Discovery and Hit-to-lead Evaluation of Piperazine Amides as Selective, State-dependent \( \text{Na}_\text{V}1.7 \) Inhibitors

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General Electrophysiological Procedures

**Stable Cell Lines.** Human Na\(_{V}\)1.5 (HEK293) and 1.7 (HEK293) stable cell lines were purchased from Eurofins Pharma Bioanalytics Services US Inc., St. Charles, MO. Mouse Na\(_{V}\)1.7 sequence was sub-cloned into the antibiotic selection vector pSLX240h, linearized and stably transfected using Lipofectamine LTX (Life Technologies, Grand Island, NY) into HEK293 cells grown in DMEM/F12, +10% Serum, +1X P/S/G, +1X NEAA, +15 mM HEPES. Following 48-72 h of transfection, media containing 80 µg/mL hygromycin was added and selection was continued for 24-30 d. When the stable pools formed good-sized colonies,
individual colonies were picked into 96 well plates, expanded, and expression was confirmed by electrophysiology testing and Western blot.

**IonWorks Barracuda Electrophysiology.** Sodium currents were recorded in population patch-clamp mode with the IonWorks® Barracuda automated electrophysiology system (Molecular Devices, LLC, Sunnyvale, CA). This system utilizes perforated patch-clamp technology to gain electrical access to cells. External saline consisted of (in mM): NaCl 140, KCl 5, CaCl2 2, MgCl2 1, HEPES 10 and glucose 11; pH 7.4 with N-methyl-D-glucamine; 320 mOsmol. Internal solution for human NaV1.5, human NaV1.7, and mouse NaV1.7 consisted of (in mM): of KCl 70, KF 70, MgCl2 0.25, HEDTA 5 and HEPES 10; pH 7.25 with N-methyl-D-glucamine; 300 mOsmol. For NaV1.7 measurements, cells were voltage clamped to -110 mV for 3 sec and sodium currents were elicited by a train of 26 depolarization pulses of 150 msec duration at 5 Hz to -20 mV at a frequency of 5 Hz. Compound was added and the cells were then held at -20mV for 5 min. Following compound incubation the cells were re-clamped to -110 mV for 3 sec to recover unbound channels and the 26 pulse voltage protocol was repeated. The ratio between peak inward current in the presence and absence of compound during pulse 26 was used to determine percent inhibition. For NaV1.5 measurements, cells were voltage clamped to -50 mV and sodium currents were elicited by a voltage step protocol repeated at a frequency of 0.1 Hz. The voltage step protocol included a step from the holding voltage to -120 mV for a 50 msec duration to recover from channel both fast and slow inactivation followed by a step to 0 mV for a duration of 15 msec. Currents were elicited by this voltage protocol for 1 min prior to compound addition. Compound was added and sodium currents were elicited by the voltage step protocol in the presence of compound at a 0.1 Hz frequency for a total duration of 6 min. The ratio between peak inward currents in the presence and absence of compound during the last step to 0 mV was used to determine percent inhibition. Concentration-response curves of percent inhibition as a function of concentration were generated to calculate IC50 values. For IC50 determination, data was fitted to a 4-parameter equation (y = A + ((B-A)/(1 + ((x/C)^D))), where A is the minimum y (POC) value, B is the maximum y (POC), C is the x (compound concentration) at the point of inflection and D is the slope factor). Non-linear regression curve-fitting was performed using Screener (Genedata AG, Basel, Switzerland) data analysis software.

**PatchXpress 7000A Electrophysiology.** Sodium currents were recorded in whole cell voltage clamp mode with the PatchXpress automated electrophysiology system (Molecular Devices, LLC, Sunnyvale, CA). Adherent cells were isolated from tissue culture flasks using trypsin-EDTA treatment for 2-3 minutes and then incubated in complete culture medium containing 10% fetal bovine serum for at least 15 minutes prior to resuspension in external solution consisting of 70 mM NaCl, 140 mM D-Mannitol, 5 mM KCl, 11 mM Glucose, 10 mM HEPES, 2 mM CaCl2, 1 mM MgCl2, pH 7.4 with NaOH or 140mM NaCl, 5 mM KCl, 11 mM Glucose, 10 mM HEPES, 2 mM CaCl2, 1 mM MgCl2, pH 7.4 with NaOH. Internal solution consisted of 62.5 mM CsCl, 75 mM CsF, 10 HEPES, 2.5 mM MgCl2, pH 7.25 with CsOH. Cells were voltage clamped at room temperature at a holding potential of -125 mV with test potentials to -10 mV (NaV1.7) or -20 mV (hNaV1.5). Compound effects were measured on a partially inactivated state of sodium channels. For human, mouse, and rat NaV1.7, cells were clamped to a holding potential yielding 20-50% inactivation. To elicit sodium current, channels were activated by pulsing to -10 mV for 15 msec. This voltage protocol was repeated at a rate of 0.1 Hz throughout the experiment. For human NaV1.5, cells were held at a holding potential of -50 mV.
To elicit sodium currents the voltage was changed to -120 mV for a period of 50 msec before a test pulse to -20 mV of 20 msec duration. These voltage protocols were repeated at a rate of 0.1 Hz throughout the experiment. A single concentration of test compound was applied to cells for 3-5 minutes. Peak sodium current was measured at the end of the compound addition period to determine percent inhibition. Cells were used for additional compound testing if currents recovered to >80% of starting values following compound washout. At least three different concentrations of test compound at half log units were applied individually, with washout, recovery of current, and resetting of holding voltage between each individual concentration. Percent inhibition as a function of compound concentration was and fit with a Hill (4-parameter logistic) fit in DataXpress 2.0 software to produce a single IC$_{50}$ curve.
General Biological Assay Procedures

**Human Liver Microsomal Assay.** Test compounds (1 μM) were incubated at 37 °C in phosphate buffer (66.7 mM, pH 7.4) with pooled human liver microsomes (0.25 mg/mL protein) and 1 mM NADPH. After 1, 5, 10, 20, 30 and 40 minutes, the reaction was stopped by the addition of acetonitrile containing 0.5% formic acid and internal standard. The quenched samples were centrifuged at 1650 g for 20 min. The supernatants were analysed directly for unchanged test compound using liquid chromatography and tandem mass spectrometric detection (LC-MS/MS).

**Solubility Determination.** Solubilities in PBS were determined according to an automated procedure.1

**Pharmacokinetic Studies.** All pharmacokinetic studies described in this manuscript were approved by the Institutional Animal Care and Use Committee at Amgen (Cambridge, MA).

**Histamine-Induced Scratching in Mice.** All procedures were approved and carried out in accordance with Amgen Inc.’s Institutional Animal Care and Use Committee. Subjects were C57Bl/6 male mice (Charles River Labs, Kingston, NY) aged between 9-10 weeks and housed 1-4 per cage with ad libitum access to food and water. Animals were kept on a 12/12 h light/dark cycle with lights on at 6:30 a.m. Following arrival from the vendor, mice were allowed to acclimate to the animal facility for 1 week prior to the start of the experiment. One day prior to behavioural testing, mice were anesthetized under 3% isoflurane and the area at the nape of the neck was shaved. Immediately afterward, mice were transported to the testing room and acclimated to individual sound-attenuated chambers (12”l X 9.5”w X 8.25”h, Med Associates VFC-008, NIR-022MD, St. Albans, VT) for 15-20 minutes. Testing was performed the following day between the hours of 8:00 am and 3:00 pm. Four hours prior to histamine treatment, mice were orally administered Compound 14 (300 mg/kg body weight), a vehicle control formulation (30% Hydroxypropyl beta-cyclodextrin, 70% H2O, pH 10), or the antihistamine Diphenhydramine (30 mg/kg in phosphate-buffered saline, Sigma D3630) which served as a positive control. Histamine dichloride (8.15 mM in a volume of 100 μL, Sigma Aldrich H7250) was injected intradermally to the shaved area, mice were placed into the sound-attenuated testing chambers, and behaviour was recorded on digital video files for a period of 30 minutes. Video recordings were later reviewed, and individual scratching bouts scored, by trained experimenters blinded to test article treatment. A scratching bout was defined as a rapid head tilt accompanied by a hind paw directed at the site of intradermal injection. Termination of a scratching bout was deemed to have occurred when the hind paw was placed back on the chamber floor or into the animal’s mouth. Data was analysed statistically via GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA) using a one-way ANOVA to assess the overall test article treatment effect and followed by Dunnett’s multiple comparison post-hoc tests.

**Open-Field Locomotor Activity in Mice.** All procedures were approved and carried out in accordance with Amgen Inc.’s Institutional Animal Care and Use Committee. Subjects were C57Bl/6 male mice (Charles River Labs, Kingston, NY) aged between 9-10 weeks and housed 1-4 per cage with ad libitum access to food and water. Animals were kept on a 12/12 h light/dark cycle with lights on at 6:30 a.m. Following arrival from the vendor, mice were allowed to

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acclimate to the animal facility for 1 week prior to the start of the experiment. On the day of testing, animals were orally administered either Compound 14 (30, 100, or 300 mg/kg body weight) or a vehicle control formulation (30% Hydroxypropyl beta-cyclodextrin, 70% H2O, pH10) between the hours of 7:00 a.m. and 5:00 p.m. Four hours following test article treatment, animals were placed into dimly-lit (15-20 Lux) open-field chambers (16” x 16”, Kinder Scientific, San Diego, CA) and behaviour was monitored over a 30-minute period during which horizontal movement parameters were measured in an automated manner via infrared photo beam breaks. Data was analysed statistically via GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA) using a one-way ANOVA to assess the overall test article treatment effect and followed by Dunnett’s multiple comparison post-hoc tests. The results of this study are shown in Figure S1.

![Figure S1](image.png)

**Figure S1.** The effect of 30, 100, and 300 mg/kg oral doses of 14 on locomotor activity in the mouse open-field assay compared to vehicle. One-way ANOVA did not reveal any statistically significant treatment effect [p>0.05; F(3, 35) = 1.89].
Commercial reagents and solvents were used as received. Microwave-assisted reactions were conducted with a Biotage Initiator. NMR spectra were recorded at ambient temperature on a Bruker AV-400 400 MHz spectrometer, a Bruker AV III 500 MHz spectrometer, or a Bruker Advance III spectrometer, operating at a proton frequency of 500 MHz using a Protasis CapNMR flow-probe, equipped with Discovery Tower™ Sample management system and a Waters Liquid Handler, made by CTC, Switzerland (Model 2777). Data are reported in parts per million (δ), and are calibrated using residual non-deuterated solvent as an internal reference: CDCl₃, δ 7.26 (CHCl₃); DMSO-d₆, δ 2.50 (d₅-DMSO). Data for ¹H NMR spectra are reported as follows: chemical shift (multiplicity, coupling constants, integration). Multiplicities are reported as follows: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad, or combinations thereof. Data for ¹³C NMR spectra are reported in parts per million (δ) and are referenced from the central peak of the carbon resonance of the solvent: CDCl₃, δ 77.00; d₆-DMSO, δ 39.52.

Purifications were performed using standard column chromatography in glass columns or medium pressure liquid chromatography (MPLC) on a CombiFlash Companion (Teledyne Isco) or a Biotage Isolera with prepacked RediSep or Biotage normal-phase silica gel (35–60 μm) columns and UV detection at 254 nm. Preparative reversed-phase high-performance liquid chromatography (HPLC) and high throughput parallel purification were performed with reversed phase preparative LC/MS: Waters auto-purification system; liquid transfer system: Tecan; drying system: Genevac; preparatory column: Xbridge (19 x 100 mm, C18, 10 μm); flow rate: 40 mL/min; general gradient: 5 – 95% B, 0.1% additive in both A and B; 10 min gradient; Mobile Phase A: H₂O, Mobile Phase B, MeCN; Additive: TFA or NH₄OH. Chiral method development was performed on an analytical Thar SFC/MS. Preparative chiral separations were performed on Thar SFC Prep 80 or SFC Prep 350 instruments. All final compounds were purified to ≥ 95% purity as determined by HPLC.

Purity (3 min methods only) and reaction analyses were measured using Agilent 1100 Series HPLC systems with UV detection at 254 nm and 215 nm (System A: Agilent Zorbax SB-C18 3.0 x 50 mm, 3.5 micron, 5 to 95% MeCN in H₂O with 0.1% TFA for 3.6 min at 1.5 mL/min; System B: Waters Xbridge C18, 3 x 50 mm, 3.5 micron, 5 to 95% MeCN in H₂O with 0.1% formic acid for 3.6 min at 1.5 mL/min.

