6S_2$ 539.0826, obsd 539.0828.

Synthesis of Intermediate I-60



Reagents: (a) 3-chlorobenzaldehyde, *n*-BuLi, THF, -78 °C, 0.5 h, 67%; (b) phthalimide, DIAD, PPh_3, THF, 70 °C, 49%; (c) hydrazine hydrate, MeOH, rt, 12 h, 32%; (d) 0.2N HCl in EtOH, H_2O, rt, 12 h; (e) $(Boc)_2O$, DIPEA, THF, 45 °C, 2 h, 77% for 2 steps.

Step 1: rac-(3-Chlorophenyl)[5-(1,3-dioxolan-2-yl)-3-thienyl]methanol (I-57). To a -78 °C cooled solution of 2.50 M of *n*-BuLi in hexane (4.08 mL, 10.2 mmol) in THF (40 mL) was added a solution of 2-(4-bromothiophen-2-yl)-1,3-dioxolane (2.00 g, 8.50 mmol) in THF (4 mL) dropwise. Immediately after addition was complete 3-chlorobenzaldehyde (0.97 mL, 8.50 mmol) was added dropwise (~2 min) as a solution in THF (4 mL). The resulting mixture was allowed to stir 30 min at -78 °C. The reaction was quenched with water before warming all the way to rt. The mixture was extracted with EtOAc twice and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified on silica gel to provide **I-57** (1.7 g, 67%). ^{1}H NMR ($CDCl_3$) δ 7.42 (s, 1H), 7.35–7.24 (m, 4H), 7.21 (s, 1H), 7.06 (s, 1H), 6.04 (s, 1H), 5.82 (d, 1H, *J* = 3.6 Hz), 4.21–3.94 (m, 4H).

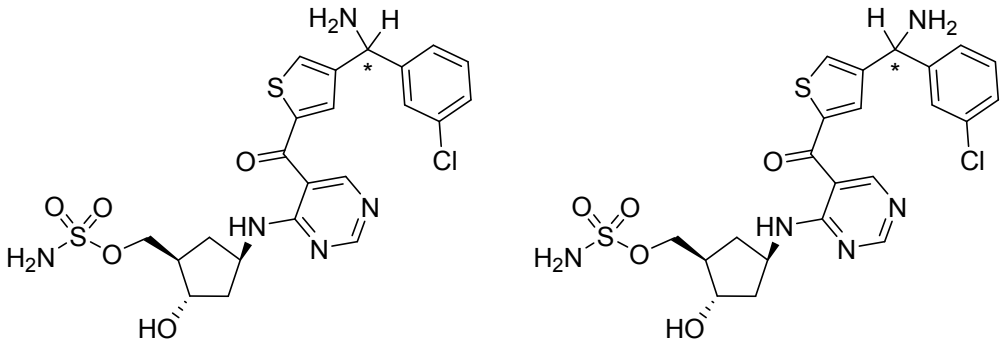
Step 2: rac-2-{(3-Chlorophenyl)[5-(1,3-dioxolan-2-yl)-3-thienyl]methyl}-1*H*-isoindole-1,3(2*H*)-dione (I-58). rac-(3-Chlorophenyl)[5-(1,3-dioxolan-2-yl)-3-thienyl]methanol (827 mg, 2.79 mmol), phthalimide (656 mg, 4.46 mmol) and triphenylphosphine (1.32 g, 5.02 mmol) were dissolved into THF (40 mL), then diisopropyl azodicarboxylate (0.99 mL, 5.02 mmol) was added to this solution at rt. The reaction was heated at 70 °C for 5 h. The reaction mixture was concentrated in vacuo and the residue was purified on silica gel to give **I-58** as a white solid (586 mg, 49%). ^{1}H NMR ($CDCl_3$) δ 7.93–7.79 (m, 2H), 7.78–7.70 (m, 2H), 7.35 (m, 1H), 7.28 (d, 1H, *J* = 1.4 Hz), 7.26 (d, 2H, *J* = 1.1 Hz), 7.21 (m, 1H), 6.63 (s, 1H), 6.05 (s, 1H), 4.14–4.11 (m, 2H), 4.04–3.96 (m, 2H).

Step 3: rac-1-(3-Chlorophenyl)-1-[5-(1,3-dioxolan-2-yl)-3-thienyl]methanamine (I-59). rac-2-{(3-Chlorophenyl)[5-(1,3-dioxolan-2-yl)-3-thienyl]methyl}-1*H*-isoindole-1,3(2*H*)-dione (432 mg, 1.01 mmol) was dissolved into MeOH (15 mL), then hydrazine hydrate (508 mg, 10.1 mmol) was added to this solution. The reaction was stirred at rt overnight. The mixture was poured into 30 mL water and extracted with DCM (20 mL) three times. The combined the organic layers were concentrated and the residue was purified on silica gel to provide **I-59** (95 mg, 32%). ^{1}H NMR ($CDCl_3$) δ 7.38 (s, 1H), 7.29–7.20 (m, 3H), 7.20–7.13 (d, 1H), 7.03 (d, 1H), 6.02 (s, 1H), 5.15 (s, 1H), 4.20–4.09 (m, 2H), 4.04–3.96 (m, 2H), 1.93–1.67 (m, 2H).

Step 4: rac-*tert*-Butyl [(3-chlorophenyl)(5-formyl-3-thienyl)methyl]carbamate (I-60). rac-1-(3-Chlorophenyl)-1-[5-(1,3-dioxolan-2-yl)-3-thienyl]methanamine (297 mg, 1.00 mmol) was dissolved into a solution of 0.2 M HCl in EtOH (15.0 mL, 3.0 mmol) and water (0.20 mL, 11 mmol). The reaction was stirred at rt overnight and then was concentrated in vacuo. The residues

were dissolved in THF (15 mL). To the solution was added *N,N*-diisopropylethylamine (0.70 mL, 4.02 mmol) followed by di-*tert*-butyldicarbonate (658 mg, 3.02 mmol) and the mixture was heated at 45 °C for 2 h. The reaction mixture was concentrated in vacuo and the residue was purified on silica gel to give **I-60** (273 mg, 77%). ^{1}H NMR ($CDCl_3$) δ 9.84 (d, 1H, *J* = 1.3 Hz), 7.57 (d, 1H, *J* = 1.4 Hz), 7.46 (q, 1H, *J* = 1.3 Hz), 7.32–7.28 (m, 2H), 7.29–7.26 (m, 1H), 7.18–7.14 (m, 1H), 5.96 (s, 1H), 5.24 (d, 1H, *J* = 8.0 Hz), 1.44 (s, 9H).

Synthesis of 32 and 33



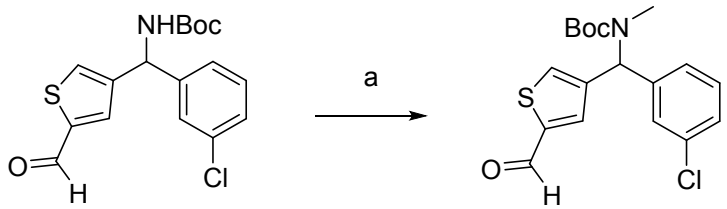
32 and 33

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(*R*)-Amino(3-chlorophenyl)methyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate and [(1*R*,2*S*,4*R*)-4-{[5-({4-[(*S*)-Amino(3-chlorophenyl)methyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (32 and 33).
Prepared by the method described for the preparation of **26** using 4-chloro-5-iodopyrimidine and intermediate **I-60** in Step 2. The following alternative conditions were employed in the reaction scheme: Step 3: oxidant used was MnO_2; Step 4: Base/solvent used was *N,N*-diisopropylethylamine/*i*-PrOH; Step 7: desilylating agent/solvent used was TFA/water. The individual diastereomers were obtained using preparative scale chiral HPLC.

32: ^{1}H NMR (MeOD) δ 8.70 (s, 1H), 8.59 (s, 1H), 7.83 (s, 1H), 7.68 (s, 1H), 7.51 (s, 1H), 7.47–7.33 (m, 3H), 5.55 (s, 1H), 4.81 (dd, 1H, *J* = 15.9, 8.0 Hz), 4.20 (qd, 3H, *J* = 9.9, 5.8 Hz), 2.57–2.45 (m, 1H), 2.29 (dt, 1H, *J* = 14.0, 7.0 Hz), 2.17 (ddd, 1H, *J* = 12.6, 11.2, 7.4 Hz), 1.94–1.80 (m, 1H), 1.48–1.39 (m, 1H). LCMS (FA): *m/z* = 538.1 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{22}H_{25}ClN_5O_5S_2$ 538.0986, obsd 538.0978.

33: ^{1}H NMR (MeOD) δ 8.73 (s, 1H), 8.61 (s, 1H), 7.85 (s, 1H), 7.68 (s, 1H), 7.51 (s, 1H), 7.47–7.33 (m, 3H), 5.55 (s, 1H), 4.81 (dd, 1H, *J* = 15.9, 8.0 Hz), 4.20 (qd, 3H, *J* = 9.9, 5.8 Hz), 2.57–2.45 (m, 1H), 2.29 (dt, 1H, *J* = 14.0, 7.0 Hz), 2.17 (ddd, 1H, *J* = 12.6, 11.2, 7.4 Hz), 1.96–1.82 (m, 1H), 1.50–1.38 (m, 1H). LCMS (FA): *m/z* = 538.1 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{22}H_{25}ClN_5O_5S_2$ 538.0986, obsd 538.0980.

Synthesis of Intermediate I-61

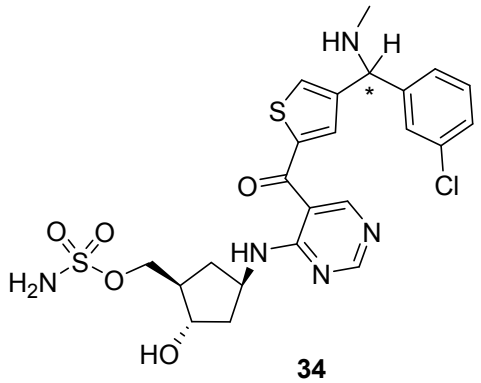


I-60 **I-61**

Reagents: (a) MeI, NaH, THF, 60 °C, 0.5 h, 48%.

Step 1: rac-*tert*-Butyl [(3-chlorophenyl)(5-formyl-3-thienyl)methyl]methylcarbamate (I-61). To a solution of rac-*tert*-butyl [(3-chlorophenyl)(5-formyl-3-thienyl)methyl]carbamate **I-60** (102 mg, 0.29 mmol) in THF (10.0 mL) was added 60% NaH in mineral oil (25.0 mg, 1.04 mmol) at rt and the mixture was stirred for 30 min. To the mixture was added methyl iodide (0.13 mL, 2.08 mmol) and the reaction was heated at 60 °C for 30 min. The reaction mixture was poured into 30 mL water and extracted with DCM (30 mL) twice. The combined organic layers were concentrated in vacuo and the residue was purified on silica gel to provide **I-61** (50 mg, 48%). ^{1}H NMR ($CDCl_3$) δ 9.92 (s, 1H), 7.61 (s, 1H), 7.54–7.45 (m, 1H), 7.35–7.32 (m, 2H), 7.25–7.22 (m, 1H), 7.16–7.11 (m, 1H), 6.61 (s, 1H), 2.73 (s, 3H), 1.49 (s, 9H).

Synthesis of 34

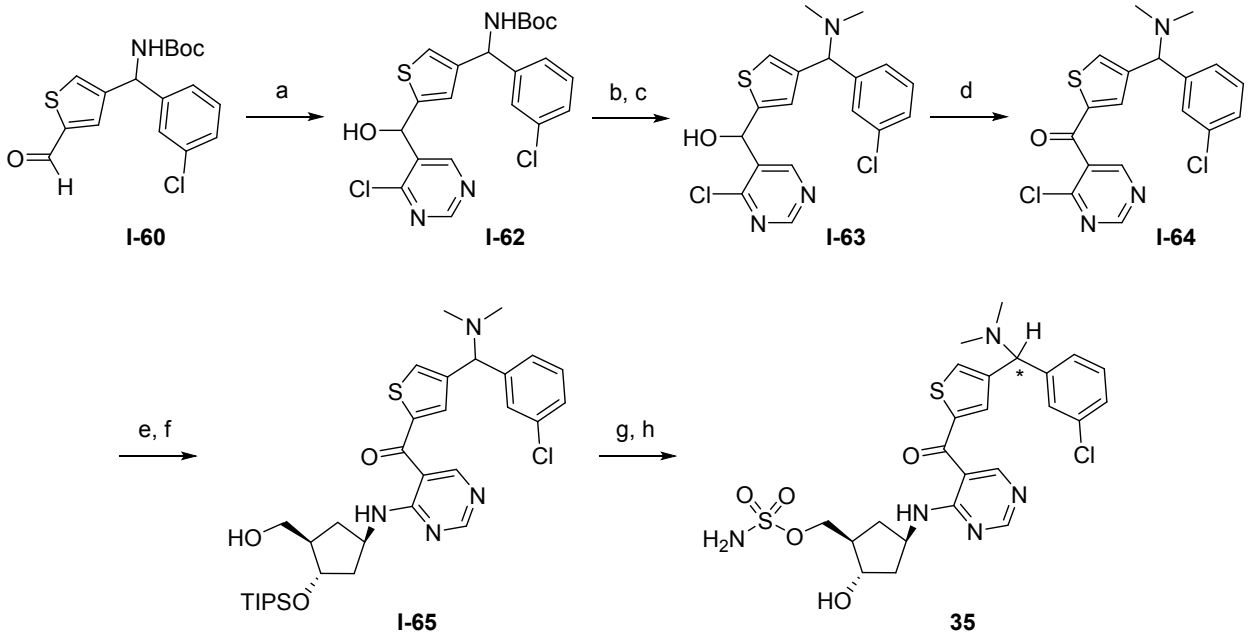


34

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(*R*)-(3-Chlorophenyl)(methylamino)methyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate and [(1*R*,2*S*,4*R*)-4-{[5-({4-[(*S*)-(3-Chlorophenyl)(methylamino)methyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (34). Prepared by the method described for the preparation of **26** using 4-chloro-5-iodopyrimidine and intermediate **I-61** in Step 2. The following alternative conditions were employed in the reaction scheme: Step 3: oxidant used was MnO_2; Step 4: Base/solvent used was *N,N*-diisopropylethylamine/*i*-PrOH; Step 7: desilylating agent/solvent used was TFA/water. Compound is a mixture of diastereomers. ^{1}H NMR (MeOD) δ 8.58 (s, 1H), 8.48 (s, 1H), 7.70 (d, 1H, *J* = 0.7 Hz), 7.54 (d, 1H, *J* = 1.3 Hz), 7.39–7.31 (m, 1H), 7.29–7.22 (m, 2H), 7.22–7.14 (m, 1H), 4.82 (s, 1H), 4.74–4.57 (m, 1H), 4.07 (ddd, 3H, *J* = 21.1, 9.0, 5.1 Hz), 2.46–2.32 (m, 1H), 2.27 (s, 3H), 2.15 (td, 1H, *J* = 8.6, 5.6 Hz), 2.08–2.00 (m, 1H), 1.84–1.74 (m, 1H), 1.35–1.29 (m,

1H). LCMS (FA): *m/z* = 552.1 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{23}H_{27}ClN_5O_5S_2$ 552.1142, obsd 552.1141.

Synthesis of 35



Reagents: (a) 4-chloro-5-iodopyrimidine, *n*-BuLi, THF, -78 °C, 0.5 h, 72%; (b) TFA, H_2O, 50 °C, 0.5 h; (c) HCHO, $NaBH(OAc)_3$, DIPEA, MeOH, rt, 12 h, 27% for 2 steps; (d) MnO_2, DCM, 40 °C, 2 h, 94%; (e) **I-31**, DIPEA, *i*-PrOH, 60 °C, 1 h; (f) 1% HCl in EtOH, rt, 0.5 h, 87% for 2 steps; (g) chlorosulfonamide, DMF, rt, 0.5 h; (h) TFA, H_2O, 50 °C, 0.5 h, 70% for 2 steps.

Step 1: *tert*-Butyl [(*S*)-(3-chlorophenyl){5-[(*S*)-(4-chloropyrimidin-5-yl)(hydroxy)methyl]-3-thienyl}methyl]carbamate, *tert*-Butyl [(*S*)-(3-chlorophenyl){5-[(*R*)-(4-chloropyrimidin-5-yl)(hydroxy)methyl]-3-thienyl}methyl]carbamate, *tert*-Butyl [(*R*)-(3-chlorophenyl){5-[(*S*)-(4-chloropyrimidin-5-yl)(hydroxy)methyl]-3-thienyl}methyl]carbamate, and *tert*-Butyl [(*R*)-(3-chlorophenyl){5-[(*R*)-(4-chloropyrimidin-5-yl)(hydroxy)methyl]-3-thienyl}methyl]carbamate (I-62). A solution of 4-chloro-5-iodopyrimidine (293 mg, 1.22 mmol) in THF (10.0 mL) was cooled at -78 °C. To the solution was added dropwise 2.50 M of *n*-BuLi in hexane (1.14 mL, 2.85 mmol) and the mixture was stirred for 10 min. To the mixture was added dropwise a solution of *tert*-butyl [(3-chlorophenyl)(5-formyl-3-thienyl)methyl]carbamate **I-60** (143 mg, 0.41 mmol) in THF (3.0 mL) at -78 °C, and the resulting mixture was stirred for 30 min at -78 °C. The reaction was quenched by addition of a solution of acetic acid (0.23 mL, 4.06 mmol) in THF (1.0 mL) at -78 °C and the mixture was warmed to rt. To the mixture was added 30mL water and extracted with EtOAc (30mL) three times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to give **I-62** (136 mg, 72%). ^1H NMR ($CDCl_3$) δ 8.95 (d, 1H, *J* = 4.9 Hz), 8.91 (s, 1H), 7.30–7.24 (m, 2H), 7.22 (s, 1H), 7.14–7.10 (m, 1H), 6.94 (s, 1H), 6.85 (s, 1H), 6.25 (s, 1H), 5.96–5.38 (m, 2H), 5.28–5.14 (br s, 1H), 1.41 (s, 9H).

Step 2: (*S*)-(4-Chloropyrimidin-5-yl){4-[(*S*)-(dimethylamino)(phenyl)methyl]-2-thienyl}methanol, (*S*)-(4-Chloropyrimidin-5-yl){4-[(*R*)-(dimethylamino)(phenyl)methyl]-2-thienyl}methanol, (*R*)-(4-Chloropyrimidin-5-yl){4-[(*S*)-(dimethylamino)(phenyl)methyl]-2-thienyl}methanol, and (*R*)-(4-Chloropyrimidin-5-yl){4-[(*R*)-(dimethylamino)(phenyl)methyl]-2-thienyl}methanol (I-63). The product mixture prepared in step 1 (723 mg, 1.55 mmol) was dissolved in a solution of TFA (24.0 mL, 311 mmol) and water (6.00 mL, 333 mmol). The mixture was heated at 50 °C for 30 min. The reaction mixture was concentrated in vacuo and the residue was diluted with 30 mL MeOH, and 1 mL *N,N*-diisopropylethylamine was added to neutralize the solution. To the solution was added formaldehyde (1.73 mL, 23.3 mmol) followed by sodium triacetoxyborohydride (2.96 g, 14.0 mmol) at rt and the resulting mixture was stirred overnight. The reaction mixture was poured into 50 mL water, and then extracted with DCM (50 mL) twice. The combined organic layers were concentrated in vacuo and the residue was purified on silica gel to give **I-63** (163 mg, 27%). ^{1}H NMR ($CDCl_3$) δ 9.01 (s, 1H), 8.89 (s, 1H), 7.37 (d, 1H, J = 1.8 Hz), 7.29–7.25 (m, 1H), 7.25–7.18 (m, 2H), 7.15 (d, 1H, J = 1.3 Hz), 7.08 (s, 1H), 6.24 (s, 1H), 6.11–5.65 (br s, 1H), 4.19 (s, 1H), 2.14 (s, 6H).

Step 3: rac-{4-[(3-Chlorophenyl)(dimethylamino)methyl]-2-thienyl}(4-chloropyrimidin-5-yl)methanone (I-64). To a solution of the products from step 2 (163 mg, 0.41 mmol) in DCM (18.0 mL) was added MnO_2 (539 mg, 6.20 mmol), and the reaction was stirred at rt overnight. To the mixture was added MnO_2 (180 mg, 2.07 mmol), and the reaction was stirred at 40 °C for 2 h. The reaction mixture was filtered through a celite pad and the filter cake was washed with DCM. The filtrate was concentrated in vacuo to give **I-64** as a white solid (152 mg, 94%). ^{1}H NMR ($CDCl_3$) δ 9.13 (s, 1H), 8.75 (s, 1H), 7.68 (s, 1H), 7.45 (s, 1H), 7.32 (s, 1H), 7.28–7.17 (m, 3H), 4.25 (s, 1H), 2.16 (s, 6H).

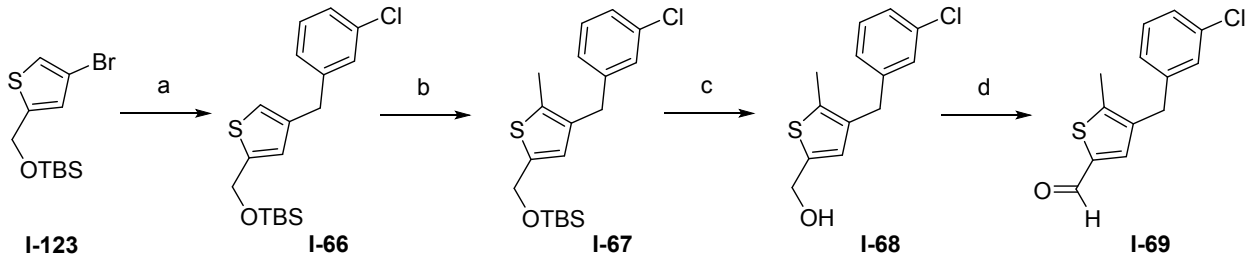
Step 4: {4-[(*S*)-(3-Chlorophenyl)(dimethylamino)methyl]-2-thienyl}[4-({(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]methanone and {4-[(*R*)-(3-Chlorophenyl)(dimethylamino)methyl]-2-thienyl}[4-({(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]methanone (I-65). To the product from step 3 (203.5 mg, 0.52 mmol), (1*R*,3*R*,4*S*)-3-({[tert-butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentanamine **I-31** (416.8 mg, 1.04 mmol) and *N,N*-diisopropylethylamine (0.36 mL, 2.08 mmol) was added *i*-PrOH (20.0 mL). The reaction was stirred at 60 °C for 1 h. The reaction was concentrated in vacuo, and then 1% HCl in EtOH solution (40 mL) was added to the residues. The reaction was stirred at rt for 30 min. The solution was poured into 1N NaOH solution (40 mL) and the mixture was extracted with DCM twice. The combined organic layers were concentrated in vacuo and purified on silica gel to provide **I-65** (289 mg, 87%). LCMS (FA): *m/z* = 645.6 (M+H).

Step 5: [(1*R*,2*S*,4*R*)-4-{[5-({4-[(*S*)-(3-Chlorophenyl)(dimethylamino)methyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate and [(1*R*,2*S*,4*R*)-4-{[5-({4-[(*R*)-(3-Chlorophenyl)(dimethylamino)methyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (35). To a solution of the products from step 4 (275 mg, 0.43 mmol) in DMF (6.0 mL) was added chlorosulfonamide (148 mg, 1.28 mmol) at rt and the reaction was stirred for 30 min. The reaction was poured into a solution of saturated $NaHCO_3$ and the mixture was extracted with

EtOAc three times. The combined organic layers were concentrated in vacuo. The residues were dissolved into a solution of TFA (16.0 mL, 208 mmol) and water (4.0 mL). The mixture was stirred at 50 °C for 30 min. The reaction mixture was concentrated in vacuo and MeOH (5 mL) was added to the residue and then 1N NaOH solution was added to the mixture to basify it to pH 12. After concentration, the residue was purified on silica gel to provide **35** (168 mg, 70%). Compound is a mixture of diastereomers. ^{1}H NMR (MeOD) δ 8.66 (s, 1H), 8.57 (s, 1H), 7.82 (s, 1H), 7.69 (s, 1H), 7.46 (s, 1H), 7.39–7.36 (m, 1H), 7.33–7.29 (m, 1H), 7.25–7.22 (m, 1H), 4.81–4.73 (m, 1H), 4.39 (s, 1H), 4.22–4.12 (m, 3H), 2.51–2.44 (m, 1H), 2.29–2.10 (m, 2H), 2.21 (s, 6H), 1.92–1.85 (m, 1H), 1.43–1.34 (m, 1H). LCMS (FA): *m/z* = 566.1 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{24}H_{29}ClN_5O_5S_2$ 566.1299, obsd 566.1318.

Procedures for the syntheses of compounds in Table 6

Synthesis of Intermediate I-69



I-123 **I-66** **I-67** **I-68** **I-69**

Reagents: (a) 3-chlorobenzylzinc chloride, $Pd_2(dba)_3$, *t*-Bu_3PBHF_4, THF, 50 °C, 1 h, 92%; (b) MeI, *n*-BuLi, THF, -78 °C, 0.5 h, 82%; (c) 1% HCl in EtOH, EtOH, rt, 0.5 h, 78%; (d) MnO_2, DCM, rt, 6 h, 90%.

Step 1: *tert*-Butyl{[4-(3-chlorobenzyl)-2-thienyl]methoxy}dimethylsilane (I-66). A 20mL microwave vessel was charged with ((4-bromothiophen-2-yl)methoxy)(*tert*-butyl)dimethylsilane (**I-123**, 425 mg, 1.38 mmol), $Pd_2(dba)_3$ (25.3 mg, 0.03 mmol), and tri-*tert*-butylphosphonium tetrafluoroborate (16.1 mg, 0.06 mmol). To the mixture was added THF (18.2 mL) and the reaction vessel was purged with argon followed by sealing with a cap. After the mixture was stirred for 5 min at rt, 0.5 M of 3-chlorobenzylzinc chloride in THF solution (3.18 mL, 1.59 mmol) was added to the mixture. The reaction was heated at 50 °C for 1 h. The reaction was cooled to rt and diluted with EtOAc. The organic layer was filtered through a celite pad and the filtrate was washed with water followed by brine. The EtOAc layer was filtered and the filtrate was concentrated in vacuo. The residue was purified on silica gel to provide **I-66** as a colorless oil (475 mg, 92%). ^{1}H NMR (DMSO-d_6) δ 7.35–7.17 (m, 4H), 7.12 (s, 1H), 6.80 (s, 1H), 4.77 (s, 2H), 3.88 (s, 2H), 0.86 (s, 9H), 0.04 (s, 6H).

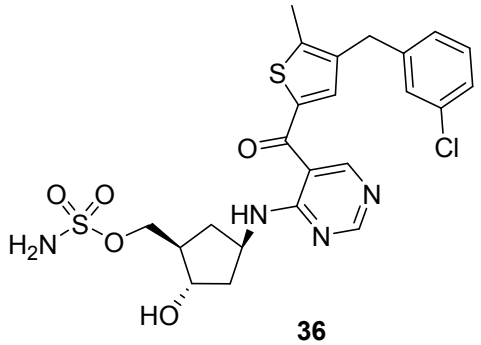
Step 2: *tert*-Butyl{[4-(3-chlorobenzyl)-5-methyl-2-thienyl]methoxy}dimethylsilane (I-67). *tert*-Butyl{[4-(3-chlorobenzyl)-2-thienyl]methoxy}dimethylsilane (0.67 g, 1.90 mmol) was dissolved into THF (10.0 mL), and then cooled down at -78 °C. 2.50 M of *n*-BuLi in hexane (6.08 mL, 15.2 mmol) was added dropwise via syringe to this solution at -78 °C and the mixture

was stirred for 30 min. To the mixture was added methyl iodide (1.18 mL, 19.0 mmol) and the reaction was stirred at -78 °C for 30 min. The reaction was quenched by addition of 30 mL water at -78 °C and the mixture was warmed to rt. The resulting mixture was extracted with DCM (30 mL) twice. The organic layers were combined and concentrated in vacuo to yield **I-67** (574 mg, 82%). ^{1}H NMR ($CDCl_3$) δ 7.12–7.08 (m, 2H), 7.03 (s, 1H), 6.94 (d, 1H, J = 7.1 Hz), 6.45 (s, 1H), 4.66 (s, 2H), 3.71 (s, 2H), 2.26 (s, 3H), 0.83 (s, 9H), 0.00 (s, 6H).

Step 3: [4-(3-Chlorobenzyl)-5-methyl-2-thienyl]methanol (I-68). *tert*-Butyl{[4-(3-chlorobenzyl)-5-methyl-2-thienyl]methoxy}dimethylsilane (0.62 g, 1.69 mmol) was dissolved into 20 mL of 1% HCl in EtOH solution and the mixture was stirred at rt for 30 min. The solution was poured into 30 mL saturated $NaHCO_3$ solution and the mixture was extracted with DCM (30 mL) twice. The combined organics were concentrated in vacuo and the mixture was purified on silica gel to yield **I-68** (332 mg, 78%). ^{1}H NMR ($CDCl_3$) δ 7.26–7.17 (m, 2H), 7.14 (s, 1H), 7.05 (dd, 1H, J = 7.1, 1.7 Hz), 6.65 (s, 1H), 4.71 (s, 2H), 3.82 (s, 2H), 2.39 (s, 3H).

Step 4: 4-(3-Chlorobenzyl)-5-methylthiophene-2-carbaldehyde (I-69). To a solution of [4-(3-chlorobenzyl)-5-methyl-2-thienyl]methanol (324 mg, 1.28 mmol) in DCM (40 mL) was added MnO_2 (1.67 g, 19.2 mmol) and the mixture was stirred at rt for 6 h. The reaction mixture was filtered through a celite pad and the filter cake was washed with DCM several times. The filtrate was concentrated in vacuo to yield **I-69** (289 mg, 90%). ^{1}H NMR ($CDCl_3$) δ 9.76 (s, 1H), 7.42 (s, 1H), 7.30–7.19 (m, 2H), 7.14 (s, 1H), 7.11–7.00 (m, 1H), 3.90 (s, 2H), 2.48 (s, 3H).

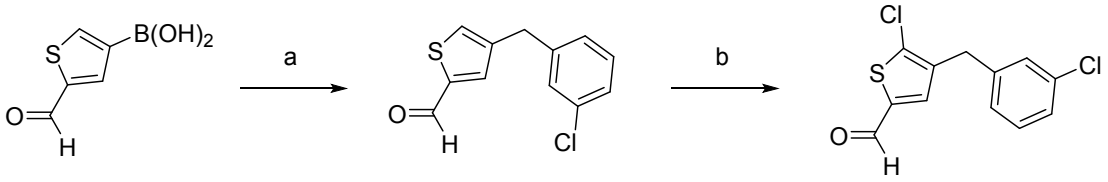
Synthesis of 36



36

{(1*R*,2*S*,4*R*)-4-[(5-{[4-(3-Chlorobenzyl)-5-methyl-2-thienyl]carbonyl}pyrimidin-4-yl)amino]-2-hydroxycyclopentyl}methyl sulfamate (36).
Prepared by the method described for the preparation of **26** using 4-chloro-5-iodopyrimidine and intermediate **I-69** in Step 2. The following alternative conditions were employed in the reaction scheme: Step 3: oxidant used was MnO_2; Step 4: Base/solvent used was *N,N*-diisopropylethylamine/*i*-PrOH; Step 7: desilylating agent/solvent used was TFA/water. ^{1}H NMR (MeOD) δ 8.63 (s, 1H), 8.53 (s, 1H), 7.39 (s, 1H), 7.27–7.23 (m, 1H), 7.19–7.17 (m, 2H), 7.11–7.09 (m, 1H), 4.79–4.71 (m, 1H), 4.22–4.15 (m, 3H), 3.94 (s, 2H), 2.50–2.44 (m, 1H), 2.45 (s, 3H), 2.26–2.24 (m, 1H), 2.16–2.10 (m, 1H), 1.90–1.83 (m, 1H), 1.42–1.37 (m, 1H). LCMS (FA): *m/z* = 537.1 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{23}H_{26}ClN_4O_5S_2$ 537.1033, obsd 537.1064.

Synthesis of Intermediate I-71

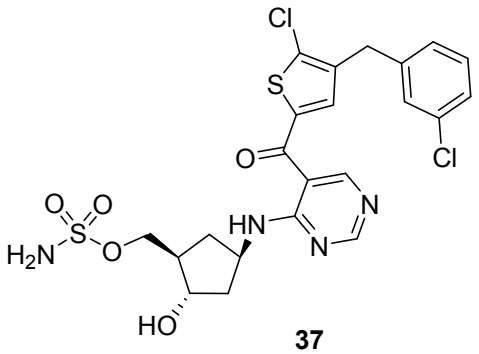


Reagents: (a) 3-chlorobenzyl bromide, $Pd(PPh_3)_4$, Na_2CO_3, 1,4-dioxane, H_2O, 80 °C, 58%; (b) NCS, DMF, 50 °C, 3 h, 48%.

Step 1: 4-(3-Chlorobenzyl)thiophene-2-carbaldehyde (I-70). A round bottom flask was charged with 2-formyl-4-thiopheneboronic acid (0.30 g, 1.92 mmol), 3-chlorobenzyl bromide (0.28 mL, 2.12 mmol), and Na_2CO_3 (0.41 g, 3.85 mmol). To the reaction vessel was added 1,4-dioxane (12 mL) and water (2 mL) followed by $Pd(PPh_3)_4$ (0.22 g, 0.19 mmol) at room temperature. The mixture was heated at 80 °C for 2 h under atmosphere of argon. After cooling to room temperature, to the reaction mixture was added water and extracted with EtOAc twice. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel to give **I-70** (0.26 g, 58%). ^{1}H NMR (DMSO-d_6) δ 9.86 (d, 1H, J = 1.0 Hz), 7.87 (s, 2H), 7.39–7.31 (m, 2H), 7.31–7.22 (m, 2H), 4.01 (s, 2H). LCMS (FA): m/z = 237.1 (M+H).

Step 2: 5-Chloro-4-(3-chlorobenzyl)thiophene-2-carbaldehyde (I-71). To a solution of 4-(3-chlorobenzyl)thiophene-2-carbaldehyde (265 mg, 1.12 mmol) and NCS (224 mg, 1.68 mmol) in DMF (5.0 mL) was stirred at 50 °C for 3 h. The reaction mixture was quenched with water and extracted with EtOAc. The combined organic layers were washed with 10% aqueous LiCl, dried over $MgSO_4$, filtered and concentrated in vacuo and the crude material was purified by silica gel to give **I-71** as a clear oil (147 mg, 48%). ^{1}H NMR (DMSO-d_6) δ 9.78 (s, 1H), 7.89 (s, 1H), 7.40–7.29 (m, 3H), 7.24–7.19 (m, 1H), 4.00 (s, 2H). LCMS (FA): m/z = 271.1 (M+H).

Synthesis of 37

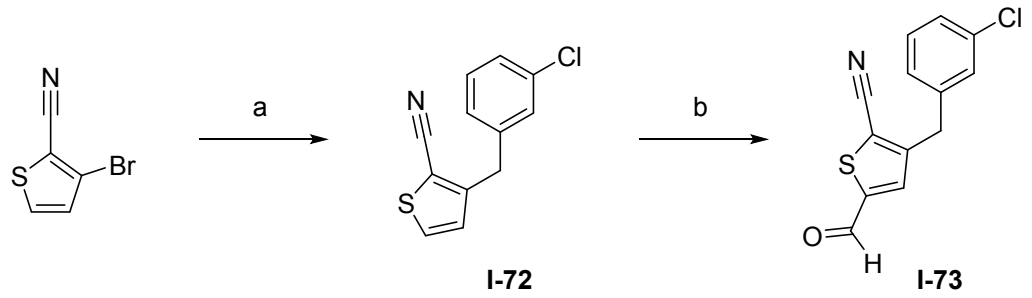


37

{(1*R*,2*S*,4*R*)-4-[(5-{[5-Chloro-4-(3-chlorobenzyl)-2-thienyl]carbonyl}pyrimidin-4-yl)amino]-2-hydroxycyclopentyl}methyl sulfamate (37).

Prepared by the method described for the preparation of **26** using 4-chloro-5-iodopyrimidine and intermediate **I-71** in Step 2. The following alternative conditions were employed in the reaction scheme: Step 3: oxidant used was MnO_2; Step 4: Base/solvent used was *N,N*-diisopropylethylamine/*i*-PrOH. ^{1}H NMR (DMSO-d_6) δ 8.70 (s, 1H), 8.66 (s, 1H), 8.20 (d, 1H, *J* = 7.5 Hz), 7.80 (s, 1H), 7.44 (s, 2H), 7.36 (d, 1H, *J* = 1.6 Hz), 7.33 (d, 1H, *J* = 7.5 Hz), 7.30–7.26 (m, 1H), 7.25–7.21 (m, 1H), 4.90 (d, 1H, *J* = 4.4 Hz), 4.75–4.64 (m, 1H), 4.11–4.03 (m, 1H), 3.99 (d, 2H, *J* = 10.4 Hz), 3.98–3.90 (m, 2H), 2.34–2.25 (m, 1H), 2.14–2.08 (m, 1H), 1.98–1.92 (m, 1H), 1.82–1.73 (m, 1H), 1.30–1.21 (m, 1H). LCMS (FA): *m/z* = 557.2 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{22}H_{23}Cl_2N_4O_5S_2$ 557.0487, obsd 557.0502.

Synthesis of Intermediate I-73

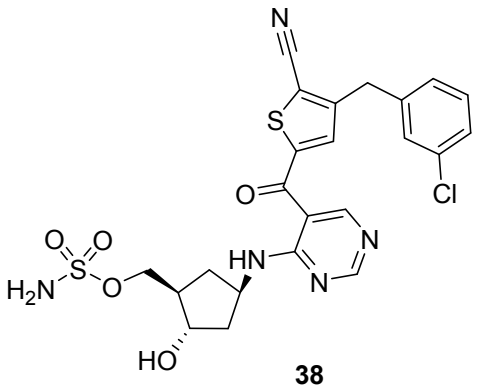


Reagents: (a) 3-chlorobenzylzinc chloride, $Pd_2(dba)_3$, *t*-Bu_3PBHF_4, THF, 50 °C, 24 h, 59%; (b) *n*-BuLi, DMF, THF, -78 °C, 1 h, 50%.

Step 1: 3-(3-Chlorobenzyl)thiophene-2-carbonitrile (I-72). To a solution of 3-bromothiophene-2-carbonitrile (1.22 g, 6.50 mmol) in THF (20 mL) was added a 0.5 M solution of 3-chlorobenzylzinc chloride in THF (15.0 mL, 7.0 mmol). To this reaction mixture was added $Pd_2(dba)_3$ (120 mg, 0.13 mmol) and tri-*t*-butylphosphonium tetrafluoroborate (74 mg, 0.26 mmol). The reaction mixture was stirred at 50 °C for 24 h then quenched with water and extracted with EtOAc. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated in vacuo. The crude material was purified on silica gel to give **I-72** as a clear oil (896 mg, 59%). ^{1}H NMR (MeOD) δ 7.83–7.73 (m, 1H), 7.38–7.13 (m, 4H), 7.12–7.02 (m, 1H), 4.15 (s, 2H).

Step 2: 3-(3-Chlorobenzyl)-5-formylthiophene-2-carbonitrile (I-73). To a -78 °C cooled solution of 3-(3-chlorobenzyl)thiophene-2-carbonitrile (150 mg, 0.64 mmol) in THF (6.04 mL) was added a 2.50 M solution of *n*-BuLi in hexane (0.39 mL, 0.96 mmol) dropwise, via syringe. The reaction mixture was allowed to stir for 20 min followed by addition of a solution of DMF (0.10 mL, 1.28 mmol) in THF (2 mL), dropwise, via syringe. The resulting mixture was stirred for an additional 1 h then quenched with water and extracted with EtOAc. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered and concentrated in vacuo. The crude material was purified on silica gel to provide **I-73** as a white solid (84 mg, 50%). ^{1}H NMR (MeOD) δ 10.76 (s, 1H), 8.81 (s, 1H), 8.29 – 8.13 (m, 3H), 8.07 (d, 1H, *J* = 7.4 Hz), 4.14 (s, 2H).

