Supporting information

Transaminases provide key chiral building blocks for the synthesis of selective M1/M4 agonists

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Section 1: General methods

Commercial reagents and solvents were purchased and used without additional purification unless otherwise noted. Oxygen and moisture sensitive chemistry were performed under an atmosphere of nitrogen. Room temperature is approximately 20-25 °C. Microwave reactions were carried out using the Biotage Initiator+ system. NMR spectra were obtained using a Bruker Avance II 400 MHz NMR spectrometer. All chemical shifts are reported in parts per million (δ) relative to tetramethylsilane. The following abbreviations are used to denote splitting patterns: s=singlet, d=doublet, t=triplet, dd=doublet of doublets, m=multiplet. Low resolution MS were obtained using an Agilent 1100 series LCMS spectrometer. Compounds were purified using Teledyne Isco Combiflash automated purification systems or Waters preparative HPLC systems. The LCMS conditions are described below:

LCMS Method 1:

Instrument: Waters Acquity UPLC, photodiode array detector; Column: AcQuity UPLC BEH C_{18} 1.7µm, 21x30 mm; 2 min run time, 2% solvent B from 0 to 0.1 min, 2 → 98% solvent B:solvent A from 0.1 to 1.8 min, 98% solvent B for 0.2 min. Solvents: Solvent A = 0.1% formic acid in water (v/v), solvent B = 0.1% formic acid in acetonitrile (v/v). Injection volume 2-5 uL; UV detection array 210-400, Mass detection 120-1250 (electrospray ionization); column at 50 °C; flow rate 1.0 mL/min.

LCMS Method 2:

Instrument: Waters Acquity UPLC, photodiode array detector; Column: AcQuity UPLC BEH C_{18} 1.7µm 21x50 mm; 2 min run time, 2% solvent B from 0 to 0.1 min, 2 → 98% solvent B:solvent A from 0.1 to 1.8 min, 98% solvent B for 0.2 min. Solvents: Solvent A = 5 mM ammonium hydroxide in water, solvent B = 5 mM ammonium hydroxide in acetonitrile. Injection volume 2-5 uL; UV detection array 210-400, Mass detection 120-1250 (electrospray ionization); column at 50 °C; flow rate 1.0 mL/min.

LCMS Method 3:

Instrument: Waters Acquity UPLC, photodiode array detector; Column AcQuity UPLC BEH C_{18} 1.7µm 21x30 mm; 5.2 min run time, 2 → 98% solvent B:solvent A from 0 to 5.15 min, 98% solvent B from 5.15 to 5.20 min. Solvents: Solvent A = 0.1% formic acid in water (v/v), solvent B = 0.1% formic acid in acetonitrile (v/v). Injection volume 2-5 uL; UV detection array 210-400, Mass detection 120-1600; column at 50 °C, flow rate 1.0 mL/min.

LCMS Method 4:

Instrument: Waters Acquity UPLC, photodiode array detector; Column AcQuity UPLC BEH C_{18} 1.7µm 21x30 mm; 5.2 min run time, 2 → 98% solvent B:solvent A from 0 to 5.15 min, 98% solvent B from 5.15 to 5.20 min. Solvents: Solvent A = 5 mM ammonium hydroxide in water, solvent B = 5 mM ammonium hydroxide in acetonitrile). Injection volume 2-5 uL; UV detection array 210-400, Mass detection 120-1600; column at 50 °C, flow rate 1.0 mL/min.

LCMS Method 5:

Instrument: Agilent 1200 LC/G1956A, diode array detector; Column: Chromolith Flash C_{18}, 1.6 micron 2x25 mm; 1.5 minute run time, 5 → 95% solvent B: solvent A from 0 → 1.2 minutes and then 95% solvent B from

1.21 → 1.5 minutes. Solvents: Solvent A= 0.0375% TFA in Water (v/v), Solvent B= 0.01875% TFA in Acetonitrile (v/v). Injection volume 2-5 uL; UV detection 220 and 254 nM, Mass detection 100-1000 (electrospray ionization); column at 50 °C; Flow rate 1.5 mL/min.

LCMS Method 6:

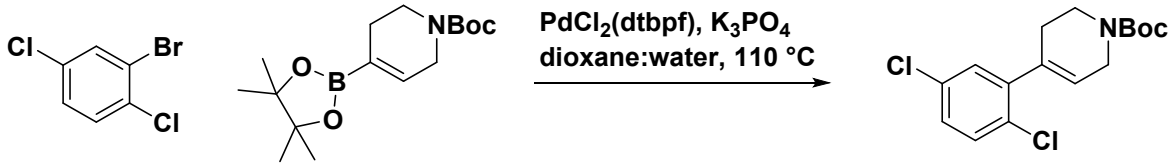
Instrument: SHIMADZU LCMS-2020, photo diode array detector; Column: Kinetex EVO C_{18}, 5 uM, 1x30 mm; 1.55 minute run time, 5 → 95% solvent B:solvent A from 0 → 1.20 minutes and then 95% solvent B from 1.21 minutes to 1.55 minutes. Solvents: Solvent A= 0.025% NH_4OH in water/v), Solvent B= acetonitrile. Injection volume 2-5 uL; UV detection 220 and 254 nM, Mass detection 100-100 (electrospray ionization); column at 40 °C; Flow rate 1.5 mL/min.

No unexpected or unusually high safety hazards were encountered.

Section 2: Synthesis of intermediates and final compounds

Synthesis of (*S*)-(6-(4-(2,5-dichlorophenyl)piperidin-1-yl)-2-azaspiro[3.4]octan-2-yl)(1-fluorocyclopropyl)methanone ((*S*)-5) and (*R*)-(6-(4-(2,5-dichlorophenyl)piperidin-1-yl)-2-azaspiro[3.4]octan-2-yl)(1-fluorocyclopropyl)methanone ((*R*)-5

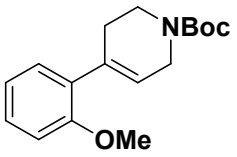
Step 1: *tert*-butyl 4-(2,5-dichlorophenyl)-3,6-dihydropyridine-1(2*H*)-carboxylate



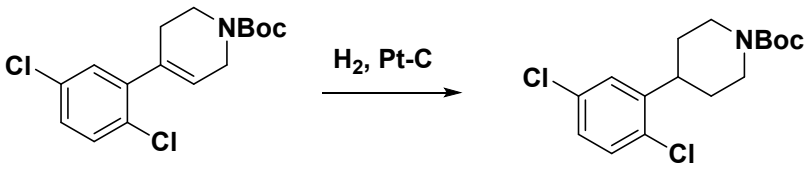
2-bromo-1,4-dichlorobenzene (2.99 g, 13.24 mmol), *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2H)-carboxylate (4.50 g, 14.56 mmol), [1,1'-Bis(di-tert-butylphosphino)ferrocene]dichloropalladium(II) (0.863 g, 1.324 mmol) and potassium phosphate, tribasic (13.68 mL, 41.0 mmol) were dissolved in a mixture of dioxane (14mL) and water (3 mL). The mixture was split into three microwave vials and each one was reacted in the microwave for 10 mins at 110 °C. The combined reactions were poured into water and the product was extracted using EtOAc (3x100 mL). The organic layer was separated and washed with brine (1x20 mL) then dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude product was purified by FCC (0- 20% heptanes:EtOAc) to yield the title intermediate (3.25 g, 8.91 mmol).

LCMS Method 1 Rt: 1.36 min, MS m/z calculated for $C_{16}H_{20}Cl_2NO_2$ $[M+H]^+$: 328.1, found: 328.2. ^1H NMR (400 MHz, DMSO-d_6) δ 7.49 (d, *J* = 8.5 Hz, 1H), 7.43 – 7.34 (m, 2H), 5.77 (s, 1H), 3.97 (q, *J* = 3.1 Hz, 2H), 3.52 (t, *J* = 4 Hz, 2H), 2.35 (m, 2.37-2.32 2H), 1.43 (s, 9H).

The following compounds were synthesized by analogy

Compound	Structure	LCMS	^{1}H NMR
SI-1		LCMS Method 1 Rt: 1.21 min MS m/z calculated for $C_{12}H_{16}NO$: 190.1 $[M\text{-}Boc+H]^+$ Found: 190.3	^{1}H NMR (400 MHz, CD_3OD) δ 7.28 - 7.17 (m, 1H), 7.11 (dd, J = 7.5, 1.8 Hz, 1H), 6.99 - 6.84 (m, 2H), 5.70 (s, 1H), 4.01 (s, 2H), 3.79 (s, 3H), 3.57 (t, J = 5.6 Hz, 2H), 2.52 - 2.38 (m, 2H), 1.49 (s, 9H).

Step 2: *tert*-butyl 4-(2,5-dichlorophenyl)piperidine-1-carboxylate

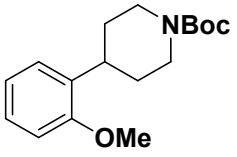


tert-butyl 4-(2,5-dichlorophenyl)-3,6-dihydropyridine-1(2*H*)-carboxylate (1.5 g, 4.57 mmol) was dissolved in EtOAc (22 mL) and was circulated through a Pt/C catalyst cartridge in an H-cube at 20 °C and 3 bar pressure for 120 min. The cartridge was replaced and the solution was circulated through the H-cube at 20 °C and 3 bar for an additional 30 min. The solvent was removed under reduced pressure to yield the title intermediate (1.34 g, 3.65 mmol) which was used without further purification.

LCMS Method 1 Rt: 1.36 min, MS m/z calculated for $C_{11}H_{14}Cl_2N$ $[M\text{-}Boc+H]^+$: 230.0, found: 229.9.

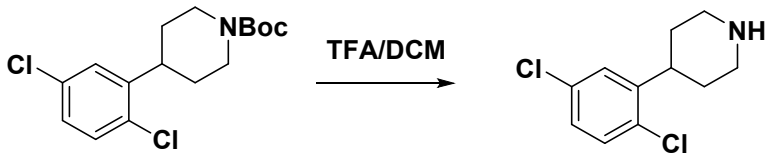
^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50 – 7.43 (m, 2H), 7.32 (dd, J = 8.7, 2.5 Hz, 1H), 4.16 – 4.05 (m, 2H), 3.07 (tt, J = 12.2, 3.4 Hz, 1H), 2.81 (bs, 2H) 1.72 (d, J = 12.9 Hz, 2H), 1.53 (qd, J = 12.6, 4.1 Hz, 2H), 1.42 (s, 9H).

The following compounds were synthesized by analogy

Compound	Structure	LCMS	^{1}H NMR
SI-2		LCMS Method 1 Rt: 1.24 min MS m/z calculated for $C_{12}H_{18}NO$: 192.1 $[M\text{-}Boc+H]^+$	^{1}H NMR (400 MHz, CD_3OD) δ 7.23 - 7.07 (m, 2H), 6.97 - 6.83 (m, 2H), 4.19 (ddd, J = 13.3, 4.3, 2.2 Hz, 2H), 3.81 (s, 3H), 3.12 (tt, J = 12.1, 3.5 Hz, 1H), 2.98 - 2.66 (m, 2H),

		Found: 192.0	1.83 - 1.68 (m, 2H), 1.66 - 1.49 (m, 2H), 1.48 (s, 9H)

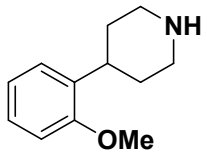
Step 3: 4-(2,5-dichlorophenyl)piperidine



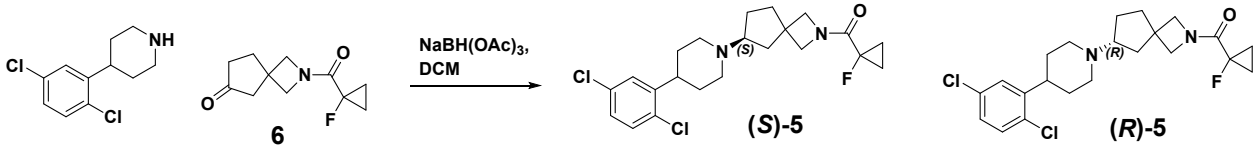
tert-butyl 4-(2,5-dichlorophenyl)piperidine-1-carboxylate (1.25 g, 3.79 mmol) was dissolved in DCM (10 mL) and TFA (5.00 mL) was added dropwise over 5 min. The reaction was stirred for 10 min at room temperature. The solvent was removed under reduced pressure and taken forward without further purification, assuming quantitative conversion.

LCMS Method 1 Rt: 0.63 min, MS m/z calculated for $C_{11}H_{14}Cl_2N$ $[M+H]^+$: 230.0, found: 230.2.

The following compounds were synthesized by analogy

Compound	Structure	LCMS	^{1}H NMR
SI-3		LCMS Method 1 Rt: 0.52 min MS m/z calculated for $C_{12}H_{18}NO$: 192.1 $[M-Boc+H]^+$ Found: 192.3	^{1}H NMR (400 MHz, DMSO-d_6) δ 7.23 - 7.08 (m, 2H), 7.01 - 6.84 (m, 2H), 3.77 (s, 3H), 3.14 - 2.89 (m, 3H), 2.66 (td, J = 12.2, 2.7 Hz, 2H), 1.73 - 1.60 (m, 2H), 1.60 - 1.42 (m, 2H).

Step 4: (*S*)-(6-(4-(2,5-dichlorophenyl)piperidin-1-yl)-2-azaspiro[3.4]octan-2-yl)(1-fluorocyclopropyl)methanone and (*R*)-(6-(4-(2,5-dichlorophenyl)piperidin-1-yl)-2-azaspiro[3.4]octan-2-yl)(1-fluorocyclopropyl)methanone



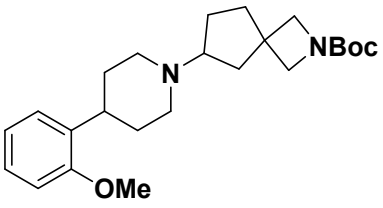
2-(1-fluorocyclopropane-1-carbonyl)-2-azaspiro[3.4]octan-6-one (**6**, 500 mg, 2.367 mmol) and 4-(2,5-dichlorophenyl)piperidine (805 mg, 3.50 mmol) were dissolved in DCM (25 ml) molecular sieves were added. The suspension was stirred at RT for 20 min, and then sodium triacetoxyborohydride (1003 mg, 4.73 mmol) was added. The suspension was stirred at RT for 16 hours. Following this, further molecular sieves and sodium triacetoxyborohydride (1003 mg, 4.73 mmol) were added and the reaction was stirred

for 60 hours. The mixture was then filtered and the residue was washed with DCM. The combined filtrate was washed with 1N NaOH (50 ml), then dried over MgSO4 and concentrated. The residue was purified by FCC (0-6% MeOH (1% NH_4OH)/DCM) to yield the racemic product (837 mg, 1.97 mmol). The two enantiomers were then separated by chiral SFC (lux cellulose-2, 21x250 mm, 5 µM, 35% MeOH/CO_2, 80 g/min) to yield the initial peak ((*S*)-5, 362 mg, 0.843 mmol) and the trailing peak ((*R*)-5, 356 mg, 0.829 mmol).

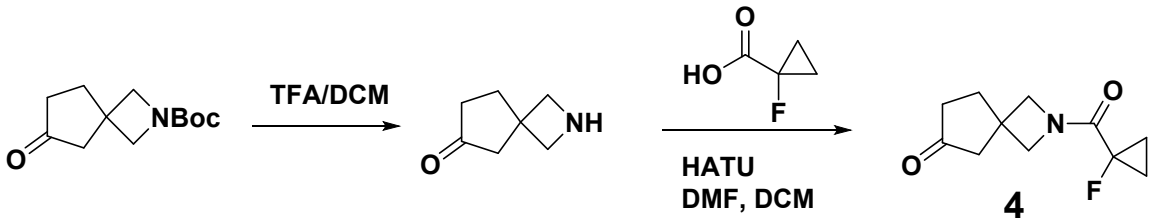
Compound(*S*)-5: SFC: Rt 3.49 min, Lux Cellulose-2 4.6x100 mm, 5 µM, 5-55% MeOH (10 mM NH_4OH/CO_2), 5 mL/min. LCMS Method 4 Rt: 3.07 min, MS m/z calculated for $C_{22}H_{28}Cl_2FN_2O$ $[M+H]^+$: 425.2, found: 425.4. ^1H NMR (400 MHz, CD_3OD) δ 7.40 - 7.30 (m, 2H), 7.20 (dd, J = 8.6, 2.5 Hz, 1H), 4.46 - 4.24 (m, 2H), 4.05 - 3.84 (m, 2H), 3.25 - 3.13 (m, 2H), 3.05 (tt, J = 12.0, 3.5 Hz, 1H), 2.73 (h, J = 7.9 Hz, 1H), 2.33 - 2.10 (m, 3H), 2.07 - 1.52 (m, 9H), 1.33 - 1.19 (m, 4H).

Compound (*R*)-5: SFC: Rt 3.97 min, Lux Cellulose-2 4.6x100 mm, 5 µM, 5-55% MeOH (10 mM NH_4OH/CO_2), 5 mL/min. LCMS Method 4 Rt: 3.07 min, MS m/z calculated for $C_{22}H_{28}Cl_2FN_2O$ $[M+H]^+$: 425.2, found: 425.3. ^1H NMR (400 MHz, CD_3OD) δ 7.39 - 7.30 (m, 2H), 7.20 (dd, J = 8.5, 2.4 Hz, 1H), 4.48 - 4.23 (m, 2H), 4.06 - 3.82 (m, 2H), 3.18 (d, J = 11.5 Hz, 2H), 3.04 (t, J = 12.1 Hz, 1H), 2.72 (q, J = 7.7 Hz, 1H), 2.21 (dq, J = 39.4, 11.3, 8.9 Hz, 3H), 2.06 - 1.53 (m, 9H), 1.34 - 1.17 (m, 4H).

The following compounds were synthesized by analogy

Compound	Structure	LCMS	^1H NMR
SI-4		LCMS Method 1 Rt: 0.82 min MS m/z calculated for $C_{24}H_{37}N_3O_3$: 401.3 $[M-Boc+H]^+$ Found: 401.5	^1H NMR (400 MHz, CD_3OD) δ 7.25 - 7.10 (m, 2H), 6.98 - 6.85 (m, 2H), 3.93 - 3.66 (m, 7H), 3.40 - 3.34 (m, 1H), 3.17 - 2.95 (m, 2H), 2.60 - 2.42 (m, 2H), 2.29 (dd, J = 13.0, 7.5 Hz, 1H), 2.14 - 1.75 (m, 9H), 1.75 - 1.55 (m, 1H), 1.43 (s, 9H)

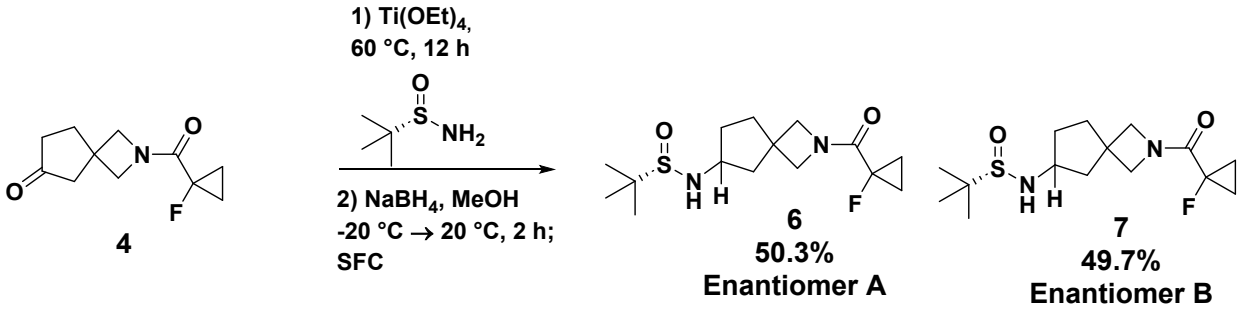
Synthesis of 2-(1-fluorocyclopropane-1-carbonyl)-2-azaspiro[3.4]octan-6-one (4)



tert-butyl 6-oxo-2-azaspiro[3.4]octane-2-carboxylate (50 g, 221.9 mmol) was dissolved in DCM (250 mL) and TFA was added (126.6 g, 85.5 mL, 1.11 mol) at 0°C and the reaction was stirred at 25 °C for 2 hours. The reaction was concentrated to give 2-azaspiro[3.4]octan-6-one (100 g, 221.89 mmol, crude) which was used for next step without purification assuming 100% yield. 2-azaspiro[3.4]octan-6-one (100 g, 221.89 mmol, crude) was dissolved in DCM (315 mL) and DIPEA was added (215.16 g, 290 mL, 1664.8 mmol) at 0 °C. After the solution was stirred for 5 min the solvent was removed and the residue was dissolved in DMF (125 mL). Separately, 1-fluorocyclopropanecarboxylic acid (24.3 g, 232.98 mmol) was dissolved in DMF (125 mL) HATU was added (89.4 g, 235.2 mmol). The solution was stirred at 25 °C for 30 min and then the DMF solution of 2-azaspiro[3.4]octan-6-one was added. The resulting solution was stirred at 25 °C for 12 h. The reaction was diluted with aqueous sodium carbonate (300 mL) and extracted with EtOAc (2x300 mL) and the combined organic layers were washed with brine (300 mL), dried over sodium sulfate, filtered and concentrated. The crude was then purified by FCC (5-50% EtOAc/petroleum ether) to yield the title compound.