Exact mass confirmation was performed on an Agilent 1200 series high performance liquid chromatography (HPLC) system (Santa Clara, S2 CA, U.S.) by flow injection analysis, eluting with a binary solvent system A and B (A, H₂O with 0.1% formic acid; B, MeCN with 0.1% formic acid) under gradient conditions (5-95% B over 3 min) at 0.3 mL/min with MS detection by an Agilent 6510-Q-TOF mass spectrometer (Santa Clara, CA, U.S.).

Optical rotations were measured on a Jasco P-2000 digital polarimeter with a sodium lamp (average of three measurements for each sample) using a 3.5 mm x 100 mm cylindrical glass cell.
(R)-N-Benzyl-2-(4-(2-chloro-5-fluorobenzoyl)piperazin-1-yl)-2-phenylacetamide (14).

**Step 1:**
To a DMF (1.0 L) solution of tert-butyl piperazine-1-carboxylate (3, 100. g, 538 mmol) was added potassium carbonate (80. g, 58 mmol) and methyl 2-bromo-2-phenylacetate (S1, 73.5 g, 320. mmol). The reaction contents were stirred at rt for 12 h. The reaction mixture was then filtered through a bed of celite, and the filtrate was concentrated in vacuo. The material was purified by column chromatography using 60-120 mesh silica gel, eluting with 20% EtOAc in heptane, to obtain tert-butyl 4-(2-methoxy-2-oxo-1-phenylethyl)piperazine-1-carboxylate (S2, 100. g, 299 mmol, 67% yield) as a colourless oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.44 (d, $J = 6.0$ Hz, 2H), 7.42 – 7.33 (m, 3H), 4.06 (s, 1H), 3.71 (d, $J = 1.1$ Hz, 3H), 3.48 (s, 4H), 2.58 – 2.30 (m, 4H), 1.46 (d, $J = 1.1$ Hz, 9H). LCMS (ESI) $m/z$: 335.2 (M+H$^+$).

**Step 2:**
To a THF (125 mL)/H$_2$O (62.5 mL) solution of tert-butyl 4-(2-methoxy-2-oxo-1-phenylethyl)piperazine-1-carboxylate (S2, 25 g, 74.8 mmol, 1.0 equiv) was added lithium hydroxide (8.9 g, 370 mmol). The reaction mixture was stirred at rt for 12 h. The reaction mixture was then concentrated in vacuo. The material was acidified to pH 5 using 0.5 N aq. HCl. The layers were separated, and the aqueous layer was extracted thrice with CH$_2$Cl$_2$. The organic extracts were combined, dried over Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to provide 2-(4-(tert-butoxycarbonylpiperazin-1-yl)-2-phenylacetic acid (S3, 20. g, 62 mmol, 84% yield) as a white amorphous solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.48 – 7.33 (m, 5H), 4.22 (s, 1H), 2.56 – 2.51 (m, 4H), 2.47 – 2.40 (m, 4H), 1.37 (s, 9H). LCMS (ESI) $m/z$: 321.2 (M+H$^+$).

**Step 3:**
To a CH$_2$Cl$_2$ (105 mL) solution of 2-(4-(tert-butoxycarbonylpiperazin-1-yl)-2-phenylacetic acid (S3, 21 g, 66 mmol) and phenylmethanamine (9.13 g, 85.0 mmol) was added triethylamine (27.4
mL, 197 mmol) and propylphosphonic anhydride (50 wt% in EtOAc, 31.3 g, 98 mmol). The reaction mixture was stirred at rt for 12 h. The reaction was diluted with CH₂Cl₂ (250 mL) and H₂O (300 mL). The layers were separated, and the organic layer was washed with brine, dried over Na₂SO₄, filtered, and concentrated in vacuo. The material was purified by column chromatography using 60-120 mesh silica gel, eluting with 20% EtOAc in hexane, to provide tert-butyl 4-(2-(benzylamino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate (S₄, 18 g, 44 mmol, 67% yield) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-d₆) δ 7.47 – 7.40 (m, 2H), 7.41 – 7.26 (m, 5H), 7.24 – 7.20 (m, 1H), 7.18 – 7.12 (m, 2H), 4.32 – 4.20 (m, 2H), 3.90 (d, J = 2.3 Hz, 1H), 3.32 (d, J = 8.6 Hz, 4H), 2.28 (q, J = 4.0, 3.2 Hz, 4H), 1.38 (d, J = 2.1 Hz, 9H). LCMS (ESI) m/z: 410.2 (M+H)+.

Step 4:

To a 1,4-dioxane (74 mL) solution of tert-butyl 4-(2-(benzylamino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate (S₄, 14.7 g, 35.9 mmol) was added hydrogen chloride (4.0 M in 1,4-dioxane, 40. mL, 180 mmol), and the reaction mixture was stirred at rt for 12 h. The reaction mixture was then concentrated in vacuo. The material was then stirred with Et₂O (50 mL) and filtered to afford racemic N-benzyl-2-phenyl-2-(piperazin-1-yl)acetamide hydrochloride as white amorphous solid. This racemate was subjected to preparatory SFC purification using a (S,S) Whelk-O column (5 μm, 5 x 15 cm), eluting with 30% MeOH containing 0.2% HNEt₂ at a flowrate of 350 mL/min with diode array detection at 225 nm, to provide (R)-N-benzyl-2-phenyl-2-(piperazin-1-yl)acetamide (S₅, 5.08 g, 16.4 mmol, 46% yield, >99% ee) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-d₆) δ 7.69 – 7.54 (m, 2H), 7.47 (dd, J = 5.3, 2.9 Hz, 3H), 7.31 – 7.18 (m, 3H), 7.16 – 7.03 (m, 2H), 5.03 (s, 1H), 4.31 (q, J = 9.1, 7.2 Hz, 2H), 3.35 (q, J = 14.6, 12.9 Hz, 4H), 3.18 (d, J = 31.3 Hz, 4H). LCMS (ESI) m/z: 310.2 (M+H)+.

Step 5:

To a CH₂Cl₂ (5.4 mL) solution of 2-chloro-5-fluorobenzoic acid (0.283 g, 1.62 mmol) and (R)-N-benzyl-2-phenyl-2-(piperazin-1-yl)acetamide (S₅, 0.502 g, 1.62 mmol) was added triethylamine (0.34 mL, 2.4 mmol) and propylphosphonic anhydride (50 wt% in EtOAc, 1.5 mL, 2.4 mmol). The solution was stirred at rt for 16 h. Sat. aq. NaHCO₃ was added. The mixture was stirred at rt for 30 min and partitioned. The aqueous layer was washed twice with CH₂Cl₂. The combined organic extracts were combined, washed with brine, dried over MgSO₄, filtered, and concentrated in vacuo. The material was absorbed onto a plug of silica gel and purified by chromatography through a Biotage Snap Ultra pre-packed silica gel column (25 g), eluting with a gradient of 10% to 70% EtOAc in heptane, to provide 14 (0.69 g, 1.5 mmol, 91% yield) as a white amorphous solid. ¹H NMR (500 MHz, DMSO-d₆) δ 8.68 (t, J = 6.1 Hz, 1H), 7.58 - 7.53 (m, 1H), 7.42 (d, J = 7.0 Hz, 2H), 7.36 - 7.28 (m, 5H), 7.27 - 7.23 (m, 2H), 7.22 - 7.17 (m, 1H), 7.14 (d, J = 7.8 Hz, 2H), 4.33 - 4.21 (m, 2H), 3.95 (s, 1H), 3.73 - 3.56 (m, 2H), 3.17 (t, J = 4.9 Hz, 2H), 2.48 - 2.38 (m, 2H), 2.38 - 2.28 (m, 2H). ¹³C NMR (101 MHz, DMSO-d₆) δ 169.98, 164.04, 161.94, 159.50, 139.38, 137.40, 137.32, 136.94, 131.42, 131.34, 128.61, 128.18, 127.76, 127.01, 126.68, 124.49, 124.46, 117.64, 117.41, 115.17, 114.93, 79.15, 73.89, 73.87, 50.43, 46.09, 41.95, 41.09, ¹⁹F NMR (376 MHz, DMSO-d₆) δ -115.02 (s, 1F). [α]D²² = −65.7° (c 1.35, CHCl₃). HPLC (System A) Rₜ 1.00 min, >95% purity. HRMS (ESI) m/z: [M+H]+ calculated for C₂₆H₂₅ClFN₃O₂: 466.1692, found 466.1697.
(R)-N-Benzyl-2-(4-(2-chloro-5-fluoronicotinoyl)piperazin-1-yl)-2-phenylacetamide (15).

To a CH$_2$Cl$_2$ (19.5 mL) solution of 2-chloro-5-fluoronicotinic acid (1.128 g, 6.43 mmol) and (R)-N-benzyl-2-phenyl-2-(piperazin-1-yl)acetamide (S5, 1.808 g, 5.84 mmol) in dichloromethane was added triethylamine (1.2 mL, 8.8 mmol) and propylphosphonic anhydride (50 wt% in EtOAc, 5.6 mL, 8.77 mmol). The solution was stirred at rt for 16 h. Sat. aq. NaHCO$_3$ was added. The mixture was stirred at rt for 30 min and partitioned. The aqueous layer was washed twice with CH$_2$Cl$_2$. The organic extracts were combined, washed with brine, dried over MgSO$_4$, filtered, and concentrated in vacuo. The material was absorbed onto a plug of silica gel and purified by chromatography through a Reveleris HP 20 µm pre-packed silica gel column (40 g), eluting with a gradient of 20% to 70% EtOAc in heptane, to provide 15 (1.90 g, 4.07 mmol, 70% yield) as a white amorphous solid. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.69 (t, $J$=6.03 Hz, 1H), 8.52 (d, $J$=2.98 Hz, 1H), 8.00 (br d, $J$=6.23 Hz, 1H), 7.42 (d, $J$=7.14 Hz, 2H), 7.18-7.36 (m, 6H), 7.14 (d, $J$=7.66 Hz, 2H), 4.22-4.33 (m, 2H), 3.96 (s, 1H), 3.58-3.72 (m, 2H), 3.19-3.26 (m, 2H), 2.30-2.46 (m, 4H). HPLC (System A) R$_t$ 1.00 min, >95% purity.

(R)-N-Benzyl-2-(4-(3,5-difluorobenzoyl)piperazin-1-yl)-2-phenylacetamide (16).

To a CH$_2$Cl$_2$ (7 mL) solution of 3,5-difluorobenzoic acid (0.333 g, 2.11 mmol) and (R)-N-benzyl-2-phenyl-2-(piperazin-1-yl)acetamide (S5, 0.652 g, 2.11 mmol) was added triethylamine (0.44 mL, 3.2 mmol) and propylphosphonic anhydride (50 wt% in EtOAc, 2.0 mL, 3.2 mmol). The solution was stirred at rt for 16 h. Sat. aq. NaHCO$_3$ was added. The mixture was stirred at rt for 30 min and partitioned. The aqueous layer was washed twice with CH$_2$Cl$_2$. The organic extracts were combined, washed with brine, dried over MgSO$_4$, filtered, and concentrated in vacuo. The material was absorbed onto a plug of silica gel and purified by chromatography through a Biotage Snap Ultra pre-packed silica gel column (25 g), eluting with a gradient of 0% to 70% EtOAc in heptane, to provide 16 (0.90 g, 2.0 mmol, 95% yield) as a white amorphous solid. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.78 (t, $J$=6.01 Hz, 1H), 7.48-7.56 (m, 2H), 7.20-7.46 (m, 9H), 7.14 (d, $J$=7.66 Hz, 2H), 4.22-4.33 (m, 2H), 3.96 (s, 1H), 3.58-3.72 (m, 2H), 3.19-3.26 (m, 2H), 2.30-2.46 (m, 4H).
(m, 11H), 4.28-4.44 (m, 2H), 4.02 (s, 1H), 3.64-3.77 (m, 2H), 3.46 (br s, 2H), 3.41-3.46 (m, 2H), 2.34-2.57 (m, 4H). HPLC (System A) Rₜ 1.02 min, >95% purity.