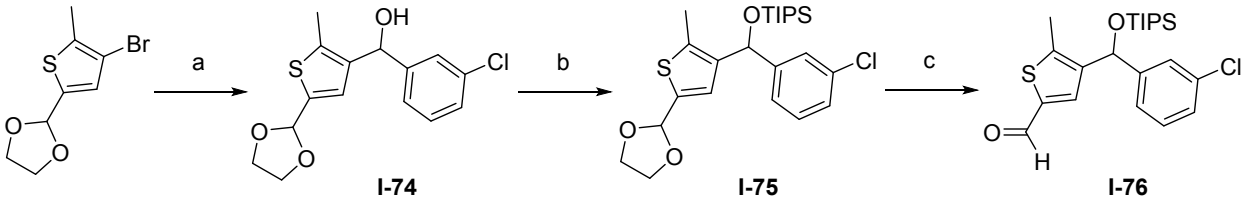
Synthesis of 38



38

{(1*R*,2*S*,4*R*)-4-[(5-{[4-(3-Chlorobenzyl)-5-cyano-2-thienyl]carbonyl}pyrimidin-4-yl)amino]-2-hydroxycyclopentyl}methyl sulfamate (38).
Prepared by the method described for the preparation of **26** using 4-chloro-5-iodopyrimidine and intermediate **I-73** in Step 2. The following alternative conditions were employed in the reaction scheme: Step 3: oxidant used was MnO_2; Step 4: Base/solvent used was *N,N*-diisopropylethylamine/*i*-PrOH. ^{1}H NMR (DMSO-d_6) δ 8.74 (s, 1H), 8.69 (s, 1H), 8.37 (d, 1H, *J* = 7.5 Hz), 7.91 (s, 1H), 7.46–7.39 (m, 2H), 7.39–7.34 (m, 1H), 7.33–7.29 (m, 1H), 7.28–7.23 (m, 1H), 4.73 (dd, 1H, *J* = 15.9, 8.0 Hz), 4.19 (s, 2H), 4.12–4.05 (m, 1H), 4.00–3.93 (m, 2H), 2.55 (s, 2H), 2.35–2.26 (m, 1H), 2.16–2.09 (m, 1H), 2.01–1.91 (m, 1H), 1.81–1.73 (m, 1H), 1.33–1.23 (m, 1H). LCMS (FA): *m/z* = 548.2 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{23}H_{23}ClN_5O_5S_2$ 548.0829, obsd 548.0849.

Synthesis of Intermediate I-76



Reagents: (a) 3-chlorobenzaldehyde, *n*-BuLi, THF, -78 °C, 15 min, 68%; (b) TIPSCl, NaH, THF, rt, 12 h, 100%; (c) 1% HCl in EtOH, rt, 2 h, 97%.

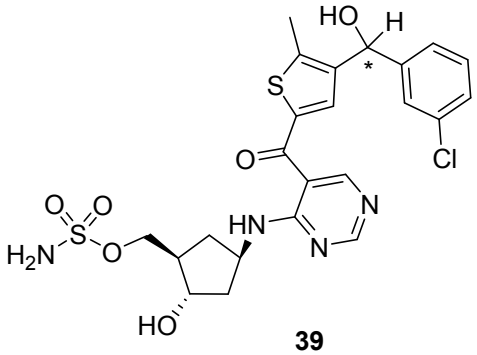
Step 1: rac-(3-Chlorophenyl)[5-(1,3-dioxolan-2-yl)-2-methyl-3-thienyl]methanol (I-74). A 100mL 2-neck round bottom flask was charged with THF (60 mL) then the flask was purged with argon, and was cooled at -78 °C. To the THF, 2.50 M of *n*-BuLi in hexane (5.40 mL, 13.5 mmol) was added dropwise via syringe and the mixture was stirred for 10 min at -78 °C. 2-(4-bromo-5-methyl-2-thienyl)-1,3-dioxolane (2.69 g, 10.8 mmol) was added dropwise at -78 °C. The solution was stirred for 30 min at -78 °C. 3-chlorobenzaldehyde (1.23 mL, 10.8 mmol) was

added to the solution at once at -78 °C and stirred for 15 min. The reaction was quenched by addition of saturated NH_4Cl (50 mL) and extracted with EtOAc (50 mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to yield **I-74** (2.3 g, 68%). ^{1}H NMR ($CDCl_3$) δ 7.14–7.06 (m, 4H), 6.79 (s, 1H), 5.81 (s, 1H), 5.70 (s, 1H), 4.00–3.91 (m, 3H), 3.88–3.77 (m, 2H), 2.32 (s, 3H).

Step 2: rac-{(3-Chlorophenyl)[5-(1,3-dioxolan-2-yl)-2-methyl-3-thienyl]methoxy}(triisopropyl)silane (I-75). rac-(3-Chlorophenyl)[5-(1,3-dioxolan-2-yl)-2-methyl-3-thienyl]methanol (927 mg, 2.98 mmol) was dissolved in THF (30.6 mL), then 60% NaH in mineral oil (329 mg, 13.7 mmol) was added to this solution. The solution was stirred at 40 °C for 30 min. TIPSCl (1.45 mL, 6.86 mmol) was added and the reaction mixture stirred at rt overnight. The solution was poured into 30 mL saturated NH_4Cl solution. The solution was extracted with EtOAc (30 mL) twice. The combined organic layers were dried over Na_2SO_4, filtered, concentrated in vacuo. The residue was purified on silica gel to provide **I-75** (1.39 g, 100%). ^{1}H NMR ($CDCl_3$) δ 7.17–7.02 (m, 4H), 6.96 (s, 1H), 5.87 (s, 1H), 5.72 (s, 1H), 4.00–3.94 (m, 2H), 3.90–3.85 (m, 2H), 2.33 (s, 3H), 1.00–0.93 (m, 21H). LCMS (FA): *m/z* = 467.1 (M+H).

Step 3: rac-4-{(3-Chlorophenyl)[(triisopropylsilyl)oxy]methyl}-5-methylthiophene-2-carbaldehyde (I-76). rac-{(3-Chlorophenyl)[5-(1,3-dioxolan-2-yl)-2-methyl-3-thienyl]methoxy}(triisopropyl)silane (1.6 g, 3.4 mmol) was dissolved in 1% HCl in EtOH (20 mL) and the reaction was stirred at rt for 2 h. The reaction mixture was diluted with water, extracted with DCM (20 mL) twice. The combined organic layers were dried over Na_2SO_4, filtered, concentrated in vacuo. The residue was purified on silica gel to give **I-76** (1.4 g, 97%). ^{1}H NMR ($CDCl_3$) δ 9.67 (s, 1H), 7.63 (s, 1H), 7.21–7.03 (m, 4H), 5.75 (s, 1H), 2.40 (s, 3H), 1.06–0.92 (m, 21H). LCMS (FA): *m/z* = 423.1 (M+H).

Synthesis of 39

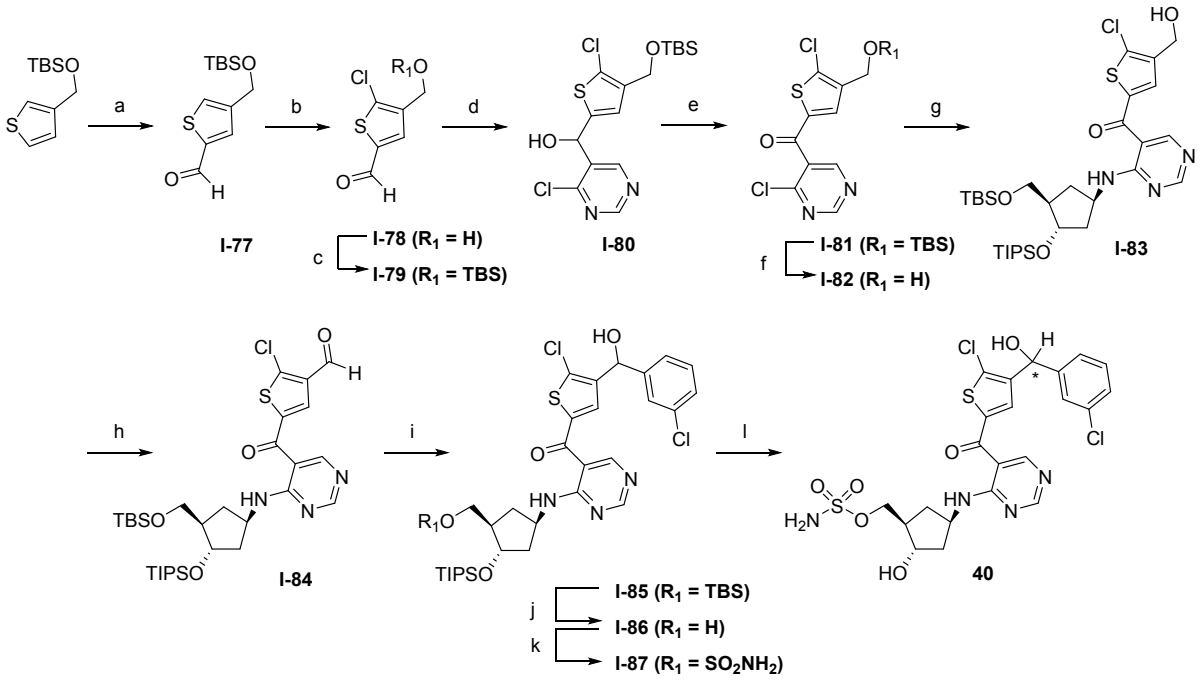


39

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(*R*)-(3-Chlorophenyl)(hydroxy)methyl]-5-methyl-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({4-[(*S*)-(3-Chlorophenyl)(hydroxy)methyl]-5-methyl-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (39).

Prepared by the method described for the preparation of **26** using 4-chloro-5-iodopyrimidine and intermediate **I-76** in Step 2. The following alternative conditions were employed in the reaction scheme: Step 3: oxidant used was MnO_2; Step 4: Base/solvent used was *N,N*-diisopropylethylamine/*i*-PrOH; Step 7: desilylating agent/solvent used was TBAF/THF. The individual diastereomers were obtained using preparative scale chiral SFC. More active epimer **39** is reported. ^{1}H NMR (MeOD) δ 8.66 (s, 1H), 8.56 (d, 1H, *J* = 4.3 Hz), 7.47 (s, 1H), 7.41 (s, 1H), 7.37–7.20 (m, 3H), 5.89 (s, 1H), 4.81–4.73 (m, 1H), 4.23–4.09 (m, 3H), 2.53 (s, 3H), 2.51–2.42 (m, 1H), 2.30–2.22 (m, 1H), 2.18–2.10 (m, 1H), 1.93–1.85 (m, 1H), 1.44–1.37 (m, 1H). LCMS (FA): *m/z* = 553.1 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{23}H_{26}ClN_4O_6S_2$ 553.0982, obsd 553.0981.

Synthesis of 40



Reagents: (a) *n*-BuLi, DMF, THF, -78 °C then rt 30 min; (b) NCS, DMF, 50 °C, 3 h; (c) TBSCl, imidazole, DCM, rt, 1 h, 86% for 2 steps; (d) 4-chloro-5-iodo-pyrimidine, *n*-BuLi, THF, -78 °C, 15 min, 84%; (e) MnO_2, DCM, rt, 12 h, 85%; (f) 2N HCl, THF, rt, h, 79%; (g) **I-31**, DIPEA, 1-PrOH, 70 °C, 2 h, 92%; (h) Dess-Martin periodinane, DCM, 0 °C, 0.5 h, %; (i) 3-chlorophenylmagnesium bromide, THF, 0 °C, 1 h, 50%; (j) 1% HCl in EtOH, EtOH, 4 °C, 12 h, 96%; (k) chlorosulfonamide, DMF, rt, 1 h, 94%; (l) H_3PO_4, CH_3CN, rt, 1 h, 39%.

Step 1: 4-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)thiophene-2-carbaldehyde (I-77). A solution of *tert*-butyl(dimethyl)(3-thienylmethoxy)silane (8.78 g, 38.4 mmol) in THF (140 mL) was cooled at -78 °C. 1.40 M of *sec*-BuLi in cyclohexane (35.7 mL, 50.0 mmol) was added dropwise via syringe to the solution at -78 °C and the mixture was stirred for 30 seconds. DMF (5.95 mL, 76.9 mmol) was added at -78 °C, and the reaction mixture was allowed to warm to rt over 30 min. Reaction was quenched with acetic acid (5 mL), and the solution was poured into 60 mL of water and extracted with EtOAc (100 mL) twice. The combined organic layers were concentrated in vacuo and the mixture was purified on silica gel to afford **I-77** as a colorless oil (6.91 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.79 (d, 1H, *J* = 1.3 Hz), 7.58 (d, 1H, *J* = 1.4 Hz), 7.47 (d, 1H, *J* = 1.2 Hz), 4.70 – 4.54 (s, 2H), 0.83 (s, 9H), -0.00 (s, 6H).

Step 2: 5-Chloro-4-(hydroxymethyl)thiophene-2-carbaldehyde (I-78). To a solution of 4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)thiophene-2-carbaldehyde (1.75 g, 6.82 mmol) in DMF (30 mL) was added NCS (1.88 g, 14.1 mmol) in one portion. The reaction mixture was then stirred at 50 °C for 3 h. The reaction mixture was allowed to cool to rt. The reaction was diluted with 50 mL water and extracted with EtOAc twice. The combined EtOAc layer was washed with brine, dried under Na_2SO_4, filtered and concentrated under reduced pressure to give **I-78** as colorless oil. ^{1}H NMR ($CDCl_3$) δ 9.76 (s, 1H), 7.74 (s, 1H), 4.65 (s, 2H), 3.41–3.18 (br s, 1H). LCMS (FA): *m/z* = 176.9 (M+H).

Step 3: 4-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-5-chlorothiophene-2-carbaldehyde (I-79). To a solution of 5-chloro-4-(hydroxymethyl)thiophene-2-carbaldehyde in DCM (30 mL), TBSCl (1.23 g, 8.19 mmol) and imidazole (0.93 g, 13.6 mmol) were added and the reaction was stirred at rt for 1 h. The reaction mixture was quenched by addition of water (60 mL) and extracted with DCM (50 mL) three times. The combined organic layers were washed by brine, dried by Na_2SO_4, filtered and concentrated in vacuo. The residue was purified on silica gel to afford **I-79** as a colorless oil (1.7 g, 86%). ^{1}H NMR ($CDCl_3$) δ 9.78 (s, 1H), 7.66 (s, 1H), 4.65 (m, 2H), 0.92 (s, 9H), 0.08 (s, 6H). LCMS (FA): *m/z* = 291.3 (M+H).

Step 4: [4-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-5-chloro-2-thienyl](4-chloropyrimidin-5-yl)methanol (I-80). A solution of 4-chloro-5-iodopyrimidine (1.2 g, 5.0 mmol) in THF (37.3 mL) was cooled at -78 °C. To the solution was added dropwise 2.50 M of *n*-BuLi in hexane (3.96 mL, 9.90 mmol) at -78 °C and the mixture was stirred for 30 min at same temp. To the mixture was added dropwise a solution of 4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-5-chlorothiophene-2-carbaldehyde (1.2 g, 4.1 mmol) in THF (7.5 mL) at -78 °C, and the reaction was stirred for 15 min. The reaction was quenched by addition of saturated NH_4Cl (150mL) and extracted with EtOAc (50 mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to give **I-80** as light yellow oil (1.4 g, 84%). ^{1}H NMR ($CDCl_3$) δ 8.97 (s, 1H), 8.91 (s, 1H), 6.88 (s, 1H), 6.21 (s, 1H), 4.56 (s, 2H), 3.48–3.09 (br s, 1H), 0.87 (s, 9H), 0.06 (s, 6H). LCMS (FA): *m/z* = 405.2 (M+H).

Step 5: [4-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-5-chloro-2-thienyl](4-chloropyrimidin-5-yl)methanone (I-81). To a solution of [4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-5-chloro-2-thienyl](4-chloropyrimidin-5-yl)methanol (1.88 g, 4.64 mmol) in DCM (97 mL) was added MnO_2 (4.03 g, 46.4 mmol) at rt, and the mixture was stirred for 12 h. The reaction was filtered through a celite pad and the residual solid was rinsed with DCM several times. The filtrate was concentrated in vacuo to give **I-81** as light yellow oil (1.59 g, 85%). ^{1}H NMR ($CDCl_3$) δ 9.13 (s, 1H), 8.75 (s, 1H), 7.32 (s, 1H), 4.65–4.58 (m, 2H), 0.86 (s, 9H), 0.07 (s, 6H). LCMS (FA): *m/z* = 403.3 (M+H).

Step 6: [5-Chloro-4-(hydroxymethyl)-2-thienyl](4-chloropyrimidin-5-yl)methanone (I-82). In a 100 mL round bottom flask , was placed [4-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-5-chloro-2-thienyl](4-chloropyrimidin-5-yl)methanone (2.07 g, 5.13 mmol), THF (24.0 mL) and 2.0 M of HCl in water (4.00 mL, 8.00 mmol). The reaction was stirred at rt. The reaction was quenched with saturated $NaHCO_3$ and the aqueous layer was extracted with EtOAc three times.

The combined organics were dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified on silica gel to afford **I-82** a yellow solid (1.17 g, 79%). ^{1}H NMR ($CDCl_3$) δ 9.14 (s, 1H), 8.76 (s, 1H), 7.38 (s, 1H), 4.67 (s, 2H), 1.87 (br s, 1H). LCMS (FA): *m/z* = 291.1 (M+H).

Step 7: [4-({(1*R*,3*R*,4*S*)-3-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl][5-chloro-4-(hydroxymethyl)-2-thienyl]methanone (I-83). In a microwave reaction vessel, to a solution of (1*R*,3*R*,4*S*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentanamine (**I-31**, 764 mg, 1.90 mmol) and [5-chloro-4-(hydroxymethyl)-2-thienyl](4-chloropyrimidin-5-yl)methanone (500 mg, 1.73 mmol) in 1-PrOH (14.0mL) was added *N,N*-diisopropylethylamine (1.00 mL, 5.74 mmol). The reaction vessel was purged with argon and then sealed. The mixture was stirred for 2 h at 70 °C, then concentrated in vacuo. To the residue was added EtOAc. The organic layer was washed with saturated NH_4Cl twice followed by water and brine. The resulting organic portion was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to provide **I-83** as a yellow residue (1.04 g, 92%). ^{1}H NMR ($CDCl_3$) δ 8.80 (s, 1H), 8.66 (s, 1H), 8.51 (d, 1H, *J* = 7.1 Hz), 7.54 (s, 1H), 4.86–4.73 (m, 1H), 4.68 (s, 2H), 4.34–4.26 (m, 1H), 3.65–3.51 (m, 2H), 2.49–2.39 (m, 1H), 2.23–2.09 (m, 2H), 1.92 (s, 1H), 1.77–1.65 (m, 1H), 1.32–1.19 (m, 1H), 1.07 (s, 21H), 0.89 (s, 9H), 0.04 (s, 6H). LCMS (FA): *m/z* = 654.7 (M+H).

Step 8: 5-{[4-({(1*R*,3*R*,4*S*)-3-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]carbonyl}-2-chlorothiophene-3-carbaldehyde (I-84). A 50mL round bottom flask under nitrogen was charged with [4-({(1*R*,3*R*,4*S*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl][5-chloro-4-(hydroxymethyl)-2-thienyl]methanone (0.57 g, 0.87 mmol) and DCM (10 mL). To the mixture was added Dess-Martin periodinane (0.55 g, 1.31 mmol) in a single portion and the mixture was stirred with cooling at 0 °C for 30 min. The reaction mixture was quenched by addition of saturated $NaHCO_3$ followed by extraction with DCM. The organic portion was dried with Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel to give **I-84** as a yellow residue (0.51 g, 90%). ^{1}H NMR ($CDCl_3$) δ 10.04 (s, 1H), 8.81 (s, 1H), 8.69 (s, 1H), 8.55 (d, 1H, *J* = 7.1 Hz), 7.79 (s, 1H), 4.88–4.76 (m, 1H), 4.34–4.27 (m, 1H), 3.66–3.53 (m, 2H), 2.51–2.39 (m, 1H), 2.24–2.09 (m, 2H), 1.78–1.66 (m, 1H), 1.34–1.21 (m, 1H), 1.07 (s, 21H), 0.89 (s, 9H), 0.04 (s, 6H). LCMS (FA): *m/z* = 652.7 (M+H).

Step 9: [[4-({(1*R*,3*R*,4*S*)-3-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]{5-chloro-4-[(*S*)-(3-chlorophenyl)(hydroxy)methyl]-2-thienyl}methanone and [[4-({(1*R*,3*R*,4*S*)-3-({[tert-Butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]{5-chloro-4-[(*R*)-(3-chlorophenyl)(hydroxy)methyl]-2-thienyl}methanone (I-85). To a solution of 5-{[4-({(1*R*,3*R*,4*S*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]carbonyl}-2-chlorothiophene-3-carbaldehyde (373 mg, 0.57 mmol) in THF (5.34 mL) was added 0.5 M of 3-chlorophenylmagnesium bromide in THF (2.29 mL, 1.14 mmol) at 0 °C, and the mixture was then stirred at 0 °C for 1 h. To the reaction was added more 0.5 M of 3-chlorophenylmagnesium bromide in THF (1.5 mL) and the mixture was stirred for 1 h. The reaction was quenched by

addition of saturated NH_4Cl and the mixture was extract with EtOAc three times. The combined organic layers were then washed with water, brine, dried over Na_2SO_4, filtered and concentrated in vacuo to give **I-85** (220 mg, 50%). ^{1}H NMR ($CDCl_3$) δ 8.69 (s, 1H), 8.63 (s, 1H), 8.54–8.48 (br d, 1H, *J*=7.3 Hz), 7.48 (d, 1H, *J* = 0.9 Hz), 7.41 (s, 1H), 7.31–7.26 (m, 3H), 5.98 (s, 1H), 4.85–4.74 (m, 1H), 4.33–4.27 (m, 1H), 3.62 (ddd, 1H, *J* = 10.0, 5.4, 2.6 Hz), 3.55 (ddd, 1H, *J* = 10.0, 5.8, 2.2 Hz), 3.16–3.09 (br s, 1H), 2.48–2.38 (m, 1H), 2.22–2.10 (m, 2H), 1.76–1.65 (m, 1H), 1.30–1.20 (m, 1H), 1.07 (m, 21H), 0.89 and 0.88 (each s, total 9H), 0.05 (s, 3H), 0.04 (s, 3H). LCMS (FA): *m/z* = 766.3 (M+H).

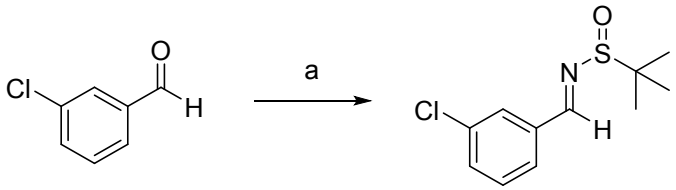
Step 10: {5-Chloro-4-[(*S*)-(3-chlorophenyl)(hydroxy)methyl]-2-thienyl}[4-({(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]methanone and {5-Chloro-4-[(*R*)-(3-chlorophenyl)(hydroxy)methyl]-2-thienyl}[4-({(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]methanone (I-86). To a solution of [[4-({(1*R*,3*R*,4*S*)-3-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]{5-chloro-4-[(*S*)-(3-chlorophenyl)(hydroxy)methyl]-2-thienyl}methanone and [[4-({(1*R*,3*R*,4*S*)-3-({[tert-butyl(dimethyl)silyl]oxy}methyl)-4-[(triisopropylsilyl)oxy]cyclopentyl}amino)pyrimidin-5-yl]{5-chloro-4-[(*R*)-(3-chlorophenyl)(hydroxy)methyl]-2-thienyl}methanone (122 mg, 0.16 mmol) in EtOH (1 mL) was added 1% HCl in EtOH solution (4.6 mL, 0.56 mmol) at 0 °C, and the reaction was stirred for 1 h at 0 °C then kept in the refrigerator for overnight. The reaction was quenched by addition of saturated $NaHCO_3$, and the mixture was concentrated in vacuo. To the residue was added water and extracted with EtOAc three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo to give **I-86** (100 mg, 96%). LCMS (FA): *m/z* = 652.1 (M+H).

Step 11: {(1*R*,2*S*,4*R*)-4-{[5-({5-Chloro-4-[(*S*)-(3-chlorophenyl)(hydroxy)methyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-[(triisopropylsilyl)oxy]cyclopentyl}methyl sulfamate and {(1*R*,2*S*,4*R*)-4-{[5-({5-Chloro-4-[(*R*)-(3-chlorophenyl)(hydroxy)methyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-[(triisopropylsilyl)oxy]cyclopentyl}methyl sulfamate (I-87). To a solution of the products from step 9 (114 mg, 0.18 mmol) in DMF (0.8 mL) was added chlorosulfonamide (40.5 mg, 0.35 mmol) at rt, and the mixture was stirred for 1 h. The reaction was quenched by addition of saturated $NaHCO_3$ and the mixture was extracted with EtOAc twice. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo to give **I-87** (120 mg, 94%). LCMS (FA): *m/z* = 731.2 (M+H).

Step 12: [(1*R*,2*S*,4*R*)-4-{[5-({5-Chloro-4-[(*S*)-(3-chlorophenyl)(hydroxy)methyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({5-Chloro-4-[(*R*)-(3-chlorophenyl)(hydroxy)methyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (40). To a solution of the products from step 10 (125 mg, 0.17 mmol) in CH_3CN (1 mL) was added H_3PO_4 (1 mL) at 0°C and the reaction was stirred at rt for 1 h. The reaction was quenched by addition of 1M Na_2CO_3 and the mixture was extracted with EtOAc three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by preparative HPLC to give 33 mg (39%) of the title compound as a mixture of diastereomers. The individual diastereomers were obtained using preparative scale chiral HPLC. More active epimer **40** is reported. ^{1}H NMR (MeOD) δ 8.64 (s, 1H), 8.55 (s, 1H), 7.51 (s, 1H),

7.40 (s, 1H), 7.29 (d, 2H, *J* = 6.7 Hz), 7.25 (dd, 1H, *J* = 5.7, 3.3 Hz), 5.88 (s, 1H), 4.80–4.74 (m, 1H), 4.19–4.09 (m, 3H), 2.50–2.42 (m, 1H), 2.26–2.19 (m, 1H), 2.14–2.06 (m, 1H), 1.88–1.82 (m, 1H), 1.43–1.34 (m, 1H). LCMS (FA): *m/z* = 573.1 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{22}H_{23}Cl2N_4O_6S_2$ 573.0436, obsd 573.0440.

Synthesis of Intermediate I-88

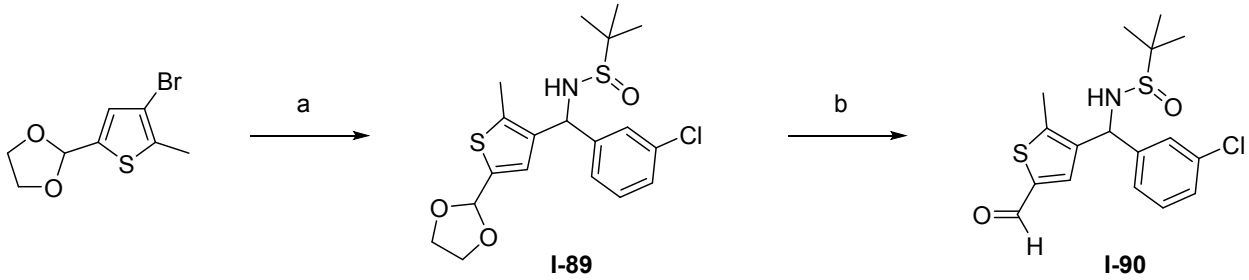


I-88

Reagents: (a) 2-methyl-2-propanesulfinamide, $CuSO_4$, DCM, rt, 15 h, 66%.

Step 1: *N*-[(*E*)-(3-Chlorophenyl)methylene]-2-methylpropane-2-sulfinamide (I-88). Copper(II) sulfate (2.9 g, 18.0 mmol) and 3-chlorobenzaldehyde (1.3 g, 9.1 mmol) were added to a solution of 2-methyl-2-propanesulfinamide (1.0 g, 8.2 mmol) in DCM (16 mL) at rt. The resulting suspension was allowed to stir for 15 h. The reaction mixture was filtered through a pad of celite and the filter cake was washed with DCM. The filtrate was concentrated and the crude mixture was purified on silica gel to afford **I-88** (1.32 g, 66%). ^{1}H NMR ($CDCl_3$) δ 8.54 (s, 1H), 7.86 (t, 1H, *J* = 1.7 Hz), 7.69 (dt, 1H, *J* = 7.5, 1.2 Hz), 7.49 (ddd, 1H, *J* = 8.0, 2.0, 1.2 Hz), 7.42 (t, 1H, *J* = 7.8 Hz), 1.27 (s, 9H); LCMS (FA): *m/z* = 244.3 (M+H).

Synthesis of Intermediate I-90



I-89 **I-90**

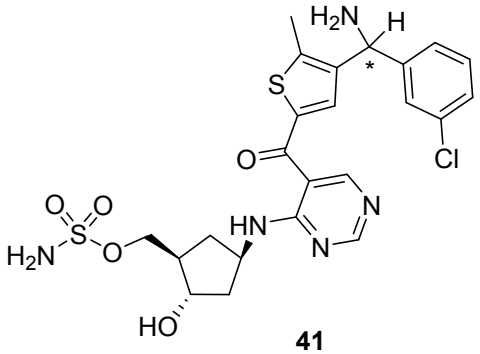
Reagents: (a) **I-88**, *n*-BuLi, THF, -78 °C, 0.5 h, 74%; (b) Dowex 50WX2, acetone, rt, 1 h, 96%.

Step 1: rac-*N*-{(3-Chlorophenyl)[5-(1,3-dioxolan-2-yl)-2-methyl-3-thienyl]methyl}-2-methylpropane-2-sulfinamide (I-89). A solution of 2-(4-bromo-5-methyl-2-thienyl)-1,3-dioxolane (3.1 g, 12.0 mmol) in THF (6 mL) was added dropwise to a solution of 2.50 M of *n*-BuLi in hexane (5.91 mL, 14.8 mmol) in THF (60 mL) at -78 °C. Immediately after addition was complete, *N*-[(*E*)-(3-chlorophenyl)methylene]-2-methylpropane-2-sulfinamide (**I-88**, 3.0 g, 12.0 mmol) was added dropwise as a solution in THF (6 mL). The resulting mixture was allowed to stir 30 min at -78 °C and then allowed to warm to near rt. The reaction was quenched with water and the resulting mixture was extracted with EtOAc twice. The combined organic layers were

dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel to afford **I-89** (3.8 g, 74%). ^{1}H NMR ($CDCl_3$) δ 7.39 and 7.33 (each s, each 0.5H), 7.29–7.20 (m, 3H), 6.95 and 6.89 (each s, each 0.5H), 5.96 and 5.93 (each s, each 0.5H), 5.61 (d, 0.5H, J = 1.8 Hz), 5.60 (d, 0.5H, J = 2.7 Hz), 4.13–4.05 (m, 2H), 4.03–3.92 (m, 2H), 3.64 (d, 0.5H, J = 2.5 Hz), 3.49 (s, 0.5H), 2.49 and 2.47 (each s, each 1.5H), 1.25 and 1.24 (each s, each 4.5H).

Step 2: rac-*N*-[(3-Chlorophenyl)(5-formyl-2-methyl-3-thienyl)methyl]-2-methylpropane-2-sulfinamide (I-90). Dowex 50WX2-200 (H) (3.80 g) was added to a solution of rac-*N*-{(3-chlorophenyl)[5-(1,3-dioxolan-2-yl)-2-methyl-3-thienyl]methyl}-2-methylpropane-2-sulfinamide (3.80 g, 9.20 mmol) in acetone (80 mL) at rt. The reaction was allowed to stir for 1 h. The reaction was filtered to remove solid resin and the crude material was purified on silica gel to provide **I-90** (3.26 g, 96%). ^{1}H NMR ($CDCl_3$) δ 9.79 and 9.77 (each s, each 0.5H), 7.65 and 7.57 (each s, each 0.5H), 7.42–7.39 (m, 0.5H), 7.37–7.34 (m, 0.5H), 7.34–7.25 (m, 3H), 5.67 and 5.66 (each s, each 0.5H), 3.75 (d, 0.5H, J = 2.2 Hz), 3.58 (d, 0.5H, J = 2.5 Hz), 2.59 and 2.58 (each s, each 1.5H), 1.29 and 1.29 (each s, each 4.5H).

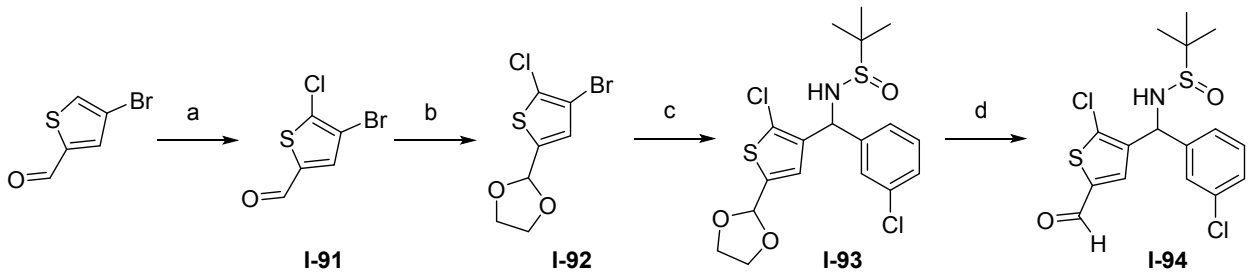
Synthesis of 41



41

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(*R*)-Amino(3-chlorophenyl)methyl]-5-methyl-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({4-[(*S*)-Amino(3-chlorophenyl)methyl]-5-methyl-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (41). Prepared by the method described for the preparation of **26** using 4-chloro-5-iodopyrimidine and intermediate **I-90** in Step 2. The following alternative conditions were employed in the reaction scheme: Step 3: oxidant used was MnO_2; Step 4: Base/solvent used was *N,N*-diisopropylethylamine/*i*-PrOH. The individual diastereomers were obtained using preparative scale chiral SFC. More active epimer **41** is reported. ^{1}H NMR (DMSO-d_6) δ 8.67 (s, 1H), 8.64 (s, 1H), 8.24 (d, 1H, J = 7.5 Hz), 7.75 (s, 1H), 7.49 (s, 1H), 7.43 (s, 2H), 7.36–7.20 (m, 3H), 5.13 (s, 1H), 4.89 (d, 1H, J = 4.5 Hz), 4.77–4.61 (m, 1H), 4.09 (dd, 1H, J = 9.8, 6.0 Hz), 4.01–3.88 (m, 2H), 2.45 (s, 3H), 2.38–2.25 (m, 1H), 2.16–2.04 (m, 1H), 2.03–1.85 (m, 1H), 1.84–1.71 (m, 1H), 1.34–1.18 (m, 1H). LCMS (FA): *m/z* = 552.2 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{23}H_{27}ClN_5O_5S_2$ 552.1142, obsd 552.1149.

Synthesis of Intermediate I-94



Reagents: (a) NCS, DMF, 50 °C, 12 h, 82%; (b) 1,2-ethanediol, *p*-TsOH, toluene, 110 °C, 12 h, 92%; (c) **I-88**, *n*-BuLi, THF, -78 °C, 15 min, 83%; (d) Dowex 50WX2, acetone, rt, 1 h, 93%.

Step 1: 4-Bromo-5-chlorothiophene-2-carbaldehyde (I-91). To a solution of 4-bromothiophene-2-carbaldehyde (20 g, 100 mmol) in DMF (49 mL, 630 mmol) was added NCS (21 g, 160 mmol), in portions. The reaction mixture was stirred at 50 °C overnight. The resulting solution was cooled to rt and then poured onto 500 mL of ice water (a light pink precipitate formed). The precipitate was collected via vacuum filtration and then dried in a vacuum oven to give **I-91** as a light tan solid (19.4 g, 82%). ^{1}H NMR ($CDCl_3$) δ 9.79 (s, 1H), 7.62 (s, 1H).

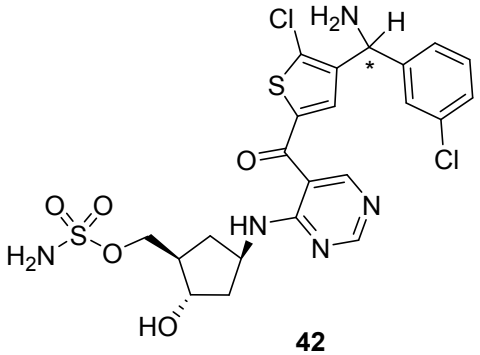
Step 2: 2-(4-Bromo-5-chloro-2-thienyl)-1,3-dioxolane (I-92). To a solution of 4-bromo-5-chlorothiophene-2-carbaldehyde (19.4 g, 85.8 mmol) in toluene (300 mL) was added 1,2-ethanediol (23.9 mL, 429 mmol) and *p*-toluenesulfonic acid monohydrate (0.82 g, 4.29 mmol). The reaction mixture was fitted with a Dean-Stark trap, stirred at reflux overnight under argon gas. The reaction was quenched with water (300mL), extracted with EtOAc (150mL) three times, washed with brine, dried over $MgSO_4$, filtered and concentrated to give ~28 g of crude product as a brown oil. The product was purified on silica gel to give **I-92** as an amber oil (21.3 g, 92%). ^{1}H NMR ($CDCl_3$) δ 6.99 (d, 1H, J = 0.5 Hz), 6.02 (s, 1H), 4.15–4.07 (m, 2H), 4.07–3.99 (m, 2H).

Step 3: rac-*N*-{[2-Chloro-5-(1,3-dioxolan-2-yl)-3-thienyl](3-chlorophenyl)methyl}-2-methylpropane-2-sulfinamide (I-93). A solution of 2-(4-bromo-5-chloro-2-thienyl)-1,3-dioxolane (900 mg, 3.30 mmol) in THF (30 mL) was cooled to -78 °C and 2.50 M of *n*-BuLi in hexane (1.66 mL, 4.15 mmol) was added dropwise. Immediately after a solution of *N*-[(*E*)-(3-chlorophenyl)methylene]-2-methylpropane-2-sulfinamide (**I-88**, 0.97 g, 3.97 mmol) in THF (4 mL) was added quickly to the reaction mixture. The resulting solution was allowed to stir for 15 min at that temperature. After warming to ~ 0 °C, the reaction was quenched by addition of water. The mixture was extracted with EtOAc three times and the combined organic extracts were dried over Na_2SO_4, filtered and concentrated. The crude product was purified on silica gel to afford **I-93** (1.2 g, 83%). ^{1}H NMR ($CDCl_3$) δ 7.41 (d, 1H, J = 17.9 Hz), 7.36–7.24 (m, 3H), 6.98 (d, 1H, J = 10.3 Hz), 5.97 (d, 1H, J = 6.8 Hz), 5.76 (dd, 1H, J = 7.5, 2.6 Hz), 4.15–4.07 (m, 2H), 4.06–3.96 (m, 2H), 3.69–3.61 (m, 1H), 1.33–1.22 (m, 9H).

Step 4: rac-*N*-[(2-Chloro-5-formyl-3-thienyl)(3-chlorophenyl)methyl]-2-methylpropane-2-sulfinamide (I-94). Dowex 50WX2-200 (H) (1 g) was added to a solution of *N*-{[2-chloro-5-(1,3-dioxolan-2-yl)-3-thienyl](3-chlorophenyl)methyl}-2-methylpropane-2-sulfinamide (0.90 g, 2.10 mmol) in acetone (20 mL) at rt. The reaction was allowed to stir for 1 h. The reaction was filtered to remove solid resin and the crude material was purified on silica gel to provide **I-94** (0.75 g, 93%). ^{1}H NMR ($CDCl_3$) δ 9.75 (d, 1H, J = 4.0 Hz), 7.63 (d, 1H, J = 16.4 Hz), 7.38 (d,

1H, *J* = 12.8 Hz), 7.34–7.27 (m, 3H), 5.78 (dd, 1H, *J* = 15.0, 3.1 Hz), 3.80–3.70 (m, 1H), 1.27 (s, 9H).