^{1}H NMR (400MHz, DMSO-d_6) δ 4.39 (m, 2H), 4.02 (m, 2H), 2.51 (s, 2H), 2.33 - 2.31 (m, 2H), 2.27 -2.25 (m, 2H), 1.38 - 1.36 (m, 2H), 1.26 - 1.21 (m, 2H).

Synthesis of 6 and 7: (*R*) or (*S*)-N-((*R*)-2-(1-fluorocyclopropane-1-carbonyl)-2-azaspiro[3.4]octan-6-yl)-2-methylpropane-2-sulfinamide and (*R*) or (*S*)-N-((*S*)-2-(1-fluorocyclopropane-1-carbonyl)-2-azaspiro[3.4]octan-6-yl)-2-methylpropane-2-sulfinamide



2-(1-fluorocyclopropane-1-carbonyl)-2-azaspiro[3.4]octan-6-one (26.5 g, 125.45 mmol) was dissolved in THF (1150 mL) and (*S*)-2-methylpropane-2-sulfinamide (45.61 g, 376.35 mmol) and $Ti(OEt)_4$ (85.85 g, 78.9 mL, 376.35 mmol) were added and the reaction was stirred at 60 °C for 12 h. Next, the reaction was cooled to -20 °C and $NaBH_4$ (14.23 g, 376.35 mmol) was added and then the reaction was stirred at 25 °C for 2 h. MeOH (60 mL) was then added in the mixture which was then diluted aq. $NaHCO_3$ (500 mL). The mixture was filtered and the filtrate was extracted with EtOAc (2x 500 mL) and the combined organic layers were washed with brine (500 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated to and purified by FCC (0-20% MeOH/DCM) to give the mixture of diastereomers (25 g, 79.0 mmol). The stereoisomers were then separated by SFC (Amycoat 21x250 mm, 5 µM, 40% MeOH (0.1% DEA)/CO_2), 80 g/min to give the initial peak **6** (21.3 g, 67.3 mmol) and trailing peak **7** (21.7 g, 68.6 mmol).

Compound 6: Rt 1.403 min, AD-H 4.6x100 mm, 5 µM, 5-55% MeOH/CO_2 5 mL/min.

LCMS Method 5 Rt: 1.001 min, MS m/z calculated for $C_{15}H_{26}FN_2O_2S$ $[M+H]^+$: 317.2, found: 317.2.

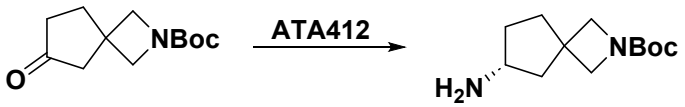
^{1}H NMR (400 MHz, DMSO-d_6) δ 5.04 (br d, J=4.4 Hz, 1H), 4.31 - 4.16 (m, 2H), 3.91 - 3.74 (m, 2H), 3.62 (br d, J=4.8 Hz,1H), 2.07 - 2.04 (m, 1H), 1.98 - 1.92 (m, 2H), 1.83 - 1.81 (m, 2H), 1.62 (m, 1H), 1.26 - 1.15 (m, 4H), 1.09 (s, 9H),

Compound 7: Rt 2.302 min, AD-H 4.6x100 mm, 5 µM, 5-55% MeOH/CO_2 5 mL/min.

LCMS Method 5 Rt: 0.985 min, MS m/z calculated for $C_{15}H_{26}FN_2O_2S$ $[M+H]^+$: 317.2, found: 317.2.

^{1}H NMR (400 MHz, DMSO-d_6) δ 5.07 (br d, J=4.4 Hz, 1H), 4.21 - 4.18 (m, 2H), 3.84 - 3.78 (m, 2H), 3.61 (br d, J=3.2 Hz,1H), 2.10 (m, 1H), 1.99 - 1.93 (m, 2H), 1.86 - 1.83 (m, 2H), 1.62 (m, 1H), 1.26 - 1.16 (m, 4H), 1.10 (s, 9H).

Synthesis of *tert*-butyl (R)-6-amino-2-azaspiro[3.4]octane-2-carboxylate (10)

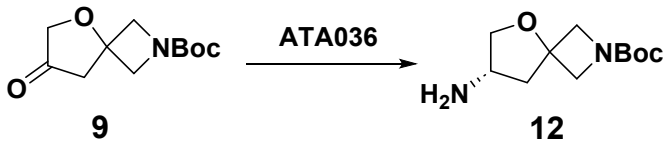


8 **10**

tert-butyl 6-oxo-2-azaspiro[3.4]octane-2-carboxylate (200 g, 0.887 mol), isopropylamine hydrochloride (845 g, 8.84 mol) and pyridoxal phosphate (10 g, 0.040 mmol) were added to a 10 L reactor and suspended in DMSO (800 mL) and 0.1M borate buffer (pH 9.0, 6200 mL). Aminotransferase ATA412 (Codexis) was dissolved in 0.1M borate buffer (400 mL) and added to the DMSO solution. The flask containing the enzyme was washed with 0.1M borate buffer (400 mL) and this was added to the DMSO solution. This wash step was repeated with 0.1M borate buffer (200 mL). The reaction was incubated at 40 °C with nitrogen bubbling through the solution until the ketone was consumed as judged by LCMS. The reaction was then cooled to 26 °C and citric acid was added until the solution pH reached 4.88. DCM (1.5 L) was added and the solution was filtered through microcrystalline cellulose. The phases were separated and the aqueous phase was added back to the reactor and NaCl (1200 g, 20.5 mol) was added and the pH was adjusted to 9.9 with 32% NaOH solution. The aqueous layer was extracted with DCM (3x2 L) and concentrated. The residue was dissolved in EtOAc (1.5 L) and washed with brine (2x100 mL). The organic layer was concentrated and the residue was dissolved in EtOAc (1.0 L) and filtered to remove NaCl and enzyme residue. The EtOAc layer was then concentrated and the residue was dissolved in EtOAc (0.87 L) and HCl in EtOAc (2M, 390 mL) was added over 1 hour. The solution was stirred for 2 hours and then filtered and washed with EtOAc to yield the title intermediate (133.9 g, 0.510 mol).

LCMS: Rt: 1.65 min (LCMS Method 4) MS m/z calculated for $C_{12}H_{23}N_2O_2$ 227.2 $[M+H]^+$ found 227.7

^{1}H NMR (400 MHz, CD_3OD) δ 3.92 – 3.81 (m, 2H), 3.77 (q, J = 8.3 Hz, 2H), 3.61 (p, J = 7.6 Hz, 1H), 2.40 (dd, J = 13.8, 8.1 Hz, 1H), 2.19 – 2.07 (m, 1H), 2.07 – 1.89 (m, 2H), 1.81 (dd, J = 13.8, 7.4 Hz, 1H), 1.71-1.58 (m, 1H), 1.43 (s, 9H).



9 12

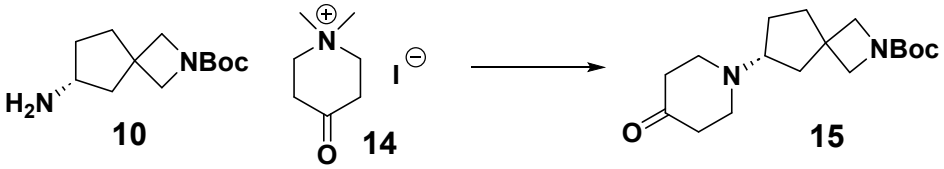
Synthesis of *tert*-butyl (S)-7-amino-5-oxa-2-azaspiro[3.4]octane-2-carboxylate (12)

tert-butyl 7-oxo-5-oxa-2-azaspiro[3.4]octane-2-carboxylate (commercially available, 129.8 g, 0.57 mol), isopropylamine hydrochloride (546 g, 5.71 mol) and pyridoxal 5′-phosphate hydrate (5.19 g, 0.02 mol) were dissolved in 0.1M Tris-HCl buffer (4200 mL, pH 8.5). The reaction was then warmed to 40 °C and a solution of ATA036 (Codexis, 5.19 g, 4 wt%) in 0.1M Tris-HCl buffer (400 mL, pH 8.5) was added. Nitrogen was bubbled through the solution and the reaction was stirred until complete conversion was achieved as determined by LCMS. The reaction was then cooled to 26 °C and 32% NaOH solution was added until pH reached 9.6. Next, NaCl (1000 g, 17.1 mol) was added followed by DCM (1500 mL) and the solution was stirred for 15 minutes and then rested for 15 minutes. The DCM layer was filtered and the cake was washed with DCM (500 mL). The aqueous layer was further extracted with DCM (2x1500 mL) and the combined organic layers were concentrated. EtOAc (500 mL) was added to the residue and the solution was filtered and the EtOAc layer was concentrated. The residue was diluted with EtOAc (450 mL) and stirred for 30 minutes and then 2M HCl in EtOAc (463 mL) was added over 1 hour. The solution was stirred for 30 minutes filtered and washed with EtOAc (100 mL). The solid was dried in a 40 °C vacuum oven to provide the amine as the hydrochloride salt (109.8 g, 0.414 mmol).

LCMS: Rt: 0.97 min (LCMS Method 4) MS m/z calculated for $C_{11}H_{21}N_2O_3$ 229.2 $[M+H]^+$, found 229.4

1H NMR (400 MHz, CD_3OD) δ 4.11 (d, J = 4.8 Hz, 2H), 4.01 – 3.89 (m, 5H), 2.71 (m, 1H), 2.27 – 2.17 (m, 1H), 1.46 (s, 9H).

Synthesis of *tert*-butyl (R)-6-(4-oxopiperidin-1-yl)-2-azaspiro[3.4]octane-2-carboxylate (15)



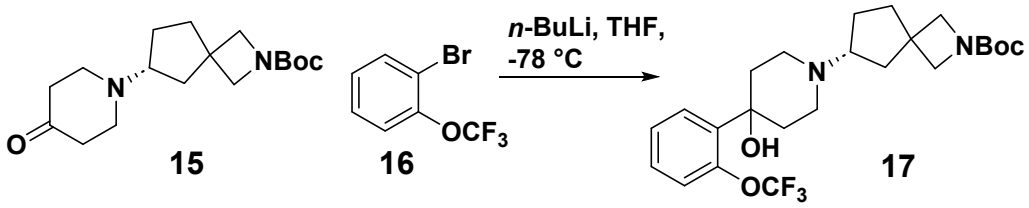
10 14 15

1,1-dimethyl-4-oxopiperidin-1-ium (5.82 g, 22.83 mmol), *tert*-butyl (*R*)-6-amino-2-azaspiro[3.4]octane-2-carboxylate (5 g, 19.03 mmol) and potassium carbonate (5.79 g, 41.9 mmol) were dissolved in EtOH (40 mL) and water (13 mL). The reaction was stirred at 60 °C for 4 hrs and then the EtOH was removed under reduced pressure. The aqueous layer was extracted with EtOAc (4x100 mL). The combined organic layers were dried over magnesium sulfate, filtered and concentrated. The crude was then purified by FCC (0-10% MeOH (1% NH_4OH)/DCM to yield the title compound (3.30 g, 10.70 mmol) as a yellow-orange solid.

LCMS Method 2: Rt: 0.86 min MS m/z calculated for $C_{17}H_{29}N_2O_3$ 309.2 $[M+H]^+$, found 309.5.

^{1}H NMR (400 MHz, CD_3OD) δ 3.93 – 3.63 (m, 4H), 2.81 (s, 7H), 2.16 (td, J = 12.6, 7.3 Hz, 1H), 2.06 – 1.45 (m, 7H), 1.43 (d, J = 1.4 Hz, 9H).

Synthesis of *tert*-butyl (*R*)-6-(4-hydroxy-4-(2-(trifluoromethoxy)phenyl)piperidin-1-yl)-2-azaspiro[3.4]octane-2-carboxylate (17)



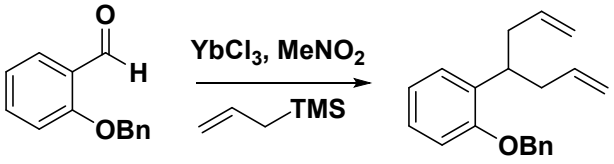
1-bromo-2-(trifluoromethoxy)benzene (1487 mg, 5.99 mmol) was dissolved in THF (5 mL) and the solution was degassed by sparging with N_2 for 3 minutes and then the reaction was cooled to -78 °C. 2.5M *n*-butyllithium in hexanes (2.394 mL, 5.99 mmol) was then added dropwise followed by a solution precooled to – 78 °C of *tert*-butyl (*R*)-6-(4-oxopiperidin-1-yl)-2-azaspiro[3.4]octane-2-carboxylate (923 mg, 2.99 mmol) in THF (7 mL) through a cannula. The reaction was stirred at -78C temperature for 1 hour and was then quenched with saturated ammonium chloride (10 mL) and warmed to room temperature. Water (10 mL) was added to the solution, which was extracted with EtOAc (3x50 mL) and the combined organic layers were washed with brine (1x10 mL), dried over sodium sulfate, filtered and concentrated. The crude was purified by FCC (0-20% MeOH/DCM) to yield the title compound (1290 mg, 2.74 mmol).

LCMS Method 4: Rt: 2.76 min MS m/z calculated for $C_{24}H_{34}F_3N_2O_4$ 471.2 $[M+H]^+$, found 471.5.

^{1}H NMR (400 MHz, CD_3OD) δ 7.74 (dd, J = 7.8, 1.9 Hz, 1H), 7.46 - 7.05 (m, 3H), 3.94 - 3.63 (m, 4H), 2.93 (m, 5H), 2.53 - 2.19 (m, 3H), 2.10 - 1.74 (m, 6H), 1.72 - 1.53 (m, 1H), 1.43 (s, 9H).

Allylation based route to3-(2-(benzyloxy)phenyl)pentane-1,5-diol (20)

Step 1: 1-(benzyloxy)-2-(hepta-1,6-dien-4-yl)benzene (19)

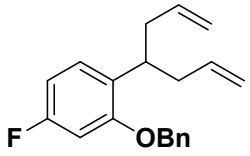
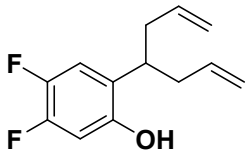


2-(Benzyloxy)benzaldehyde (10 g, 47.1 mmol) was added to a 250 mL round bottom flask followed by nitromethane (85 mL). Next, ytterbium (III) chloride (3.29 g, 11.78 mmol) was added to the reaction and the mixture was stirred for 15 minutes at room temperature. Subsequently, allyltrimethylsilane (22.46 mL, 141 mmol) was slowly added over 5 mins with an addition funnel. The reaction was stirred at room temperature for 72 hours and then it was filtered and the solution was concentrated. The crude was purified by FCC (0-20% EtOAc/heptanes) to provide the product as a colorless oil (9.52 g, 34.2 mmol) in approximately 90% purity.

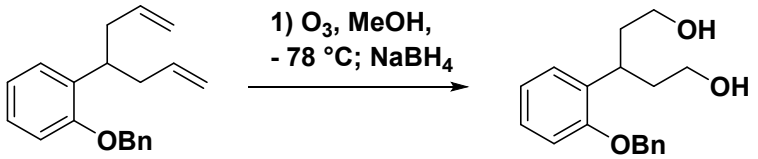
LCMS Method 2: Rt: 1.44 min MS m/z calculated for $C_{20}H_{21}O$ 277.2 $[M-H]^-$, found 277.5.

^{1}H NMR (400 MHz, CD_3OD) δ 7.47 – 7.41 (m, 2H), 7.36 (dd, J = 8.5, 6.3 Hz, 2H), 7.34 – 7.26 (m, 1H), 7.17 – 7.07 (m, 2H), 6.96 (d, J = 8.2 Hz, 1H), 6.89 (t, J = 7.4 Hz, 1H), 5.70 – 5.60 (m 2H), 4.96 – 4.80 (m, 4H), 2.39 – 2.35 (m, 4H). The methine proton is obscured by the residual solvent.

The following compounds were synthesized by analogy

Compound	Structure	LCMS	^{1}H NMR
SI-5		LCMS Method 2: Rt: 1.43 min, MS m/z calculated for $C_{20}H_{20}FO$: 295.2 $[M-H]^-$,found: 295.1	^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 - 7.19 (m, 5H), 6.97 (dd, 1H), 6.61 - 6.47 (m, 2H), 5.57 (m, 2H), 4.95 (s, 2H), 4.90 - 4.78 (m, 4H), 3.19 (t, 1H), 2.29 (m, 4H).
SI-6		Not tested	^{1}H NMR (400 MHz, $CDCl_3$) δ 6.90 (dd, J=8.9, 11.6 Hz, 1H), 6.62 (dd, J=6.8, 11.2 Hz, 1H), 5.68 (tdd, J=7.1, 10.1, 17.1 Hz, 2H), 5.56 (br s, 1H), 5.07 - 4.92 (m, 4H), 3.16 - 3.03 (m, 1H), 2.48 - 2.24 (m, 4H),

Step 2: 3-(2-(benzyloxy)phenyl)pentane-1,5-diol (20)



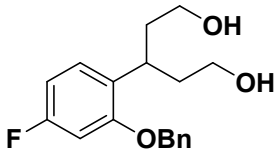
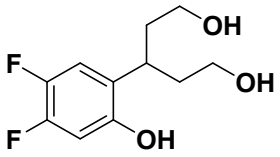
19 **20**

1-(benzyloxy)-2-(hepta-1,6-dien-4-yl)benzene (7.16 g, 20.58 mmol) was dissolved in methanol (50 mL) and DCM (25 mL) and cooled to -78 °C. Next, ozone was bubbled through the reaction mixture for 1 hour at which point, the reaction was a pale purple/violet color. Ozone was discontinued and the reaction was sparged with N_2 for 10 minutes with the reaction maintained at - 78 °C. Next, sodium borohydride (12.94 g, 342 mmol) was cautiously added to the reaction portionwise over 60 minutes. After adding sodium borohydride, the reaction was warmed to room temperature and stirred for 16 hours. Next, the solution was poured into water (200 mL) and extracted with EtOAc (3x50 mL). The combined organics were washed with brine (1x50 mL) dried over sodium sulfate, filtered and concentrated. The crude was purified by FCC (0-80% EtOAc/heptanes) to yield the title compound (3.7 g, 12.9 mmol).

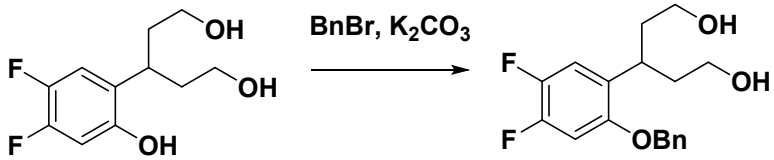
LCMS Method 4: Rt: 1.87 min MS m/z calculated for $C_{18}H_{23}O_3$ 287.2 $[M+H]^+$, found 287.3.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 - 7.26 (m, 5H), 7.16 (t, J = 7.6 Hz, 2H), 6.99 - 6.93 (m, 2H), 5.05 (s, 2H), 3.53 - 3.43 (m, 3H), 3.38 (ddd, J = 10.9, 8.3, 5.6 Hz, 2H), 2.10 (s, 2H), 1.93 (ddt, J = 13.7, 8.3, 5.7 Hz, 2H), 1.84 - 1.74 (m, 2H).

The following compounds were synthesized by analogy

Compound	Structure	LCMS	^{1}H NMR
SI-7		LCMS Method 2: Rt: 0.91 min MS m/z calculated for $C_{18}H_{22}FO_3$: 305.2 [M-H]$^-$,found: 305.3	^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50 - 7.45 (m, 2H), 7.44 - 7.38 (m, 2H), 7.36 - 7.30 (m, 1H), 7.17 (dt, 1H), 6.99 - 6.85 (m, 1H), 6.71 (td, 1H), 5.10 (s, 2H), 4.26 (t, 2H), 3.22 (td, 5H), 1.74 (q, 4H).
SI-8		LCMS Method 6: Rt: 0.609 min	^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (dd, J=5.7, 8.7 Hz, 1H), 7.25 (dd, J=2.9, 10.4 Hz, 1H), 7.01 (dt, J=2.7, 8.3 Hz, 1H), 4.44 - 4.33 (m, 2H), 3.32 - 3.18 (m, 5H), 1.86 - 1.65 (m, 4H).

Synthesis of 3-(2-(benzyloxy)-4,5-difluorophenyl)pentane-1,5-diol (SI-9)



SI-8 **SI-9**

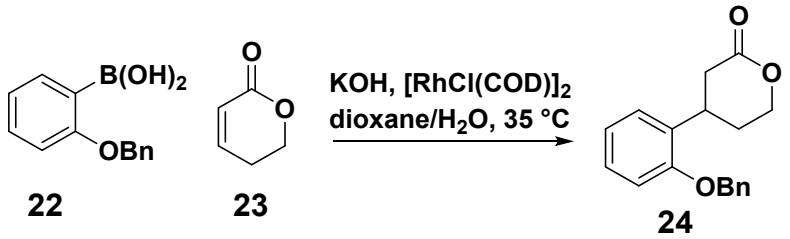
3-(4,5-difluoro-2-hydroxyphenyl)pentane-1,5-diol (3.46 g, 14.90 mmol) was added to a 250mL round bottom flask and dissolved in acetone (100 mL). K_2CO_3 (3.09 g, 22.35 mmol) and benzyl bromide (1.858 mL, 15.64 mmol) were added and the reaction was warmed to 60 °C and stirred for 3 hours. The reaction was cooled to room temperature and concentrated. The residue was dissolved in DCM (100 mL) and washed with water (1x20 mL), and the aqueous layer was back extracted with DCM (2x50 mL). The combined organics were washed with brine (1x20 mL) dried over magnesium sulfate, filtered and concentrated. The residue was purified by FCC (0-10% MeOH/DCM) to yield the title compound (4.75 g, 14.74 mmol).