(R)-N-Benzyl-2-(4-(3-cyano-5-fluorobenzoyl)piperazin-1-yl)-2-phenylacetamide (17).

\[
\begin{align*}
\text{HOC-} & \quad \text{CN} \\
\text{C}_{2}H_{5} & \quad \text{EtOAc, rt, 16 h} \\
\end{align*}
\]

To a CH₂Cl₂ (20 mL) solution of 3-cyano-5-fluorobenzonic acid (1.584 g, 9.59 mmol) and (R)-N-benzyl-2-phenyl-2-(piperazin-1-yl)acetamide (S₅, 3.0277 g, 9.79 mmol) was added triethylamine (2.0 mL, 15 mmol) and propylphosphonic anhydride (50 wt% in EtOAc, 9.3 mL, 15 mmol). The solution was stirred at rt for 16 h. Sat. aq. NaHCO₃ was added. The mixture was stirred at rt for 30 min and partitioned. The aqueous layer was washed twice with CH₂Cl₂. The organic extracts were combined, washed with brine, dried over MgSO₄, filtered, and concentrated in vacuo. The material was absorbed onto a plug of silica gel and purified by chromatography through a Reveleris HP 20 µm pre-packed silica gel column (40 g), eluting with a gradient of 20% to 80% EtOAc in heptane, to provide 17 (4.3 g, 9.4 mmol, 96% yield) as a white amorphous solid. ¹H NMR (500 MHz, DMSO-d₆) δ 8.69 (t, J=6.03 Hz, 1H), 7.94 (br d, J=8.17 Hz, 1H), 7.74 (s, 1H), 7.65 (br d, J=7.91 Hz, 1H), 7.43 (d, J=7.01 Hz, 2H), 7.17-7.37 (m, 6H), 7.13 (d, J=7.27 Hz, 2H), 4.22-4.32 (m, 2H), 3.93 (s, 1H), 3.63 (br s, 2H), 3.32 (br s, 2H), 2.38-2.45 (m, 2H), 2.33 (br s, 2H). HPLC (System A) Rₜ 0.98 min, >95% purity.

(R)-N-Benzyl-2-(4-(3-cyano-5-(trifluoromethoxy)benzoyl)piperazin-1-yl)-2-phenylacetamide (18).

\[
\begin{align*}
\text{HOC-} & \quad \text{OCF₃} \\
\text{C}_{2}H_{5} & \quad \text{EtOAc, rt, 16 h} \\
\end{align*}
\]

A CH₂Cl₂ (44 mL) solution of (R)-N-benzyl-2-phenyl-2-(piperazin-1-yl)acetamide (S₅, 8.19 g, 26.5 mmol), 3-cyano-5-(trifluoromethoxy)benzoic acid (7.34 g, 31.8 mmol), and triethylamine (7.4 mL, 53 mmol) was cooled to 0 °C and then treated with propylphosphonic anhydride (50 wt% in EtOAc, 31.5 mL, 52.9 mmol). After stirring the yellow slurry 16 h while slowly warming to rt, the reaction was quenched with sat. aq. NaHCO₃, stirred for 1 h and subsequently extracted thrice with CH₂Cl₂. The organic extracts were combined, filtered through a phase separator, and concentrated in vacuo. The material was absorbed onto a plug of silica gel and purified by
column chromatography through a Biotage Snap Ultra column (100 g), eluting with 0% to 100% EtOAc in heptane with 10% CH₂Cl₂ cosolvent, to provide 18 (7.5114 g, 14.38 mmol, 54% yield) as a white amorphous solid. ¹H NMR (500 MHz, DMSO-d₆) δ 10.46 (s, 1H), 8.38-8.46 (m, 2H), 8.02 (dd, J=9.0, 2.3 Hz, 1H), 7.79-7.90 (m, J=8.0 Hz, 2H), 7.64-7.73 (m, J=8.0 Hz, 2H), 7.27-7.33 (m, 2H), 7.19-7.26 (m, 3H), 6.95 (d, J=9.0 Hz, 1H), 4.94 (q, J=9.1 Hz, 2H), 4.21-4.31 (m, 3H), 2.78-3.10 (m, 1H), 2.77 (t, J=8.2 Hz, 1H), 2.59 (t, J=7.7 Hz, 1H), 2.45-2.49 (m, 1H), 1.94 ppm (d, J=6.1 Hz, 2H). HPLC (System A) Rₜ 1.07 min, >95% purity.

(R)-N-Benzyl-2-(4-(2-chloro-5-fluorobenzoyl)piperazin-1-yl)-2-(4-cyanophenyl)acetamide (19).

\[
\text{3} \xrightarrow{\text{MeCN, 0 °C to rt, 3 h}} \text{S6} \xrightarrow{TFA, CH}_2\text{Cl}_2, 0 °C to rt, 18 h} \xrightarrow{\text{HOAc, TFE, rt, 16 h, chiral separation}} \text{19}
\]

**Step 1:**

To a MeCN (70 mL) slurry of 2-chloro-5-fluorobenzoic acid (3.00 g, 17.2 mmol), tert-butyl piperazine-1-carboxylate (3, 3.20 g, 17.2 mmol), and triethylamine (7.2 ml, 52 mmol) cooled in an ice water bath was added (1-cyano-2-ethoxy-2-oxoethylidenaminoxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU, 7.73 g, 18.1 mmol). The resulting yellow solution was stirred for 3 h while allowing the reaction to slowly warm to rt. The mixture was then concentrated in vacuo and purified by column chromatography using a Silicycle HP column (120 g), eluting with 0% to 100% EtOAc in heptane with 5% CH₂Cl₂ cosolvent, to afford tert-butyl 4-(2-chloro-5-fluorobenzoyl)piperazine-1-carboxylate (S6, 5.53 g, 16.1 mmol, 94% yield) as a white flocculent solid. HPLC (System A) Rₜ 1.22 min. LCMS (ESI) m/z: 365.0 (M+Na)⁺.

**Step 2:**

To a CH₂Cl₂ (65 mL) solution of tert-butyl 4-(2-chloro-5-fluorobenzoyl)piperazine-1-carboxylate (S6, 5.53 g, 16.1 mmol) cooled in an ice water bath was added trifluoroacetic acid (8.7 mL, 110 mmol). The reaction was allowed to warm to rt while stirring for 18 h. The reaction was concentrated in vacuo and absorbed on a Biotage Isolute SCX-2 strong cation exchange column (20 g), washing with MeOH prior to eluting with 2 M NH₃ in MeOH, to afford
(2-chloro-5-fluorophenyl)(piperazin-1-yl)methanone (S7, 3.83 g, 15.78 mmol, 98% yield) as an off-white amorphous solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.57 (dd, $J = 4.77$, 8.81 Hz, 1H), 7.26-7.38 (m, 2H), 3.55 (dt, $J = 2.93$, 5.09 Hz, 2H), 3.05 (dd, $J = 4.20$, 5.86 Hz, 2H), 2.68-2.82 (m, 2H), 2.59-2.68 (m, 2H). HPLC (System A) R_t 0.57 min. LCMS (ESI) $m/z$: 243.0 (M+H)$^+$.  

**Step 3:**

To a TFE (2 mL) solution of (2-chloro-5-fluorophenyl)(piperazin-1-yl)methanone (S7, 86 mg, 0.25 mmol) was added 4-formylbenzonitrile (S8, 33 mg, 0.25 mmol) and acetic acid (17 $\mu$L, 0.30 mmol) dropwise. To this solution was added benzyl isocyanide (1, 30. $\mu$L, 0.25 mmol). The reaction was stirred 16 h at rt. The mixture was then concentrated in vacuo. The material was purified by preparative reversed-phase high-performance liquid chromatography to afford rac-19 (32.5 mg, 0.0662 mmol, 26% yield) as a white amorphous solid. This racemate was subjected to preparatory SFC purification using a (S,S) Whelk-O column (5 $\mu$m, 2 x 15 cm), eluting with 50% MeOH at a flowrate of 80 mL/min with diode array detection at 215 nm, to provide 19 (14.2 mg, >99%ee) as a white amorphous solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.66 (d, $J = 8.3$ Hz, 2H), 7.45 (d, $J = 8.3$ Hz, 2H), 7.39 - 7.30 (m, 4H), 7.20 (dd, $J = 1.9$, 7.7 Hz, 2H), 7.11 (br t, $J = 5.8$ Hz, 1H), 7.04 (ddd, $J = 3.0$, 7.8, 8.9 Hz, 1H), 6.97 (td, $J = 2.5$, 7.8 Hz, 1H), 4.57 - 4.38 (m, 2H), 4.02 (s, 1H), 3.90 - 3.79 (m, 1H), 3.78 - 3.69 (m, 1H), 3.35 - 3.25 (m, 1H), 3.25 - 3.15 (m, 1H), 2.75 (br dd, $J = 1.3$, 4.7 Hz, 1H), 2.64 - 2.53 (m, 1H), 2.49 - 2.42 (m, 1H), 2.39 - 2.28 (m, 1H). HPLC (System A) R_t 1.09 min, >95% purity. LCMS (ESI) $m/z$: 491.0 (M+H)$^+$.  

(R)-N-Benzyl-2-(4-cyano-3-methylphenyl)-2-(4-(3,5-difluorobenzoyl)piperazin-1-yl)acetamide (20).

To a vial charged with 4-formyl-2-methylbenzonitrile (S9, 0.051 g, 0.35 mmol) was sequentially added (2-chloro-5-fluorophenyl)(piperazin-1-yl)methanone (S7, 0.085 g, 0.350 mmol), TFE (2.8 mL), acetic acid (0.024 ml, 0.42 mmol), and benzyl isocyanide (1, 0.043 mL, 0.35 mmol). The vial was shaken for 18 h at 40 °C. The reaction was absorbed on a Biotage Isolute SCX-2 strong cation exchange column (2 g), washing with MeOH prior to eluting with 2 M NH$_3$ in MeOH. The filtrate was concentrated in vacuo and purified via preparatory reverse-phase purification, eluting with 10% to 30% MeCN in H$_2$O containing 0.1% NH$_3$OH, to afford rac-20 (0.0995 g, 0.197 mmol, 56% yield) as a white amorphous solid. A portion of this racemate (0.094 g) was subjected to preparatory SFC purification using a Chiralpak AD-H column (5 $\mu$m, 2 x 15 cm), eluting with 40% i-PrOH containing 0.2% HNEt$_2$ at a flowrate of 80 mL/min with diode array detection at 215 nm, to provide 20 (0.0152 g, >99%ee) as a white amorphous solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.81 (t, $J = 5.96$ Hz, 1H), 7.70 (d, $J = 7.88$ Hz, 1H), 7.57 (dd, $J = 4.96$, 8.9 Hz, 2H), 7.40 (dd, $J = 6.20$, 7.8 Hz, 2H), 7.30 (d, $J = 7.78$ Hz, 2H), 7.20 (dd, $J = 7.78$, 8.9 Hz, 2H), 7.11 (br t, $J = 4.78$ Hz, 1H), 2.82 (br s, 1H), 2.65 - 2.53 (m, 1H), 2.49 - 2.42 (m, 1H), 2.37 - 2.28 (m, 1H). HPLC (System A) R_t 1.09 min, >95% purity. LCMS (ESI) $m/z$: 491.0 (M+H)$^+$.  

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\( J = 4.82, 7.67 \text{ Hz, } 1H), 7.18-7.35 \text{ (m, } 6H), 7.09-7.17 \text{ (m, } 3H), 4.20-4.36 \text{ (m, } 2H), 4.07 \text{ (s, } 1H), 3.87 \text{ (s, } 3H), 3.58-3.74 \text{ (m, } 2H), 3.12-3.23 \text{ (m, } 2H), 2.27-2.48 \text{ (m, } 4H). \) HPLC (System A) \( R_t \) 1.10 min, >95% purity. LCMS (ESI) \( m/z: 505.0 \text{ (M+H)}^+ \).