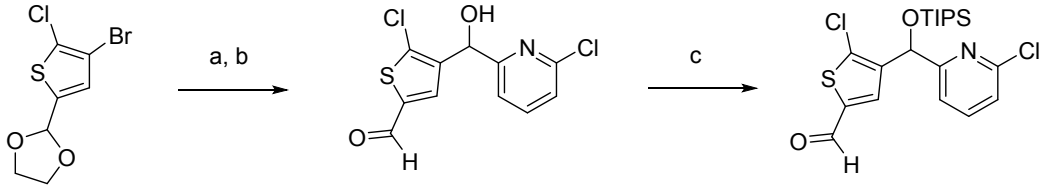
Synthesis of 42



42

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(*R*)-Amino(3-chlorophenyl)methyl]-5-chloro-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({4-[(*S*)-Amino(3-chlorophenyl)methyl]-5-chloro-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (42). Prepared by the method described for the preparation of **26** using 4-chloro-5-iodopyrimidine and intermediate **I-94** in Step 2. The following alternative conditions were employed in the reaction scheme: Step 3: oxidant used was MnO_2. The individual diastereomers were obtained using preparative scale chiral HPLC. More active epimer **42** is reported. ^{1}H NMR (DMSO-d_6) δ 8.72 (s, 1H), 8.68 (s, 1H), 8.22 (d, 1H, *J* = 7.5 Hz), 7.93 (s, 1H), 7.51 (s, 1H), 7.42 (s, 2H), 7.39–7.21 (m, 3H), 5.14 (s, 1H), 4.89 (d, 1H, *J* = 4.3 Hz), 4.79–4.63 (m, 1H), 4.10 (dd, 1H, *J* = 9.7, 6.0 Hz), 4.03–3.88 (m, 2H), 2.39–2.26 (m, 1H), 2.18–2.03 (m, 1H), 2.02–1.87 (m, 1H), 1.84–1.68 (m, 1H), 1.34–1.22 (m, 1H). LCMS (FA): *m/z* = 572.4 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{23}Cl_2N_6O_5S_2$ 572.0596, obsd 572.0599.

Synthesis of Intermediate I-96



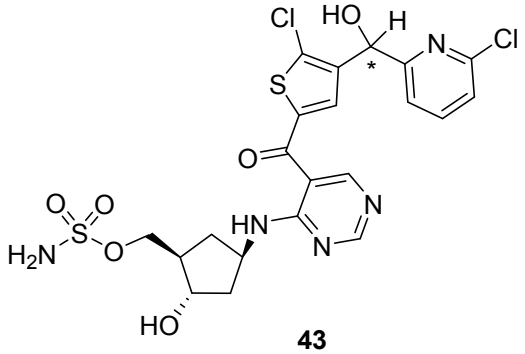
I-92 **I-95** **I-96**

Reagents: (a) 6-chloropyridine-2-carbaldehyde, *n*-BuLi, THF, -78 °C, 1 h; (b) 1% HCl in EtOH, rt, 2 h; 61% for 2 steps; (c) TIPSCl, NaH, THF, rt, 12 h, 90%.

Step 1: rac-5-Chloro-4-[(6-chloropyridin-2-yl)(hydroxy)methyl]thiophene-2-carbaldehyde (I-95). 2-(4-Bromo-5-chloro-2-thienyl)-1,3-dioxolane (**I-92**, 854 mg, 3.17 mmol) was placed in a 50mL 2-neck round bottom flask under an atmosphere of argon. THF (30 mL) was added and the reaction was cooled at -78 °C. 2.50 M of *n*-BuLi in hexane (1.58 mL, 3.96 mmol) was added dropwise and the solution was stirred for 30 min. 6-Chloropyridine-2-carbaldehyde (449 mg, 3.17 mmol) was added in one portion and the reaction was stirred for 30 min at -78 °C. The reaction was quenched with saturated NH_4Cl and the mixture was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified on silica gel to give the desired protected intermediate. This was then dissolved into a 1% HCl/EtOH solution (50 mL) and allowed to stir at rt for 2 h. The reaction was neutralized with 1N NaOH and extracted with DCM (30 mL) twice. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo to afford **I-95** (0.55g, 61%). ^{1}H NMR ($CDCl_3$) δ 9.73 (s, 1H), 7.68 (t, 1H, *J* = 7.8 Hz), 7.59 (s, 1H), 7.32 (d, 1H, *J* = 7.8 Hz), 7.17 (d, 1H, *J* = 7.5 Hz), 5.96 (s, 1H), 4.64 (brs, 1H).

Step 2: rac-5-Chloro-4-{(6-chloropyridin-2-yl)[(triisopropylsilyl)oxy]methyl}thiophene-2-carbaldehyde (I-96). rac-5-Chloro-4-[(6-chloropyridin-2-yl)(hydroxy)methyl]thiophene-2-carbaldehyde (0.56 g, 1.95 mmol) was dissolved in THF (20.0 mL), then 60% NaH in mineral oil (0.14 g, 5.85 mmol) was added to this solution. The solution was stirred at 40 °C for 30 min. TIPSCl (0.62 mL, 2.93 mmol) was added and the reaction mixture stirred at rt overnight. The solution was poured into 30 mL saturated NH_4Cl solution. The solution was extracted with EtOAc (30 mL) twice. The combined organic layers were dried over Na_2SO_4, filtered, concentrated in vacuo. The residue was purified on silica gel to give **I-96** (780 mg, 90%). ^{1}H NMR ($CDCl_3$) δ 9.73 (s, 1H), 7.71–7.65 (m, 2H), 7.60 (d, 1H, *J* = 7.6 Hz), 7.22–7.17 (m, 1H), 6.03 (s, 1H), 1.20–1.08 (m, 3H), 1.02–0.96 (m, 18H).

Synthesis of 43

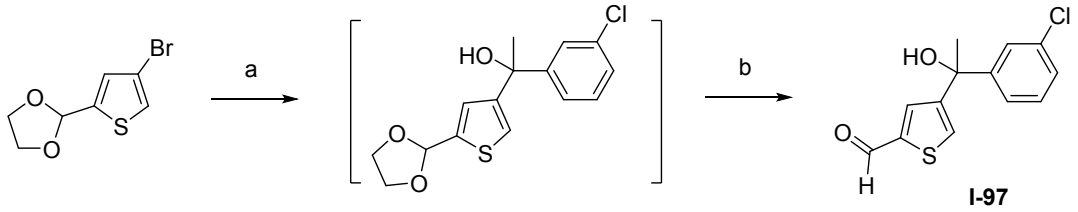


43

[(1*R*,2*S*,4*R*)-4-{[5-({5-Chloro-4-[(*S*)-(6-chloropyridin-2-yl)(hydroxy)methyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({5-Chloro-4-[(*R*)-(6-chloropyridin-2-yl)(hydroxy)methyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (43). Prepared by the method described for the preparation of **26** using 4-chloro-5-iodopyrimidine and intermediate **I-96** in Step 2. The following alternative conditions were employed in the reaction

scheme: Step 3: oxidant used was MnO_2; Step 4: Base/solvent used was *N,N*-diisopropylethylamine/*i*-PrOH; Step 7: desilylating agent/solvent used was TFA/water. The individual diastereomers were obtained using preparative scale chiral SFC. More active epimer **43** is reported. ^{1}H NMR (MeOD) δ 8.72 (s, 1H), 8.64 (s, 1H), 7.84 (t, 1H, *J* = 7.8 Hz), 7.62 (dd, 2H, *J* = 8.0, 3.6 Hz), 7.35 (d, 1H, *J* = 7.9 Hz), 5.93 (s, 1H), 4.86–4.70 (m, 1H), 4.28–4.10 (m, 3H), 2.57–2.41 (m, 1H), 2.21 (dd, 2H, *J* = 46.9, 5.5 Hz), 1.92 (dt, 1H, *J* = 15.2, 7.0 Hz), 1.53–1.35 (m, 1H). LCMS (FA): *m/z* = 574.0 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{21}H_{22}Cl_2N_5O_6S_2$ 574.0389, obsd 574.0389.

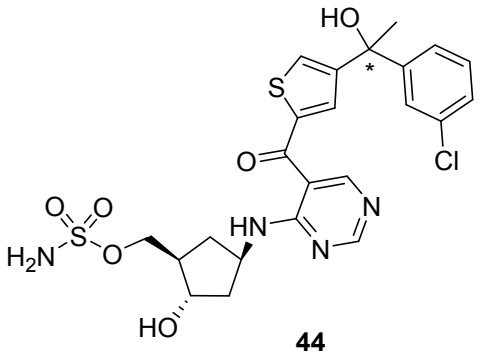
Synthesis of Intermediate I-97



Reagents: (a) 3-chloroacetophenone, *n*-BuLi, THF, -78 °C, 10 min; (b) Dowex 50WX2, acetone, rt, 2 h, 86% for 2 steps.

Step 1: rac-4-[1-(3-Chlorophenyl)-1-hydroxyethyl]thiophene-2-carbaldehyde (I-97). To a round bottom flask was added THF (100 mL) and 2.5 M *n*-BuLi in hexane (7.0 mL, 17.5 mmol) at -78 °C. 2-(4-Bromothiophen-2-yl)-1,3-dioxolane (3.16 g, 13.4 mmol) in 5 mL THF was added and the mixture was stirred for 30 seconds. To the mixture was added 3-chloroacetophenone (1.82 mL, 14.1 mmol) and the reaction was stirred at -78 °C for 10 min. The reaction was quenched by addition of saturated NH_4Cl and the reaction was warmed to rt. The mixture was extracted with EtOAc twice and the combined organic layers were washed with brine, dried under $MgSO_4$, filtered, and concentrated in vacuo. To the residue was added 40 mL of acetone and 3 g of Dowex 50WX2-200 ion-exchange resin and the mixture was stirred for 2 h at rt. The mixture was filtered and the filtrate was concentrated in vacuo. The residue was purified on silica gel to provide **I-97** (3.09 g, 86%). ^{1}H NMR ($CDCl_3$) δ 9.80 (d, 1H, *J* = 1.2 Hz), 7.62 (dt, 2H, *J* = 2.7, 1.5 Hz), 7.47–7.44 (m, 1H), 7.30–7.23 (m, 3H), 2.68–2.30 (br s, 1H), 1.94 (s, 3H).

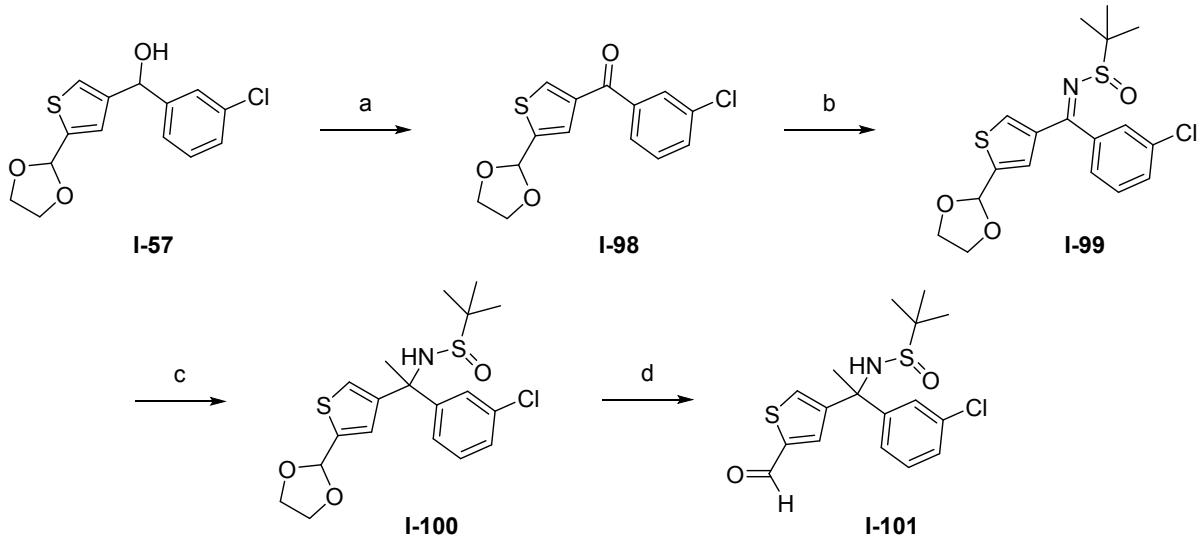
Synthesis of 44



44

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*R*)-1-(3-Chlorophenyl)-1-hydroxyethyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*S*)-1-(3-Chlorophenyl)-1-hydroxyethyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (44). Prepared by the method described for the preparation of **26** using 4-chloro-5-iodopyrimidine and intermediate **I-97** in Step 2. The following alternative conditions were employed in the reaction scheme: Step 3: oxidant used was MnO_2; Step 4: Base/solvent used was *N,N*-diisopropylethylamine/*i*-PrOH; Step 7: desilylating agent/solvent used was TBAF/THF. The individual diastereomers were obtained using preparative scale chiral HPLC. More active epimer **44** is reported. ^{1}H NMR (MeOD) δ 8.65 (s, 1H), 8.55 (s, 1H), 7.80 (s, 1H), 7.59 (s, 1H), 7.52 (s, 1H), 7.36 (d, 1H, *J* = 7.8 Hz), 7.31 (t, 1H, *J* = 7.7 Hz), 7.25 (d, 1H, *J*=7.7 Hz), 4.84–4.73 (m, 1H), 4.19 (tt, 3H, *J* = 15.8, 7.8 Hz), 2.55–2.42 (m, 1H), 2.27 (td, 1H, *J* = 14.3, 5.7 Hz), 2.21–2.11 (m, 1H), 1.98–1.87 (m, 4H), 1.46 (ddd, 1H, *J* = 14.5, 9.2, 4.5 Hz). LCMS (FA): *m/z* = 553.1 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{23}H_{26}ClN_4O_6S_2$ 553.0982, obsd 553.0994.

Synthesis of Intermediate I-101



Reagents: (a) MnO_2, DCM, rt, 14 h, 95%; (b) 2-methyl-2-propanesulfinamide, $Ti(OEt)_4$, THF, 80 °C, 5 h, 91%; (c) MeLi, THF, -78 °C, 40 min, 96%; (d) Dowex 50WX2, acetone, rt, 1 h, 96%.

Step 1: (3-Chlorophenyl)[5-(1,3-dioxolan-2-yl)-3-thienyl]methanone (I-98). To a solution of **I-57** (1.8 g, 6.10 mmol) in DCM (54.5 mL) was added MnO_2 (5.80 g, 66.7 mmol). The mixture was stirred at rt for 14 h. The reaction was then filtered through a celite pad and the residual solid was washed with DCM several times. The filtrate was concentrated in vacuo to yield **I-98** (1.7 g, 95%). ^{1}H NMR ($CDCl_3$) δ 7.93 (d, 1H, *J* = 1.4 Hz), 7.80 (t, 1H, *J* = 1.8 Hz), 7.70 (dt, 1H, *J* = 7.7, 1.3 Hz), 7.64–7.61 (m, 1H), 7.56 (ddd, 1H, *J* = 8.0, 2.1, 1.1 Hz), 7.43 (t, 1H, *J* = 7.8 Hz), 6.11 (s, 1H), 4.20–4.11 (m, 2H), 4.10–4.01 (m, 2H).

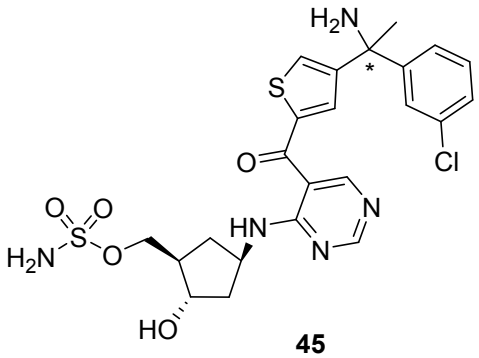
Step 2: *N*-{(*E*)-(3-Chlorophenyl)[5-(1,3-dioxolan-2-yl)-3-thienyl]methylene}-2-methylpropane-2-sulfinamide (I-99). (3-Chlorophenyl)[5-(1,3-dioxolan-2-yl)-3-

thienyl]methanone (1.70 g, 5.77 mmol) was added to a solution of $Ti(OEt)_4$ (5.30 g, 23.2 mmol) in THF (23 mL) under an atmosphere of argon. 2-methyl-2-propanesulfinamide (1.10 g, 9.08 mmol) was then added and the solution heated at 80 °C for 3 h. Additional 2-methyl-2-propanesulfinamide (0.46 g, 3.80 mmol) was added and the solution stirred at 80 °C for an additional 5 h. The reaction was allowed to cool to rt, and the mixture was poured into an equal volume of brine while rapidly stirring. The resulting suspension was filtered through a medium frit filter, and the filter cake was washed with EtOAc. The filtrate was transferred to a separatory funnel where the organic layer was washed with brine. The combined aqueous layers were extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified on silica gel to afford **I-99** as a white solid (2.1 g, 91%). ^{1}H NMR ($CDCl_3$) δ 7.63 (s, 1H), 7.55–7.28 (m, 5H), 6.09 (s, 1H), 4.20–4.09 (m, 2H), 4.09–4.00 (m, 2H), 1.28 (s, 9H).

Step 3: rac-*N*-{1-(3-Chlorophenyl)-1-[5-(1,3-dioxolan-2-yl)-3-thienyl]ethyl}-2-methylpropane-2-sulfinamide (I-100). 1.6 M of MeLi in ether (4.12 mL, 6.60 mmol) was added dropwise to a solution of *N*-{(*E*)-(3-chlorophenyl)[5-(1,3-dioxolan-2-yl)-3-thienyl]methylene}-2-methylpropane-2-sulfinamide (2.10 g, 5.28 mmol) in THF (20 mL) at -78 °C and the resulting solution allowed to stir for 40 min. After the reaction was warmed to 0 °C, the mixture was diluted with ether and quenched with water. The mixture was extracted with EtOAc three times and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to yield 2.1 g (96%) of **I-100**. ^{1}H NMR ($CDCl_3$) δ 7.40–7.37 (m, 1H), 7.28–7.22 (m, 3H), 7.20 (d, 1H, *J* = 1.6 Hz), 6.95 (d, 1H, *J* = 1.2 Hz), 6.00 (s, 1H), 4.13–4.08 (m, 2H), 4.02–3.96 (m, 2H), 3.74 (s, 1H), 2.06 (s, 3H), 1.23 (s, 9H).

Step 4: rac-*N*-[1-(3-Chlorophenyl)-1-(5-formyl-3-thienyl)ethyl]-2-methylpropane-2-sulfinamide (I-101). Dowex 50WX2-200 (H) (1.0 g) was added to a solution of rac-*N*-{1-(3-chlorophenyl)-1-[5-(1,3-dioxolan-2-yl)-3-thienyl]ethyl}-2-methylpropane-2-sulfinamide (2.10 g, 5.07 mmol) in acetone (39 mL) at rt. The reaction was allowed to stir for 1 h. The reaction was filtered to remove solid resin and the filtrate was concentrated in vacuo. The crude material was purified on silica gel to yield **I-101** (1.8 g, 96%). ^{1}H NMR ($CDCl_3$) δ 9.87 (d, 1H, *J* = 1.2 Hz), 7.72 (t, 1H, *J* = 1.4 Hz), 7.62 (d, 1H, *J* = 1.6 Hz), 7.37–7.35 (m, 1H), 7.34–7.29 (m, 2H), 7.28–7.23 (m, 1H), 3.81 (s, 1H), 2.10 (s, 3H), 1.28 (s, 9H).

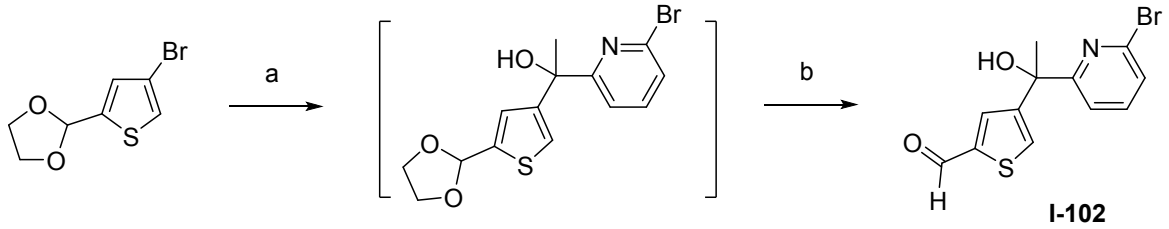
Synthesis of 45



45

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*S*)-1-Amino-1-(3-chlorophenyl)ethyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*R*)-1-Amino-1-(3-chlorophenyl)ethyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (45). Prepared by the method described for the preparation of **26** using 4-chloro-5-iodopyrimidine and intermediate **I-101** in Step 2. The following alternative conditions were employed in the reaction scheme: Step 3: oxidant used was MnO_2. The individual diastereomers were obtained using preparative scale chiral HPLC. More active epimer **45** is reported. ^{1}H NMR (DMSO-d_6) δ 8.63 (s, 1H), 8.63 (s, 1H), 8.28 (d, 1H, J = 7.5 Hz), 7.88 (s, 1H), 7.73 (s, 1H), 7.55 (s, 1H), 7.39–7.15 (m, 3H), 4.78–4.61 (m, 1H), 4.17–4.02 (m, 1H), 4.02–3.85 (m, 2H), 2.63 (s, 2H), 2.37–2.22 (m, 1H), 2.16–2.08 (m, 1H), 2.00–1.84 (m, 1H), 1.81–1.66 (m, 4H), 1.35–1.17 (m, 1H). LCMS (FA): *m/z* = 552.4 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{23}H_{27}ClN_5O_5S_2$ 552.1142, obsd 552.1148.

Synthesis of Intermediate I-102

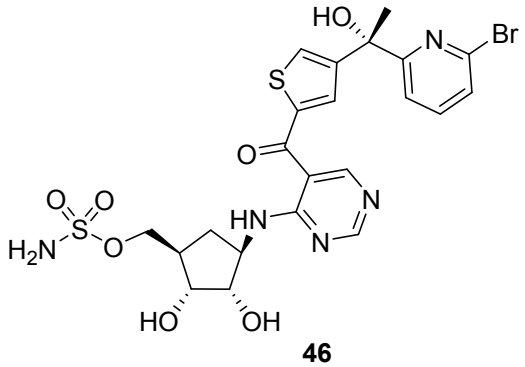


I-102

Reagents: (a) 2-acetyl-6-bromopyridine, *n*-BuLi, THF, -78 °C, 10 min; (b) Dowex 50WX2, acetone, rt, 2 h, 66% for 2 steps.

Step 1: rac-4-[1-(6-Bromopyridin-2-yl)-1-hydroxyethyl]thiophene-2-carbaldehyde (I-102). Prepared by the method described for the preparation of intermediate **I-97** using 2-(4-bromothiophen-2-yl)-1,3-dioxolane and 2-acetyl-6-bromopyridine in Step 1. ^{1}H NMR ($CDCl_3$) δ 9.85 (d, 1H, J = 1.2 Hz), 7.73 (d, 1H, J = 1.5 Hz), 7.69 (t, 1H, J = 1.4 Hz), 7.56 (t, 1H, J = 7.8 Hz), 7.43 (dd, 1H, J = 7.8, 0.7 Hz), 7.28 (dd, 1H, J = 7.7, 0.7 Hz), 2.05–1.95 (br s, 1H), 1.92 (s, 3H).

Synthesis of 46

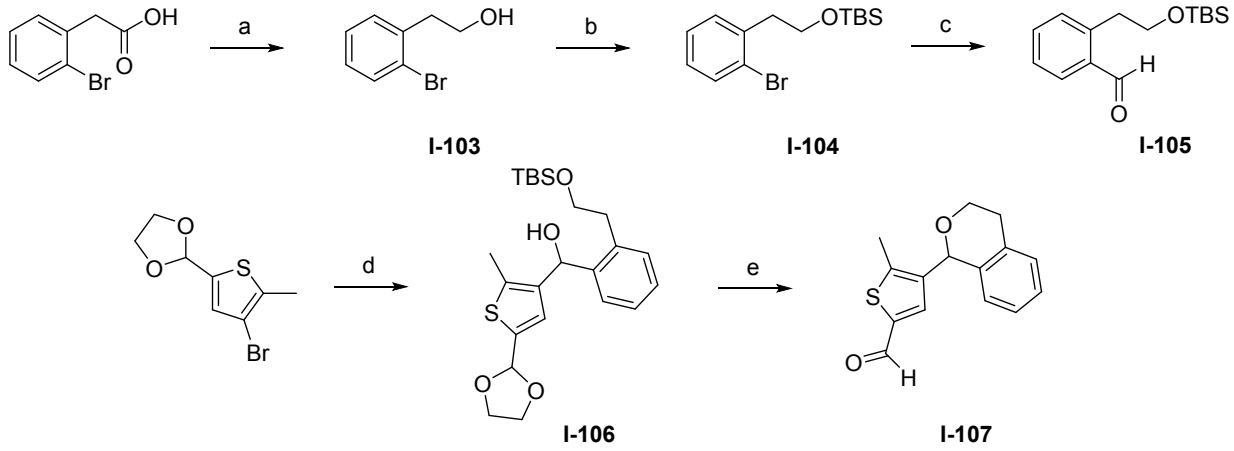


46

[(1*R*,2*R*,3*S*,4*R*)-4-{[5-({4-[(1*S*)-1-(6-Bromopyridin-2-yl)-1-hydroxyethyl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2,3-dihydroxycyclopentyl]methyl sulfamate (46). Prepared by the method described for the preparation of **1** using 4-chloro-5-iodopyrimidine and intermediate **I-102** in Step 2 of the synthesis of intermediate **I-3**. The individual diastereomers were obtained using preparative scale chiral SFC. More active epimer **46** is reported. ^{1}H NMR (MeOD) δ 8.75 (s, 1H), 8.61 (s, 1H), 7.86 (s, 1H), 7.74 (s, 2H), 7.67 (t, 1H, *J* = 7.7 Hz), 7.44 (dd, 1H, *J* = 7.7, 1.0 Hz), 4.59 (dd, 1H, *J* = 14.6, 8.4 Hz), 4.25–4.14 (m, 2H), 3.98–3.88 (m, 2H), 2.56–2.44 (m, 1H), 2.38 (d, 1H, *J* = 4.9 Hz), 1.97 (s, 3H), 1.36 (dt, 1H, *J* = 13.1, 8.7 Hz). LCMS (AA): *m/z* = 614.0 (M+H).

Procedures for the syntheses of compounds in Table 7

Synthesis of Intermediate I-107



Reagents: (a) 1M BH_3 in THF, THF, rt to 60 °C, 12 h; (b) TBSCl, imidazole, DCM, rt, 2 h, 87% for 2 steps; (c) *n*-BuLi, DMF, THF, -78 °C, 10 min, 99%; (d) *n*-BuLi, **I-105**, THF, -78 °C, 10 min, 86%; (e) TFA, rt, 12 h, 89%.

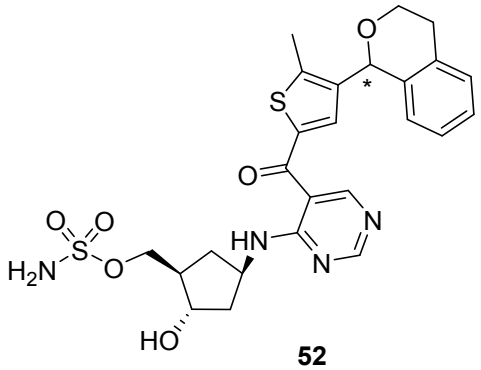
Step 1 and 2: (2-Bromophenethoxy)(*tert*-butyl)dimethylsilane (I-104). To a solution of 2-(2-bromophenyl)acetic acid (21.5 g, 100 mmol) in THF (400 mL) was added slowly 1.0 M of borane in THF (120 mL, 120 mmol) at rt. When bubbling ceased the resulting reaction mixture was heated at 60 °C overnight. Reaction was quenched via slow careful addition of 1.0 M of HCl in water (300 mL). THF was removed in vacuo and the resulting residue was partitioned between Et_2O and water. Layers were separated, and the aqueous layer was extracted with Et_2O twice. The combined organic solvents were dried over $MgSO_4$, filtered and concentrated in vacuo. To a solution of the crude alcohol produced above (**I-103**, 12.3 g, 61.2 mmol) in DCM (154 mL) was added 1*H*-imidazole (8.3 g, 120 mmol) followed by TBSCl (10.1 g, 67.3 mmol) at rt, and the reaction was stirred for 2 h. The reaction was quenched by addition of water (250 mL) and extracted with DCM (200 mL) three times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified on silica gel to provide **I-104** (16.9 g, 87%). ^{1}H NMR ($CDCl_3$) δ 7.54–7.52 (m, 1H), 7.28–7.21 (m, 2H), 7.11–7.05 (m, 1H), 3.84 (t, 2H, *J* = 7.0 Hz), 2.99 (t, 2H, *J* = 7.0 Hz), 0.88 (s, 9H), -0.01 (s, 6H).

Step 3: 2-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)benzaldehyde (I-105). A solution of (2-bromophenethoxy)(*tert*-butyl)dimethylsilane (2.53 g, 8.03 mmol) in THF (36 mL) was cooled to -78 °C, at which point was added 2.50 M of *n*-BuLi in hexane (4.49 mL, 11.2 mmol). After stirring for 5 min, DMF (0.93 mL, 12.0 mmol) was added, and the reaction mixture was stirred at -78 °C for 10 min. The reaction was quenched by adding saturated aq. NH_4Cl (10 mL) and then was warmed to rt. Reaction mixture was further diluted with water (20 mL) and THF was removed in vacuo. Aqueous residue was diluted with Et_2O (30 mL), the layers were separated, and the aqueous layer was extracted Et_2O (30 mL) twice. Combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude residue was purified on silica gel to afford **I-105** (2.11 g, 99%). ^{1}H NMR ($CDCl_3$) δ 10.38 (s, 1H), 7.94 (dd, 1H, *J* = 7.7, 1.2 Hz), 7.63–7.56 (m, 1H), 7.50–7.44 (m, 1H), 7.40 (d, 1H, *J* = 7.6 Hz), 3.94 (t, 2H *J* = 6.4 Hz), 3.34 (t, 2H, *J* = 6.4 Hz), 0.91 (s, 9H), 0.00 (s, 6H).

Step 4: [2-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)phenyl][5-(1,3-dioxolan-2-yl)- 2-methyl-3-thienyl]methanol (I-106). A solution of 2-(4-bromo-5-methyl-2-thienyl)-1,3-dioxolane (1.70 g, 6.82 mmol) in THF (26.6 mL) was cooled to -78 °C, and then 2.50 M of *n*-BuLi in hexane (2.94 mL, 7.35 mmol) was added and the mixture was stirred for 10 min at -78 °C. A solution of 2-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)benzaldehyde (**I-105**, 1.39 g, 5.25 mmol) in THF (13.3 mL) was then added, and the reaction was stirred for 10 min at -78 °C. The reaction was quenched by adding brine and then warmed to rt. The aqueous mixture was extracted with EtOAc twice. The combined organic solvents were washed with brine, dried and concentrated in vacuo. The residue was purified on silica gel to provide **I-106** (1.96 g, 86%). ^{1}H NMR ($CDCl_3$) δ 7.30–7.26 (m, 2H), 7.26–7.21 (m, 2H), 7.07 (s, 1H), 6.09 (d, 1H, *J* = 2.7 Hz), 6.03 (s, 1H), 4.19–4.13 (m, 2H), 4.06–4.00 (m, 2H), 3.96–3.89 (m, 1H), 3.87–3.77 (m, 1H), 3.52 (d, 1H, *J* = 2.9 Hz), 3.06 (ddd, 1H, *J* = 14.3, 8.4, 6.2 Hz), 2.87 (dt, 1H, *J* = 13.9, 5.2 Hz), 2.37 (s, 3H), 0.86 (s, 9H), -0.00 (s, 3H), -0.01 (s, 3H).

Step 5: 4-(3,4-Dihydro-1*H*-isochromen-1-yl)-5-methylthiophene-2-carbaldehyde (I-107). A 100 mL round bottom flask was charged with [2-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)phenyl][5-(1,3-dioxolan-2-yl)-2-methyl-3-thienyl]methanol (1.96 g, 4.51 mmol) and TFA (6.60 mL, 85.7 mmol) at rt. The resulting purple solution was stirred at rt overnight. The reaction mixture was carefully poured into saturated aqueous $NaHCO_3$ (50 mL). The layers were separated, and the aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with brine, then dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified on silica gel to yield **I-107** (1.03 g, 89%). ^{1}H NMR ($CDCl_3$) δ 9.73 (s, 1H), 7.41 (s, 1H), 7.27–7.19 (m, 2H), 7.18–7.12 (m, 1H), 6.75 (d, 1H, *J* = 7.7 Hz), 5.86 (s, 1H), 4.21 (ddd, 1H, *J* = 11.3, 5.5, 3.7 Hz), 3.97 (ddd, 1H, *J* = 11.4, 9.7, 4.0 Hz), 3.16 (ddd, 1H, *J* = 15.9, 9.4, 5.4 Hz), 2.85 (dt, 1H, *J* = 16.6, 3.6 Hz), 2.59 (s, 3H).

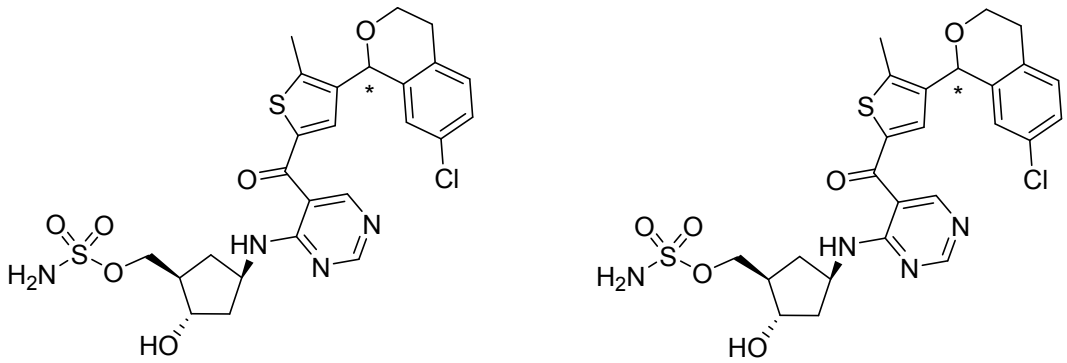
Synthesis of 52



52

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*R*)-3,4-Dihydro-1*H*-isochromen-1-yl]-5-methyl-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*S*)-3,4-Dihydro-1*H*-isochromen-1-yl]-5-methyl-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (52).
Prepared by the method described for the preparation of **23** using 4-chloro-5-iodopyrimidine and intermediate **I-107** in Step 3. The individual diastereomers were obtained using preparative scale chiral SFC. More active epimer **52** is reported. ^{1}H NMR (400 MHz, MeOD) δ 8.59 (s, 1H), 8.53 (s, 1H), 7.24 (s, 1H), 7.22–7.15 (m, 2H), 7.16–7.07 (m, 1H), 6.76 (d, 1H, *J* = 7.6 Hz), 5.93 (s, 1H), 4.83–4.70 (m, 1H), 4.24–4.10 (m, 4H), 4.01–3.89 (m, 1H), 3.16–3.03 (m, 1H), 2.87–2.76 (m, 1H), 2.54 (s, 3H), 2.52–2.43 (m, 1H), 2.31–2.19 (m, 1H), 2.13 (ddd, 1H, *J* = 12.7, 7.4, 4.2 Hz), 1.88 (dt, 1H, *J* = 13.6, 7.2 Hz), 1.40 (dt, 1H, *J* = 13.1, 9.1 Hz); LCMS (FA): *m/z* = 545.2 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{25}H_{28}N_4O_6S_2$ 545.1529, obsd 545.1550.

Synthesis of ML-93 and 47



ML-93 and 47

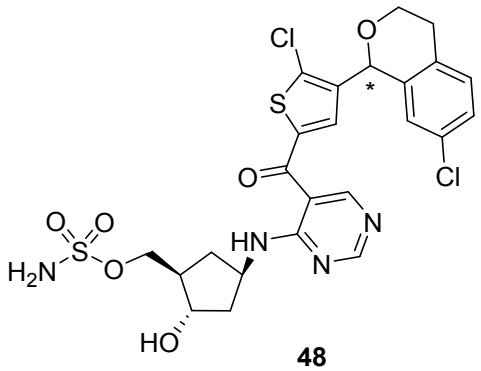
[(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*R*)-7-Chloro-3,4-dihydro-1*H*-isochromen-1-yl]-5-methyl-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate and [(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*S*)-7-Chloro-3,4-dihydro-1*H*-isochromen-1-yl]-5-methyl-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (ML-93 and 47).
Prepared by the method described for the preparation of **23**. The following alternative conditions were employed in the reaction scheme: Step 3: The required aldehyde used was prepared

following the procedure outlined for the preparation of intermediate **I-107** starting with 2-(2-bromo-4-chlorophenyl)acetic acid in Step 1; Step 5: Base/solvent used was triethylamine/DMF. The individual diastereomers were obtained using preparative scale chiral SFC.

ML-93: ^{1}H NMR (DMSO-d_6) δ 8.60 (d, 2H, J = 9.1 Hz), 8.19 (d, 1H, J = 7.5 Hz), 7.44 (s, 2H), 7.36 (s, 1H), 7.29–7.22 (m, 2H), 6.75 (s, 1H), 5.91 (s, 1H), 4.88 (d, 1H, J = 4.6 Hz), 4.75–4.63 (m, 1H), 4.16–4.06 (m, 2H), 4.00–3.92 (m, 2H), 3.88–3.79 (m, 1H), 3.08–2.97 (m, 1H), 2.81–2.73 (m, 1H), 2.48 (s, 3H), 2.37–2.26 (m, 1H), 2.16–2.06 (m, 1H), 1.98–1.89 (m, 1H), 1.80–1.70 (m, 1H), 1.32–1.22 (m, 1H); LCMS (FA): *m/z* = 579.2 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{25}H_{28}ClN_4O_6S_2$ 579.1139, obsd 579.1166.

47: ^{1}H NMR (MeOD) δ 8.62 (s, 1H), 8.53 (s, 1H), 7.29 (s, 1H), 7.24–7.14 (m, 2H), 6.77–6.69 (m, 1H), 5.88 (s, 1H), 4.83–4.70 (m, 1H), 4.25–4.09 (m, 4H), 3.98–3.85 (m, 1H), 3.15–2.98 (m, 1H), 2.86–2.71 (m, 1H), 2.56–2.42 (m, 4H), 2.31–2.19 (m, 1H), 2.19–2.08 (m, 1H), 1.95–1.83 (m, 1H), 1.46–1.33 (m, 1H); LCMS (FA): *m/z* = 579.1 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{25}H_{28}ClN_4O_6S_2$ 579.1139, obsd 579.1168.

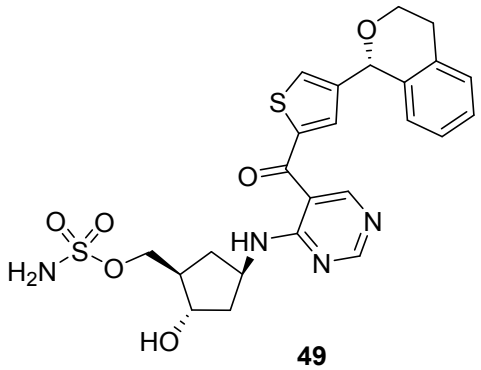
Synthesis of 48



48

[(1*R*,2*S*,4*R*)-4-{[5-({5-Chloro-4-[(1*R*)-7-chloro-3,4-dihydro-1*H*-isochromen-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({5-Chloro-4-[(1*S*)-7-chloro-3,4-dihydro-1*H*-isochromen-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (48). Prepared by the method described for the preparation of **23**. The following alternative conditions were employed in the reaction scheme: Step 3: The required aldehyde used was prepared following the procedure outlined for the preparation of intermediate **I-107** starting with 2-(2-bromo-4-chlorophenyl)acetic acid in Step 1 and using intermediate **I-92** in Step 3; Step 6: Desilylating agent/solvent used was H_3PO_4/CH_3CN. The individual diastereomers were obtained using preparative scale chiral SFC. More active epimer **48** is reported. ^{1}H NMR (MeOD) δ 8.61 (s, 1H), 8.55 (s, 1H), 7.30 (s, 1H), 7.21 (d, 2H J = 1.5 Hz), 6.77 (s, 1H), 5.95 (s, 1H), 4.83–4.73 (m, 1H), 4.27–4.13 (m, 4H), 3.98–3.88 (m, 1H), 3.15–3.06 (m, 1H), 2.83–2.74 (m, 1H), 2.53–2.43 (m, 1H), 2.30–2.21 (m, 1H), 2.17–2.07 (m, 1H), 1.92–1.83 (m, 1H), 1.47–1.36 (m, 1H); LCMS (FA): *m/z* = 599.2 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{24}H_{25}Cl_2N_4O_6S_2$ 599.0593, obsd 599.0596.