LCMS Method 2: Rt: 0.89 min MS m/z calculated for $C_{18}H_{21}F_2O_3$: 323.1 [M+H]$^+$,found: 323.4

^{1}H NMR (400 MHz, CD_3OD) δ 7.51 – 7.42 (m, 2H), 7.42 – 7.27 (m, 3H), 7.10 (dd, J = 11.7, 9.2 Hz, 1H), 6.96 (dd, J = 12.6, 6.9 Hz, 1H), 5.05 (s, 2H), 3.40 (t, J = 7.0 Hz, 4H), 3.34 (s, 1H), 1.86 (q, J = 7.0 Hz, 4H).

Rhodium based route to 3-(2-(benzyloxy)phenyl)pentane-1,5-diol (20)

Step 1: 4-(2-(benzyloxy)phenyl)tetrahydro-2*H*-pyran-2-one (24)



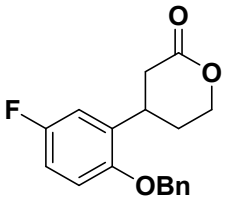
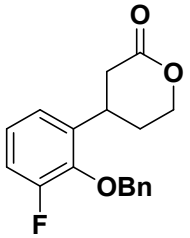
22 23 24

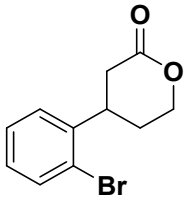
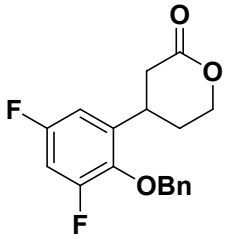
Potassium hydroxide (3.95 g, 70.3 mmol) in water (17.50 mL) was added dropwise to a solution of $[RhCl(COD)]_2$ (0.347 g, 0.703 mmol), (2-(benzyloxy)phenyl)boronic acid (commercially available, 22.46 g, 98 mmol) and 5,6-dihydro-2H-pyran-2-one (commercially available, 6.06 mL, 70.3 mmol) in 1,4-dioxane (175 mL) at 0 °C over a period of 2 mins. The temperature of reaction was then raised to 35 °C and stirred for 16 h. The reaction was diluted with EtOAc (200 mL) and 2M HC (50 mL). The aqueous solution was separated and back extracted with EtOAc (50 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude mixture was then purified by FCC (0 → 60% EtOAc/heptanes) to yield the title compound (19.49 g, 68.3 mmol).

LCMS Method 1: Rt: 1.03 min MS m/z calculated for $C_{18}H_{19}O_3$: 283.2 $[M+H]^+$, found 283.5.

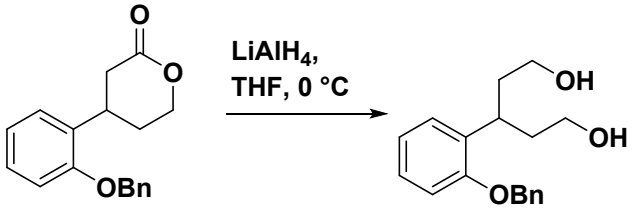
^{1}H NMR (400 MHz, CD_3OD) δ 7.49 - 7.27 (m, 5H), 7.20 (ddd, J = 14.8, 7.5, 1.8 Hz, 2H), 7.05 (dd, J = 8.1, 1.2 Hz, 1H), 6.99 - 6.89 (m, 1H), 5.13 (s, 2H), 4.49 - 4.27 (m, 2H), 3.71 - 3.50 (m, 1H), 2.85 (dd, J = 17.2, 6.4 Hz, 1H), 2.68 (dd, J = 17.2, 9.8 Hz, 1H), 2.10 (m, 2H).

The following compounds were synthesized by analogy

Compound	Structure	LCMS	^{1}H NMR
SI-10		LCMS Method 3 Rt: 2.31 min MS m/z calculated for $C_{18}H_{18}FO_3$: 301.1 $[M+H]^+$ Found: 301.2	^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52 - 7.26 (m, 5H), 7.17 - 6.99 (m, 3H), 5.13 (s, 2H), 4.43 - 4.25 (m, 2H), 3.58 (m, 1H), 2.83 - 2.54 (m, 2H), 1.97 (m, 2H).
SI-11		LCMS Method 2 Rt: 1.11 min MS m/z calculated for $C_{18}H_{18}FO_3$: 301.1 $[M+H]^+$ Found: 301.2	^{1}H NMR (400 MHz, DMSO-d_6) δ 7.47 – 7.24 (m, 5H), 7.14 – 7.03 (m, 2H), 6.99 (dd, J = 6.4, 3.2 Hz, 1H), 5.13 (s, 2H), 4.37 (m, 1H), 4.23 (m, 1H), 3.46 (m, 1H), 2.64 – 2.41 (m,

			2H), 1.90 (m, 1H), 1.81 – 1.69 (m, 1H).
SI-12		LCMS Method 2 Rt: 0.91 min MS m/z calculated for $C_{11}H_{12}BrO_2$: 255.0 $[M+H]^+$ Found: 254.9	^{1}H NMR (400 MHz, DMSO-d_6) δ 7.63 (d, J = 7.9 Hz, 1H), 7.42 (d, J = 4.0 Hz, 2H), 7.21 (dq, J = 8.7, 4.2 Hz, 1H), 4.45 - 4.34 (m, 2H), 3.62 (tt, J = 10.6, 5.4 Hz, 1H), 2.79 (ddd, J = 17.1, 6.1, 1.6 Hz, 1H), 2.63 (dd, J = 16.9, 10.4 Hz, 1H), 2.17 - 1.87 (m, 2H).
SI-13		LCMS Method 2 Rt: 1.07 min MS m/z calculated for $C_{18}H_{17}F_2O_3$: 319.1 $[M+H]^+$ Found: 319.1	1H NMR (400 MHz, CD_3OD) δ 7.43 – 7.30 (m, 5H), 6.96 (ddd, J = 11.3, 8.4, 3.0 Hz, 1H), 6.83 (ddd, J = 9.4, 2.9, 1.8 Hz, 1H), 5.09 (d, J = 2.3 Hz, 2H), 4.38 (ddd, J = 11.5, 5.1, 3.3 Hz, 1H), 4.22 (td, J = 11.2, 3.7 Hz, 1H), 3.52 – 3.38 (m, 1H), 2.58 – 2.40 (m, 2H), 1.88 (m, 1H), 1.78 – 1.63 (m, 1H).

Step 2: 3-(2-(benzyloxy)phenyl)pentane-1,5-diol (20)



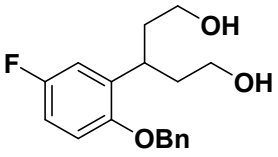
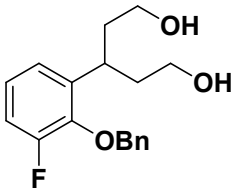
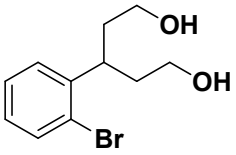
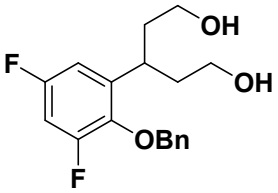
24 **20**

Lithium aluminum hydride (76 mL, 76 mmol, 1M in THF) was added to a stirred solution of 4-(2-(benzyloxy)phenyl)tetrahydro-2H-pyran-2-one (19.49 g, 69.0 mmol) in anhydrous THF (400 mL) at 0 °C and then the reaction mixture was stirred for 2 h at 0 °C. The reaction was quenched by water (10 mL) at -5 °C until gas production ceased and then a solution of NaOH (25 g) in water (25 mL) was portion-wise added to the mixture at 0 °C. Na_2SO_4 (300 g) was next added to the reaction mixture and was stirred for 60 min. The mixture was filtered and the solvent was removed under reduced pressure. The crude product was purified by FCC (0-10% MeOH/DCM) to yield the title compound (19.3 g, 64.1 mmol).

LCMS Method 1: Rt: 0.85 min MS m/z calculated for calculated for $C_{18}H_{23}O_3$ 287.2 $[M+H]^+$, found 287.3.

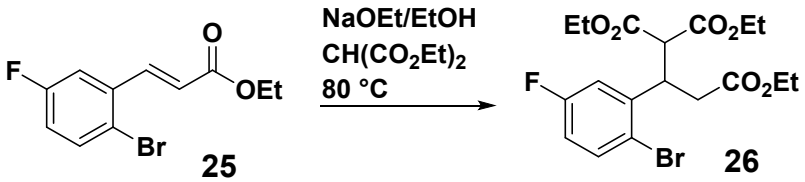
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 - 7.31 (m, 5H), 7.26 - 7.16 (m, 2H), 7.07 - 6.95 (m, 2H), 5.11 (s, 2H), 3.61 - 3.35 (m, 5H), 1.92 (m, 4H), 1.66 (s, 2H).

The following compounds were synthesized by analogy

Compound	Structure	LCMS	^{1}H NMR
SI-14		LCMS Method 3 Rt: 1.88 min MS m/z calculated for $C_{18}H_{22}FO_3$: 305.2 $[M+H]^+$ Found: 305.1	^{1}H NMR (400 MHz, CD_3OD) δ 7.46 (d, J = 7.1 Hz, 2H), 7.41 – 7.27 (m, 3H), 6.97 (ddd, J = 15.2, 9.3, 3.8 Hz, 2H), 6.86 (td, J = 8.5, 3.1 Hz, 1H), 5.05 (s, 2H), 3.47 – 3.32 (m, 5H), 1.88 (q, J = 7.2 Hz, 4H).
SI-15		LCMS Method 3 Rt: 1.89 min	^{1}H NMR (400 MHz, CD_3OD) δ 7.52 - 7.46 (m, 2H), 7.41 - 7.30 (m, 3H), 7.12 - 6.97 (m, 3H), 5.05 (s, 2H), 3.40 - 3.33 (m, 5H), 1.92 - 1.73 (m, 4H).
SI-16		LCMS Method 1 Rt: 0.69 min	^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (d, J = 7.9 Hz, 1H), 7.35 (d, J = 4.4 Hz, 2H), 7.12 (dq, J = 8.5, 4.2 Hz, 1H), 4.35 (t, J = 5.0 Hz, 2H), 3.26 (dd, J = 10.0, 4.5 Hz, 5H), 1.94 - 1.57 (m, 4H).
SI-17		LCMS Method 1 Rt: 0.90 min MS m/z calculated for $C_{18}H_{21}F_2O_3$: 323.2 $[M+H]^+$ Found: 323.5	^{1}H NMR (400 MHz, CD_3OD) δ 7.55 – 7.47 (m, 2H), 7.45 – 7.32 (m, 3H), 6.96 – 6.82 (m, 2H), 5.04 (s, 2H), 3.50 – 3.35 (m, 5H), 1.90 (m, 2H), 1.76 (m, 2H).

Decarboxylative based route to 3-(2-bromo-5-fluorophenyl)pentane-1,5-diol (29)

Step 1: triethyl 2-(2-bromo-5-fluorophenyl)propane-1,1,3-tricarboxylate (27)

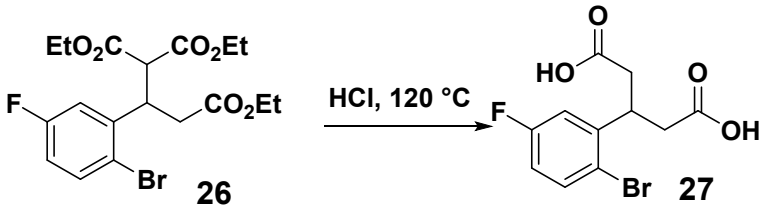


Solid sodium metal (9.85 g, 428 mmol) was added into EtOH (400 mL) and the reaction mixture was stirred at 25 °C until the solid was disappeared. Then diethylmalonate (46 g, 286 mmol) and ethyl (*E*)-3-(2-bromo-

5-fluorophenyl)acrylate (39 g, 143 mmol) was added to the reaction mixture. The mixture was then warmed to 80 °C and stirred for 16 hours. The reaction mixture was then concentrated and the residue was purified by FCC (0-25% EtOAc/petroleum ether) to provide the title compound as a light yellow liquid (32 g, 74 mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 - 7.42 (m, 1H), 7.01 (dd, J=2.7, 9.8 Hz, 1H), 6.88 - 6.71 (m, 1H), 4.37 (d, J=7.2 Hz, 1H), 4.22 - 4.14 (m, 2H), 4.10 - 3.97 (m, 4H), 3.91 (d, J=8.1 Hz, 1H), 2.90 (d, J=7.0 Hz, 2H), 1.27 - 1.20 (m, 3H), 1.11 (m, 6H).

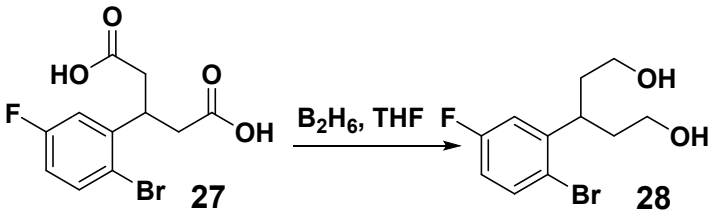
Step 2: 3-(2-bromo-5-fluorophenyl)pentanedioic acid (28)



triethyl 2-(2-bromo-5-fluorophenyl)propane-1,1,3-tricarboxylate (32 g, 74 mmol) was dissolved in concentrated HCl (300 mL), and the reaction mixture was stirred at 120 °C for 16 hours. The reaction mixture was concentrated and then coevaporated with toluene (2x50 mL) to give 3-(2-bromo-5-fluorophenyl)pentanedioic acid (21 g, 68.8 mmol) as a yellow solid that was used without further purification.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (dd, J=5.6, 8.6 Hz, 1H), 7.38 - 7.29 (m, 1H), 7.41 - 7.29 (m, 1H), 7.09 - 6.98 (m, 1H), 3.87 (m, 1H), 2.68 - 2.54 (m, 4H)

Step 3: 3-(2-bromo-5-fluorophenyl)pentane-1,5-diol (29)

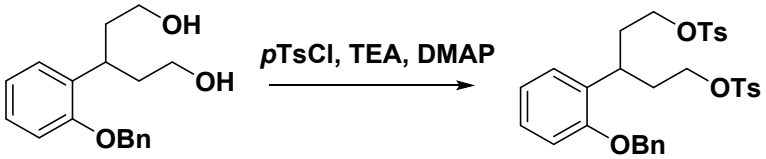


3-(2-bromo-5-fluorophenyl)pentanedioic acid (21 g, 68.8 mmol) was dissolved in THF (200 mL) and cooled to 0 °C. Next, B_2H_6 (34.4 mL, 344 mmol, 10 M in dimethyl sulfide) was added dropwise and following the addition, the reaction was warmed to 25 °C and the reaction was stirred for 16 hours. The reaction was then quenched with MeOH (100 mL) and HCl (2N, 100 mL) amd the solution was concentrated. The residue was purified by FCC (0-50% EtOAc/petroleum ether) to yield the title intermediate (10.78 g, 38.89 mmol) as a white solid.

LCMS Method 6: Rt: 0.819 min, MS m/z calculated for $C_{11}H_{13}BrFO_2$: 275.0 $[M-H]^-$,found: 275.4

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.59 (dd, J=5.7, 8.7 Hz, 1H), 7.25 (dd, J=2.9, 10.4 Hz, 1H), 7.01 (dt, J=2.7, 8.3 Hz, 1H), 4.44 - 4.33 (m, 2H), 3.32 - 3.18 (m, 5H), 1.86 - 1.65 (m, 4H).

Synthesis of 3-(2-(benzyloxy)phenyl)pentane-1,5-diyl bis(4-methylbenzenesulfonate) (SI-18)



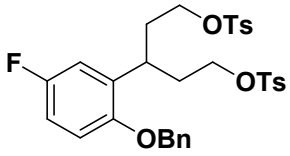
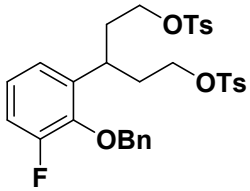
20 **SI-18**

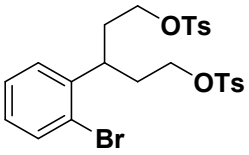
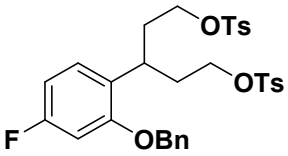
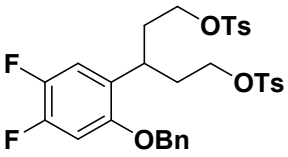
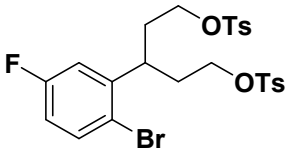
3-(2-(benzyloxy)phenyl)pentane-1,5-diol (19.31 g, 67.4 mmol) was dissolved in MeCN (40 mL) and triethylamine (41.4 mL, 297 mmol) was added and the reaction was cooled to at -5 °C. Next, TsCl (28.3 g, 148 mmol) and DMAP (0.824 g, 6.74 mmol) were added. After addition, the reaction was stirred at room temperature for 16 hours. Subsequently, the solvent was removed under reduced pressure and the crude product was dissolved in DCM (200 mL) and washed with water (25 mL) and brine (25 mL) then dried over Na_2SO_4 and filtered. The DCM was removed under reduced pressure and the crude was purified by FCC (0 → 50% EtOAc/heptanes) to yield the title compound (35.41 g, 53.6 mmol)

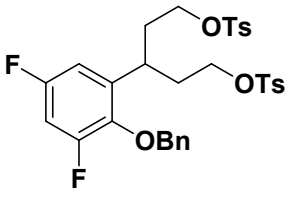
LCMS Method 3: Rt= 3.25 min MS m/z calculated for $C_{32}H_{35}O_7S_2$: $[M+H]^+$ 595.2, found: 595.1

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 - 7.63 (m, 4H), 7.46 - 7.33 (m, 5H), 7.30 (s, 4H), 7.20 - 7.13 (m, 1H), 6.99 - 6.72 (m, 3H), 5.00 (s, 2H), 4.01 - 3.61 (m, 4H), 3.15 (m, 1H), 2.45 (s, 6H), 2.03 (m, 2H), 1.89 (m, 2H).

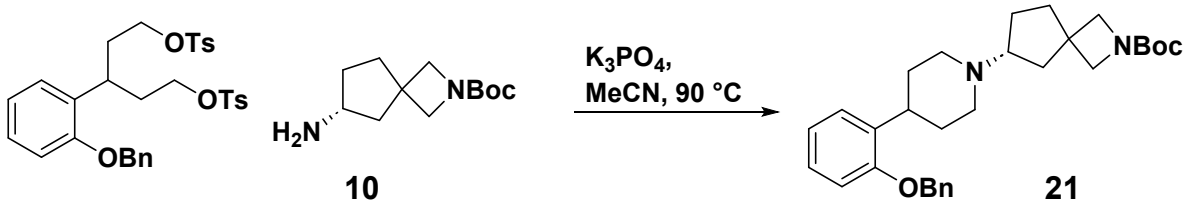
The following compounds were synthesized in analogy

Compound	Structure	LCMS	^{1}H NMR
SI-19		LCMS Method 3 Rt: 3.26 min MS m/z calculated for $C_{32}H_{34}FO_7S_2$: 613.2 $[M+H]^+$ Found: 613.4	^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.67 (d, J = 8.3 Hz, 4H), 7.47 - 7.37 (m, 5H), 7.33 (d, J = 8.1 Hz, 4H), 6.91 - 6.80 (m, 2H), 6.56 (dd, J =9.2, 2.7 Hz, 1H), 4.99 (s, 2H), 3.95 - 3.86 (m, 2H), 3.77 (ddd, J = 9.9,7.9, 6.3 Hz, 2H), 3.14 (dt, J = 9.5, 4.6 Hz, 1H), 2.46 (s, 6H), 2.03 - 1.84 (m, 3H).
SI-20		LCMS Method 1 Rt: 1.37 min MS m/z calculated for $C_{32}H_{37}FNO_7S_2$: 630.2 $[M+NH_4]^+$ Found: 630.7.	^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 - 7.64 (m, 4H), 7.42 - 7.33 (m, 5H), 7.31 - 7.28 (m, 4H), 7.08 - 6.84 (m, 2H), 6.75 - 6.64 (m, 1H), 5.02 (s, 2H), 3.83 (dt, J = 10.0, 6.2 Hz, 2H), 3.72 (dt, J = 10.1, 7.0 Hz, 2H),

			3.18 (p, J = 7.5 Hz, 1H), 2.45 (s, 6H), 1.86 (m 4H).
SI-21		LCMS Method 3 Rt: 3.02 min MS m/z calculated for $C_{25}H_{31}BrNO_6S_2$: 586.1 $[M+NH_4]^+$ Found: 586.0	^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.76 - 7.68 (m, 4H), 7.50 (dd, J = 8.0, 1.2 Hz, 1H), 7.36 (d, J = 8.2 Hz, 4H), 7.28 - 7.20 (m, 1H), 7.12 - 7.01 (m, 2H), 3.92-3.86 (m, J = 10.0, 6.2 Hz, 2H), 3.84-3.78 (m, J = 9.9, 7.0 Hz, 2H), 3.47 - 3.31 (m, 1H), 2.47 (s, 6H), 2.04-1.90 (m, 4H).
SI-22		LCMS Method 2 Rt: 1.35 min MS m/z calculated for $C_{32}H_{37}FNO_7S_2$: 630.2 $[M+NH_4]^+$ Found: 630.2.	1H NMR (400 MHz, CDCl3) δ 7.68 (d, J = 8.3 Hz, 4H), 7.47 – 7.34 (m, 5H), 7.29 (d, J = 8.4 Hz, 4H), 6.79 (dd, J = 8.5, 6.6 Hz, 1H), 6.60 (dd, J = 11.0, 2.6 Hz, 1H), 6.49 (m, 1H), 3.88 (m, 2H), 3.77 (m, 2H), 3.10 (m, 1H), 2.46 (s, 6H), 2.01 (m, 2H), 1.94 – 1.79 (m, 2H), 1.30-1.26 (m, 2H)
SI-23		LCMS Method 2 Rt: 1.34 min MS m/z calculated for $C_{32}H_{36}F_2NO_7S_2$: 648.2 $[M+NH_4]^+$ Found: 648.6	NT
SI-24		LCMS Method 3 Rt: 3.01 min MS m/z calculated for $C_{25}H_{30}BrFNO_6S_2$: 604.1 $[M+NH_4]^+$ Found: 604.1	^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 - 7.67 (m, 4H), 7.42 (dd, J = 8.8, 5.5 Hz, 1H), 7.31 (d, J = 8.0 Hz, 4H), 6.78 (m, 1H), 6.73 - 6.62 (m, 1H), 3.88 (m, 2H), 3.80 (dt, J = 10.1, 6.9 Hz, 2H), 3.34 (s, 2H), 2.44 (s, 6H), 2.05 - 1.93 (m, 2H), 1.89 (bs, 2H).