\((R)-\text{N-Benzyl-2-(4-(3,5-difluorobenzoyl)piperazin-1-yl)-N-methyl-2-phenylacetamide (21).}\)

**Step 1:**

To a CH\(_2\text{Cl}_2\) (60 mL) solution of tert-butyl 4-(2-methoxy-2-oxo-1-phenylethyl)piperazine-1-carboxylate (S2, 6.05 g, 18.09 mmol) was added 2,2,2-trifluoroacetic acid (34.6 mL, 452 mmol). The reaction was stirred at rt for 2 h. The reaction was then basified with 6 N NaOH and extracted with thrice with CH\(_2\text{Cl}_2\). The organic extracts were combined, dried over MgSO\(_4\), filtered, and concentrated in vacuo to provide methyl 2-phenyl-2-(piperazin-1-yl)acetate (S10) as a yellow oil that was used without further purification. \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \( \delta \ 7.19-7.45 \text{ (m, } 9H), 7.02-7.16 \text{ (m, } 4H), 4.60-4.74 \text{ (m, } 1H), 4.47-4.56 \text{ (m, } 2H), 3.49-3.67 \text{ (m, } 2H), 3.20-3.31 \text{ (m, } 2H), 2.75-2.91 \text{ (m, } 3H), 2.51-2.68 \text{ (m, } 2H), 2.31-2.48 \text{ (m, } 2H). \) HPLC (System A) \( R_t \) 0.67 min. LCMS (ESI) \( m/z: 234.2 \text{ (M+H)}^+ \).

**Step 2:**

To a CH\(_2\text{Cl}_2\) (36 mL) solution of 3,5-difluorobenzoic acid (2.86 g, 18.10 mmol) and methyl 2-phenyl-2-(piperazin-1-yl)acetate (S10) from the prior step was added triethylamine (5.0 mL, 36 mmol) and propylphosphonic anhydride (50 wt% in EtOAc, 9.3 mL, 15 mmol). The solution was stirred at rt for 16 h. Sat. aq. NaHCO\(_3\) was added. The mixture was stirred at rt for 30 min and partitioned. The aqueous layer was washed twice with CH\(_2\text{Cl}_2\). The organic extracts were combined, washed with brine, dried over MgSO\(_4\), filtered, and concentrated in vacuo to provide methyl 2-(4-(3,5-difluorobenzoyl)piperazin-1-yl)-2-phenylacetate (S11, 6.71 g, 17.9 mmol, 99% yield over 2 steps) as a tan amorphous solid. HPLC (System A) \( R_t \) 0.92 min. LCMS (ESI) \( m/z: 375.2 \text{ (M+H)}^+ \).

**Step 3:**
A THF (70 mL)/MeOH (12 mL)/H₂O (12 mL) solution of methyl 2-(4-(3,5-difluorobenzoyl)piperazin-1-yl)-2-phenylacetate (S11, 6.70 g, 17.90 mmol) and lithium hydroxide (2.14 g, 89.0 mmol) was stirred at rt for 4 h. The mixture was cooled using an ice-H₂O bath and then acidified to ca. pH 3 with 2 N HCl. The mixture was then extracted thrice with CH₂Cl₂. The organic extracts were combined, washed with brine, dried over MgSO₄, filtered, and concentrated in vacuo to afford racemic 2-(4-(3,5-difluorobenzoyl)piperazin-1-yl)-2-phenylacetic acid (rac-S12, 5.30 g, 14.7 mmol, 82% yield) as a white amorphous solid. This racemate was subjected to preparatory SFC purification using a Chiralpak AD-H column (5 µm, 2 x 25 cm), eluting with 25% MeOH at a flowrate of 70 mL/min with diode array detection at 215 nm, to provide (R)-2-(4-(3,5-difluorobenzoyl)piperazin-1-yl)-2-phenylacetic acid (S12, 1.82 g, >99% ee). HPLC (System A) Rt 1.09 min. LCMS (ESI) m/z: 361.2 (M+H)+.

Step 4:

To a CH₂Cl₂ (1 mL) mixture of N-methyl-1-phenylmethanamine (0.024 g, 0.20 mmol), (R)-2-(4-(3,5-difluorobenzoyl)piperazin-1-yl)-2-phenylacetic acid (S12, 0.072 g, 0.20 mmol) was sequentially added triethylamine (0.056 mL, 0.40 mmol) and propylphosphonic anhydride (50 wt% in EtOAc, 0.26 mL, 0.40 mmol). The mixture was shaken at rt for 16 h. Sat. aq. NaHCO₃ was added. The mixture was shaken at rt for 30 min and partitioned in a phase separator, and the filtrate was dried in vacuo. The material was purified via high throughput parallel purification to provide 21 (56.0 mg, 0.121 mmol, 60% yield) as a white amorphous solid. ¹H NMR (500 MHz, DMSO-d₆) δ 7.45 - 7.29 (m, 7H), 7.28 - 7.21 (m, 2H), 7.13 - 7.03 (m, 4H), 4.74 - 4.63 (m, 1H), 4.56 - 4.48 (m, 2H), 3.58 (br d, J=2.3 Hz, 2H), 3.31 - 3.20 (m, 2H), 2.87 (s, 3H), 2.77 (s, 1H), 2.62 (br d, J=3.5 Hz, 1H), 2.46 - 2.28 (m, 2H). HPLC (System B) Rₜ 2.01 min, >95% purity. LCMS (ESI) m/z: 464.2 (M+H)+.

(R)-2-(4-(3,5-Difluorobenzoyl)piperazin-1-yl)-2-phenyl-N-((6-(trifluoromethyl)pyridin-3-yl)methyl)acetamide (22).

To a DMF (1 mL) mixture of (6-(trifluoromethyl)pyridin-3-yl)methanamine (0.044 g, 0.25 mmol), 2-(4-(3,5-difluorobenzoyl)piperazin-1-yl)-2-phenylacetic acid (rac-S12, 0.080 g, 0.22 mmol) was added triethylamine (0.062 mL, 0.44 mmol) and propylphosphonic anhydride (50 wt% in DMF, 0.28 mL, 0.44 mmol). The mixture was stirred at rt for 16 h. The reaction was purified via preparatory reverse-phase purification, eluting with 5% to 95% MeCN in H₂O using 0.1% NH₄OH additive, to provide rac-22 (63.4 mg, 0.122 mmol, 55% yield) as a white amorphous solid. This racemate was subjected to preparatory SFC purification using a (S,S) Whelk-O column (5 µm, 2 x 15 cm), eluting with 35% MeOH containing 0.2% HNEt₂ at a
flowrate of 80 mL/min with diode array detection at 254 nm, to provide 22 (21.5 mg, >99%ee) as a white amorphous solid. \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \(\delta\) 8.90 (br t, \(J=5.81\) Hz, 1H), 8.52 (s, 1H), 7.78 (s, 2H), 7.27-7.41 (m, 6H), 7.09 (br d, \(J=5.38\) Hz, 2H), 6.43-4.43 (m, 2H), 3.92-3.93 (s, 1H), 3.28-3.37 (m, 4H), 2.93 (br s, 2H), 2.30 (br s, 2H). HPLC (System A) \(R_t\) 1.00 min, >95% purity. LCMS (ESI) \(m/z\): 519.2 (M+H).\(^7\)

\((R)-2-(4-(3,5-Difluorobenzoyl)piperazin-1-yl)-2-phenyl-N-(4-(trifluoromethyl)phenyl)acetamide (23).\)

To a DMF (0.5 mL) mixture of 4-(trifluoromethyl)aniline (0.040 g, 0.25 mmol) and 2-(4-(3,5-difluorobenzoyl)piperazin-1-yl)-2-phenylacetic acid (rac-S12, 0.090 g, 0.25 mmol) was added triethylamine (0.070 mL, 0.50 mmol) and propylphosphonic anhydride (50 wt% in DMF, 0.32 mL, 0.50 mmol). The mixture was stirred at rt for 16 h. The reaction was then purified via high throughput parallel purification to provide rac-23 (35.7 mg, 0.0709 mmol, 28% yield) as a white amorphous solid. This racemate was subjected to preparatory SFC purification using a (S,S) Whelk-O column (5 \(\mu\)m, 2 x 15 cm), eluting with 30% MeOH containing 0.2% HNEt\(_2\) at a flowrate of 80 mL/min with diode array detection at 250 nm to provide 23 (16.1 mg, >99%ee) as a white amorphous solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 9.10 (s, 1H), 7.70 (d, \(J=8.55\) Hz, 2H), 7.60 (d, \(J=8.66\) Hz, 2H), 7.28-7.44 (m, 5H), 6.84-6.96 (m, 3H), 4.09 (s, 1H), 3.82 (br s, 2H), 3.52 (br s, 2H), 2.64 (s, 4H). HPLC (System A) \(R_t\) 1.17 min, >95% purity. LCMS (ESI) \(m/z\): 504.2 (M+H).\(^7\)

\((R)-2-(4-(3,5-Difluorobenzoyl)piperazin-1-yl)-2-phenyl-N-(6-(2,2,2-trifluoroethoxy)pyridin-3-yl)acetamide (24).\)

To a DMF (0.5 mL) mixture of 6-(2,2,2-trifluoroethoxy)pyridin-3-amine (0.048 g, 0.25 mmol), 2-(4-(3,5-difluorobenzoyl)piperazin-1-yl)-2-phenylacetic acid (rac-S12, 0.090 g, 0.25 mmol) was added triethylamine (0.070 mL, 0.50 mmol) and propylphosphonic anhydride (50 wt% in DMF, 0.32 mL, 0.50 mmol). The mixture was stirred at rt for 16 h. The reaction was purified
via high throughput parallel purification to provide rac-24 (27.3 mg, 0.0511 mmol, 20% yield) as a white amorphous solid. This racemate was subjected to preparatory SFC purification using a (S,S) Whelk-O column (5 μm, 2 x 15 cm), eluting with 35% MeOH containing 0.2% HNEt₂ at a flow rate of 80 mL/min with diode array detection at 215 nm, to provide 24 (8.0 mg, >99% ee) as a white amorphous solid. ¹H NMR (400 MHz, CDCl₃) δ 8.87 (s, 1H), 8.24 (d, J=2.49 Hz, 1H), 8.02 (d, J=2.72, 8.89 Hz, 1H), 7.30-7.47 (m, 5H), 6.85-6.96 (m, 4H), 4.76 (q, J=8.55 Hz, 2H), 4.09 (s, 1H), 3.45-4.00 (m, 4H), 2.43-2.77 (m, 4H). HPLC (System A) Rₜ 1.12 min, >95% purity. LCMS (ESI) m/z: 535.2 (M+H)+.

(R)-N-(Cyclopropylmethyl)-2-(4-(3,5-difluorobenzoyl)piperazin-1-yl)-2-phenylacetamide (25).

![chemical structure](image)

To a CH₂Cl₂ (1 mL) mixture of cyclopropylmethanamine (0.014 g, 0.20 mmol) and (R)-2-(4-(3,5-difluorobenzoyl)piperazin-1-yl)-2-phenylacetic acid (S12, 0.072 g, 0.20 mmol) was sequentially added triethylamine (0.056 mL, 0.40 mmol) and propylphosphonic anhydride (50 wt% in EtOAc, 0.26 mL, 0.40 mmol). The mixture was shaken at rt for 16 h. Sat. aq. NaHCO₃ was added.  The mixture was shaken at rt for 30 min and partitioned in a phase separator, and the filtrate was dried in vacuo. The material was purified via high throughput parallel purification to provide 25 (14.3 mg, 0.0344 mmol, 17% yield) as a white amorphous solid. ¹H NMR (500 MHz, DMSO-d₆) δ 8.21 (br s, 1H), 7.41 (br d, J=7.20 Hz, 2H), 7.26-7.37 (m, 4H), 7.13 (br d, J=5.32 Hz, 2H), 3.85 (s, 1H), 3.54-3.72 (m, 2H), 3.28-3.40 (m, 2H), 2.89-3.00 (m, 2H), 2.23-2.44 (m, 4H), 0.88 (br s, 1H), 0.34 (br d, J=7.91 Hz, 2H), 0.06-0.16 (m, 2H). HPLC (System B) Rₜ 1.94 min, >95% purity. LCMS (ESI) m/z: 416.2 (M+H)+.

(R)-N-(2-Cyclopropyl-2,2-difluoroethyl)-2-(4-(3,5-difluorobenzoyl)piperazin-1-yl)-2-phenylacetamide (26).