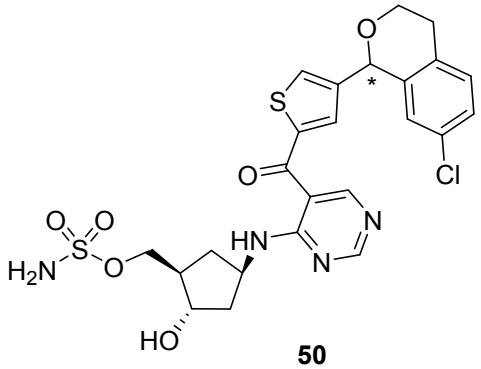
Synthesis of 49



49

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*R*)-3,4-Dihydro-1*H*-isochromen-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (49). Prepared by the method described for the preparation of **23**. The following alternative conditions were employed in the reaction scheme: Step 3: The required aldehyde used was prepared following the procedure outlined for the preparation of intermediate **I-107** starting with 2-(4-bromothiophen-2-yl)-1,3-dioxolane in Step 3; Step 5: Base/solvent used was K_2CO_3/DMF. The individual diastereomers were obtained using preparative scale chiral SFC. More active epimer **49** is reported. ^{1}H NMR (MeOD) δ 8.70 (s, 1H), 8.58 (s, 1H), 7.82–7.74 (m, 1H), 7.59–7.52 (m, 1H), 7.26–7.12 (m, 3H), 6.96–6.87 (m, 1H), 5.92 (s, 1H), 4.85–4.75 (m, 1H), 4.28–4.07 (m, 4H), 4.00–3.89 (m, 1H), 3.10–2.97 (m, 1H), 2.95–2.82 (m, 1H), 2.60–2.47 (m, 1H), 2.34–2.22 (m, 1H), 2.22–2.11 (m, 1H), 1.98–1.84 (m, 1H), 1.51–1.37 (m, 1H); LCMS (FA): *m/z* = 531.6 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{24}H_{27}N_4O_6S_2$ 531.1372, obsd 531.1396.

Synthesis of 50

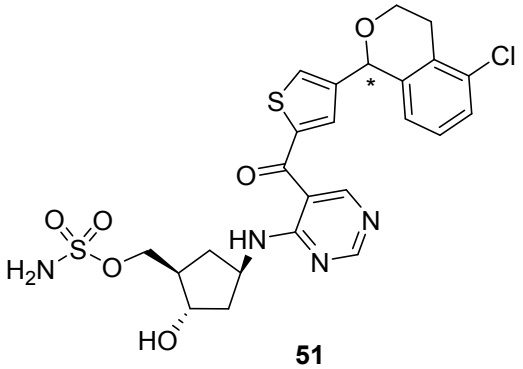


50

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*R*)-7-Chloro-3,4-dihydro-1*H*-isochromen-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*S*)-7-Chloro-3,4-dihydro-1*H*-isochromen-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (50). Prepared by the method described for the preparation of **23**. The following alternative conditions were employed in the reaction scheme: Step 3: The required aldehyde used was prepared

following the procedure outlined for the preparation of intermediate **I-107** starting with 2-(2-bromo-4-chlorophenyl)acetic acid in Step 1 and using 2-(4-bromothiophen-2-yl)-1,3-dioxolane in Step 3; Step 5: Base/solvent used was triethylamine/DMF. The individual diastereomers were obtained using preparative scale chiral SFC. More active epimer **50** is reported. ^{1}H NMR (DMSO-d_6) δ 8.65 (s, 1H), 8.64 (s, 1H), 8.26 (d, 1H, J = 7.5 Hz), 7.95 (s, 1H), 7.64 (d, 1H, J = 1.2 Hz), 7.44 (s, 2H), 7.30–7.22 (m, 2H), 6.91 (s, 1H), 5.91 (s, 1H), 4.89 (d, 1H, J = 4.5 Hz), 4.77–4.65 (m, 1H), 4.13–4.01 (m, 2H), 4.01–3.91 (m, 2H), 3.87–3.78 (m, 1H), 3.01–2.91 (m, 1H), 2.83–2.74 (m, 1H), 2.37–2.28 (m, 1H), 2.18–2.09 (m, 1H), 1.99–1.91 (m, 1H), 1.81–1.72 (m, 1H), 1.34–1.23 (m, 1H); LCMS (FA): *m/z* = 565.1 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{24}H_{26}ClN_4O_6S_2$ 565.0982, obsd 565.0995.

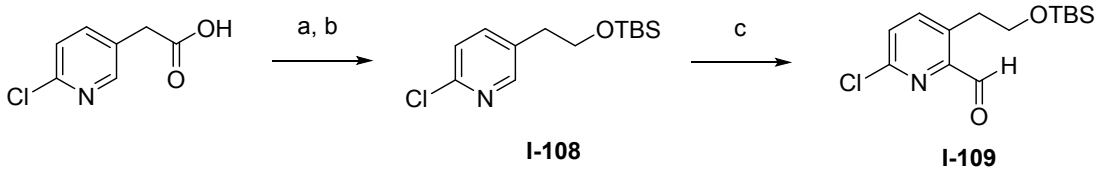
Synthesis of 51



51

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*R*)-5-Chloro-3,4-dihydro-1*H*-isochromen-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*S*)-5-Chloro-3,4-dihydro-1*H*-isochromen-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (51). Prepared by the method described for the preparation of **23**. The following alternative conditions were employed in the reaction scheme: Step 3: The required aldehyde used was prepared following the procedure outlined for the preparation of intermediate **I-107** starting with 2-(2-bromo-6-chlorophenyl)acetic acid in Step 1 and using 2-(4-bromothiophen-2-yl)-1,3-dioxolane in Step 3; Step 5: Base/solvent used was K_2CO_3/DMF. The individual diastereomers were obtained using preparative scale chiral HPLC. More active epimer **51** is reported. ^{1}H NMR (MeOD) δ 8.70 (s, 1H), 8.59 (s, 1H), 7.83–7.75 (m, 1H), 7.60–7.52 (m, 1H), 7.38–7.29 (m, 1H), 7.20–7.12 (m, 1H), 6.94–6.85 (m, 1H), 5.92 (s, 1H), 4.85–4.76 (m, 1H), 4.28–4.09 (m, 4H), 3.96 (d, 1H, J = 12.1, 7.6, 5.1 Hz), 3.04–2.84 (m, 2H), 2.61–2.45 (m, 1H), 2.34–2.22 (m, 1H), 2.17 (m, 1H), 1.99–1.85 (m, 1H), 1.53–1.37 (m, 1H); LCMS (FA): *m/z* = 565.1 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{24}H_{26}ClN_4O_6S_2$ 565.0982, obsd 565.1003.

Synthesis of Intermediate I-109



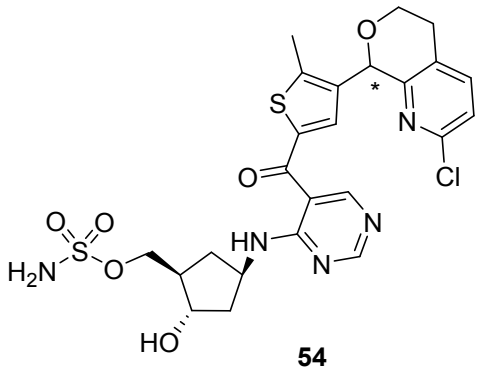
I-108 **I-109**

Reagents: (a) BH_3-THF, THF, 80 °C, 0.5 h; (b) TBSCl, imidazole, DCM, rt, 0.5 h, 89% for 2 steps; (c) *n*-BuLi, *N,N*-dimethylaminoethanol, DMF, hexane, -78 °C, 1 h, 55%.

Step 1: 5-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)-2-chloropyridine (I-108). The title compound was prepared as described in Step 1 and 2 of the synthesis of intermediate **I-107** starting with 2-(6-chloropyridin-3-yl)acetic acid.

Step 2: 3-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)-6-chloropyridine-2-carbaldehyde (I-109). To a solution of *N,N*-dimethylaminoethanol (4.43 mL, 44.1 mmol) in hexane (25 mL) was added a 2.5 M solution of *n*-BuLi in hexane (36.7 mL, 91.7 mmol), dropwise over 30 min via syringe at 0 °C. The reaction mixture was stirred at 0 °C then cooled to -78 °C. To the resulting mixture was added a solution of 5-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)-2-chloropyridine (4.0 g, 14.7 mmol) in hexane (25 mL), dropwise over 15 min, via syringe. The reaction mixture was stirred at -78 °C for 1 hour followed by addition of a solution of DMF (5.13 mL, 66.2 mmol) in THF (26 mL), dropwise over 15 min, via syringe. The resulting solution was stirred at -78 °C for 1 hour then quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The crude material was purified on silica gel to provide **I-109** (2.44 g, 55%). ^{1}H NMR ($CDCl_3$) δ 10.15 (s, 1H), 7.77 (d, 1H, *J* = 8.1 Hz), 7.50 (d, 1H, *J* = 8.1 Hz), 3.91 (t, 2H, *J* = 5.9 Hz), 3.32 (t, 2H, *J* = 5.9 Hz), 0.89 (s, 9H), -0.00 (s, 6H); LCMS (FA): *m/z* = 300.1 (M+H).

Synthesis of 54

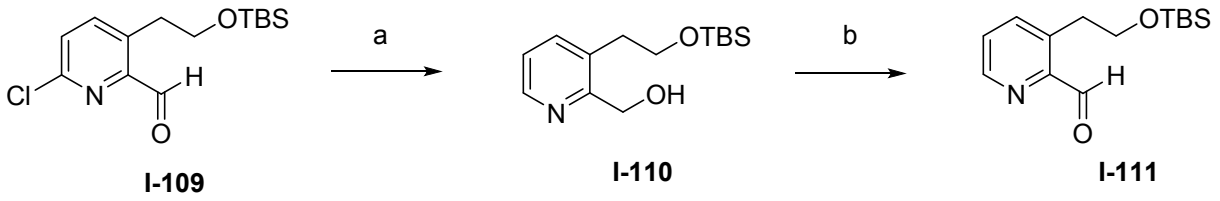


54

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(8*S*)-2-Chloro-5,8-dihydro-6*H*-pyrano[3,4-b]pyridin-8-yl]-5-methyl-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({4-[(8*R*)-2-Chloro-5,8-dihydro-6*H*-pyrano[3,4-b]pyridin-8-yl]-5-methyl-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (54). Prepared by the method described for the preparation of **23**. The following alternative conditions were employed in the reaction scheme: Step 3: The required aldehyde used was prepared

following the procedure outlined for the preparation of intermediate **I-107** using intermediate **I-109** in Step 3; Step 5: Base/solvent used was K_2CO_3/DMF; Step 6: Desilylating agent/solvent used was TBAF/THF. The individual diastereomers were obtained using preparative scale chiral SFC. More active epimer **54** is reported. ^{1}H NMR (DMSO-d_6) δ 8.59 (s, 1H), 8.51 (s, 1H), 8.17 (d, 1H, J = 7.5 Hz), 7.72 (d, 1H, J = 8.1 Hz), 7.42 (s, 2H), 7.36 (d, 1H, J = 8.0 Hz), 7.29 (s, 1H), 5.88 (s, 1H), 4.87 (d, 1H, J = 4.6 Hz), 4.74–4.61 (m, 1H), 4.14–4.04 (m, 2H), 3.98–3.82 (m, 3H), 3.12–3.00 (m, 1H), 2.86–2.75 (m, 1H), 2.50 (s, 3H), 2.32–2.24 (m, 1H), 2.16–2.05 (m, 1H), 1.97–1.88 (m, 1H), 1.79–1.68 (m, 1H), 1.31–1.20 (m, 1H); LCMS (FA): *m/z* = 580.1 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{24}H_{27}ClN_5O_6S_2$ 580.1091, obsd 580.1092.

Synthesis of Intermediate I-111



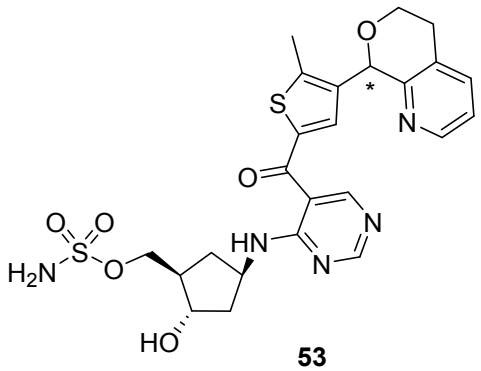
I-109 **I-110** **I-111**

Reagents: (a) $Pd(OH)_2$, H_2, EtOH, rt, 15 h, 95%; (b) MnO_2, DCM, rt, 20 h, 66%.

Step 1: [3-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)pyridin-2-yl]methanol (I-110). To a solution of 3-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)-6-chloropyridine-2-carbaldehyde (**I-109**, 2.30 g, 7.67 mmol) in ethanol (75 mL) was added $NaHCO_3$ (2.58 g, 30.7 mmol) and $Pd(OH)_2$ on carbon (10:90, palladium hydroxide:carbon, 0.54 g, 0.38 mmol). The resulting mixture was purged with hydrogen gas, and then stirred at rt under a balloon of hydrogen gas for 15 h. The reaction mixture was filtered over a pad of celite and the filtrate was concentrated to give crude product as a grey residue. The crude material was purified on silica gel to give **I-110** as a clear oil (1.96 g, 95%). ^{1}H NMR ($CDCl_3$) δ 8.54–8.49 (m, 1H), 7.66–7.54 (m, 1H), 7.31–7.22 (m, 1H), 4.84 (s, 2H), 3.87 (t, 2H, J = 6.4 Hz), 2.81 (t, 2H, J = 6.4 Hz), 0.88 (s, 9H), -0.00 (s, 6H); LCMS (FA): *m/z* = 268.2 (M+H).

Step 2: 3-(2-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)pyridine-2-carbaldehyde (I-111). To a solution of [3-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)pyridin-2-yl]methanol (2.0 g, 7.46 mmol) in DCM (72 mL) was added MnO_2 (6.50 g, 74.7 mmol). The reaction mixture was stirred at rt for 20 h then filtered over a pad of celite. The resulting filtrate was concentrated in vacuo. The crude material was purified on silica gel to afford **I-111** as a clear oil (1.31 g, 66%). ^{1}H NMR ($CDCl_3$) δ 10.28 (d, 1H, J = 0.5 Hz), 8.83–8.64 (m, 1H), 7.91–7.72 (m, 1H), 7.55–7.44 (m, 1H), 3.95 (t, 2H, J = 6.1 Hz), 3.37 (t, 2H, J = 6.1 Hz), 0.90 (s, 9H), -0.00 (s, 6H); LCMS (FA): *m/z* = 266.2 (M+H).

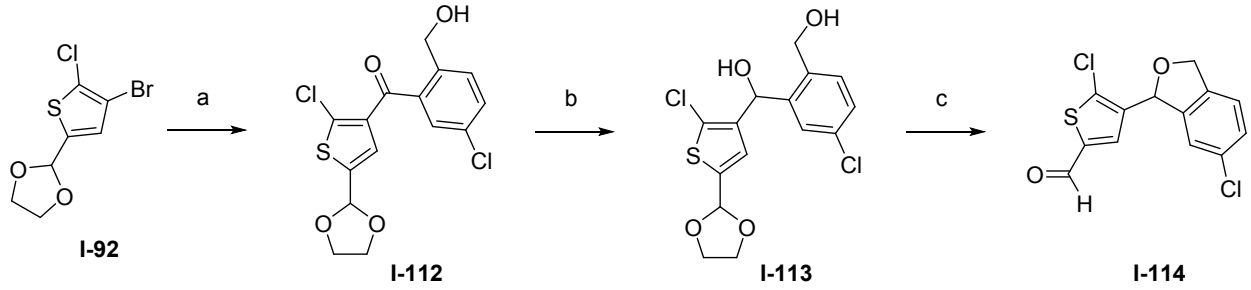
Synthesis of 53



53

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(8*S*)-5,8-Dihydro-6*H*-pyrano[3,4-b]pyridin-8-yl]-5-methyl-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({4-[(8*R*)-5,8-Dihydro-6*H*-pyrano[3,4-b]pyridin-8-yl]-5-methyl-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (53). Prepared by the method described for the preparation of **23**. The following alternative conditions were employed in the reaction scheme: Step 3: The required aldehyde used was prepared following the procedure outlined for the preparation of intermediate **I-107** using intermediate **I-111** in Step 3; Step 5: Base/solvent used was K_2CO_3/DMF; Step 6: Desilylating agent/solvent used was TBAF/THF. The individual diastereomers were obtained using preparative scale chiral HPLC. More active epimer **53** is reported. ^{1}H NMR (MeOD) δ 8.63 (s, 1H), 8.54 (s, 1H), 8.34 (dd, 1H, *J* = 4.8, 1.5 Hz), 7.70 (d, 1H, *J* = 7.8 Hz), 7.31 (dd, 1H, *J* = 7.8, 4.8 Hz), 7.13 (s, 1H), 5.93 (s, 1H), 4.83–4.69 (m, 1H), 4.25–4.11 (m, 4H), 4.02–3.91 (m, 1H), 3.22–3.08 (m, 1H), 2.99–2.88 (m, 1H), 2.55 (s, 3H), 2.52–2.45 (m, 1H), 2.33–2.21 (m, 1H), 2.20–2.09 (m, 1H), 1.93–1.84 (m, 1H), 1.50–1.36 (m, 1H); LCMS (FA): *m/z* = 546.0 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{24}H_{28}N_5O_6S_2$ 546.1481, obsd 546.1483.

Synthesis of Intermediate I-114



Reagents: (a) 6-Chlorophthalide, *n*-BuLi, THF, -78 °C, 10 min, 99%; (b) $NaBH_4$, THF, MeOH, rt, 1 h, 67%; (c) TFA, DCM, rt, 1 h, 76%.

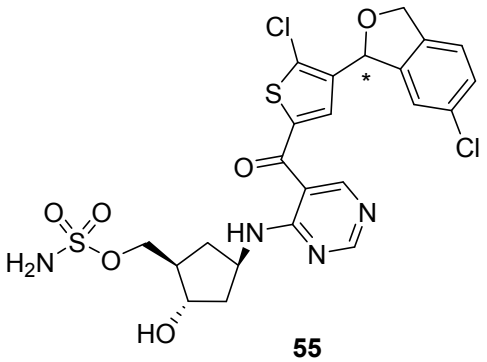
Step 1: [2-Chloro-5-(1,3-dioxolan-2-yl)-3-thienyl][5-chloro-2-(hydroxymethyl)phenyl]methanone (I-112). To a two-neck 100 mL round-bottom flask containing THF (40 mL) cooled at -78 °C was added 2.50 M of *n*-BuLi in hexane (1.90 mL, 4.75 mmol). The solution was stirred for 10 min under an atmosphere of argon at -78 °C. A solution of intermediate **I-92** (0.96 g, 3.55 mmol) in THF (3.0 mL) was added dropwise quickly and a light yellow precipitate formed. The mixture was stirred at -78 °C for 10 min. A solution of 6-

chlorophthalide (0.57 g, 3.38 mmol) in THF (2.0 mL) was added quickly and the reaction turned to a dark yellow/orange solution. The solution was allowed to stir for 10 min. The reaction was removed from the dry ice bath and quenched with saturated NH_4Cl. After warming to rt, the reaction was diluted with EtOAc and saturated NH_4Cl. The layers were separated, and the organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo to afford **I-112** (1.21 g, 99%).

Step 2: [2-Chloro-5-(1,3-dioxolan-2-yl)-3-thienyl][5-chloro-2-(hydroxymethyl)phenyl]methanol (I-113). To a solution of [2-chloro-5-(1,3-dioxolan-2-yl)-3-thienyl][5-chloro-2-(hydroxymethyl)phenyl]methanone (1.21 g, 3.38 mmol) in THF (40 mL) and methanol (10 mL) was added sodium tetrahydroborate (0.26 g, 6.77 mmol) at rt portion wise, and the mixture was stirred for 1 h. The reaction was concentrated in vacuo to remove the methanol. The residue was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, filtered and concentrated in vacuo. Purification was accomplished on silica gel to provide **I-113** as a clear colorless oil (0.82 g, 67%). LCMS (FA): *m/z* = 343.3 (M+H).

Step 3: 5-Chloro-4-(6-chloro-1,3-dihydro-2-benzofuran-1-yl)thiophene-2-carbaldehyde (I-114). A 100 mL round bottom flask was charged with [2-chloro-5-(1,3-dioxolan-2-yl)-3-thienyl][5-chloro-2-(hydroxymethyl)phenyl]methanol (0.63 g, 1.74 mmol) in DCM (4.0 mL) and TFA (15 mL) was added at rt. The reaction mixture was stirred at rt for 1 h. The reaction was carefully poured into saturated $NaHCO_3$, extracted with EtOAc two times, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified on silica gel to give **I-114** as a clear colorless oil (0.39 g, 76%). ^{1}H NMR ($CDCl_3$) δ 9.72 (s, 1H), 7.43 (s, 1H), 7.35 (dd, 1H, *J* = 8.1, 1.5 Hz), 7.28 (d, 1H, *J* = 2.9 Hz), 7.13 (s, 1H), 6.37 (s, 1H), 5.32 (dd, 1H, *J* = 12.4, 2.5 Hz), 5.20 (dd, 1H, *J* = 12.4, 1.4 Hz).

Synthesis of 55

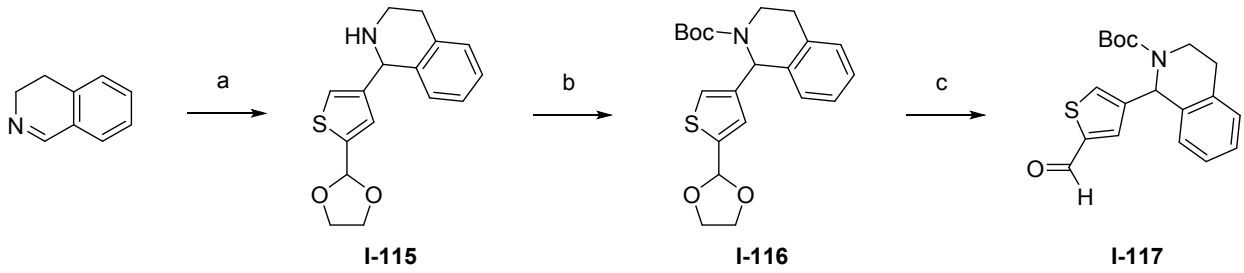


55

[(1*R*,2*S*,4*R*)-4-{[5-({5-Chloro-4-[(1*R*)-6-chloro-1,3-dihydro-2-benzofuran-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({5-Chloro-4-[(1*S*)-6-chloro-1,3-dihydro-2-benzofuran-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (55). Prepared by the method described for the preparation of **23** using 4-chloro-5-iodopyrimidine and intermediate **I-114** in Step 3. The individual diastereomers were obtained using preparative scale chiral SFC. More active epimer **55** is reported. ^{1}H NMR (MeOD) δ 8.62 (s, 1H), 8.55 (s, 1H),

7.40–7.27 (m, 3H), 7.14 (s, 1H), 6.36 (s, 1H), 5.28 (dd, 1H, J = 12.5, 2.2 Hz), 5.13 (d, 1H, J = 12.4 Hz), 4.81–4.72 (m, 1H), 4.22–4.08 (m, 3H), 2.55–2.36 (m, 1H), 2.31–2.19 (m, 1H), 2.18–2.07 (m, 1H), 1.93–1.81 (m, 1H), 1.48–1.34 (m, 1H); LCMS (FA): *m/z* = 585.0 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{23}H_{23}Cl_2N_4O_6S_2$ 585.0436, obsd 585.0456.

Synthesis of Intermediate I-117



Reagents: (a) BF_3-OEt_2, THF, -30 °C, 20 min, then 2-(4-Bromo-2-thienyl)-1,3-dioxolane, *n*-BuLi, THF, -78 °C, 20 min; (b) $(Boc)_2O$, DMAP, CH_3CN, rt, 2 h, 27% for 2 steps; (c) Dowex 50WX-2, acetone, rt, 18 h, 91%.

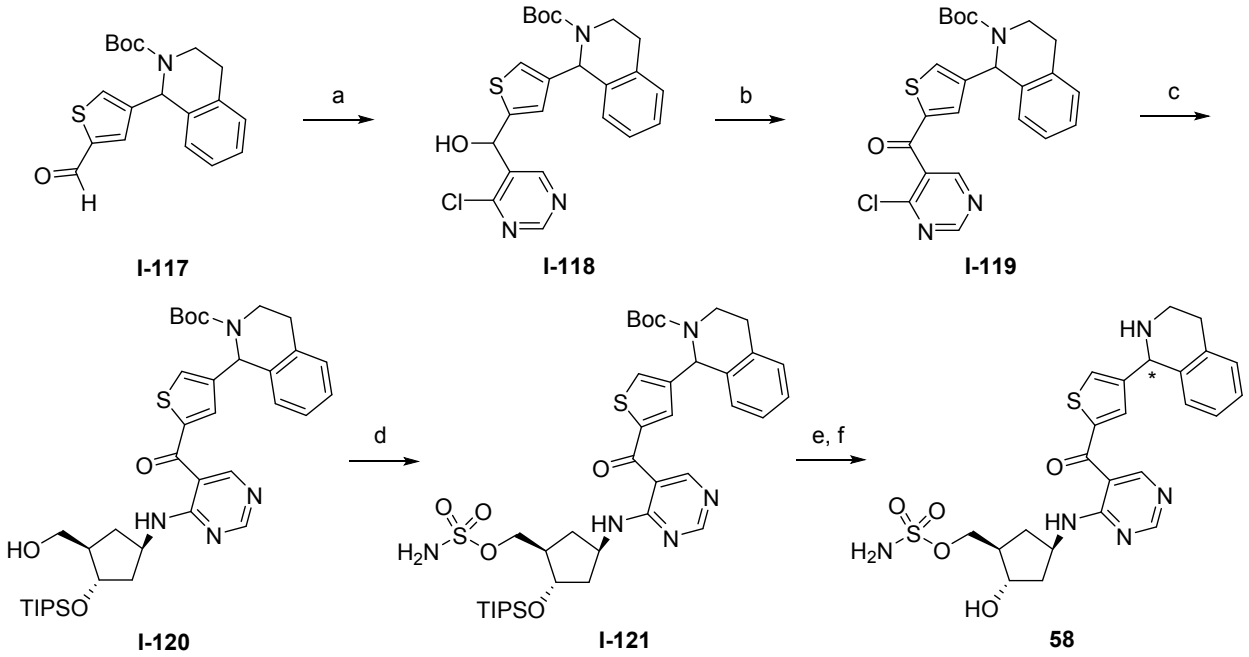
Step 1: 1-[5-(1,3-Dioxolan-2-yl)-3-thienyl]-1,2,3,4-tetrahydroisoquinoline (I-115). A solution of 3,4-dihydroisoquinoline (500 mg, 3.81 mmol) in THF (15.4 mL) was cooled at -30 °C. To this solution was added dropwise boron trifluoride etherate (0.53 mL, 4.19 mmol) at -30 °C, and the mixture was stirred for 20 min. Into a separate 50 mL 2-neck flask 2.50 M of *n*-BuLi in hexane (1.83 mL, 4.57 mmol) was added at -78 °C followed by a solution of 2-(4-bromo-2-thienyl)-1,3-dioxolane (1.08 g, 4.57 mmol) in THF (10 mL). After 5 min, lithiated thiophene suspension was added to the above solution of dihydroisoquinoline BF_3-OEt_2 complex at -78 °C. The reaction was stirred for 20 min at -78 °C and then quenched by addition of water. The layers were separated and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to provide **I-115** (513 mg) and some impurities as a red amorphous solid. This mixture was used for the next step without further purification.

Step 2: *tert*-Butyl 1-[5-(1,3-dioxolan-2-yl)-3-thienyl]-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (I-116). The crude mixture from step 1was dissolved in MeCN (6.97 mL), to which was added $(Boc)_2O$ (1.25 g, 5.72 mmol) and DMAP (2.33 mg, 19.1 μmol) at rt. After stirring for 2 h, the reaction was quenched by adding water. The layers were separated and the aqueous layer was extracted with EtOAc twice. The combined organics were washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified on silica gel to provide **I-116** (393 mg, 27% for 2 steps). ^1H NMR ($CDCl_3$) δ 7.26–7.03 (m, 6H), 6.82 (s, 1H), 6.01 (s, 1H), 4.17–3.93 (m, 5H), 3.20–3.04 (m, 1H), 3.04–2.86 (m, 1H), 2.79–2.68 (m, 1H), 1.57–1.46 (m, 9H); LCMS (FA): m/z = 388.3 (M+H).

Step 3: *tert*-Butyl 1-(5-formyl-3-thienyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (I-117). To a solution of *tert*-butyl 1-[5-(1,3-dioxolan-2-yl)-3-thienyl]-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (393 mg, 1.01 mmol) in acetone (7.72 mL) was added 500 mg of Dowex 50WX-2-200 (H)(acid resin), and the mixture was shaken for 18 h at rt. The reaction was filtered through a glass frit funnel and the residual resin was rinsed with acetone several times. To the filtrate was

added saturated aqueous $NaHCO_3$ (25 mL) and the mixture was concentrated to half volume in vacuo. The residue was diluted with EtOAc, the layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to provide **I-117** as a colorless amorphous solid (319 mg, 91%). ^{1}H NMR ($CDCl_3$) δ 9.84 (s, 1H), 7.64 (s, 1H), 7.30–7.07 (m, 5H), 6.48–6.23 (br s, 1H), 4.09–3.88 (m, 1H), 3.22–3.06 (m, 1H), 3.05–2.89 (m, 1H), 2.81–2.69 (m, 1H), 1.52 (s, 9H).

Synthesis of 58



Reagents: (a) 4-chloro-5-iodopyrimidine, *n*-BuLi, THF, -78 °C, 0.5 h, 69%; (b) MnO_2, DCM, rt, 14 h, 98%; (c) **I-8**, K_2CO_3, DMF, rt, 2 h, 86%; (d) chlorosulfonamide, DMF, rt, 3 h, 92%; (e) TBAF monohydrate, THF, rt, 12 h, 78%; (f) TFA, rt, 15 min, 95%.

Step 1: *tert*-Butyl 1-{5-[(4-chloropyrimidin-5-yl)(hydroxy)methyl]-3-thienyl}-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (I-118). The title compound was prepared in an analogous fashion to method described for the preparation of **23** using 4-chloro-5-iodopyrimidine and intermediate **I-117** in Step 3. ^{1}H NMR ($CDCl_3$) δ 9.05 (s, 1H), 8.96 (s, 1H), 7.26–7.17 (m, 3H), 7.12 (s, 1H), 7.02 (s, 1H), 6.78 (s, 1H), 6.40–6.10 (m, 2H), 3.10 (s, 1H), 2.97 (s, 1H), 2.89–2.69 (m, 2H), 1.50 (s, 9H).

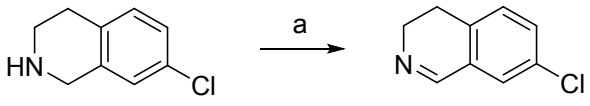
Step 2: *tert*-Butyl 1-{5-[(4-chloropyrimidin-5-yl)carbonyl]-3-thienyl}-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (I-119). The title compound was prepared by the method described for the preparation of **23** in Step 4 using intermediate **I-118**. ^{1}H NMR ($CDCl_3$) δ 9.13 (s, 1H), 8.76 (s, 1H), 7.46 (s, 1H), 7.39 (s, 1H), 7.28–7.21 (m, 3H), 7.11 (d, 1H, J = 7.6 Hz), 6.36 (s, 1H), 3.12 (s, 1H), 3.06–2.91 (m, 2H), 2.76 (d, 1H, J = 16.0 Hz), 1.47 (s, 9H).

Step 3: *tert*-Butyl (1*S*)-1-[5-[4-[[(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-triisopropylsilyloxy-cyclopentyl]amino]pyrimidine-5-carbonyl]-3-thienyl]-3,4-dihydro-1*H*-isoquinoline-2-carboxylate and *tert*-butyl (1*R*)-1-[5-[4-[[(1*R*,3*R*,4*S*)-3-(hydroxymethyl)-4-triisopropylsilyloxy-cyclopentyl]amino]pyrimidine-5-carbonyl]-3-thienyl]-3,4-dihydro-1*H*-isoquinoline-2-carboxylate (I-120). The title compounds were prepared in an analogous fashion for the synthesis of **23** in Step 5 using intermediate **I-119** and **I-8**. The following alternative conditions were employed in the reaction: Base/solvent used was K_2CO_3/DMF. LCMS (FA): m/z = 708.1 (M+H).

Step 4: *tert*-Butyl (1*S*)-1-[5-[4-[[(1*R*,3*R*,4*S*)-3-(sulfamoyloxymethyl)-4-triisopropylsilyloxy-cyclopentyl]amino]pyrimidine-5-carbonyl]-3-thienyl]-3,4-dihydro-1*H*-isoquinoline-2-carboxylate and *tert*-Butyl (1*R*)-1-[5-[4-[[(1*R*,3*R*,4*S*)-3-(sulfamoyloxymethyl)-4-triisopropylsilyloxy-cyclopentyl]amino]pyrimidine-5-carbonyl]-3-thienyl]-3,4-dihydro-1*H*-isoquinoline-2-carboxylate (I-121). The title compounds were prepared in a similar fashion to the method described for the preparation of **23** in Step 6 using **I-120** without the final deprotection step. LCMS (FA): m/z = 787.1 (M+H).

Step 5: [(1*R*,2*S*,4*R*)-2-Hydroxy-4-{[5-({4-[(1*R*)-1,2,3,4-tetrahydroisoquinolin-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}cyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-2-Hydroxy-4-{[5-({4-[(1*S*)-1,2,3,4-tetrahydroisoquinolin-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}cyclopentyl]methyl sulfamate (58).
To a solution of the product mixture from Step 4 (**I-121**, 392 mg, 0.50 mmol) in THF (7.7 mL) was added a solution of TBAF hydrate (278 mg, 1.0 mmol) in THF (7.7 mL) at rt, and the mixture was stirred for 3h. The reaction was quenched by addition of water and extracted with EtOAc three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to provide the Boc-protected intermediate as a light yellow amorphous solid (245 mg, 78%). LCMS (FA): m/z = 630.9 (M+H). To a flask containing the product mixture from the above reaction (245 mg, 0.39 mmol) was added TFA (3.1 mL, 39.9 mmol) and the mixture was stirred at rt for 15 min. The mixture was concentrated in vacuo and a small amount of saturated $NaHCO_3$ was added to the residue. The resulting mixture was concentrated in vacuo and the residue was purified on silica gel [eluting with 50% DCM in mixed solution of (2% NH_4OH: 5% MeOH: 43% CH_3CN in 50% DCM) for 3min then gradient to 100% of mixed solution (2% NH_4OH: 5% MeOH: 43% CH_3CN in 50% DCM)] to provide the final compound as a mixture of diastereomers (196 mg, 95%). The individual diastereomers were obtained using preparative scale chiral HPLC. More active epimer **58** is reported. ^{1}H NMR (MeOD) δ 8.73 (s, 1H), 8.56 (s, 1H), 7.64 (s, 1H), 7.57 (s, 1H), 7.21–7.13 (m, 2H), 7.14–7.05 (m, 1H), 6.87 (d, 1H, *J* = 7.7 Hz), 5.25 (s, 1H), 4.82–4.74 (m, 1H), 4.25–4.10 (m, 3H), 3.23–3.13 (m, 1H), 3.10–2.81 (m, 3H), 2.50 (dt, 1H, *J* = 13.9, 7.7 Hz), 2.32–2.20 (m, 1H), 2.14 (ddd, 1H, *J* = 12.5, 7.4, 4.4 Hz), 1.89 (dt, 1H, *J* = 14.9, 7.5 Hz), 1.42 (dt, 1H, *J* = 13.0, 9.1 Hz); LCMS (FA): *m/z* = 530.6 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{24}H_{28}N_5O_5S_2$ 530.1532, obsd 530.1531.

Synthesis of Intermediate I-122

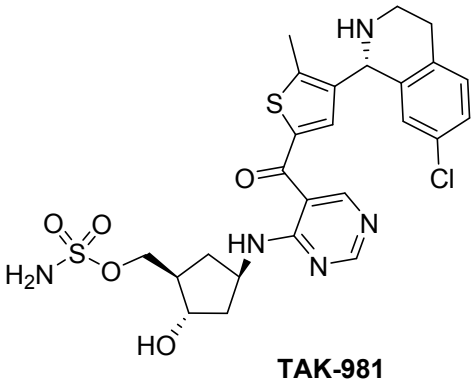


I-122

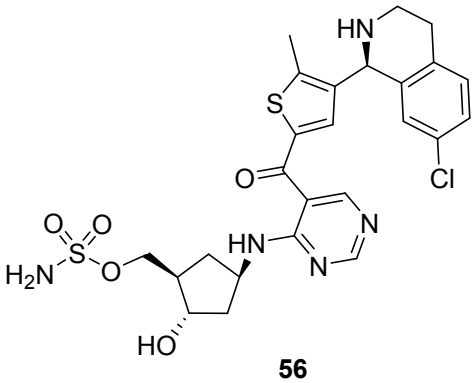
Reagents: (a) MnO_2, DCM, rt, 16 h, 83%.

Step 1: 7-Chloro-3,4-dihydroisoquinoline (I-122). To a solution of 7-chloro-1,2,3,4-tetrahydro-isoquinoline (1.15 g, 6.86 mmol) in DCM (70 mL) was added MnO_2 (5.96 g, 68.6 mmol) at rt, and the mixture was stirred for 16 h. The reaction was filtered through a celite pad and the residual solid was rinsed with DCM several times. The filtrate was concentrated in vacuo and the residue was purified on silica gel to provide **I-122** as a colorless solid (915 mg, 83%). ^{1}H NMR (DMSO-d_6) δ 8.35 (t, 1H, J = 2.1 Hz), 7.52 (d, 1H, J = 2.2 Hz), 7.46 (dd, 1H, J = 8.0, 2.3 Hz), 7.28 (d, 1H, J = 8.0 Hz), 3.70–3.63 (m, 2H), 2.71–2.65 (m, 2H).

Synthesis of TAK-981 and 56



TAK-981



56

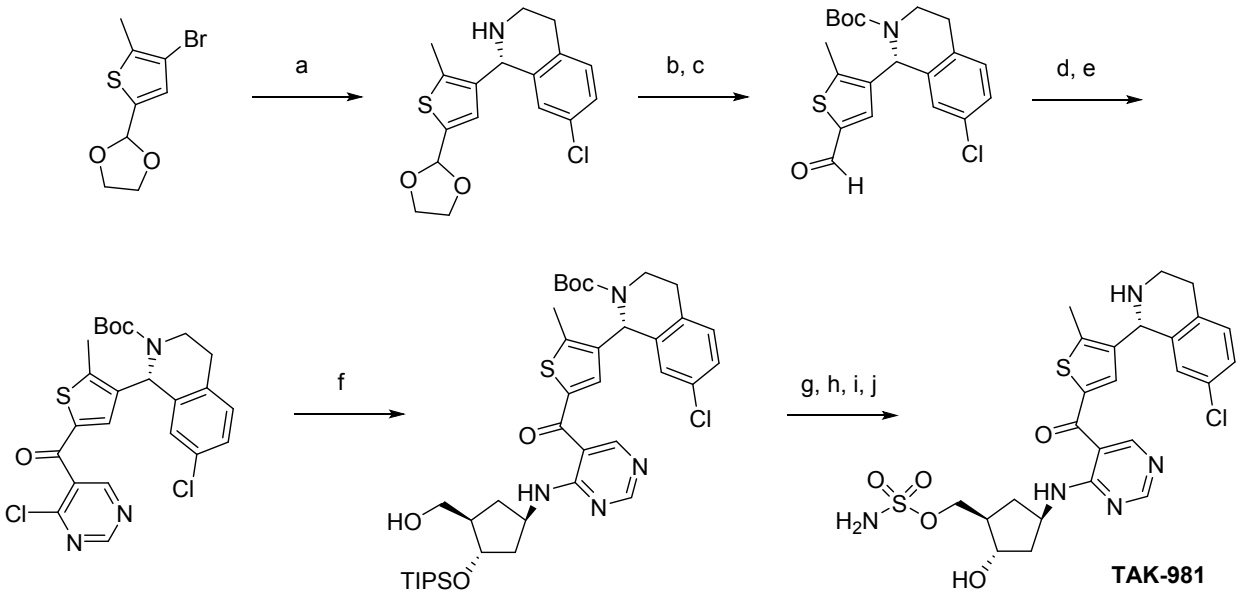
[(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*R*)-7-Chloro-1,2,3,4-tetrahydroisoquinolin-1-yl]-5-methyl-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (TAK-981) and [(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*S*)-7-Chloro-1,2,3,4-tetrahydroisoquinolin-1-yl]-5-methyl-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (56). Prepared by the method described for the preparation of **58**. The required aldehyde used in Step 1 was prepared following the procedure outlined for the preparation of intermediate **I-117** starting with 2-(4-bromo-5-methyl-2-thienyl)-1,3-dioxolane and intermediate **I-122**. The individual diastereomers were resolved using silica gel column chromatography in Step 3.