SI-25		LCMS Method 1 Rt: 1.39 min MS m/z calculated for $C_{32}H_{36}F_2NO_7S_2$: 648.2 $[M+NH_4]^+$ Found: 648.5	^{1}H NMR (400 MHz, CD_3OD) δ 7.69 – 7.62 (m, 4H), 7.47 – 7.32 (m, 9H), 6.88 (m, 1H), 6.58 (m, 1H), 4.94 (s, 2H), 3.83 (dt, J = 10.2, 5.9 Hz, 2H), 3.69 (m, 2H), 3.29 – 3.18 (m, 1H), 2.45 (s, 6H), 1.91 – 1.67 (m, 4H).

***tert*-butyl (R)-6-(4-(2-(benzyloxy)phenyl)piperidin-1-yl)-2-azaspiro[3.4]octane-2-carboxylate (21)**

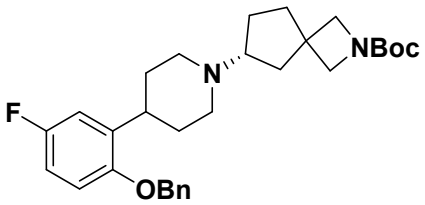


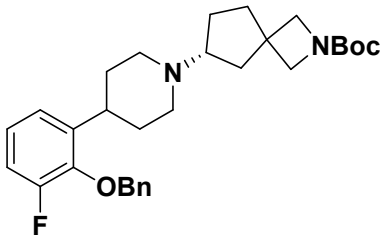
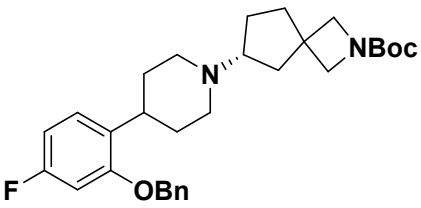
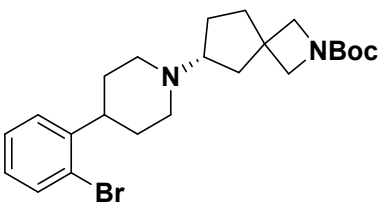
tert-butyl (*R*)-6-amino-2-azaspiro[3.4]octane-2-carboxylate (4.85 g, 18.46 mmol) and 3-(2-(benzyloxy)phenyl)pentane-1,5-diyl bis(4-methylbenzenesulfonate) (10.98 g, 18.46 mmol) were suspended in MeCN (120 mL) and K_3PO_4 (12.54 g, 59.1 mmol) was added. The reaction was then warmed to 90 °C and stirred for 16 hours. The reaction was then cooled to room temperature and EtOAc (200 mL) was added and the solid was filtered and washed with EtOAc. The combined organics were concentrated and purified by FCC (0-10% MeOH (10% NH_4OH)/DCM) to yield the title compound (8.28 g, 17.37 mmol).

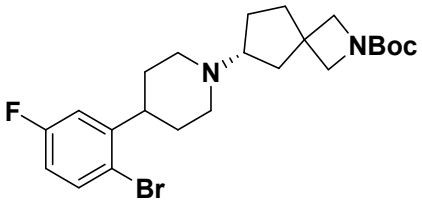
LCMS Method 2: Rt: 1.40 min MS m/z calculated for $C_{30}H_{41}N_2O_3$ 477.3 $[M+H]^+$, found 477.5.

^{1}H NMR (400 MHz, CD_3OD) δ 7.46 – 7.28 (m, 5H), 7.22 – 7.11 (m, 2H), 6.99 (dd, *J* = 8.3, 1.0 Hz, 1H), 6.91 (td, *J* = 7.6, 1.3 Hz, 1H), 5.10 (s, 2H), 3.85-3.70 (m, 4H), 3.19 – 3.12 (m, 2H), 3.07 (tt, *J* = 12.1, 3.7 Hz, 1H), 2.70 (p, *J* = 8.2 Hz, 1H), 2.20-2.10 (m, 3H), 2.03 – 1.67 (m, 8H), 1.63 – 1.48 (m, 1H), 1.43 (s, 9H).

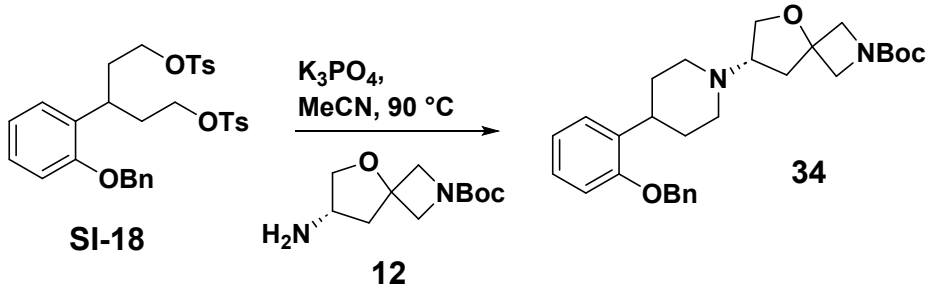
The following compounds were synthesized in analogy

Compound	Structure	LCMS	^{1}H NMR
29		LCMS Method 2 Rt: 1.42 min MS m/z calculated for $C_{30}H_{40}FN_2O_3$: 495.3 $[M+H]^+$ Found: 495.5.	^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.50 – 7.40 (m, 4H), 7.40 – 7.34 (m, 1H), 6.98 (dd, *J* = 10.0, 2.8 Hz, 1H), 6.94 – 6.83 (m, 2H), 5.10 (s, 2H), 3.87 – 3.66 (m, 4H), 3.18 – 2.97 (m, 3H), 2.70 – 2.55 (m, 1H),

			2.18 – 1.98 (m, 3H), 1.98 – 1.77 (m, 5H), 1.77 – 1.49 (m, 4H), 1.45 (s, 9H).
30		LCMS Method 4 Rt: 3.46 min MS m/z calculated for $C_{30}H_{40}FN_2O_3$: 495.3 $[M+H]^+$ Found: 495.4.	^{1}H NMR (400 MHz, CD_3OD) 7.45 – 7.29 (m, 5H), 7.12 – 6.89 (m, 3H), 5.06 (s, 2H), 3.92 – 3.61 (m, 4H), 3.06 (d, J = 11.3 Hz, 2H), 2.94 – 2.77 (m, 1H), 2.64 (s, 1H), 2.15 (dd, J = 12.8, 7.3 Hz, 1H), 2.07 – 1.77 (m, 5H), 1.75 – 1.46 (m, 6H), 1.43 (s, 9H).
31		LCMS Method 4 Rt: 3.59 min MS m/z calculated for $C_{30}H_{40}FN_2O_3$: 495.3 $[M+H]^+$ Found: 495.4.	^{1}H NMR (400 MHz, CD_3OD) δ 7.54 – 7.27 (m, 5H), 7.17 (dd, J = 8.5, 6.6 Hz, 1H), 6.79 (dd, J = 11.2, 2.5 Hz, 1H), 6.64 (td, J = 8.4, 2.5 Hz, 1H), 5.09 (s, 2H), 3.95 – 3.64 (m, 4H), 3.22 – 3.09 (m, 2H), 3.01 (t, J = 12.1 Hz, 1H), 2.71 (s, 1H), 2.18 (dd, J = 12.8, 7.4 Hz, 3H), 2.05 – 1.49 (m, 9H), 1.43 (s, 9H).
32		LCMS Method 2 Rt: 1.42 min MS m/z calculated for $C_{23}H_{34}BrN_2O_2$: 449.2 $[M+H]^+$ Found: 449.5.	^{1}H NMR (400 MHz, CD_3OD) δ 7.54 (d, J = 8.0 Hz, 1H), 7.32 (d, J = 4.4 Hz, 2H), 7.09 (dt, J = 8.7, 4.5 Hz, 1H), 3.91 – 3.65 (m, 4H), 3.16 (m, 2H), 3.04 (m, 1H), 2.77 – 2.60

			(m, 1H), 2.16 (m, 3H), 2.04 – 1.82 (m, 5H), 1.73 (m, 3H), 1.63 – 1.50 (m, 1H), 1.43 (s, 9H).
33		LCMS Method 1 Rt: 0.78 min MS m/z calculated for $C_{23}H_{33}BrFN_2O_2$: 467.2 $[M+H_4]^+$ Found: 467.5	^1H NMR (400 MHz, CD_3OD) δ 7.57 (dd, J = 8.8, 5.4 Hz, 1H), 7.09 (dd, J = 10.2, 3.0 Hz, 1H), 6.90 (td, J = 8.3, 3.0 Hz, 1H), 3.91 – 3.66 (m, 4H), 3.17 (d, J = 11.4 Hz, 2H), 3.02 (dd, J = 14.0, 10.3 Hz, 1H), 2.70 (p, J = 8.4 Hz, 1H), 2.26 – 2.08 (m, 3H), 2.05 – 1.80 (m, 6H), 1.81 – 1.62 (m, 3H), 1.62 – 1.52 (m, 1H), 1.44 (s, 9H).

Synthesis of *tert*-butyl (S)-7-(4-(2-(benzyloxy)phenyl)piperidin-1-yl)-5-oxa-2-azaspiro[3.4]octane-2-carboxylate (34)

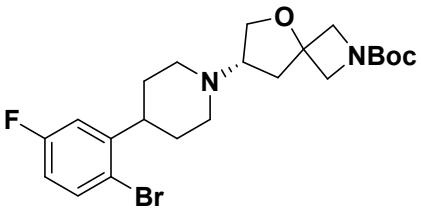
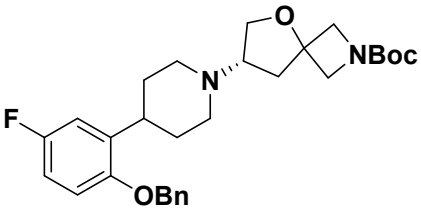


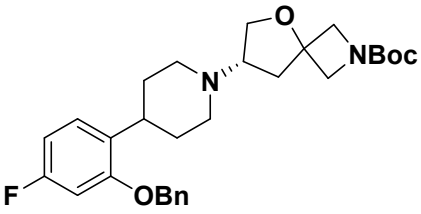
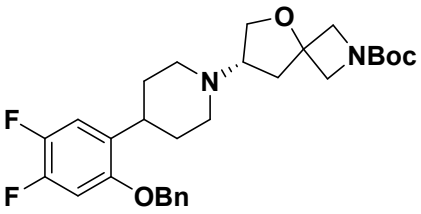
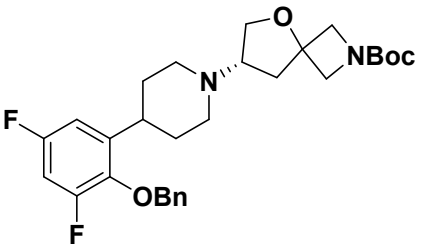
3-(2-(benzyloxy)phenyl)pentane-1,5-diyl bis(4-methylbenzenesulfonate) (35.41 g, 54.8 mmol) and *tert*-butyl (*S*)-7-amino-5-oxa-2-azaspiro[3.4]octane-2-carboxylate (14.50 g, 54.8 mmol) were suspended in MeCN (500 mL) and potassium phosphate (34.9 g, 164 mmol) was added to the solution. The reaction was warmed to 90 °C and stirred for 60 hours. The reaction was cooled to room temperature and concentrated and the residue was suspended in EtOAc (500 mL). The slurry was filtered and washed with EtOAc (100 mL) and the combined organics were concentrated to afford an orange oil. The crude was purified by FCC (0-10% MeOH (1% NH_4OH)/DCM) to yield the title compound (17.09 g, 33.9 mmol).

LCMS Method 2: Rt: 0.78 min, MS m/z calculated for $C_{29}H_{39}N_2O_4$: 479.3 $[M+NH_4]^+$, found: 479.2

^{1}H NMR (400 MHz, CD_3OD) δ 7.47 - 7.26 (m, 5H), 7.23 - 7.09 (m, 2H), 7.04 - 6.84 (m, 2H), 5.09 (s, 2H), 4.11 - 3.78 (m, 5H), 3.70 (dd, J = 8.7, 7.4 Hz, 1H), 3.05 (tt, J = 14.0, 4.4 Hz, 3H), 2.96 - 2.72 (m, 1H), 2.42 (dd, J = 12.9, 7.5 Hz, 1H), 2.18 (qd, J = 11.5, 2.8 Hz, 2H), 2.03 (dd, J = 12.9, 8.5 Hz, 1H), 1.91 - 1.60 (m, 4H), 1.43 (s, 9H).

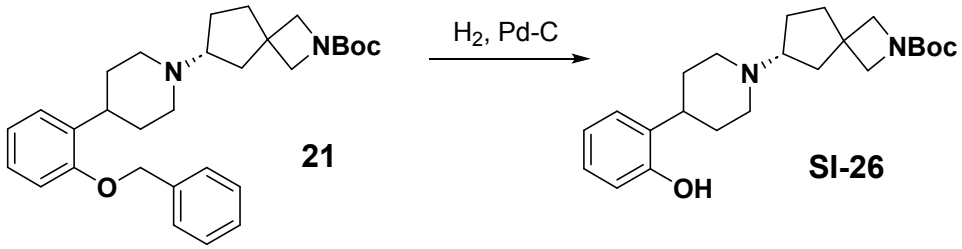
The following compounds were synthesized in analogy to 31

Compound	Structure	LCMS	^{1}H
34		LCMS Method 4 Rt: 2.96 min MS m/z calculated for $C_{22}H_{31}BrFN_2O_3$: 469.1 $[M+H]^+$ Found: 469.5	^{1}H NMR (400 MHz, CD_3OD) δ 7.58 (dd, J = 8.8, 5.4 Hz, 1H), 7.11 (dd, J = 10.2, 3.0 Hz, 1H), 6.97 – 6.87 (m, 1H), 4.11 – 3.97 (m, 3H), 3.95 – 3.84 (m, 2H), 3.75 (dd, J = 8.6, 7.4 Hz, 1H), 3.19 – 2.98 (m, 3H), 2.92 (d, J = 10.9 Hz, 1H), 2.46 (dd, J = 12.9, 7.5 Hz, 1H), 2.25 (m, 2H), 2.08 (dd, J = 12.9, 8.3 Hz, 1H), 1.89 (m, 2H), 1.71 (m, 2H), 1.46 (s, 9H).
35		LCMS Method 2 Rt: 1.33 min MS m/z calculated for $C_{29}H_{38}FN_2O_4$: 497.3 $[M+H]^+$ Found: 497.2	^{1}H NMR (400 MHz, CD_3OD) δ 7.47 - 7.26 (m, 5H), 7.23 - 7.09 (m, 2H), 7.04 - 6.84 (m, 1H), 5.09 (s, 2H), 4.11 - 3.78 (m, 5H), 3.70 (dd, J = 8.7, 7.4 Hz, 2H), 3.05 (m, 3H), 2.96 - 2.72 (m, 1H), 2.42 (dd, J = 12.9, 7.5 Hz, 1H), 2.18 (m, 2H), 2.03 (dd, J = 12.9, 8.5 Hz, 1H), 1.91 - 1.60 (m, 4H), 1.43 (s, 9H).

36		LCMS Method 4 Rt: 3.59 min MS m/z calculated for $C_{29}H_{37}FN_2O_4$: 497.3 $[M+H]^+$ Found: 497.4	^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 4.4 Hz, 4H), 7.35 (m, 1H), 7.13 (t, J = 7.7 Hz, 1H), 6.71 – 6.58 (m, 2H), 5.06 (s, 2H), 4.12 – 3.90 (m, 4H), 3.83 (d, J = 8.8 Hz, 1H), 3.71 (t, J = 8.2 Hz, 1H), 2.97 (dd, J = 15.5, 9.1 Hz, 3H), 2.81 (d, J = 11.1 Hz, 1H), 2.35 (dd, J = 12.6, 7.3 Hz, 1H), 2.09 (m, 3H), 1.82 (d, J = 12.2 Hz, 2H), 1.69 (d, J = 12.9 Hz, 2H), 1.43 (s, 9H).
37		LCMS Method 1 Rt: 0.90 min MS m/z calculated for $C_{29}H_{37}F_2N_2O_4$: 515.3 $[M+H]^+$ Found: 515.4	^1H NMR (400 MHz, CD_3OD) δ 7.46 – 7.29 (m, 5H), 7.07 (dd, J = 11.9, 9.1 Hz, 1H), 6.96 (dd, J = 12.6, 6.9 Hz, 1H), 5.07 (s, 2H), 4.04 – 3.92 (m, 3H), 3.92 – 3.82 (m, 2H), 3.70 (dd, J = 8.7, 7.2 Hz, 1H), 3.12 – 2.91 (m, 3H), 2.85 (dd, J = 12.4, 3.4 Hz, 1H), 2.41 (dd, J = 12.9, 7.4 Hz, 1H), 2.15 (m, 2H), 2.02 (dd, J = 12.8, 8.3 Hz, 1H), 1.87 – 1.72 (m, 2H), 1.65 (m, 2H), 1.43 (s, 9H).
38		LCMS Method 2 Rt: 1.35 min MS m/z calculated for $C_{29}H_{37}F_2N_2O_4$: 515.3 $[M+H]^+$ Found: 515.3	^1H NMR (400 MHz, CD_3OD) δ 7.42 – 7.30 (m, 5H), 6.87 (ddd, J = 11.6, 8.4, 3.0 Hz, 1H), 6.74 (dt, J = 9.7, 2.4 Hz, 1H), 5.02 (s, 2H), 4.06 –

			3.93 (m, 3H), 3.93 – 3.82 (m, 2H), 3.68 (dd, J = 8.7, 7.1 Hz, 1H), 3.05 – 2.94 (m, 2H), 2.90 – 2.69 (m, 2H), 2.40 (dd, J = 12.8, 7.4 Hz, 1H), 2.08 – 1.93 (m, 3H), 1.62 – 1.46 (m, 4H), 1.44 (s, 9H).

Synthesis of (*R*)-tert-butyl 6-(4-(2-hydroxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octane-2-carboxylate (SI-26)

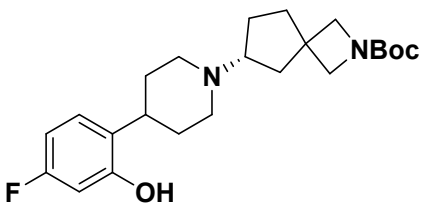


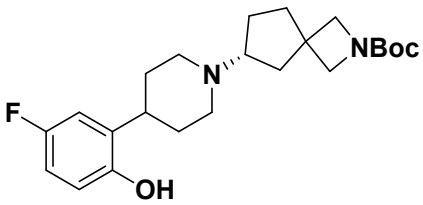
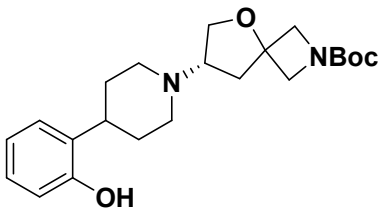
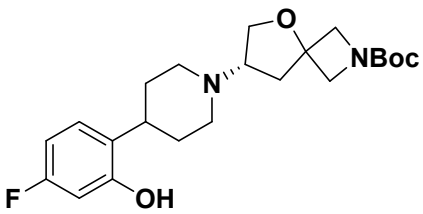
tert-butyl (*R*)-6-(4-(2-(benzyloxy)phenyl)piperidin-1-yl)-2-azaspiro[3.4]octane-2-carboxylate (8.28 g, 17.37 mmol) was dissolved in MeOH (100 mL) and Pd-C (10% Pd, 830 mg) was added. The flask was purged with H_2 three times and then the reaction was stirred under a balloon of hydrogen for 16 hours. The reaction was then filtered and concentrated to give the title intermediate as a white solid that was used without further purification (5.73 g, 14.84 mmol).