![chemical structure](image)

To a DMF (0.75 mL) mixture of 2-cyclopropyl-2,2-difluoroethanamine (0.047 g, 0.30 mmol), (R)-2-(4-(3,5-difluorobenzoyl)piperazin-1-yl)-2-phenylacetic acid (S12, 0.054 g, 0.15 mmol) was sequentially added triethylamine (0.042 mL, 0.30 mmol) and propylphosphonic anhydride (50
wt% in DMF, 0.19 mL, 0.30 mmol). The mixture was shaken at rt for 16 h. The material was filtered and purified via high throughput parallel purification to provide 26 (38.1 mg, 0.0822 mmol, 55% yield) as a white amorphous solid. 1H NMR (500 MHz, DMSO-d6) δ 8.49 (br t, J=5.97 Hz, 1H), 7.42 (br d, J=7.01 Hz, 2H), 7.25-7.38 (m, 4H), 7.12 (br d, J=5.32 Hz, 2H), 3.98 (s, 1H), 3.52-3.73 (m, 4H), 3.25-3.31 (m, 2H), 2.25-2.44 (m, 4H), 1.13-1.33 (m, 1H), 0.37-0.53 (m, 4H). HPLC (System B) Rt 1.92 min, >95% purity. LCMS (ESI) m/z: 464.2 (M+H)+.

(R)-N-Benzyl-2-(6-(2-chloro-5-fluorobenzoyl)-3,6-diazabicyclo[3.1.1]heptan-3-yl)-2-phenylacetamide (27).

![Chemical diagram]

**Step 1:**

A TFE (8 mL) solution of tert-butyl 3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (S13, 203 mg, 1.024 mmol) was treated with benzaldehyde (S14, 0.10 mL, 1.0 mmol), acetic acid (0.070 mL, 1.2 mmol), and benzyl isocyanide (1, 125 µl, 1.02 mmol). The solution was stirred for 18 h at rt. The reaction was then filtered through a Biotage Isolute SCX-2 strong cation exchange column (5 g), washing with MeOH prior to eluting with 2 M NH3 in MeOH. The filtrate was concentrated in vacuo and purified by column chromatography through a Biotage Snap Ultra column (25 g), eluting with 0% to 100% EtOAc in heptane with 5% CH2Cl2 cosolvent, to afford tert-butyl 3-(2-(benzylamino)-2-oxo-1-phenylethyl)-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (S15, 252.8 mg, 0.600 mmol, 59% yield) as a white amorphous solid. HPLC (System A) Rt 1.05 min. LCMS (ESI) m/z: 422.2 (M+H)+.

**Step 2:**

A CH2Cl2 (3 mL) solution of tert-butyl 3-(2-(benzylamino)-2-oxo-1-phenylethyl)-3,6-diazabicyclo[3.1.1]heptane-6-carboxylate (S15, 252.8 mg, 0.600 mmol) was treated with trifluoroacetic acid (1.5 mL, 20. mmol). The solution was stirred at rt for 1 h. The reaction was then quenched with sat. aq. NaHCO3 until the aqueous layer pH >8. The reaction was extracted thrice with CH2Cl2. The organic extracts were passed through a phase separator, combined, and concentrated in vacuo to afford N-benzyl-2-(3,6-diazabicyclo[3.1.1]heptan-3-yl)-2-
phenylacetamide (S16) as an off-white amorphous solid that was used without further purification. HPLC (System A) R\text{t} 0.83 min. LCMS (ESI) \(m/z: 322.2\) (M+H)^+.  

**Step 3:**

To a CH\textsubscript{2}Cl\textsubscript{2} (1.3 mL) mixture of \(N\)-benzyl-2-(3,6-diazabicyclo[3.1.1]heptan-3-yl)-2-phenylacetamide (S16) from the previous step and 2-chloro-5-fluorobenzoic acid (160 mg, 0.917 mmol) was sequentially added triethylamine (0.21 mL, 1.5 mmol) and propylphosphonic anhydride (50 wt% in EtOAc, 0.91 mL, 1.5 mmol). The mixture was stirred at rt for 18 h. Sat. aq. NaHCO\textsubscript{3} was added. The mixture was shaken at rt for 1 h and then extracted thrice with CH\textsubscript{2}Cl\textsubscript{2}. The organic extracts were combined, partitioned through a phase separator, and the organic filtrate was concentrated \textit{in vacuo}. The material was purified via column chromatography through a Biotage Snap Ultra column (25 g), eluting with 0% to 50% EtOAc in heptane, to provide rac-27 (201.7 mg, 0.422 mmol, 70% yield over 2 steps) as a white amorphous solid. HPLC (System A) R\text{t} 1.94 min. LCMS (ESI) \(m/z: 416.2\) (M+H)^+. This racemate was subjected to preparatory SFC purification using a (S,S) Whelk-O column (5 μm, 2 x 25 cm), eluting with 40% MeOH at a flowrate of 80 mL/min with diode array detection at 215 nm, to provide 27 (76.0 mg, >99% ee) as a white amorphous solid. \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) δ ppm 2.02 - 2.18 (m, 1 H) 2.37 (d, \(J=10.90\) Hz, 1 H) 2.45 (br. s., 1 H) 2.67 - 2.77 (m, 1 H) 2.68 - 2.74 (m, 1 H) 2.82 - 2.97 (m, 1 H) 3.01 - 3.24 (m, 1 H) 3.94 - 4.09 (m, 1 H) 4.21 - 4.27 (m, 2 H) 4.30 - 4.42 (m, 2 H) 7.13 - 7.16 (m, 2 H) 7.19 - 7.36 (m, 8 H) 7.43 (t, \(J=7.22\) Hz, 2 H) 7.50 - 7.60 (m, 1 H) 8.61 - 8.70 (m, 1 H). HPLC (System A) R\text{t} 1.05 min, >95% purity. LCMS (ESI) \(m/z: 478.2\) (M+H)^+.  

(R)-\(N\)-Benzyl-2-(8-(2-chloro-5-fluorobenzoyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-2-phenylacetamide (28).  

![Chemical structures](image)

**Step 1:**

A DMF (1.4 mL) slurry of \(tert\)-butyl 3,8-diazabicyclo[3.2.1]octane-8-carboxylate (S17, 299.2 mg, 1.409 mmol), potassium carbonate (234 mg, 1.69 mmol), and methyl 2-bromo-2-phenylacetate (S1, 323 mg, 1.41 mmol) was stirred for 3 d at rt. The reaction was then quenched
with H₂O and extracted thrice with EtOAc. The organic extracts were combined, washed with H₂O, filtered through a phase separator, and concentrated \textit{in vacuo} to afford \textit{tert}-butyl 3-(2-methoxy-2-oxo-1-phenylethyl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (S₁₈, 498.4 mg, 1.383 mmol, 98% yield) as a light yellow oil that was used without further purification. HPLC (System A) Rᵣ 1.24 min. LCMS (ESI) \textit{m/z}: 361.2 (M+H)⁺.

**Step 2:**

A THF (2.8 mL)/MeOH (1 mL)/H₂O (1 mL) solution of \textit{tert}-butyl 3-(2-methoxy-2-oxo-1-phenylethyl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (S₁₈, 498.4 mg, 1.383 mmol) was treated with lithium hydroxide monohydrate (296 mg, 7.05 mmol). The reaction was stirred for 18 h at rt. The reaction was then acidified with 2 N HCl until the pH was < 2 and then extracted four times with CH₂Cl₂. The organic extracts were combined, filtered through a phase separator, and concentrated \textit{in vacuo} to afford 2-(8-\{\textit{tert}-butoxycarbonyl\}-3,8-diazabicyclo[3.2.1]octan-3-yl)-2-phenylacetic acid (S₁₉, 407.0 mg, 1.175 mmol, 85 % yield) as a white amorphous solid that was used without further purification. HPLC (System A) Rᵣ 0.89 min. LCMS (ESI) \textit{m/z}: 347.2 (M+H)⁺.

**Step 3:**

A CH₂Cl₂ (2.0 mL) solution of 2-(8-\{\textit{tert}-butoxycarbonyl\}-3,8-diazabicyclo[3.2.1]octan-3-yl)-2-phenylacetic acid (S₁₉, 407.0 mg, 1.175 mmol), benzylamine (0.14 mL, 1.29 mmol), and triethylamine (0.33 mL, 2.4 mmol) was treated with propylphosphonic anhydride (50 wt% in EtOAc, 1.4 mL, 2.4 mmol). After stirring the slurry at rt for 18 h, the reaction was quenched with sat. aq. NaHCO₃, and the mixture was stirred for 1 h and subsequently extracted thrice with CH₂Cl₂. The organic extracts were combined, filtered through a phase separator, and concentrated \textit{in vacuo}. The material was purified via column chromatography through a Biotage Snap Ultra column (100 g), eluting with 0% to 100% EtOAc in heptane with 5% CH₂Cl₂ cosolvent, to afford \textit{tert}-butyl 3-(2-{benzylamino}-1-phenylethyl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (S₂₀, 384.6 mg, 0.883 mmol, 75% yield) as a white amorphous solid. HPLC (System A) Rᵣ 1.16 min. LCMS (ESI) \textit{m/z}: 436.2 (M+H)⁺.

**Step 4:**

A CH₂Cl₂ (5 mL) solution of \textit{tert}-butyl 3-(2-{benzylamino}-1-phenylethyl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (S₂₀, 384.6 mg, 0.883 mmol) was treated with trifluoroacetic acid (1.7 mL, 23 mmol). After stirring for 1 h at rt, sat. aq. NaHCO₃ was added until pH > 7. The mixture was extracted thrice with CH₂Cl₂. The organic extracts were filtered through a phase separator, combined, and concentrated \textit{in vacuo} to afford N-benzyl-2-(3,8-diazabicyclo[3.2.1]octan-3-yl)-2-phenylacetamide (S₂₁) as an off-white amorphous solid that was used without further purification. HPLC (System A) Rᵣ 0.87 min. LCMS (ESI) \textit{m/z}: 336.2 (M+H)⁺.

**Step 5:**
A CH$_2$Cl$_2$ (1.0 mL) solution of $N$-benzyl-2-(3,8-diazabicyclo[3.2.1]octan-3-yl)-2-phenylacetamide (S21, 0.200 g, 0.596 mmol), 2-chloro-5-fluorobenzoic acid (125 mg, 0.715 mmol), and triethylamine (0.17 mL, 1.2 mmol) was treated with propylphosphonic anhydride (50 wt% in EtOAc, 0.71 mL, 1.2 mmol). After stirring the solution at rt for 18 h, the reaction was quenched with sat. aq. NaHCO$_3$, and the mixture was stirred for 1 h and subsequently extracted thrice with CH$_2$Cl$_2$. The organic extracts were combined, filtered through a phase separator, and concentrated in vacuo. The material was purified via column chromatography through a Biotage Snap Ultra column (100 g), eluting with 0% to 50% EtOAc in heptane, to afford rac-28 (144.7 mg, 0.294 mmol, 33% yield over 2 steps) as a white amorphous solid. This racemate was subjected to preparatory SFC purification using a (S,S) Whelk-O column (5 μm, 2 x 15 cm), eluting with 55% MeOH at a flowrate of 80 mL/min with diode array detection at 220 nm to provide 28 (41.4 mg, >99%ee) as a white amorphous solid. $^1$H NMR (500 MHz, DMSO-d$_6$) δ ppm 1.76 - 1.90 (m, 2 H) 1.93 - 2.08 (m, 2 H) 2.13 (d, $J$=9.73 Hz, 1 H) 2.30 - 2.37 (m, 1 H) 2.44 (d, $J$=10.25 Hz, 1 H) 2.66 - 2.90 (m, 1 H) 3.47 - 3.62 (m, 1 H) 3.94 (d, $J$=4.15 Hz, 1 H) 4.19 - 4.34 (m, 2 H) 4.51 - 4.67 (m, 1 H) 7.10 (d, $J$=7.27 Hz, 1 H) 7.14 (d, $J$=7.53 Hz, 1 H) 7.17 - 7.36 (m, 8 H) 7.43 (d, $J$=7.40 Hz, 1 H) 7.46 (d, $J$=7.01 Hz, 1 H) 7.50 - 7.60 (m, 1 H) 8.55 (ddd, $J$=17.97, 5.97, 5.77 Hz, 1 H). HPLC (System A) R$_t$ 1.21 min, >95% purity. LCMS (ESI) m/z: 492.2 (M+H)$^+$. 

(R)-2-(4-(2-Chloro-5-fluoronicotinoyl)piperazin-1-yl)-2-phenyl-$N$-(6-(2,2,2-trifluoroethoxy)pyridin-3-yl)acetamide (29).