TAK-981: ^{1}H NMR (MeOD) δ 8.63 (s, 1H), 8.53 (s, 1H), 7.29 (s, 1H), 7.17 (s, 2H), 6.71 (s, 1H), 5.23 (s, 1H), 4.83–4.69 (m, 1H), 4.32–4.07 (m, 3H), 3.40–3.23 (m, 1H), 3.12–2.94 (m, 2H), 2.90–2.75 (m, 1H), 2.61 (s, 3H), 2.55–2.39 (m, 1H), 2.33–2.19 (m, 1H), 2.18–2.09 (m, 1H), 1.96–1.78 (m, 1H), 1.50–1.34 (m, 1H); LCMS (FA): *m/z* = 578.4 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{25}H_{29}ClN_5O_5S_2$ 578.1299, obsd 578.1302.

56: ^{1}H NMR (MeOD) δ 8.63 (s, 1H), 8.53 (s, 1H), 7.29 (s, 1H), 7.17 (s, 2H), 6.71 (s, 1H), 5.23 (s, 1H), 4.83–4.70 (m, 1H), 4.27–4.10 (m, 3H), 3.41–3.24 (m, 1H), 3.15–2.95 (m, 2H), 2.87–2.76 (m, 1H), 2.61 (s, 3H), 2.55–2.38 (m, 1H), 2.33–2.20 (m, 1H), 2.18–2.08 (m, 1H), 1.96–1.80 (m,

1H), 1.48–1.33 (m, 1H); LCMS (FA): *m/z* = 578.4 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{25}H_{29}ClN_5O_5S_2$ 578.1299, obsd 578.1296.

An Alternative Synthesis of TAK-981

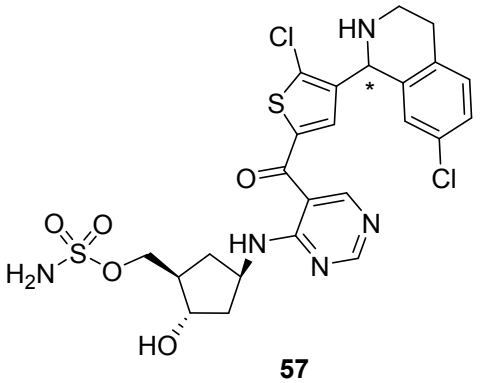


Reagents: (a) *n*-BuLi, THF, -78 °C, 20 min, then **I-122**, BF_3-OEt_2, THF, 0 °C, 1 h, then chiral SFC chromatography, 21%; (b) $(Boc)_2O$, DCM, rt, 3 h; (c) 1M HCl, THF, rt, 1 h, 81% for 2 steps; (d) 4-chloro-5-iodopyrimidine, *n*-BuLi, THF, -40 °C, 10 min, then rt, 30 min; (e) MnO_2, DCM, 30 °C, 4 h, 58% for 2 steps; (f) **I-8**, K_2CO_3, DMF, rt, 5 h, 84%; (g) chlorosulfonamide, DMF, rt, 5 min; (h) TBAF hydrate, THF, 40 °C, 2 h, 88% for 2 steps; (i) TFA, DCM, rt, 1 h; (j) MeOH/CH_3CN recrystallization, 81%, for 2 steps.

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*R*)-7-Chloro-1,2,3,4-tetrahydroisoquinolin-1-yl]-5-methyl-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (TAK-981).

For a detailed synthesis see: Huszar, D. et al. "A small molecule SUMOylation inhibitor activates antitumor immune responses and potentiates immune therapies in preclinical models." *Science Translational Medicine*, submitted, manuscript under revision.

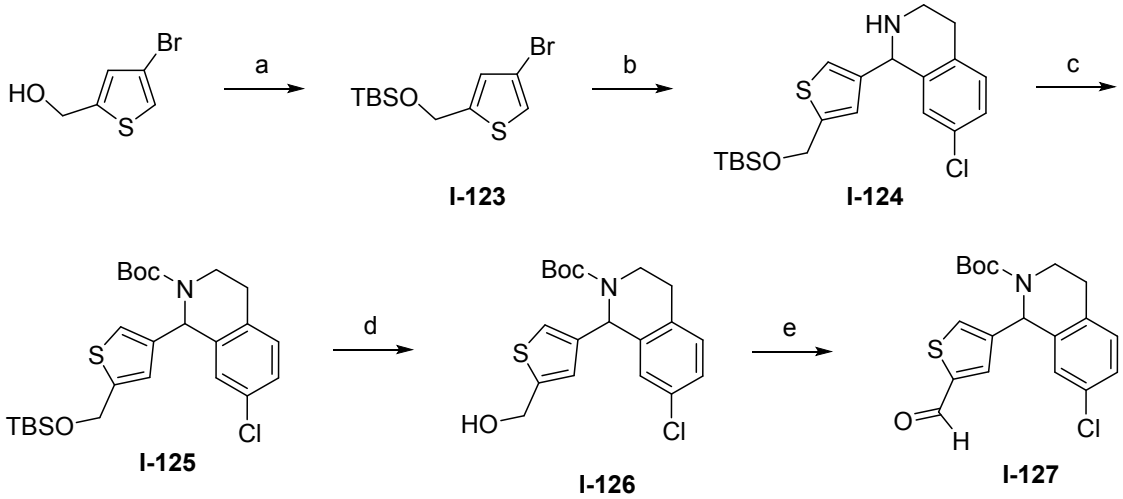
Synthesis of 57



57

[(1*R*,2*S*,4*R*)-4-{[5-({5-Chloro-4-[(1*R*)-7-chloro-1,2,3,4-tetrahydroisoquinolin-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({5-Chloro-4-[(1*S*)-7-chloro-1,2,3,4-tetrahydroisoquinolin-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (57). Prepared by the method described for the preparation of **58**. The required aldehyde used in Step 1 was prepared following the procedure outlined for the preparation of intermediate **I-117** starting with intermediate **I-92** and **I-122**. The individual diastereomers were resolved using silica gel column chromatography in Step 3. More active epimer **57** is reported. ^{1}H NMR (DMSO-d_6) δ 8.61 (s, 1H), 8.57 (s, 1H), 8.15 (d, 1H, J = 7.0 Hz), 7.49–7.36 (m, 3H), 7.25–7.12 (m, 2H), 6.67 (s, 1H), 5.17 (s, 1H), 4.86 (d, 1H, J = 4.3 Hz), 4.74–4.60 (m, 1H), 4.13–4.03 (m, 1H), 3.99–3.88 (m, 2H), 3.23–3.02 (m, 2H), 2.98–2.83 (m, 2H), 2.75–2.63 (m, 1H), 2.36–2.23 (m, 1H), 2.15–2.03 (m, 1H), 1.95–1.83 (m, 1H), 1.78–1.65 (m, 1H), 1.33–1.17 (m, 1H); LCMS (FA): *m/z* = 598.3 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{24}H_{26}Cl_2N_5O_5S_2$ 598.0752, obsd 598.0757.

Synthesis of Intermediate I-127



Reagents: (a) TBSCl, imidazole, DCM, rt, 12 h, 95%; (b) *n*-BuLi, THF, -78 °C, 10 min, then **I-122**, BF_3-OEt_2, THF, -30 °C, 0.5 h; (c) $(Boc)_2O$, DMAP, CH_3CN, rt, 0.5 h, 50% for 2 steps; (d) 1N HCl, THF, 40 °C, 2 h, 91%; (e) Dess-Martin periodinane, DCM, rt, 0.5 h, 88%.

Step 1: [(4-Bromo-2-thienyl)methoxy](*tert*-butyl)dimethylsilane (I-123). To a solution of (4-bromo-2-thienyl)methanol (5.0 g, 26 mmol) in DCM (83 mL) was added 1*H*-imidazole (3.53 g, 51.8 mmol) followed by TBSCl (4.29 g, 28.5 mmol) at rt, and the reaction was stirred overnight. The reaction was quenched by addition of water (50 mL) and extracted with DCM (30 mL) twice. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude residue was purified on silica gel to provide **I-123** (7.59 g, 95%). ^{1}H NMR ($CDCl_3$) δ 7.02 (d, 1H, *J* = 1.4 Hz), 6.73–6.70 (m, 1H), 4.72 (d, 2H, *J* = 0.9 Hz), 0.82 (s, 9H), 0.00 (s, 6H).

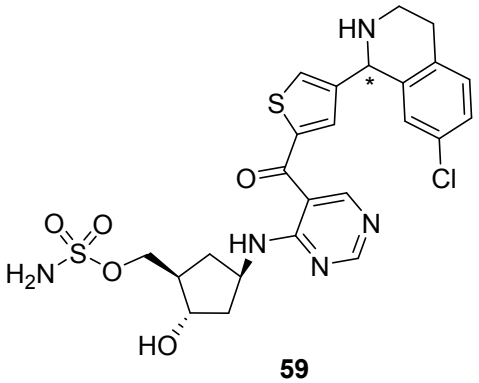
Step 2: 1-[5-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-3-thienyl]-7-chloro-1,2,3,4-tetrahydroisoquinoline (I-124). The title compound was prepared by the method described for the preparation of intermediate **I-117** in Step 1 starting with intermediates **I-123** and **I-122**.

Step 3: *tert*-Butyl 1-[5-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-3-thienyl]-7-chloro-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (I-125). The title compound was prepared by the method described for the preparation of intermediate **I-117** in Step 2 using intermediate **I-124**. ^{1}H NMR (DMSO- d_6) δ 7.29–7.23 (m, 3H), 6.91 (br s, 1H), 6.82 (s, 1H), 6.18 (br s, 1H), 4.78 (s, 2H), 3.97–3.80 (m, 1H), 3.17–3.08 (m, 1H), 2.81–2.71 (m, 2H), 1.43 (s, 9H), 0.85 (s, 6H), 0.04 (s, 3H).

Step 4: *tert*-Butyl 7-chloro-1-[5-(hydroxymethyl)-3-thienyl]-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (I-126). To a solution of *tert*-butyl 1-[5-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-3-thienyl]-7-chloro-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (1.72 g, 3.48 mmol) in THF (15 mL) was added a 1M HCl solution (15 mL, 15 mmol). The reaction mixture was stirred at 40 °C for 2 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (100 mL) and extracted with EtOAc (100 mL) three times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified on silica gel to provide **I-126** (1.2 g, 91%). LCMS (FA): (M+1) 380.3.

Step 5: *tert*-Butyl 7-chloro-1-(5-formyl-3-thienyl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (I-127). To a solution of *tert*-butyl 7-chloro-1-[5-(hydroxymethyl)-3-thienyl]-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (980 mg, 2.6 mmol) in DCM (20 mL) was added Dess-Martin periodinane (1.64 g, 3.87 mmol) and the reaction mixture was stirred at rt for 30 min. The reaction was quenched by addition of a saturated aqueous solution of $NaHCO_3$ (60 mL) and extracted with DCM (60 mL) three times. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified on silica gel to provide **I-127** (860 mg, 88%). ^{1}H NMR ($CDCl_3$) δ 9.88 (d, 1H, *J* = 1.2 Hz), 7.65 (s, 1H), 7.38 (s, 1H), 7.25 (dd, 1H, *J* = 8.2, 2.1 Hz), 7.18 (d, 1H, *J* = 8.2 Hz), 7.13 (s, 1H), 6.37 (s, 1H), 4.15 (d, 1H, *J* = 7.3 Hz), 3.11 (s, 1H), 2.96 (s, 1H), 2.75 (d, 1H, *J* = 16.1 Hz), 1.54 (s, 9H). LCMS (FA): (M+1) 378.3.

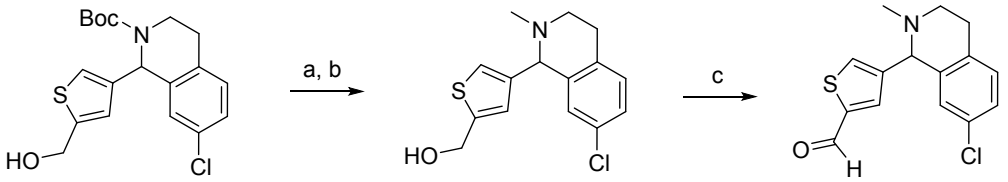
Synthesis of 59



59

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*R*)-7-Chloro-1,2,3,4-tetrahydroisoquinolin-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*S*)-7-Chloro-1,2,3,4-tetrahydroisoquinolin-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (59). Prepared by the method described for the preparation of **58** starting with intermediate **I-127**. The individual diastereomers were obtained using preparative scale chiral SFC. More active epimer **59** is reported. ^{1}H NMR (DMSO-d_6) δ 8.68 (s, 1H), 8.64 (s, 1H), 8.27 (d, 1H, J = 7.6 Hz), 7.72 (s, 1H), 7.67 (d, 1H, J = 1.1 Hz), 7.48–7.12 (m, 4H), 6.87 (d, 1H, J = 1.6 Hz), 5.14 (s, 1H), 4.88 (d, 1H, J = 4.2 Hz), 4.77–4.63 (m, 1H), 4.09 (dd, 1H, J = 9.8, 6.0 Hz), 4.02–3.89 (m, 2H), 3.11–2.94 (m, 2H), 2.93–2.64 (m, 3H), 2.37–2.27 (m, 1H), 2.17–2.08 (m, 1H), 1.94 (ddd, 1H, J = 11.8, 7.4, 3.7 Hz), 1.82–1.70 (m, 1H), 1.28 (dt, 1H, J = 12.7, 9.2 Hz); LCMS (FA): *m/z* = 564.4 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{24}H_{27}ClN_5O_5S_2$ 564.1142, obsd 564.1148.

Synthesis of Intermediate I-129



I-126 **I-128** **I-129**

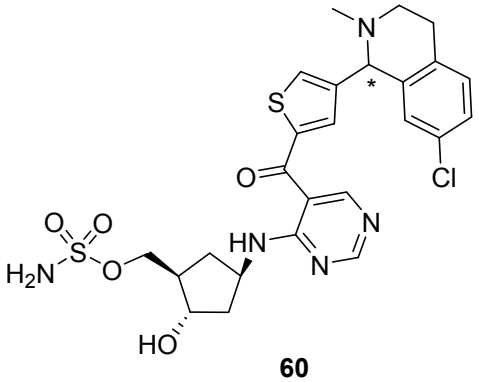
Reagents: (a) TFA, DCM, rt, 5 min; (b) 10 M of formaldehyde in water, $NaBH(OAc)_3$, CH_3CN, rt, 0.5 h, 88% for 2 steps; (c) Dess-Martin periodinane, rt, 0.5 h, 60%.

Step 1: [4-(7-Chloro-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)-2-thienyl]methanol (I-128). A solution of *tert*-butyl 7-chloro-1-[5-(hydroxymethyl)-3-thienyl]-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (**I-126**, 220 mg, 0.58 mmol) in TFA (4.00 mL, 51.9 mmol) was stirred for 5 min at rt and then concentrated in vacuo. The residue was azeotroped with toluene twice and the residue was dried under high vacuum for 2 h. The residue was dissolved in CH_3CN (5.0 mL), at which point was added 10 M of formaldehyde in water solution (0.24 mL, 2.90 mmol) followed by sodium triacetoxyborohydride (246 mg, 1.16 mmol) at rt, and the mixture was stirred for 30 min. The reaction was quenched by addition of saturated $NaHCO_3$ (60 mL) and extracted with EtOAc (60 mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to provide **I-128** (150 mg, 88%).

^{1}H NMR ($CDCl_3$) δ 7.14–7.02 (m, 3H), 6.78–6.72 (m, 2H), 4.75 (s, 2H), 4.30 (s, 1H), 3.15–3.02 (m, 2H), 2.84–2.75 (m, 1H), 2.61 (s, 1H), 2.60–2.53 (m, 1H), 2.27 (s, 3H).

Step 2: 4-(7-Chloro-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)thiophene-2-carbaldehyde (I-129). To a solution of [4-(7-chloro-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl)-2-thienyl]methanol (145 mg, 0.49 mmol) in DCM (3.0 mL) was added Dess-Martin periodinane (314 mg, 0.74 mmol) at rt, and the mixture was stirred for 30 min. The reaction was quenched by addition of saturated $NaHCO_3$ (50 mL) and extracted with DCM (50 mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to yield **I-129** (80 mg, 60%). ^{1}H NMR (MeOD) δ 9.83 (d, 1H, *J* = 1.2 Hz), 7.85 (s, 1H), 7.68 (d, 1H, *J* = 1.4 Hz), 7.21–7.12 (m, 2H), 6.73 (s, 1H), 4.61 (s, 1H), 3.15–3.05 (m, 2H), 2.95–2.85 (m, 1H), 2.72–2.62 (m, 1H), 2.29 (s, 3H). LCMS (FA): m/z = 292.3 (M+H).

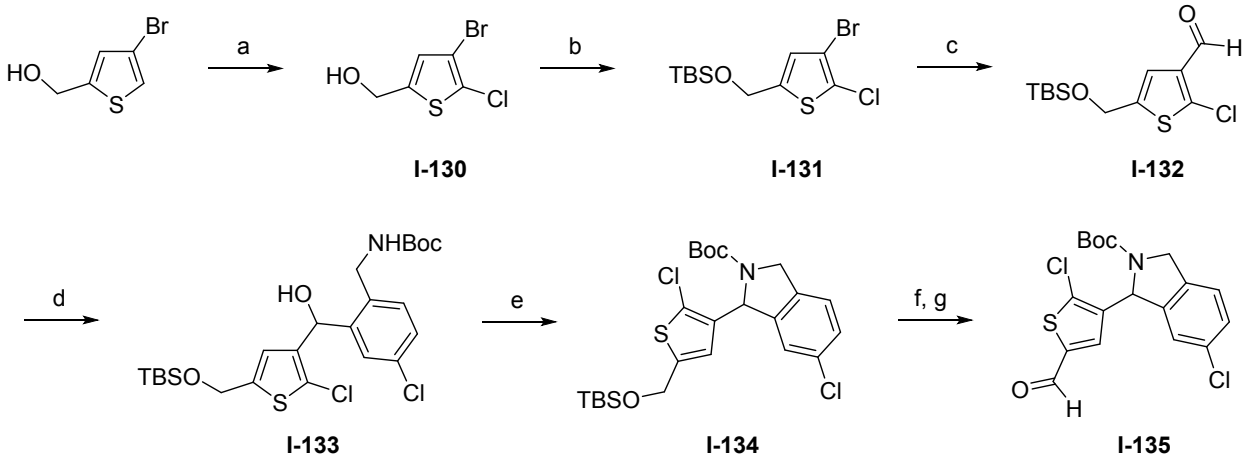
Synthesis of 60



60

[(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*R*)-7-Chloro-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({4-[(1*S*)-7-Chloro-2-methyl-1,2,3,4-tetrahydroisoquinolin-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (60). Prepared by the method described for the preparation of **59** starting with intermediate **I-129**. The following alternative conditions were employed in the reaction scheme: Step 5: Silyl deprotection agent/solvent used was HCl/MeOH. The individual diastereomers were obtained using preparative scale chiral SFC. More active epimer **60** is reported. ^{1}H NMR (DMSO-d_6) δ 8.62 (s, 1H), 8.59 (s, 1H), 8.22 (d, 1H, *J* = 7.5 Hz), 7.93 (d, 1H, *J* = 1.0 Hz), 7.50 (d, 1H, *J* = 1.1 Hz), 7.45–7.36 (br s, 2H), 7.19 (s, 2H), 6.79 (s, 1H), 4.87 (d, 1H, *J* = 4.5 Hz), 4.75–4.62 (m, 1H), 4.56 (s, 1H), 4.09 (dd, 1H, *J* = 9.7, 6.0 Hz), 4.00–3.89 (m, 2H), 3.06–2.91 (m, 2H), 2.87–2.73 (m, 1H), 2.56–2.52 (m, 1H), 2.36–2.27 (m, 1H), 2.21 (s, 3H), 2.17–2.06 (m, 1H), 1.98–1.88 (m, 1H), 1.73 (dt, 1H, *J* = 13.3, 6.9 Hz), 1.27 (dt, 1H, *J* = 12.3, 9.1 Hz); LCMS (FA): *m/z* = 578.4 (M+H). HRMS *m/z* $[M+H]^+$ calcd for $C_{25}H_{29}ClN_5O_5S_2$ 578.1299, obsd 578.1303.

Synthesis of Intermediate I-135



Reagents: (a) NCS, DMF, 60 °C, 3 h, 62%; (b) TBSCl, imidazole, DCM, rt, 2 h, 95%; (c) *n*-BuLi, DMF, THF, -78 °C, 15 min, 90%; (d) *tert*-butyl (2-bromo-4-chlorobenzyl)carbamate, *n*-BuLi, THF, -78 °C, 15 min, 43%; (e) MsCl, DIPEA, DCM, rt, 0.5 h, 60%; (f) TBAF, THF, rt, 15 min, 89%; (g) MnO_2, DCM, rt, 20 h, 84%.

Step 1: (4-Bromo-5-chloro-2-thienyl)methanol (I-130). To a solution of (4-bromo-2-thienyl)methanol (2.5 g, 12.9 mmol) in DMF (5.0 mL) was added NCS (2.42 g, 18.1 mmol) and the reaction was heated to 60 °C and stirred for 3 h. Water was added and the mixture was extracted with EtOAc three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to provide **I-130** (1.8 g, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82 (s, 1H), 4.73 (s, 2H), 2.00–1.91 (m, 1H).

Step 2: [(4-Bromo-5-chloro-2-thienyl)methoxy](*tert*-butyl)dimethylsilane (I-131). The title compound was prepared by the method described for the synthesis of intermediate **I-127** in Step 1 using intermediate **I-130**. ^{1}H NMR ($CDCl_3$) δ 6.71 (s, 1H), 4.76 (d, 2H, J = 1.0 Hz), 0.93 (s, 9H), 0.11 (s, 6H).

Step 3: 5-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-2-chlorothiophene-3-carbaldehyde (I-132). To a solution of [(4-bromo-5-chloro-2-thienyl)methoxy](*tert*-butyl)dimethylsilane (4.0 g, 11.7 mmol) in THF (100 mL) cooled to -78 °C was added 2.50 M of *n*-BuLi in hexane (5.15 mL, 12.9 mmol) dropwise and mixture was stirred for 30 min at -78 °C. To this solution was then added DMF (1.36 mL, 17.6 mmol) and the reaction mixture was stirred for 15 min at -78 °C. The reaction was warmed to 0 °C and quenched by the addition of saturated NH_4Cl (100 mL) and extracted with EtOAc (100 mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to provide **I-132** (3.06 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.86 (s, 1H), 6.99 (s, 1H), 4.67 (d, 2H, J = 1.0 Hz) 0.82 (s, 9H), 0.00 (s, 6H).

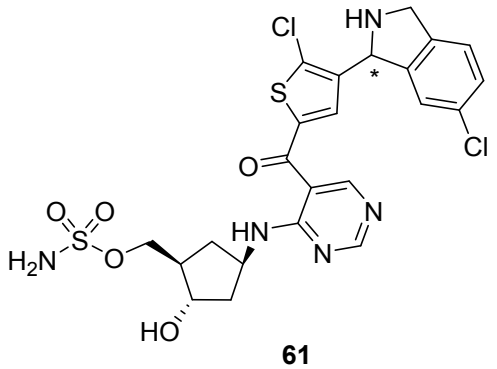
Step 4: *tert*-Butyl (2-{[5-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-2-chloro-3-thienyl](hydroxy)methyl}-4-chlorobenzyl)carbamate (I-133). To a solution of *tert*-butyl (2-bromo-4-chlorobenzyl)carbamate (700 mg, 2.18 mmol) in THF (20 mL) cooled to -78 °C was added 2.50 M of *n*-BuLi in hexane (1.75 mL, 4.37 mmol) dropwise and mixture was stirred for 20 min at -78 °C. To this solution was then added 5-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-2-chlorothiophene-3-carbaldehyde (605 mg, 2.08 mmol) in THF (5.0 mL) and the reaction mixture

was stirred for 15 min at -78 °C. The reaction was quenched by the addition of saturated NH_4Cl (100 mL) and extracted with EtOAc (100 mL) three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to provide **I-133** (501 mg, 43%). LCMS (FA): *m/z* = 554.3 (M+Na).

Step 5: *tert*-Butyl 1-[5-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-2-chloro-3-thienyl]-6-chloro-1,3-dihydro-2*H*-isoindole-2-carboxylate (I-134). To a solution of *tert*-butyl (2-{[5-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-2-chloro-3-thienyl](hydroxy)methyl}-4-chlorobenzyl)carbamate (475 mg, 0.89 mmol) in DCM (10 mL) and *N,N*-diisopropylethylamine (0.39 mL, 2.23 mmol) was added MsCl (0.073 mL, 0.94 mmol) and the reaction mixture was stirred for 30 min at rt. The reaction was quenched by the addition of water and extracted with EtOAc three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to provide **I-134** (302 mg, 60%).

Step 6: *tert*-Butyl 6-chloro-1-(2-chloro-5-formyl-3-thienyl)-1,3-dihydro-2*H*-isoindole-2-carboxylate (I-135). To a solution of *tert*-butyl 1-[5-({[*tert*-butyl(dimethyl)silyl]oxy}methyl)-2-chloro-3-thienyl]-6-chloro-1,3-dihydro-2*H*-isoindole-2-carboxylate (295 mg, 0.57 mmol) in THF (5.0 mL) was added a solution of TBAF hydrate (208 mg, 0.745 mmol) in THF (2.0 mL) at rt, and the mixture was stirred for 15 min. The reaction was quenched by addition of water and extracted with EtOAc three times. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica gel to provide the primary alcohol (205 mg, 89%). LCMS (FA): m/z = 422.0 (M+Na). To a flask containing the product from the above reaction (205 mg, 0.51 mmol) in DCM (20 mL) was added MnO_2 (445 mg, 5.12 mmol) at rt, and the mixture was stirred for 20 h. The reaction was filtered through a celite pad and the residual solid was rinsed with DCM several times. The filtrate was concentrated in vacuo and the residue was purified on silica gel to provide **I-135** as a colorless solid (171 mg, 84%). LCMS (FA): m/z = 398.0 (M+H).

Synthesis of 61



61

[(1*R*,2*S*,4*R*)-4-{[5-({5-Chloro-4-[(1*R*)-6-chloro-2,3-dihydro-1*H*-isoindol-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate or [(1*R*,2*S*,4*R*)-4-{[5-({5-Chloro-4-[(1*S*)-6-chloro-2,3-dihydro-1*H*-isoindol-1-yl]-2-thienyl}carbonyl)pyrimidin-4-yl]amino}-2-hydroxycyclopentyl]methyl sulfamate (62).

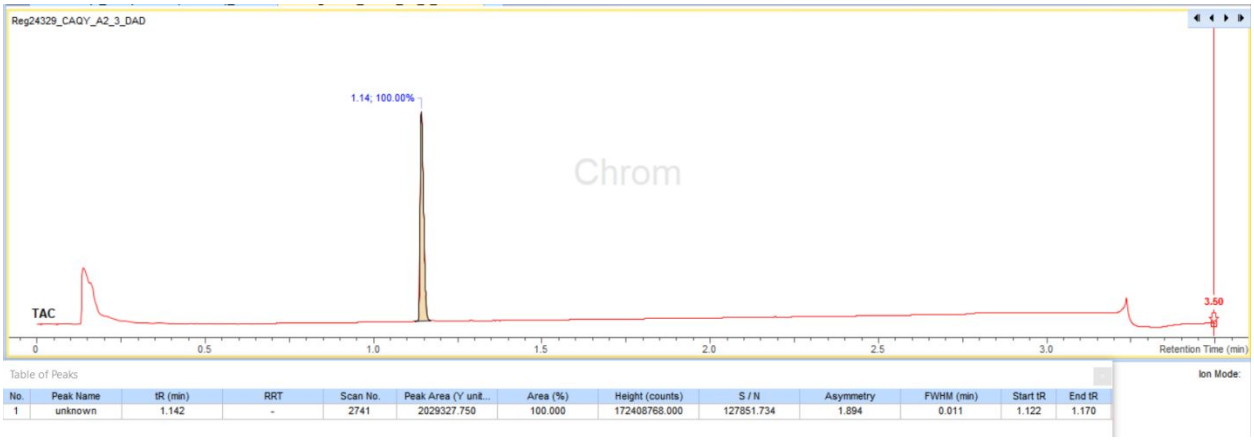
Prepared by the method described for the preparation of **58** starting with intermediate **I-135**. The individual diastereomers were obtained using preparative scale chiral HPLC. More active epimer **61** is reported. ^{1}H NMR (DMSO-d_6) δ 8.60 (d, 2H, J = 6.1 Hz), 8.14 (d, 1H, J = 7.5 Hz), 7.51–7.38 (m, 3H), 7.36 (d, 1H, J = 8.1 Hz), 7.30 (dd, 1H, J = 8.1, 1.6 Hz), 7.04 (s, 1H), 5.59 (s, 1H), 4.94–4.81 (br s, 1H), 4.74–4.61 (m, 1H), 4.30–4.14 (m, 2H), 4.13–4.03 (m, 1H), 4.00–3.86 (m, 2H), 3.80–3.56 (br s, 1H), 2.36–2.20 (m, 1H), 2.15–2.08 (m, 1H), 2.00–1.86 (m, 1H), 1.80–1.69 (m, 1H), 1.31–1.18 (m, 1H); LCMS (FA): m/z = 584.2 (M+H). HRMS m/z $[M+H]^+$ calcd for $C_{23}H_{24}Cl_2N_5O_5S_2$ 584.0596, obsd 584.0602.

HPLC traces of Compounds

Compound purity was determined by analysis of the diode array UV trace of an LC-MS spectrum using the following procedure: compounds were dissolved in DMSO, methanol, or acetonitrile, and the solutions were analyzed using a Hewlett-Packard HP1100 or Agilent 1100 Series LC system connected to a Micromass mass spectrometer using reverse phase C18 columns. One of two gradients was used to elute the compounds: either a formic acid (FA) gradient (acetonitrile containing 0-100% 0.1% formic acid in water) or an ammonium acetate (AA) gradient (acetonitrile containing 0-100% 10 mM ammonium acetate in water). All compounds were determined to be >95% pure unless otherwise noted.

HPLC Chromatogram

Compound **2**: Method (FA), Purity: 100%



HPLC Chromatogram

Compound **3**: Method (FA), Purity: 99.5%

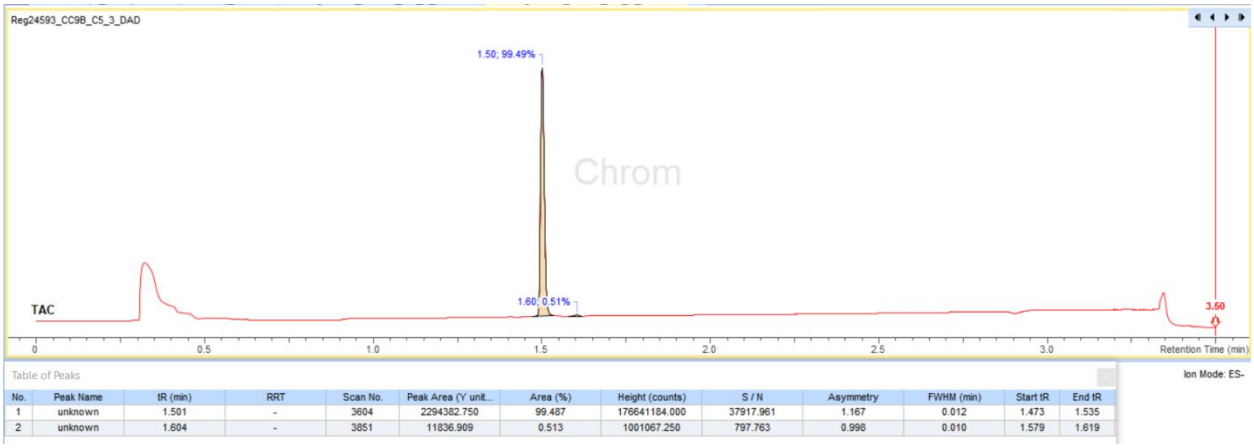


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.501	-	3604	2294382.750	99.487	176641184.000	37917.961	1.167	0.012	1.473	1.535
2	unknown	1.604	-	3851	11836.909	0.513	1001067.250	797.763	0.998	0.010	1.579	1.619

HPLC Chromatogram

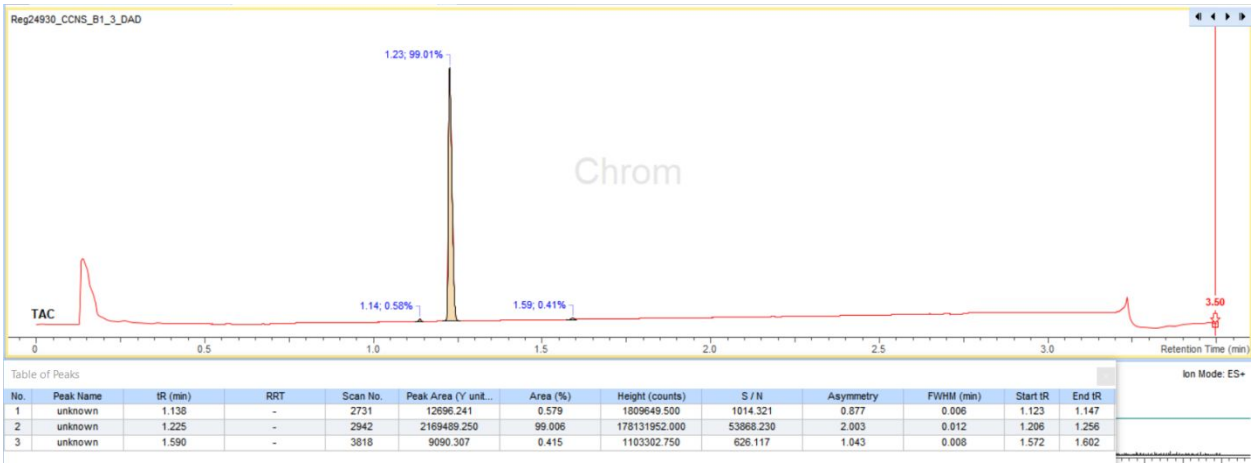
Compound **4**: Method (FA), Purity: 92.5%

Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	0.898	-	2155	42191.125	2.320	5609394.500	1630.445	1.022	0.007	0.887	0.915
2	unknown	0.980	-	2353	1682337.625	92.495	157438128.000	30977.055	1.716	0.010	0.973	0.995
3	unknown	1.000	-	2401	94306.133	5.185	11696892.000	2064.570	-	0.003	0.995	1.006

HPLC Chromatogram

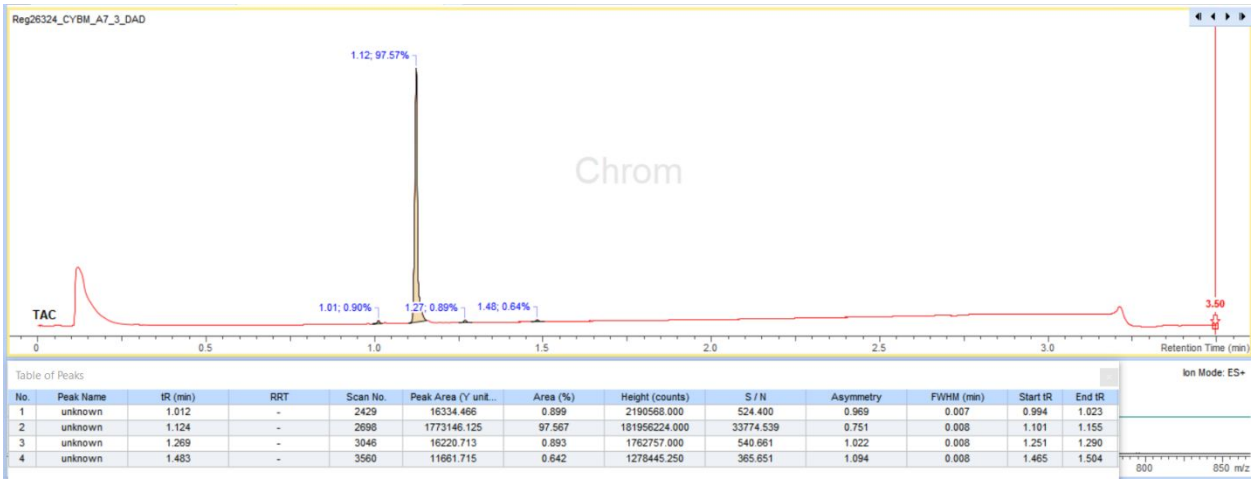
Compound **5**: Method (FA), Purity: 99.0%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.138	-	2731	12696.241	0.579	1809649.500	1014.321	0.877	0.006	1.123	1.147
2	unknown	1.225	-	2942	2169489.250	99.006	178131952.000	53868.230	2.003	0.012	1.206	1.256
3	unknown	1.590	-	3818	9090.307	0.415	1103302.750	626.117	1.043	0.008	1.572	1.602

HPLC Chromatogram

Compound **6**: Method (FA), Purity: 97.6%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.012	-	2429	16334.466	0.899	2190568.000	524.400	0.969	0.007	0.994	1.023
2	unknown	1.124	-	2698	1773146.125	97.567	181956224.000	33774.539	0.751	0.008	1.101	1.155
3	unknown	1.269	-	3046	16220.713	0.893	1762757.000	540.661	1.022	0.008	1.251	1.290
4	unknown	1.483	-	3560	11661.715	0.642	1278445.250	365.651	1.094	0.008	1.465	1.504

HPLC Chromatogram

Compound **7**: Method (FA), Purity: 97.4%

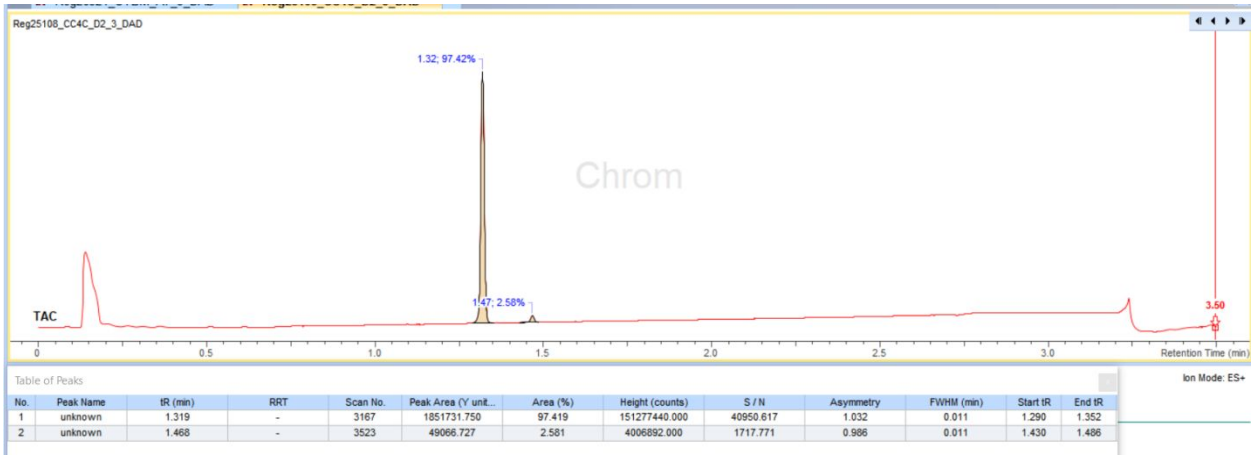


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.319	-	3167	1851731.750	97.419	151277440.000	40950.617	1.032	0.011	1.290	1.352
2	unknown	1.468	-	3523	49066.727	2.581	4006892.000	1717.771	0.986	0.011	1.430	1.486

HPLC Chromatogram

Compound **8**: Method (FA), Purity: 97.2%

Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.301	-	3124	55877.133	2.358	7017681.500	3352.581	1.017	0.007	1.283	1.316
2	unknown	1.404	-	3371	2303185.500	97.202	234913520.000	56626.902	0.748	0.009	1.377	1.425
3	unknown	1.504	-	3610	10414.494	0.440	1244354.250	574.413	0.931	0.008	1.488	1.517

HPLC Chromatogram

Compound **9**: Method (FA), Purity: 96.1%

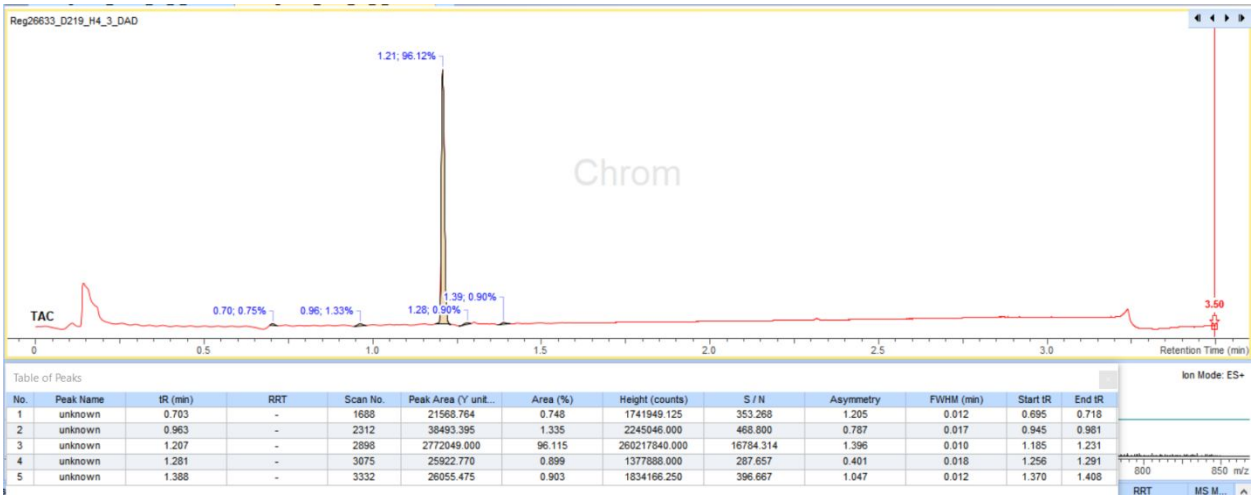


Table of Peaks

Ion Mode: ES+

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	0.703	-	1688	21568.764	0.748	1741949.125	353.268	1.205	0.012	0.695	0.718
2	unknown	0.963	-	2312	38493.395	1.335	2245046.000	468.800	0.787	0.017	0.945	0.981
3	unknown	1.207	-	2898	2772049.000	96.115	260217840.000	16784.314	1.396	0.010	1.185	1.231
4	unknown	1.281	-	3075	25922.770	0.899	1377888.000	287.657	0.401	0.018	1.256	1.291
5	unknown	1.388	-	3332	26055.475	0.903	1834166.250	396.667	1.047	0.012	1.370	1.408

800 850 m/z

RRT MS M...