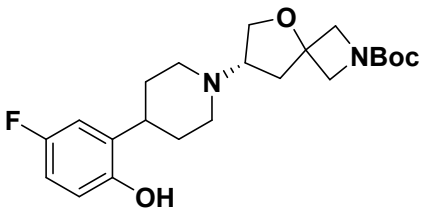
LCMS Method 2: Rt: 1.08 min, MS m/z calculated for $C_{23}H_{35}N_2O_3$: 387.3 $[M+H]^+$, found: 387.4

^{1}H NMR (400 MHz, CD_3OD) δ 7.13 - 7.06 (m, 1H), 7.03 - 6.93 (m, 1H), 6.83 - 6.69 (m, 2H), 3.93 - 3.67-(m, 4H), 3.24 - 3.12 (m, 2H), 3.03 - 2.90 (m, 1H), 2.85 - 2.63 (m, 1H), 2.32 - 2.09 (m, 3H), 2.01 – 1.91 (m, 2H), 1.89 - 1.72 (m, 6H), 1.66 – 1.51 (m, 1H), 1.44 (s, 9H).

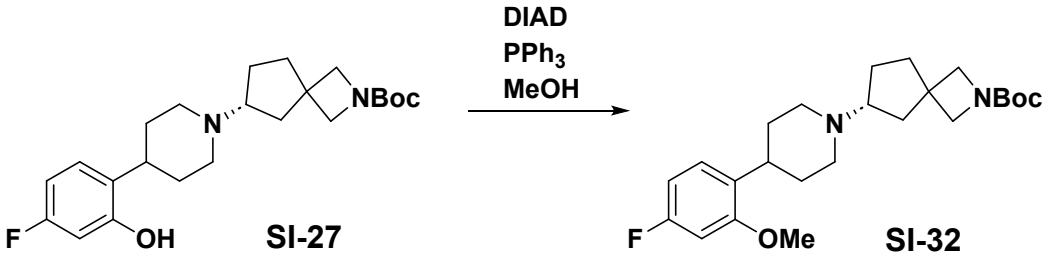
The following compounds were synthesized by analogy

Compound	Structure	LCMS	^{1}H
SI-27		LCMS Method 4 Rt: 2.57 min MS m/z calculated for $C_{23}H_{34}FN_2O_3$: 405.3 $[M+H]^+$ Found: 405.8	^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (dd, J = 8.4, 6.7 Hz, 1H), 6.55 – 6.48 (m, 2H), 3.89 – 3.67 (m, 4H), 3.11 (t, J = 10.8 Hz, 2H), 2.88 – 2.81 (m, 1H), 2.62 (s, 1H), 2.19 – 2.00 (m, 3H), 1.99 – 1.69

			(m, 8H), 1.64 - 1.57 (m, 1H), 1.44 (s, 9H).
SI-28		LCMS Method 1 Rt: 0.78 min MS m/z calculated for $C_{23}H_{34}FN_2O_3$: 405.3 $[M+H]^+$ Found: 405.4	^1H NMR (400 MHz, $CDCl_3$) δ 6.83 (dd, J = 9.9, 2.6 Hz, 1H), 6.77 – 6.67 (m, 2H), 3.89 – 3.68 (m, 4H), 3.14 (t, J = 10.9 Hz, 2H), 2.95 (tt, J = 11.9, 3.8 Hz, 1H), 2.66 – 2.60 (m, 1H), 2.22 – 2.03 (m, 3H), 2.07 – 1.56 (m, 10H), 1.46 (s, 9H).
SI-29		LCMS Method 1 Rt: 0.68 min MS m/z calculated for $C_{22}H_{33}N_2O_4$: 389.2 $[M+H]^+$ Found: 389.4	^1H NMR (400 MHz, $CDCl_3$) δ 7.15 (dd, J = 7.4, 1.8 Hz, 1H), 7.05 (t, J = 7.8, 1H), 6.87 (t, J = 7.5 Hz, 1H), 6.74 (dd, J = 7.8, 1.3 Hz, 1H), 4.12 – 3.92 (m, 4H), 3.84 (d, J = 9.0 Hz, 1H), 3.82 – 3.72 (m, 1H), 3.05 (d, J = 12.4 Hz, 2H), 2.94 - 2.83 (m, 2H), 2.35 (dd, J = 12.9, 7.3 Hz, 1H), 2.21 – 2.06 (m, 3H), 1.93 – 1.72 (m, 4H), 1.43 (s, 9H).
SI-30		LCMS Method 3 Rt: 1.23 min MS m/z calculated for $C_{22}H_{32}FN_2O_4$: 407.2 $[M+H]^+$ Found: 407.4	^1H NMR (400 MHz, $CDCl_3$) δ 7.11 (dd, J = 8.6, 6.6 Hz, 1H), 6.62 (td, J = 8.5, 2.5 Hz, 1H), 6.52 (dd, J = 9.8, 2.5 Hz, 1H), 4.16 – 3.94 (m, 4H), 3.87 (d, J = 8.9 Hz, 1H), 3.76 (t, J = 8.1 Hz, 1H), 3.05 - 2.99 (m, 2H), 2.87 – 2.79 (m, 2H), 2.40 – 2.34 (t, m, 1H), 2.20 – 2.05 (m, 3H),

			1.85 - 1.75 (m, 4H), 1.47 (s, 9H).
SI-31		LCMS Method 4 Rt: 2.24 min MS m/z calculated for $C_{22}H_{32}FN_2O_4$: 407.2 $[M+H]^+$ Found: 407.2	^{1}H NMR (400 MHz, CD_3OD) δ 6.90 - 6.80 (m, 1H), 6.79 - 6.66 (m, 2H), 4.15 - 3.81 (m, 5H), 3.74 (dd, J = 8.7, 7.4 Hz, 1H), 3.16 - 2.81 (m, 4H), 2.45 (dd, J = 12.9, 7.5 Hz, 1H), 2.23 (qd, J = 11.3, 2.7 Hz, 2H), 2.07 (dd, J = 12.9, 8.5 Hz, 1H), 1.93 - 1.61 (m, 4H), 1.46 (s, 9H).

Synthesis of (*R*)-tert-butyl 6-(4-(4-fluoro-2-methoxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octane-2-carboxylate SI-32

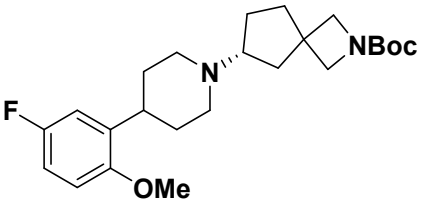
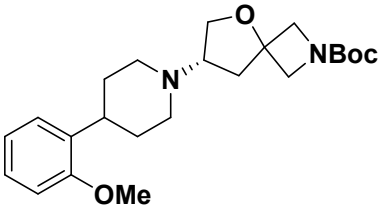
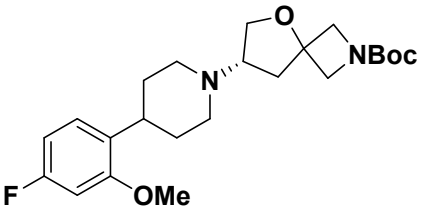
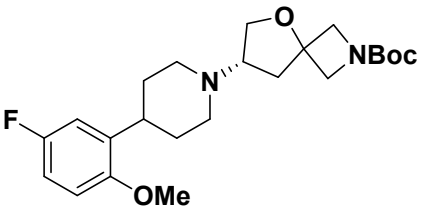


(*R*)-tert-butyl 6-(4-(4-fluoro-2-hydroxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octane-2-carboxylate (300 mg, 0.742 mmol) was dissolved in THF (7 mL) and methanol (0.6 mL, 14.83 mmol) was added. Resin bound triphenyl phosphine (3 mmol/g, 1236 mg, 3.71 mmol) and (E)-di-tert-butyl diazene-1,2-dicarboxylate (854 mg, 3.71 mmol) were then added. The reaction was shaken for 16 hours and then filtered and rinsed with EtOAc. The filtrate was concentrated and purified by FCC (0-10% MeOH (1% NH_4OH)/DCM) to yield the title intermediate (268 mg, 0.640 mmol).

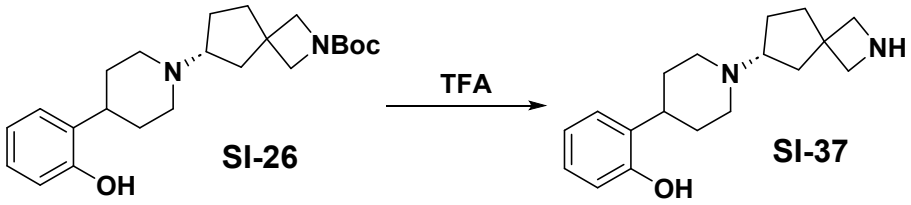
LCMS Method 2: Rt: 1.31 min, MS m/z calculated for $C_{24}H_{36}FN_2O_3$: 419.3 $[M+H]^+$, found: 419.4

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (s, 1H), 6.65 – 6.57 (m, 2H), 3.87 – 3.80 (m, 6H), 3.81 – 3.71 (m, 3H), 3.13 – 3.07 (m, 2H), 2.93 – 2.86 (m, 1H), 2.59 (br s, 1H), 2.17 – 2.13 (m, 1H), 2.09 – 2.02 (m, 2H), 1.93 – 1.70 (m, 8H), 1.58 (s, 3H), 1.46 (s, 9H).

The following compounds were synthesized by analogy

Compound	Structure	LCMS	^{1}H
SI-33		LCMS Method 1 Rt: 0.82 min MS m/z calculated for $C_{24}H_{36}FN_2O_3$: 419.3 $[M+H]^+$ Found: 419.6	Not tested
SI-34		LCMS Method 1 Rt: 0.77 min MS m/z calculated for $C_{23}H_{35}N_2O_4$: 403.3 $[M+H]^+$ Found: 403.4	^{1}H NMR (400 MHz, CD_3OD) δ 7.26 – 7.04 (m, 2H), 6.99 – 6.79 (m, 2H), 4.09 – 3.93 (m, 3H), 3.95 – 3.83 (m, 2H), 3.81 (s, 3H), 3.72 (dd, J = 8.6, 7.4 Hz, 1H), 3.12 – 2.92 (m, 3H), 2.92 – 2.81 (m, 1H), 2.43 (dd, J = 12.9, 7.5 Hz, 1H), 2.28 – 2.13 (m, 2H), 2.05 (dd, J = 12.9, 8.5 Hz, 1H), 1.88 – 1.64 (m, 4H), 1.44 (s, 9H).
SI-35		LCMS Method 2 Rt: 1.17 min MS m/z calculated for $C_{23}H_{34}FN_2O_4$: 421.4 $[M+H]^+$ Found: 421.6	Not tested
SI-36		LCMS Method 2 Rt: 1.18 min MS m/z calculated for $C_{23}H_{34}FN_2O_4$: 421.4 $[M+H]^+$ Found: 421.4	Not tested

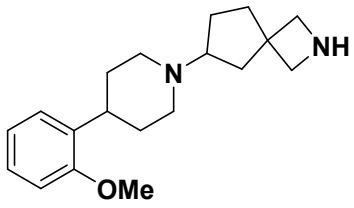
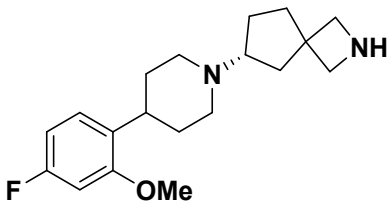
Synthesis of (*R*)-tert-butyl 6-(4-(2-(2-methoxyethoxy)phenyl)piperidin-1-yl)-2-azaspiro[3.4]octane-2-carboxylate (SI-37)

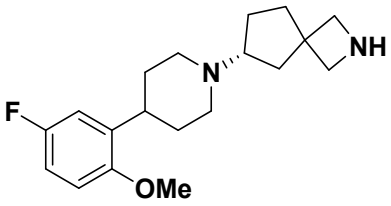
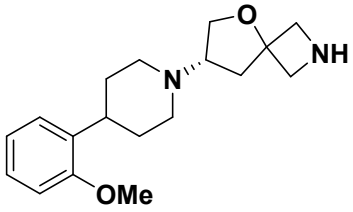
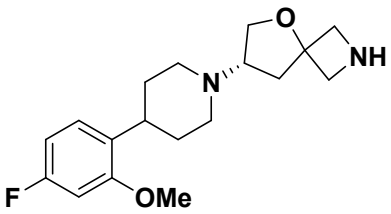
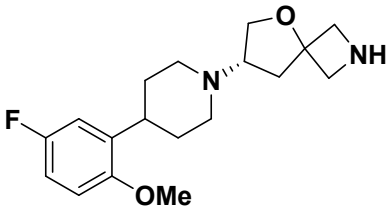


tert-butyl (*R*)-6-(4-(2-hydroxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octane-2-carboxylate (1.40 g, 3.62 mmol) was dissolved in DCM (6 mL) and TFA (2.5 mL, 32.4 mmol) was added. The reaction is stirred at room temperature for 4 hours and then the reaction was concentrated *in vacuo* and used without further purification assuming 100% yield.

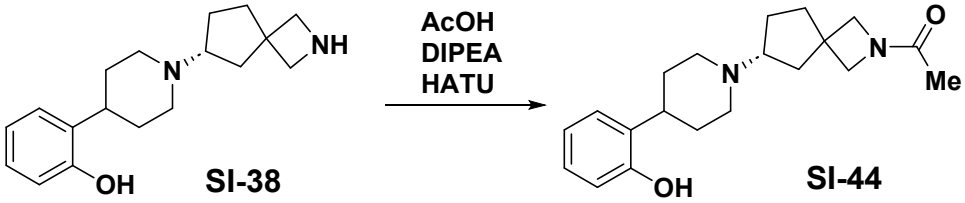
LCMS Method 1: Rt: 0.34 min, MS m/z calculated for $C_{18}H_{37}N_2O$: 287.2 $[M+H]^+$, found: 287.2

The following compounds were synthesized by analogy

Compound	Structure	LCMS	^{1}H
SI-38		LCMS Method 1 Rt: 0.43 min MS m/z calculated for $C_{19}H_{29}N_2O$: 301.2 $[M+H]^+$ Found: 301.3	^{1}H NMR (400 MHz, CD_3OD) δ 7.22 – 7.06 (m, 2H), 6.95 – 6.82 (m, 2H), 3.81 (s, 3H), 3.73 – 3.66 (m, 1H), 3.66 – 3.59 (m, 2H), 3.56 (d, J = 8.7 Hz, 1H), 3.19 – 3.08 (m, 2H), 3.03 – 2.90 (m, 1H), 2.63 (tt, J = 9.1, 7.2 Hz, 1H), 2.25 (dd, J = 12.8, 7.3 Hz, 1H), 2.20 – 2.04 (m, 2H), 2.04 – 1.66 (m, 8H), 1.59 – 1.46 (m, 1H).
SI-39		LCMS Method 1 Rt: 0.44 min MS m/z calculated for $C_{19}H_{28}FN_2O$: 319.2 $[M+H]^+$ Found: 319.5	Not tested

SI-40		LCMS Method 1 Rt: 0.43 min MS m/z calculated for $C_{19}H_{28}FN_2O$: 319.2 $[M+H]^+$ Found: 319.4	Not tested
SI-41		LCMS Method 1 Rt: 0.44 min MS m/z calculated for $C_{18}H_{27}N_2O_2$: 303.2 $[M+H]^+$ Found: 303.0	Not tested
SI-42		LCMS Method 1 Rt: 0.48 min MS m/z calculated for $C_{18}H_{27}FN_2O_2$: 321.2 $[M+H]^+$ Found: 321.3	Not tested
SI-43		LCMS Method 2 Rt: 0.93 min MS m/z calculated for $C_{18}H_{27}FN_2O_2$: 321.2 $[M+H]^+$ Found: 321.1	Not tested

Synthesis of (*R*)-1-(6-(4-(2-hydroxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octan-2-yl)ethan-1-one (SI-44)



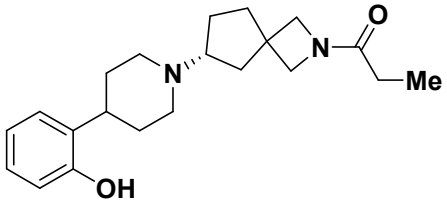
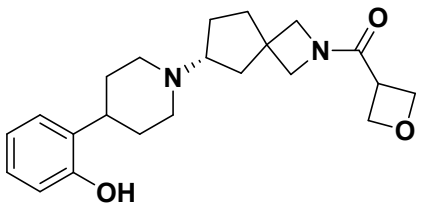
Acetic acid (83 mg, 1.382 mmol) was added to DMF (3 mL) followed by added DIPEA (1.14 mL, 6.55 mmol) and HATU (1073 mg, 2.82 mmol). The solution was stirred for 10 min and then (*R*)-2-(1-(2-azaspiro[3.4]octan-6-yl)piperidin-4-yl)phenol (675 mg, 0.909 mmol) in DMF (3 mL) was added. The resulting solution was stirred for 16 hours. Water (50 mL) was then added and the aqueous layer was

extracted with EtOAc (3x50 mL). Both layers contained product, so they were combined and concentrated and the residue was purified by FCC (0-30% MeOH (1% NH_4OH)) to provide the title intermediate (117 mg, 0.331 mmol) and a double acetylated byproduct (260 mg. 0.702 mmol). The byproduct was dissolved in MeOH (10 mL) and water (1 mL) and potassium carbonate (116 mg, 0.842 mmol) was added. The reaction was stirred for 2 hours and then the solvent was removed and the material was purified by FCC (0-10% MeOH (1% NH_4OH)/DCM) to provide additional desired product (130 mg, 0.396 mmol) which was combined with the initially isolated material.

LCMS Method 2: Rt: 0.74 min, MS m/z calculated for $C_{20}H_{29}N_2O_2$: 329.2 $[M+H]^+$, found: 329.9.

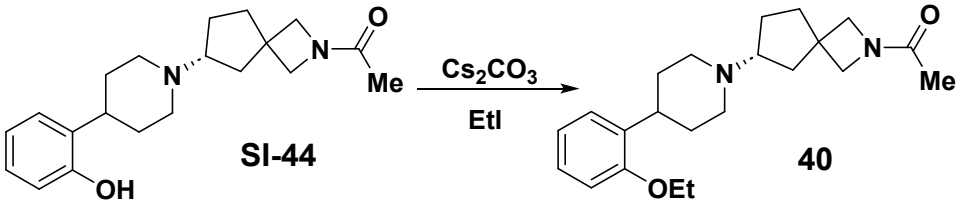
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (s, 1H), 7.06 (dd, J = 7.7, 1.7 Hz, 1H), 6.97 (td, J = 7.5, 1.6 Hz, 1H), 6.84 - 6.65 (m, 2H), 4.13 - 3.83 (m, 3H), 3.75 - 3.56 (m, 2H), 3.01 (s, 2H), 2.81 (s, 1H), 2.16 -2.00 (m, 2H), 1.91 - 1.76 (m, 4H), 1.72 (d, J = 2.8 Hz, 8H), 1.48 (s, 1H).

The following compounds were synthesized by analogy

Compound	Structure	LCMS	^{1}H
SI-45		LCMS Method 2 Rt: 0.83 min MS m/z calculated for $C_{21}H_{30}N_2O_2$: 343.2 $[M+H]^+$ Found: 343.2	^{1}H NMR (400 MHz, CD_3OD) δ 7.00 (dd, J = 7.6, 1.6 Hz, 1H), 6.92 (td, J = 7.7, 1.7 Hz, 1H), 6.68 (ddd, J = 14.7, 7.8, 1.3 Hz, 2H), 4.14 - 3.91 (m, 2H), 3.87 - 3.58 (m, 2H), 3.44 - 3.31 (m, 2H), 3.15 (q, J =8.6 Hz, 1H), 3.00 (tt, J = 10.2, 5.0 Hz, 1H), 2.61 (d, J = 13.7 Hz, 2H), 2.27 (dt, J = 13.8, 7.2 Hz, 1H), 2.12 - 1.79 (m, 10H), 1.67 (dd, J = 12.6, 8.6 Hz, 1H), 0.99 (t, J = 7.5 Hz, 3H).
SI-46		LCMS Method 2 Rt: 0.77 min MS m/z calculated for $C_{22}H_{31}N_2O_3$: 371.2 $[M+H]^+$ Found: 371.4	^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03-7.19 (m, 2H), 6.88 (br d, J=7.82 Hz, 2H), 4.85-4.94 (m, 2H), 4.71-4.79 (m, 2H), 4.01-4.18 (m, 1H), 3.54-3.99 (m, 6H), 3.18-3.41 (m, 1H), 2.98-3.17 (m,

			1H), 2.66-2.81 (m, 1H), 1.93-2.43 (m, 9H), 1.80-1.91 (m, 1H), 1.54-1.71 (m, 1H), 1.39-1.50 (m, 1H).