**Step 1:**

A CH$_2$Cl$_2$ (50 mL) solution of 2-(4-(tert-butoxycarbonyl)piperazin-1-yl)-2-phenylacetic acid (S3, 9.87 g, 30.8 mmol), 6-(2,2,2-trifluoroethoxy)pyridin-3-amine (5.89 g, 30.8 mmol), and triethylamine (8.6 mL, 62 mmol) was cooled to 0 °C and then treated with propylphosphonic anhydride (50 wt% in EtOAc, 37 mL, 61.6 mmol) portionwise over 1 min. After stirring the solution at rt for 18 h while allowing to slowly warm to rt, the reaction was quenched with sat. aq. NaHCO$_3$, and the mixture was stirred for 1 h and subsequently extracted thrice with CH$_2$Cl$_2$. The organic extracts were combined, filtered through a phase separator, and concentrated in
The material was purified via column chromatography through a Biotage Snap Ultra column (100 g), eluting 0% to 100% EtOAc in heptane with 5% CH₂Cl₂ cosolvent, to afford tert-butyl 4-(2-oxo-1-phenyl-2-((6-(2,2,2-trifluoroethoxy)pyridin-3-yl)amino)ethyl)piperazine-1-carboxylate (S22, 12.51 g, 25.3 mmol, 82% yield) as a sepiamorphous solid. HPLC (System A) Rᵣ 1.11 min. LCMS (ESI) m/z: 495.2 (M+H)⁺.

**Step 2:**

A CH₂Cl₂ (25 mL) solution of tert-butyl 4-(2-oxo-1-phenyl-2-((6-(2,2,2-trifluoroethoxy)pyridin-3-yl)amino)ethyl)piperazine-1-carboxylate (S22, 12.51 g, 25.3 mmol) was treated with hydrogen chloride (4.0 M in dioxane, 28.4 ml, 114 mmol). After stirring solution for 30 min at rt, sat. aq. NaHCO₃ was added until pH > 7. The mixture was extracted thrice with CH₂Cl₂. The organic extracts were combined, filtered through a phase separator, and concentrated in vacuo to afford racemic 2-phenyl-2-(piperazin-1-yl)-N-(6-(2,2,2-trifluoroethoxy)pyridin-3-yl)acetamide hydrochloride (rac-S23·HCl) as a brown amorphous solid. This racemate was subjected to preparatory SFC purification using a Chiralpak AS-H column (5 μm, 5 x 15 cm), eluting with 20% i-PrOH at a flow rate of 350 mL/min with diode array detection at 215 nm to provide (R)-2-phenyl-2-(piperazin-1-yl)-N-(6-(2,2,2-trifluoroethoxy)pyridin-3-yl)acetamide (S23, 6.75 g, 17.1 mmol, 15% yield, >99% ee) as a brown amorphous solid. HPLC (System A) Rᵣ 0.95 min. LCMS (ESI) m/z: 395.2 (M+H)⁺.

**Step 3:**

A CH₂Cl₂ (4.2 mL) solution of (R)-2-phenyl-2-(piperazin-1-yl)-N-(6-(2,2,2-trifluoroethoxy)pyridin-3-yl)acetamide (S23, 1.00 g, 2.54 mmol), 2-chloro-5-fluoronicotinic acid (0.534 g, 3.04 mmol), and triethylamine (0.71 ml, 5.1 mmol) was treated with propylphosphonic anhydride (50 wt% in EtOAc, 3.0 mL, 5.1 mmol). After stirring the slurry at rt for 18 h, the reaction was quenched with sat. aq. NaHCO₃, and the mixture was stirred for 1 h and subsequently extracted thrice with CH₂Cl₂. The organic extracts were combined, filtered through a phase separator, and concentrated in vacuo. The material was purified via column chromatography through a Biotage Snap Ultra column (50 g), eluting 0% to 100% EtOAc in heptane with 5% CH₂Cl₂ cosolvent, to afford 29 (0.6982 g, 1.265 mmol, 50% yield) as a white amorphous solid. ¹H NMR (DMSO-d₆, 500 MHz): δ 10.28 (br d, J = 4.4 Hz, 1H), 8.53 (d, J = 2.9 Hz, 1H), 8.41 (s, 1H), 8.01 (ddd, J = 14.7, 8.4, 2.3 Hz, 2H), 7.49 (d, J = 7.5 Hz, 2H), 7.35-7.42 (m, 2H), 7.29-7.35 (m, 1H), 6.95 (d, J = 8.8 Hz, 1H), 4.94 (q, J = 9.1 Hz, 2H), 4.11 (s, 1H), 3.53-3.80 (m, 3H), 3.26 (br d, J = 3.9 Hz, 2H), 2.54-2.68 (m, 1H), 2.82-2.67 ppm (m, 2H). HPLC (System A) Rᵣ 1.09 min, >95% purity. LCMS (ESI) m/z: 552.0 (M+H)⁺.

(R)-2-(4-Cyanophenyl)-2-(4-(3,5-difluorobenzoyl)piperazin-1-yl)-N-(6-(2,2,2-trifluoroethoxy)pyridin-3-yl)acetamide (30).
**Step 1:**

To a CCl₄ (2 L) solution of methyl 2-(4-cyanophenyl)acetate (S₂₄, 200. g, 1.14 mol) was added N-bromosuccinimide (224 g, 1.25 mol) under N₂ atmosphere. Azobisisobutyronitrile (9.37 g, 57.1 mmol) was added to the reaction mixture, and the reaction mixture was heated at 85 °C for 16 h. The reaction mixture was then cooled to rt, filtered through celite, washed twice with hexane, and concentrated in vacuo. The material was absorbed onto a plug of silica gel and purified by column chromatography through a Redi-Sep pre-packed silica gel column (330 g), eluting 0% to 5% EtOAc in hexane, to provide methyl 2-bromo-2-(4-cyanophenyl)acetate (S₂₅, 110. g, 433 mmol, 38% yield) as an orange oil. ¹H NMR (400 MHz, DMSO-d₆) δ 7.89 (dd, J = 8.3, 1.7 Hz, 2H), 7.78 – 7.71 (m, 2H), 6.10 (d, J = 1.5 Hz, 1H), 3.77 – 3.70 (m, 3H). LCMS (ESI) m/z: 254.0 (M+H)+.

**Step 2:**

To a DMF (3 L) solution of tert-butyl piperazine-1-carboxylate (3, 55 g, 240 mmol) was added potassium carbonate (44.5 g, 325 mmol) and methyl 2-bromo-2-(4-cyanophenyl)acetate (S₂₅, 60. g, 240 mmol). The reaction mixture was stirred at rt for 12 h. The reaction was then filtered through a bed of celite, and the filtrate was concentrated in vacuo. The material was purified by column chromatography through a Redi-Sep pre-packed silica gel column (330 g), eluting 0% to 10% EtOAc in hexane, to provide tert-butyl 4-(1-(4-cyanophenyl)-2-methoxy-2-oxoethyl)piperazine-1-carboxylate (S₂₆, 40. g, 110 mmol, 38% yield) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-d₆) δ 7.98 – 7.75 (m, 2H), 7.69 – 7.42 (m, 2H), 6.10 (d, J = 1.5 Hz, 1H), 3.77 – 3.70 (m, 3H), 4.40 (s, 1H), 2.22 (s, 3H).
3.63 (s, 3H), 3.23 – 3.17 (m, 4H), 2.34 – 2.31 (m, 4H), 1.36 (s, 9H). LCMS (ESI) m/z: 360.1 (M+H)⁺.

Step 3:

To a THF (400 mL)/H₂O (200 mL) solution of tert-butyl 4-(1-(4-cyanophenyl)-2-methoxy-2-oxoethyl)piperazine-1-carboxylate (S26, 40. g, 110 mmol) was added lithium hydroxide (13.33 g, 557.1 mmol), and the reaction mixture was stirred at rt for 2 h. Volatiles were then removed in vacuo. The concentrate was cooled to 0 °C and acidified with HOAc to adjust to about pH 6. The aqueous layer was extracted twice with CH₂Cl₂. The organic extracts were combined, washed with brine, dried over Na₂SO₄, filtered, and concentrated in vacuo to afford 2-(4-(tert-butoxycarbonyl)piperazin-1-yl)-2-(4-cyanophenyl)acetic acid (S27, 30. g, 87 mmol, 78% yield) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-d₆) δ = 8.02 – 7.73 (m, 2H), 7.69 – 7.47 (m, 2H), 4.16 (s, 1H), 3.30 (d, J = 6.7 Hz, 5H), 2.43 – 2.20 (m, 3H), 1.37 (t, J = 2.2 Hz, 9H). LCMS (ESI) m/z: 346.0 (M+H)⁺.

Step 4:

To a CH₂Cl₂ (160 mL) solution of 2-(4-(tert-butoxycarbonyl)piperazin-1-yl)-2-(4-cyanophenyl)acetic acid (S27, 15.82 g, 45.8 mmol) was added 6-(2,2,2-trifluoroethoxy)pyridin-3-amine (8.0 g, 42 mmol). Triethylamine (11.6 mL, 83.2 mmol) and propylphosphonic anhydride (50 wt% in EtOAc, 26.5 g, 83.2 mmol) was added to the reaction mixture after cooling to 0 °C. The reaction mixture was stirred at rt for 16 h. The reaction mixture was then quenched with sat. aq. NaHCO₃ and extracted thrice with CH₂Cl₂. The organic extracts were combined, dried over Na₂SO₄, filtered, and concentrated in vacuo. The material was purified by column chromatography through a Redi-Sep pre-packed silica gel column (120 g), eluting 0% to 50% EtOAc in hexane, to provide tert-butyl 4-(2-oxo-1-phenyl-2-((6-(2,2,2-trifluoroethoxy)pyridin-3-yl)amino)ethyl)piperazine-1-carboxylate (S28, 17 g, 34 mmol, 79% yield) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-d₆) δ 10.38 (s, 1H), 8.40 (d, J = 2.5 Hz, 1H), 7.99 (dd, J = 9.0, 2.7, 1.1 Hz, 1H), 7.86 (dd, J = 8.3, 1.5 Hz, 2H), 7.72 – 7.64 (m, 2H), 7.07 – 6.83 (m, 1H), 5.19 – 4.80 (m, 2H), 4.23 (s, 1H), 3.37 (d, J = 4.8 Hz, 4H), 2.37 (tt, J = 11.7, 5.7 Hz, 4H), 1.38 (d, J = 1.4 Hz, 9H). LCMS (ESI) m/z: 520.0 (M+H)⁺.

Step 5:

To a CH₂Cl₂ (340 mL) solution of tert-butyl 4-(2-oxo-1-phenyl-2-((6-(2,2,2-trifluoroethoxy)pyridin-3-yl)amino)ethyl)piperazine-1-carboxylate (S28, 17 g, 33 mmol) was added trifluoroacetic acid (12.6 mL, 164 mmol). The reaction mixture was stirred at rt for 2 h. The reaction mixture was then concentrated in vacuo. The residue was dissolved in CH₂Cl₂ and basified with 20% NaHCO₃ (aq.) solution to adjust the pH to about 9. The organic layer was separated, washed with brine, dried over Na₂SO₄, filtered, and concentrated in vacuo to afford racemic 2-(4-cyanophenyl)-2-(piperazin-1-yl)-N-(6-(2,2,2-trifluoroethoxy)pyridin-3-yl)acetamide (rac-S29) as an off-white amorphous solid. This racemate was subjected to preparatory SFC purification using a YMC Amylose SA column (5 μm, 3 x 25 cm), eluting with 30% 20 mM NH₃ in MeOH at a flowrate of 120 mL/min with diode array detection at 230 nm, to provide (R)-2-(4-cyanophenyl)-2-(piperazin-1-yl)-N-(6-(2,2,2-trifluoroethoxy)pyridin-3-
yl)acetamide (S29, 1.18 g, 2.81 mmol, 8.5% yield, 96.1%ee) as an off-white amorphous solid.

$\text{\textsuperscript{1}H NMR (400 MHz, DMSO-d$_6$)} \delta 10.35 \text{ (s, 1H), } 8.41 \text{ (d, } J = 2.7 \text{ Hz, 1H), } 7.99 \text{ (dd, } J = 8.8, 2.8 \text{ Hz, 1H), } 7.85 \text{ (d, } J = 7.9 \text{ Hz, 2H), } 7.68 \text{ (d, } J = 7.8 \text{ Hz, 2H), } 6.96 \text{ (d, } J = 8.7 \text{ Hz, 1H), } 4.95 \text{ (q, } J = 9.1 \text{ Hz, 2H), } 4.13 \text{ (d, } J = 5.1 \text{ Hz, 1H), } 2.72 \text{ (t, } J = 4.7 \text{ Hz, 4H), } 2.39 - 2.08 \text{ (m, 4H). LCMS (ESI) m/z: 420.1 (M+H)$^+$}.