HPLC Chromatogram

Compound **10**: Method (FA), Purity: 97.6%

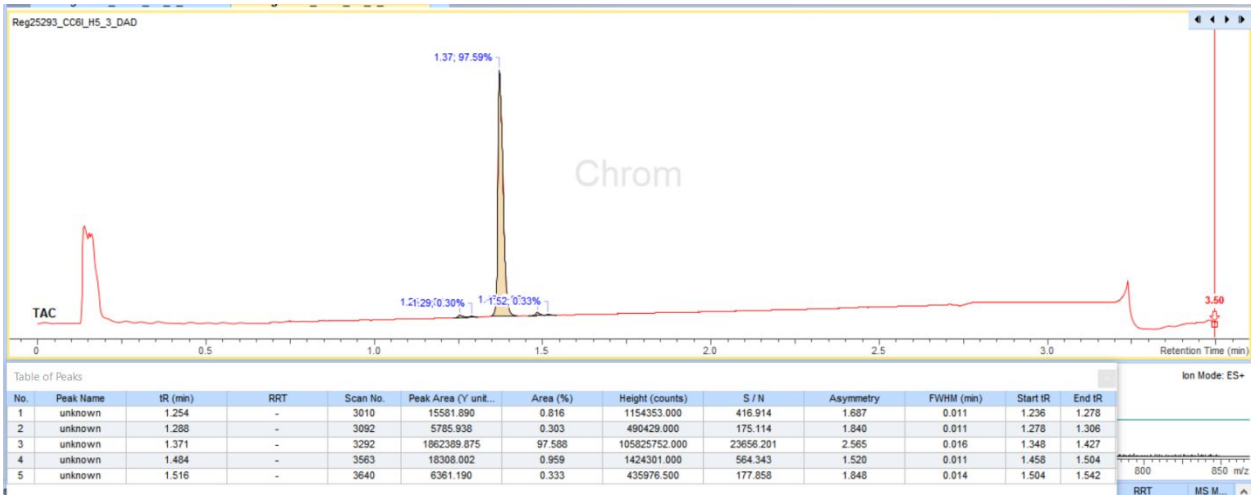


Table of Peaks

Ion Mode: ES+

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.254	-	3010	15581.890	0.816	1154353.000	416.914	1.687	0.011	1.236	1.278
2	unknown	1.288	-	3092	5785.938	0.303	490429.000	175.114	1.840	0.011	1.278	1.306
3	unknown	1.371	-	3292	1862389.875	97.588	105825752.000	23656.201	2.565	0.016	1.348	1.427
4	unknown	1.484	-	3563	18308.002	0.959	1424301.000	564.343	1.520	0.011	1.458	1.504
5	unknown	1.516	-	3640	6361.190	0.333	435976.500	177.858	1.848	0.014	1.504	1.542

800 850 m/z

RRT MS M...

HPLC Chromatogram

Compound **12**: Method (FA), Purity: 98.2%

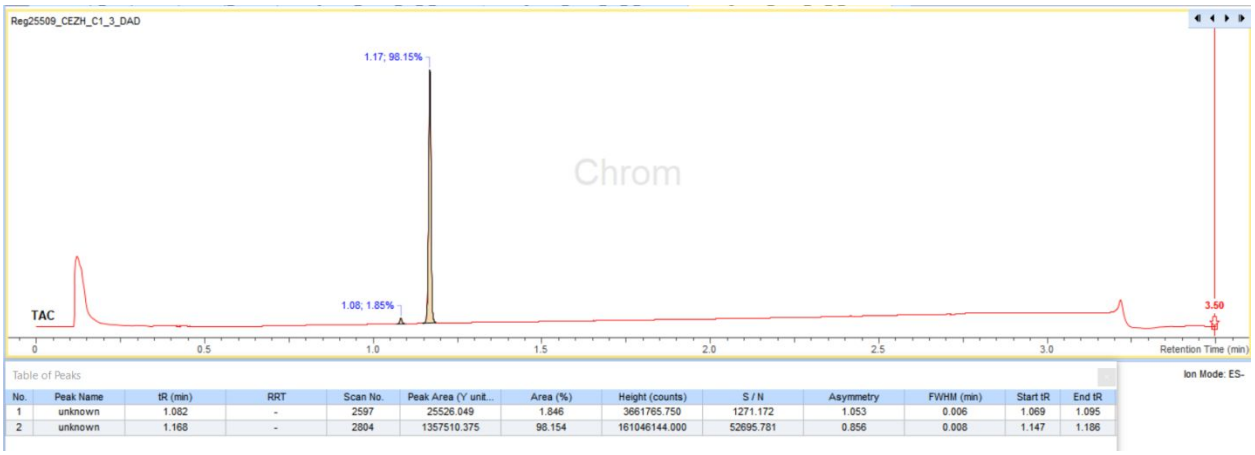


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.082	-	2597	25526.049	1.846	3661765.750	1271.172	1.053	0.006	1.069	1.095
2	unknown	1.168	-	2804	1357510.375	98.154	161046144.000	52695.781	0.856	0.008	1.147	1.186

HPLC Chromatogram

Compound **ML-792**: Method (FA), Purity: 100%

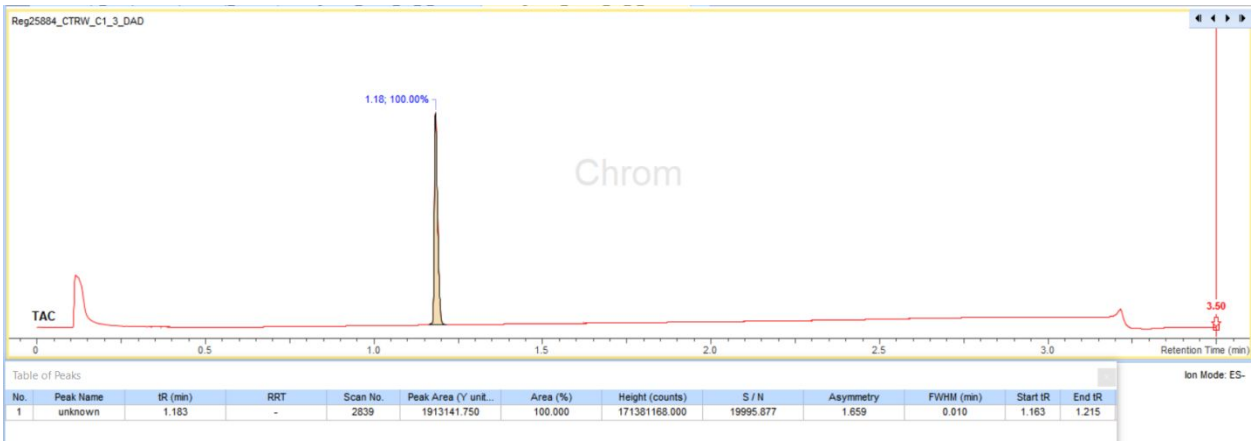


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.183	-	2839	1913141.750	100.000	171381168.000	19995.877	1.659	0.010	1.163	1.215

HPLC Chromatogram

Compound **15**: Method (FA), Purity: 99.3%

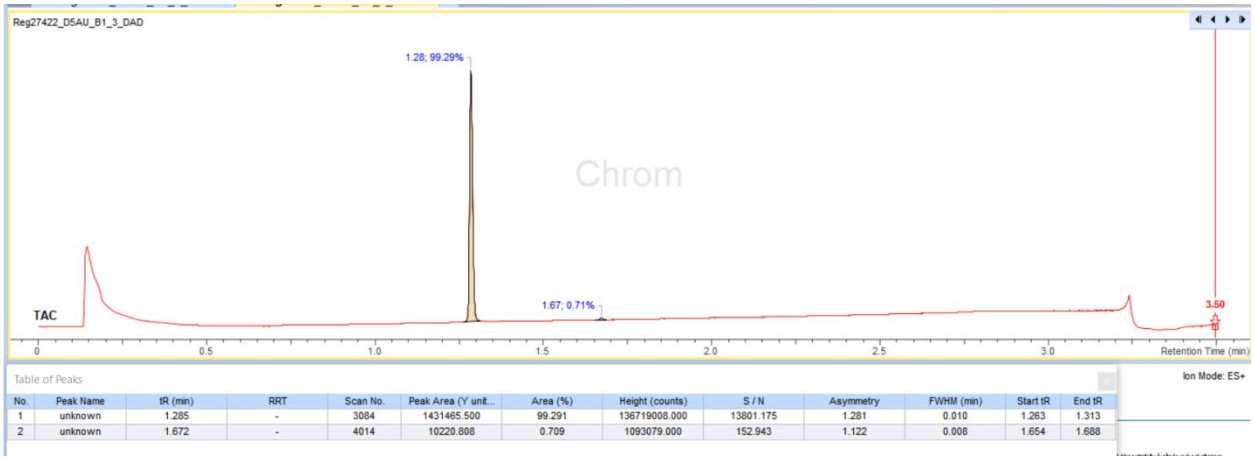


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.285	-	3084	1431465.500	99.291	136719008.000	13801.175	1.281	0.010	1.263	1.313
2	unknown	1.672	-	4014	10220.808	0.709	1093079.000	152.943	1.122	0.008	1.654	1.688

HPLC Chromatogram

Compound **16**: Method (FA), Purity: 99.6%

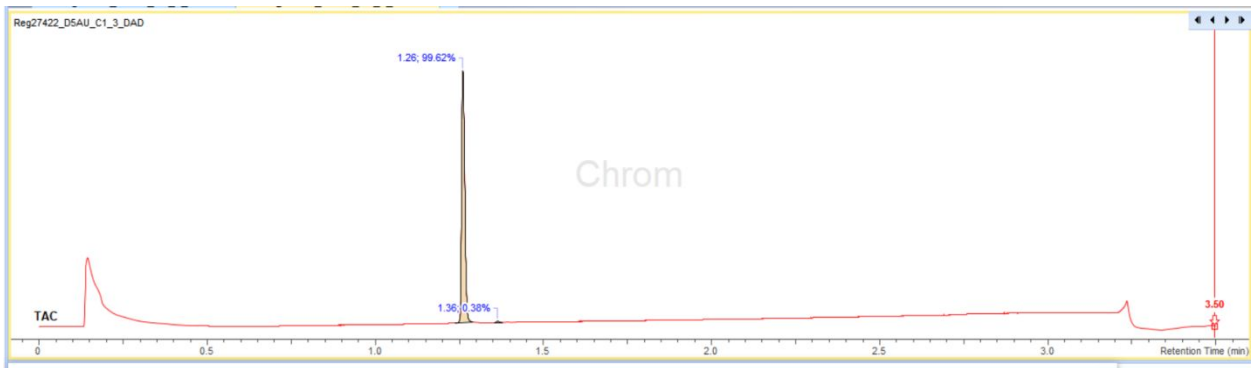


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.260	-	3026	1735983.250	99.624	157783904.000	16753.902	1.359	0.010	1.238	1.291
2	unknown	1.364	-	3274	6550.918	0.376	819054.750	86.754	1.229	0.007	1.355	1.378

HPLC Chromatogram

Compound **17**: Method (FA), Purity: 99.0%

Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.229	-	2950	1530819.875	99.001	17778040.000	38042.551	0.784	0.008	1.208	1.247
2	unknown	1.281	-	3075	10235.702	0.662	978954.500	328.187	1.422	0.009	1.262	1.304
3	unknown	1.339	-	3215	5207.018	0.337	730689.500	267.344	1.069	0.006	1.330	1.352

HPLC Chromatogram

Compound **18**: Method (FA), Purity: 97.1%

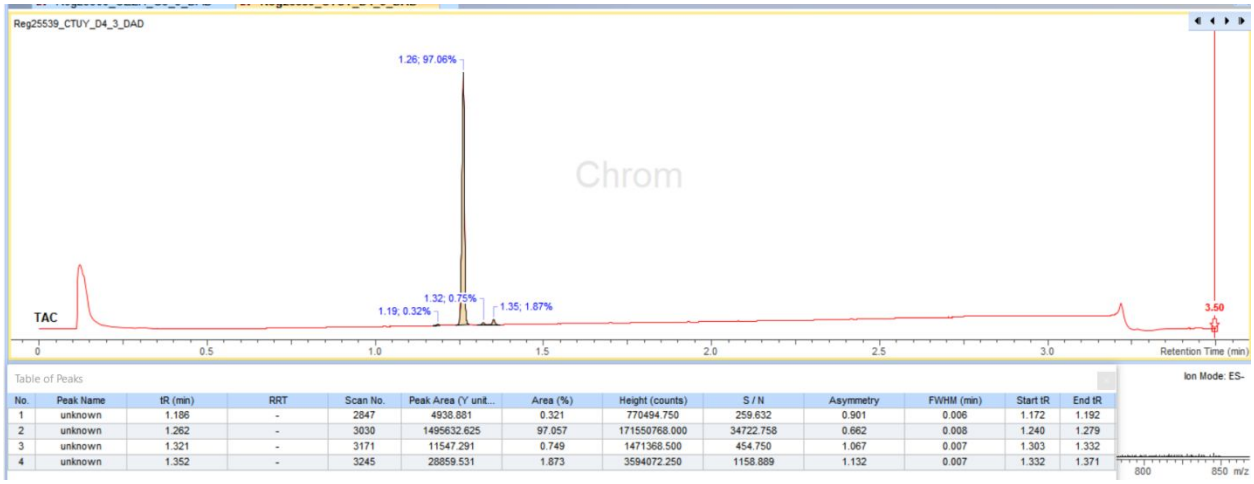
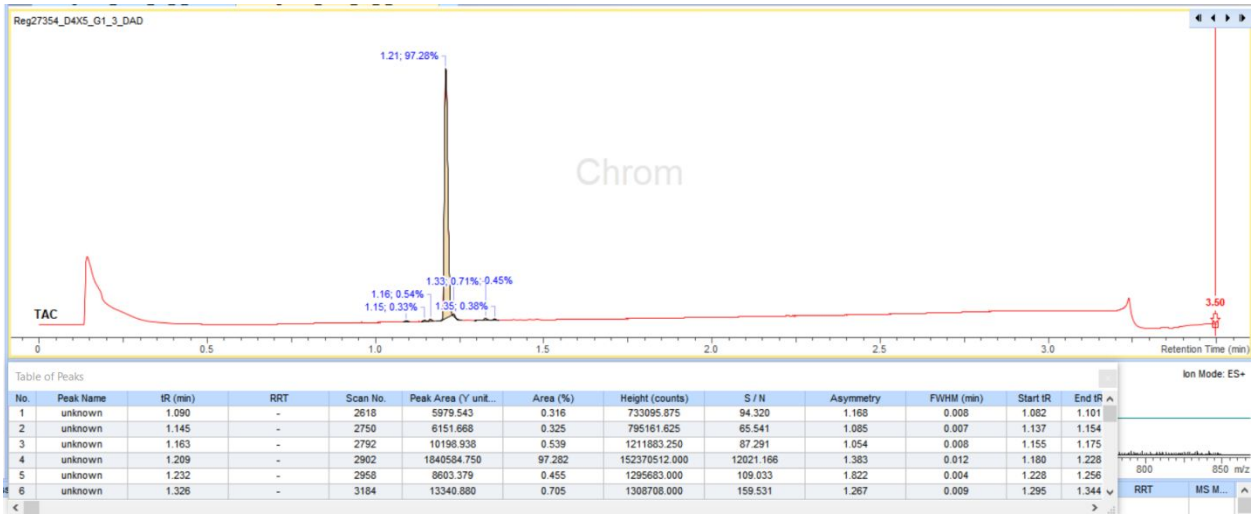


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.186	-	2847	4938.881	0.321	770494.750	259.632	0.901	0.006	1.172	1.192
2	unknown	1.262	-	3030	1495632.625	97.057	171550768.000	34722.758	0.662	0.008	1.240	1.279
3	unknown	1.321	-	3171	11547.291	0.749	1471368.500	454.750	1.067	0.007	1.303	1.332
4	unknown	1.352	-	3245	28859.531	1.873	3594072.250	1158.889	1.132	0.007	1.332	1.371

HPLC Chromatogram

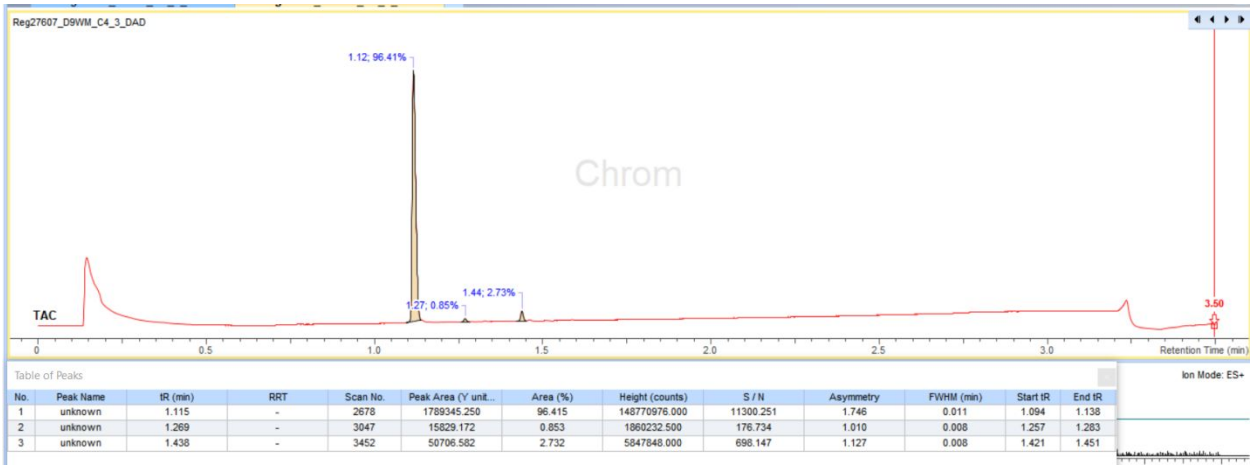
Compound **19**: Method (FA), Purity: 97.3%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.090	-	2618	5979.543	0.316	733095.875	94.320	1.168	0.008	1.082	1.101
2	unknown	1.145	-	2750	6151.668	0.325	795161.625	65.541	1.085	0.007	1.137	1.154
3	unknown	1.163	-	2792	10198.938	0.539	1211883.250	87.291	1.054	0.008	1.155	1.175
4	unknown	1.209	-	2902	1840584.750	97.282	152370512.000	12021.166	1.383	0.012	1.180	1.228
5	unknown	1.232	-	2958	8603.379	0.455	1295683.000	109.033	1.822	0.004	1.228	1.256
6	unknown	1.326	-	3184	13340.880	0.705	1308708.000	159.531	1.267	0.009	1.295	1.344

HPLC Chromatogram

Compound **21**: Method (FA), Purity: 96.4%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.115	-	2678	1789345.250	96.415	148770976.000	11300.251	1.746	0.011	1.094	1.138
2	unknown	1.269	-	3047	15829.172	0.853	1860232.500	176.734	1.010	0.008	1.257	1.283
3	unknown	1.438	-	3452	50706.582	2.732	5847848.000	698.147	1.127	0.008	1.421	1.451

HPLC Chromatogram

Compound **22**: Method (FA), Purity: 99.8%

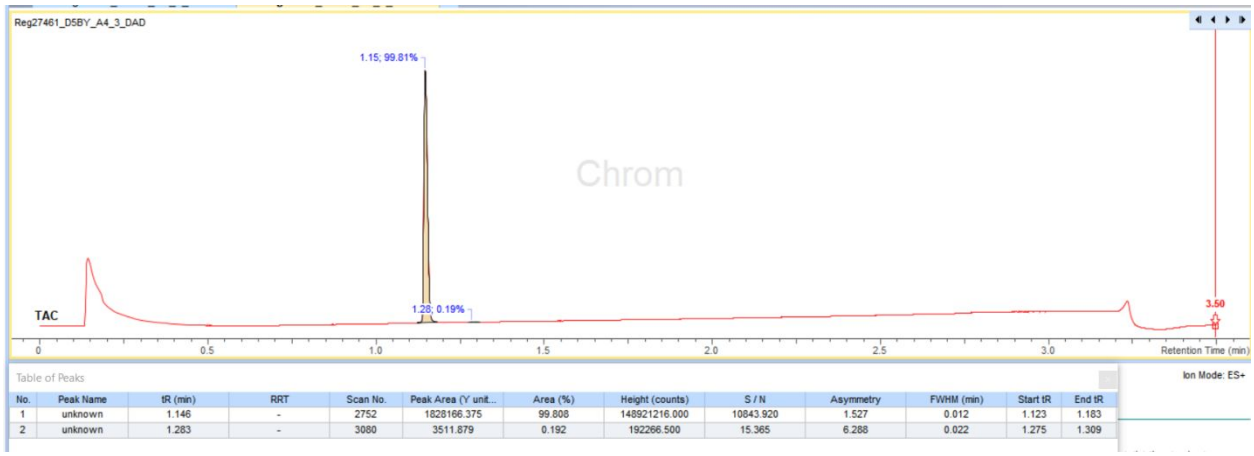


Table of Peaks

Ion Mode: ES+

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.146	-	2752	1828166.375	99.808	148921216.000	10843.920	1.527	0.012	1.123	1.183
2	unknown	1.283	-	3080	3511.879	0.192	192266.500	15.365	6.288	0.022	1.275	1.309

HPLC Chromatogram

Compound **23**: Method (FA), Purity: 98.3%

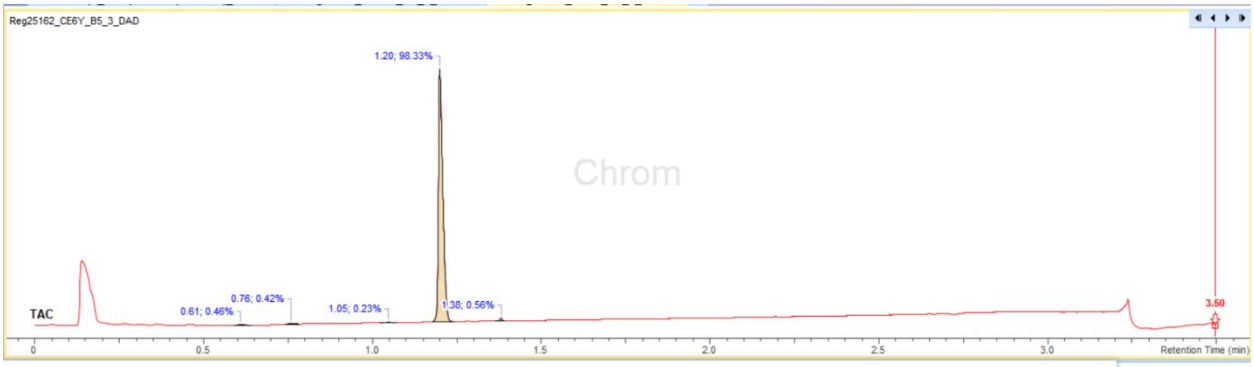


Table of Peaks

Ion Mode: ES+

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	0.609	-	1463	12022.328	0.459	548037.688	258.400	1.399	0.020	0.590	0.641
2	unknown	0.759	-	1823	10943.568	0.418	544250.313	229.044	1.109	0.020	0.740	0.782
3	unknown	1.048	-	2517	5991.782	0.229	452343.500	165.233	0.839	0.009	1.023	1.071
4	unknown	1.199	-	2879	2574647.750	98.335	172048704.000	28426.254	2.280	0.014	1.178	1.242
5	unknown	1.380	-	3314	14639.810	0.559	1914963.500	719.296	0.956	0.007	1.364	1.391

HPLC Chromatogram

Compound **24**: Method (FA), Purity: 99.4%

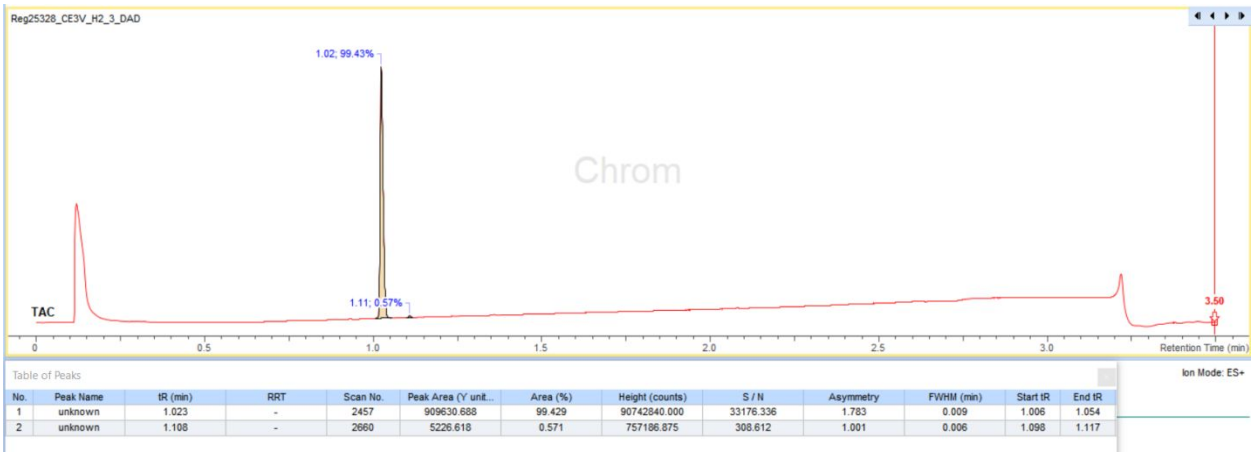


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.023	-	2457	909630.688	99.429	90742840.000	33176.336	1.783	0.009	1.006	1.054
2	unknown	1.108	-	2660	5226.618	0.571	757186.875	308.612	1.001	0.006	1.098	1.117

HPLC Chromatogram

Compound **25**: Method (FA), Purity: 95.3%

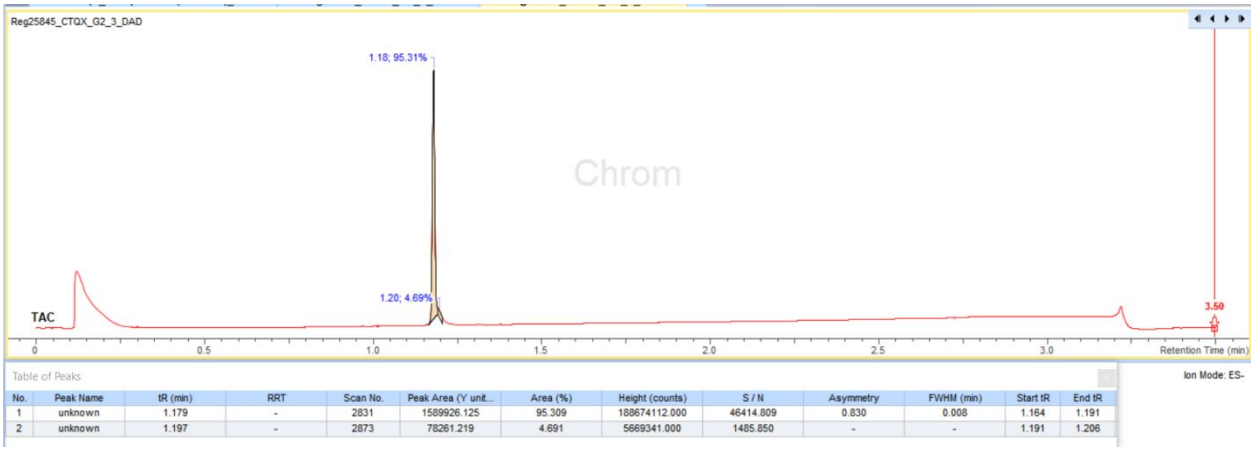


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.179	-	2831	1589926.125	95.309	188674112.000	46414.809	0.830	0.008	1.164	1.191
2	unknown	1.197	-	2873	78261.219	4.691	5669341.000	1485.850	-	-	1.191	1.206

HPLC Chromatogram

Compound **26**: Method (FA), Purity: 98.9%

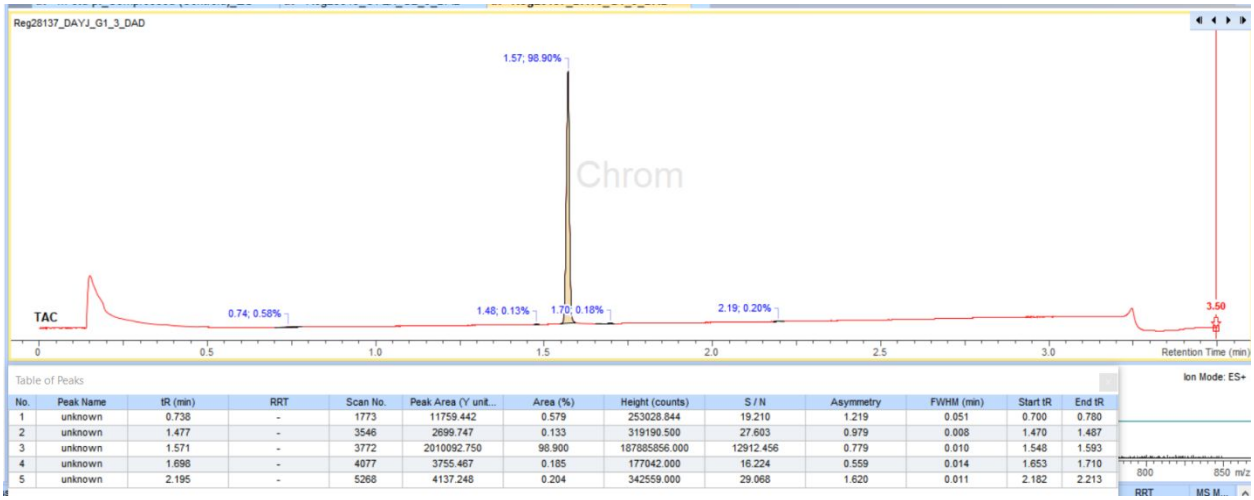


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	0.738	-	1773	11759.442	0.579	253028.844	19.210	1.219	0.051	0.700	0.780
2	unknown	1.477	-	3546	2699.747	0.133	319190.500	27.603	0.979	0.008	1.470	1.487
3	unknown	1.571	-	3772	2010092.750	98.900	187885856.000	12912.456	0.779	0.010	1.548	1.593
4	unknown	1.698	-	4077	3755.467	0.185	177042.000	16.224	0.559	0.014	1.653	1.710
5	unknown	2.195	-	5268	4137.248	0.204	342559.000	29.068	1.620	0.011	2.182	2.213

HPLC Chromatogram

Compound **28**: Method (FA), Purity: 95.3%

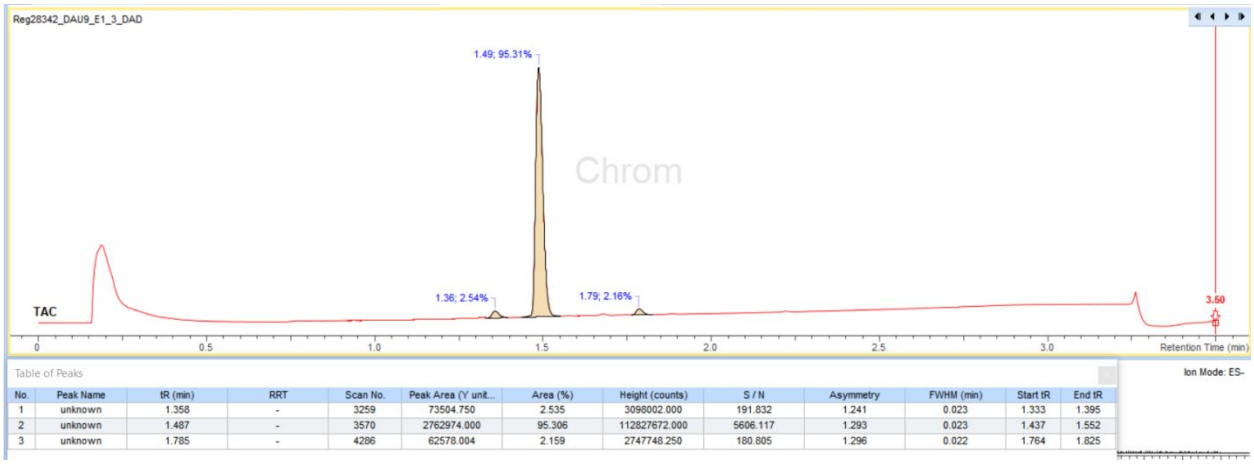
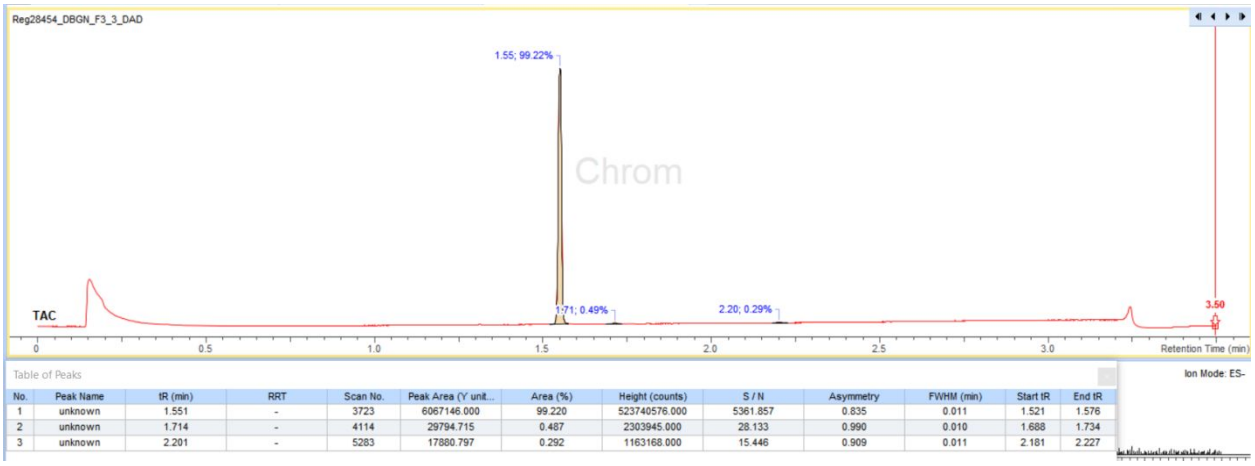


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.358	-	3259	73504.750	2.535	3098002.000	191.832	1.241	0.023	1.333	1.395
2	unknown	1.487	-	3570	2762974.000	95.306	112827672.000	5606.117	1.293	0.023	1.437	1.552
3	unknown	1.785	-	4286	62578.004	2.159	2747748.250	180.805	1.296	0.022	1.764	1.825

HPLC Chromatogram

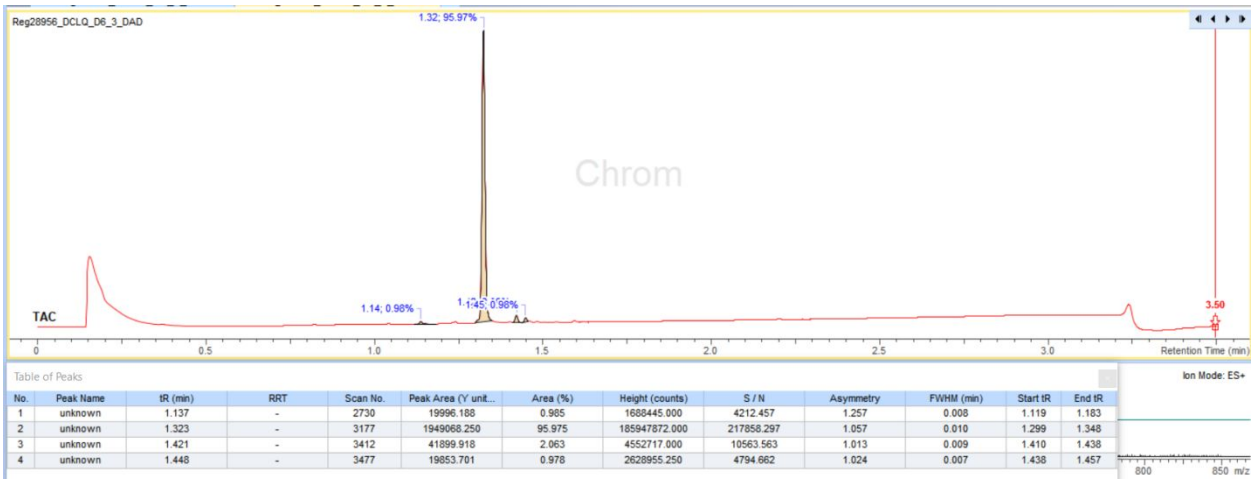
Compound **29**: Method (FA), Purity: 99.2%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.551	-	3723	6067146.000	99.220	523740576.000	5361.857	0.835	0.011	1.521	1.576
2	unknown	1.714	-	4114	29794.715	0.487	2303945.000	28.133	0.990	0.010	1.688	1.734
3	unknown	2.201	-	5283	17880.797	0.292	1163168.000	15.446	0.909	0.011	2.181	2.227

HPLC Chromatogram

Compound **30**: Method (FA), Purity: 96.0%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.137	-	2730	19996.188	0.985	1688445.000	4212.457	1.257	0.008	1.119	1.183
2	unknown	1.323	-	3177	1949068.250	95.975	185947872.000	217858.297	1.057	0.010	1.299	1.348
3	unknown	1.421	-	3412	41899.918	2.063	4552717.000	10563.563	1.013	0.009	1.410	1.438
4	unknown	1.448	-	3477	19853.701	0.978	2628955.250	4794.662	1.024	0.007	1.438	1.457