Synthesis of (*R*)-1-(6-(4-(2-ethoxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octan-2-yl)ethanone (40)

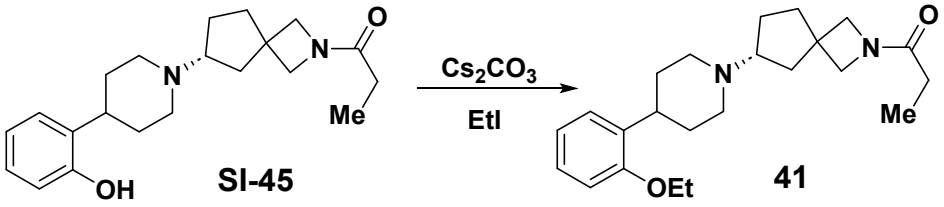


(R)-1-(6-(4-(2-hydroxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octan-2-yl)ethan-1-one (45 mg, 0.137 mmol) was dissolved in MeCN (10 mL) and cesium carbonate (134 mg, 0.411 mmol) and iodoethane (64 mg, 0.411 mmol) were added. The reaction was warmed to 82 °C and stirred for 4 hours. The reaction was then cooled to RT and concentrated. The residue was dissolved in DCM (50 mL) and washed with water (2x10 mL) and brine (1x10 mL) dried over magnesium sulfate, filtered and concentrated. The crude was then purified by HPLC (65-95% MeCN/H_2O (5 mM NH_4OH), X-bridge C_{18} 30x50 mm 5 µM, 75 mL/min) to provide the title compound (35 mg, 0.098 mmol).

LCMS Method 4: Rt: 2.28 min, MS m/z calculated for $C_{22}H_{33}N_2O_2$: 357.2 $[M+H]^+$, found: 357.4.

^{1}H NMR (400 MHz, CD_3OD) δ 7.14 (ddd, J = 18.4, 7.7, 1.7 Hz, 2H), 6.94 - 6.66 (m, 2H), 4.23 - 3.98 (m, 4H), 3.93 - 3.67 (m, 2H), 3.23 - 3.10 (m, 2H), 3.00 (tt, J = 11.8, 3.9 Hz, 1H), 2.79 - 2.56 (m, 1H), 2.27 - 2.07 (m, 3H), 2.06 - 1.67 (m, 11H), 1.59 (m, 1H), 1.40 (t, J = 6.9 Hz, 3H).

Synthesis of (*R*)-1-(6-(4-(2-ethoxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octan-2-yl)propan-1-one (41)



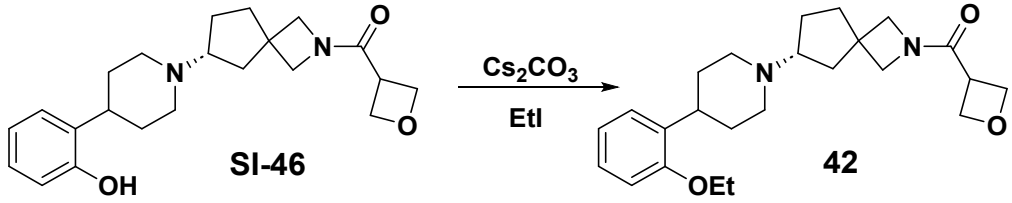
(*R*)-1-(6-(4-(2-hydroxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octan-2-yl)propan-1-one (120 mg, 0.175 mmol) was dissolved in MeCN (10 mL) and cesium carbonate (171 mg, 0.526 mmol) and iodoethane (82 mg, 0.526 mmol) were added. The reaction was warmed to 82 °C and stirred for 4 hours. The reaction was cooled to RT and concentrated. The residue was dissolved in DCM (50 mL) and washed with water (2x10 mL) and brine, (1x10 mL) dried over magnesium sulfate, filtered and concentrated. The crude was then

purified by HPLC (35-60% MeCN/H_2O (5 mM NH_4OH) X-bridge C_{18} 30x50 mm 5 µM 75 mL/min) to provide the title compound (15 mg, 0.040 mmol).

LCMS Method 4: Rt: 2.50 min, MS m/z calculated for $C_{23}H_{35}N_2O_2$: 371.3 $[M+H]^+$, found: 371.4.

^{1}H NMR (400 MHz, CD_3OD) δ 7.24 - 7.03 (m, 2H), 6.99 - 6.70 (m, 2H), 4.19 - 3.97 (m, 4H), 3.94 - 3.70 (m, 2H), 3.24 - 3.12 (m, 2H), 3.01 (m, 1H), 2.75 (q, J = 8.7, 8.1 Hz, 1H), 2.18 (m, 5H), 2.08 - 1.71 (m, 8H), 1.61 (m, 1H), 1.40 (t, J = 6.9 Hz, 3H), 1.08 (t, J = 7.6 Hz, 3H).

Synthesis of (*R*)-(6-(4-(2-ethoxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octan-2-yl)(oxetan-3-yl)methanone (42)

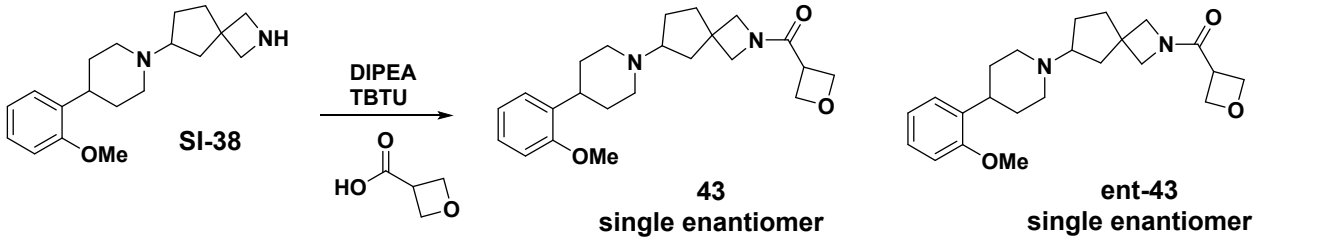


(*R*)-(6-(4-(2-hydroxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octan-2-yl)(oxetan-3-yl)methanone (16.7 mg, 0.045 mmol), iodoethane (5 µL, 0.068 mmol) and cesium carbonate (44 mg, 0.135 mmol) were dissolved in DMF (0.75 mL) and the reaction was stirred for 16 hours. The reaction was then diluted with MeOH (3 mL) and directly purified by HPLC (35-60% MeCN/H_2O (5 mM NH_4OH), X-bridge C_{18} 30x50 mm, 5 µM, 75 mL/min) to yield the title compound (11.6 mg, 0.029 mmol).

LCMS Method 4: Rt: 0.66 min, MS m/z calculated for $C_{24}H_{35}N_2O_3$: 399.3 $[M+H]^+$, found: 399.4.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.14 (m, 2H), 6.93 (t, *J* = 7.4 Hz, 1H), 6.86 (d, *J* = 8.0 Hz, 1H), 4.95-4.91 (m, 2H), 4.77-4.73 (m, 2H), 4.06 (q, *J* = 6.9 Hz, 2H), 4.01 – 3.76 (m, 5H), 3.11 (t, *J* = 13.1 Hz, 2H), 3.04-2.96 (m, 1H), 2.63 (p, *J* = 7.7 Hz, 1H), 2.20-2.04 (m, 3H), 2.03 – 1.56 (m, 9H), 1.44 (t, *J* = 6.9 Hz, 3H).

Synthesis of (*R*)-(6-(4-(2-ethoxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octan-2-yl)(oxetan-3-yl)methanone and (*S*)-(6-(4-(2-ethoxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octan-2-yl)(oxetan-3-yl)methanone (43)



Oxetane-3-carboxylic acid (183 mg, 1.79 mmol), DIPEA (632 mg, 4.89 mmol) and TBTU (786 mg, 2.45 mmol) were dissolved in DCM (6.5 mL) and DMF (2 mL) and stirred until the solution became clear. Next, 6-(4-(2-methoxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octane (490 mg, 1.63 mmol), was added and the

reaction was stirred for 3 hours and then concentrated and purified by FCC (0-10% MeOH (5% NH_4OH)/DCM) to yield 276 mg of racemic product. The enantiomers were then separated by chiral SFC (OJ-H, 2x25 cm, 10% MeOH (0.1% DEA)/CO2, 75 mL/min) to yield the faster eluting enantiomer Example 41 as a white solid (89 mg, 1.63 mmol) and Example *ent*-41, the slower eluting enantiomer was also isolated as a white solid (88 mg, 1.63 mmol). For ease of handling, compound 41 (89 mg, 0.231 mmol) was dissolved in MeOH (2 mL) and citric acid (44.5 mg, 0.231 mmol) in MeOH (2 mL) was added. The reaction was stirred for 1 hour and then concentrated to yield the product as a citrate salt. A similar procedure was conducted with *ent*-41. The analytical data below is for the free base.

Compound 41

SFC: Rt: 3.40 min (Chiralpak® IB 4.6x100 mm, 5 uM, 5-55% 1:1 MeOH/IPA (10 mM NH4OH)/CO2 5 mL/min)

LCMS Method 4: Rt: 2.04 min MS m/z calculated for $C_{23}H_{33}N_2O_3$: 385.2 $[M+H]^+$, found 385.2

1H NMR (400 MHz, CD3OD) δ 7.14 - 7.21 (m, 2H) 6.87 - 6.96 (m, 2H) 4.76 - 4.83 (m, 4H) 3.83 - 4.07 (m, 5H) 3.83 (s, 3H) 3.12 - 3.21 (m, 2H) 2.95 - 3.05 (m, 1H) 2.67 - 2.78 (m, 1H) 2.10 - 2.28 (m, 3H) 1.87 - 2.06 (m, 3H) 1.73 - 1.87 (m, 5H) 1.53 - 1.67 (m, 1H).

Compound *ent*-41

SFC: Rt: 3.55 min (Chiralpak® IB 4.6x100 mm, 5 uM, 5-55% 1:1 MeOH/IPA (10 mM NH4OH)/CO2 5 mL/min)

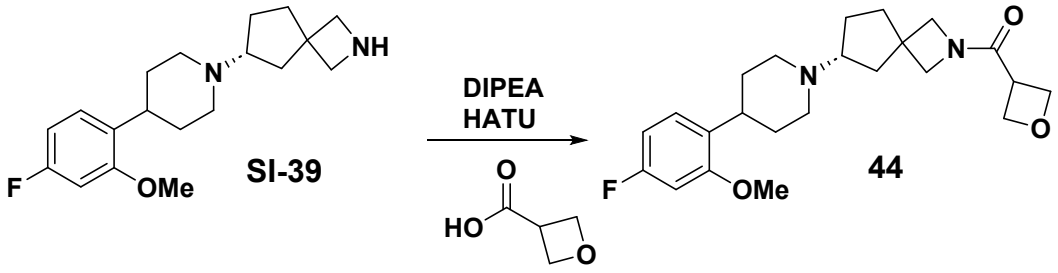
LCMS Method 4: Rt: 2.06 min MS m/z calculated for $C_{23}H_{33}N_2O_3$: 385.2 $[M+H]^+$, found 385.4

1H NMR (400 MHz, CD3OD) δ 7.14 - 7.21 (m, 2H) 6.87 - 6.96 (m, 2H) 4.77 - 4.83 (m, 4H) 3.83 - 4.07 (m, 5H) 3.83 (s, 3H) 3.11 - 3.21 (m, 2H) 2.94 - 3.05 (m, 1H) 2.67 - 2.77 (m, 1H) 2.10 - 2.26 (m, 3H) 1.86 - 2.07 (m, 3H) 1.71 - 1.86 (m, 5H) 1.54 - 1.67 (m, 1H).

Biological data for 43 and *ent*-43

Compound	M4 EC_{50} (nM)	% activity
43	6.1	65
ent-43	350	43

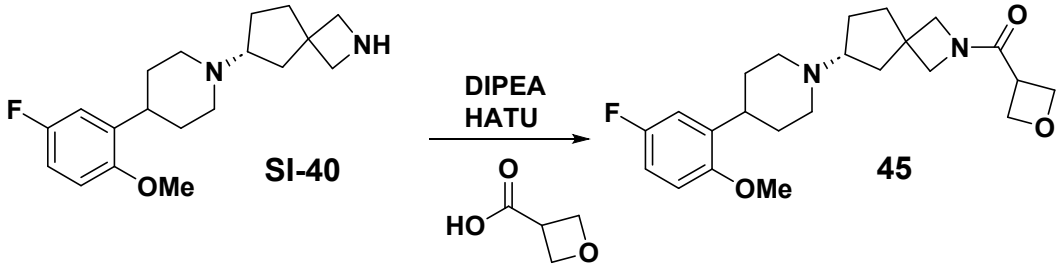
Synthesis of (*R*)-(6-(4-(4-fluoro-2-methoxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octan-2-yl)(oxetan-3-yl)methanone (44)



Oxetane-3-carboxylic acid (115 mg, 0.961 mmol) was dissolved in DMF (2 mL) and DCM (2.5 mL) and TBTU (308 mg, 0.961 mmol) was added. The reaction was stirred for 15 minutes and then this solution was

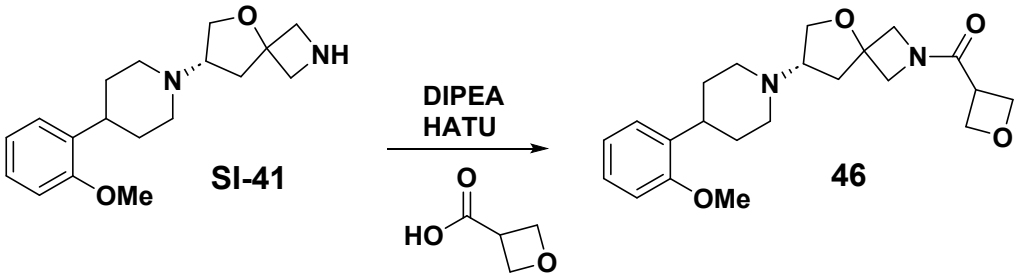
added to a solution of (*R*)-6-(4-(4-fluoro-2-methoxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octane (277 mg, 0.641 mmol) and DIPEA (0.56 mL, 3.20 mmol) in DCM (10 mL). The reaction was then stirred for 16 hours and the DCM was concentrated and the residue was diluted with EtOAc (50 mL) and washed with saturated sodium bicarbonate (1x10 mL), water (2x10 mL) and brine (1x10 mL) dried over sodium sulfate filtered and concentrated. The residue was dissolved in MeOH and purified by HPLC (35-60% MeCN/H_2O (5 mM NH_4OH), X-bridge C_{18} 30x50 mm, 5 µM, 75 mL/min) to yield the title compound (23 mg, 0.54 mmol).
LCMS Method 4: Rt: 2.12 min MS m/z calculated for $C_{23}H_{32}FN_2O_3$: 403.2 $[M+H]^+$, found 403.6.
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (dd, J = 8.5, 6.7 Hz, 1H), 6.60 - 6.43 (m, 2H), 4.82 (ddd, J = 6.8, 5.6, 3.0 Hz, 2H), 4.72 - 4.58 (m, 2H), 3.93 - 3.64 (m, 8H), 3.00 (s, 2H), 2.80 (m, 1H), 2.52 (s, 1H), 2.15 - 1.47 (m, 12H).

Synthesis of (*R*)-(6-(4-(5-fluoro-2-methoxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octan-2-yl)(oxetan-3-yl)methanone (45)



Oxetane-3-carboxylic acid (105 mg, 0.871 mmol) was dissolved in DMF (5 mL) and TBTU (280 mg, 0.871 mmol) was added. The reaction was stirred for 15 minutes and it was then added to a solution of (*R*)-6-(4-(5-fluoro-2-methoxyphenyl)piperidin-1-yl)-2-azaspiro[3.4]octane (185 mg, 0.581 mmol) and DIPEA (0.51 mL, 2.90 mmol) in DCM (10 mL). The reaction was stirred for 16 hours and the DCM was concentrated and the residue was diluted with EtOAc (50 mL) and the organic layer was washed with saturated sodium carbonate (1x10 mL), water (2x10 mL) and brine (1x10 mL), dried over sodium sulfate, filtered and concentrated. The residue was dissolved in MeOH and purified by HPLC (35-60% MeCN/H_2O (5 mM NH_4OH), X-bridge C_{18} 30x50 mm, 5 µM, 75 mL/min) to yield the title compound (18 mg, 0.042 mmol).
LCMS Method 4: Rt: 2.10 min MS m/z calculated for $C_{23}H_{32}FN_2O_3$: 403.3 $[M+H]^+$, found 403.6.
^{1}H NMR (400 MHz, CD_3OD) δ 7.00 - 6.72 (m, 3H), 4.77 (dd, J = 8.2, 2.0 Hz, 4H), 4.09 - 3.73 (m, 8H), 3.21 - 3.04 (m, 2H), 2.96 (m, 1H), 2.68 (m, 1H), 2.30 - 1.47 (m, 12H).

Synthesis of (*S*)-(7-(4-(2-methoxyphenyl)piperidin-1-yl)-5-oxa-2-azaspiro[3.4]octan-2-yl)(oxetan-3-yl)methanone (46)

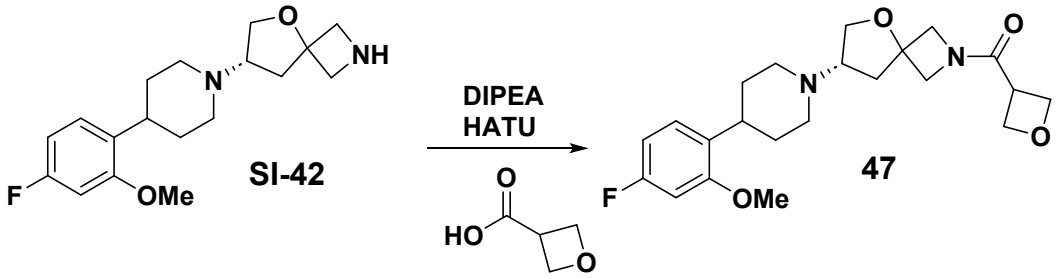


Oxetane-3-carboxylic acid (69 mg, 0.675 mmol) was dissolved in DMF (1 mL) and TBTU was added. The reaction was stirred for 20 minutes and it was then added to a solution of (*S*)-(7-(4-(2-methoxyphenyl)piperidin-1-yl)-5-oxa-2-azaspiro[3.4]octan-2-yl)(oxetan-3-yl)methanone (51 mg, 0.169 mmol) and DIPEA (0.120 mL, 0.675 mmol) in DCM (1 mL). The reaction was stirred for 16 hours and the solution was concentrated and the residue was dissolved in MeCN and purified by HPLC (10-30% MeCN/H_2O (5 mM NH_4OH), X-bridge C_{18} 30x50 mm, 5 µM, 75 mL/min) to yield the title compound (4.7 mg, 0.012 mmol).

LCMS Method 3: Rt:0.83 min MS m/z calculated for $C_{22}H_{31}N_2O_4$: 387.2 $[M+H]^+$, found 387.4.

^1H NMR (400 MHz, $CDCl_3$) δ 7.15-7.13 (m, 1H), 7.07-7.06 (m, 1H), 6.87 (t, *J* = 7.5 Hz, 1H), 6.79 (d, *J* = 8.1 Hz, 1H), 4.82-4.77 (m, 1H), 4.70-4.64 (m, 1H), 4.59 – 4.10 (m, 5H), 4.10 – 3.83 (m, 4H), 3.74 (s, 3H), 3.66-3.55 (m, 2H), 3.42 (d, *J* = 11.5 Hz, 1H), 3.07 (t, *J* = 12.6 Hz, 1H), 2.93 – 2.71 (m, 3H), 2.70 – 2.41 (m, 1H), 2.29-2.18 (d, *J* = 18.7 Hz, 2H), 1.98-1.94 (s, 2H).

Synthesis of (S)-(7-(4-(4-fluoro-2-methoxyphenyl)piperidin-1-yl)-5-oxa-2-azaspiro[3.4]octan-2-yl)(oxetan-3-yl)methanone (47)

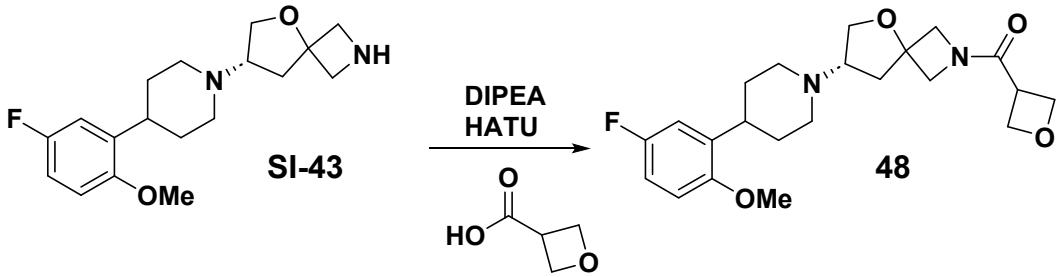


Oxetane-3-carboxylic acid (78 mg, 0.762 mmol) was dissolved in DMF (1 mL) and TBTU (122 mg, 0.381 mmol) was added. The reaction was stirred for 20 minutes and it was then added to a solution of (*S*)-7-(4-(4-fluoro-2-methoxyphenyl)piperidin-1-yl)-5-oxa-2-azaspiro[3.4]octane (61 mg, 0.190 mmol) and DIPEA (0.133 mL, 0.762 mmol) in DCM (1 mL). The reaction was stirred for 16 hours and the solution was then concentrated and the residue was dissolved in MeCN and purified by HPLC (10-30% MeCN/H_2O (5 mM NH_4OH), X-bridge C_{18} 30x50 mm, 5 µM, 75 mL/min) to yield the title compound (17 mg, 0.038 mmol).