**Step 6:**

A DMF (0.45 mL) solution of (R)-2-(4-cyanophenyl)-2-(piperazin-1-yl)-N-(6-(2,2,2-trifluoroethoxy)pyridin-3-yl)acetamide (S29, 75 mg, 0.18 mmol) and 3,5-difluorobenzoic acid (34 mg, 0.21 mmol) was treated with a DMF (0.9 mL) solution of 1-hydroxybenzotriazole hydrate (35 mg, 0.23 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (44 mg, 0.23 mmol), and Hünig's base (91 µL, 0.53 mmol). The reaction was shaken at rt for 18 h and then purified via high throughput parallel purification to provide 30 (60.5 mg, 0.108 mmol, 60% yield) as a white amorphous solid. $\text{\textsuperscript{1}H NMR (500 MHz, DMSO-d$_6$)} \delta 10.37 \text{ (s, 1H), } 8.39 \text{ (br d, } J = 2.3 \text{ Hz, 1H), } 7.98 \text{ (br dd, } J = 2.5, 9.0 \text{ Hz, 1H), } 7.86 \text{ (br d, } J = 8.3 \text{ Hz, 2H), } 7.69 \text{ (br d, } J = 8.0 \text{ Hz, 2H), } 7.38 - 7.29 \text{ (m, 1H), } 7.13 \text{ (br d, } J = 5.2 \text{ Hz, 2H), } 6.96 \text{ (br d, } J = 8.8 \text{ Hz, 1H), } 4.94 \text{ (q, } J = 9.0 \text{ Hz, 2H), } 4.27 \text{ (s, 1H), } 3.67 \text{ (br d, } J = 2.3 \text{ Hz, 2H), } 3.35 \text{ (br s, 2H), } 2.60 - 2.53 \text{ (m, 1H), } 2.48 - 2.30 \text{ (m, 3H). HPLC (System B) R$_t$ 2.10 min, >95% purity. LCMS (ESI) m/z: 560.2 (M+H)$^+$}.

(R)-2-(4-Cyanophenyl)-N-(cyclopropylmethyl)-2-(8-(3,5-difluorobenzoyl)-3,8-diazabicyclo[3.2.1]octan-3-yl)acetamide (31).

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**Step 1:**

To a THF (250 mL)/MeOH (250 mL)/H$_2$O (250 mL) solution of methyl 2-bromo-2-(4-cyanophenyl)acetate (S25, 50 g, 200 mmol) was added lithium hydroxide (4.71 g, 197 mmol) after cooling to 0 °C. The reaction mixture was stirred at 0 °C for 2 min. The reaction mixture was then acidified to about pH 2 with 1.5 N HCl (aq.) and extracted twice with CH$_2$Cl$_2$. The organic extracts were combined, washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo to afford 2-bromo-2-(4-cyanophenyl)acetic acid (S30, 45 g, 190 mmol,
95% yield) as a colourless syrup. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta = 13.84$ (d, $J = 30.0$ Hz, 1H), 8.03 – 7.85 (m, 2H), 7.81 – 7.67 (m, 2H), 5.93 (d, $J = 3.2$ Hz, 1H).

Step 2:

To a CH$_2$Cl$_2$ (200 mL) solution of 2-bromo-2-(4-cyanophenyl)acetic acid (S30, 47.3 g, 197 mmol), cyclopropylmethanamine (14 g, 200 mmol), and triethylamine (55 mL, 390 mmol) was added propylphosphonic anhydride (50 wt% in EtOAc, 125 g, 394 mmol) after cooling to 0 °C. The reaction mixture was stirred for 16 h while allowing to slowly warm to rt. The reaction mixture was then quenched with sat. aq. NaHCO$_3$ and extracted twice with CH$_2$Cl$_2$. The organic extracts were combined, washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo. The material was purified by column chromatography through a Redi-Sep pre-packed silica gel column (330 g), eluting 0% to 20% EtOAc in hexane, to provide 2-bromo-2-(4-cyanophenyl)-N-(cyclopropylmethyl)acetamide (S31, 26 g, 89 mmol, 45% yield) as an off-white amorphous solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta 8.60$ (t, $J = 5.7$ Hz, 1H), 7.97 – 7.82 (m, 2H), 7.82 – 7.68 (m, 2H), 2.97 (ddt, $J = 19.0$, 13.6, 7.2 Hz, 2H), 1.05 – 0.81 (m, 1H), 0.54 – 0.30 (m, 2H), 0.26 – 0.01 (m, 2H). LCMS (ESI) $m/z$: 293.0 (M+H)$^+$. 

Step 3:

A DMF (12 mL) slurry of tert-butyl 3,8-diazabicyclo[3.2.1]octane-8-carboxylate (S17, 2.5 g, 11.8 mmol), potassium carbonate (3.58 g, 25.9 mmol), and 2-bromo-2-(4-cyanophenyl)-N-(cyclopropylmethyl)acetamide (S31, 3.62 g, 12.4 mmol) was stirred at rt for 18 h. The reaction was then quenched with H$_2$O and extracted thrice with EtOAc. The organic extracts were combined, washed twice with H$_2$O and once with brine, filtered through a phase separator, and concentrated in vacuo to afford tert-butyl 3-(1-(4-cyanophenyl)-2-((cyclopropylmethyl)amino)-2-oxoethyl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (S32) as a light yellow amorphous solid that was used without further purification. HPLC (System A) R$_t$ 1.21 min. LCMS (ESI) $m/z$: 425.2 (M+H)$^+$. 

Step 4:

A dioxane (40 mL) solution of tert-butyl 3-(1-(4-cyanophenyl)-2-((cyclopropylmethyl)amino)-2-oxoethyl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (S32) from the previous step was treated with hydrogen chloride (4.0 M in dioxane, 13.25 ml, 53.0 mmol). The reaction was stirred for 5 h at 50 °C. The slurry was then concentrated in vacuo to afford racemic 2-(3,8-diazabicyclo[3.2.1]octan-3-yl)-2-(4-cyanophenyl)-N-(cyclopropylmethyl)acetamide hydrochloride (rac-S33·HCl) as an off-white amorphous solid. This racemate was subjected to preparatory SFC purification using a Chiralcel OZ-H column (5 µm, 2 x 25 cm), eluting with 35% MeOH containing 0.2% HNEt$_3$ at a flowrate of 80 mL/min with diode array detection at 239 nm to provide (R)-2-(3,8-diazabicyclo[3.2.1]octan-3-yl)-2-(4-cyanophenyl)-N-(cyclopropylmethyl)acetamide (S33, 1.820 g, 5.04 mmol, 43% yield, >99%ee) as a white amorphous solid. HPLC (System A) R$_t$ 0.77 min. LCMS (ESI) $m/z$: 325.2 (M+H)$^+$. 

Step 5:
A DMF (0.45 mL) solution of \((R)-2-(3,8\text{-diazabicyclo}[3.2.1]\text{octan-3-yl})-2-(4\text{-cyanophenyl})-N\text{-}(cyclopropylmethyl)acetamide (S33, 57 mg, 0.18 mmol) and 3,5-difluorobenzoic acid (34 mg, 0.21 mmol) was treated with a DMF (0.45 mL) solution of 1-hydroxybenzotriazole hydrate (35 mg, 0.23 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (44 mg, 0.23 mmol), and Hünig's base (91 µL, 0.53 mmol). The reaction was shaken at rt for 18 h and then purified via high throughput parallel purification to provide 31 (47.6 mg, 0.103 mmol, 59% yield) as a white amorphous solid.  

1H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 8.27 - 8.14 (m, 1H), 7.82 (br d, \(J = 6.2\) Hz, 2H), 7.63 (br d, \(J = 7.9\) Hz, 2H), 7.47 - 7.29 (m, 1H), 7.14 (br s, 2H), 4.70 - 4.42 (m, 1H), 4.02 (s, 1H), 3.98 - 3.83 (m, 1H), 2.94 (br d, \(J = 2.1\) Hz, 2H), 2.87 - 2.66 (m, 1H), 2.47 - 2.43 (m, 1H), 2.40 - 2.29 (m, 1H), 2.23 - 2.05 (m, 1H), 2.04 - 1.74 (m, 4H), 0.98 - 0.81 (m, 1H), 0.36 (br s, 2H), 0.11 (br s, 2H).  

13C NMR (101 MHz, DMSO-\(d_6\)) \(\delta\) 168.69, 163.94, 163.91, 163.31, 143.24, 132.14, 129.39, 118.71, 110.63, 110.56, 110.46, 110.37, 105.46, 105.21, 79.15, 72.68, 56.63, 51.94, 42.64, 10.70, 3.04, 2.92.  

19F NMR (376 MHz, DMSO-\(d_6\)) \(\delta\) –109.42 (br d, \(J = 14.3\) Hz, 1F).  

\([\alpha]_D^{26} = -68.4^\circ\) (c 0.36, CHCl₃).  

HPLC (System B) R₂ 1.84 min, >95% purity.  

HRMS (ESI) \(m/z\): [M+H]+ calculated for C₂₆H₂₆F₂N₄O₂: 465.2097, found 465.2095.  

\(\text{(R)-2-(4-Cyanophenyl)-N-(cyclopropylmethyl)-2-(8-(2,5-difluorobenzoyl)-3,8-diazabicyclo}[3.2.1]\text{octan-3-yl})\text{-acetamide (32).}\)

A DMF (0.45 mL) solution of \((R)-2-(3,8\text{-diazabicyclo}[3.2.1]\text{octan-3-yl})-2-(4\text{-cyanophenyl})-N\text{-}(cyclopropylmethyl)acetamide (S33, 57 mg, 0.18 mmol) and 2,5-difluorobenzoic acid (34 mg, 0.21 mmol) was treated with a DMF (0.45 mL) solution of 1-hydroxybenzotriazole hydrate (35 mg, 0.23 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (44 mg, 0.23 mmol), and Hünig's base (91 µL, 0.53 mmol). The reaction was shaken at rt for 18 h and then purified via high throughput parallel purification to provide 32 (47.9 mg, 0.103 mmol, 59% yield) as a white amorphous solid.  

1H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 8.23 (td, \(J = 5.8, 9.5\) Hz, 1H), 7.81 (t, \(J = 8.8\) Hz, 2H), 7.62 (t, \(J = 8.2\) Hz, 2H), 7.41 - 7.30 (m, 2H), 7.29 - 7.24 (m, 1H), 4.64 - 4.51 (m, 1H), 4.02 (d, \(J = 3.1\) Hz, 1H), 3.81 - 3.62 (m, 1H), 3.29 (s, 1H), 3.03 - 2.88 (m, 2H), 2.86 - 2.68 (m, 1H), 2.47 - 2.41 (m, 1H), 2.38 - 2.26 (m, 1H), 2.19 - 1.86 (m, 3H), 1.84 - 1.77 (m, 2H), 0.95 - 0.80 (m, 1H), 0.41 - 0.31 (m, 2H), 0.16 - 0.06 (m, 2H).  

13C NMR (101 MHz, DMSO-\(d_6\)) \(\delta\) 168.62, 168.60, 159.62, 159.60, 143.19, 132.14, 132.10, 129.38, 119.50, 118.70, 117.98, 117.94, 110.46, 110.43, 79.15, 72.57, 72.53, 56.62, 56.36, 56.32, 56.19, 55.22, 54.90, 51.73, 51.67, 42.66, 42.64, 27.88, 27.71, 26.81, 26.59, 10.71, 10.67, 3.04, 2.92.  

19F NMR (376 MHz, DMSO-\(d_6\)) \(\delta\) –118.68 (br dd, \(J = 5.7, 18.6\) Hz, 1F), –122.75—–123.03 (m, 1F).  

\([\alpha]_D^{26} = -72.6^\circ\) (c 0.35, CHCl₃).  

HPLC (System B) R₂ 1.78 min.  

HRMS (ESI) \(m/z\): [M+H]+ calculated for C₂₆H₂₆F₂N₄O₂: 465.2097, found 465.2098.
(R)-2-(4-(3-Cyano-5-fluorobenzoyl)piperazin-1-yl)-N-(2-cyclopropyl-2,2-difluoroethyl)-2-phenylacetamide (33).