HPLC Chromatogram

Compound **31**: Method (FA), Purity: 95.1%

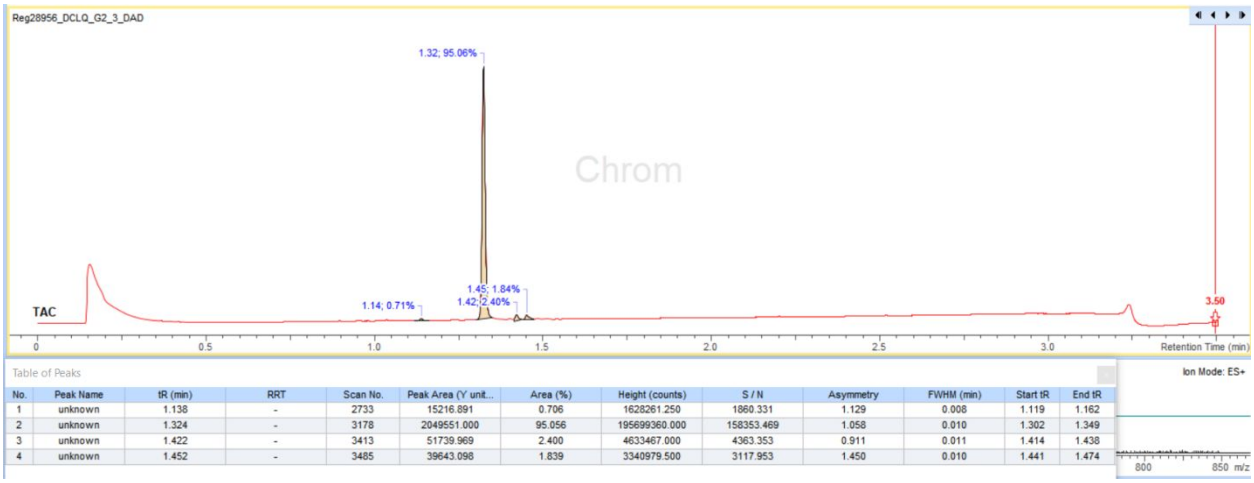


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.138	-	2733	15216.891	0.706	1628261.250	1860.331	1.129	0.008	1.119	1.162
2	unknown	1.324	-	3178	2049551.000	95.056	195699360.000	158353.469	1.058	0.010	1.302	1.349
3	unknown	1.422	-	3413	51739.969	2.400	4633467.000	4363.353	0.911	0.011	1.414	1.438
4	unknown	1.452	-	3485	39643.098	1.839	3340979.500	3117.953	1.450	0.010	1.441	1.474

HPLC Chromatogram

Compound **32**: Method (FA), Purity: 94.9%

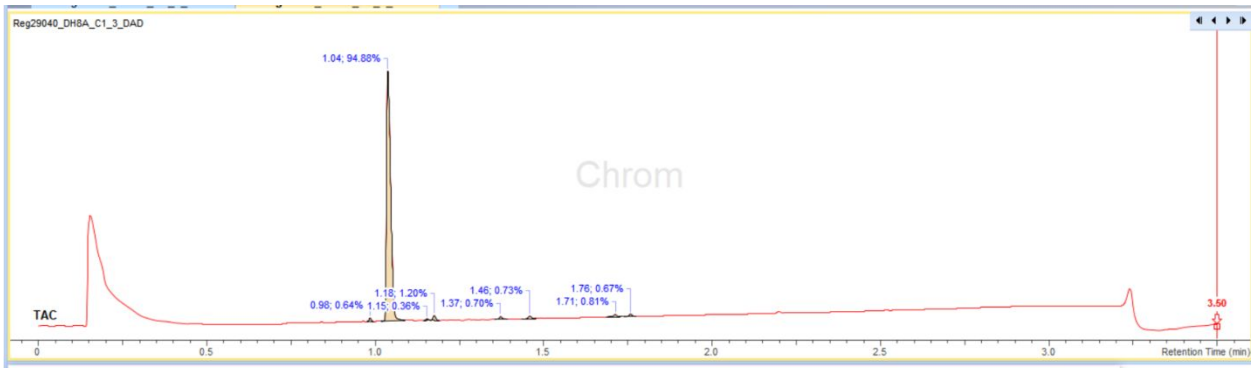
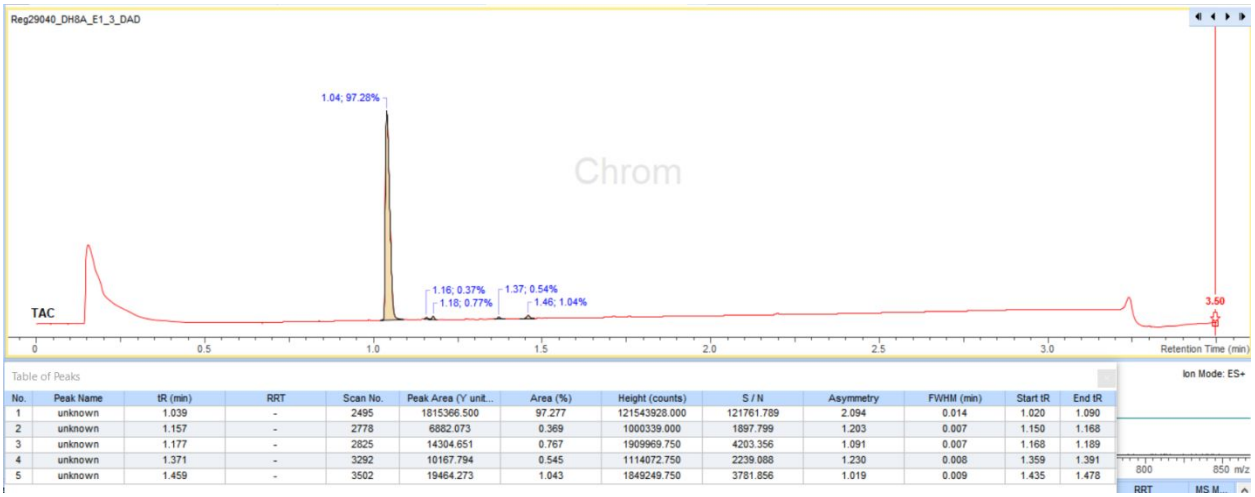


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	0.985	-	2364	9916.237	0.643	1435054.250	4200.828	1.052	0.006	0.976	0.995
2	unknown	1.037	-	2490	1463381.500	94.879	103548248.000	104646.273	2.134	0.014	1.018	1.089
3	unknown	1.154	-	2770	5577.096	0.362	699659.000	1814.192	1.076	0.007	1.144	1.165
4	unknown	1.175	-	2821	18578.051	1.205	2000470.000	4227.586	1.063	0.009	1.165	1.190
5	unknown	1.372	-	3294	10795.813	0.700	1063413.250	2242.028	1.191	0.008	1.355	1.393
6	unknown	1.459	-	3502	11287.454	0.732	1088742.500	2628.172	1.064	0.009	1.435	1.477
7	unknown	1.712	-	4111	12558.794	0.814	917818.500	1800.414	0.953	0.010	1.686	1.734
8	unknown	1.758	-	4220	10275.283	0.666	1002717.000	2220.367	1.062	0.009	1.738	1.774

HPLC Chromatogram

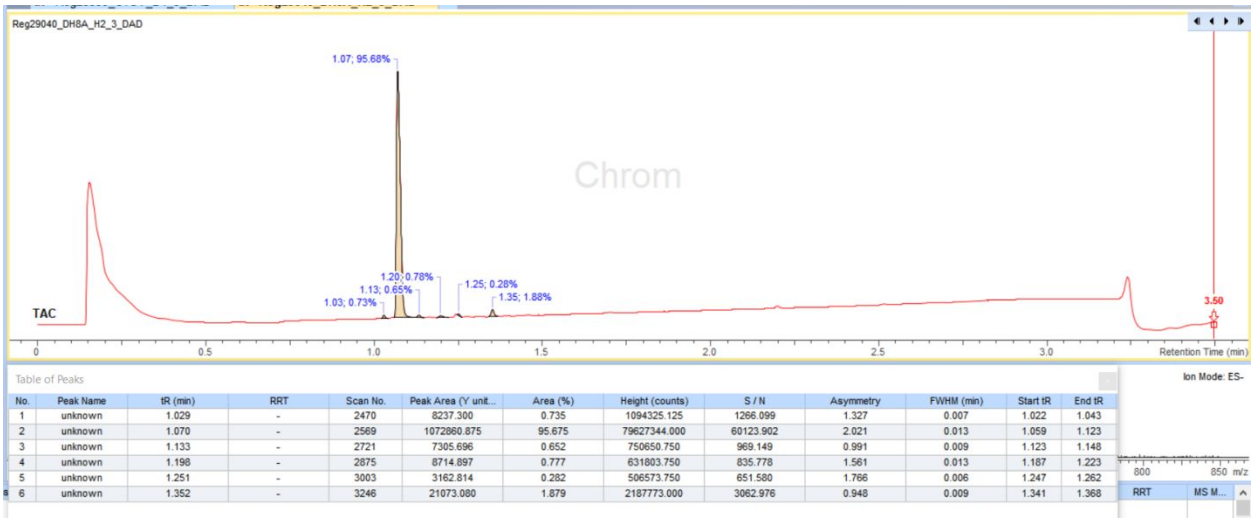
Compound **33**: Method (FA), Purity: 97.3%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.039	-	2495	1815366.500	97.277	121543928.000	121761.789	2.094	0.014	1.020	1.090
2	unknown	1.157	-	2778	6882.073	0.369	1000339.000	1897.799	1.203	0.007	1.150	1.168
3	unknown	1.177	-	2825	14304.651	0.767	1909969.750	4203.356	1.091	0.007	1.168	1.189
4	unknown	1.371	-	3292	10167.794	0.545	1114072.750	2239.088	1.230	0.008	1.359	1.391
5	unknown	1.459	-	3502	19464.273	1.043	1849249.750	3781.856	1.019	0.009	1.435	1.478

HPLC Chromatogram

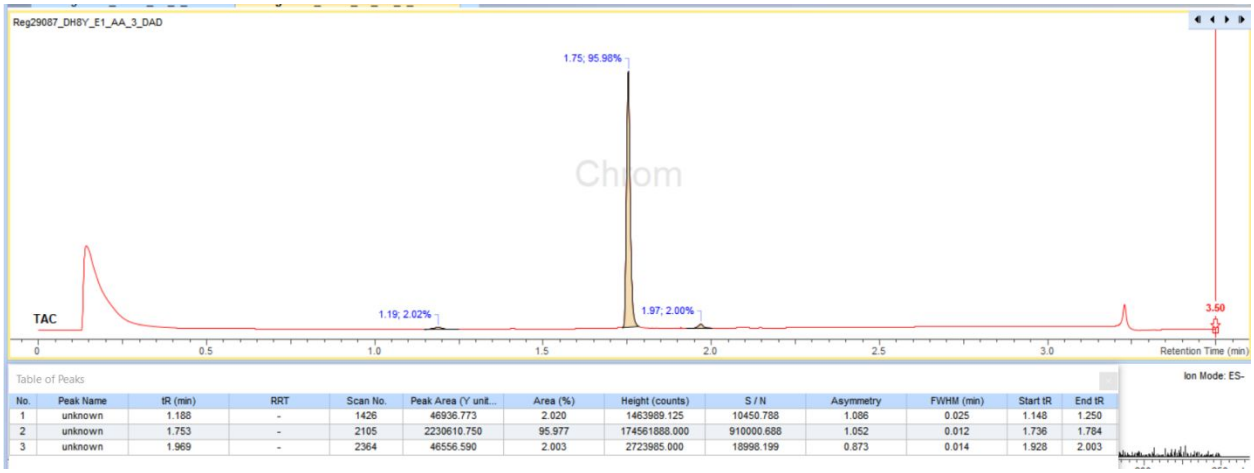
Compound **34**: Method (FA), Purity: 95.7%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.029	-	2470	8237.300	0.735	1094325.125	1266.099	1.327	0.007	1.022	1.043
2	unknown	1.070	-	2569	1072860.875	95.675	79627344.000	60123.902	2.021	0.013	1.059	1.123
3	unknown	1.133	-	2721	7305.696	0.652	750650.750	969.149	0.991	0.009	1.123	1.148
4	unknown	1.198	-	2875	8714.897	0.777	631803.750	835.778	1.561	0.013	1.187	1.223
5	unknown	1.251	-	3003	3162.814	0.282	506573.750	651.580	1.766	0.006	1.247	1.262
6	unknown	1.352	-	3246	21073.080	1.879	2187773.000	3062.976	0.948	0.009	1.341	1.368

HPLC Chromatogram

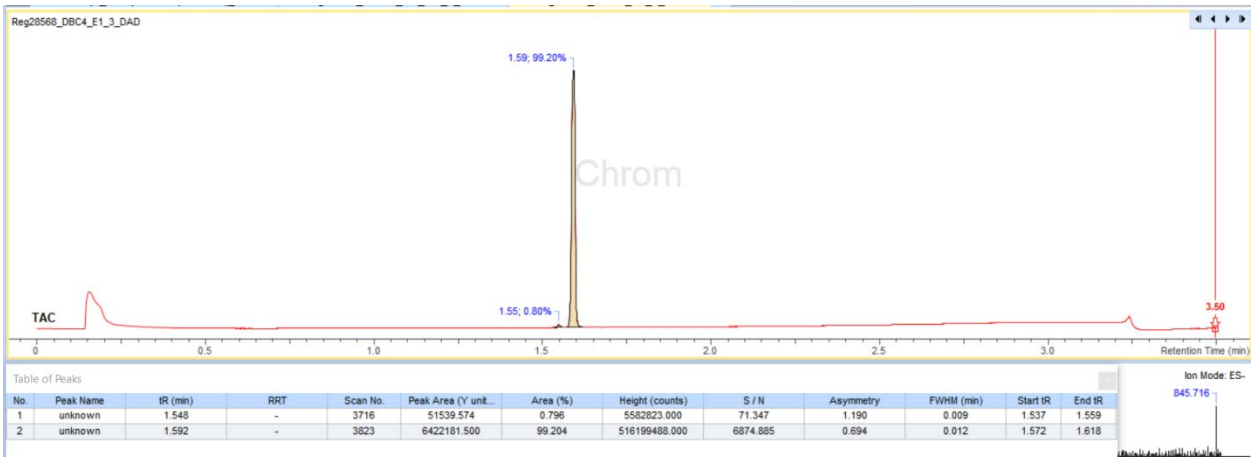
Compound **35**: Method (AA), Purity: 96.0%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.188	-	1426	46936.773	2.020	1463989.125	10450.788	1.086	0.025	1.148	1.250
2	unknown	1.753	-	2105	2230610.750	95.977	174561888.000	910000.688	1.052	0.012	1.736	1.784
3	unknown	1.969	-	2364	46556.590	2.003	2723985.000	18998.199	0.873	0.014	1.928	2.003

HPLC Chromatogram

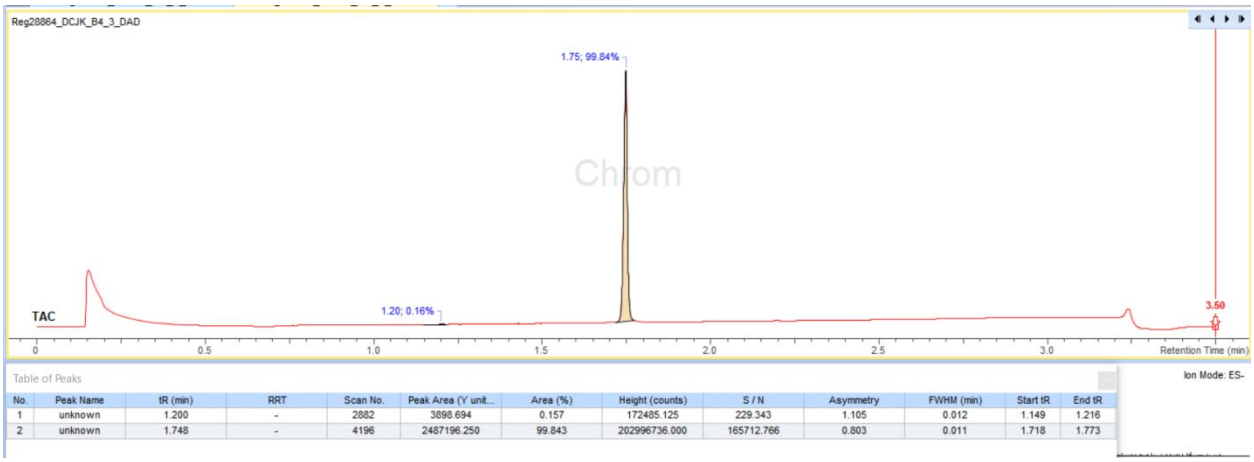
Compound **36**: Method (FA), Purity: 99.2%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.548	-	3716	51539.574	0.796	5582823.000	71.347	1.190	0.009	1.537	1.559
2	unknown	1.592	-	3823	6422181.500	99.204	516199488.000	6874.885	0.694	0.012	1.572	1.618

HPLC Chromatogram

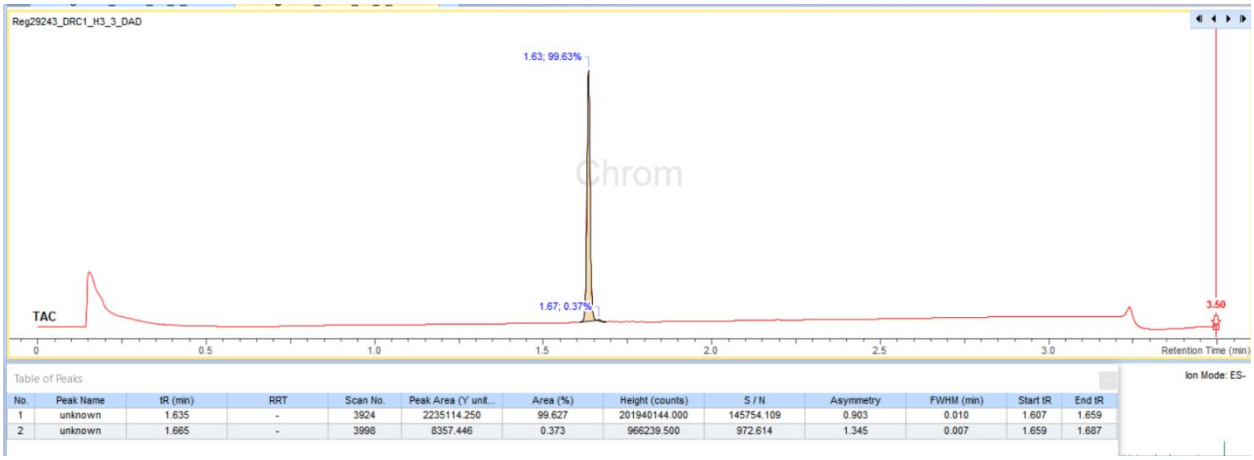
Compound **37**: Method (FA), Purity: 99.8%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.200	-	2882	3898.694	0.157	172485.125	229.343	1.105	0.012	1.149	1.216
2	unknown	1.748	-	4196	2487196.250	99.843	202996736.000	165712.766	0.803	0.011	1.718	1.773

HPLC Chromatogram

Compound **38**: Method (FA), Purity: 99.6%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.635	-	3924	2235114.250	99.627	201940144.000	145754.109	0.903	0.010	1.607	1.659
2	unknown	1.665	-	3998	8357.446	0.373	966239.500	972.614	1.345	0.007	1.659	1.687

HPLC Chromatogram

Compound **39**: Method (FA), Purity: 98.5%

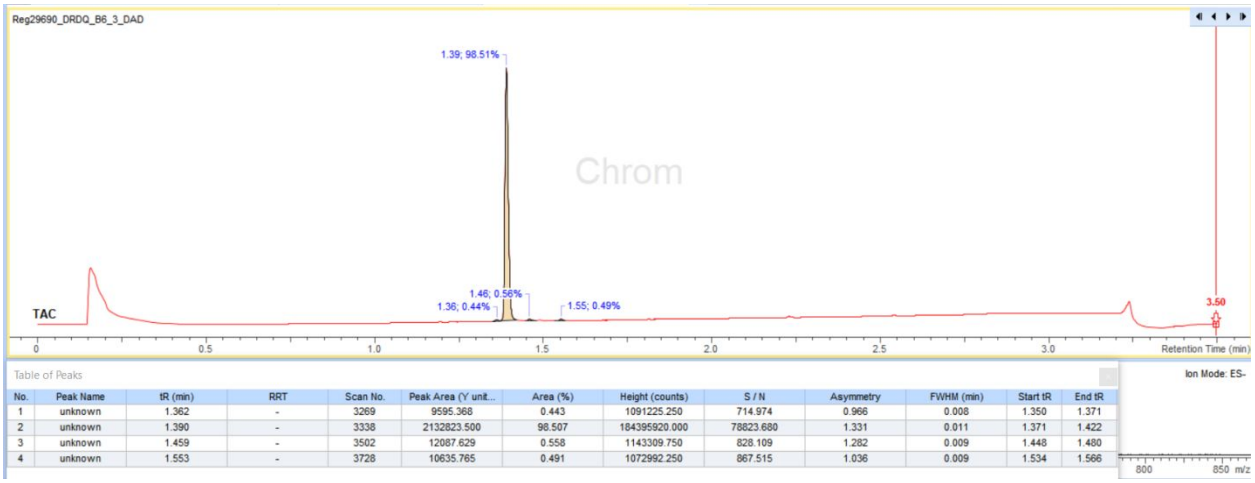


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.362	-	3269	9595.368	0.443	1091225.250	714.974	0.966	0.008	1.350	1.371
2	unknown	1.390	-	3338	2132823.500	98.507	184395920.000	78823.680	1.331	0.011	1.371	1.422
3	unknown	1.459	-	3502	12087.629	0.558	1143309.750	828.109	1.282	0.009	1.448	1.480
4	unknown	1.553	-	3728	10635.765	0.491	1072992.250	867.515	1.036	0.009	1.534	1.566

HPLC Chromatogram

Compound **40**: Method (FA), Purity: 99.7%

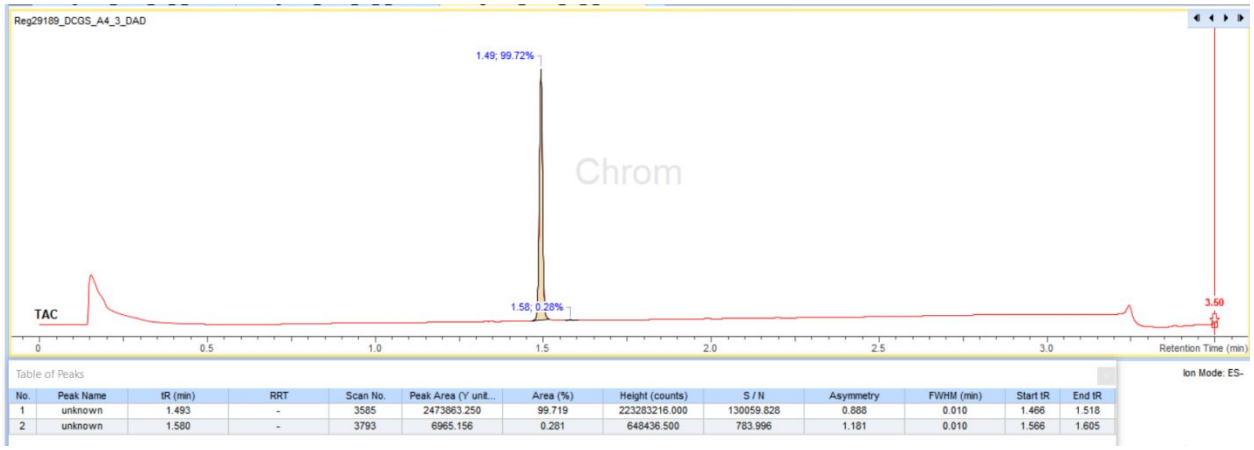


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.493	-	3585	2473863.250	99.719	223283216.000	130059.828	0.888	0.010	1.466	1.518
2	unknown	1.580	-	3793	6965.156	0.281	648436.500	783.996	1.181	0.010	1.566	1.605

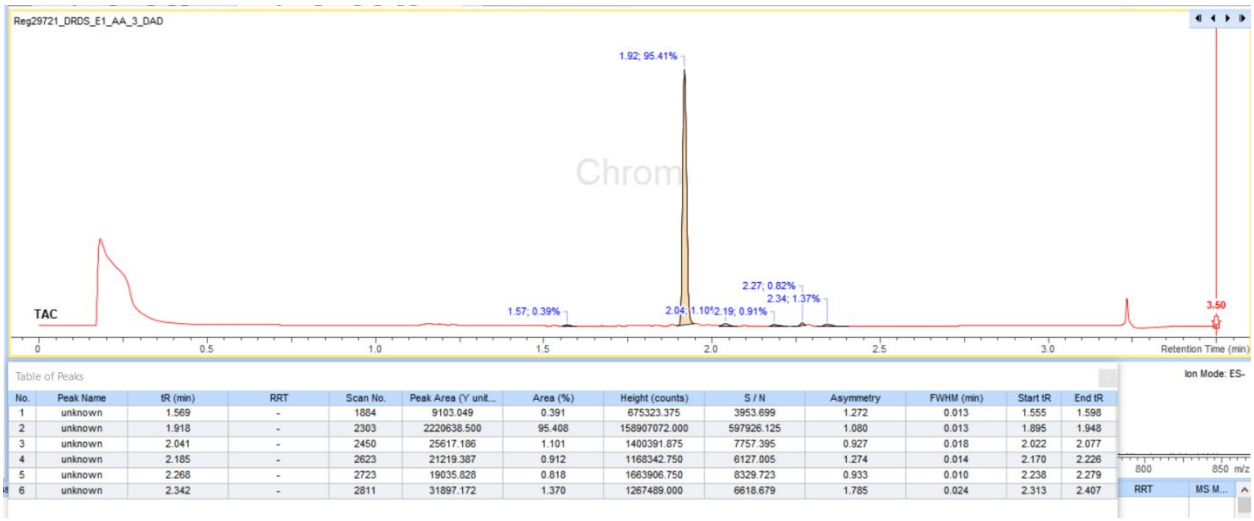
HPLC Chromatogram

Compound **41**: Method (FA), Purity: 95.1%

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	0.988	-	2373	2345930.750	95.079	106263176.000	33603.633	1.767	0.021	0.943	1.052
2	unknown	1.121	-	2691	20979.541	0.850	1671432.000	916.321	1.618	0.010	1.112	1.144
3	unknown	1.156	-	2776	40436.742	1.639	3198626.250	1716.853	1.105	0.011	1.144	1.196
4	unknown	1.281	-	3075	60001.098	2.432	5080903.000	3631.550	1.186	0.010	1.251	1.310

HPLC Chromatogram

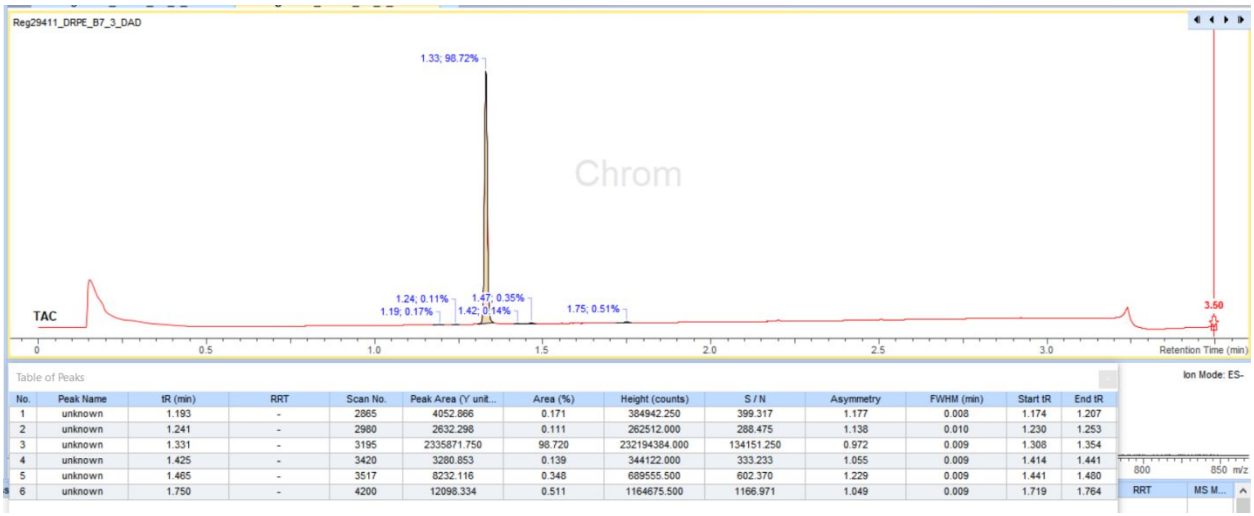
Compound **42**: Method (AA), Purity: 95.4%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.569	-	1884	9103.049	0.391	675323.375	3953.699	1.272	0.013	1.555	1.598
2	unknown	1.918	-	2303	2220638.500	95.408	158907072.000	597926.125	1.080	0.013	1.895	1.948
3	unknown	2.041	-	2450	25617.186	1.101	1400391.875	7757.395	0.927	0.018	2.022	2.077
4	unknown	2.185	-	2623	21219.387	0.912	1168342.750	6127.005	1.274	0.014	2.170	2.226
5	unknown	2.268	-	2723	19035.828	0.818	1663906.750	8329.723	0.933	0.010	2.238	2.279
6	unknown	2.342	-	2811	31897.172	1.370	1267489.000	6618.679	1.785	0.024	2.313	2.407

HPLC Chromatogram

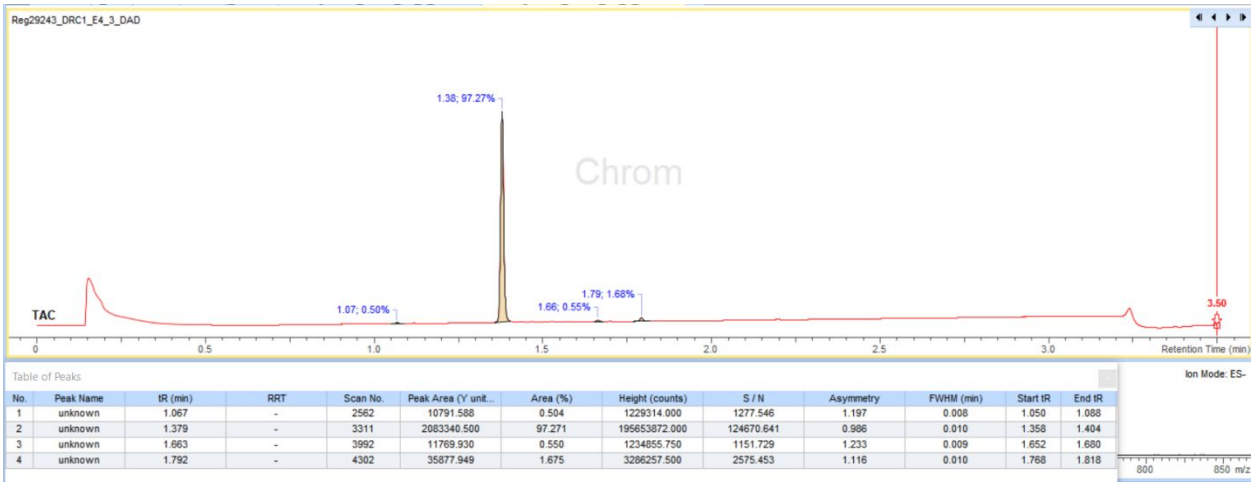
Compound **43**: Method (FA), Purity: 98.7%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.193	-	2865	4052.866	0.171	384942.250	399.317	1.177	0.008	1.174	1.207
2	unknown	1.241	-	2980	2632.298	0.111	262512.000	288.475	1.138	0.010	1.230	1.253
3	unknown	1.331	-	3195	2335871.750	98.720	232194384.000	134151.250	0.972	0.009	1.308	1.354
4	unknown	1.425	-	3420	3280.853	0.139	344122.000	333.233	1.055	0.009	1.414	1.441
5	unknown	1.465	-	3517	8232.116	0.348	689555.500	602.370	1.229	0.009	1.441	1.480
6	unknown	1.750	-	4200	12098.334	0.511	1164675.500	1166.971	1.049	0.009	1.719	1.764

HPLC Chromatogram

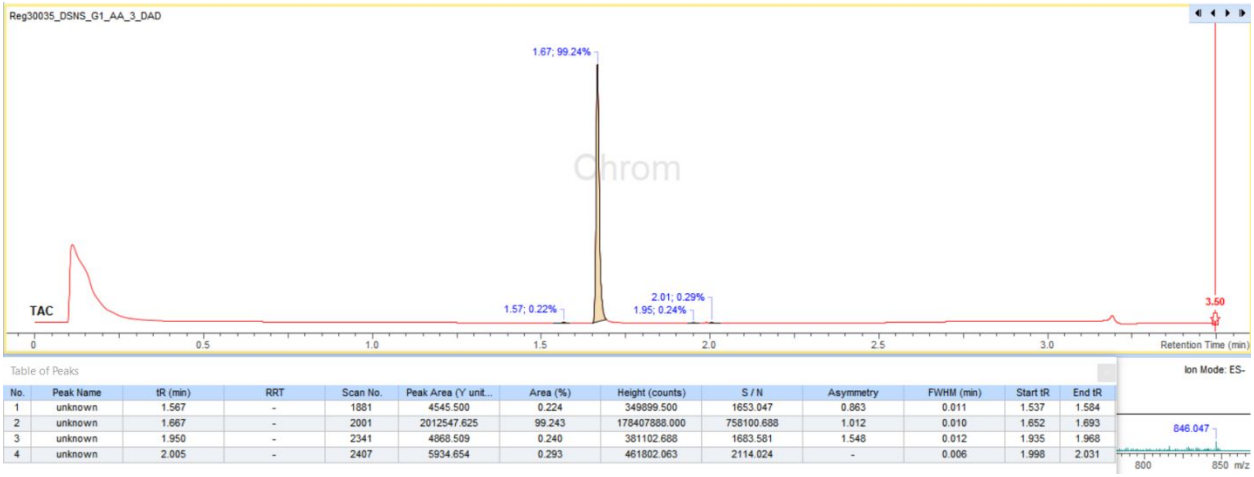
Compound **44**: Method (FA), Purity: 97.3%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.067	-	2562	10791.588	0.504	1229314.000	1277.546	1.197	0.008	1.050	1.088
2	unknown	1.379	-	3311	2083340.500	97.271	195653872.000	124670.641	0.986	0.010	1.358	1.404
3	unknown	1.663	-	3992	11769.930	0.550	1234855.750	1151.729	1.233	0.009	1.652	1.680
4	unknown	1.792	-	4302	35877.949	1.675	3286257.500	2575.453	1.116	0.010	1.768	1.818

HPLC Chromatogram

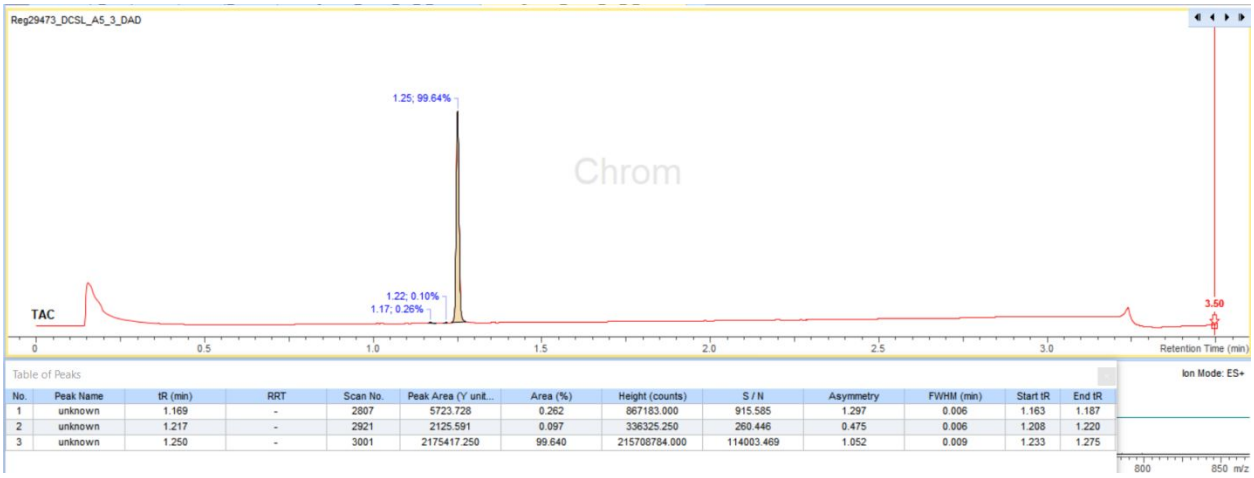
Compound **45**: Method (AA), Purity: 99.2%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.567	-	1881	4545.500	0.224	349899.500	1653.047	0.863	0.011	1.537	1.584
2	unknown	1.667	-	2001	2012547.625	99.243	17847888.000	758100.688	1.012	0.010	1.652	1.693
3	unknown	1.950	-	2341	4868.509	0.240	381102.688	1683.581	1.548	0.012	1.935	1.968
4	unknown	2.005	-	2407	5934.654	0.293	461802.063	2114.024	-	0.006	1.998	2.031

HPLC Chromatogram

Compound **46**: Method (FA), Purity: 99.6%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.169	-	2807	5723.728	0.262	867183.000	915.585	1.297	0.006	1.163	1.187
2	unknown	1.217	-	2921	2125.591	0.097	336325.250	260.446	0.475	0.006	1.208	1.220
3	unknown	1.250	-	3001	2175417.250	99.640	215708784.000	114003.469	1.052	0.009	1.233	1.275

HPLC Chromatogram

Compound **ML-93**: Method (FA), Purity: 98.5%

Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.397	-	3353	2303513.000	98.477	196807296.000	80959.680	0.925	0.011	1.374	1.423
2	unknown	1.490	-	3576	21144.961	0.904	1876479.750	967.694	1.059	0.010	1.475	1.515
3	unknown	1.945	-	4670	14477.651	0.619	964826.000	581.924	1.049	0.014	1.926	1.965

HPLC Chromatogram

Compound **47**: Method (FA), Purity: 94.5%

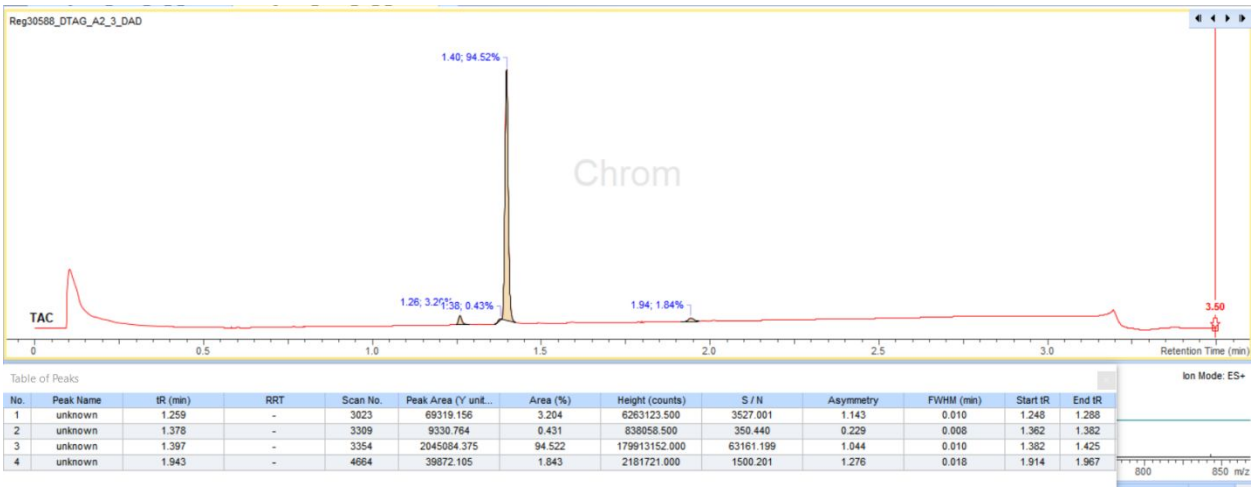
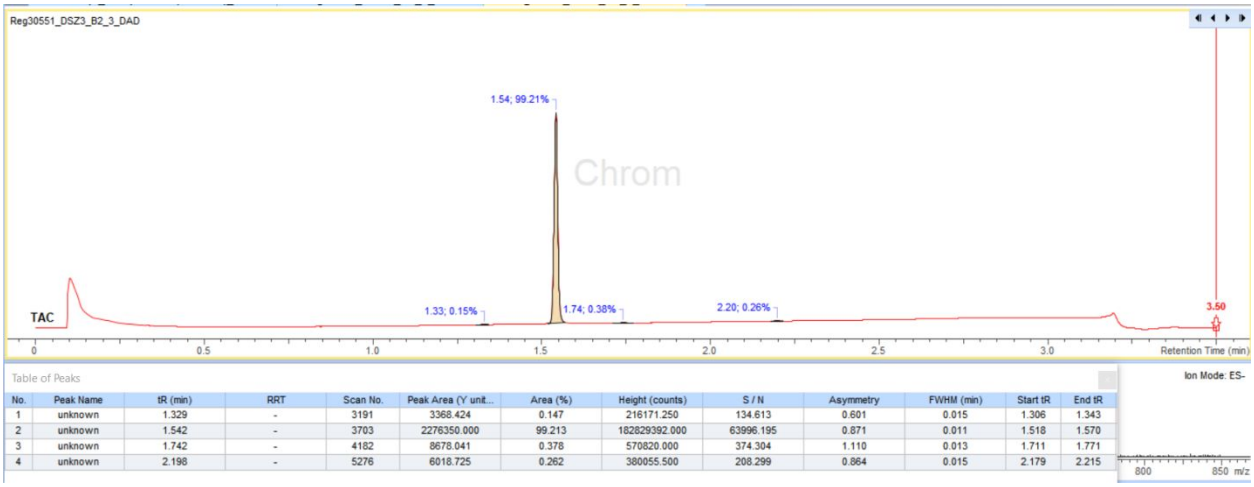