LCMS Method 3: Rt:0.91 min MS m/z calculated for $C_{22}H_{30}FN_2O_4$: 405.2 $[M+H]^+$, found 405.4.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (dt, J = 8.3, 6.4 Hz, 1H), 6.58 (m, 2H), 4.86 - 4.68 (m, 4H), 4.37 - 4.25 (m, 1H), 4.17 (m, 3H), 4.04 (dd, J = 10.0, 7.4 Hz, 2H), 3.78 (s, 5H), 3.62 (d, J = 9.9 Hz, 1H), 3.43 (d, J = 9.6 Hz, 1H), 3.10 (tt, J = 12.2, 3.7 Hz, 1H), 2.95 (t, J = 12.3 Hz, 2H), 2.82 - 2.65 (m, 2H), 2.36 - 2.12 (m, 2H), 2.08 - 1.96 (m, 2H).

Synthesis of (*S*)-(7-(4-(5-fluoro-2-methoxyphenyl)piperidin-1-yl)-5-oxa-2-azaspiro[3.4]octan-2-yl)(oxetan-3-yl)methanone (48)



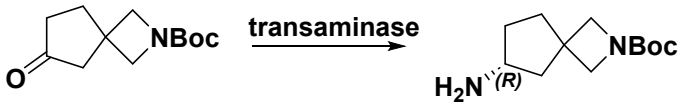
Oxetane-3-carboxylic acid (70 mg, 0.687 mmol) and TBTU (110 mg, 0.343 mmol) were dissolved in DMF (1 mL) and stirred for 20 minutes. This was then added to a solution of (*S*)-7-(4-(5-fluoro-2-methoxyphenyl)piperidin-1-yl)-5-oxa-2-azaspiro[3.4]octane (55 mg, 0.172 mmol) and DIPEA (0.120 mL, 0.687 mmol) in DCM (1 mL) and the reaction was stirred for 16 hours. The solution was then concentrated and the residue was dissolved in MeCN and purified by HPLC (10-30% MeCN/H_2O (5 mM NH_4OH), X-bridge C_{18} 30x50 mm, 5 µM, 75 mL/min) to yield the title compound (10 mg, 0.024 mmol).

LCMS Method 3: Rt:0.91 min MS m/z calculated for $C_{22}H_{30}FN_2O_4$: 405.2 $[M+H]^+$, found 405.5.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89-6.80 (m, 2H), 6.70 (dd, *J* = 8.9, 4.4 Hz, 1H), 4.83-4.77 (m, 2H), 4.69-4.66 (m, 2H), 4.39 – 4.25 (m, 2H), 4.17 – 3.95 (m, 4H), 3.73 (s, 3H), 3.64 – 3.46 (m, 2H), 3.36 – 3.32 (d, *J* = 11.6 Hz, 1H), 3.10-3.04 (m, 1H), 2.98 (dd, *J* = 13.8, 6.6 Hz, 1H), 2.76 (bs, 2H), 2.54 – 2.33 (m, 3H), 1.96-1.92 (d, *J* = 14.4 Hz, 3H).

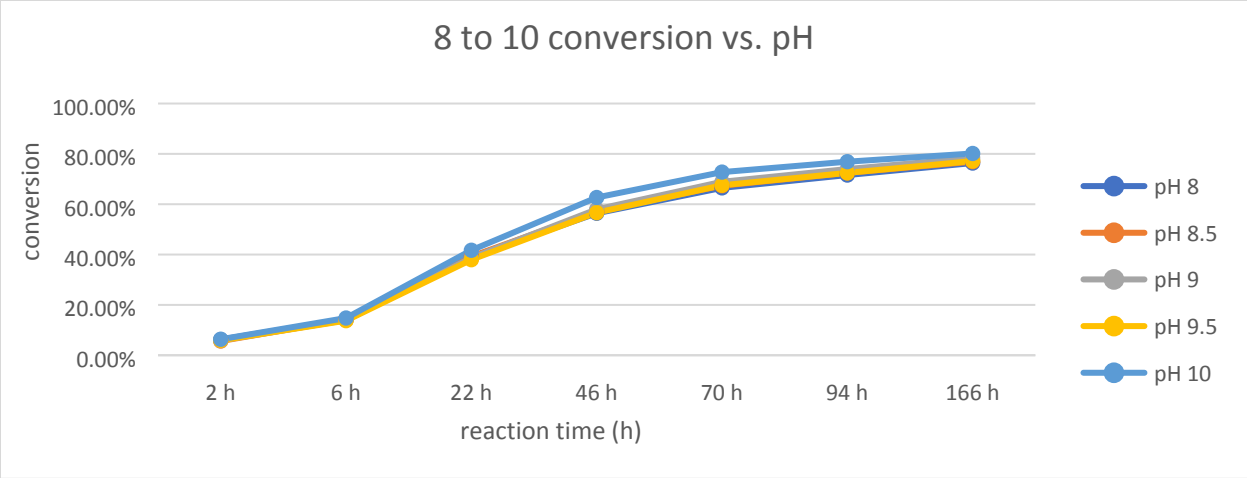
Section 3: Full details of enzymatic screen and reaction optimization

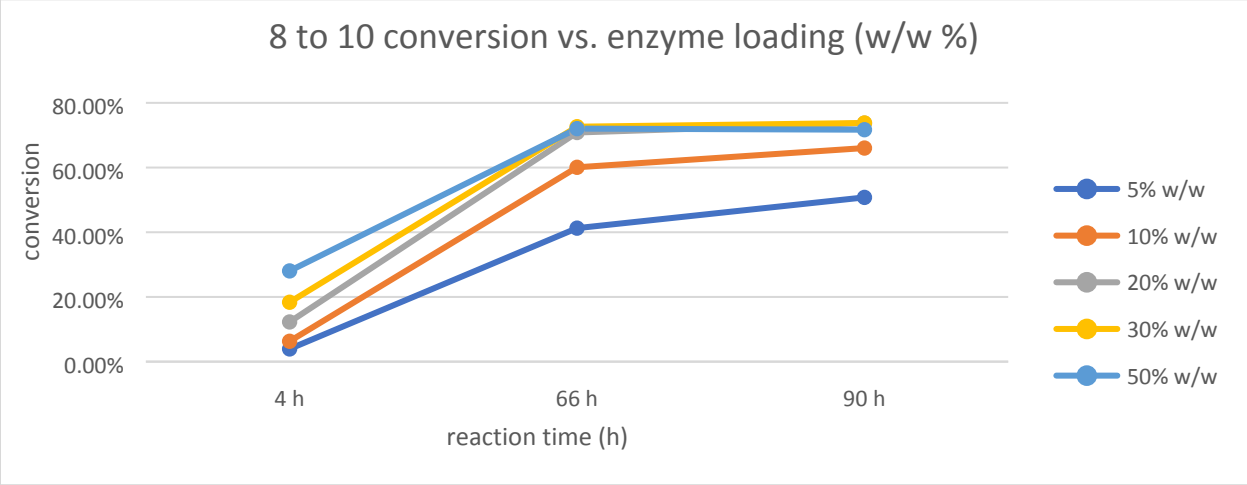
In each vial of 96-well plates were dissolved corresponding transaminases (3 mg) in Tris-HCl buffer 100 mM pH 8.5 (270 uL, 4.5 mM PLP, 1 M isopropyl amine), then ketone (3 mg) dissolved in DMSO (30 µL, 10% v/v) were added. The reaction was shaken at 35 °C and 600 rpm for 24 h. For workup, 100 uL of reaction mixture was taken and diluted with acetonitrile (800 µL), then centrifuged to remove the deactivated enzyme, finally supernatant liquor was taken for analysis.

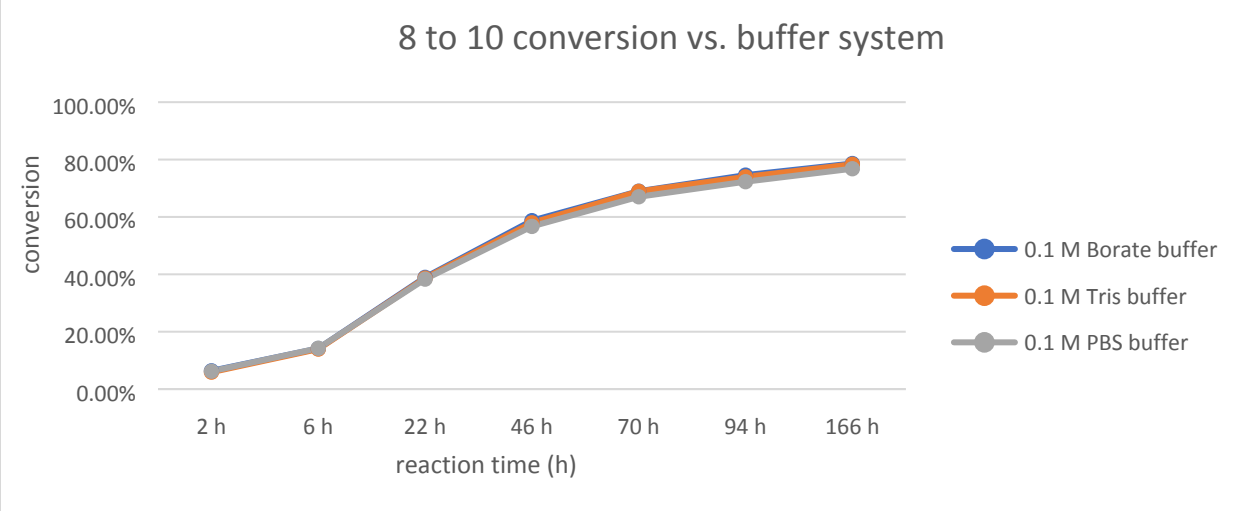


8 **10**

enzyme	enzyme name from vendor	results	
		conv.	ee (*R*) enantiomer
Codexis	ATA 013	75.77%	17.81%
	ATA 025	86.71%	3.89%
	ATA 200	83.04%	-9.15%
	ATA 217	77.54%	-4.17%
	ATA 234	81.67%	5.79%
	ATA 237	79.29%	-86.41%
	ATA 238	81.70%	-22.66%
	ATA 251	87.20%	-6.40%
	ATA 254	86.31%	-71.81%
	ATA 256	82.72%	2.43%
	ATA 260	84.02%	-32.75%
	ATA 412	**77.12%**	**100.00%**
	ATA 415	72.61%	84.35%
	ATA P1 B04	84.12%	-27.48%
	ATA P1 F03	76.68%	-96.61%
	ATA 032	85.67%	23.41%
	ATA036	60.83%	47.00%
C-Lecta	ATA 82	76.99%	1.66%
	ATA 85	72.47%	100.00%
	ATA 86	76.03%	100.00%
J.M.	TA 118	79.84%	-51.14%

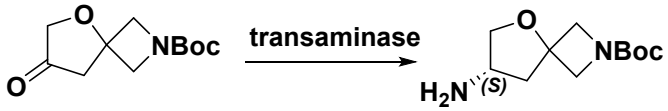






Conversions and enantioselectivity were determined using the following UPLC conditions:

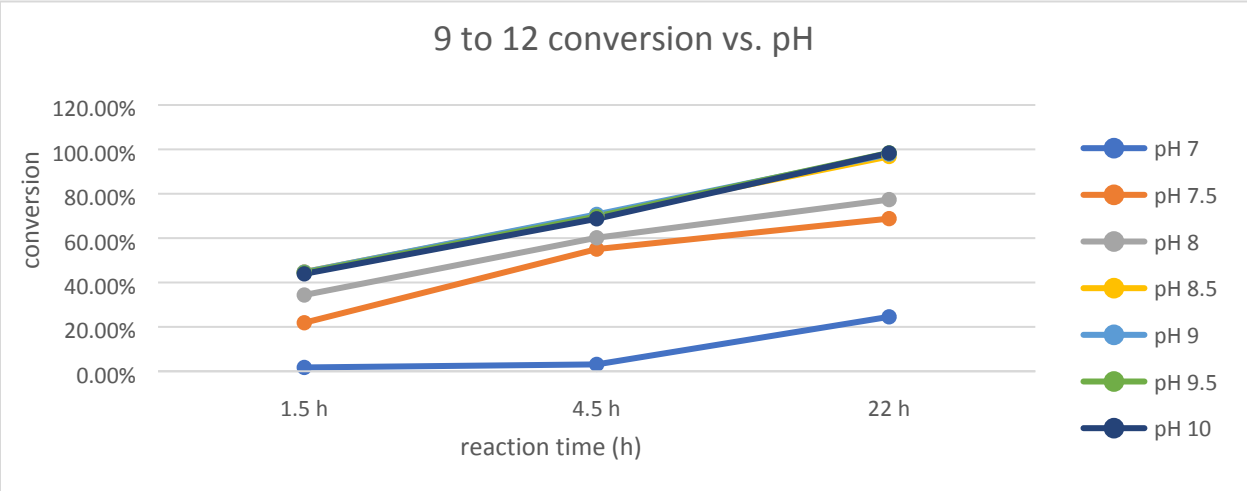
Instrument	Agilent 1200 HPLC		
Column	IE-3, 3.0 um 4.6x150 mm		
Mobile Phase	A: 0.1% DEA in heptane（v/v） B: EtOH		
Solvent	EtOH		
Flow	1.0 mL/min		
Detection	UV 254nm 210nm 230nm		
Injection volume	5 μL		
Column temperature	25 °C		
Gradient	Time (Min)	% B	Comment
	0.0	5	Start of acquisition
	15	60	
	20	60	
	20.1	5	
	25	5	End of acquisition

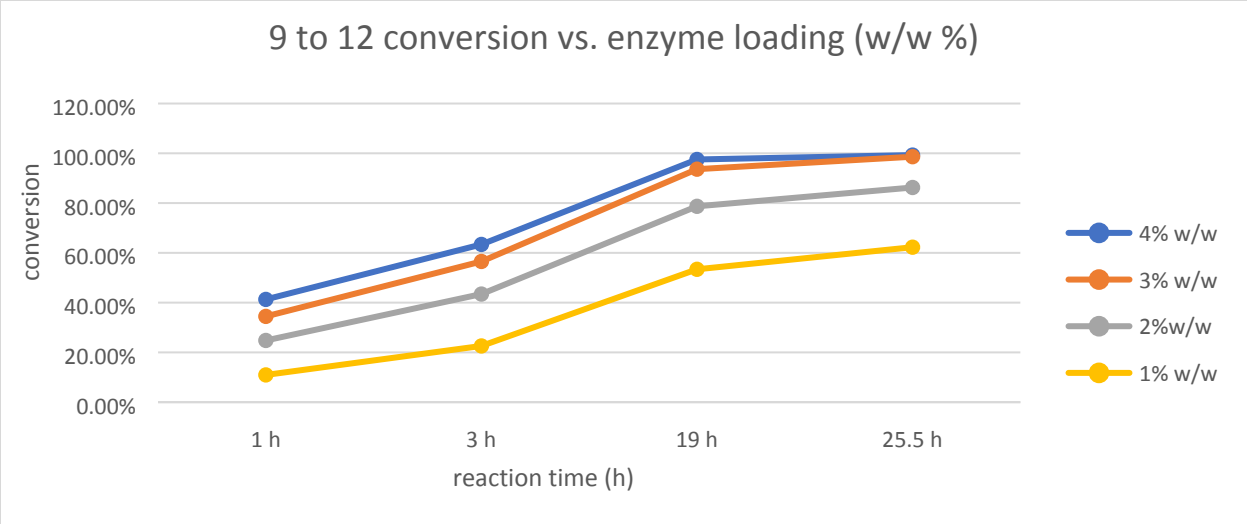


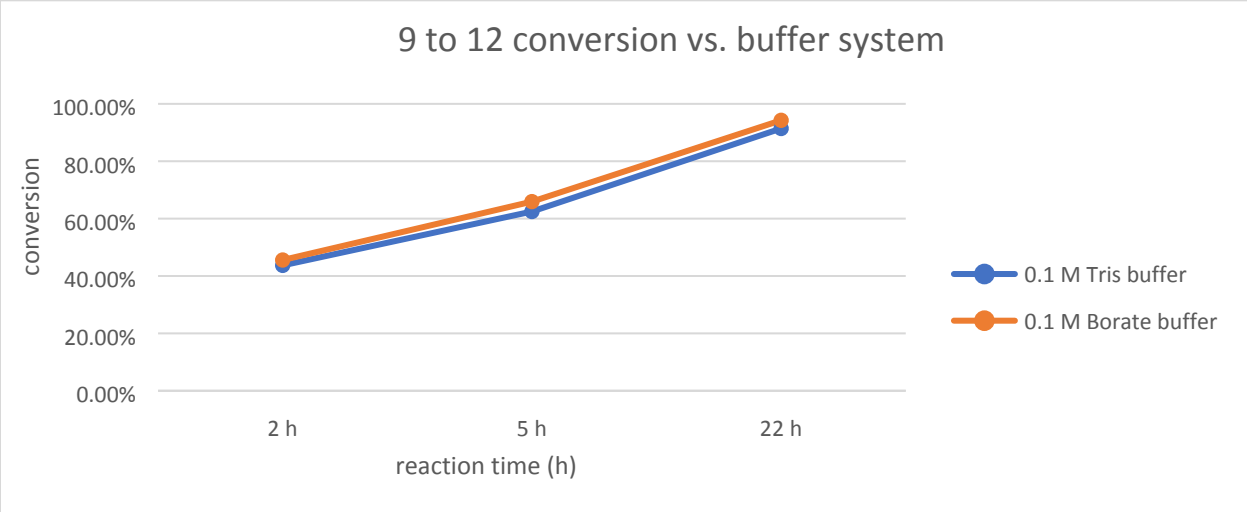
9 **12**

vendor	enzymes	results	
		conv.	ee (*S*) enantiomer
Codexis	ATA 007	37.60%	NA
	ATA 013	100.00%	83.25%
	ATA 025	97.62%	64.22%
	ATA 113	93.21%	-75.32%
	ATA 117	98.73%	95.47%
	ATA 200	98.39%	-72.21%

	ATA 217	99.41%	-52.31%
	ATA 234	97.43%	-7.35%
	ATA 237	96.00%	-67.32%
	ATA 238	97.20%	-48.37%
	ATA 251	95.33%	1.83%
	ATA 254	98.80%	-32.05%
	ATA 256	97.92%	-31.58%
	ATA 260	99.45%	-23.28%
	ATA 301	98.66%	80.95%
	ATA 303	100.00%	85.11%
	ATA 412	98.11%	90.18%
	ATA 415	98.93%	87.54%
	ATA P1 B04	99.12%	-90.17%
	ATA P1 F03	98.67%	-86.27%
	ATA P1 G05	99.49%	-91.32%
	ATA P2 A01	98.58%	96.64%
	ATA P2 A07	96.94%	96.25%
	ATA P2 B01	97.68%	98.17%
	ATA 032	95.22%	77.42%
	ATA036	**98.60%**	**98.00%**







Conversions and reactivity were determined using the following UPLC method:

Instrument	Agilent 1200 HPLC
Column	IE-3, 3.0 um 4.6x150 mm
Mobile Phase	A: 0.1%TFA in heptane（v/v） B: IPA
Solvent	EtOH
Flow	1.0 mL/min
Detection	UV 254nm 210nm 230nm

Injection volume	5 µL		
Column temperature	25 °C		
Gradient	Time (Min)	% B	Comment
	0.0	5	Start of acquisition
	15	60	
	20	60	
	20.1	5	
	25	5	End of acquisition

Section 4: Details of small molecule x-ray crystallography

Small molecule X-ray structures of **(S)-5**, **11**, and **13**. Single-crystal X-ray diffraction studies were carried out on our two Bruker three-circle diffractometers using Cu Kα radiation (λ = 1.5478) from either a microsource or a rotating anode. Crystals of the subject compounds were grown by dissolving approximately 1 mg of the sample in 200 µL of solvent (see table below), which were then evaporated. Data were collected in a nitrogen gas stream at 100 K, integrated using the Bruker SAINT software and scaled using SADABS. Structure solution and refinement was carried out with standard SHELX modules. The experimental values are tabulated in the table below. The refined coordinates have been deposited with Cambridge Crystallographic Data Centre (CCDC).