Step 1:
To a CH$_2$Cl$_2$ (6.4 mL) solution of 2-(4-((tert-butoxycarbonyl)piperazin-1-yl)-2-phenylacetic acid (S3, 0.618 g, 1.93 mmol) was added 2-cyclopropyl-2,2-difluoroethanamine hydrochloride (0.304 g, 1.93 mmol). Triethylamine (0.94 mL, 6.8 mmol) and propylphosphonic anhydride (50 wt% in EtOAc, 2.5 mL, 3.9 mmol) was added to the reaction mixture. The reaction mixture was stirred at rt for 16 h. The reaction mixture was then quenched with sat. aq. NaHCO$_3$, stirred for 30 min, and extracted twice with CH$_2$Cl$_2$. The organic extracts were combined, dried over MgSO$_4$, filtered, and concentrated in vacuo to provide tert-butyl 4-(2-((2-cyclopropyl-2,2-difluoroethyl)amino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate (S34) as an off-white amorphous solid that was used without further purification. HPLC (System A) Rt 1.00 min. LCMS (ESI) m/z: 424.2 (M+H)$^+$.  

Step 2:
A CH$_2$Cl$_2$ (5 mL) of 4-(2-((2-cyclopropyl-2,2-difluoroethyl)amino)-2-oxo-1-phenylethyl)piperazine-1-carboxylate (S34) from the previous step was treated with trifluoroacetic acid (2.9 ml, 39 mmol). The mixture was stirred at rt for 2 h. The mixture was basified with 6 N NaOH until pH 9. The mixture was then extracted thrice with CH$_2$Cl$_2$. The organic extracts were combined, washed with brine, dried over MgSO$_4$, filtered, and concentrated in vacuo to provide racemic N-(2-cyclopropyl-2,2-difluoroethyl)-2-phenyl-2-(piperazin-1-yl)acetamide (rac-S35, 0.555 g, 1.72 mmol, 89% yield over 2 steps) as an off-white amorphous solid. This racemate was subjected to preparatory SFC purification using a Chiralpak AD-H column (5 μm, 2 x 25 cm), eluting with 25% MeOH containing 0.2% HNEt$_2$ at a flowrate of 60 mL/min with diode array detection at 215 nm to provide (R)-N-(2-cyclopropyl-2,2-difluoroethyl)-2-phenyl-2-(piperazin-1-yl)acetamide (S35, 191 mg, >99%ee). HPLC (System A) Rt 0.79 min. LCMS (ESI) m/z: 324.2 (M+H)$^+$.  

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Step 3:

To a DMF (0.5 mL) solution of (R)-N-(2-cyclopropyl-2,2-difluoroethyl)-2-phenyl-2-(piperazin-1-yl)acetamide (S35, 0.047 g, 0.15 mmol) was added 3-cyano-5-fluorobenzoic acid (0.048 g, 0.291 mmol), triethylamine (0.041 mL, 0.291 mmol), and propylphosphonic anhydride (50 wt% in DMF, 0.19 mL, 0.291 mmol). The reaction mixture was stirred at rt for 16 h and then purified via high throughput parallel purification to provide 33 (51.6 mg, 0.110 mmol, 73% yield) as a white amorphous solid. 1H NMR (500 MHz, DMSO-d6) δ 8.49 (br s, 1H), 7.93 (br d, J=8.17 Hz, 1H), 7.73 (s, 1H), 7.64 (br d, J=7.92 Hz, 1H), 7.42 (br d, J=6.88 Hz, 2H), 7.27-7.37 (m, 3H), 3.96-4.00 (m, 1H), 3.63 (br s, 4H), 3.25-3.31 (m, 2H), 2.29-2.45 (m, 4H), 1.20-1.29 (m, 1H), 0.39-0.51 (m, 4H). 13C NMR (101 MHz, DMSO-d6) δ 170.41, 165.49, 165.47, 162.61, 160.14, 139.53, 139.45, 136.74, 128.63, 128.20, 127.82, 127.09, 127.06, 122.09, 120.41, 120.16, 119.52, 119.50, 119.30, 117.16, 117.13, 113.30, 113.20, 79.15, 73.58, 43.02, 13.64, 0.77. 19F NMR (376 MHz, DMSO-d6) δ –106.59 (s, 2F), –110.47 (s, 1F). [α]D^22 = –56.2° (c 0.34, CHCl3). HPLC (System B) R, 1.83 min, >95% purity. HRMS (ESI) m/z: [M+H]^+ calculated for C25H25F3N4O2: 471.2002, found 471.2002.

(R)-2-(4-(2-Chloro-5-fluorobenzoyl)piperazin-1-yl)-2-(4-cyanophenyl)-N-(2-cyclopropyl-2,2-difluoroethyl)acetamide (34).

Step 1:

To a CH2Cl2 (800 mL) solution of tert-butyl 4-(1-(4-cyanophenyl)-2-methoxy-2-oxoethyl)piperazine-1-carboxylate (S26, 80.0 g, 223 mmol) was added trifluoroacetic acid (86.0 mL, 1.12 mol). The reaction mixture was stirred at rt for 2 h. The reaction mixture was then concentrated in vacuo. The material was dissolved in H2O and washed twice with MTBE. The aqueous layer was basified with 20% NaHCO3 (aq.) to adjust to about pH 9 and then extracted twice with 10% MeOH in CH2Cl2. The organic extracts were combined, washed with brine,
dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo to afford methyl 2-(4-cyanophenyl)-2-(piperazin-1-yl)acetate (S36, 50.0 g, 193 mmol, 87% yield) as a colourless syrup. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.98 – 7.78 (m, 2H), 7.66 – 7.56 (m, 2H), 4.45 (s, 1H), 3.64 (s, 3H), 3.56 (s, 1H), 2.90 (t, $J$ = 4.9 Hz, 4H), 2.48 – 2.41 (m, 4H). LCMS (ESI) $m/z$: 260.1 (M+H)$^+$.  

**Step 2:**

To a CH$_2$Cl$_2$ (300 mL) solution of methyl 2-(4-cyanophenyl)-2-(piperazin-1-yl)acetate (S36, 20.0 g, 77.0 mmol) was added 2-chloro-5-fluorobenzooic acid (8.0 g, 77 mmol). Triethylamine (21.5 mL, 154 mmol) and propylphosphonic anhydride (50 wt% in EtOAc, 49.1 g, 154 mmol) was added to the reaction mixture after cooling to 0 °C. The reaction mixture was then stirred for 16 h while slowly warming to rt. The reaction mixture was then quenched with sat. aq. NaHCO$_3$ solution and was extracted twice with CH$_2$Cl$_2$. The organic extracts were combined, dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo. The material was purified by column chromatography through a Redi-Sep pre-packed silica gel column (120 g), eluting 0% to 50% EtOAc in hexane, to provide methyl 2-(4-(2-chloro-5-fluorobenzoyl)piperazin-1-yl)-2-(4-cyanophenyl)acetate (S37, 20.0 g, 48.1 mmol, 62% yield) as a white amorphous solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.96 – 7.78 (m, 2H), 7.68 – 7.50 (m, 3H), 7.36 – 7.22 (m, 2H), 4.46 (d, $J$ = 2.9 Hz, 1H), 3.64 (d, $J$ = 2.1 Hz, 3H), 3.14 (t, $J$ = 4.8 Hz, 4H), 2.47 – 2.30 (m, 4H). LCMS (ESI) $m/z$: 416.1 (M+H)$^+$.  

**Step 3:**

To a THF (100 mL)/MeOH (100 mL)/H$_2$O (200 mL) solution of methyl 2-(4-(2-chloro-5-fluorobenzoyl)piperazin-1-yl)-2-(4-cyanophenyl)acetate (S37, 20.0 g, 48.1 mmol) was added lithium hydroxide (5.76 g, 240 mmol), and the reaction mixture was stirred at rt for 2 h. Volatiles were then removed in vacuo. The concentrate was cooled to 0 °C and acidified with 1.5 N HCl to about pH 6. The aqueous layer was then extracted thrice with CH$_2$Cl$_2$. The organic extracts were combined, washed with brine, dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo to afford racemic 2-(4-(2-chloro-5-fluorobenzoyl)piperazin-1-yl)-2-(4-cyanophenyl)acetic acid (rac-S38, 15.0 g, 37.3 mmol, 78% yield) as a white amorphous solid. This racemate was subjected to preparatory SFC purification using a Chiralpak AD-H column (5 $\mu$m, 3 x 25 cm), eluting with 30% MeOH containing 0.5% HNEt$_2$ at a flowrate of 120 mL/min with diode array detection at 230 nm, to provide (R)-2-(4-(2-chloro-5-fluorobenzoyl)piperazin-1-yl)-2-(4-cyanophenyl)acetic acid (S38, 4.11 g, 96.7%ee) as a white amorphous solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.70 (d, $J$ = 7.9 Hz, 2H), 7.65 – 7.49 (m, 3H), 7.31 (dd, $J$ = 8.4, 6.6 Hz, 2H), 3.84 – 3.56 (m, 4H), 3.22 – 3.11 (m, 2H), 2.75 (q, $J$ = 7.1 Hz, 1H), 2.43 – 2.31 (m, 1H), 2.24 (dt, $J$ = 10.6, 5.1 Hz, 1H). LCMS (ESI) $m/z$: 402.1 (M+H)$^+$.  

**Step 4:**

To a CH$_2$Cl$_2$ (0.6 mL) mixture of 2-cyclopropyl-2,2-difluoroethanamine (0.035 g, 0.25 mmol) and (R)-2-(4-(2-chloro-5-fluorobenzoyl)piperazin-1-yl)-2-(4-cyanophenyl)acetic acid (S38, 0.050 g, 0.12 mmol) was added triethylamine (0.035 mL, 0.25 mmol) and propylphosphonic anhydride (50 wt% in EtOAc, 0.16 ml, 0.25 mmol). The mixture was shaken for 16 h at rt. The mixture was filtered and purified via high throughput parallel purification to provide 34 (36.1
mg, 0.0715 mmol, 60% yield) as a white amorphous solid. $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.64 (br s, 1H), 7.82 (br d, $J$=8.30 Hz, 2H), 7.61 (br d, $J$=7.92 Hz, 2H), 7.56 (br s, 1H), 7.25-7.37 (m, 2H), 4.18 (br s, 1H), 3.53-3.74 (m, 4H), 3.14-3.20 (m, 2H), 2.26-2.47 (m, 4H), 1.27 (br s, 1H), 0.39-0.54 (m, 4H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 169.35, 164.05, 161.95, 159.50, 142.39, 137.33, 137.26, 132.16, 131.44, 131.36, 129.56, 124.48, 124.45, 122.00, 118.67, 117.68, 117.45, 115.19, 114.94, 110.60, 72.72, 50.53, 50.38, 50.01, 49.80, 46.07, 43.38, 43.08, 41.05, 13.92, 13.64, 13.37, 0.82. $^{19}$F NMR (376 MHz, DMSO-$d_6$) δ = –105.61—107.76 (m, 2F), –115.01 (s, 1F). $[\alpha]_D^{22} = -47.3^\circ$ (c 0.63, CHCl$_3$). HPLC (System A) $R_t$ 1.07 min, >95% purity. HRMS (ESI) m/z: [M+H]$^+$ calculated for C$_{25}$H$_{24}$ClF$_3$N$_4$O$_2$: 505.1613, found 505.1621.
Copies of Selected HPLC and NMR Spectra

HPLC spectra of 14:

Signal 1: MSD1 TIC, MS File

Signal 2: MWD1 A, Sig=254,4 Ref=off

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Signal 3: MWD1 B, Sig=215,8 Ref=off

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Chiral SFC spectra of \textit{rac-14, 14} (peak 1), and its enantiomer (peak 2):
HPLC spectra of 33:

Signal 1: MSD1 TIC, MS File

Signal 2: MWD1 A, Sig=254,4 Ref-off

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Chiral SFC spectra of S35 (peak 1, penultimate precursor to 33), its enantiomer (peak 2), and rac-S35.
HPLC spectra of 34:

Signal 1: MSD1 TIC, MS File

Signal 2: MWD1 A, Sig=254,4 Ref=off

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Totals: 900.80750 1174.11877
Chiral SFC spectra of \textit{rac-S38} and \textit{S38} (penultimate precursor to 34).