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.259	-	3023	69319.156	3.204	6263123.500	3527.001	1.143	0.010	1.248	1.288
2	unknown	1.378	-	3309	9330.764	0.431	838058.500	350.440	0.229	0.008	1.362	1.382
3	unknown	1.397	-	3354	2045084.375	94.522	179913152.000	63161.199	1.044	0.010	1.382	1.425
4	unknown	1.943	-	4664	39872.105	1.843	2181721.000	1500.201	1.276	0.018	1.914	1.967

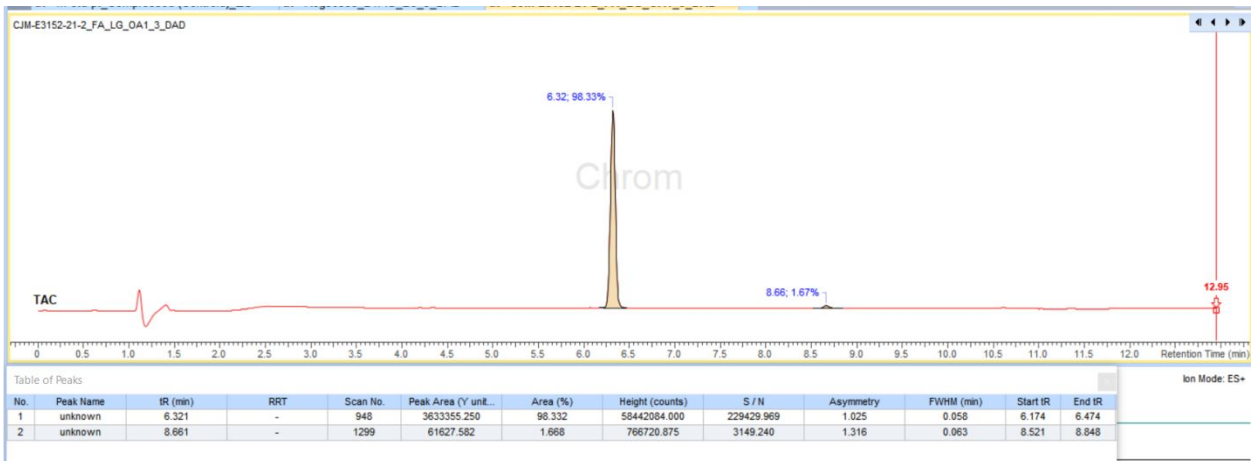
HPLC Chromatogram

Compound **48**: Method (FA), Purity: 99.2%



HPLC Chromatogram

Compound **49**: Method (FA), Purity: 98.3%



HPLC Chromatogram

Compound **50**: Method (FA), Purity: 95.4%

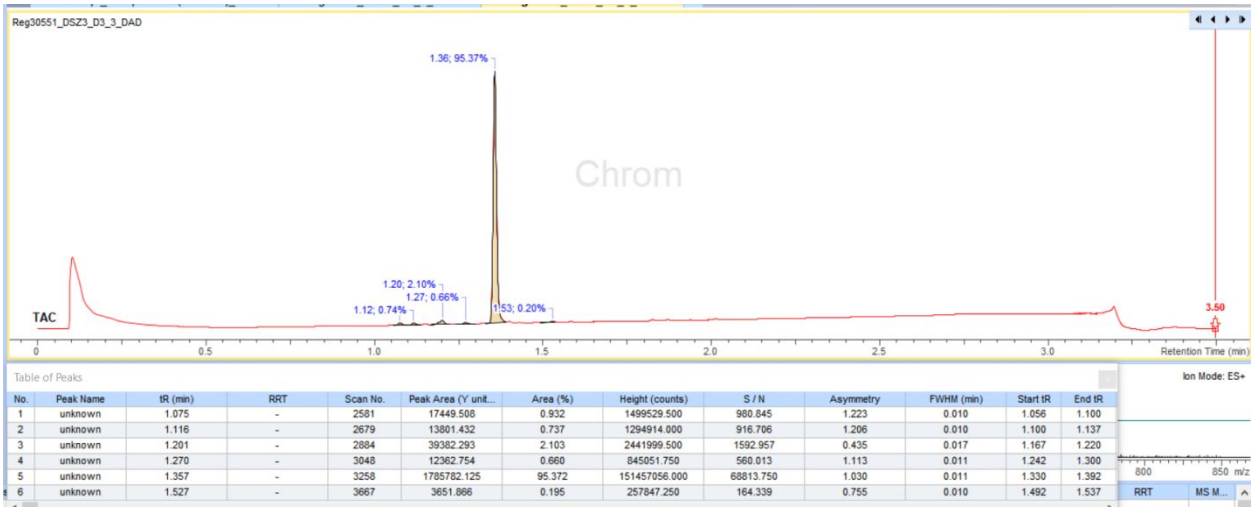


Table of Peaks

Ion Mode: ES+

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.075	-	2581	17449.508	0.932	1499529.500	980.845	1.223	0.010	1.056	1.100
2	unknown	1.116	-	2679	13801.432	0.737	1294914.000	916.706	1.206	0.010	1.100	1.137
3	unknown	1.201	-	2884	39382.293	2.103	2441999.500	1592.957	0.435	0.017	1.167	1.220
4	unknown	1.270	-	3048	12362.754	0.660	845051.750	560.013	1.113	0.011	1.242	1.300
5	unknown	1.357	-	3258	1785782.125	95.372	151457056.000	68813.750	1.030	0.011	1.330	1.392
6	unknown	1.527	-	3667	3651.866	0.195	257847.250	164.339	0.755	0.010	1.492	1.537

800 850 m/z

RRT MS M...

HPLC Chromatogram

Compound **51**: Method (AA), Purity: 99.0%

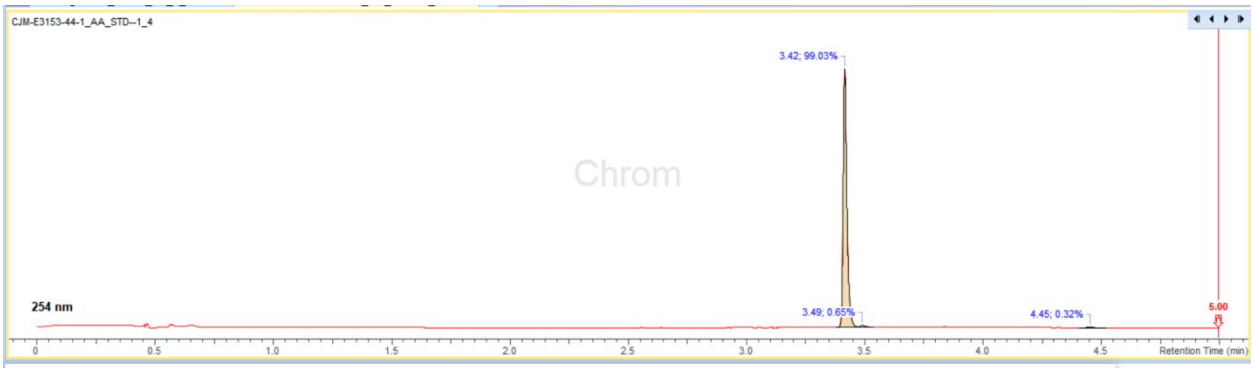


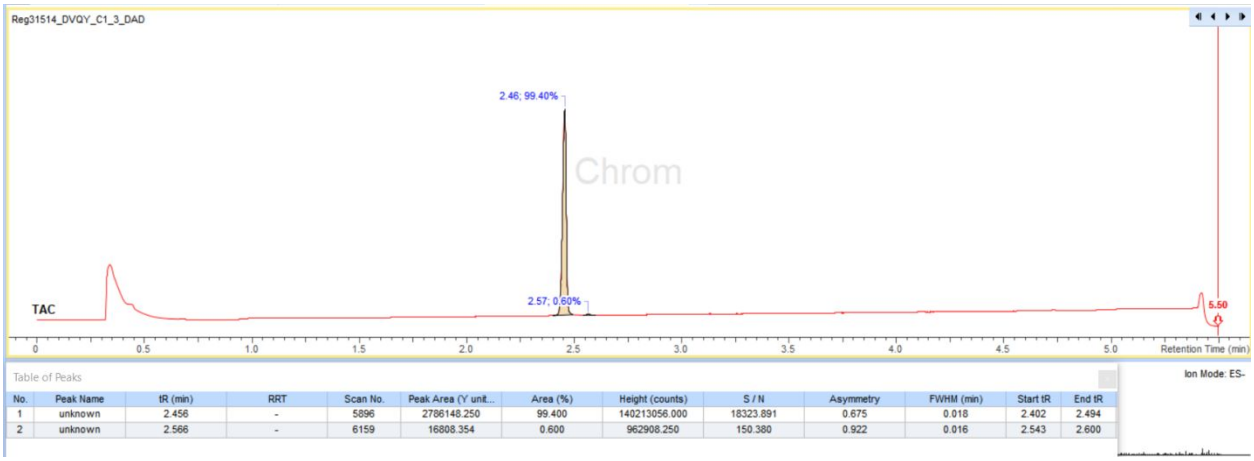
Table of Peaks

Ion Mode: ES+

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	3.415	-	16394	13.916	99.028	766.223	227757.781	1.284	0.016	3.381	3.473
2	unknown	3.490	-	16753	0.092	0.655	4.924	1486.575	1.294	0.017	3.473	3.538
3	unknown	4.454	-	21381	0.045	0.317	1.213	456.836	0.753	0.030	4.408	4.519

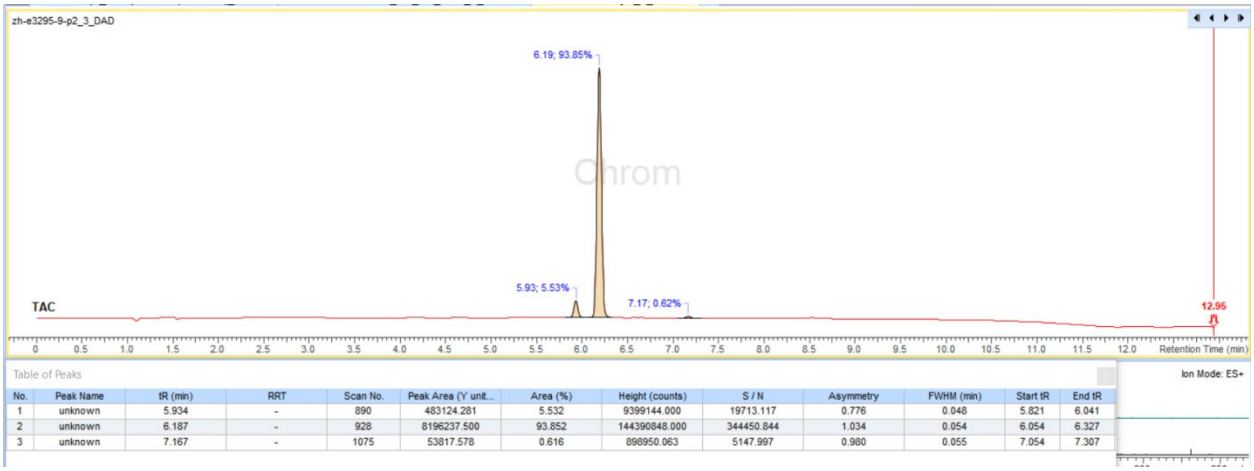
HPLC Chromatogram

Compound **52**: Method (FA), Purity: 99.4%



HPLC Chromatogram

Compound **53**: Method (FA), Purity: 93.9%



HPLC Chromatogram

Compound **54**: Method (FA), Purity: 100%

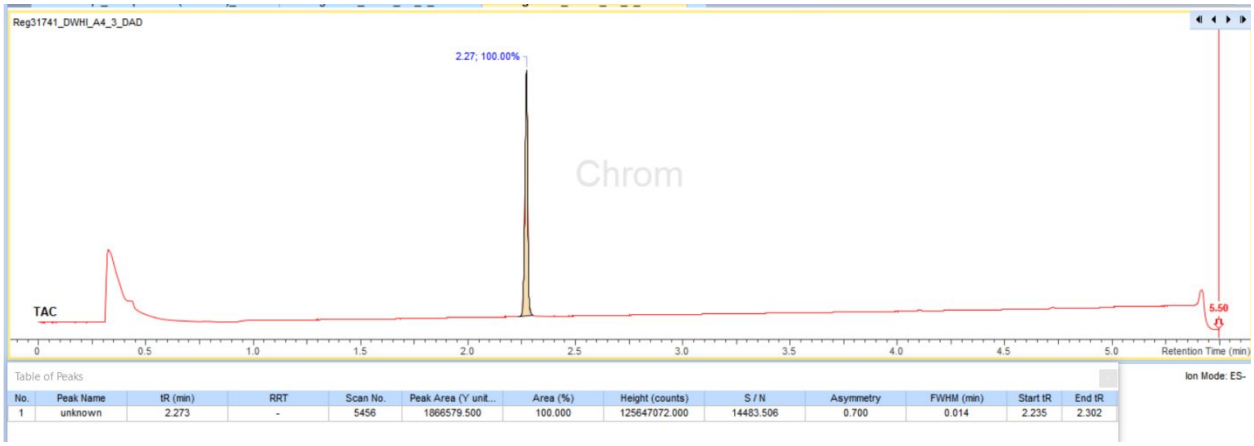


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	2.273	-	5456	1866579.500	100.000	125647072.000	14483.506	0.700	0.014	2.235	2.302

HPLC Chromatogram

Compound **55**: Method (FA), Purity: 96.7%

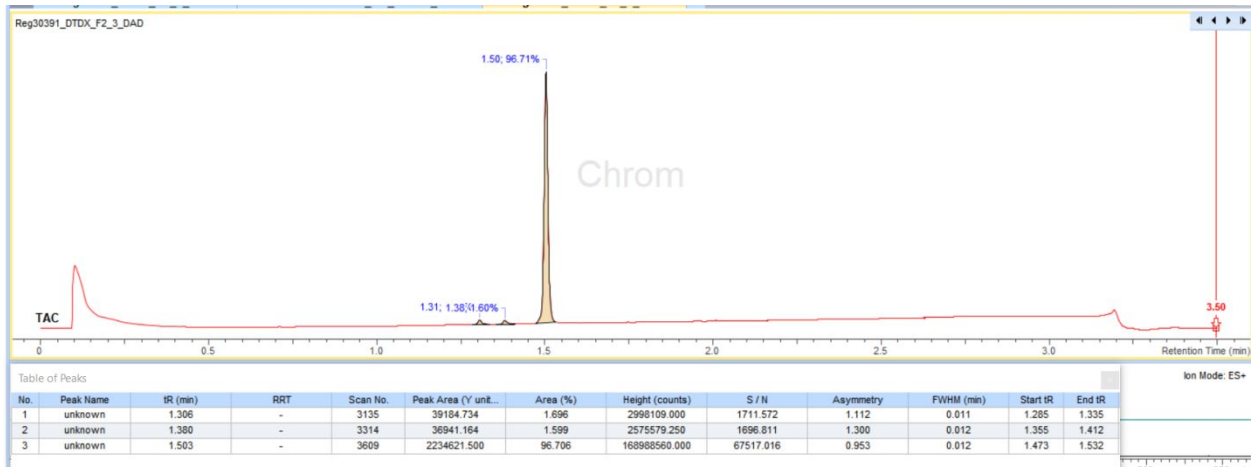


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.306	-	3135	39184.734	1.696	2998109.000	1711.572	1.112	0.011	1.285	1.335
2	unknown	1.380	-	3314	36941.164	1.599	2575579.250	1696.811	1.300	0.012	1.355	1.412
3	unknown	1.503	-	3609	2234621.500	96.706	168988560.000	67517.016	0.953	0.012	1.473	1.532

HPLC Chromatogram

Compound **TAK-981**: Method (FA), Purity: 96.8%

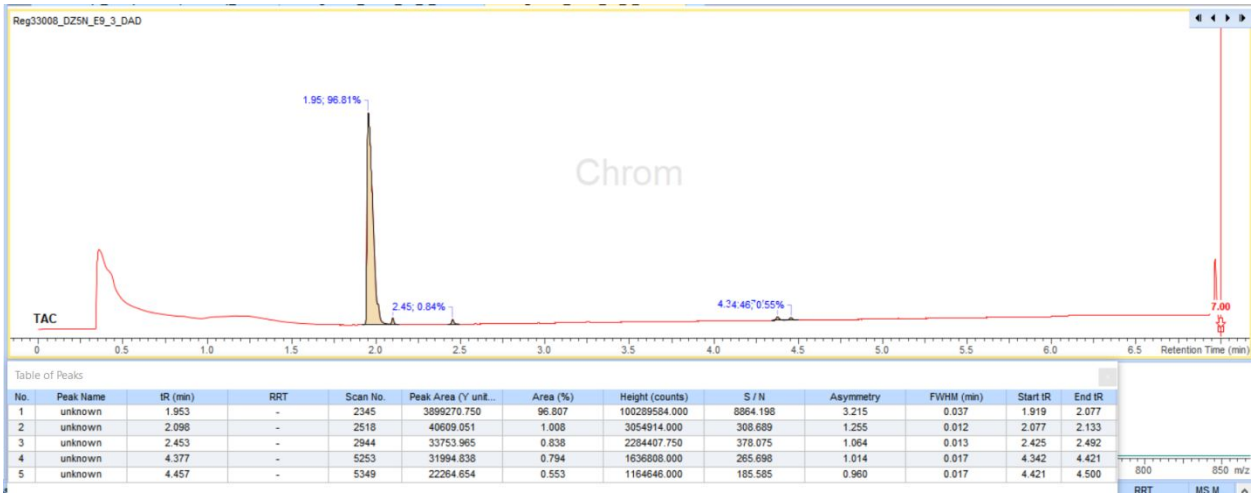


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.953	-	2345	3899270.750	96.807	100289584.000	8864.198	3.215	0.037	1.919	2.077
2	unknown	2.098	-	2518	40609.051	1.008	3054914.000	308.689	1.255	0.012	2.077	2.133
3	unknown	2.453	-	2944	33753.965	0.838	2284407.750	378.075	1.064	0.013	2.425	2.492
4	unknown	4.377	-	5253	31994.838	0.794	1636808.000	265.698	1.014	0.017	4.342	4.421
5	unknown	4.457	-	5349	22264.654	0.553	1164646.000	185.585	0.960	0.017	4.421	4.500

HPLC Chromatogram

Compound **56**: Method (FA), Purity: 95.5%

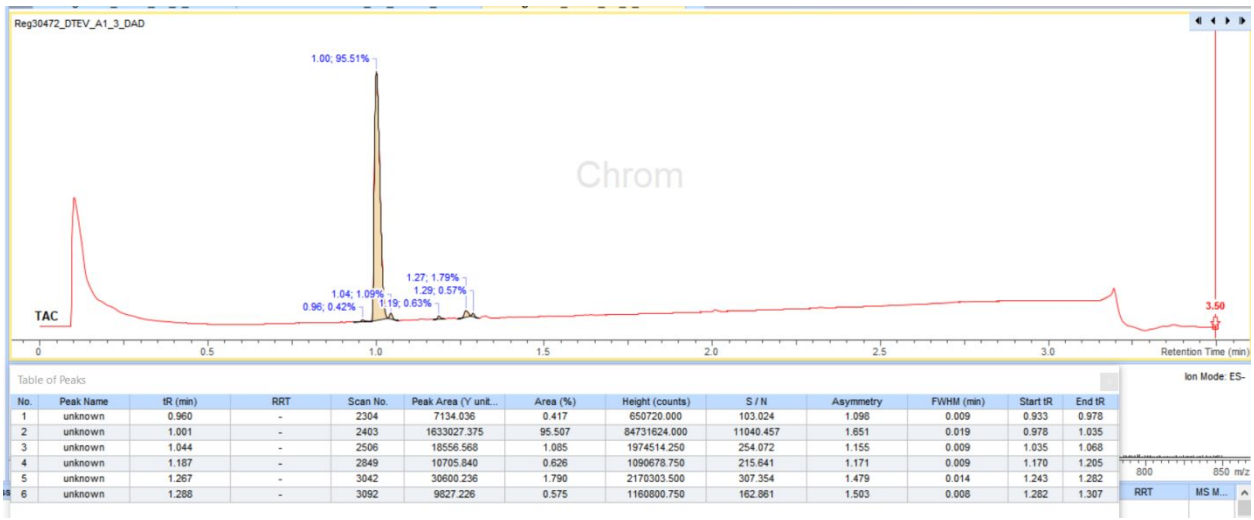
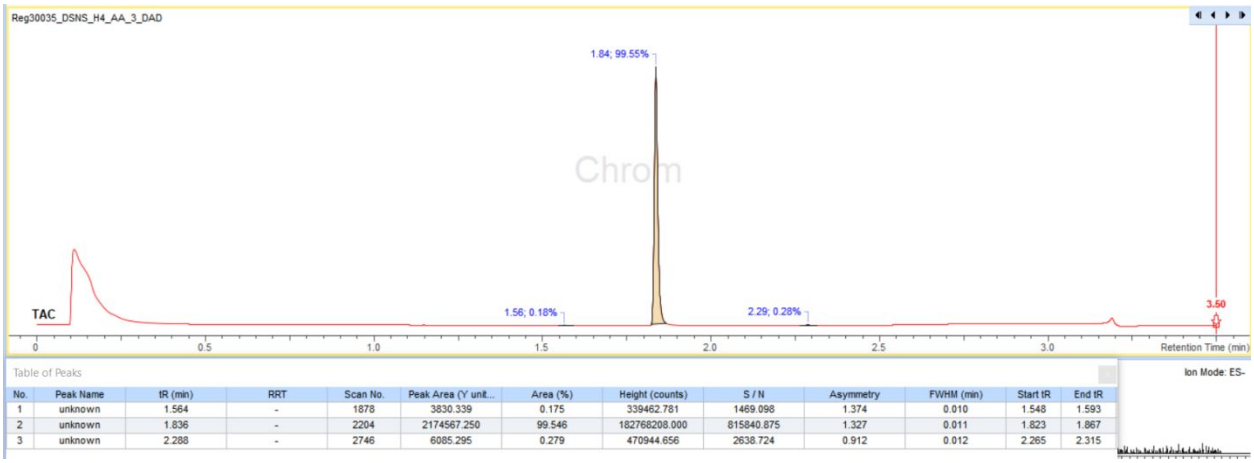


Table of Peaks

No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	0.960	-	2304	7134.036	0.417	650720.000	103.024	1.098	0.009	0.933	0.978
2	unknown	1.001	-	2403	1633027.375	95.507	84731624.000	11040.457	1.651	0.019	0.978	1.035
3	unknown	1.044	-	2506	18556.568	1.085	1974514.250	254.072	1.155	0.009	1.035	1.068
4	unknown	1.187	-	2849	10705.840	0.626	1090678.750	215.641	1.171	0.009	1.170	1.205
5	unknown	1.267	-	3042	30600.236	1.790	2170303.500	307.354	1.479	0.014	1.243	1.282
6	unknown	1.288	-	3092	9827.226	0.575	1160800.750	162.861	1.503	0.008	1.282	1.307

HPLC Chromatogram

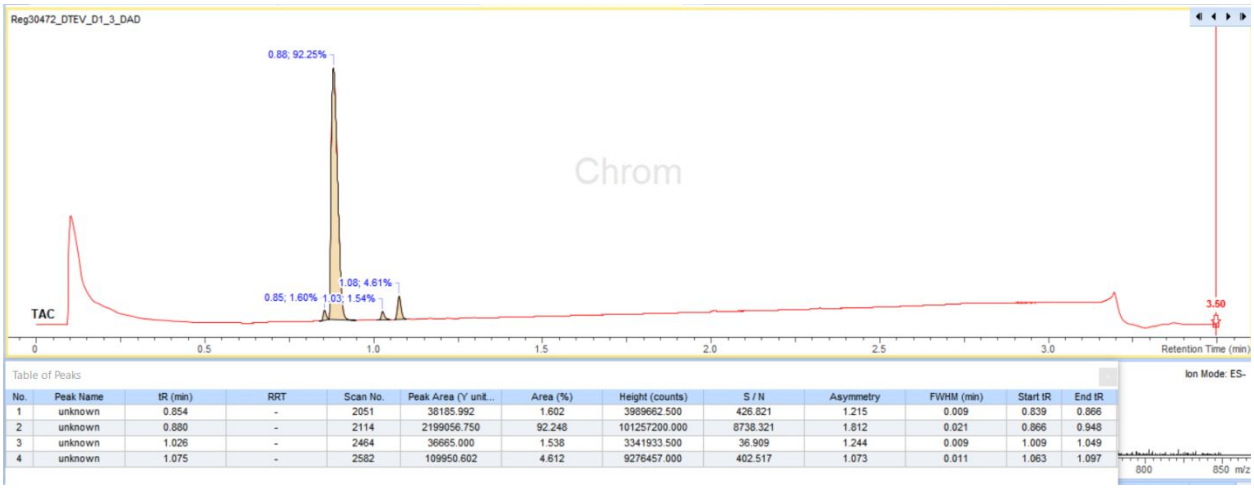
Compound **57**: Method (AA), Purity: 99.6%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.564	-	1878	3830.339	0.175	339462.781	1469.098	1.374	0.010	1.548	1.593
2	unknown	1.836	-	2204	2174567.250	99.546	182768208.000	815840.875	1.327	0.011	1.823	1.867
3	unknown	2.288	-	2746	6085.295	0.279	470944.656	2638.724	0.912	0.012	2.265	2.315

HPLC Chromatogram

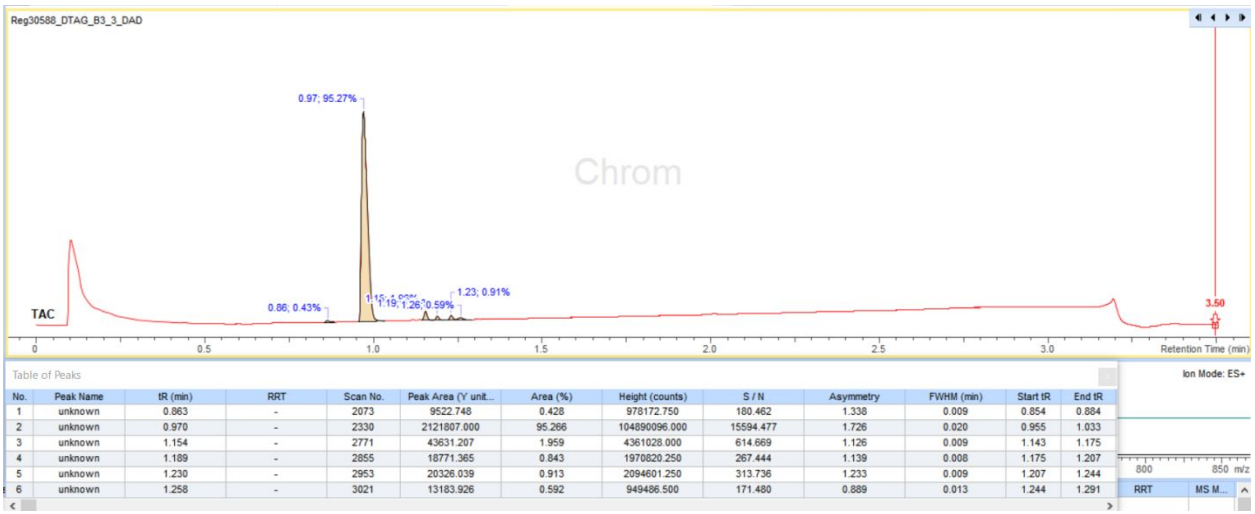
Compound **58**: Method (FA), Purity: 92.3%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	0.854	-	2051	38185.992	1.602	3989662.500	426.821	1.215	0.009	0.839	0.866
2	unknown	0.880	-	2114	2199056.750	92.248	101257200.000	8738.321	1.812	0.021	0.866	0.948
3	unknown	1.026	-	2464	36665.000	1.538	3341933.500	36.909	1.244	0.009	1.009	1.049
4	unknown	1.075	-	2582	109950.602	4.612	9276457.000	402.517	1.073	0.011	1.063	1.097

HPLC Chromatogram

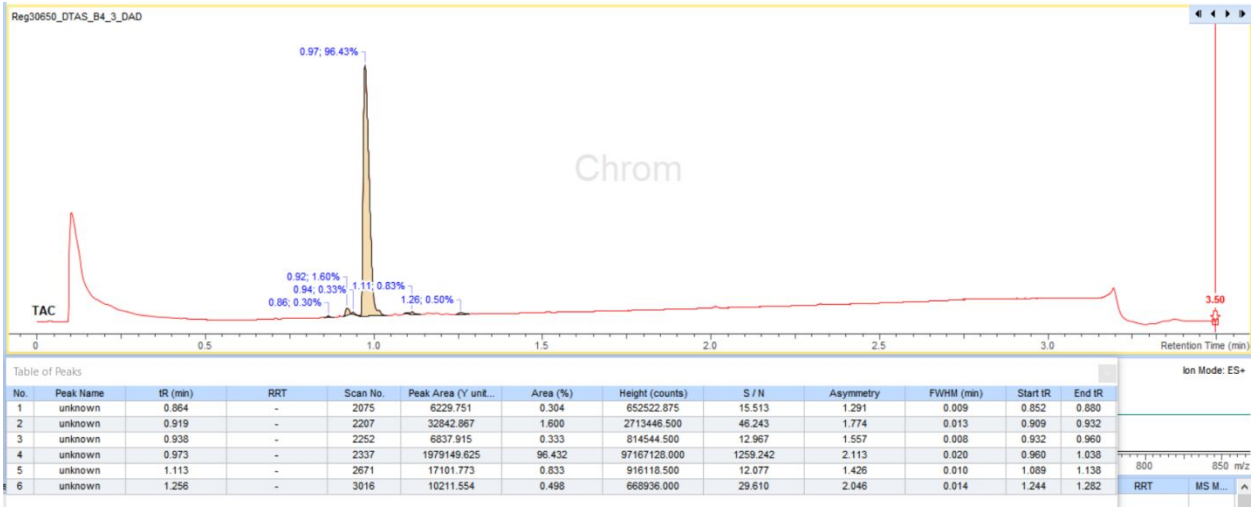
Compound **59**: Method (FA), Purity: 95.3%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	0.863	-	2073	9522.748	0.428	978172.750	180.462	1.338	0.009	0.854	0.884
2	unknown	0.970	-	2330	2121807.000	95.266	104890096.000	15594.477	1.726	0.020	0.955	1.033
3	unknown	1.154	-	2771	43631.207	1.959	4361028.000	614.669	1.126	0.009	1.143	1.175
4	unknown	1.189	-	2855	18771.365	0.843	1970820.250	267.444	1.139	0.008	1.175	1.207
5	unknown	1.230	-	2953	20326.039	0.913	2094601.250	313.736	1.233	0.009	1.207	1.244
6	unknown	1.258	-	3021	13183.926	0.592	949486.500	171.480	0.889	0.013	1.244	1.291

HPLC Chromatogram

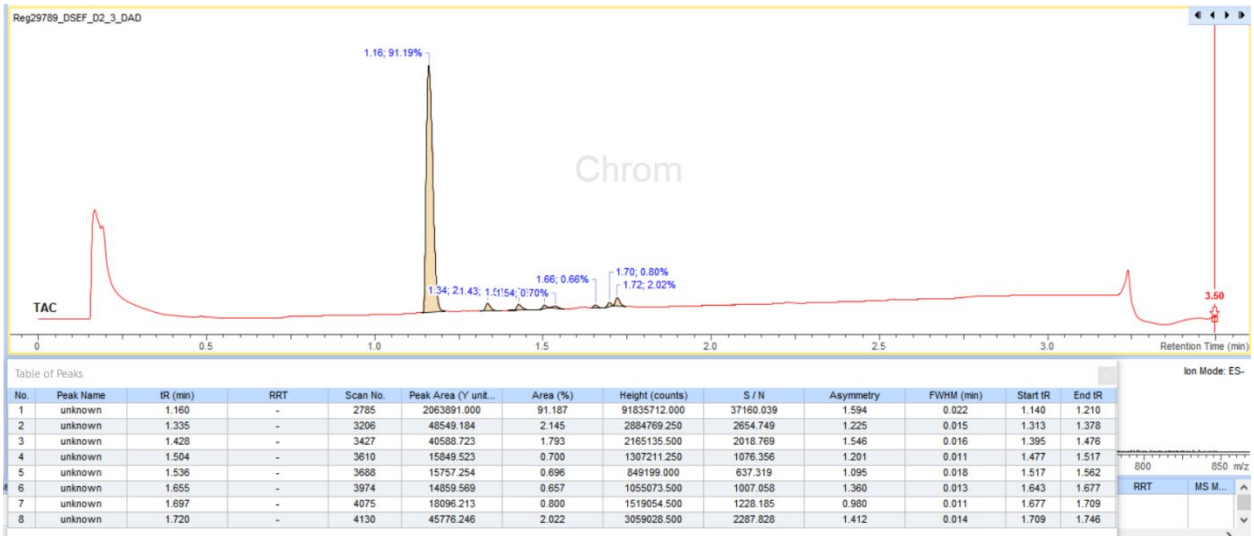
Compound **60**: Method (FA), Purity: 96.4%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	0.864	-	2075	6229.751	0.304	652522.875	15.513	1.291	0.009	0.852	0.880
2	unknown	0.919	-	2207	32842.867	1.600	2713446.500	46.243	1.774	0.013	0.909	0.932
3	unknown	0.938	-	2252	6837.915	0.333	814544.500	12.967	1.557	0.008	0.932	0.960
4	unknown	0.973	-	2337	1979149.625	96.432	97167128.000	1259.242	2.113	0.020	0.960	1.038
5	unknown	1.113	-	2671	17101.773	0.833	916118.500	12.077	1.426	0.010	1.089	1.138
6	unknown	1.256	-	3016	10211.554	0.498	668936.000	29.610	2.046	0.014	1.244	1.282

HPLC Chromatogram

Compound **61**: Method (FA), Purity: 91.2%



No.	Peak Name	tR (min)	RRT	Scan No.	Peak Area (Y unit...	Area (%)	Height (counts)	S / N	Asymmetry	FWHM (min)	Start tR	End tR
1	unknown	1.160	-	2785	2063891.000	91.187	91835712.000	37160.039	1.594	0.022	1.140	1.210
2	unknown	1.335	-	3206	48549.184	2.145	2884769.250	2654.749	1.225	0.015	1.313	1.378
3	unknown	1.428	-	3427	40588.723	1.793	2165135.500	2018.769	1.546	0.016	1.395	1.476
4	unknown	1.504	-	3610	15849.523	0.700	1307211.250	1076.356	1.201	0.011	1.477	1.517
5	unknown	1.536	-	3688	15757.254	0.696	849199.000	637.319	1.095	0.018	1.517	1.562
6	unknown	1.655	-	3974	14859.569	0.657	1055073.500	1007.058	1.360	0.013	1.643	1.677
7	unknown	1.697	-	4075	18096.213	0.800	1519054.500	1228.185	0.980	0.011	1.677	1.709
8	unknown	1.720	-	4130	45776.246	2.022	3059028.500	2287.828	1.412	0.014	1.709	1.746

Crystallography of SUMO-inhibitor adduct bound in SAE

Baculoviruses were made using the pDEST8 for N terminally His-tagged SAE1 (full-length construct; UniProt entry Q9UBE0) and untagged SAE2 (construct containing residues 1–550; UniProt entry Q9UBT2). Protein was expressed by coinfecting Sf9 cells and harvesting 72 hr postinfection. SAE1/2 were purified by affinity chromatography (HisTrap HP, GE Healthcare) followed by TEV protease cleavage of the His tag, ion exchange (MonoQ, GE Healthcare), and size exclusion (Superdex-200, GE Healthcare) chromatography. N-terminally His-tagged SUMO1 (full length construct; UniProt entry P63165) was cloned into pDEST8 and expressed in Sf9 cells. SUMO1 was purified by affinity (HisTrap HP) and size exclusion (Superdex-200) chromatography. Prior to crystallization, SAE1/2 was concentrated to 12 mg/ml and SUMO1 to 50 mg/ml by ultrafiltration in 10 mM Tris pH 7.5, 100 mM NaCl, 1 mM TCEP.

Crystals of SAE1/2 with SUMO1-inhibitor adducts bound were grown by mixing 50µl SAE1/2 and 3.0µl SUMO1 with 1.25-2.5µl of 10 mM inhibitor, incubating for 15 minutes at 25°C, and followed by hanging-drop vapor diffusion against a reservoir containing Hampton Index 57 (50 mM BisTris, pH 6.5, 50 mM AmSulfate, 30% pentaerythritol ethoxylate) at a 1:1 ratio. Crystallization condition was used as cryoprotection and crystals were flash cooled in liquid nitrogen.

Data were collected at LRL-CAT 31ID beamline of the Advanced Photon Source and CMCF-08ID beamline at the Canadian Light Source. Data processed using HKL2000 (Z. Otwinowski and W. Minor, "Processing of X-ray Diffraction Data Collected in Oscillation Mode", *Methods in Enzymology*, Volume **276**: Macromolecular Crystallography, part A, p.307-326, 1997,C.W. Carter, Jr. & R. M. Sweet, Eds., Academic Press (New York).) and XDS (Kabsch, W. *XDS. Acta Cryst.* D**66**, 125-132 (2010)). The structure was solved by molecular replacement using existing SAE1/2-SUMO1 coordinates (PDB entry 1Y8R) as a starting model. Manual rebuilding of the model was accomplished using the program Coot (P. Emsley, K. Cowtan Coot: model-building tools for molecular graphics, Acta Crystallogr. D Biol. Crystallogr., 60 (2004), pp. 2126-213), and refinement was carried out with the CCP4i

(E. Potterton, P. Briggs, M. Turkenburg, E. Dodson, A graphical user interface to the CCP4

program suite Acta Crystallogr. D Biol. Crystallogr., 59 (2003), pp. 1131-1137) graphical interface to Refmac (G.N. Murshudov, A.A. Vagin, E.J. Dodson, Refinement of macromolecular structures by the maximum-likelihood method, Acta Crystallogr. D Biol. Crystallogr., 53 (1997), pp. 240-255). The final model contains one SAE1/2:SUMO1-inhibitor complexes per asymmetric unit.

Micro electron diffraction of TAK-981

Electron diffraction was performed using a Thermo Fisher Scientific (Hillsboro, Oregon) Glacios Cryo Transmission Electron Microscope (Cryo-TEM) operated at 200kV, equipped with a Ceta-D detector and operated at cryogenic temperature. Microscope data collection settings were as follows: camera length 1100mm; exposure time 222 ms; oscillation 89º/frame; dose rate ~0.1 e-/s/Å^2, -193ºC. Diffraction data was processed using HKL2000 (Z. Otwinowski and W. Minor, **"**Processing of X-ray Diffraction Data Collected in Oscillation Mode**",** *Methods in Enzymology*, Volume **276**: Macromolecular Crystallography, part A, p.307-326, 1997,C.W. Carter, Jr. & R. M. Sweet, Eds., Academic Press (New York)) and solved/refined with Shelx (Sheldrick, G. M. (2015). *Acta Cryst.* C**71**, 3-8.).