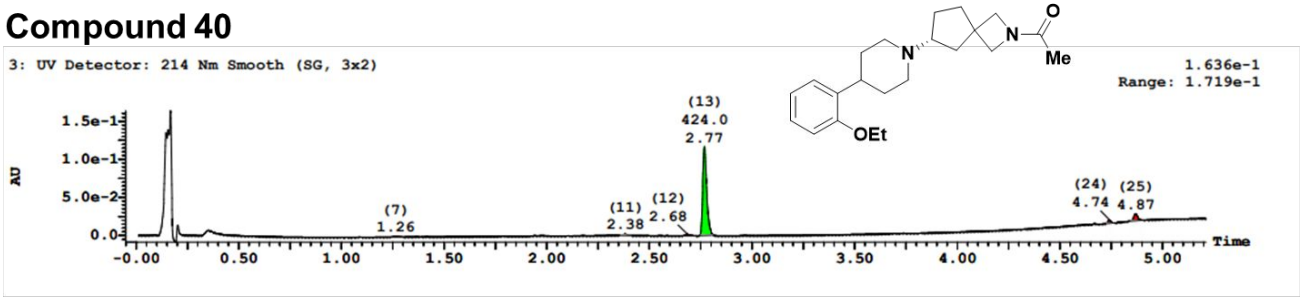
Cpmpound [x-ray ID]	**(S)-5 [DAP06b]**	**10 [VMO04a]**	**12 [VMO05a]**
CCDC deposition code	2285271	2285272	2285273
Empirical formula	C12 H23 Cl N2 O2	C28 H33 Cl2 F N2 O4 S	C11 H21 Cl N2 O3
Formula weight	583.52	262.77	264.75
Solvent(s) of crystallization	Acetonitrile	Dichloromethane / heptane	Acetone
Temperature	100(2) K	100(2) K	100(2) K
Wavelength	1.54178 Å	1.54178 Å	1.54178 Å
Crystal system	monoclinic	monoclinic	orthorhombic
Space group	P21	P21	C2221
Unit cell dimensions	a = 6.082(3) Å	a = 6.2788(2) Å	a = 8.3417(4) Å

	b = 33.338(13) Å c = 13.536(6) Å α = 90.00° β = 90.44(2)° γ = 90.00°	b = 6.0343(2) Å c = 19.8391(8) Å α = 90° β = 93.510(2)° γ = 90°	b = 9.1291(4) Å c = 35.9696(15) Å α = 90° β = 90° γ = 90°
Volume	2744(2) $Å^3$	750.26(5) $Å^3$	2739.2(2) $Å^3$
Z	4	2	8
Density (calculated)	1.412 g/cm^3	1.163 g/cm^3	1.284 g/cm^3
Absorption coefficient	3.214 mm^{-1}	2.209 mm^{-1}	2.483 mm^{-1}
F(000)	1224	284	1136
Crystal size	0.10 x 0.08 x 0.02 mm^3	0.35 x 0.10 x 0.02 mm^3	0.08 x 0.04 x 0.02 mm^3
Theta range for data collection	2.65 to 68.37°	7.281 to 68.074°	2.457 to 68.775°
Index ranges	-7 <= h <= 7 -40 <= k <= 40 -16 <= l <= 16	-7 <= h <= 7 -7 <= k <= 7 -23 <= l <= 23	-10 <= h <= 10 -10 <= k <= 10 -43 <= l <= 43
Reflections collected	58200	19705	37241
Independent reflections	10037 [R(int)=0.0583]	2674 [R(int)=0.0323]	2528 [R(int)=0.1537]
Completeness to theta	Θ = 68.37°, 99.9%	Θ = 68.074°, 98.1 %	Θ = 68.775°, 99.7 %
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents	Semi-empirical from equivalents
Max. and min. transmission	0.9385 and 0.7394	0.7531 and 0.7531	0.7531 and 0.6033
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	10037 / 388 / 738	2674 / 190 / 265	2528 / 0 / 159
Goodness-of-fit on F^2	1.034	1.045	1.074
Final R indices [I>2sigma(I)]	R1 = 0.0352, wR2 = 0.0867	R1 = 0.0354, wR2 = 0.0920	R1 = 0.0497, wR2 = 0.1177
R indices (all data)	R1 = 0.0388, wR2 = 0.0884	R1 = 0.0364, wR2 = 0.0929	R1 = 0.0576, wR2 = 0.1211

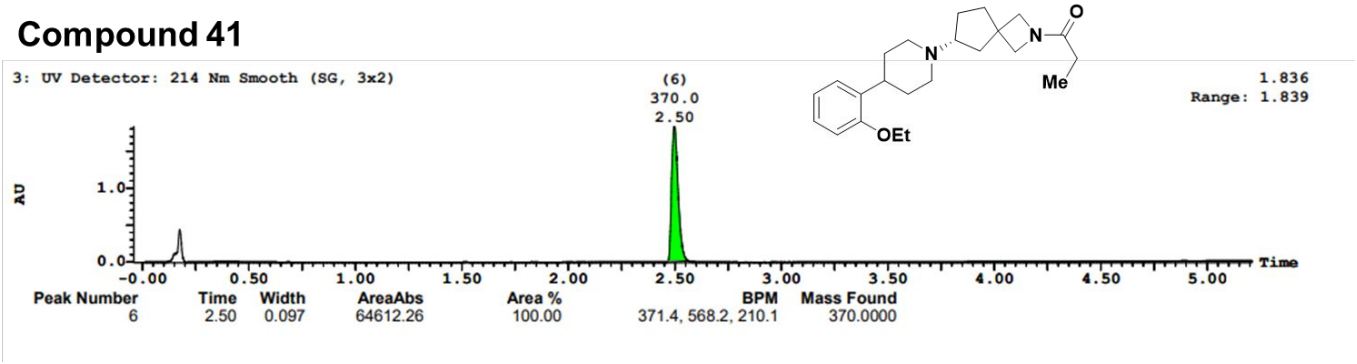
Absolute structure parameter	0.021(8)	0.058(7) using [1123 quotients]	0.037(15) using [847 quotients]
Largest diff. peak and hole	0.71 and -0.34 e·Å^{-3}	0.30 and -0.17 e·Å^{-3}	0.41 and -0.41 e·Å^{-3}

Section 5: LCMS data for compounds tested in vitro

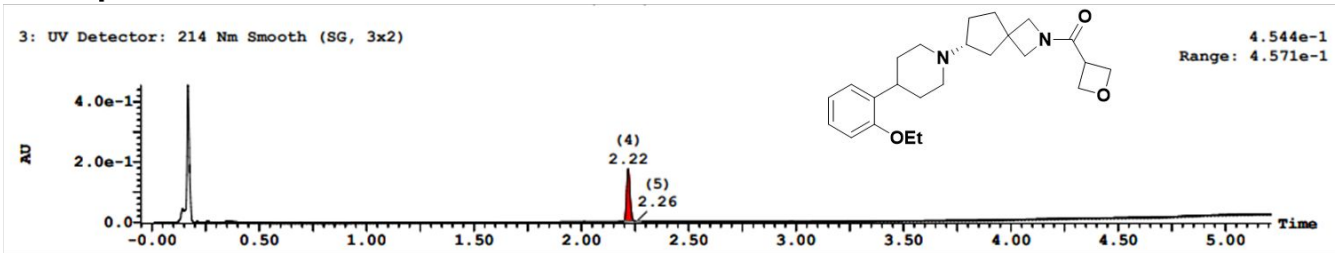
Compound 40



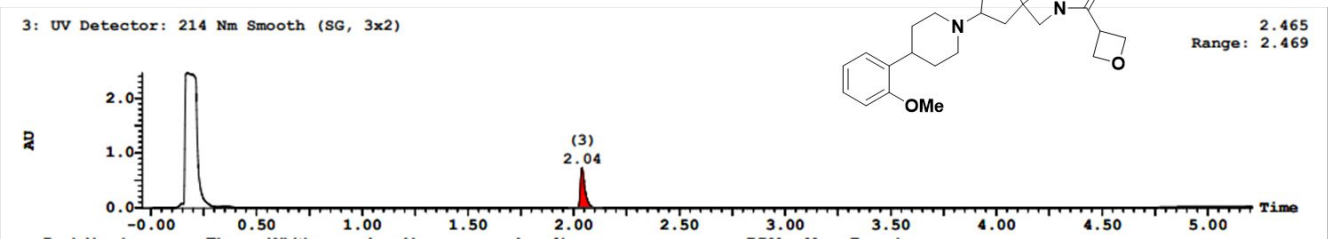
Compound 41



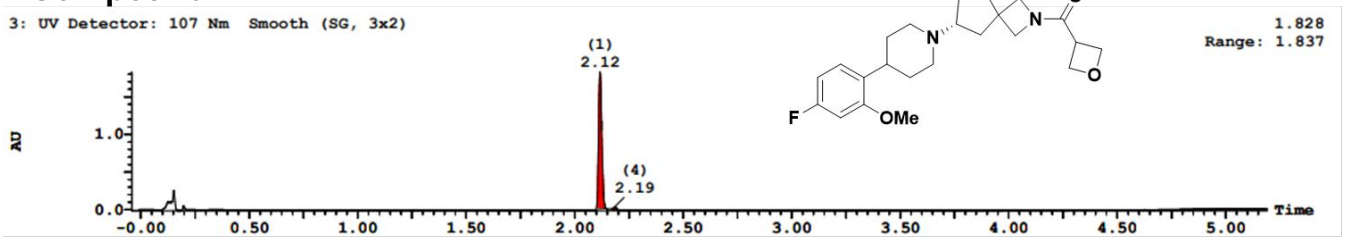
Compound 42



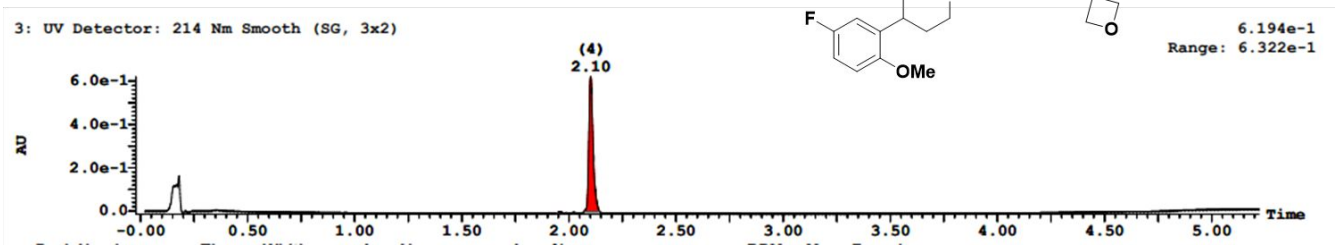
Compound 43



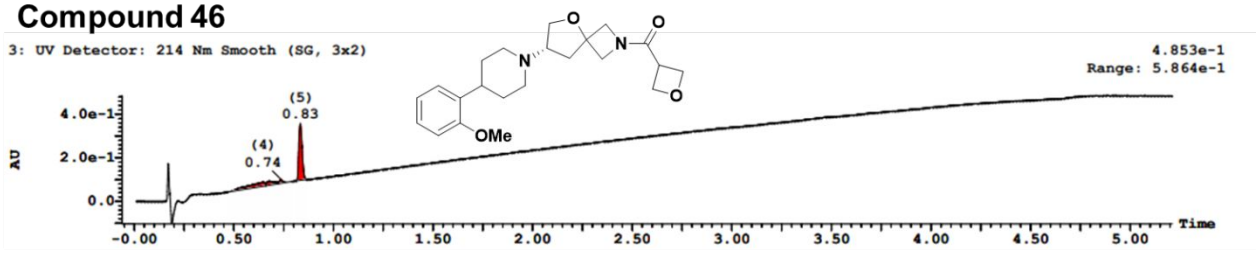
Compound 44



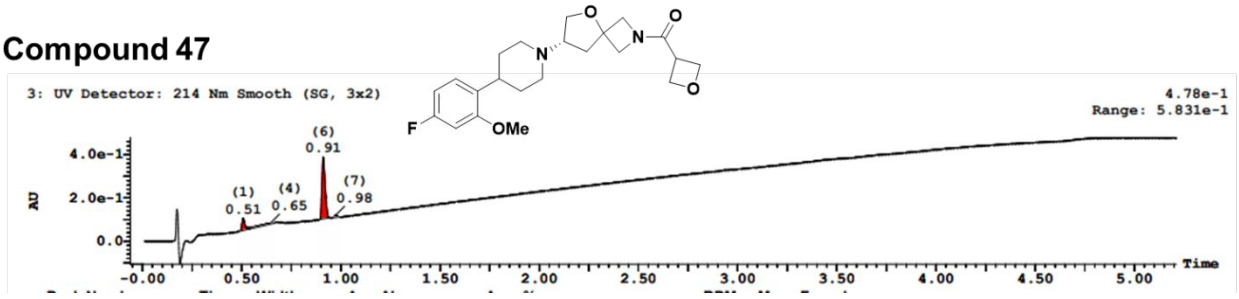
Compound 45



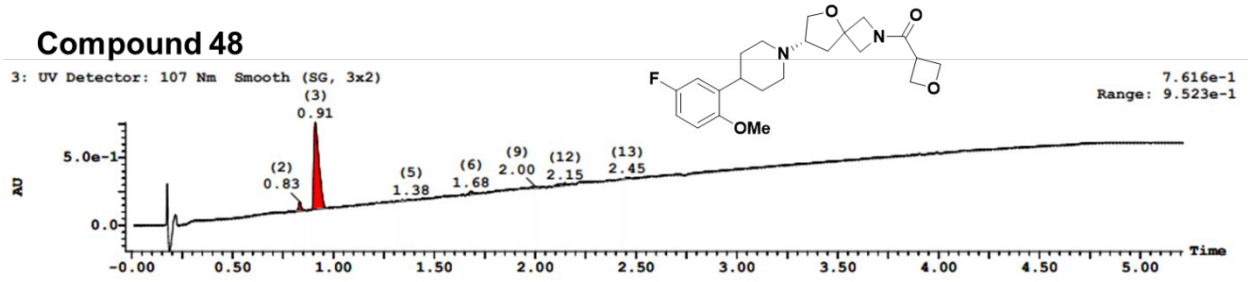
Compound 46



Compound 47



Compound 48



Section 6: Muscarinic FDSS assays

Examples were characterized by measuring the intracellular mobilization of Ca++ ions caused by signaling events mediated by the receptor. The Intra-cellular Calcium flux levels were captured by the highly sensitive Ca++ indicator, Calcium Assay Kit (BD Biosciences) The fluorescent activity the all receptor was monitored by the fluorescent imager, FDSS 7000EX (Hamamatsu) over a span of 3 minutes. The change in Calcium flux was readily captured upon activation with the muscarinic orthosteric agonist, carbachol.

CHRM1 Cell Line Maintenance

Cloned human M1 receptor (CHRM1) was stably expressed in HEK293 cells and were grown and maintained in a monolayer culture with DMEM/High Glucose (Life Technologies) supplemented with 10% Fetal Bovine Serum, 1X Pen-Strep, and 0.5mg/mL Geneticin in a humidified atmosphere (5% CO_2) at 37 °C. The cultures were grown to 90% confluency in T150 flasks (Corning) and washed with 1X DPBS and lifted with 0.05% Trypsin (Life Technologies). The cells were then spun (1K rpm, 3 minutes) and frozen using Recovery Cell Culture Freezing Media (Gibco Technologies). Cells were stored in liquid nitrogen and thawed a day before the assay.

CHRM2 Cell Line Maintenance

CHO-K1 cells stably expressing the human cloned CHRM2 receptor (M2_CHO cells) were grown and maintained in a monolayer culture with F12/HAM (Life Technologies) supplemented with 10% Fetal Bovine Serum, 1X Pen-Strep, and 0.4mg/mL Geneticin in a humidified atmosphere (5% CO_2) at 37 °C. The cultures were grown to 80-90% confluency in T150 flasks (Corning) and washed with 1X DPBS and lifted with 0.05% Trypsin (Life Technologies). The cells were then spun (1K rpm, 3 minutes) and frozen using Recovery Cell Culture Freezing Media (Gibco Technologies). Cells were stored in liquid nitrogen and thawed a day before the assay.

CHRM3 and CHRM5 Cell Line Maintenance

CHO-K1 cells stably expressing the human cloned CHRM3 receptor (M3_CHO cells) were grown and maintained in a monolayer culture with F12/HAM (Life Technologies) supplemented with 10% Fetal Bovine Serum, 1X Pen-Strep, and 0.4mg/mL Geneticin in a humidified atmosphere (5% CO_2) at 37 °C. The cultures were grown to 80-90% confluency in T150 flasks (Corning) and washed with 1X DPBS and lifted with 0.05% Trypsin (Life Technologies). The cells were then spun (1K rpm, 3 minutes) and frozen using Recovery Cell Culture Freezing Media (Gibco Technologies). Cells were stored in liquid nitrogen and thawed a day before the assay. A similar procedure was used for cells stably expressing the human cloned CHRM5 receptor (M5_CHO).

CHRM4 Cell Line Maintenance

CHO-K1 cells stably expressing the human cloned CHRM4 receptor (M4_CHO cells) were grown and maintained in a monolayer culture with F12/HAM (Life Technologies) supplemented with 10% Fetal Bovine Serum, 1X Pen-Strep, and 0.4mg/mL Geneticin in a humidified atmosphere (5% CO_2) at 37 °C. The cultures were grown to 80-90% confluency in T150 flasks (Corning) and washed with 1X DPBS then lifted with

0.05% Trypsin (Life Technologies). The cells were harvested in growth media then spun (1K rpm, 3 minutes) and cryopreserved using Recovery Cell Culture Freezing Media (Gibco Technologies). Cells were stored in liquid nitrogen and thawed a day before the assay.

CHRM1 Ca^{++} Flux Assay

Prior to the day of the assay, stable HEK293 M1 cells were thawed and plated on 384 well black walled clear bottom TC treated plates (Greiner Cat#781091) at 25K cells/well with DMEM/High Glucose supplemented with 10% FBS (Hyclone) Pen-Strep (Life Technologies) and kept overnight in a humidified atmosphere (5% CO_2) at 37 °C. The following day, cells were loaded with 20 µL Ca^{++} dye (BD Biosciences) using Loading Buffer (HBSS +Ca/+Mg, 20 mM HEPES) and placed back in cell incubator for a minimum of 1 hour. The dye was replaced with 45 µL Assay Buffer (HBSS -Ca/-Mg, 20 mM HEPES) and kept at room temperature prior to running on cell imager. Compounds were prepared in Assay Buffer and 5 µL was added to the cells. The FDSS 7000EX (Hamamatsu) was used to acquire Ca^{++} traces for 3 minutes from cells treated with 11 point dose in triplicate in order to generate dose response curves in agonist mode. All compounds were serially diluted in DMSO then prepared in Assay Buffer for Ca^{++} flux studies. The dose response curves were generated from the average of triplicate wells obtained from each data point and used a non-linear regression of four parameter dose response algorithm. The Percent Activity (PA) was measured to EC_{100} of Carbachol.

CHRM2 Ca^{++} Flux Assay

Prior to the day of the assay, stable M2 CHO cells were thawed and plated on Greiner 384 well TC treated plate at a density of 12K cells/well and kept overnight in a humidified atmosphere (5% CO_2) at 37 °C. The following day, the cells were loaded with Ca^{++} dye (BD Biosciences) using Loading Buffer (HBSS +Ca/+Mg, 20 mM HEPES, 2.5 mM Probenecid) and placed back in cell incubator for a minimum of 1 hour and maximum of 2 hours. After incubation, the dye was replaced with Assay Buffer (HBSS -Ca/-Mg, 20 mM HEPES, 2.5mM Probenecid) supplemented with 20 µM ATP (Sigma Aldrich) and kept at room temperature for 60 minutes prior before running on cell imager. The FDSS 7000EX (Hamamatsu) was used to acquire Ca^{++} traces from cells in response to compound treatment and the data was used to generate dose response curves in agonist mode. All compounds were serially diluted in DMSO then prepared in Assay Buffer for Ca^{++} flux studies. The dose response curves were generated from the average of triplicate wells obtained from each data point and used a non-linear regression of four parameter dose response algorithm. The Percent Activity (PA) was measured to EC_{100} of Carbachol.

CHRM3 and CHRM5 Ca^{++} Flux Assay

Prior to the day of the assay, stable M3_CHO or M5_CHO cells were thawed and plated on Greiner 384 well black TC treated plates at 12K cells/well in F12/DMEM supplemented with 10% FBS (Hyclone) and kept overnight in a humidified atmosphere (5% CO_2) at 37 °C. The next day, cells were loaded with Ca^{++} dye (BD Biosciences) using Loading Buffer (HBSS +Ca/+Mg, 20 mM HEPES, 2.5 mM Probenecid) and placed back in cell incubator for a minimum of 1 hour. After incubation, the dye was replaced with Assay Buffer (HBSS -Ca/-Mg, 20 mM HEPES, 2.5 mM Probenecid) and kept at room temperature in the dark

before running on cell imager. The FDSS 7000EX (Hamamatsu) was used to acquire Ca^{++} traces from cells treated with 11 point dose response of compounds in triplicate in order to generate dose response curves in agonist mode. All compounds were serially diluted in DMSO then prepared in Assay Buffer for Ca^{++} flux studies. The dose response curves were generated from the average of triplicate wells obtained from each data point and used a non-linear regression of four parameter dose response algorithm. The Percent Activity (PA) was measured to EC_{100} of Carbachol.

CHRM4 Ca^{++} Flux Assay

Prior to the day of the assay, stable M4_CHO cells were thawed and plated on 384 well black walled clear bottom TC treated plates (Greiner Cat#781091) at 12K cells/well using F12/HAM Media supplemented with 10% FBS (Life Technologies) and kept overnight in a humidified atmosphere (5% CO_2) at 37 °C. The next day, cells were loaded with 20 µL Ca^{++} dye (BD Biosciences) using Loading Buffer (HBSS +Ca/+Mg, 20mM HEPES, 2.5 mM Probenecid) and placed back in cell incubator for a minimum of 1 hour. After incubation, the dye was replaced with 45 µL Assay Buffer (HBSS -Ca/-Mg, 20mM HEPES, 2.5mM Probenecid) supplemented with 20 µM ATP (Sigma Aldrich) and kept at room temperature in the dark for 60 minutes before running on a cell imager. The FDSS 7000EX (Hamamatsu) was used to capture Ca^{++} traces for a span of 3 minutes from cells treated with 11 point dose of compound in triplicate in order to generate dose response curves in agonist mode. All compounds were serially diluted in DMSO then prepared in Assay Buffer for Ca^{++} flux studies. The dose response curves were generated from the average of triplicate wells obtained from each data point and used a non-linear regression of four parameter dose response algorithm. The Percent Activity (PA) was measured to EC_{100} of Carbachol.